STATE OF MAINE
DEPARTMENT OF ENVIRONMENTAL PROTECTION

IN THE MATTER OF

ARIES PINE TREE, LLC SANFORD, YORK COUNTY AIR EMISSION LICENSE APPLICATION (A-1195-71-A-N) AND SOLID WASTE PROCESSING FACILITY LICENSE APPLICATION (S-100033)	**APPENDIX TO PRE-FILED TESTIMONY OF KYLE DOUDRICK, PH.D., P.E.**

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NOTICE OF IDLING OF OPERATIONS

UNION COUNTY IMPROVEMENT AUTHORITY

Solid Waste Disposal Revenue Bonds
(Aries Linden, LLC Project), Series 2019 (AMT) (Green Bonds)
CUSIPS-906352AA7*, 906352AB5*

Solid Waste Disposal Subordinated Revenue Bonds
(Aries Linden, LLC Project), Series 2021 (AMT) (Green Bonds)
CUSIP -960352AC3*

Solid Waste Disposal Subordinated Revenue Bonds
(Aries Linden, LLC Project), Series 2023 (AMT)
CUSIP -960352AE9*

Solid Waste Disposal Subordinated Revenue Bonds
(Aries Linden, LLC Project), Series 2024 (AMT)
CUSIP -960352AF6*

Commencing on November 20, 2025, Aries Linden, LLC (the "Company") began the implementation of a plan to "idle" operations at its plant in Linden, New Jersey by terminating the employment of substantially all of its employees at the plant. Pursuant to its plan, the Company anticipates that the plant will be in "idle" status by December 31, 2025.

In the past several years, the Company and its sponsors have invested significant amounts of money into the plant with the goal of achieving sustained routine continuous operations. Although the Company has made significant progress advancing the plant, there is still a significant amount of repair work that needs to be done before it can achieve sustained continuous operations. Over the past year, the Company has been attempting to raise additional funding from outside investors to help support the necessary repairs of the plant, but, despite its efforts, the Company has been unable to attract new investors, and the Company is running out of money to sustain operations at the plant. Accordingly, the Company needs to idle all operations at the plant to conserve cash and buy time to work on potentially restructuring its debt and completing a plan for repair work to the plant to permit sustained continuous operations.

Given that operations at the plant will be in "idle" status, the Company has not prepared or filed, and will not be preparing or filing, with the Trustee for the Bonds or on EMMA, any operating and/or capital budgets for the plant for fiscal year 2026, as required by the Loan Agreements and the Continuing Disclosure Agreements relating to the Bonds.

ARIES LINDEN, LLC

Dated: December 4, 2025

* No representation is made as to the accuracy of the CUSIP numbers which are included solely for the convenience of the holders of the Bonds.

Toxicological Profile for Perfluoroalkyls

Released May 2021

Last Updated March 2020

U.S. Department of Health and Human Services
Agency for Toxic Substances and Disease Registry

KD0002 TS274127-A

DISCLAIMER

Use of trade names is for identification only and does not imply endorsement by the Agency for Toxic Substances and Disease Registry, the Public Health Service, or the U.S. Department of Health and Human Services.

FOREWORD

This toxicological profile is prepared in accordance with guidelines* developed by the Agency for Toxic Substances and Disease Registry (ATSDR) and the Environmental Protection Agency (EPA). The original guidelines were published in the *Federal Register* on April 17, 1987. Each profile will be revised and republished as necessary.

The ATSDR toxicological profile succinctly characterizes the toxicologic and adverse health effects information for these toxic substances described therein. Each peer-reviewed profile identifies and reviews the key literature that describes a substance's toxicologic properties. Other pertinent literature is also presented, but is described in less detail than the key studies. The profile is not intended to be an exhaustive document; however, more comprehensive sources of specialty information are referenced.

The focus of the profiles is on health and toxicologic information; therefore, each toxicological profile begins with a relevance to public health discussion which would allow a public health professional to make a real-time determination of whether the presence of a particular substance in the environment poses a potential threat to human health. The adequacy of information to determine a substance's health effects is described in a health effects summary. Data needs that are of significance to the protection of public health are identified by ATSDR.

Each profile includes the following:

(A) The examination, summary, and interpretation of available toxicologic information and epidemiologic evaluations on a toxic substance to ascertain the levels of significant human exposure for the substance due to associated acute, intermediate, and chronic exposures;

(B) A determination of whether adequate information on the health effects of each substance is available or in the process of development to determine levels of exposure that present a significant risk to human health of acute, intermediate, and chronic health effects; and

(C) Where appropriate, identification of toxicologic testing needed to identify the types or levels of exposure that may present significant risk of adverse health effects in humans.

The principal audiences for the toxicological profiles are health professionals at the Federal, State, and local levels; interested private sector organizations and groups; and members of the public.

This profile reflects ATSDR's assessment of all relevant toxicologic testing and information that has been peer-reviewed through September 2018. New studies were added in 2019 following public comment, and NHANES data were updated. Staff from the Centers for Disease Control and Prevention and other Federal scientists have also reviewed the profile. In addition, this profile has been peer-reviewed by a nongovernmental panel and was made available for public review. Final responsibility for the contents and views expressed in this toxicological profile resides with ATSDR.



Patrick N. Breysse, Ph.D., CIH
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*Legislative Background

The toxicological profiles are developed under the Comprehensive Environmental Response, Compensation, and Liability Act of 1980, as amended (CERCLA or Superfund). CERCLA section 104(i)(1) directs the Administrator of ATSDR to "…effectuate and implement the health related authorities" of the statute. This includes the preparation of toxicological profiles for hazardous substances most commonly found at facilities on the CERCLA National Priorities List (NPL) and that pose the most significant potential threat to human health, as determined by ATSDR and the EPA. Section 104(i)(3) of CERCLA, as amended, directs the Administrator of ATSDR to prepare a toxicological profile for each substance on the list. In addition, ATSDR has the authority to prepare toxicological profiles for substances not found at sites on the NPL, in an effort to "…establish and maintain inventory of literature, research, and studies on the health effects of toxic substances" under CERCLA Section 104(i)(1)(B), to respond to requests for consultation under section 104(i)(4), and as otherwise necessary to support the site-specific response actions conducted by ATSDR.

VERSION HISTORY

Date	Description
May 2021	Final toxicological profile released
March 2020	Toxicological profile last updated
June 2018	Draft for public comment toxicological profile released
August 2015	Draft for public comment toxicological profile released
May 2009	Draft for public comment toxicological profile released

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ATSDR scientists review peer reviewers' comments and determine whether changes will be made to the profile based on comments. The peer reviewers' comments and responses to these comments are part of the administrative record for this compound.

The listing of peer reviewers should not be understood to imply their approval of the profile's final content. The responsibility for the content of this profile lies with ATSDR.

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CHAPTER 1. RELEVANCE TO PUBLIC HEALTH

This toxicological profile on perfluoroalkyls discusses information on 10 perfluoroalkyls that have been measured in the serum collected from a representative U.S. population 12 years of age and older in the National Health and Nutrition Examination Survey (NHANES) 2003–2004 (Calafat et al. 2007b), as well as 2 compounds (PFBA and PFHxA) that have been identified in other monitoring studies. More recent NHANES monitoring studies have not evaluated additional perfluoroalkyl compounds (CDC 2019). The perfluoroalkyl compounds discussed in the profile include:

Compound	Acronym	CAS Registry Number
Perfluorobutanoic acid	PFBA	375-22-4
Perfluorohexanoic acid	PFHxA	307-24-4
Perfluoroheptanoic acid	PFHpA	375-85-9
Perfluorooctanoic acid	PFOA	335-67-1
Perfluorononanoic acid	PFNA	375-95-1
Perfluorodecanoic acid	PFDA	335-76-2
Perfluoroundecanoic acid	PFUnA	2058-94-8
Perfluorododecanoic acid	PFDoDA	307-55-1
Perfluorobutane sulfonic acid	PFBS	375-73-5
Perfluorohexane sulfonic acid	PFHxS	355-46-4
Perfluorooctane sulfonic acid	PFOS	1763-23-1
Perfluorooctane sulfonamide	FOSA	754-91-6

Perfluoroalkyls can exist in several ionic forms, most commonly as the anionic form or acidic form. In the environment, perfluoroalkyls are found in the anionic form (ITRC 2017). The names for the anionic and acidic forms (e.g., perfluorooctanoate and perfluorooctanoic acid) are often used interchangeably even though there are differences in physical and chemical properties and behavior in the environment, and the same acronym is used for both forms (e.g., PFOA). ATSDR has opted to the utilize the same terminology as NHANES (i.e., the acidic form names).

The term “perfluoroalkyls” used throughout the toxicological profile is referring to at least one of these 12 compounds and the information may not be applicable to other perfluoroalkyl compounds.

1.1 OVERVIEW AND U.S. EXPOSURES

The perfluoroalkyls discussed in this profile primarily consist of perfluorinated aliphatic carboxylic acids (PFCAs) and perfluorinated aliphatic sulfonic acids (PFSAs). These substances have been used extensively in surface coating and protectant formulations due to their unique surfactant properties (Kissa

2001; Schultz et al. 2003). Major applications have included protectants for paper and cardboard packaging products, carpets, leather products, and textiles that enhance water, grease, and soil repellency (3M 1999; Hekster et al. 2003; Kissa 2001; Schultz et al. 2003), and in firefighting foams (Schultz et al. 2003). Perfluoroalkyls such as PFOA have also been used as processing aids in the manufacture of fluoropolymers such as nonstick coatings on cookware (DuPont 2008; EPA 2008a).

Perfluoroalkyls are human-made substances that do not occur naturally in the environment. The perfluoroalkyl substances discussed in this profile, especially PFOS and PFOA, have been detected in air, water, and soil in and around fluorochemical facilities. However, these industrial releases have been declining since eight companies began voluntarily phasing out the production and use of several perfluoroalkyls in the early 2000s (3M 2007b, 2008a, 2008b; Barton et al. 2007; Davis et al. 2007; DuPont 2008; EPA 2007a, 2008a, 2016a). PFOA and PFOS may still be produced domestically, imported, and used by companies not participating in the PFOA Stewardship program. Under the Toxic Substances Control Act (TSCA), EPA has proposed a significant new use rule (SNUR) for long-chain perfluoroalkyl carboxylate (LCPFAC) chemical substances and sulfonates to ensure that the manufacture, import, or processing of LCPFAC chemical substances for any discontinued uses cannot begin without EPA review. EPA essentially excluded the use or import of all LCPFAC chemical substances by proposing a SNUR for LCPFACs and sulfonates (EPA 2015). Data are becoming more available regarding current releases of shorter-chain perfluoroalkyls (perfluorinated carboxylic acids with six or fewer carbons and perfluorosulfonic acids with five or fewer carbons) that are now being used in surface treatment products or perfluoropolyethers that are used as a replacement for PFOA in emulsion polymerization. Environmental fate and toxicity research of newer replacement substances is ongoing (De Silva et al. 2016; Gomis et al. 2018; Kabore et al. 2018).

In the environment, some of the perfluoroalkyls discussed in this profile can also be formed from environmental degradation of precursor compounds released during the manufacture and use of consumer products containing perfluoroalkyls (D'eon and Mabury 2007; D'eon et al. 2009; Martin et al. 2006; Prevedouros et al. 2006). Under the PFOA Stewardship Program with the U.S. Environmental Protection Agency (EPA), eight major fluoropolymer producers have phased out PFOA, precursor substances that can degrade to long-chain perfluoroalkyls such as PFOA, and higher homologues from emissions and products (EPA 2008a, 2016a).

1. RELEVANCE TO PUBLIC HEALTH

Due to the strength of the carbon-fluorine bonds, perfluoroalkyls are very stable in the environment and are resistant to biodegradation, photooxidation, direct photolysis, and hydrolysis (3M 2000; EPA 2008a; OECD 2002, 2007; Schultz et al. 2003). The perfluoroalkyl carboxylic acids and sulfonic acids have very low volatility due to their ionic nature (Kissa 2001; Prevedouros et al. 2006; SPARC 2008). As a group, perfluoroalkyls are persistent in soil and water (3M 2000; Prevedouros et al. 2006). Perfluoroalkyls are mobile in soil and leach into groundwater (Davis et al. 2007). Volatile fluorotelomer alcohols may be broken down into substances like PFOA, and atmospheric deposition can lead to contamination of soils and leaching into groundwater away from point sources. Perfluoroalkyls have been detected in many parts of the world, including oceans and the Arctic, indicating that long-range transport is possible (Armitage et al. 2006; Barber et al. 2007; Prevedouros et al. 2006; Wania 2007; Wei et al. 2007a; Yamashita et al. 2005, 2008).

Perfluoroalkyls have been detected in all environmental media including air, surface water, groundwater (including drinking water), soil, and food. Human exposure may occur from all of these media. Contaminated drinking water led to increased levels of exposure to PFOA, PFOS, and other perfluoroalkyls for some populations residing near fluoropolymer manufacturing facilities (ATSDR 2008; Emmett et al. 2006a; Steenland et al. 2009b). Median PFOA serum levels (measured in 2005–2006) of 45,276 non-occupationally exposed individuals residing in southeastern Ohio and West Virginia who were exposed to PFOA via contaminated drinking water (Shin et al. 2011b) were approximately 6 times greater than the median serum PFOA concentration in a representative sample of the U.S. general population (2005–2006 NHANES data; CDC 2018). Serum levels of PFOA and PFOS in the general population of the United States have sharply declined in recent years as U.S. production of these substances ceased (CDC 2019). For example, the geometric mean concentrations of PFOA and PFOS in the general population were 5.2 and 30.4 ng/mL (ppb), respectively, in 1999–2000; in 2015–2016, PFOA declined by 70% to 1.56 ng/mL and PFOS declined 84% to 4.72 ng/mL (CDC 2018, 2019).

Based on environmental measurements and theoretical models, one study has proposed that the major exposure pathways for PFOS for the general population in Europe and North America are food and water ingestion, dust ingestion, and hand-to-mouth transfer from mill-treated carpets (Trudel et al. 2008). For PFOA, major exposure pathways were proposed to be oral exposure resulting from migration from paper packaging and wrapping into food, general food and water ingestion, inhalation from impregnated clothes, and dust ingestion. This includes exposure to 8:2 fluorotelomer alcohol in food packaging and air, which can be broken down into PFOA. PFOS and PFOA exposure pathways are proposed to be similar for children except that exposure from hand-to-mouth transfer from treated carpets is expected to

be much greater in children. Based on these exposure pathways, adult uptake doses estimated for high-exposure scenarios were approximately 30 and 47 ng/kg/day for PFOS and PFOA, respectively (Trudel et al. 2008). PFOS and PFOA doses estimated for children under the age of 12 under high exposure scenarios were 101–219 and 65.2–128 ng/kg/day, respectively. Since PFOA and PFOS are no longer produced or used in the United States, current exposure levels may be lower than those predicted by Trudel et al. (2008). A study by Vestergren and Cousins (2009) evaluated potential exposure to perfluorocarboxylate homologues for different populations and also concluded that dietary intake was the primary background exposure pathway for the general population, while inhalation of indoor air was the main exposure pathway for occupationally exposed individuals with estimated intakes >150 ng/kg/day. Although not well studied, the available absorption data (Fasano et al. 2005; Franko et al. 2012) suggest that dermal contact may also contribute to the overall perfluoroalkyl body burden.

Perfluoroalkyls have been detected in human breast milk and umbilical cord blood. The reported maximum concentrations of PFOS and PFOA measured in human breast milk samples from women living in Massachusetts (samples were collected in 2004) were 0.617 and 0.161 ng/mL, respectively (Tao et al. 2008b). Maximum concentrations of other perfluoroalkyls were <0.06 ng/mL. In most umbilical cord samples collected in 2004–2005 in Maryland, the maximum concentrations of PFOS and PFOA were 34.8 and 7.1 ng/mL, respectively (Apelberg et al. 2007a, 2007b). Other perfluoroalkyls have been detected less frequently.

1.2 SUMMARY OF HEALTH EFFECTS

The toxicity of PFOA and PFOS has been evaluated in a large number of studies of humans and laboratory animals; less toxicity data are available for other perfluoroalkyls. However, comparison of the toxicity of perfluoroalkyls across species is problematic due to differences in elimination half-lives, lack of adequate mechanistic data, species differences in the mechanism of toxicity for some endpoints, and differences in measurement of exposure levels between epidemiological and experimental studies. Table 1-1 lists half-lives for PFOA, PFOS, PFHxS, PFNA, PFBS, and PFBA for human, nonhuman primates, rats, and mice to illustrate some of the species differences. For example, for PFOA, the estimated elimination half-life is measured in years in humans and in hours in female rats.

Table 1-1. Summary of Estimated Elimination Half-lives for Select Perfluoroalkyls[a]

	Humans	Nonhuman primates	Rats	Mice
PFOA	2.1–10.1 years	20.1–32.6 days	Males: 44–322 hours Females: 1.9–16.2 hours	
PFOS	3.3–27 years	110–170 days	179–1,968 hours	731–1,027 hours
PFHxS	4.7–35 years	87–141 days	Males: 382–688 hours Females: 1.03–41.28 hours	597–643 hours
PFNA	2.5–4.3 years		Males: 710–1,128 hours Females: 33.6–58.6 hours	619.2– 1,653 hours
PFBS	665 hours	8.0–95.2 hours	2.1–7.42 hours	
PFBA	72–81 hours	40.3–41.0 hours	1.03–9.22 hours	2.79–13.34 hours

[a]See Table 3-5 for additional information and citations.

The mechanisms of toxicity of perfluoroalkyls have not been fully elucidated. There is strong evidence that many of the adverse effects observed in laboratory animals involve the activation of peroxisome proliferator-activated receptor-α (PPARα), which can mediate a broad range of biological responses (Issemann and Green 1990). There are species differences in the activation of PPARα; rats and mice are the most sensitive species and guinea pigs, nonhuman primates, and humans are less responsive. Although humans are less responsive to PPARα agonists, they do have functional PPARα. This may explain some of the species differences in perfluoroalkyl toxicity. PPARα-dependent mechanisms have been associated with a variety of effects, including hepatocellular hypertrophy, alterations in lipid metabolism, decreased pup survival, and some immune effects. However, there is evidence that PPARα-independent mechanisms are also involved in PFOA and PFOS toxicity, including liver and immune toxicity; it is not known if species differences exist for these mechanisms. In general, epidemiological studies use serum perfluoroalkyl levels as a biomarker of exposure, which contrasts with experimental studies that utilize dose, expressed in mg/kg body weight/day units, or air concentrations as the dose metric. Although physiologically based pharmacokinetic (PBPK) models have been developed for rodents and humans, these models are not sufficient to allow for direct comparisons between administered doses in laboratory animals and serum concentrations in humans.

***Effects in Humans*.** Perfluoroalkyls have been detected in the serum of workers, residents living near perfluoroalkyl facilities, and the general population. A large number of epidemiological studies have evaluated possible associations between perfluoroalkyl exposure and a wide range of adverse health outcomes. However, most of the studies have focused on PFOA and/or PFOS; fewer studies have evaluated a smaller number of potential health outcomes for the remaining 10 perfluoroalkyls included in

this toxicological profile. Most of the epidemiological studies lack exposure monitoring data, and there is a potential for multiple routes of exposure (inhalation and oral); however, most of the studies used serum perfluoroalkyl level as a biomarker of exposure. The three primary sources of this information are occupational exposure studies, studies of communities living near a PFOA manufacturing facility with high levels of PFOA in the drinking water, and studies of populations exposed to background levels of perfluoroalkyls (referred to as general population studies). In the studies examined, workers have the highest potential exposure to a specific perfluoroalkyl, followed by the highly-exposed residents such as residents in the Mid-Ohio Valley who have elevated levels of PFOA and background levels of other perfluoroalkyls, and then the general population. In one study of workers at the Washington Works facility in West Virginia, the arithmetic mean serum PFOA level in 2001–2004 was 1,000 ng/mL (Sakr et al. 2007a); the arithmetic mean PFOA level in highly-exposed residents (without occupational exposure) near this facility was 423 ng/mL in 2004–2005 (Emmett et al. 2006a). By comparison, the arithmetic mean concentration of PFOA in the U.S. population was 4.91 ng/mL in 2005–2006 (calculated by ATSDR from NHANES data reported in CDC 2013). Although a large number of epidemiological studies have examined the potential of perfluoroalkyls to induce adverse health effects, most of the studies are cross-sectional in design and do not establish causality. Based on a number of factors (described in Section 2.1), the available epidemiological studies suggest associations between perfluoroalkyl exposure and several health outcomes; however, cause-and-effect relationships have not been established for these outcomes:

- Pregnancy-induced hypertension/pre-eclampsia (PFOA, PFOS)
- Increases in serum hepatic enzymes, particularly alanine aminotransferase (ALT), and decreases in serum bilirubin levels (PFOA, PFOS, PFHxS)
- Increases in serum lipids, particularly total cholesterol and low-density lipoprotein (LDL) cholesterol (PFOA, PFOS, PFNA, PFDA)
- Decreased antibody response to vaccines (PFOA, PFOS, PFHxS, PFDA)
- Small (<20-g or 0.7-ounce decrease in birth weight per 1 ng/mL increase in either PFOA or PFOS blood level) decreases in birth weight (PFOA, PFOS)

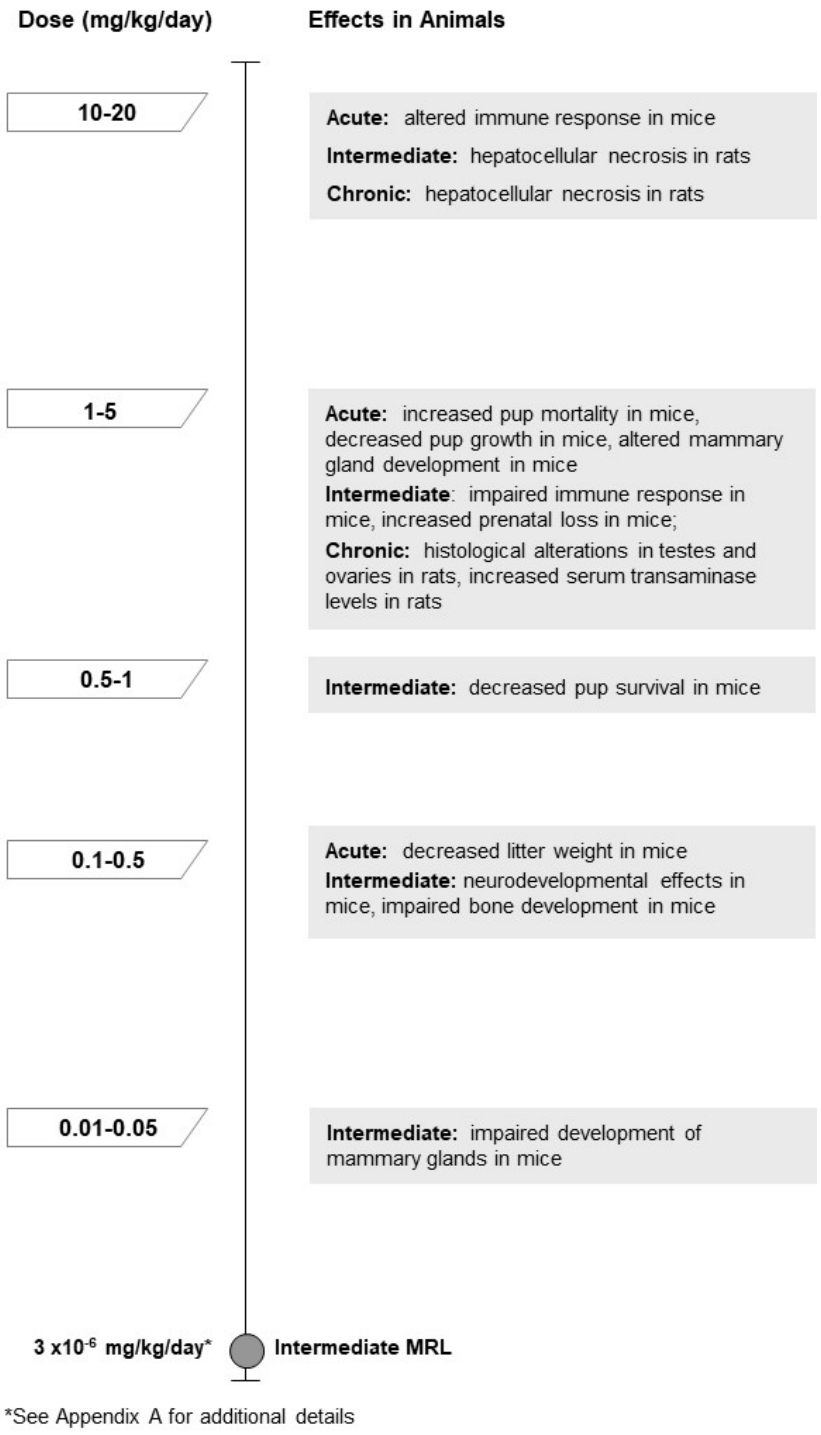
The International Agency for Research on Cancer (IARC 2017) concluded that PFOA is possibly carcinogenic to humans (Group 2B), and EPA (2016e, 2016f) concluded that there was suggestive evidence of the carcinogenic potential of PFOA and PFOS in humans. Increases in testicular and kidney cancer have been observed in highly exposed humans.

There is also some suggestive evidence for associations between perfluoroalkyls and additional health outcomes; there is less certainty in these associations due to inconsistencies across studies and/or a smaller number of studies examining a specific outcome. These health outcomes include osteoarthritis in women under 50 years of age (PFOA, PFOS) and decreased antibody response to vaccines (PFNA, PFUnA, PFDoDA). Additionally, associations between serum PFOA and PFOS and decreases in glomerular filtration rate and increases in serum uric acid levels and between serum PFOA, PFOS, PFHxS, and PFNA and increased risk of early menopause have been observed; these effects may be due to reverse causation, where the effect (disease) causes the change in serum perfluoroalkyl levels (exposure).

Effects in Laboratory Animals. Most of the information regarding the effects of perfluoroalkyls in animals is derived from oral studies; considerably less information is available from inhalation and dermal exposure studies. PFOA and PFOS are the most studied perfluoroalkyls, with considerably less data for the other compounds. Of the 233 animal studies reviewed in this toxicological profile, 42% examined PFOA, 31% examined PFOS, and 27% examined other perfluoroalkyls (8 studies on PFHxS, 17 studies on PFNA, 1 study on PFUnA, 5 studies on PFBS, 6 studies on PFBA, 9 studies on PFDA, 8 studies on PFDoDA, 1 study on FOSA, and 8 studies on PFHxA). The primary effects observed in rats and mice exposed to perfluoroalkyls are liver toxicity, developmental toxicity, and immune toxicity (see Figures 1-1, 1-2, and 1-3); not all of these effects have been observed or examined for all perfluoroalkyls. Based on limited data, the toxicity of perfluoroalkyls does not appear to be specific to the route of administration. It should be noted that, for the most part, adverse health effects in studies in animals have been associated with exposure concentrations or doses that resulted in blood levels of perfluoroalkyls that were significantly higher than those reported in perfluoroalkyl workers or in the general population. Furthermore, there are profound differences in the toxicokinetics of perfluoroalkyls between humans and experimental animals. The elimination $t_{1/2}$ of PFOA is approximately 4 years in humans compared with days or hours in rodents. These factors, plus issues related to the mode of action of perfluoroalkyls (see below), make it somewhat difficult at this time to determine the true relevance of some effects reported in animal studies to human health.

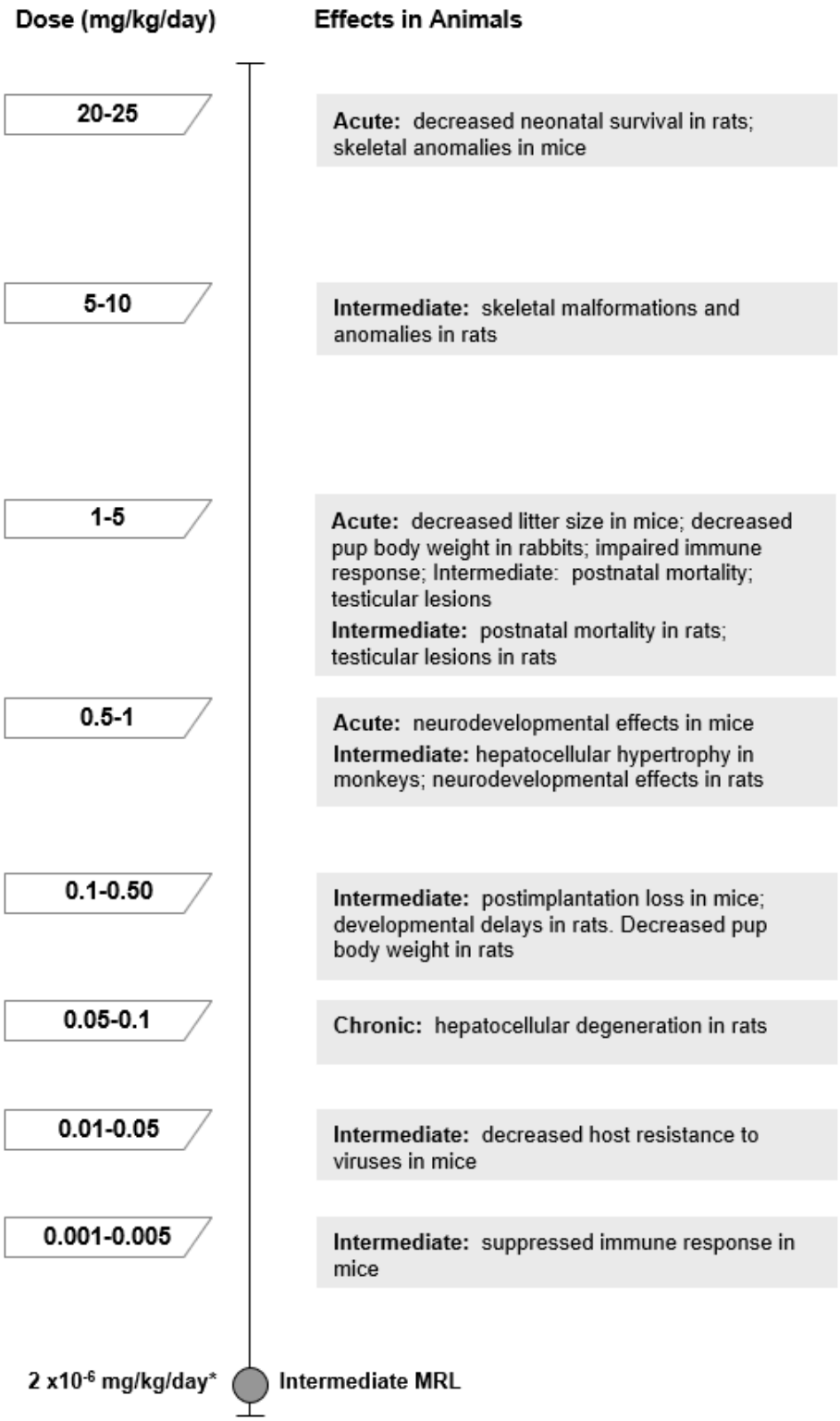
Liver Effects. Many studies have described morphological and biochemical alterations in the liver from rodents following acute and longer-term oral exposure to PFOA. Some of the effects observed in rats include increases in liver weight, hepatocellular hypertrophy, and decreases in serum cholesterol and triglyceride levels (e.g., Butenhoff et al. 2004b; Liu et al. 1996; Pastoor et al. 1987; Yang et al. 2001; see Section 2.9 for a complete list of citations). The observed hepatomegaly and hypertrophy are likely due

Figure 1-1. Health Effects Found in Animals Following Oral Exposure to PFOA



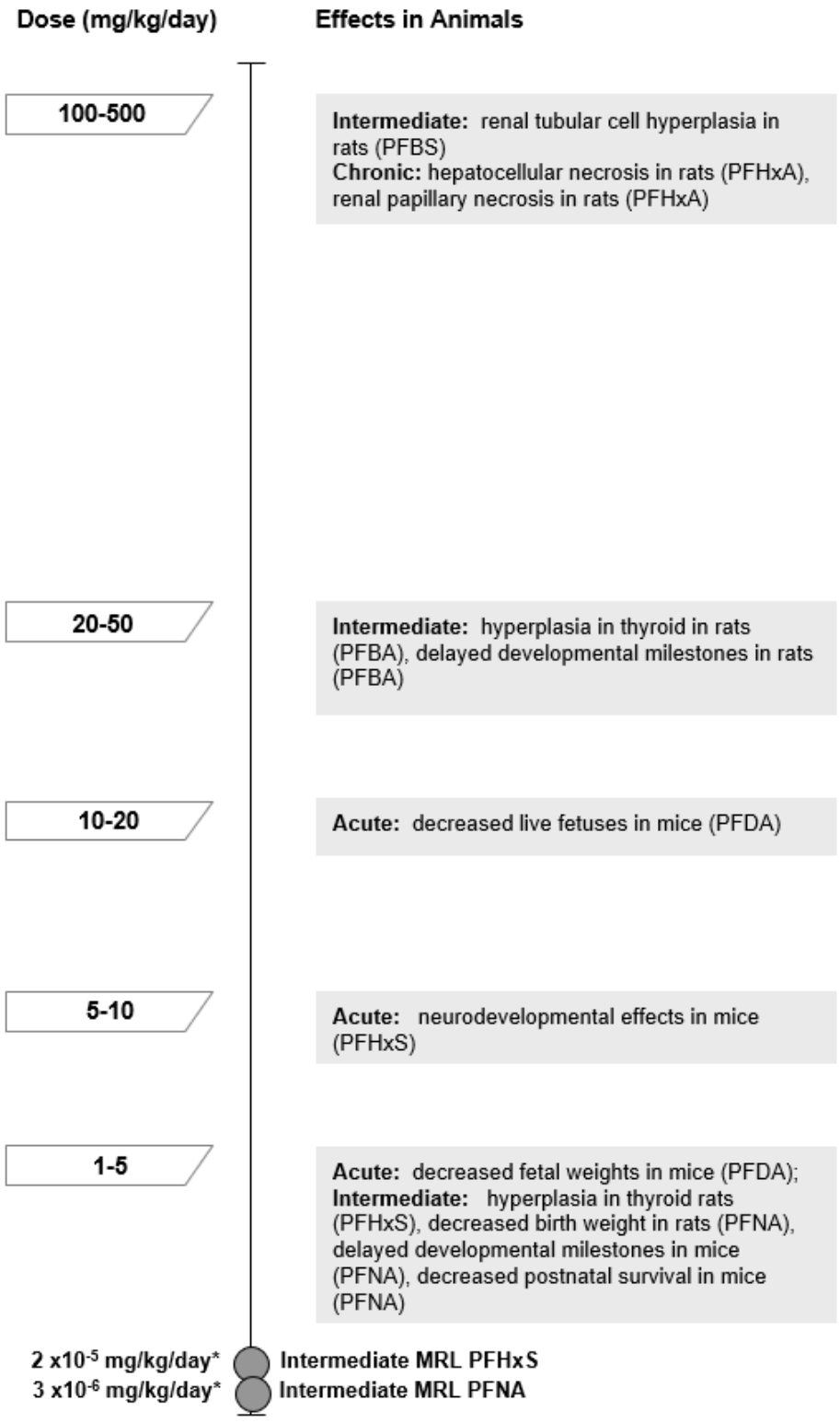
*See Appendix A for additional details

Figure 1-2. Health Effects Found in Animals Following Oral Exposure to PFOS



*See Appendix A for additional details

Figure 1-3. Health Effects Found in Animals Following Oral Exposure to Other Perfluoroalkyls



*See Appendix A for additional details

to expansion of the smooth endoplasmic reticulum and proliferation of peroxisomes, as confirmed by increased activity of biochemical markers and light and electron microscopy (Pastoor et al. 1987). It is important to note also that there appear to be different sensitivities for different endpoints. For example, in male rats dosed with PFOA for 14 days, absolute liver weight and fatty acid β-oxidation activity were significantly increased at 2 mg/kg/day, whereas hepatic microsomal concentration of total cytochrome P450 was significantly increased at 20 mg/kg/day (Liu et al. 1996). In general, longer-term studies with PFOA have shown that the hepatic effects are reversible once dosing ceases and that recovery tends to parallel the decline in blood levels of PFOA (Perkins et al. 2004). Studies in mice have provided similar results. However, studies in PPARα-null mice suggest that hepatomegaly may also be due to a PPARα-independent process in mice (Yang et al. 2002b), since PFOA induced hepatomegaly to the same extent in wild-type mice and PPARα-null mice, but failed to increase acyl-CoA oxidase activity in PPARα-null mice. PFOA exposure also resulted in increases in absolute liver weight in monkeys treated with ≥3 mg/kg/day for 26 weeks, an effect that was partly associated with significant mitochondrial proliferation, but not peroxisome proliferation (Butenhoff et al. 2002).

Similar to PFOA, PFOS exposure results in increases in liver weight, hepatocellular hypertrophy, and decreases in serum cholesterol and triglyceride levels in rodents (e.g., Elcombe et al. 2012a, 2012b; Era et al. 2009; Seacat et al. 2003; Thibodeaux et al. 2003). PFOS induced an increase in absolute liver weight, a decrease in serum cholesterol, and hepatocellular hypertrophy and lipid vacuolation in monkeys in a 26-week study (Seacat et al. 2002). Not unexpectedly, there was no evidence of peroxisome proliferation and no increase in hepatic palmitoyl-CoA oxidase, consistent with the fact that monkeys (and humans) seem to be refractory to peroxisome proliferative responses (Cattley et al. 1998; Klaunig et al. 2003).

Studies with other perfluoroalkyls have shown that, in general, liver weight and parameters of fatty acid β-oxidation are more severely affected as the carbon length increases up to about a 10-carbon chain length (Butenhoff et al. 2009a, 2012a; Goecke-Flora and Reo 1996; Goecke et al. 1992; Kudo et al. 2000, 2006; Permadi et al. 1992, 1993; van Otterdijk 2007a, 2007b). Significant peroxisome activity seems to require a carbon length >7 (Goecke-Flora and Reo 1996; Goecke et al. 1992), but increases over control levels have been reported with a four-carbon chain length (Permadi et al. 1993; Wolf et al. 2008a). In an *in vitro* study in COS-1 cells transfected with mouse PPARα, PFOA had the lowest effective concentration needed for PPARα activation followed by PFNA and PFDA, PFHxA, and PFBA (Wolf et al. 2008a). This pattern was not found for the sulfonates; the lowest effective concentration was for PFHxS followed by PFOS and PFBS. Wolf et al. (2008a) also found that carboxylate perfluoroalkyls activated PPARα at lower concentrations than the sulfonate perfluoroalkyls. In COS-1 cells transfected

with human PPARα, PFNA had the lowest effective concentration followed by PFOA PFHxA, PFHxS, PFOS, PFBS, PFBA, and PFDA (Wolf et al. 2008a). Studies have shown that the differential activity is also related to differential accumulation of the perfluoroalkyls in the liver (Kudo and Kawashima 2003; Kudo et al. 2000, 2006). Hydrophobicity, which increases as carbon length increases, seems to favor biliary enterohepatic recirculation, resulting in a more protracted toxicity (Goecke-Flora and Reo 1996). As discussed in greater detail in Section 2.9, the increases in liver weight and hepatocellular hypertrophy observed in the rat and mouse studies were considered rodent-specific adaptive responses and were not considered relevant to humans. However, other liver effects including biliary effects and hepatocellular necrosis were considered relevant to humans.

Developmental Effects. PFOA and PFOS have induced developmental effects in rodents. Most studies with PFOA have been conducted in mice. Specific effects reported include prenatal loss, reduced neonate weight and viability, neurodevelopment toxicity, altered bone development, and delays in mammary gland differentiation, eye opening, vaginal opening, and first estrus (Abbott et al. 2007; Albrecht et al. 2013; Cheng et al. 2013; Johansson et al. 2008; Koskela et al. 2016; Lau et al. 2006; Macon et al. 2011; Ngo et al. 2014; Onishchenko et al. 2011; Sobolewski et al. 2014; White et al. 2007, 2009, 2011; Wolf et al. 2007; Yahia et al. 2010). These effects occurred generally in the absence of overt maternal toxicity. Some of these effects, such as reduced pup survival from birth to weaning, have been observed in mice treated with as low as 0.6 mg/kg/day PFOA on gestation days (GDs) 1–17 (Abbott et al. 2007). This dose level resulted in mean serum PFOA concentrations of 5,200 and 3,800 ng/mL in dams and pups, respectively, on postnatal day (PND) 22. A cross-fostering study in mice showed that *in utero*, lactation only, and *in utero* and lactation exposure resulted in significant decreases in postnatal growth (Wolf et al. 2007). Alterations in spontaneous behavior were reported in 2- or 4-month-old male mice that were administered a single gavage dose of PFOA at the age of 10 days (Johansson et al. 2008). Increases in motor activity were also observed following *in utero* exposure to PFOA (Cheng et al. 2013; Onishchenko et al. 2011). Gestational exposure resulted in altered bone morphology and bone mineral density in the mature offspring (Koskela et al. 2016). Delays in ossification were found in another gestational exposure study in mice (Lau et al. 2006). A cross-fostering study showed that the delays in mammary gland development were observed following *in utero* exposure and following lactation-only exposure (White et al. 2009); however, the results of a 2-generation study showed that the delayed development did not appear to affect lactational support (White et al. 2011). No fetal toxicity or teratogenicity was reported in offspring of rabbits exposed to up to 50 mg/kg/day PFOA on GDs 6–18 (Gortner et al. 1982), suggesting that rabbits are less susceptible than mice to the developmental effects of PFOA, although comparing administered doses is probably not very informative due to toxicokinetic differences between species.

There were significant increases in body weight gain in mice aged 10–40 weeks that were exposed to low levels of PFOA (0.01–0.3 mg/kg/day) on GDs 1–17 (Hines et al. 2009). Increases in serum insulin and leptin levels were also observed, but there was no change in serum glucose or the response to a glucose challenge. A comparison of the effects of *in utero* exposure (GDs 1–17) to adult exposure (17 days at age 8 weeks) demonstrated that *in utero* exposure resulted in higher body weights, white fat weight, and brown fat weight at age 18 months (Hines et al. 2009).

Studies conducted with wild-type and PPARα knockout mice showed that PPARα was required for PFOA-induced postnatal lethality and that the expression of one copy of the gene was sufficient to mediate this effect (Abbott et al. 2007). Strain or PPARα expression did not affect serum PFOA levels. The mechanism of reduced postnatal viability has not been elucidated. Alterations in gene expression in both fetal liver and lung have been reported following exposure of mice to PFOA during pregnancy (Rosen et al. 2007). In the liver, PFOA altered the expression of genes linked to fatty acid catabolism, lipid transport, ketogenesis, glucose metabolism, lipoprotein metabolism, cholesterol biosynthesis, steroid metabolism, bile acid biosynthesis, phospholipid metabolism, retinol metabolism, proteosome activation, and inflammation. In the lung, transcriptional-related changes were predominantly associated with fatty acid catabolism. Although decreased pup survival appears to be linked to PPARα expression, there are insufficient data to determine whether other developmental effects observed in rats and mice are PPARα-independent.

PFOS significantly decreased birth weight and survival in neonatal rats exposed *in utero* (Chen et al. 2012b; Lau et al. 2003; Xia et al. 2011), and cross-fostering exposed pups with unexposed dams failed to improve survival rates (Lau et al. 2003). PFOS serum levels of pups at birth associated with significant decreased survival were approximately ≥70,000 ng/mL. In contrast to PFOA, the results of a study in wild-type and PPARα-null mice suggest that the decrease in pup survival was not dependent on PPARα activation (Abbott et al. 2009). Dosing rats late during gestation (GDs 17–20) caused significantly more lethality than dosing early (GDs 2–5) (Grasty et al. 2003). Since pups had difficulty breathing within minutes of birth and their lungs showed evidence of delayed lung maturation and other histological alterations (Grasty et al. 2003, 2005; Yahia et al. 2008), the possibility that this caused the early death has been suggested. Other effects included decreases in birth weight or pup body weight, delays in eye opening, cleft palate, and neurodevelopmental alterations (Butenhoff et al. 2009b; Case et al. 2001; Chen et al. 2012b; Era et al. 2009; Fuentes et al. 2006, 2007a, 2007b; Lau et al. 2003; Luebker et al. 2005a, 2005b; Onishchenko et al. 2011; Thibodeaux et al. 2003; Wang et al. 2015c; Yahia et al. 2008). Alterations in spontaneous motor activity were observed in mice. A decrease in activity was observed

when mice were placed in a novel environment (Fuentes et al. 2007a; Onishchenko et al. 2011); another study found a decrease in motor activity followed by increased activity (Johansson et al. 2009). Evaluation of immunological parameters in 8-week-old pups from mice exposed to PFOS during gestation showed reduced natural killer (NK) cell activity, suppressed IgM response to immunization, and alterations in splenic and thymic lymphocyte subpopulations (Keil et al. 2008).

Similar to PFOA and PFOS, increases in fetal mortality were observed in mice exposed to PFDA on GDs 6–15 (Harris and Birnbaum 1989) and decreases in litter size and pup survival were observed in mice exposed to PFNA (Wolf et al. 2010). In contrast, gestational exposure to PFBA, PFBS, or PFHxS did not result in alterations in pup survival or pup body weight (Das et al. 2008; Hoberman and York 2003; Lieder et al. 2009b). Decreases in spontaneous activity followed by an increase in activity were observed in mice exposed to PFHxS on PND 10 (Viberg et al. 2013); no alterations were observed in mice similarly exposed to PFDA (Johansson et al. 2008).

Immunological Effects. A number of studies have examined the immunotoxicity of perfluoroalkyls in rats and mice; these data suggest that mice are considerably more sensitive than rats. PFOA- and PFOS-induced immunological alterations in adult mice are characterized by thymus and spleen atrophy, alterations in thymic and splenic lymphocyte phenotypes, and impaired response to T-dependent antigens (DeWitt et al. 2008, 2009; Dong et al. 2009; Guruge et al. 2009; Lefebvre et al. 2008; Loveless et al. 2008; Qazi et al. 2012; Yang et al. 2000, 2002a; Zheng et al. 2009). The lowest lowest-observed-adverse-effect level (LOAEL) for immune effects in mice exposed to PFOA was 3.75 mg/kg/day administered for 15 days; this dosing level resulted in a mean PFOA serum level of 75,000 ng/mL (DeWitt et al. 2008). For PFOS, several studies identified LOAELs of 0.02–0.8 mg/kg/day (Dong et al. 2009, 2011; Zheng et al. 2009) and one study identified a LOAEL of 0.00166 mg/kg/day for suppressed response to a T-dependent antigen (Peden-Adams et al. 2008). PFOA applied to the skin of mice increased serum IgE levels following a challenge with ovalbumin relative to mice treated with ovalbumin alone, which led the investigators to suggest that PFOA may increase the IgE response to environmental allergens (Fairley et al. 2007). More limited data are available for other perfluoroalkyls. Thymic and/or splenic alterations were observed in rats and mice administered ≥1 mg/kg/day PFNA (Fang et al. 2008, 2009, 2010). No histological alterations were observed in rodents exposed to PFHxS (Butenhoff et al. 2009a), PFDA (Harris et al. 1989), PFBS (3M 2001), or PFBA (3M 2007a; Butenhoff et al. 2012a; van Otterdijk 2007a, 2007b).

Cancer Effects. PFOA, as many other PPARα agonists, induced hepatocellular adenomas, Leydig cell adenomas, and pancreatic acinar cell adenomas in rats (Biegel et al. 2001). An increase in hepatocellular adenomas was also observed in rats chronically exposed to PFOS (Butenhoff et al. 2012b). Liver tumors induced by PFOA are believed to be mediated largely through PPARα activation, and considered to be of limited or no relevance to humans (EPA 2016h), based on species differences in response to PPARα activation. Although Leydig cell tumors are also commonly induced by peroxisome proliferating agents, the mode of action by which these tumors are induced by PFOA, and thus their relevance to humans, is much less clear (Corton et al. 2014; EPA 2016h; Klaunig et al. 2003). One mode of action proposed for the induction of Leydig cell tumors involves PFOA-induced decreases in circulating testosterone levels, leading to increased production of gonadotropin releasing hormone and circulating luteinizing hormone (LH), which promotes Leydig cell proliferation. Reduced testosterone levels may occur through decreased biosynthesis, or via the conversion of testosterone to estradiol via the enzyme aromatase, both of which may be related to PPARα activation (EPA 2016h). However, the data supporting a PPARα-dependent mode of action for Leydig cell tumors is not sufficiently established to rule out human relevance (EPA 2016h). Likewise, the mechanism of PFOA-induced pancreatic acinar cell tumors may include a PPARα-dependent component, but the mechanism has not been fully elucidated, and relevant data are limited. A proposed mode of action involves stimulation of PPARα leading to reduced bile flow and/or changes in bile acid composition with subsequent increase in cholecystokinin (CCK), which stimulates pancreatic cell proliferation and tumor formation (EPA 2016h). Support for this mode of action is limited to information demonstrating increased biliary excretion of PFOA in wild-type and PPARα null mice (Minata et al. 2010) and data showing altered expression of bile acid transporters (OATPs and MRPs) in exposed laboratory animals (Cheng and Klassen 2008a; Maher et al. 2008). The limitations in available data on the mode of action for pancreatic tumor development preclude a conclusion regarding the human relevance of PFOA-induced pancreatic tumors (EPA 2016h).

1.3 MINIMAL RISK LEVELS (MRLs)

ATSDR develops MRLs as screening tools to help identify chemicals that may be of concern. An MRL is an estimate of the daily human exposure to a hazardous substance that is likely to be without appreciable risk of adverse noncancer health effects over a specified route and duration of exposure. MRLs are derived when reliable and sufficient data exist to identify the target organ(s) of effect or the most sensitive health effect(s) for a specific duration for a given route of exposure. These substance-specific estimates, which are intended to serve as screening levels, are used by ATSDR health assessors to determine areas and populations potentially at risk for health effects from exposure to a particular substance. Exposure above the MRLs does not mean that health problems will occur. Instead, it may act

as a signal to health assessors to look more closely at a particular site where exposures may be identified. MRLs do not define regulatory or action levels for ATSDR.

ATSDR uses the point of departure (POD)/uncertainty factor approach to derive MRLs. Potential PODs are no-observed-adverse-effect levels (NOAELs), LOAELs, or the lower limit of the benchmark dose (BMDL). MRLs are set below levels that, based on current information, might cause adverse health effects in the people most sensitive to such substance-induced effects. MRLs are generally based on the most sensitive substance-induced endpoint considered to be of relevance to humans. Serious health effects (such as irreparable damage to the liver or kidneys) are not used as a basis for establishing MRLs. MRLs are derived for acute (1–14 days), intermediate (15–364 days), and chronic (≥365 days) durations and for the oral and inhalation routes of exposure. ATSDR does not extrapolate across exposure durations to derive MRLs for durations with limited databases.

Most MRLs contain a degree of uncertainty because of the lack of precise toxicological information on the people who might be most sensitive (e.g., infants, elderly, nutritionally or immunologically compromised) to the effects of hazardous substances. ATSDR uses a conservative (i.e., protective) approach to address this uncertainty consistent with the public health principle of prevention. Although human data are preferred, MRLs often must be based on animal studies because relevant human studies are lacking. In the absence of evidence to the contrary, ATSDR assumes that humans are more sensitive to the effects of hazardous substance than animals and that certain persons may be particularly sensitive. Thus, the resulting MRL may be as much as 100-fold below levels that have been shown to be nontoxic in laboratory animals. ATSDR utilizes uncertainty factors to account for uncertainties associated with: (1) extrapolating from a LOAEL to a NOAEL; (2) extrapolating from animals to humans; and (3) to account for human variability. Default values of 10 are used for each of these categories of uncertainty factors; a value of 1 can be used if complete certainty exists for a particular uncertainty factor category. A partial uncertainty factor of 3 can be used when chemical-specific data decreases the uncertainty. On a case-by-case basis, ATSDR also utilizes modifying factors to account for MRL-specific database deficiencies.

Oral MRLs have been derived for several perfluoroalkyls. A summary of the MRLs derived for perfluoroalkyls is presented in Table 1-2 and detailed discussions of MRLs are provided in Appendix A. The database was not considered adequate for derivation of inhalation MRLs. Though inhalation data are available for PFOA and PFNA, these studies examined a limited number of endpoints and the data are not adequate for identifying the most sensitive targets of toxicity or establishing dose-response relationships. No inhalation data are available for other perfluoroalkyls.

Table 1-2. Overview of Minimal Risk Levels Derived for Perfluoroalkyls

	Inhalation MRLs			Oral MRLs		
Compound	Acute	Intermediate	Chronic	Acute	Intermediate	Chronic
PFOA	X[a]	X	X	X	$3x10^{-6}$ mg/kg/day (Table 1-3)	X
PFOS	X	X	X	X	$2x10^{-6}$ mg/kg/day (Table 1-4)	X
PFHxS	X	X	X	X	$2x10^{-5}$ mg/kg/day (Table 1-5)	X
PFNA	X	X	X	X	$3x10^{-6}$ mg/kg/day (Table 1-6)	X
PFDA	X	X	X	X	X	X
PFUnA	X	X	X	X	X	X
PFHpA	X	X	X	X	X	X
PFBS	X	X	X	X	X	X
PFBA	X	X	X	X	X	X
PFDoDA	X	X	X	X	X	X
PFHxA	X	X	X	X	X	X
FOSA	X	X	X	X	X	X

[a]X indicates that no MRL was derived.

FOSA = perfluorooctane sulfonamide; PFBA = perfluorobutanoic acid; PFBS = perfluorobutane sulfonic acid; PFDA = perfluorodecanoic acid; PFDoDA = perfluorododecanoic acid; PFHpA = perfluoroheptanoic acid; PFHxA = perfluorohexanoic acid; PFHxS = perfluorohexane sulfonic acid; PFNA = perfluorononanoic acid; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid; PFUnA = perfluoroundecanoic acid

The oral databases were considered adequate for derivation of intermediate-duration oral MRLs for PFOA, PFOS, PFHxS, and PFNA based on laboratory animal data. The databases were not considered adequate for derivation of MRLs for the other perfluoroalkyls. Hepatic, immune, and developmental endpoints were the most sensitive targets in laboratory animals exposed to PFOA (see Figure 1-4) and PFOS (see Figure 1-5), respectively. The most sensitive targets were hepatic and thyroid endpoints for PFHxS and body weight and developmental endpoints for PFNA. As discussed in Section 1.2, toxicokinetic and mechanistic differences exist between humans and laboratory animals, in particular differences in elimination rates and the relevance of effects associated with activation of PPARα. The uncertainties in the relevance of animal data for developing screening levels are decreased by focusing on health outcomes also reported in epidemiological studies or involving PPARα-independent mechanisms of action and estimating a POD using serum perfluoroalkyl concentrations. The MRL values for PFOA, PFOS, PFHxS, and PFNA are summarized in Tables 1-3, 1-4, 1-5, and 1-6 and discussed in greater detail in Appendix A.

Figure 1-4. Summary of Sensitive Targets of PFOA – Oral

Developmental endpoints are the most sensitive target of PFOA.
Numbers in circles are the lowest LOAELs for all health effects in animals.

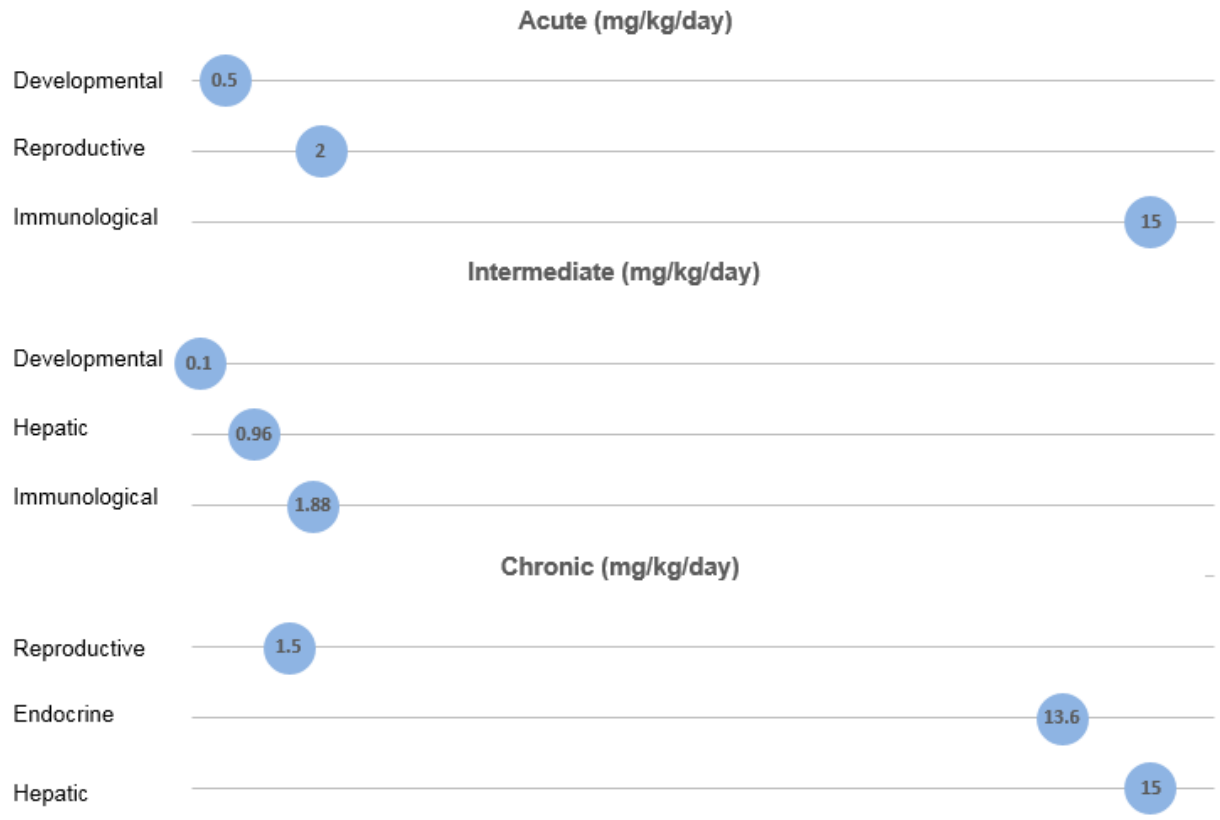


Figure 1-5. Summary of Sensitive Targets of PFOS – Oral

The immune system and developing organism are the most sensitive targets of PFOS.
Numbers in circles are the lowest LOAELs for all health effects in animals.

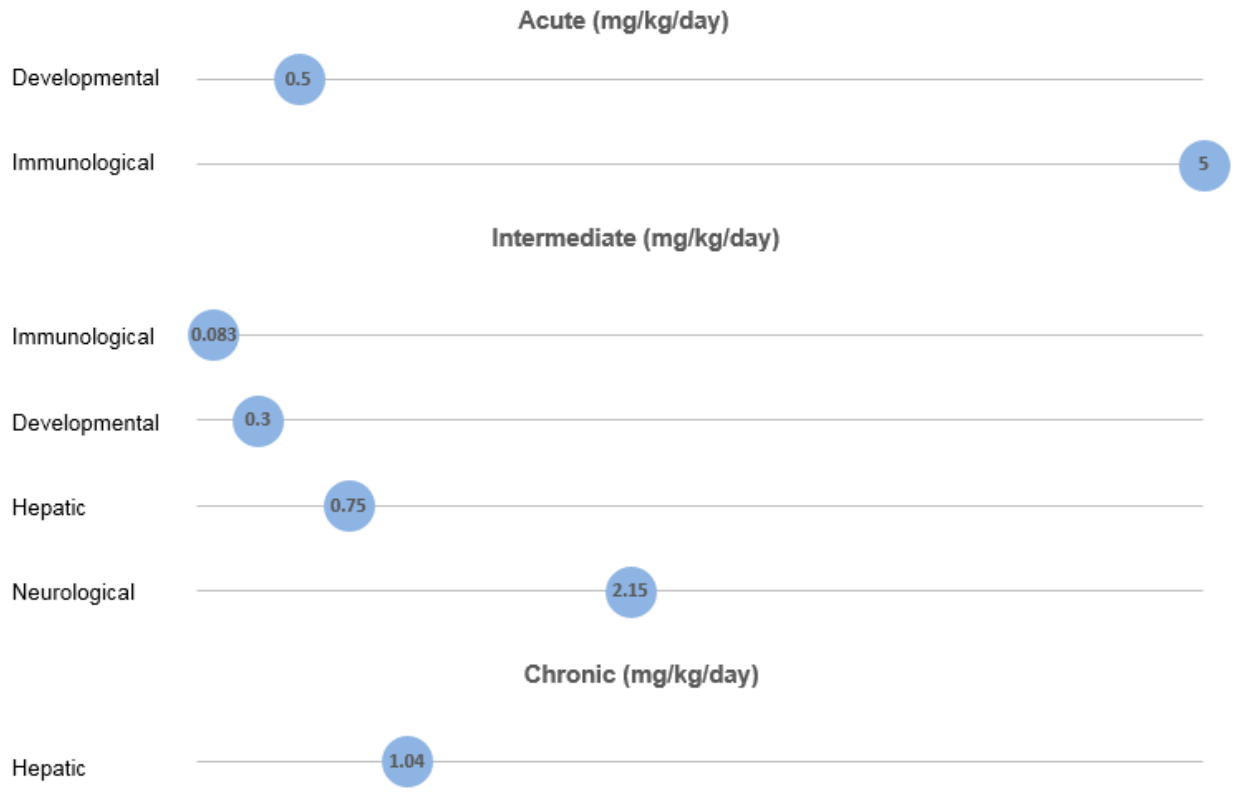


Table 1-3. Minimal Risk Levels (MRLs) for PFOA[a]

Exposure duration	MRL	Critical effect	Point of departure	Uncertainty factor	Reference
Inhalation exposure					
Acute	Inadequate acute-duration study (exposure ≤14 days)				
Intermediate	Inadequate intermediate-duration study (exposure 15–364 days)				
Chronic	Inadequate chronic-duration study (exposure ≥365 days)				
Oral exposure (mg/kg/day)					
Acute	Inadequate acute-duration study (exposure ≤14 days)				
Intermediate	$3x10^{-6}$	Skeletal effects in mice	0.000821 ($LOAEL_{HED}$)	300	Koskela et al. 2016
Chronic	Inadequate chronic-duration study (exposure ≥365 days)				

[a]See Appendix A for additional information.

HED = human equivalent dose; LOAEL = lowest-observed-adverse-effect level; PFOA = perfluorooctanoic acid

Table 1-4. Minimal Risk Levels (MRLs) for PFOS[a]

Exposure duration	MRL	Critical effect	Point of departure	Uncertainty and modifying factors	Reference
Inhalation exposure					
Acute	Inadequate acute-duration study (exposure ≤14 days)				
Intermediate	Inadequate intermediate-duration study (exposure 15–364 days)				
Chronic	Inadequate chronic-duration study (exposure ≥365 days)				
Oral exposure (mg/kg/day)					
Acute	Inadequate acute-duration study (exposure ≤14 days)				
Intermediate	$2x10^{-6}$	Delayed eye opening and decreased pup weight in rats	0.000515 ($NOAEL_{HED}$)[b]	30 10	Luebker et al. 2005a
Chronic	Inadequate chronic-duration study (exposure ≥365 days)				

[a]See Appendix A for additional information.

HED = human equivalent dose; NOAEL = no-observed-adverse-effect level; PFOS = perfluorooctane sulfonic acid

Table 1-5. Minimal Risk Levels (MRLs) for PFHxS[a]

Exposure duration	MRL	Critical effect	Point of departure	Uncertainty and modifying factors	Reference
Inhalation exposure					
Acute	Inadequate acute-duration study (exposure ≤14 days)				
Intermediate	Inadequate intermediate-duration study (exposure 15–364 days)				
Chronic	Inadequate chronic-duration study (exposure ≥365 days)				
Oral exposure (mg/kg/day)					
Acute	Inadequate acute-duration study (exposure ≤14 days)				
Intermediate	$2x10^{-5}$	Thyroid follicular epithelial hypertrophy/ hyperplasia in rats	0.0047 ($NOAEL_{HED}$)	30 10	Butenhoff et al. 2009a
Chronic	Inadequate chronic-duration study (exposure ≥365 days)				

[a]See Appendix A for additional information.

HED = human equivalent dose; NOAEL = no-observed-adverse-effect level; PFHxS = perfluorohexane sulfonic acid

Table 1-6. Minimal Risk Levels (MRLs) for PFNA[a]

Exposure duration	MRL	Critical effect	Point of departure	Uncertainty and modifying factors	Reference
Inhalation exposure					
Acute	Inadequate acute-duration study (exposure ≤14 days)				
Intermediate	Inadequate intermediate-duration study (exposure 15–364 days)				
Chronic	Inadequate chronic-duration study (exposure ≥365 days)				
Oral exposure (mg/kg/day)					
Acute	Inadequate acute-duration study (exposure ≤14 days)				
Intermediate	$3x10^{-6}$	Decreased body weight and developmental delays in mice	0.001 ($NOAEL_{HED}$)	30 10	Das et al. 2015
Chronic	Inadequate chronic-duration study (exposure ≥365 days)				

[a]See Appendix A for additional information.

HED = human equivalent dose; NOAEL = no-observed-adverse-effect level; PFNOA = perfluorononanoic acid

CHAPTER 2. HEALTH EFFECTS

2.1 INTRODUCTION

The primary purpose of this chapter is to provide public health officials, physicians, toxicologists, and other interested individuals and groups with an overall perspective on the toxicology of perfluoroalkyls. It contains descriptions and evaluations of toxicological studies and epidemiological investigations and provides conclusions, where possible, on the relevance of toxicity and toxicokinetic data to public health.

This document discusses information on perfluoroalkyls that have been measured in the serum collected from a representative U.S. population ≥12 years of age in the 2003–2004 NHANES (Calafat et al. 2007b), as well as two compounds (PFBA and PFHxA) that have been identified in other monitoring studies. More recent NHANES monitoring studies have not evaluated additional perfluoroalkyl compounds (CDC 2019). The perfluoroalkyl compounds discussed in the profile are listed below. They are discussed in the profile in following order, based on the abundance of epidemiological data:

Perfluorooctanoic acid (PFOA, CAS Registry Number 335-67-1)
Perfluorooctane sulfonic acid (PFOS, CAS Registry Number 1763-23-1)
Perfluorohexane sulfonic acid (PFHxS, CAS Registry Number 355-46-4)
Perfluorononanoic acid (PFNA, CAS Registry Number 375-95-1)
Perfluorodecanoic acid (PFDA, CAS Registry Number 335-76-2)
Perfluoroundecanoic acid (PFUnA, CAS Registry Number 2058-94-8)
Perfluoroheptanoic acid (PFHpA, CAS Registry Number 375-85-9)
Perfluorobutane sulfonic acid (PFBS, CAS Registry Number 375-73-5)
Perfluorobutanoic acid (PFBA, CAS Registry Number 375-22-4)
Perfluorododecanoic acid (PFDoDA, CAS Registry Number 307-55-1)
Perfluorohexanoic acid (PFHxA, CAS Registry Number 307-24-4)
Perfluorooctane sulfonamide (FOSA, CAS Registry Number 754-91-6)

The term perfluoroalkyls used throughout the profile is referring to at least one of these 12 compounds and may not be applicable to other perfluoroalkyl compounds.

A glossary and list of acronyms, abbreviations, and symbols can be found at the end of this profile.

To help public health professionals and others address the needs of persons living or working near hazardous waste sites, the information in this section is organized by health effect. These data are discussed in terms of route of exposure (inhalation, oral, and dermal) and three exposure periods: acute (≤14 days), intermediate (15–364 days), and chronic (≥365 days).

As discussed in Appendix B, a literature search was conducted to identify relevant studies examining health effect endpoints. Figures 2-1, 2-2, 2-3, 2-4, and 2-5 provide an overview of the database of studies in humans or experimental animals included in this chapter of the profile. These studies evaluate the potential health effects associated with inhalation, oral, or dermal exposure to perfluoroalkyls, but may not be inclusive of the entire body of literature. ATSDR's approach for assessing study quality and weight-of-evidence evaluation is described in the Agency's Guidance for the Preparation of Toxicological Profile document (https://www.atsdr.cdc.gov/toxprofiles/guidance/profile_development_guidance.pdf).

Summaries of the epidemiological studies, including details on the study design and results, are presented in tables in the *Supporting Document for Epidemiological Studies for Perfluoroalkyls*; briefer summaries of the studies are presented in summary tables for each endpoint. For studies in which the population was divided into perfluoroalkyl exposure categories, such as quartiles, the risk ratio reported in the summary table is for the lowest exposure category with a statistically significant association; risk ratios for higher exposure categories are presented in the *Supporting Document for Epidemiological Studies for Perfluoroalkyls* tables. In general, associations were also found for higher exposure categories.

Summaries of experimental studies are separated by exposure route and are presented in Tables 2-1, 2-2, 2-3, 2-4, 2-5, and 2-6. The inhalation data for PFOA and other perfluoroalkyls are presented in Tables 2-1 and 2-2, respectively. A large number of experimental studies have evaluated the oral toxicity of PFOA and PFOS, the results of these studies are presented in Tables 2-3 and 2-4, respectively. Lesser amounts of data are available for the remaining 10 perfluoroalkyl compounds; the study results for these compounds are presented in Table 2-5. Table 2-5 is divided by exposure duration and by compound. The dermal data for PFOA is presented in Table 2-6. In addition, the NOAEL and LOAEL values from inhalation and oral studies are graphically presented in Figures 2-6, 2-7, 2-8, 2-9, and 2-10.

Levels of significant exposure (LSEs) for each route and duration are presented in tables and illustrated in figures. The points in the figures showing NOAELs or LOAELs reflect the actual doses (levels of exposure) used in the studies. LOAELs have been classified into "less serious" or "serious" effects. "Serious" effects are those that evoke failure in a biological system and can lead to morbidity or mortality (e.g., acute respiratory distress or death). "Less serious" effects are those that are not expected to cause significant dysfunction or death, or those whose significance to the organism is not entirely clear. ATSDR acknowledges that a considerable amount of judgment may be required in establishing whether an endpoint should be classified as a NOAEL, "less serious" LOAEL, or "serious" LOAEL, and that in some cases, there will be insufficient data to decide whether the effect is indicative of significant

dysfunction. However, the Agency has established guidelines and policies that are used to classify these endpoints (ATSDR 2003). ATSDR believes that there is sufficient merit in this approach to warrant an attempt at distinguishing between "less serious" and "serious" effects. The distinction between "less serious" effects and "serious" effects is considered to be important because it helps the users of the profiles to identify levels of exposure at which major health effects start to appear. LOAELs or NOAELs should also help in determining whether or not the effects vary with dose and/or duration, and place into perspective the possible significance of these effects to human health.

A User's Guide has been provided at the end of this profile (see Appendix C). This guide should aid in the interpretation of the tables and figures for LSEs and MRLs.

The discussion of the available data for each health effect is divided into several subsections. Each health effect section begins with an overview, which contains a brief discussion of the available data and conclusions that can be drawn from the data. Compound-specific discussions follow the overview; the perfluoroalkyls are discussed in the following order: PFOA, PFOS, PFHxS, PFNA, PFDA, PFUnA, PFHpA, PFBS, PFBA, PFDoDA, PFHxA, and FOSA. It is noted that for most health effects, there are no data for a number of the perfluoroalkyls. The health effect endpoints examined in epidemiological and experimental data for each perfluoroalkyl is summarized in Figures 2-1 and 2-2, respectively. The compound-specific discussions are further divided into Epidemiological Studies and Laboratory Animal Studies; for data-rich endpoints, a compound-specific summary is also included. Each perfluoroalkyl is treated separately in this chapter. Although there is some evidence of similar health outcomes for some compounds, there is evidence of qualitative and mechanistic differences (Peters and Gonzalez 2011).

The health effects of perfluoroalkyls have been evaluated in a large number of epidemiological and animal studies; the literature search framework for identifying these studies is discussed in Appendix B. As illustrated in Figures 2-3, 2-4, and 2-5, most of the health effects data come from epidemiological studies. For PFOA, PFOS, and other perfluoroalkyls, 74, 76, and 70%, respectively, of the health effect studies were in humans; it is noted that most epidemiological studies examined more than one perfluoroalkyl. More than half (52%) of the epidemiological studies were cross-sectional studies, 29% were prospective studies, and the remainder were retrospective, case-control, cohort, or longitudinal studies. Three population categories were examined in epidemiological studies: workers at facilities involved in the production or use of perfluoroalkyls (most of the studies involved workers at two U.S. facilities and typically involved higher than background exposure to PFOA and PFOS), communities living near a PFOA manufacturing facility with high levels of PFOA in the drinking water (almost all of

the studies involved residents living near a PFOA production facility in West Virginia; elevated PFOA exposure and background exposure to other perfluoroalkyls), and populations exposed to background levels of perfluoroalkyls (referred to as general population studies). Most of the studies of communities living near perfluoroalkyl manufacturing facilities are part of the C8 Health Project and C8 Health Study (C8 is a synonym for PFOA). The C8 Health Project was a population study of Ohio and West Virginia residents living near the DuPont Washington Works facility in West Virginia and was funded by DuPont as part of a class action settlement agreement. The Washington Works facility began using PFOA in 1951 and peak use was in the late 1990s. At the time of enrollment (2005–2006), blood samples were collected from over 69,000 participants who lived, worked, or attended school in six contaminated water districts surrounding the facility for at least 12 months between 1950 and December 2004 (Frisbee et al. 2009); the six water districts were Little Hocking Water Association, Tuppers Plains Chester Water District, Village of Pomeroy, Lubeck Public Service District, Mason County Public Service District, or private water sources within these areas. The participants ranged in age from 1.5 to >100 years, with an average age of 39.1 years.

Serum perfluoroalkyl levels were used as the biomarker of exposure in almost all of the epidemiological studies since most of the studies did not provide external exposure levels. The highest levels of serum PFOA were found among workers, followed by the community members, and then the general population. One study of PFOA workers in 2004–2005 reported an average serum PFOA level of 1,000 ng/mL (Sakr et al. 2007a). A study of community members living near this same facility reported a mean serum PFOA level of 423 ng/mL in 2004–2005. In the United States, the mean geometric mean serum PFOA level in 2005–2006 was 3.92 ng/mL (CDC 2018). In a study of two PFOS facilities, mean serum PFOS levels in workers were 960–1,400 ng/mL in 2000 (Olsen et al. 2003a); the geometric mean serum PFOS levels in the U.S. general population in 1999–2000 was 30.4 ng/mL (CDC 2018). Bach et al. (2015b) investigated whether transport of blood samples under ambient temperature conditions and processing delays impact serum perfluoroalkyl concentrations. Using the conditions of the Danish National Birth Cohort study, Bach et al. (2015a) found relative differences between serum samples that were transported with processing delays and those processed immediately of 1% (winter sampling) to 3% (summer sampling) for PFOA, -29–2% for PFOS, 12–11% for PFHxS, -5–3% for PFNA, -39–0% for PFDA, -77 to -7% for PFUnA, and 38–17% for PFHpA. This discrepancy has not been verified for other Danish National Birth Cohort studies or for other studies.

Most of the epidemiological studies provided a single serum perfluoroalkyl concentration, which has been shown to be a reliable biomarker of recent exposure; however, it does not provide information on

historical exposure. The lack of historical exposure data is a particular limitation of the occupational and community population studies where past exposures were typically higher than current exposures.

Another limitation of the epidemiological studies involves co-exposure to multiple perfluoroalkyls. A number of the epidemiological studies have found strong correlations between serum levels of different perfluoroalkyls. *In vitro* studies (Carr et al. 2013; Wolf et al. 2014) have shown that at lower concentrations, binary pairs of perfluoroalkyls demonstrate concentration and response additivity, but deviate from additivity at higher concentrations (Wolf et al. 2014). These possible interactions (or dose additivity) complicate the interpretation of the epidemiological data.

Although a large number of epidemiological studies have examined the potential of perfluoroalkyls to induce adverse health effects, most of the studies are cross-sectional in design and do not establish causality. ATSDR evaluated the available epidemiological data to assess whether the preponderance of the evidence suggested a possible association between perfluoroalkyl exposure and a particular health effect. This approach took into consideration the consistency of the findings across studies, the quality of the studies, dose-response, and plausibility. It should be noted that although the data may provide evidence for an association, it does not always imply that the observed effect is biologically relevant because the magnitude of the change may be within the normal limits or not indicative of an adverse health outcome. Plausibility depends primarily on experimental toxicology studies that establish a plausible biological mechanism for the observed effects. ATSDR's toxicological profile development guidance (https://www.atsdr.cdc.gov/toxprofiledocs/additional_resources.html/#Profile_Development) describes in detail the weight-of-evidence approach that includes quality assessment of every study included in the profile.

The available epidemiological studies suggest associations between perfluoroalkyl exposure and several health outcomes; however, cause-and-effect relationships have not been established for these outcomes:

- **Hepatic effects.** Increases in serum enzymes and decreases in serum bilirubin, observed in studies of PFOA, PFOS, and PFHxS, are suggestive of liver alterations. In addition, the results of epidemiological studies of PFOA, PFOS, PFNA, and PFDA suggest an association between perfluoroalkyl exposure and increases in serum lipid levels, particularly total cholesterol and LDL cholesterol; see Section 2.9 for detailed discussion and citations.

- **Cardiovascular effects.** There is suggestive epidemiological evidence for an association between serum PFOA and PFOS and pregnancy-induced hypertension and/or pre-eclampsia; see Section 2.5 for detailed discussion and citations.

- **Immune effects.** Evidence is suggestive of an association between serum PFOA, PFOS, PFHxS, and PFDA levels and decreased antibody responses to vaccines; there is also limited evidence for PFNA, PFUnA, and PFDoDA; see Section 2.14 for detailed discussion and citations.

- **Developmental effects.** Evidence is suggestive of an association between serum PFOA and PFOS and small decreases in birth weight; the decrease in birth weight is <20 g (0.7 ounces) per 1 ng/mL increase in blood PFOA or PFOS level; see Section 2.17 for detailed discussion and citations.

As presented in Figures 2-3, 2-4, and 2-5, most of the available literature on the health effects of perfluoroalkyls in laboratory animals was conducted in oral studies, with a few inhalation and dermal exposure studies identified. The most commonly examined endpoints were liver, body weight, developmental, reproductive, and immunological.

The results of the animal studies suggest the following:

- **Hepatic effects.** Evidence from acute, intermediate, and/or chronic oral studies in rats, mice, and monkeys indicates that the liver is a sensitive target of PFOA, PFOS, PFHxS, PFNA, PFDA, PFUnA, PFBA, PFBS, PFDoDA, and PFHpA toxicity. The effects include increases in liver weight, hepatocellular hypertrophy, and decreases in serum lipid levels. These effects were considered specific to rodents and were not considered relevant to humans. Some degenerative and necrotic effects that are likely relevant to humans have also been observed for PFOA, PFOS, and PFHpA. See Section 2.9 for detailed discussion and citations.

- **Immune effects.** Evidence from acute and intermediate oral studies in mice indicates that immune endpoints are sensitive targets of PFOA and PFOS toxicity. The most commonly reported effect was an impaired response to antigens. No alteration in antigen response was observed in the one study of PFNA. Immune function has not been tested for the other perfluoroalkyls examined in this profile. See Section 2.14 for detailed discussion and citations.

- **Reproductive effects.** Impaired mammary gland development has been observed in mice orally exposed to PFOA. In general, studies of PFOA and PFOS have not found alterations in fertility. See Section 2.16 for detailed discussion and citations.

- **Developmental effects.** Evidence from acute and intermediate oral studies in rats and/or mice indicates that developmental endpoints are targets of PFOA, PFOS, PFHxS, PFNA, PFDA, PFUnA, and PFBA toxicity. The developmental effects include decreases in pup body weight, decreases in pup survival, and alterations in locomotor activity. See Section 2.17 for detailed discussion and citations.

Figure 2-1. Health Effect Endpoints Examined in Epidemiological Studies

Health Effect Endpoint	Perfluoroalkyl											
	PFOA	PFOS	PFHxS	PFNA	PFDA	PFUnA	PFHpA	PFBS	PFBA	PFDoDA	PFHxA	FOSA
Body weight	•	•	•	•	•	•				•		•
Respiratory	•											
Cardiovascular	•	•	•	•	•	•	•	•	•	•	•	•
Gastrointestinal		•										
Hematological	•	•										
Musculoskeletal	•	•	•	•								
Hepatic	•	•	•	•	•	•	•	•	•	•		
Renal	•	•	•	•	•			•		•	•	
Dermal												
Ocular												
Endocrine	•	•	•	•	•	•				•		
Immunological	•	•	•	•	•	•	•	•		•	•	•
Neurological	•	•	•	•								
Reproductive	•	•	•	•	•	•		•		•	•	•
Developmental	•	•	•	•	•	•	•		•	•		•
Other noncancer	•	•	•	•	•	•	•					•
Cancer	•	•	•	•	•	•	•			•		•

FOSA = perfluorooctane sulfonamide; PFBA = perfluorobutanoic acid; PFBS = perfluorobutane sulfonic acid; PFDA = perfluorodecanoic acid; PFDoDA = perfluorododecanoic acid; PFHpA = perfluoroheptanoic acid; PFHxA = perfluorohexanoic acid; PFHxS = perfluorohexane sulfonic acid; PFNA = perfluorononanoic acid; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid; PFUnA = perfluoroundecanoic acid

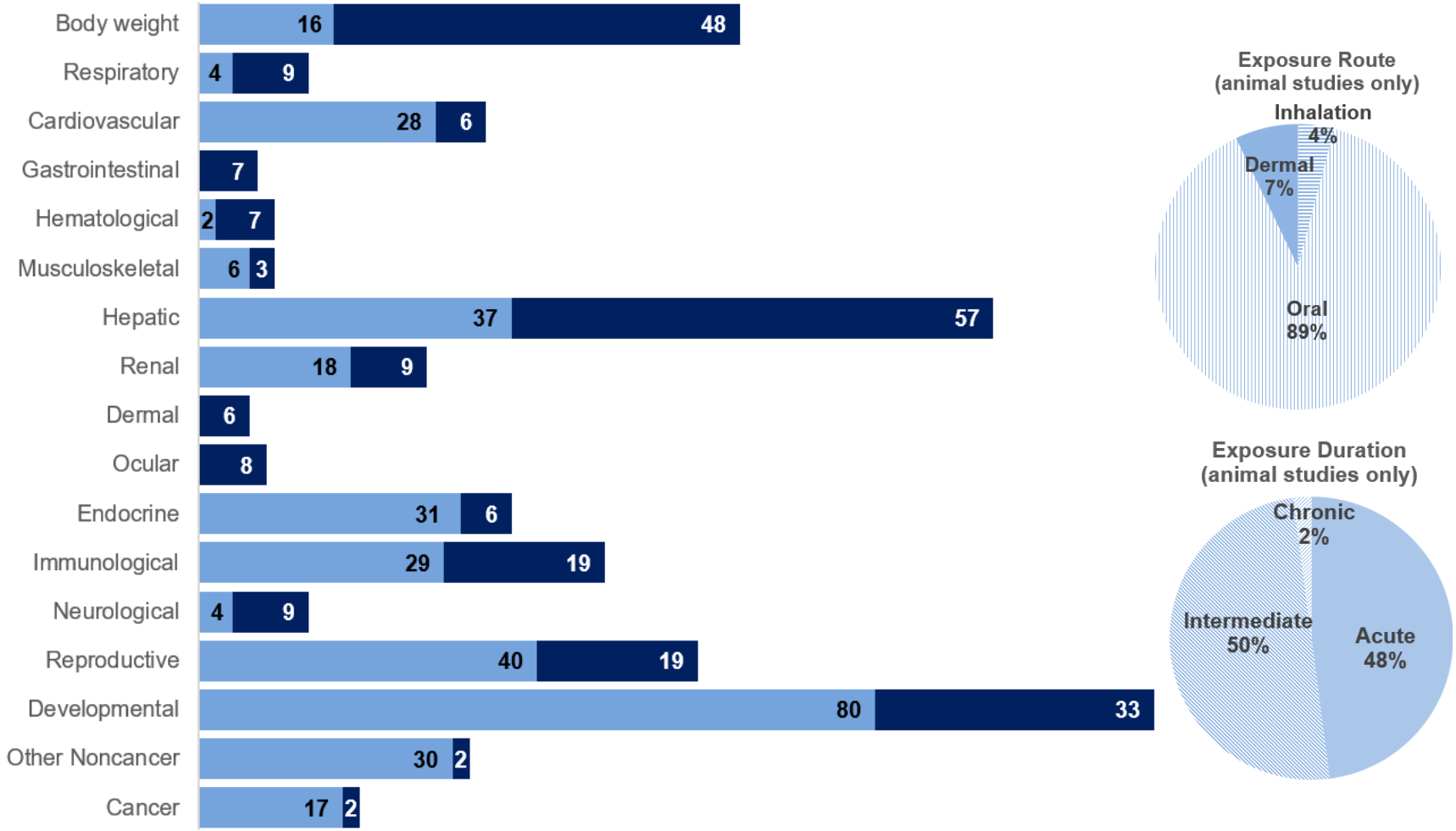
Figure 2-2. Health Effect Endpoints Examined in Laboratory Animal Studies

Health Effect Endpoint	PFOA	PFOS	PFHxS	PFNA	PFDA	PFUnA	PFHpA	PFBS	PFBA	PFDoDA	PFHxA	FOSA
	Perfluoroalkyl											
Body weight	•	•	•	•	•	•		•	•	•	•	•
Respiratory	•	•	•	•	•			•	•		•	
Cardiovascular	•	•	•		•			•	•	•	•	
Gastrointestinal	•	•	•		•			•	•	•	•	
Hematological	•	•	•		•	•		•	•	•	•	
Musculoskeletal	•	•	•					•	•		•	
Hepatic	•	•	•	•	•	•		•	•	•	•	•
Renal	•	•	•		•	•		•	•	•	•	
Dermal	•	•							•			
Ocular	•	•						•	•		•	
Endocrine	•	•	•		•			•	•	•	•	
Immunological	•	•	•	•	•			•	•		•	
Neurological	•	•	•		•			•	•	•	•	
Reproductive	•	•	•	•				•	•	•	•	
Developmental	•	•	•	•	•	•		•	•	•	•	
Other noncancer	•			•								
Cancer	•	•										

FOSA = perfluorooctane sulfonamide; PFBA = perfluorobutanoic acid; PFBS = perfluorobutane sulfonic acid; PFDA = perfluorodecanoic acid; PFDoDA = perfluorododecanoic acid; PFHpA = perfluoroheptanoic acid; PFHxA = perfluorohexanoic acid; PFHxS = perfluorohexane sulfonic acid; PFNA = perfluorononanoic acid; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid; PFUnA = perfluoroundecanoic acid

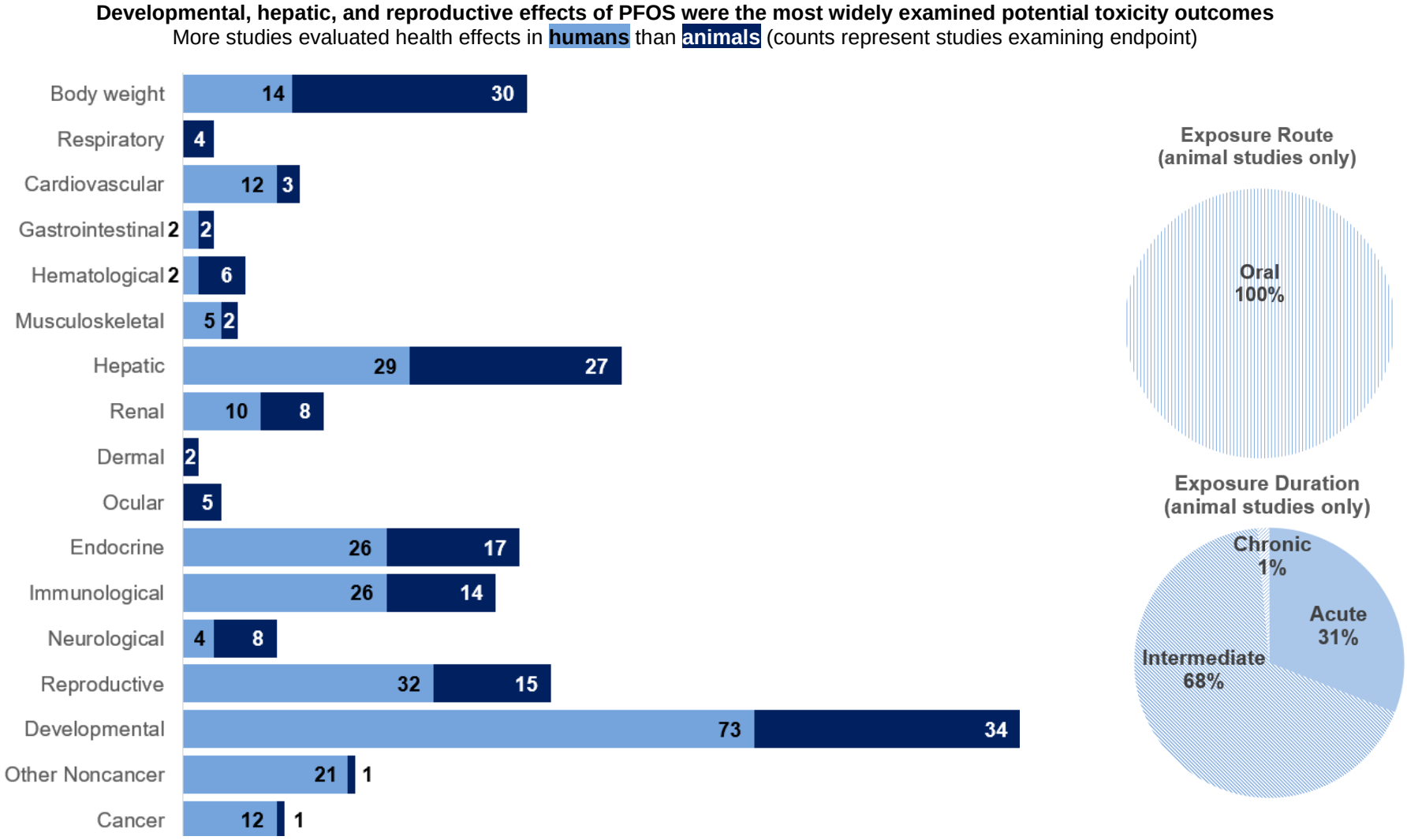
Figure 2-3. Overview of the Number of Studies Examining PFOA Health Effects*

Developmental, hepatic, and body weight effects of PFOA were the most widely examined potential toxicity outcomes
More studies evaluated health effects in **humans** than **animals** (counts represent studies examining endpoint)



*Includes studies discussed in Chapter 2. A total of 363 studies (including those finding no effect) have examined toxicity; most animal studies examined multiple endpoints. In this figure, the number of human studies is referring to the number of publications; most human studies examined multiple endpoints.

Figure 2-4. Overview of the Number of Studies Examining PFOS Health Effects*

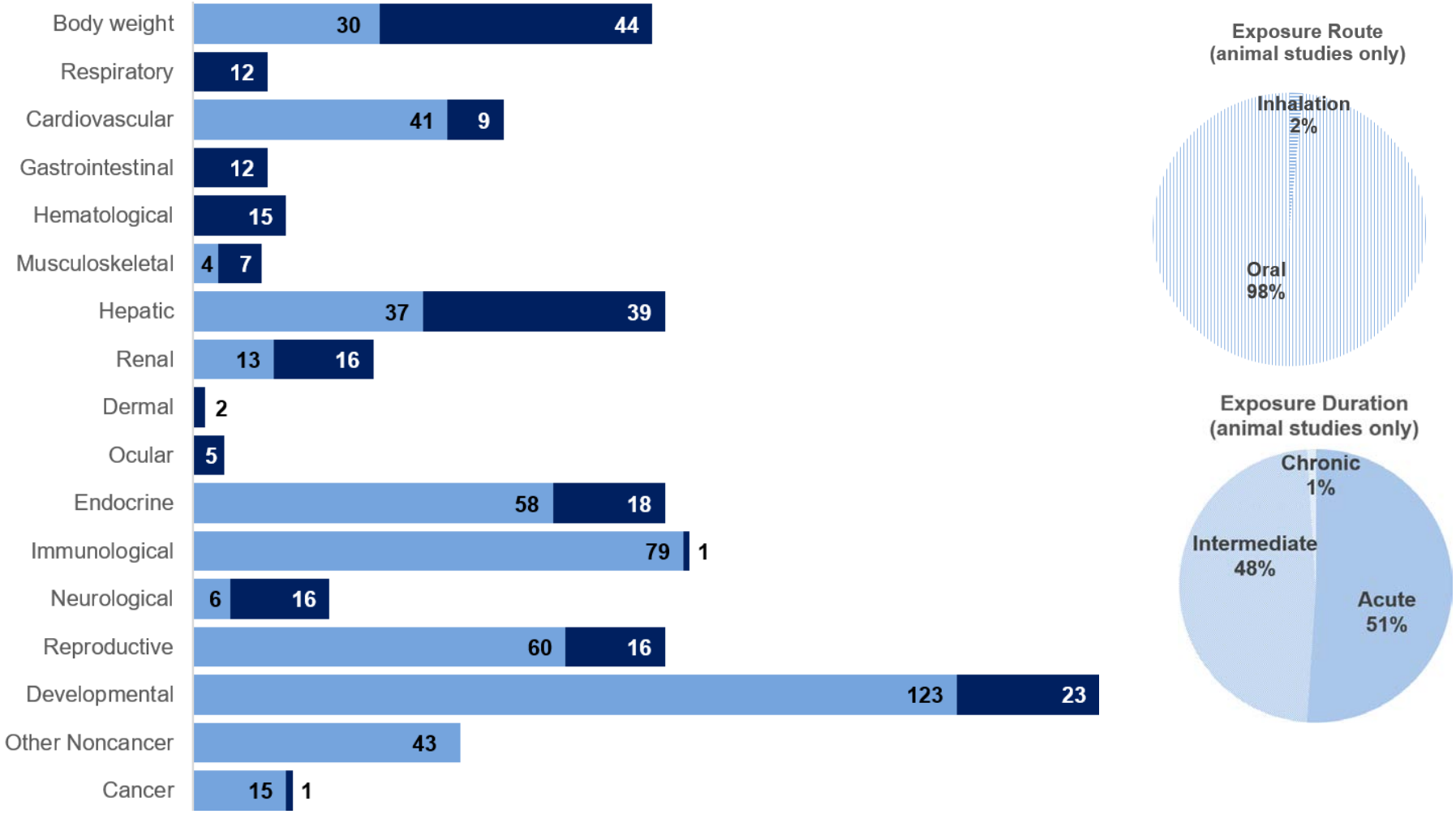


*Includes studies discussed in Chapter 2. A total of 301 studies (including those finding no effect) have examined toxicity; most animal studies examined multiple endpoints. In this figure, the number of human studies is referring to the number of publications; most human studies examined multiple perfluoroalkyls.

Figure 2-5. Overview of the Number of Studies Examining Other Perfluoroalkyls Health Effects*

Developmental, hepatic, and body weight effects of other perfluoroalkyls were the most widely examined potential toxicity outcomes

More studies evaluated health effects in **humans** than **animals** (counts represent studies examining endpoint)



*Includes studies discussed in Chapter 2. A total of 213 studies (including those finding no effect) have examined toxicity; most animal studies examined multiple endpoints. Most human studies examined multiple perfluoroalkyls; within each publication, the results for each perfluoroalkyl is counted as a study.

KD0050

Table 2-1. Levels of Significant Exposure to PFOA – Inhalation

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/m^3)	Parameters monitored	Endpoint	NOAEL (mg/m^3)	Less serious LOAEL (mg/m^3)	Serious LOAEL (mg/m^3)	Effect
ACUTE EXPOSURE									
1	Rat (albino) 5 M,F	1 hour (NS)	18,600	CS, BW, GN, HP	Resp		18,600		Red nasal discharge; dry rales
					Ocular		18,600		Red material around the eyes; lacrimation
					Neuro		18,600		Excessive salivation
Griffith and Long 1980									
APFO									
2	Rat (CD) 36 M	4 hours	380, 810, 830, 2,200, 4,800, 5,700	CS, HE, BI, GN, HP	Death			980 M	LC_{50}
					Bd wt			380 M	Weight loss for 1–2 days after exposure (data not shown)
					Resp			380 M	Pulmonary edema
					Gastro		380 M		Stomach irritation
					Hepatic	810			Liver enlargement at 810 mg/m^3; no histological alterations
					Ocular	380 M		810 M	Corneal opacity and corrosion
Kennedy et al. 1986									
APFO									
3	Rat (CD) 24 M	2 weeks 6 hours/day 5 days/week	0, 1, 7.6, 84	CS, HE, BI, GN, HP	Bd wt	7.6	84		7% lower body weight on exposure day 5
					Resp	84			
					Cardio	84			
					Gastro	84			
					Hemato	84			
					Musc/skel	84			
					Hepatic	84			Increased absolute and relative liver weight; hepatocellular hypertrophy at 7.6 mg/m^3
					Renal	84			
					Dermal	84			
					Ocular	84			
					Endocr	84			
					Neuro	84			No histological alterations
					Repro	84			No histological alterations
Kennedy et al. 1986									
APFO									

Table 2-1. Levels of Significant Exposure to PFOA – Inhalation

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/m^3)	Parameters monitored	Endpoint	NOAEL (mg/m^3)	Less serious LOAEL (mg/m^3)	Serious LOAEL (mg/m^3)	Effect
4	Rat (Sprague-Dawley) 12 F	GDs 6–15 6 hours/day	0, 0.1, 1, 10, 25	MX, DX, OW, CS, HP	Death			25	3/12 deaths on GDs 12, 13, and 17
					Bd wt	1	10		12% decrease weight gain on GDs 6–15
					Hepatic	25			18% increase absolute liver weight at 25 mg/m^3
					Develop	10	25		10% decreased neonatal body weight on PND 1
Staples et al. 1984 APFO									

[a]The number corresponds to entries in Figure 2-6.

APFO = ammonium perfluorooctanoate (ammonia salt of PFOA); BI = biochemical changes; BW or Bd wt = body weight; F = female(s); Cardio = cardiovascular; CS = clinical signs; Develop = developmental; DX = developmental toxicity; Endocr = endocrine; Gastro = gastrointestinal; GD = gestation day; GN = gross necropsy; HE or Hemato = hematological; HP = histopathology; LC_{50} = lethal concentration, 50% kill; LE = lethality; LOAEL = lowest-observed-adverse-effect level; M = male(s); Musc/skel = musculoskeletal; MX = maternal toxicity; Neuro = neurological; NOAEL = no-observed-adverse-effect level; NS = not specified; OW = organ weight; PFOA = perfluorooctanoic acid; PND = postnatal day; Repro = reproductive; Resp = respiratory

Figure 2-6. Levels of Significant Exposure to PFOA – Inhalation
Acute (≤14 days)

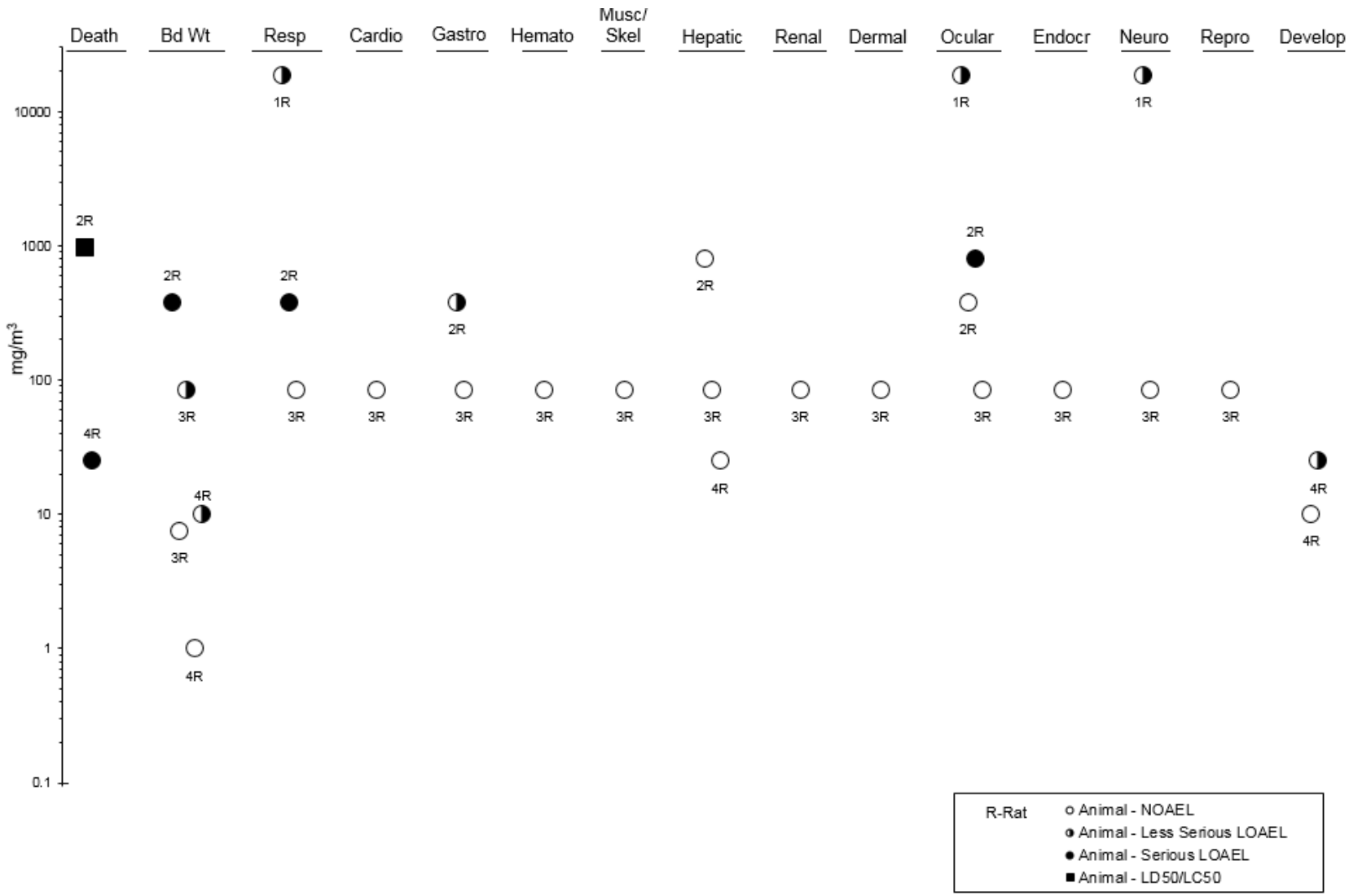


Table 2-2. Levels of Significant Exposure to Other Perfluoroalkyls – Inhalation

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/m^3)	Parameters monitored	Endpoint	NOAEL (mg/m^3)	Less serious LOAEL (mg/m^3)	Serious LOAEL (mg/m^3)	Effect
ACUTE EXPOSURE									
PFNA									
1	Rat (CD) 6 M	4 hours	67–4,600	LE	Death			820	14-day LC_{50}
Kinney et al. 1989									
Exposure was nose-only.									
2	Rat (CD) 10 M	4 hours	0, 67, 590	BW, OW	Bd wt	67	590		Reduced 18% 5 days after exposure
					Resp	67	590		Lung noise; labored breathing during and after exposure
					Hepatic	67			28% increase in absolute liver weight 5 days after exposure to ≥67 mg/m^3
Kinney et al. 1989									
Exposure was nose-only.									

[a]The number corresponds to entries in Figure 2-7.

BW or Bd wt = body weight; LC_{50} = lethal concentration, 50% kill; LE = lethality; LOAEL = lowest-observed-adverse-effect level; M = male(s); NOAEL = no-observed-adverse-effect level; OW = organ weight; PFNA = perfluorononanoic acid; Resp = respiratory

Figure 2-7. Levels of Significant Exposure to Other Perfluoroalkyls – Inhalation
Acute (≤14 days)

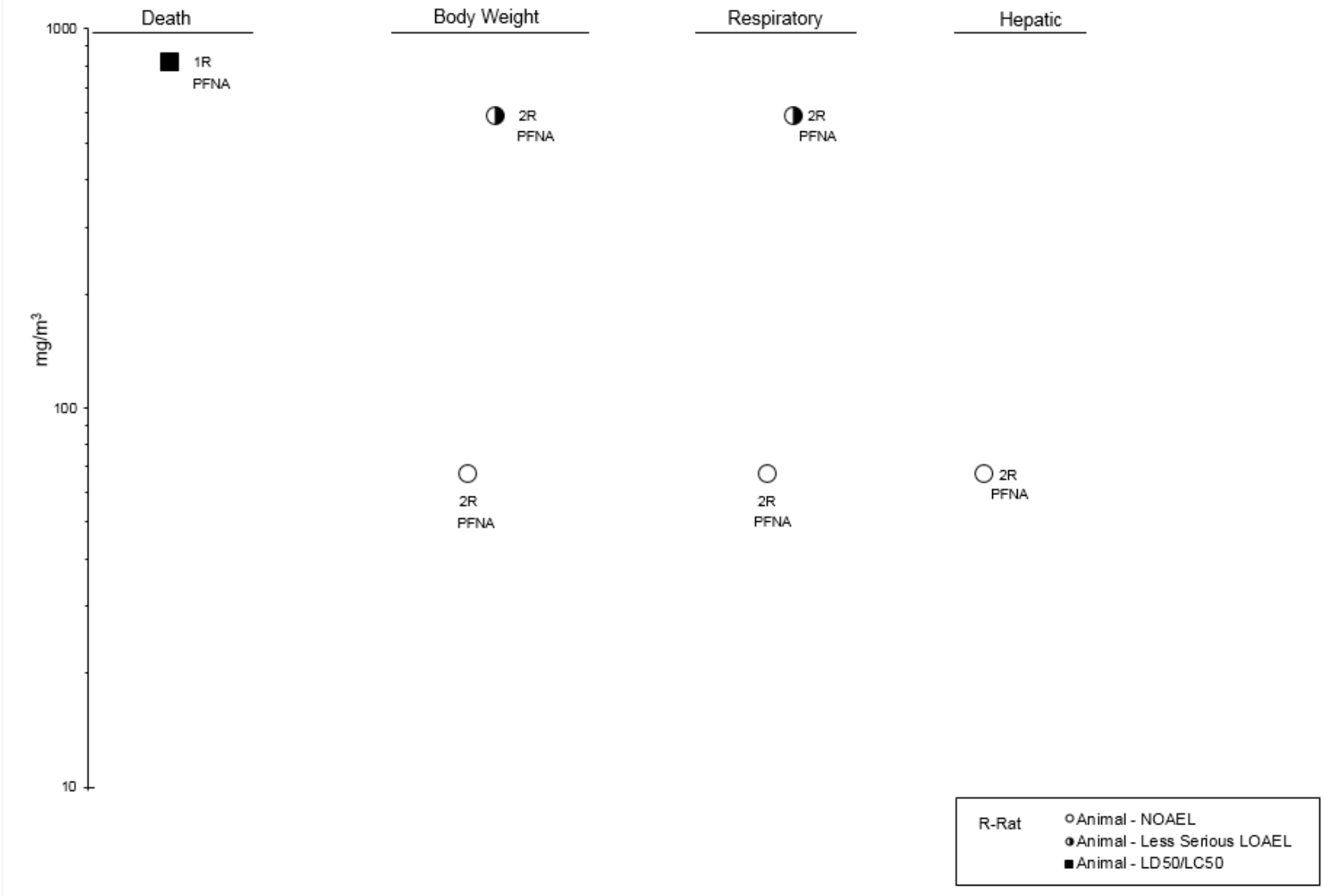


Table 2-3. Levels of Significant Exposure to PFOA – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
ACUTE EXPOSURE									
1	Monkey (Rhesus) 10 M,F	2 weeks (G)	0, 3, 10, 30, 100	LE, CS, HE, BI, GN, HP	Death			100	Unspecified number out of four died on week 2
Griffith and Long 1980									
APFO									
2	Rat (CD) 10 M	14 days (GW)	0, 25	BI, OW	Repro		25		184% increase in serum estradiol
Biegel et al. 1995									
APFO									
3	Rat (CD) 75 M	14 days (GW)	0, 1, 10, 25, 50	BW, OW, BI, HP	Bd wt	10	25		14% reduction in final body weight
					Hepatic	50			46% increase in relative liver weight at ≥10 mg/kg/day
					Repro	1	10		63% increase in serum estradiol
Cook et al. 1992									
APFO									
4	Rat (Sprague-Dawley) 18 M	1 or 7 days (F)	0, 18, 23		Hepatic	23			Increased liver weight, decreased serum cholesterol, triglyceride, hepatocellular hypertrophy at ≥18 mg/kg/day
Elcombe et al. 2010									
APFO									
5	Rat (albino) 25 M,F	Once (GO)	100, 215, 464, 1,000, 2,150		Death			680 M 430 F	LD_{50} LD_{50}
Griffith and Long 1980									
APFO									

Table 2-3. Levels of Significant Exposure to PFOA – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
6	Rat (albino) 40 M,F	28 days (F)	M: 0, 3, 10, 30, 10, 300, 1,000, 3,000; F: 0, 3.4, 11.3, 34, 113, 340, 1,130, 3,400		Death			1,000 M 1,130 F	5/5 males and 5/5 females died before end of 1st week of study
Griffith and Long 1980									
APFO									
7	Rat (Wistar) 8 M	7 days ad lib (F)	0, 16	BW, OW, BI, EA	Bd wt	16			
					Hepatic	16			66% increase in absolute liver weight
Haughom and Spydevold 1992									
APFO									
8	Rat (Sprague-Dawley) 3 M	14 days (F)	0, 20	OW, EA	Hepatic	20			45% increase in relative liver weight
Ikeda et al. 1985									
PFOA									
9	Rat (Sprague-Dawley) 16 M	14 days (GW)	0, 0.5, 5, 50	BW, OW, CS, HE, BI	Bd wt	50			
					Hepatic	50			2-fold increased mean relative liver weight at 50 mg/kg/day
					Immuno	50			No alterations in spleen weight or splenocyte phenotype
Iwai and Yamashita 2006									
APFO									

Table 2-3. Levels of Significant Exposure to PFOA – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
10	Rat (Wistar) 5–12 M	Once (GO)	0, 50	BW, FX	BW	50			
					Neuro	50			No alteration in performance on novel object recognition test
Kawabata et al. 2017									
PFOA									
11	Rat (Wistar) 30 M	1 week (F)	0, 1.2, 2.4, 4.7, 9.5	BW, OW, EA, HP	Bd wt	38			
					Hepatic	9.5			Significant increase in absolute and relative liver weight at ≥4.7 mg/kg/day
Kawashima et al. 1995									
PFOA									
12	Rat (SD-IGS BR) 10 M	14 days (GW)	0, 0.3, 1, 3, 10, 30,	BW, OW, BC	Bd wt	1	3		24% decrease in overall body weight gain
					Hepatic	30			Decreased serum cholesterol levels at ≥0.3 mg/kg/day
Loveless et al. 2006									
APFO									
13	Rat (CD) 15 M	14 days (G)	0, 0.2, 2, 20, 40	BW, OW, EA	Bd wt	2	20		14% lower final body weight
					Hepatic	40			34% increase in absolute and relative liver weight at ≥2 mg/kg/day
					Repro	0.2	2		2-fold increase in serum estradiol
Liu et al. 1996									
APFO									
14	Rat (Sprague-Dawley) 24 M	1, 3, 7 days (GW)	0, 50	BW, BI, EA, HP	Bd wt		50		17% weight loss
					Hepatic	50			2-fold increase in relative and absolute liver weight
Pastoor et al. 1987									
APFO									

Table 2-3. Levels of Significant Exposure to PFOA – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
15	Rat (Sprague-Dawley) 12 or 25 F	GDs 6–15 (GO)	0, 100	DX, MX, BW	Bd wt			100	33% reduced maternal body weight gain
					Develop	100			No alterations in fetal body weight or teratology
Staples et al. 1984									
APFO									
16	Mouse (Kunming) 12 F	GDs 1–7 or 13 (GW)	0, 2.5, 5, 10	BC, OW, RX	Repro		2.5		Decreases in progesterone levels at ≥5 mg/kg in GD 13 group; increased serum estradiol level at 10 mg/kg/day on GD 7; decreases in number of corpora lutea at ≥2.5 mg/kg/day in GD 1–7 groups and at ≥5 mg/kg/day in GD 13 groups
					Develop	5	10		Increased resorbed embryos at 10 mg/kg/day in GD 13 group
Chen et al. 2017b									
PFOA									
17	Mouse (SV129 wild type) 4 M	7 days (G)	0, 10	BW, HP	Bd wt	10			
					Hepatic	10			Hepatocellular hypertrophy, steatosis, and increased hepatic triglyceride levels
Das et al. 2017									
PFOA									
18	Mouse (C57BL/6N) 6 F	10 days (W)	0, 3.75, 7.5, 15	FX	Immuno	7.5	15		Altered response to sRBC
DeWitt et al. 2009									
PFOA									

Table 2-3. Levels of Significant Exposure to PFOA – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
19	Mouse (CD) 40 M,F	28 days (F)	M: 0, 5.4, 18.0, 54, 180, 540, 1,800, 5,400; F: 0, 5.8, 19.5, 58, 195, 580, 1,950, 5,800		Death			180 M 195 F	5/5 died before 2nd week of study 5/5 died before 2nd week of study
Griffith and Long 1980									
APFO									
20	Mouse (C57BL/6N) 16 F	GDs 6–17 (DW)	0, 0.5, 1		Bd wt	1			
					Develop		0.5		7–10% decrease in litter weight on PND 2
Hu et al. 2010									
APFO									
21	Mouse (CD-1) 10 M	Once (G)	0, 0.58, 8.70	CS, OF, DX	Develop		0.58		Decreased spontaneous activity and altered response to cholinergic stimulant
Johansson et al. 2008									
APFO									
10-day-old mice were administered a single dose of PFOA; neurodevelopmental testing was conducted when the pups were 2 or 4 months of age									
22	Mouse (CD-1) 5 M,F	14 days (F)	0, 5.3, 54, 537	OW	Hepatic	537			123–155% increase in absolute liver weight in 14 days at ≥5.3 mg/kg/day
Kennedy 1987									
APFO									
23	Mouse (CD-1) 10 M	14 days (GW)	0, 0.3, 1, 3, 10, 30,	BW, OW, BC	Bd wt	3		10	6–12% decreased in body weight gain
					Hepatic	30			Decreased serum cholesterol levels at ≥0.3 mg/kg/day
Loveless et al. 2006									
APFO									

Table 2-3. Levels of Significant Exposure to PFOA – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
24	Mouse (C57BL/6N) 3 M	2–10 days (F)	0, 78, 390	BW, OW, EA	Bd wt			78	25% body weight loss after 10 days of treatment
					Hepatic	390			74% increase in absolute liver weight at ≥78 mg/kg/day
Permadi et al. 1992									
PFOA									
25	Mouse (C57BL/6N) 4 M	2–10 days (F)	0, 78, 390	BW, OW, EA	Bd wt			78	25% body weight loss after 5 days of treatment
					Hepatic	390			74% increase in absolute liver weight in 5 days at ≥78 mg/kg/day
Permadi et al. 1993									
PFOA									
26	Mouse (C57BL/6) 4 M	10 days (F)	0, 2	BW, FI, BC, HP	Bd wt	2			
					Hemato	2			
					Hepatic	2			68% increase in liver weight, hepatocellular hypertrophy, and decreased serum cholesterol levels
					Immuno	2			
Qazi et al. 2010a									
PFOA									
27	Mouse (BALB/c) 5 F	7 days (GW)	20	BW, OW, BC	Bd wt		20		36% decrease in body weight gain
					Hepatic	20			32% increase in relative liver weight
					Immuno		20		Inhibition of T-and B-lymphocyte proliferation in response to sRBC; decreased phagocytosis by peripheral blood cells and NK cell activity; decreased IgM antibody formation in response to OVA
Vetvicka and Vetvickova 2013									
PFOA									

Table 2-3. Levels of Significant Exposure to PFOA – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
28	Mouse (CD-1) 14 F	GDs 8–17 GDs 1217 (GW)	0, 5	DX, GN	Develop		5		Altered mammary gland development in female pups; reduced pup weight on PND 20
White et al. 2007									
APFO									
29	Mouse (CD-1) 56 F	GDs 8–17 (GW)	0, 5		Bd wt	5			
					Hepatic	5			40–120% increased relative liver weight in lactating dams on PNDs 1–10
					Repro		5		Immature mammary gland morphology in lactating dams on PNDs 1–10
					Develop		5		Delayed mammary gland development (30–60%) in female pups on PNDs 1–10
White et al. 2009									
APFO									
30	Mouse (CD-1) 12–14 F	GDs 7–17 GDs 10–17 GDs 13–17 GDs 15–17	0, 5		Develop		5		Delayed mammary gland development (31–47%) in female pups on PNDs 22–32 and at 18 months
White et al. 2009									
APFO									
31	Mouse (CD-1) 6–14 F	GDs 7–17, GDs 10–17 GDs 13–17 GDs 15–17 (GW)	0, 5, 20	DX, MX, BW, OW	Bd wt	20			No alterations in dams dosed on GDs 15–17
					Hepatic	20			Increase in relative liver weight in dams dosed on GDs 13–17, 10–17, or 7–17 at ≥5 mg/kg/day
					Develop		5		Reduced pup body weight at weaning, 43% in males and 35% in females
Wolf et al. 2007									
APFO									

Table 2-3. Levels of Significant Exposure to PFOA – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
32	Mouse (C57BL/6N) 8 M	7 days (F)	0, 24	CS, BW, OW, BI	Bd wt		24		>10% reduced final body weight
					Hepatic	24			2-fold increase in absolute liver weight
Xie et al. 2003									
PFOA									
33	Mouse (C57BL/6N) 8 M	10 days (F)	0, 30	BW, OW, BI, CS	Bd wt		30		17% decrease in final body weight
					Hepatic	30			>90% increase in absolute and relative liver weight
					Immuno			30	86% reduction in absolute thymus weight; 30% reduction in absolute spleen weight
Yang et al. 2000									
PFOA									
34	Mouse (C57BL/6N) 8 M	10 days (F)	0, 1, 3.5, 11.5, 23, 58	CS, BW, OW, OF, BI	Hepatic	1			35% increase in absolute liver weight at ≥1 mg/kg/day
					Immuno		11.5		40–50% decrease in spleen and thymus weights
Yang et al. 2001									
PFOA									
35	Mouse (C57BL/6N) 8–12 M	7 days (F)	0, 24	CS, BW, BI, OF	Immuno		24		Decreased humoral response to immunization with horse red blood cells
Yang et al. 2002a									
PFOA									
36	Mouse (C57BL/6N) 16 M	7 days ad lib (F)	0, 33	BW, OW, BI, OF	Bd wt		33		14% decreased mean body weight
					Hepatic	33			86% increase in absolute liver weight
					Immuno		33		40% reduction in spleen weight and 79% reduction in thymus weight
Yang et al. 2002b									
PFOA									
Experiments with PPARα-null mice suggested PPARα-dependent and -independent immune effects									

Table 2-3. Levels of Significant Exposure to PFOA – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
37	Rabbit (New Zealand) 18 F	GDs 6–18 1 time/day (GW)	0, 1.5, 5, 50	CS, MX, DX, BW	Develop	50			
Gortner et al. 1982									
PFOA									
INTERMEDIATE EXPOSURE									
38	Monkey (Cynomolgus) 4–6 M	26 weeks 1 time/day (C)	0, 3, 10, 30/20	BC, BW, CS, EA, FI, GN, HE, HP, LE, OP, OW, UR	Bd wt	10	20		12% decrease by week 10
					Resp	20			
					Cardio	20			
					Gastro	20			
					Hemato	20			
					Musc/skel	20			
					Hepatic		3		36% increase in absolute liver weight at ≥3 mg/kg/day; increased serum triglyceride levels at 30/20 mg/kg/day
					Renal	20			
					Dermal	20			
					Ocular	20			
					Endocr	3	10		Significant decrease in serum TT4 (27–35%) and FT4 (30–38%)
					Immuno	20			No histological alterations
					Neuro	20			No histological alterations
					Repro	20			No histological alterations
Butenhoff et al. 2002									
APFO									

Table 2-3. Levels of Significant Exposure to PFOA – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
39	Monkey (Rhesus) 10 M,F	90 days 1 time/day (G)	0, 3, 10, 30, 100	LE, CS, HE, BI, GN, HP	Death			30	One male and two females died during weeks 7–12
					Bd wt	10		30	33% body weight loss by week 6
					Cardio	10			
					Gastro	10	30		Emesis
					Hemato	30			
					Hepatic	10			
					Renal	10			
					Immuno	10	30		Atrophy of lymphoid follicles in spleen and lymph nodes
					Neuro	10	30		Hypoactivity and prostration
					Repro	100			No histological alterations in testes or ovaries
Griffith and Long 1980									
APFO									
40	Monkey (Cynomolgus) 8 M	30 day 1 time/day (C)	0, 2, 20	CS, BW, FI, BI, HE, EA, GN, HP	Bd wt	20			
					Hemato	20			
					Hepatic	20			
					Endocr	20			No alterations in serum levels of thyroid hormones and TSH and histopathology of adrenals
					Repro	20			No alterations in serum estradiol, estriol, or histopathology of the testes
Thomford 2001									
APFO									

Table 2-3. Levels of Significant Exposure to PFOA – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
41	Rat (CD) 156 M	1 year ad lib (F)	0, 13.6	CS, BW, FI, OW, GN, HP	Bd wt		13.6		>10% reduced weight gain
					Hepatic	13.6			Increased relative liver weight
					Repro		13.6		Significant increase in serum estradiol at 1, 3, 6, 9, and 12 months (~100–180%); prolactin was decreased at all time points, but not always significantly
Biegel et al. 2001									
APFO									
42	Rat (Sprague-Dawley) 30 M,F	70–90 days 1 time/day (GW)	0, 1, 3, 10, 30	MX, DX, OF, BW, GN, HP	Bd wt	3	10		>11% reduced body weight
					Hepatic	1	3		Increased absolute and relative liver weight in P-generation (36%) and F1-generation (30%) males; hepatocellular hypertrophy and less commonly necrosis in F1 males (incidence not reported)
					Renal		3		Increased absolute and relative kidney weight in P-generation (14%) and F1-generation (11%) males
					Endocr	10	30		Vacuolation of zona glomerulosa of adrenal gland
					Repro	30			No alteration in reproductive performance in P0 or F1 generation
					Develop	10		30	Increased number of dead pups on PNDs 6–8
Butenhoff et al. 2004b									
APFO									
43	Rat (Wistar) 5 F	GD 1 to PND 21 ad lib (W)	0, 1.6		Develop		1.6		17–18% reduced motor coordination and increased locomotor activity in pups on PNDs 34–35
Cheng et al. 2013									
PFOA									

Table 2-3. Levels of Significant Exposure to PFOA – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
44	Rat (Sprague-Dawley) 10 M	Daily 28 days (G)	0, 5, 20	OW, HP	Resp		5		
					Hepatic		5		Cytoplasmic vacuolization, necrosis, hypertrophy, increased liver weight at ≥5 mg/kg/day; fatty degeneration, angiectasis and congestion in the hepatic sinusoid or central vein at 20 mg/kg/day
					Neuro		5		Cachexia and lethargy
Cui et al. 2009									
PFOA									
45	Rat (Sprague-Dawley) 10 M	28 days (F)	0, 18		Hepatic		18		Increased liver weight (43% on day 29), decreased serum cholesterol (39% on day 29) and triglyceride (73% on day 29), hepatocellular hypertrophy and hyperplasia
Elcombe et al. 2010									
APFO									
46	Rat (CD) 40 M,F	28 days ad lib (F)	M: 0, 3, 10, 30, 100, 300, 1,000, 3,000; F: 0, 3.4, 11.3, 34, 113, 340, 1,130, 3,400	BW, FI, HP	Bd wt	10 M	30 M	100 M	30 mg/kg/day: 11% reduction in final body weight; 100 mg/kg/day: 33% reduction in final body weight
					Hepatic	3 M			Hepatocyte hypertrophy
Griffith and Long 1980									
APFO									
47	Rat (CD) 30 M,F	90 days ad lib (F)	M: 0, 1, 3, 10, 30, 100; F: 0, 1.1, 3.4, 11, 34, 110	CS, BW, FI, HE BI, GN, HP, OW	Bd wt	30 M 110 F	100 M		33% reduction in final mean body weight
					Resp	100 F			
					Cardio	110 F			
					Gastro	110 F			
					Musc/skel	110 F			
					Hepatic	100 M			Hepatocyte hypertrophy; 50% increase in absolute liver weight
					Renal	110 F			

Table 2-3. Levels of Significant Exposure to PFOA – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
					Dermal	110 F			
					Ocular	110 F			
					Endocr	110 F			
					Immuno	110 F			
					Neuro	110 F			No histological alterations
					Repro	100 M 110 F			No histological alterations
Griffith and Long 1980									
APFO									
48	Rat (CD) 10 M	28 days (G)	0, 0.29, 0.96, 9.6, 29		Bd wt	0.96	9.6		10% decrease in final body weight
					Hemato	29			
					Hepatic	0.29	29		34% decrease in serum triglyceride levels, minimal hepatocellular hypertrophy at ≥0.29 mg/kg/day; minimal focal necrosis at 29 mg/kg/day
					Immuno	29			
Loveless et al. 2008									
APFO									
49	Rat (CD) 55 M	13 weeks ad lib (F)	0, 0.06, 0.64, 1.94, 6.5	CS, BW, FI, OW, GN, HP	Bd wt	6.5			
					Resp	6.5			
					Hepatic	6.5			Minimal to moderate hepatocellular hypertrophy at ≥0.64 mg/kg/day
					Neuro	6.5			No histological alterations
					Repro	6.5			No histological alterations
Perkins et al. 2004									
APFO									

Table 2-3. Levels of Significant Exposure to PFOA – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
50	Mouse (129S1/SvlmJ WT) 17 F	GDs 1–17 (GW)	0, 0.1, 0.3, 0.6, 1, 3, 5, 10, 20	MX, DX, BW	Bd wt	10 F			
					Hepatic	20			Increased absolute and relative liver weight of dams on PND 22 at ≥1 mg/kg/day
					Develop	0.3		0.6	Significantly reduced pup survival (46%) from birth to weaning
Abbott et al. 2007									
APFO									
Body weight NOAEL is for changes during pregnancy.									
51	Mouse (wild-type Sv/129) 5–6 F	GDs 1–17 1 time/day (GW)	0, 3		Hepatic	3			28% increased liver weight, hepatocellular hypertrophy with increased peroxisomes
					Develop			3	31.5% reduced pups per litter on PND 20
Albrecht et al. 2013									
PFOA									
52	Mouse (C57BL/6N) 8 F	15 days ad lib (W)	0, 0.94, 1.88, 3.75, 7.5, 15, 30	BW, OW, OF	Bd wt	7.5		15	Weight loss (~5%)
					Immuno	1.88	3.75		Reduced sRBC-specific response to IgM antibody titers
DeWitt et al. 2008									
APFO									
53	Mouse (wild-type C57BL/6-Tac and PPARα knockout) 8 F	15 days (W)	0, 7.5, 30	BW, OW, BC	Bd wt		30		14–20% decrease in body weight in wild-type mice
					Immuno	7.5	30		16 and 14% reduction in sRBC-specific antibody response in wild-type and PPARα knockout mice, respectively; 29.8% decrease in relative spleen weight in wild-type mice
DeWitt et al. 2016									
PFOA									

Table 2-3. Levels of Significant Exposure to PFOA – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
54	Mouse (C57BL/6N) 8 F	15 days (W)	0, 0.94, 1.88, 3.75, 7.5	BW, OW, BC	Bd wt	7.5			
					Immuno	0.94	1.88		Decrease in DNP-specific IgM antibody responses; decreases in relative spleen (17%) and thymus weights (14%) at 7.5 mg/kg/day
DeWitt et al. 2016									
PFOA									
55	Mouse (C57BL/6N) 8 F	10, 13, 15 days (W)	0, 3.75, 7.5	BC	Bd wt	7.5			
DeWitt et al. 2016									
PFOA									
56	Mouse (CD-1) 6–14 dams; 21–37 F offspring	GDs 1–17 (GW)	0, 0.01, 0.1, 0.3, 1, 5	HP	Hepatic	0.3	1		Increase in severity of chronic inflammation at ≥1 mg/kg/day; Ito cell hypertrophy and centrilobular hepatocellular hypertrophy at 5 mg/kg/day
Filgo et al. 2015a, 2015b									
PFOA									
Animals exposed *in utero* on GDs 1–17 and examined at 18 months of age; the offspring were also examined by Hines et al. 2009									
57	Mouse (129/Sv WT) 3–7 dams; 6–10 F offspring	GDs 1–17 (GW)	0, 0.1, 0.3, 0.6, 1	HP	Hepatic	1			Increased severity of centrilobular hepatocyte hypertrophy at ≥0.3 mg/kg/day
Filgo et al. 2015a, 2015b									
PFOA									
Animals exposed *in utero* on GDs 1–17 and examined at 18 months of age; the offspring were also examined by Abbott et al. 2007									
58	Mouse (129/Sv PPARα-knockout) 5–9 dams; 6–10 F offspring	GDs 1–17 (GW)	0, 0.1, 0.3, 1, 3	HP	Hepatic	1	3		Centrilobular hepatocyte hypertrophy and bile duct hyperplasia at 3 mg/kg/day
Filgo et al. 2015a, 2015b									
PFOA									
Animals exposed *in utero* on GDs 1–17 and examined at 18 months of age; the offspring were also examined by Abbott et al. 2007									

Table 2-3. Levels of Significant Exposure to PFOA – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
59	Mouse (CD-1) NS F	GDs 1–17 (GW)	0, 0.1, 0.3, 1	NX	Develop	0.3	1		Increased ambulatory activity and decreased methamphetamine-induced activity
Goulding et al. 2017									
PFOA									
60	Mouse (CD) 40 M,F	28 days ad lib (F)	M: 0, 5.4, 18.0, 54, 180, 540, 1,800, 5,400; F: 0, 5.8, 19.5, 58, 195, 580, 1,950, 5,800	BW, FI, HP	Death			54 M 58 F	4/5 died before end of 4th week 5/5 died before 4th week of study
					Bd wt		5.4 M	5.8 F	Males: final body weight 20% lower than controls; females: final body weight 25% lower than controls
					Hepatic	18			3-fold or greater increased absolute and relative liver weight and hepatocellular hypertrophy at ≥5.4 mg/kg/day
Griffith and Long 1980									
APFO									
61	Mouse (CD-1) 5–14F	GDs 1–17 (GW)	0, 0.01, 0.1, 0.3, 1, 3, 5	BW FX	Develop		5 F		Decreased birth weight (approximately 8%) and body weight at weaning (24%)
Hines et al. 2009									
APFO									
62	Mouse (CD-1) 5 M,F	21 days ad lib (F)	0, 0.0018, 0.0054, 0.018, 0.054, 0.18, 0.54, 1.8, 5.4	OW	Hepatic	5.4			39–41% increase in absolute liver weight at ≥5.4 mg/kg/day
Kennedy 1987									
APFO									
63	Mouse (C57BL/6) 10 F	GDs 1–21 (F)	0.3	BW, DX	Develop		0.3[b]		Altered femur and tibial bone morphology, decreased tibial mineral density
Koskela et al. 2016									
PFOA									
Offspring were examined at 13 and 17 months of age									

Table 2-3. Levels of Significant Exposure to PFOA – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
64	Mouse (CD-1) 9–45 M	GDs 1–17 1 time/day (GW)	0, 1, 3, 5, 10, 20, 40	MX, DX, BW, OW	Bd wt	5		10	32% reduced weight gain during pregnancy
					Hepatic	1			38% increase in absolute liver weight at ≥1 mg/kg/day
					Develop		1	5	Reduced ossification of proximal phalanges and advanced preputial separation at ≥1 mg/kg/day; 20% decrease in pup body weight on PND 23 at ≥3 mg/kg/day; increased number of dams with full litter resorptions, decrease in neonatal survival, tail and limb defects, delay in eye opening, and delay in first estrus at ≥5 mg/kg/day
Lau et al. 2006									
APFO									
65	Mouse (CD) 20 M	28 days (G)	0, 0.29, 0.96, 9.6, 29		Bd wt	0.96		9.6	Weight loss (86% of controls)
					Hepatic	0.29	0.96		Mild hepatocellular hypertrophy at ≥0.29, moderate to severe hypertrophy and single cell necrosis at ≥0.96 mg/kg/day; decreased serum cholesterol (31%) and triglyceride (53%) at ≥9.6 mg/kg/day
					Immuno	0.96 M	9.6 M		Decreased response to sRBC, decreased number of splenic and thymic lymphocytes
Loveless et al. 2008									
APFO									
66	Mouse (CD-1) 13 F	GDs 1–17 (G)	0, 0.3, 1.0, 3.0	DX	Develop		0.3		Impaired development of mammary glands in offspring
Macon et al. 2011									
PFOA									

Table 2-3. Levels of Significant Exposure to PFOA – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
67	Mouse (CD-1) 13 F	GDs 10–17 (G)	0, 0.01, 0.1, 1.0	DX	Develop		0.01		Developmental delays in mammary gland development
Macon et al. 2011									
PFOA									
68	Mouse (C57BL/6J-Apc+/+) 20–21 F	GDs 1–17 (GW)	0, 0.01, 0.1, 3.0	BC, BW, FI, HE, OW	Develop	0.1	3		Decrease in number of successful births
Ngo et al. 2014									
PFOA									
69	Mouse (C57BL/6/Bk1) 6 M, 10 F	GDs 1–21 ad lib (F)	0, 0.3		Develop		0.3		Altered exploratory behavior in adult offspring (increased in males and decreased in females); increased global activity in males
Onishchenko et al. 2011									
PFOA									
70	Mouse (CD-1) 17–21 F dams; 4–6 M,F pups	GDs 1–17 (GW)	0, 0.01, 0.1, 0.3, 1	BI, BW, HP, OF, OW	Hepatic		0.01		Increased severity of hepatocellular hypertrophy at PND 91 and periportal inflammation on PND 21 at ≥0.01 mg/kg/day (incidence was not reported); decreased serum total cholesterol, LDL, and HDL levels in high-fat fasted animals on PND 91 at ≥0.3 mg/kg/day
Quist et al. 2015a, 2015b									
PFOA									
Subgroup of female offspring were fed a high-fat diet (50% calories from diet) for 6 weeks									
71	Mouse (C57BL/6) 6 M,F	6 weeks (F)	0.55	BC, BW, FI, OW	Hepatic	0.55			66–67% increase in relative liver weight in males; increased plasma cholesterol levels in males (35%) and females (70%)
Rebholz et al. 2016									
PFOA									

Table 2-3. Levels of Significant Exposure to PFOA – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
72	Mouse (BALB/c) 6 M,F	6 weeks (F)	0.55	BC, BW, FI, OW	Hepatic	0.55			54–65% increase in relative liver weight; 20% increase in plasma cholesterol in males
Rebholz et al. 2016									
PFOA									
73	Mouse (C57BL/6) 6 F	GD 7 to PND 21 (F)	0, 0.1	BH, BW	Develop		0.1		Increased horizontal and ambulatory locomotor activity and decreased resting time in males; decrease in novel object recognition in males and females
Sobolewski et al. 2014									
PFOA									
74	Mouse (ICR) 10 M	21 days ad lib (W)	0, 0.5, 2.6, 18, 47	BW, OW, GN, HP	Bd wt	2.6		18	17% decrease in weight gain
					Hepatic	18	47		27% increase in relative liver weight at ≥0.5 mg/kg/day; increases in ALT at ≥2.6 mg/kg/day; hepatocytomegaly at 18 mg/kg/day; necrosis at 47 mg/kg/day
					Renal	47			
Son et al. 2008									
APFO									
75	Mouse (ICR) 10 M	21 days (W)	0, 0.49, 2.64, 17.63, 47.21	FX HP	Immuno		47.21		Marked hyperplasia in spleen white pulp and thymic atrophy
Son et al. 2009									
PFOA									
76	Mouse (C57BL/6N) 7–8 M	3 weeks (F)	0, 5	HP	Hepatic		5		Hepatocellular hypertrophy and degeneration
Tan et al. 2013									
PFOA									

Table 2-3. Levels of Significant Exposure to PFOA – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
77	Mouse (CD-1) 4–12 F	GDs 1–17 (GW)	0, 0.01, 0.1, 0.3, 1.0	BW, DX, OW	Develop		0.01		Developmental delays in the mammary glands on PNDs 35 (26%) and 56 (30%)
Tucker et al. 2015 PFOA									
78	Mouse (C57BL/6) 2–6 F	GDs 1–17 (GW)	0, 0.01, 0.1, 0.3, 1.0	BW, DX, OW	Develop	0.1	0.3		Developmental delays in the mammary glands on PNDs 21 (38%) and 61 (25%)
Tucker et al. 2015 PFOA									
79	Mouse (CD-1) 14–16 F	GDs 1–17 GDs 8–17 1 time/day GDs 12–17 (GW)	0, 5	MX, DX, GN, HP	Repro		5		Delayed mammary gland differentiation
White et al. 2007 APFO									
80	Mouse (CD-1) 14 F	GDs 1–17 1 time/day (GW)	0, 5	DX, GN	Develop			5	Increased prenatal loss; 40% reduced neonatal body weight on PNDs 5 and 10
White et al. 2007 APFO									
81	Mouse (CD-1) 28–48 F	GDs 1–17 1 time/day (GW)	0, 3, 5		Develop		3		Delayed mammary gland development in female pups on PNDs 22–63 and at 18 months
White et al. 2009 APFO									
82	Mouse (CD-1) 10–12 F	GDs 1–17 1 time/day (GW)	0, 1, 5		Repro		1		Delayed mammary gland lactational differentiation in dams on PND 22
					Develop	1		5	323% increased prenatal loss, 16.7% decreased live fetuses, 24.3% decreased neonatal survival
White et al. 2011 APFO									

Table 2-3. Levels of Significant Exposure to PFOA – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
83	Mouse (CD-1) 10–12 F	GDs 1–17 1 time/day (GW)	0, 0.0024, 1.0024		Repro		0.0024		Delayed mammary gland lactational differentiation in dams on PND 22
		GD 7 to PND 22 (W); 3-generation study			Develop		0.0024		Delayed mammary gland development in female pups on PNDs 22–63
White et al. 2011									
APFO									
84	Mouse (CD-1) 28–48 F	GDs 1–17 1 time/day (GW)	0, 3, 5	CS, BW, MX, DX, OW	Bd wt	5			
					Hepatic	5			Significant increase in relative and absolute maternal liver weight on PND 22 at ≥3 mg/kg/day
					Develop		3	5	3 mg/kg/day: reduced weight gain through lactation (14.8% in males and 20.6% in females); delayed eye opening and hair growth at ≥3 mg/kg/day; decreased pup survival from birth to weaning at 5 mg/kg/day
Wolf et al. 2007									
APFO									
85	Mouse (ICR) 5–10 F	GDs 0–17 or GDs 0–18 (GW)	0, 1, 5, 10	BW, FI, WI, OW, HP, DX	Hepatic	1	10		35% increased maternal relative liver weight with hepatocellular hypertrophy at ≥1 mg/kg/day; single cell necrosis and mild calcification at 10 mg/kg/day
					Renal	1			35% increased maternal relative kidney weight with renal hypertrophy
					Develop	1		5	14% increased neonatal mortality, 9.5% reduced fetal body weight
Yahia et al. 2010									
PFOA									

Table 2-3. Levels of Significant Exposure to PFOA – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
86	Mouse (BALB/c) 5 F	5 days/week 4 weeks starting at PND 21 (GW)	0, 1, 5, 10	BW, OW, HP	Bd wt	5	10		13% decrease in body weight gain
					Hepatic	10			Increases in liver weight and hepatocellular hypertrophy at ≥1 mg/kg
					Develop		1		Delay in vaginal opening at 1 mg/kg and mammary gland growth inhibition at 5 and 10 mg/kg
Yang et al. 2009									
PFOA									
87	Mouse (C57BL/6)) 5 F	5 days/week 4 weeks starting at PND 21 (GW)	0, 1, 5, 10	BW, OW, HP	Bd wt	5	10		10% decrease in body weight gain
					Hepatic	10			Increases in liver weight and hepatocellular hypertrophy at ≥1 mg/kg
					Develop	1	5		Delay in vaginal opening at 5 mg/kg and mammary gland growth stimulation a 5 mg/kg and inhibition at 10 mg/kg
Yang et al. 2009									
PFOA									
88	Mouse (C57BL/6)) 2–5 F	5 days/week 4 weeks (GW)	0, 5	BC	Repro		5		Increased progesterone levels
Zhao et al. 2010									
PFOA									
89	Mouse (PPARα knockout) NR F	5 days/week 4 weeks (GW)	0, 5	HP	Develop		5		Mammary gland growth stimulation
Zhao et al. 2010									
PFOA									

Table 2-3. Levels of Significant Exposure to PFOA – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
CHRONIC EXPOSURE									
90	Rat (CD) 156 M	2 years ad lib (F)	0, 13.6	CS, BW, FI, OW, GN, HP	Bd wt		13.6		>10% reduction in weight gain most of the study
					Hepatic	13.6			Increased relative liver weight
					Repro		13.6		Increased incidence of Leydig cell hyperplasia; elevated serum LH at 18 months
					Other noncancer		13.6		Increased incidence of acinar cell hyperplasia in pancreas
					Cancer			13.6	CEL: testicular Leydig cell adenomas and pancreatic acinar cell adenomas
Biegel et al. 2001									
APFO									
91	Rat (Sprague-Dawley) 50–65 M, 50–65 F	2 years ad lib (F)	0, 1.5, 15	CS, FI, BW, OW, HE, BI, GN, HP	Bd wt	1.5 F	15 F		10.3% lower terminal body weight
					Resp	15			
					Cardio	15			
					Gastro	15			
					Hemato	15			
					Hepatic	1.5	15		Increased serum ALT and AST at ≥1.5 mg/kg/day; hepatocellular hypertrophy at 15 mg/kg/day; hepatocellular necrosis at 15 mg/kg/day only at 1 year
					Renal	15			
					Ocular	15			
					Endocr	15			
					Immuno	15			
					Neuro	15			

Table 2-3. Levels of Significant Exposure to PFOA – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
					Repro	1.5 M 15 F	15 M		Vascular mineralization in the testes
					Other noncancer		1.5 M		Inflammation of the salivary gland
					Cancer			15	CEL: testicular Leydig cell adenomas

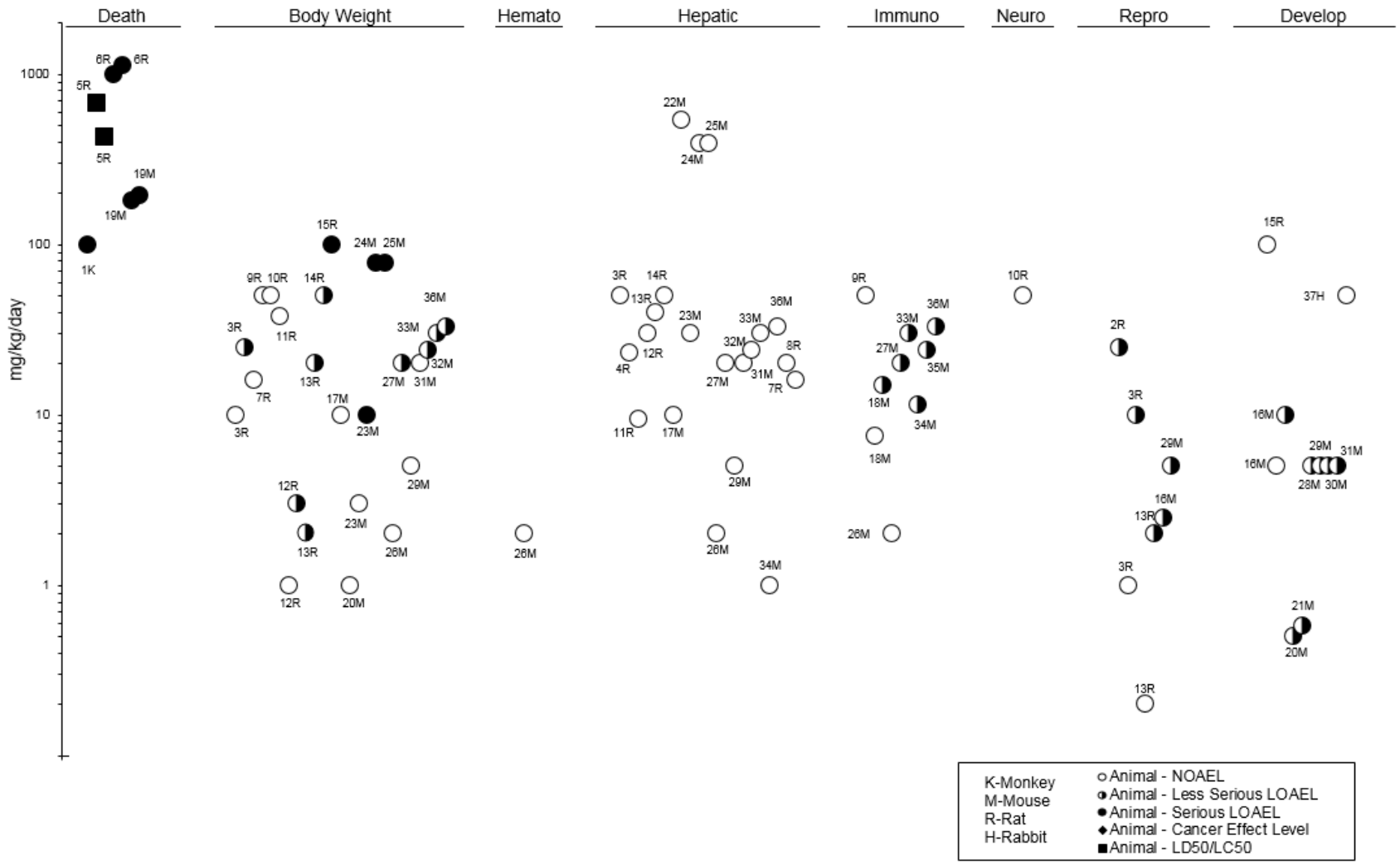
3M 1983; Butenhoff et al. 2012c

APFO

[a]The number corresponds to entries in Figure 2-8.
[b]Used to derive an intermediate-duration oral MRL of $3x10^{-6}$ mg/kg/day based on the predicted TWA serum PFOA level of 8.29 µg/mL at the LOAEL dose and an empirical clearance model to estimate a HED. The $LOAEL_{HED}$ of 0.000821 mg/kg/day was divided by an uncertainty factor of 300 (10 for the use of a LOAEL, 3 for extrapolation from animals to humans with dosimetric adjustment, and 10 for human variability).

ad lib = *ad libitum*; ALT = alanine aminotransferase; APFO = ammonium perfluorooctanoate (ammonium salt of PFOA); AST = aspartate aminotransferase; BC = biochemistry; BI = biochemical changes; BW or Bd wt = body weight; C = capsule; Cardio = cardiovascular; CS = clinical signs; Develop = developmental; DW = drinking water; DX = developmental toxicity; EA = enzyme activity; Endocr = endocrine; (F) = feed; F = female(s); FI = food intake; FT4 = free thyroxine; FX = fetal toxicity; G = gavage; Gastro = gastrointestinal; GD = gestation day; GN = gross necropsy; GO = gavage in oil vehicle; GW = gavage in water vehicle; HDL = high-density lipoprotein; HE or Hemato = hematological; HED = human equivalent dose; HP = histopathology; Immuno = immunotoxicological; LD_{50} = lethal dose, 50% kill; LE = lethality; LDL = low-density lipoprotein; LH = luteinizing hormone; LOAEL = lowest-observed-adverse-effect level; M = male(s); MRL = Minimal Risk Level; Musc/skel = musculoskeletal; MX = maternal toxicity; Neuro = neurological; NK = natural killer; NOAEL = no observed-adverse-effect level; NX = neurotoxicity; OF = organ function; OP = ophthalmology; OW = organ weight; PFOA = perfluorooctanoic acid; PND = postnatal day; PPARα = peroxisome proliferator-activated receptor-α; Repro = reproductive; Resp = respiratory; RX = reproductive toxicity; sRBC = sheep red blood cell; TSH = thyroid-stimulating hormone; TT4 = total thyroxine; TWA = time-weighted average; UR = urinalysis; W = water; WI = water intake

Figure 2-8. Levels of Significant Exposure to PFOA – Oral
Acute (≤14 days)



KD0080

Figure 2-8. Levels of Significant Exposure to PFOA – Oral
Intermediate (15–364 days)

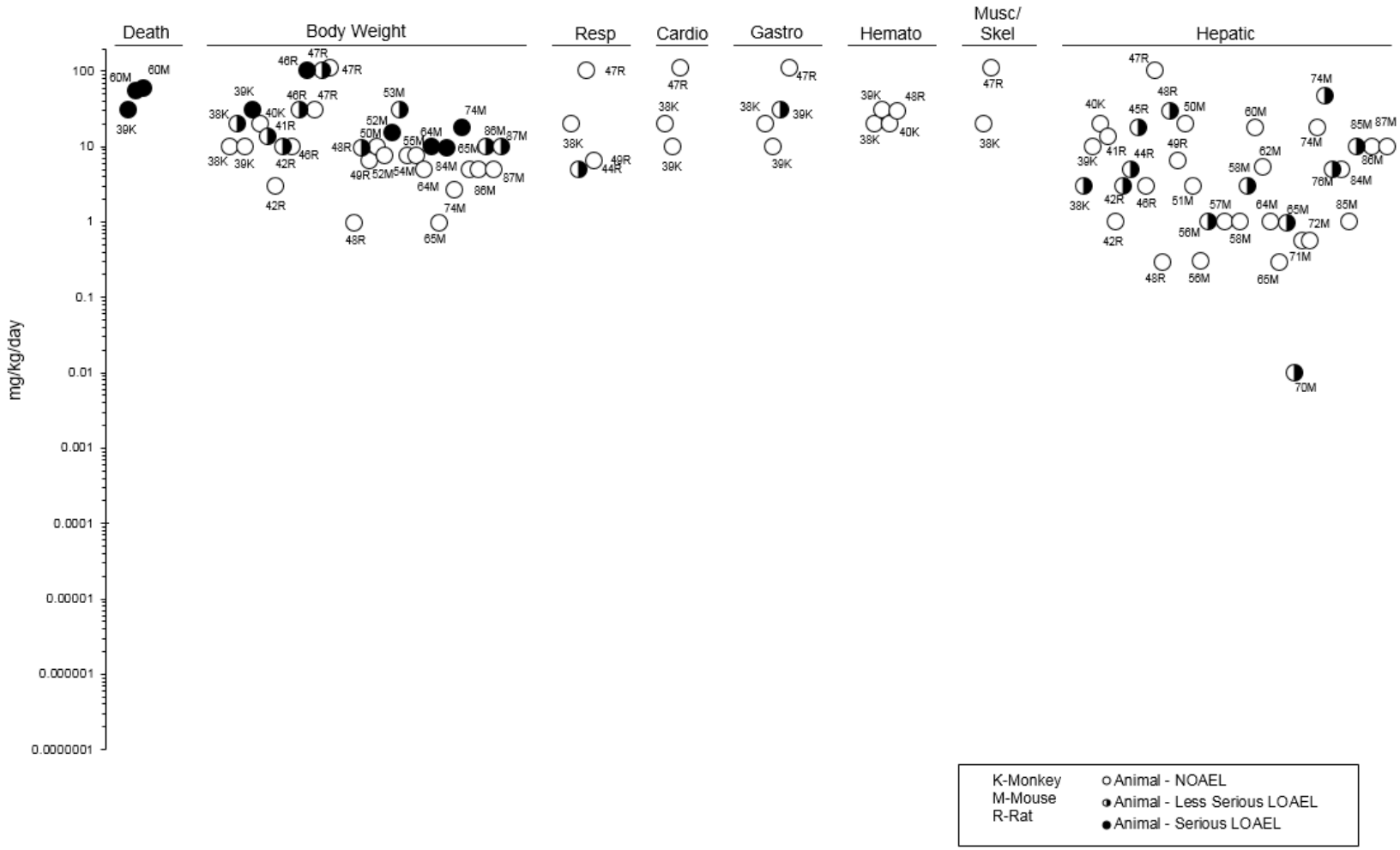


Figure 2-8. Levels of Significant Exposure to PFOA – Oral
Intermediate (15–364 days)

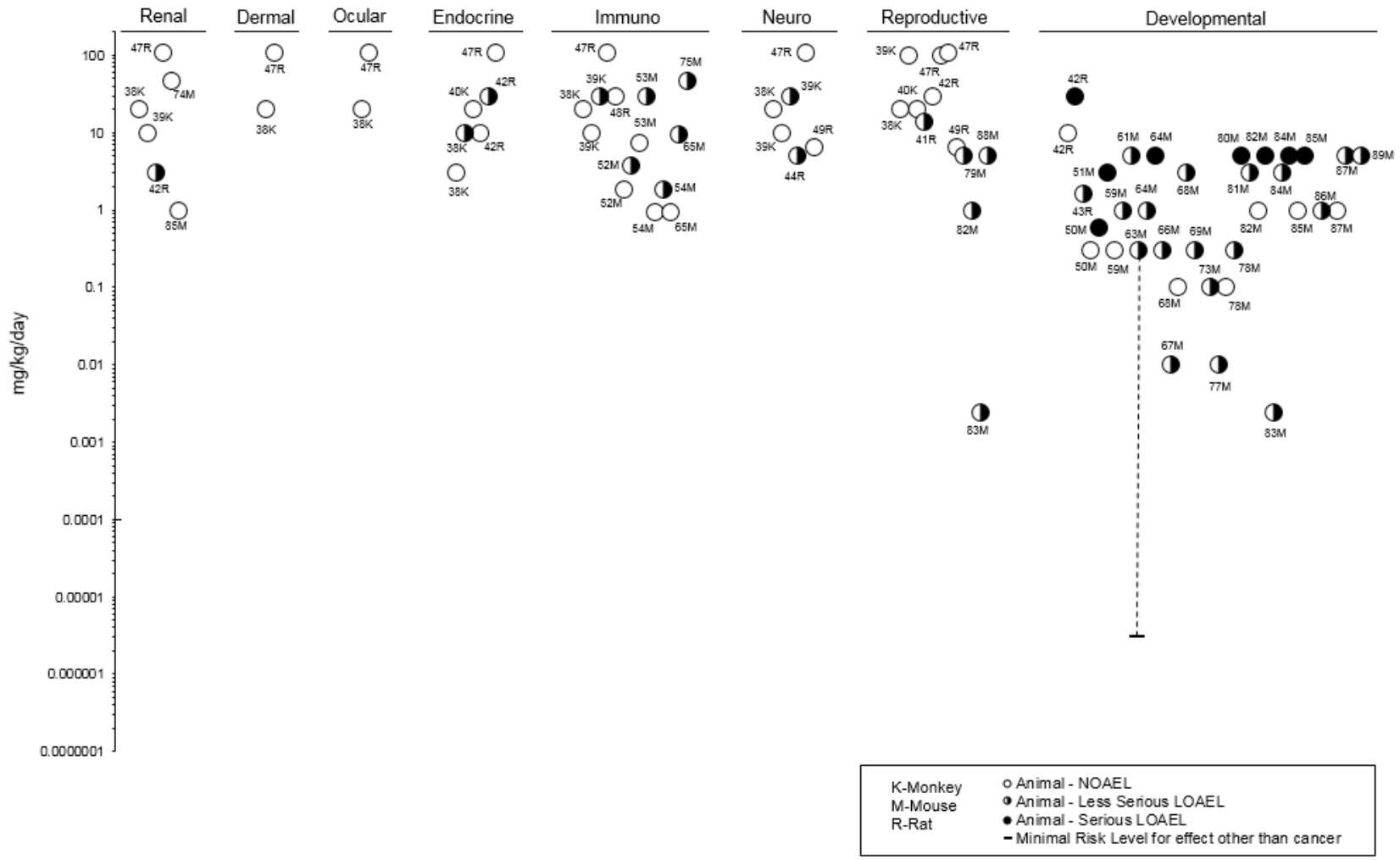


Figure 2-8. Levels of Significant Exposure to PFOA – Oral
Chronic (≥365 days)



Table 2-4. Levels of Significant Exposure to PFOS – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
ACUTE EXPOSURE									
1	Monkey (cynomolgus) 4–6M, 4–6F	Once or 3 times in 315 days (G)	0, 9, 13.3 (M), 14 (F)	BW, BC	Bd wt	13.3 M 14 F			
					Hepatic		13.3 M 14 F		Decrease in HDL cholesterol; values still within normal range
					Renal	13.3 M 14 F			
					Endocr		13.3 M 14 F		Decreased serum T4; values still within normal range
Chang et al. 2017									
PFOS potassium salt									
2	Rat (Sprague-Dawley) 5–15 F	Once (GW)	0, 15	BI, OF	Endocr		15		Transient decrease in serum TT4 (24, 38, and 53% after 2, 6, and 24 hours, respectively)
Chang et al. 2008b									
PFOS potassium salt									
3	Rat (Sprague-Dawley) 30 M	1 day (F)	0, 1.97, 10.3	HP	Hepatic	10.3			
					Endocr	10.3			
Elcombe et al. 2012a									
PFOS potassium salt									
4	Rat (Sprague-Dawley) 30 M	7 day (F)	0, 0, 1.72, 8.17	HP	Hepatic	8.17			Decreased serum cholesterol (38%) and triglyceride (55%) levels at 8.17 mg/kg/day
					Endocr	8.17			
Elcombe et al. 2012a									
PFOS potassium salt									
5	Rat (Sprague-Dawley) 10 M	7 day (F)	0, 1.79, 8.96	HP	Hepatic	8.96			Hepatocellular hypertrophy, increased liver weight, decreased serum cholesterol at ≥1.79 mg/kg/day
					Endocr	8.96			
Elcombe et al. 2012b									
PFOS potassium salt									

Table 2-4. Levels of Significant Exposure to PFOS – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
6	Rat (Sprague-Dawley) NS F	2 day GDs 19–20 1 time/day (GW)	0, 25, 50	BW, MX, DX	Develop			25	Decreased neonatal survival (82% of controls on PND 1)
Grasty et al. 2003									
PFOS potassium salt									
7	Rat (Sprague-Dawley) NS F	4 days GDs 2–5, 6–9, 10–13, 14–17, or 17–20 (GW)	0, 25	MX, DX	Bd wt			25	Weight loss during treatment when treated on GDs 2–5 (22%) or 6–9 (17%)
					Develop			25	Decreased neonatal survival (90% survival on GDs 2–5; 30% survival on GDs 17–20)
Grasty et al. 2003									
PFOS potassium salt									
8	Rat (Sprague-Dawley) NS F	2 days GDs 19–20 1 time/day (G)	0, 25, 50		Develop			25	Increased neonatal mortality
Grasty et al. 2005									
PFOS potassium salt									
9	Rat (Wistar) 8 M	7 days ad lib (F)	0, 15	BW, OW, BI, EA	Hepatic	15			40% increase in absolute liver weight
Haughom and Spydevold 1992									
PFOS potassium salt									
10	Mouse (wild-type 129S1/Svlm) 8–20 F	GDs 15–18 1 time/day (GW)	0, 4.5, 6.5, 8.5, 10.5	DX	Develop			4.5	31% reduced percentage of live pups per litter on PND 15
Abbott et al. 2009									
PFOS potassium salt									

Table 2-4. Levels of Significant Exposure to PFOS – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
11	Mouse (ICR) 5–7 F	GDs 11–15 1 time/day (GW)	0, 50	BW, OW, DX	Hepatic	50			103% increased maternal relative liver weight
					Develop		50		6.1% increased cleft palate and 12.7% reduced body weight in fetuses
Era et al. 2009									
PFOS potassium salt									
12	Mouse (CD-1) 10–11 F	GDs 6–18 1 time/day (GW)	0, 1.5, 3, 6	MX, DX, BW, CS, OW	Bd wt	6			
					Hepatic	6			21% increase in absolute liver weight at ≥3 mg/kg/day
					Endocr	6			No alterations in serum T3 or T4 levels
					Develop	6			
Fuentes et al. 2006									
PFOS potassium salt									
13	Mouse (CD-1) 8–10 F	GDs 12–18 (GW)	0, 6	CS, BW, BH, MX, DX	Bd wt	6			
					Develop		6		Reduced body weight of pups on PNDs 4 and 8
Fuentes et al. 2007b									
PFOS potassium salt									
14	Mouse (CD-1) 8–10 F	GDs 12–18 (GW)	0, 6	DX	Develop		6		Decreased distance traveled in open field test at 3 months of age
Fuentes et al. 2007a									
PFOS potassium salt									
15	Mouse (NMRI) 12 M pups	Single dose (GO)	0, 11.3	BH, BW, OF	Develop		11.3		Altered spontaneous behavior (≤60, 87.5, or 60% changes in total activity, rearing, and locomotion)
Hallgren et al. 2015									
PFOS									
16	Mouse (CD-1) 10 M pups	Once (G)	0, 0.75, 11.3	CS, OF, DX	Develop		0.75 M		24% decreased total spontaneous activity at 2 months of age; no significant alteration at 4 months
Johansson et al. 2008									
PFOS potassium salt									

Table 2-4. Levels of Significant Exposure to PFOS – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
17	Mouse (CD-1) 10 F	GDs 11–16 (G)	0, 0.5, 2.0, 8.0	BC, BW, DX, HP	Bd wt		8.0		21% reduction in maternal body weight gain on GDs 14–17
					Repro		0.5		Decreases in mean fetal placental weight and placental capacity
					Develop		0.5	2.0	Post-implantation losses at ≥0.5 mg/kg/day; 24 and 35% reduction fetal body weight and 31 and 52% reduction in the number of live fetuses at 2.0 and 8.0 mg/kg/day
Lee et al. 2015a									
PFOS									
18	Mouse (C57BL/6) 4M	10 days (F)	0, 6	BW FI BC HP	Bd wt	6			
					Hemato	6			
					Hepatic	6			41% increase in liver weight, hepatocellular hypertrophy, and decreased serum cholesterol levels
					Immuno	6			
Qazi et al. 2010a									
PFOS									
19	Mouse (BALB/c) 5 F	7 days (GW)	20	BW, OW, BC	Bd wt		20		41% decreased body weight gain
					Hepatic	20			59% increase in relative liver weight
					Immuno		20		Inhibition of T lymphocyte proliferation in response to sRBC; decreased phagocytosis by peripheral blood cells and NK cell activity; decreased IgM antibody formation in response to OVA
Vetvicka and Vetvickova 2013									
PFOS									
20	Mouse (CD-1) 4 M	14 days 1 time/day (GO)	0, 1, 5, 10		Bd wt	10			
					Hepatic	10			~70% increased absolute liver weight at ≥5 mg/kg/day
					Repro	10			
Wan et al. 2011									
PFOS potassium salt									

Table 2-4. Levels of Significant Exposure to PFOS – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
21	Mouse (C57BL/6) 10 M	1 day (GO)	0, 300, 400, 500, 600, 700	BW, CS, HP, LE	Death			579	LD_{50}
Xing et al. 2016									
PFOS									
22	Mouse (C57BL/6N) 12 M	7 days (G)	0, 5, 20, 40	FX	Immuno		5		Impaired response to T-cell mitogens; suppressed response to sRBC
Zheng et al. 2009									
PFOS									
23	Rabbit (New Zealand) 22 F	GDs 6–20 1 time/day (GW)	0, 0.1, 1.0, 2.5, 3.75	MX, DX, BW, CS	Bd wt	0.1		1 F	21% decreased mean maternal body weight gain on GDs 7–21; no effect on food consumption
					Develop	1	2.5	3.75	Decreased fetal body weight; 10% at 2.5 mg/kg/day and 24% at 3.75 mg/kg/day; 10/22 does aborted between GD 22 and 28 at 3.75 mg/kg/day
Case et al. 2001									
PFOS potassium salt									
INTERMEDIATE									
24	Monkey (Cynomolgus) 4–6 M, 4–6 F	26 weeks 1 time/day (C)	0, 0.03, 0.15, 0.75	CS, BW, OW, HE, BI, GN, HP	Bd wt	0.15 M 0.75 F	0.75 M		13.5% reduction in final body weight
					Resp	0.75			
					Cardio	0.75			
					Gastro	0.75			
					Hemato	0.75			
					Musc/skel	0.75			
					Hepatic	0.15	0.75		47–55% increased absolute liver weight; 50–60% decreased serum cholesterol; hepatocellular hypertrophy, mild bile stasis, and lipid vacuolation at 0.75 mg/kg/day
					Renal	0.75			
					Dermal	0.75			

Table 2-4. Levels of Significant Exposure to PFOS – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
					Ocular	0.75			
					Endocr	0.15	0.75		Increased TSH and decreased total T3
					Immuno	0.75			
					Neuro	0.75			No histological alterations
					Repro	0.15	0.75 M		Significant decrease in serum estradiol on days 62 (48%), 91 (42%), and 182 (96%); no histological alterations
Seacat et al. 2002									
PFOS potassium salt									
25	Monkey (Cynomolgus) 6 M,F	4 weeks 1 time/day (C)	0, 0.02, 2	CS, BW, FC, HE, BI, GN, HP, EA	Bd wt	2			
					Resp	2			
					Hemato	2			
					Hepatic	2			
					Renal	2			
					Ocular	2			
					Endocr	2			
					Immuno	2			No histological alteration
					Repro	2			No histological alterations
Thomford 2002a									
PFOS potassium salt									
26	Rat (Sprague-Dawley) 25 F	GD 0 to PND 20 1 time/day (GW)	0, 0.1, 0.3, 1		Repro	1			
					Develop	0.3	1		~30% increased locomotor activity and concurrent failure to habituate to test environment in male pups on PND 17
Butenhoff et al. 2009b									
PFOS potassium salt									
27	Rat (Sprague-Dawley) 25 F	GD 0 to PND 20 1 time/day (GW)	0, 0.1, 0.3, 1		Develop		1		2.1-fold increased fetal thyroid cell proliferation on GD 20
Chang et al. 2009									
PFOS potassium salt									

Table 2-4. Levels of Significant Exposure to PFOS – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
28	Rat (Sprague-Dawley) 10 F	GDs 1–21 1 time/day (GW)	0, 0.1, 2		Develop	0.1		2	~5-fold increased postnatal mortality and severe lung histopathology in pups
Chen et al. 2012b PFOS									
29	Rat (Sprague-Dawley) 10 M	Daily 28 days (G)	0, 5, 20	HP	Death			20	100% by day 26
					Resp		5		Pulmonary congestion
					Hepatic		5		Hepatocellular hypertrophy and focal necrosis at ≥5 mg/kg/day; fatty degeneration at 20 mg/kg/day
					Neuro		5		Cachexia and lethargy
Cui et al. 2009 PFOS									
30	Rat (Sprague-Dawley) 15M, 15F	Daily 28 days (F)	M: 0, 0.13, 1.23, 2.98, 5.89 F: 0, 0.14, 1.33, 3.47, 7.01		Cardio	5.89 M			
					Hemato	3.47 F	7.01 F		Decreased red blood cells (8.9%), hemoglobin (10%), hematocrit (8.8%)
					Hepatic	5.89 M			Increased relative liver weight; hepatocellular hypertrophy in males at 5.89 mg/kg/day
					Renal	5.89 M			
					Endocr	0.14	1.23		Decreased T4 level (82% in males; 48% in females)
Curran et al. 2008 PFOS potassium salt									
31	Rat (Sprague-Dawley) 30 M	28 days (F)	0, 1.54, 7.34	HP	Hepatic	7.34			Hepatocellular hypertrophy and decreased serum cholesterol levels at ≥1.54 mg/kg/day
					Endocr	7.34			
Elcombe et al. 2012a PFOS potassium salt									

Table 2-4. Levels of Significant Exposure to PFOS – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
32	Rat (Wistar) 5–6 M	Daily 28 days (G)	0, 0.12, 0.5, 2.0, 8.5	FX	Neuro	2	8.5		Tonic convulsions in response to stimuli
Kawamoto et al. 2011									
PFOS									
33	Rat (Sprague-Dawley) NS	GDs 2–21 (GW)	0, 1, 2, 3, 5, 10		Develop	1		2	Reduced serum T4 in pups at 1 mg/kg/day; approximately 60% survival at weaning versus 80% in controls at 2 mg/kg/day
Lau et al. 2003									
PFOS potassium salt									
34	Rat (Sprague-Dawley) 15M, 15F	28 days (F)	M: 0, 0.14, 1.33, 3.21, 6.34; F: 0, 0.15, 1.43, 3.73, 7.58	OW, BW	Bd wt Hepatic Immuno	1.33 M 6.34 M 6.34 M	3.21 M		12% decrease in terminal body weight Increased relative liver weight at ≥0.14 mg/kg/day
Lefebvre et al. 2008									
PFOS									
35	Rat (Sprague-Dawley) 10 dams; 12–13 pups	GDs 12–18 (GO)	0, 5, and 20	BC, BW, DX, OW	Bd wt Develop	5 5	20 20		30% reduction in body weight of dams 13% reduction in body weight of male pups
Li et al. 2016									
PFOS									
36	Rat (Sprague-Dawley) 5M	21 days (G)	0, 5, 10	BW, BC, OW, HP	Bd wt Repro	10	5		Delayed maturation of testicular Leydig cells, decreased seminal vesicle weight, decreased epididymal sperm count, decreased serum testosterone levels
Li et al. 2018									
PFOS									

Table 2-4. Levels of Significant Exposure to PFOS – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
37	Rat (Sprague-Dawley) 19 M	28 days (GO)	0, 0.5, 1.0, 3.0, 6.0	BC, BW, HP, OF, OW	Neuro	0.5	1		Degeneration of gonadotropic cells of the pituitary gland at ≥1.0 mg/kg/day; dense chromatin, condensed ribosomes, loss of morphology in the hypothalamus at ≥3.0 mg/kg/day
					Repro	0.5	1		Loss/degeneration of spermatozoids, marked edema in the testes
Lopez-Doval et al. 2014									
PFOS									
38	Rat (Sprague-Dawley) 35 M,F	84 days (6 weeks prior to mating GD 0 to PND 21) 1 time/day (GW)	0, 0.1, 0.4, 1.6, 3.2	MX, DX, BW, OW, OF, GN, HP, FC	Bd wt	1.6	3.2		>10% reduction in body weight
					Repro	3.2			No alterations in mating and fertility parameters
					Develop	0.1[b]	0.4	1.6	Delayed eye opening and transient decrease in F2 pup body weight (13%) on LDs 7–14 at ≥0.4 mg/kg/day; decreased pup survival to postpartum day 21 at ≥1.6 mg/kg/day
Luebker et al. 2005a									
PFOS potassium salt									
39	Rat (Sprague-Dawley) 50 F	90 day 1 time/day	0, 1.6	MX, DX, BW, OW, OF, GN		Develop		1.6	Increased pup mortality during PNDs 1–4
Luebker et al. 2005a									
PFOS potassium salt									
Cross-foster study									
40	Rat (Sprague-Dawley) 20 F	62–67 days 42 days prior to mating through GD 20 or PND 4 (G)	0, 0.4, 0.8, 1, 1.2, 1.6, 2	MX, DX, BW, CS, BI	Bd wt	1.6	2		22% reduction in body weight gain during premating; food consumption reduced 5.8%
					Hepatic	2			16% reduction in serum total cholesterol on PND 5 at ≥0.4 mg/kg/day; increased liver weight in dams at ≥0.8 mg/kg/day
					Endocr		0.4		46% reduction in total T4 on PND 5
					Repro	2			No alteration in fertility

Table 2-4. Levels of Significant Exposure to PFOS – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
					Develop		0.4	1.6	>10% decrease in mean pup weight per litter on PND 5 at ≥0.4 mg/kg/day; ~50% decrease mean pup survival per litter on PND 5 at ≥1.6 mg/kg/day
Luebker et al. 2005b									
PFOS potassium salt									
41	Rat (Sprague-Dawley) 10 M	25 days (GW)	0, 0.5, 1.0, 3.0 and 6.0	OF, OW	Endocr		0.5		Decreases in serum corticosterone (~58%) and ACTH levels (~11%), decrease in corticotrophin releasing hormone levels in hypothalamus (~8%); decrease in relative adrenal weight (~43%),
Pereiro et al. 2014									
PFOS									
42	Mouse (C57) 12 M	5 weeks (GO)	0, 0.5, 10	BW, OW, HP, RX	Bd wt	0.5	10		17% decrease in body weight
					Repro	0.5	10		Decreases in sperm concentration, serum testosterone levels; vacuolation in testicular spermatogonia, spermatocyte, and Leydig cells
Qu et al. 2016									
PFOS									
43	Rat (Sprague-Dawley) 21 dams; 10–12 M,F pups/litter	GDs 2–6 (G)	0, 18.75	BW, OF, OW	Bd wt		18.75		Reduced body weight in dams; approximately 98% on GD 8 and 33% on GD 20
					Develop		18.75		Decreased birth weight in females only (approximately 11%); increased systolic blood pressure in male offspring at 7 and 52 weeks and in female offspring at 37 and 65 weeks; reduced nephron endowment
Rogers et al. 2014									
PFOS									

Table 2-4. Levels of Significant Exposure to PFOS – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
44	Rat (Sprague-Dawley) 7 M	28 days (GW)	0, 3.0, and 6.0	BI, BW, OW	Bd wt	6			
					Endocr		3		Decreased serum prolactin (~78%) and estradiol concentrations (~18%)
Salgado et al. 2015 PFOS									
45	Rat (Sprague-Dawley) 25 M,F	4 weeks ad lib (F)	M: 0, 0.05, 0.18, 0.37, 1.51; F: 0, 0.05, 0.22, 0.47, 1.77	CS, CO, OW, HE, BI, GN, HP	Bd wt	1.77 F			
					Hemato	1.77 F			
					Hepatic	1.77 F			
					Renal	1.77 F			
					Ocular	1.77 F			
					Immuno	1.77 F			No histological alterations
					Neuro	1.77 F			No histological alterations
					Repro	1.51 M 1.77 F			No histological alterations
Seacat et al. 2003 PFOS potassium salt									
46	Rat (Sprague-Dawley) 25 M,F	14 weeks ad lib (F)	M:0, 0.03, 0.13, 0.34, 1.33; F:0, 0.04, 0.15, 0.4, 1.56	CS, CO, OW, HE, BI, GN, HP	Bd wt	1.56 F			
					Hemato	0.34 M	1.33 M		45% increase in non-segmented neutrophils
					Hepatic	1.33 M			Increased absolute and relative liver weight; increased serum ALT; hepatocyte hypertrophy and vacuolation at 1.33/1.55 mg/kg/day
					Renal	1.56 F			
					Ocular	1.56 F			
					Endocr	1.56 F			
					Immuno	1.56 F			No histological alterations
					Neuro	1.56 F			No histological alterations
					Repro	1.33 M 1.56 F			No histological alterations
Seacat et al. 2003 PFOS potassium salt									

Table 2-4. Levels of Significant Exposure to PFOS – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
47	Rat (Sprague-Dawley) 25–50 F	GDs 2–20 (GW)	0, 1, 2, 3, 5, 10	MX, DX, BW, FI, WI, OW, BI	Bd wt	1	2		Decreased mean body weight gain, 10% at 2 mg/kg/day and 33% at 5 mg/kg/day
					Hepatic	10			
					Endocr		1		Reduced total and free T4 and T3
					Develop		10		Increased incidences of cleft palate
Thibodeaux et al. 2003									
PFOS potassium salt									
48	Rat (Wistar) 10 or 15 dams; 6–10 M,F pups	GD 1 to PND 1, PNDs 1–7 or 35, or GD 1 to PND 7 or 35	0, 0.8, 2.4	DX	Develop		0.8		Decreased spatial learning ability in prenatally or postnatally exposed offspring at ≥0.8 mg/kg/day and in offspring exposure pre- and postnatally at 2.4 mg/kg/day; decreased memory ability in offspring exposure pre- and postnatally at 2.4 mg/kg/day
Wang et al. 2015c									
PFOS									
49	Rat (Sprague-Dawley) 10 F	GDs 2–21 1 time/day (GW)	0, 0.1, 0.6, 2		Develop	0.6		2	5-fold increased neonatal mortality on PNDs 1–3
Xia et al. 2011									
PFOS									
50	Rat (Sprague-Dawley) 8–10 M	91 days (W)	0, 0.27, 0.79, 2.37	BC	Endocr		0.27 M		42% decrease in total T4 levels
					Develop		3.2 F		19–36% reduced serum T4 levels in pups on PNDs 21–35 after gestation- and/or postnatal-only exposure
Yu et al. 2009a									
PFOS potassium salt									

Table 2-4. Levels of Significant Exposure to PFOS – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
51	Mouse (E3L CETP) 6–8 M	4–6 weeks (F)	0, 3	BW, FI, BC, OW	Bd wt	3			
					Hepatic	3			Decreased plasma triglyceride, total cholesterol, non-HDL cholesterol, and HDL cholesterol levels; increased hepatic triglyceride levels, increased liver weight
Bijland et al. 2011									
PFOS									
52	Mouse (C57BL/6N) 10 M	60 days (G)	0, 0.00833, 0.08333, 0.41667, 0.83333, 2.0833	FX	Immuno	0.0083	0.0833		Impaired response to sRBC
Dong et al. 2009									
PFOS potassium salt									
53	Mouse (C57BL/6N) 12 M	60 days (G)	0, 0.00833, 0.0167, 0.0833, 0.4167, 0.8333	FX	Immuno	0.0167	0.0833		Impaired response to sRBC
Dong et al. 2011									
PFOS potassium salt									
54	Mouse (ICR) 6–8 F	GDs 1–17 1 time/day (GW)	0, 20		Bd wt		20		35% reduced maternal body weight
					Develop		20		89% increased cleft palate, 25% reduced body weight in fetuses
Era et al. 2009									
PFOS potassium salt									
55	Mouse (CD-1) 10 M	4 weeks (GW)	0, 3, 6	CS, BW, BH, MX, DX	Develop		3		Impaired retention of the task in the water maze test
Fuentes et al. 2007c									
PFOS potassium salt									
56	Mouse (B6C3F1) 30 F	21 days (G)	0, 0.005, 0.025		Immuno	0.005	0.025		Decreased host resistance to influenza virus
Guruge et al. 2009									
PFOS									

Table 2-4. Levels of Significant Exposure to PFOS – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
57	Mouse (B6C3F1) 10–12 F	GDs 1–17 1 time/day (GW)	0, 0.1, 1, 5	MX, DX, BW, CS, OF	Develop	0.1	1		42.5% reduced NK cell activity in male pups at 8 weeks of age
Keil et al. 2008									
PFOS potassium salt									
58	Mouse (CD-1) 21–22 F	GDs 1–17 1 time/day (GW)	0, 1, 5, 10, 15, 20	DX, OW, BI	Develop		1	10	1 mg/kg/day: delayed eye opening at ≥1 mg/kg/day, ~ 0% postnatal survival at weaning versus 90% in controls at 10 mg/kg/day
Lau et al. 2003									
PFOS potassium salt									
59	Mouse (CD-1) 4 dams; 8 fetuses	GDs 1–17 (GO)	3	BC, EA, OF	Hepatic	3			No alteration in maternal hepatic lipid levels
					Develop		3		Significant increase in cholesterol levels in fetal livers
Lee et al. 2015b									
PFOS									
60	Mouse (C57BL/6) 15 M,F	Daily 3 months (G)	0, 0.43, 2.15, 10.75	FX	Neuro	0.43	2.15		Impaired spatial learning and memory
Long et al. 2013									
PFOS									
61	Mouse (BALB/c) 28 M	3 weeks	0, 2.5, 5 and 10	BW, OF, OW	Bd wt		10 M		~15% reduction in body weight during the recovery period
					Immuno	2.5 M	5 M		~36% decrease in spleen index during recovery; ~15% inhibition in Con A-induced T-cell proliferation during treatment; 32% increase in CD3+ cells after recovery; ~60% increase in CD3+CD8+ cells and ~56% increase in CD3+CD4+ cells on week 2; 15% inhibition in Con A-induced T-cell proliferation during recovery
Lv et al. 2015									
PFOS									

Table 2-4. Levels of Significant Exposure to PFOS – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
62	Mouse (C57BL/6J-Apc+/+) 20–21 F	GDs 1–17 (GW)	0, 0.01, 0.1, 3.0	BC, BW, FI, HE, OW	Develop	0.1	3		Decrease in number of successful births
Ngo et al. 2014									
PFOS									
63	Mouse (C57BL/6/Bk1) 6 F	GDs 1–21 ad lib	0, 0.3		Develop		0.3		Decreased locomotion, muscle strength, and motor coordination in adult offspring
Onishchenko et al. 2011									
PFOS, potassium salt									
64	Mouse (B6C3F1) 5M, 5F	28 days (G)	0, 0.000166, 0.00166, 0.00331, 0.0166, 0.0331, 0.166	OW, FX	Immuno	0.000166 M	0.00166 M		Suppressed response to sRBC (~60%)
Peden-Adams et al. 2008									
PFOS potassium salt									
65	Mouse (B6C3F1) 5 M	28 days (F)	0.20	NS	Bd wt		0.2		21% reduction in body weight
					Immuno	0.2			No alterations in thymic lymphocyte phenotypes, response to sRBC, or IgM antibodies to LPS
Qazi et al. 2010b									
PFOS									
66	Mouse (CD-1) 5 F	GDs 1–17 1 time/day (GW)	0, 5, 10		Develop	5			Peroxisome proliferation in fetal liver at ≥5 mg/kg/day
Rosen et al. 2009									
PFOS potassium salt									
67	Mouse (CD-1) 60–80 F	GDs 1–17 (GW)	0, 1, 5, 10, 15, 20	BW, OW, BI, DX	Bd wt	20			
					Hepatic	20			Increase in absolute and relative liver weight and decreased serum triglycerides at ≥5 mg/kg/day
					Endocr	15	20		Decreased total T4 on GD 6

Table 2-4. Levels of Significant Exposure to PFOS – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
					Develop	1	5	20	Increased incidences of sternal defects at ≥5 mg/kg/day; reduced percentage of live fetuses (9%) at 20 mg/kg/day
Thibodeaux et al. 2003									
PFOS potassium salt									
68	Mouse (CD-1) 4 M	21 days 1 time/day (GO)	0, 1, 5, 10	BW, BI, OW, HP	Bd wt	5	10		~15% reduced body weight
					Hepatic	10			Increased absolute liver weight at ≥5 mg/kg/day
					Repro	5	10		~17% reduced serum testosterone, ~38% reduced epididymal sperm count
Wan et al. 2011									
PFOS potassium salt									
69	Mouse (CD-1) 6 F (dams)	GD 3 to PND 21 or GD 3 to PND 63 (GO)	0, 0.3, 3	BW, OF, OW	Hepatic	3			~24% increase in relative liver weight in dams at 3 mg/kg/day
					Develop	3			Increase in relative liver weight at 3 mg/kg/day in male and female pups on PND 21 (~20–32%), in male STD-fed adults (~11%), and in male HDF-fed adults (~33%)
					Develop		0.3		In PND 63 offspring fed a high fat diet, increased serum glucose levels at ≥0.3 mg/kg/day in females (~40%) and 3 mg/kg/day in males (~8%); increased serum insulin in males (~109%) and females (~85%); increased response to oral glucose tolerance test, increased HOMA-IR index, and 33% increased relative liver weight at 3 mg/kg/day
					Other noncancer	3 F			No significant alteration in fasting serum insulin or glucose levels; significant increase in HOMA-IR index at 0.3 and 3 mg/kg/day
Wan et al. 2014b									
PFOS									

Table 2-4. Levels of Significant Exposure to PFOS – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
70	Mouse (C57BL/6) 10 M	30 days (GO)	0, 2.5, 5, 10	BW, HP, OF, OW	Bd wt		10		31% reduction in body weight (correlated with 68% reduction in feed consumption)
					Hepatic		2.5		Increased liver weight (35%) and serum AST(~12%) and GGT levels (~98%) at ≥2.5 mg/kg/day; increases in ALT (~45%) and ALP (~36%) at ≥5 mg/kg/day; cytoplasmic vacuolation, focal or flake-like necrosis, and hepatocellular hypertrophy observed, but no incidence data provided
					Renal	10			
Xing et al. 2016									
PFOS									
71	Mouse (ICR) 5 F	GDs 0–17 GDs 0–18 (GW)	0, 1, 10, 20		Hepatic	20			60% increased absolute liver weight at ≥10 mg/kg/day
					Develop		1	20	GDs 0–17: 15.8% increased sternal defects in fetuses at ≥1 mg/kg/day; 8.8% decrease in number of live fetuses at 20 mg/kg/day
					Develop			10	GDs 0–18: decreased survival (55.2%) at 10 mg/kg/day on PND 4, decreased neonatal BW, intracranial blood vessel dilatation, lung atelectasis
Yahia et al. 2008									
PFOS potassium salt									
CHRONIC EXPOSURE									
72	Rat (Sprague-Dawley) 70 M,F	104 weeks ad lib (F)	0, 0.025, 0.10, 0.25, 1.04	CS, BW, FC, GN, HP, BI	Bd wt	0.25 F	1.04 F		14% reduction in final body weight
					Resp	1.04			
					Cardio	1.04			
					Gastro	1.04			
					Hemato	1.04			
					Musc/skel	1.04			

Table 2-4. Levels of Significant Exposure to PFOS – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
					Hepatic	0.25 M	1.04 M		Hepatocellular hypertrophy at ≥0.1 mg/kg/day; single cell necrosis and cystic degeneration at 1.04 mg/kg/day
					Renal	1.04			
					Dermal	1.04			
					Ocular	1.04			
					Endocr	1.04			
					Immuno	1.04			No histological alterations
					Neuro	1.04			No histological alterations
					Repro	1.04			No histological alterations
Butenhoff et al. 2012b; Thomford 2002b									
PFOS potassium salt									

[a]The number corresponds to entries in Figure 2-9.
[b]Used to derive an intermediate-duration oral MRL of $2x10^{-6}$ mg/kg/day based on the predicted TWA serum PFOA level of 29.7 µg/mL at the NOAEL dose and an empirical clearance model to estimate a HED. The $NOAEL_{HED}$ of 0.000515 mg/kg/day was divided by an uncertainty factor of 30 (3 for extrapolation from animals to humans with dosimetric adjustment and 10 for human variability) and a modifying factor of 10 for concern that immunotoxicity may be a more sensitive endpoint than developmental toxicity.

ad lib = *ad libitum*; ALP = alkaline phosphatase; ALT = alanine aminotransferase; AST = aspartate aminotransferase; BC = biochemistry; BH = behavioral; BI = biochemical changes; BW or Bd wt = body weight; C = capsule; Cardio = cardiovascular; CS = clinical signs; Develop = developmental; DX = developmental toxicity; EA = enzyme activity; Endocr = endocrine; (F) = feed; F = female(s); FX = fetal toxicity; FI = food intake; FX = fetal toxicity; G = gavage; Gastro = gastrointestinal; GD = gestation day; GGT = gamma-glutamyl transferase; GN = gross necropsy; GO = gavage in oil vehicle; GW = gavage in water vehicle; HDL = high-density lipoprotein; HE or Hemato = hematological; HED = human equivalent dose; HOMA IR = Homeostatic Model Assessment of Insulin Resistance; HP = histopathology; Immuno = immunotoxicological; LD = lactation day; LD50 = lethal dose, 50% kill; LE = lethality; LOAEL = lowest-observed-adverse-effect level; LPS = lipopolysaccharide; M = male(s); MRL = Minimal Risk Level; Musc/skel = musculoskeletal; MX = maternal toxicity; Neuro = neurological; NK = natural killer; NOAEL = no observed-adverse-effect level; NS = not specified; OF = organ function; OP = ophthalmology; OW = organ weight; PFOS = perfluorooctane sulfonic acid; PND = postnatal day; Repro = reproductive; Resp = respiratory; RX = reproductive toxicity; sRBC = sheep red blood cell; T3 = triiodothyronine; T4 = thyroxine; TSH = thyroid-stimulating hormone; TT4 = total thyroxine; TWA = time-weighted average; W = water

Figure 2-9. Levels of Significant Exposure to PFOS – Oral
Acute (≤14 days)

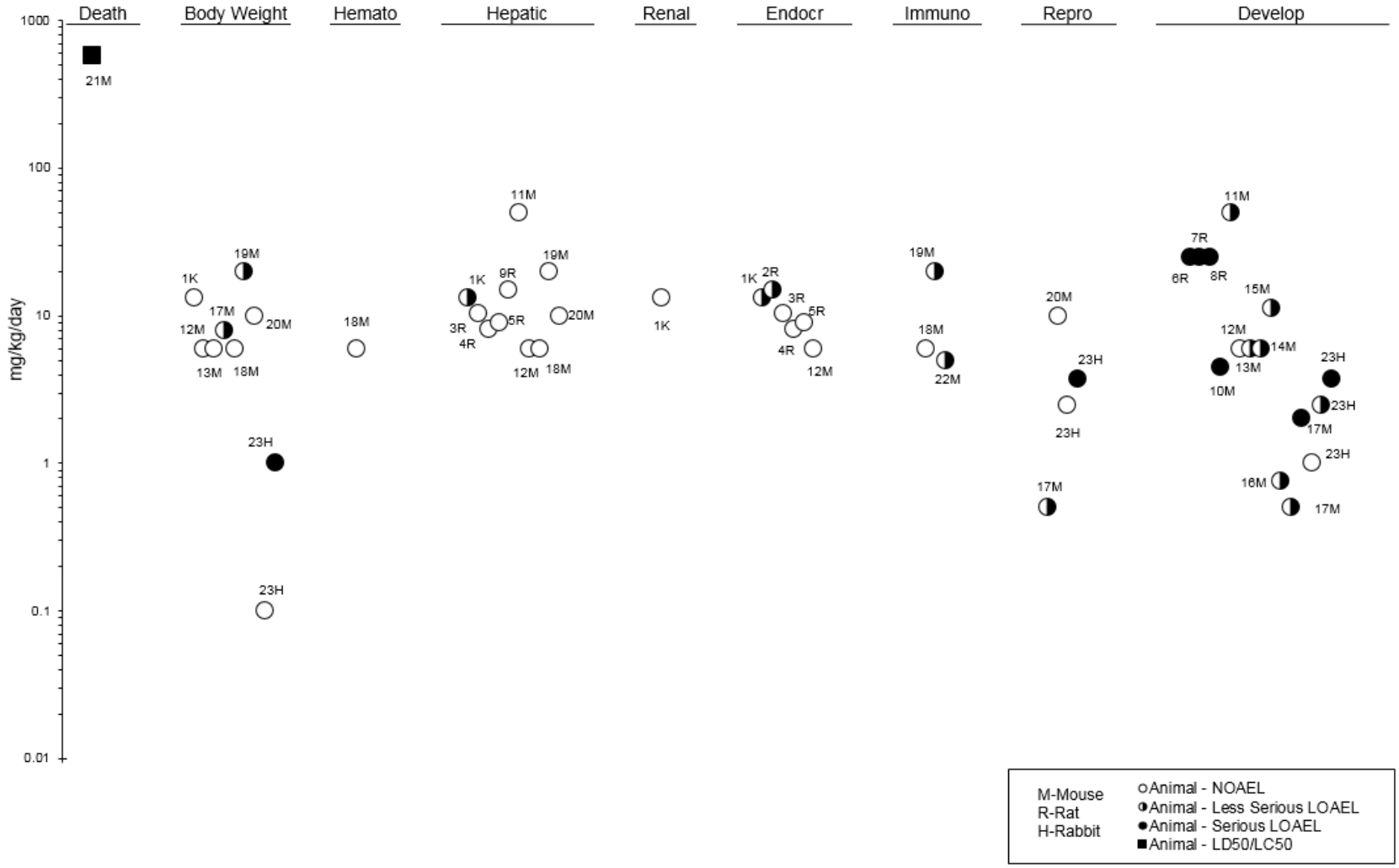
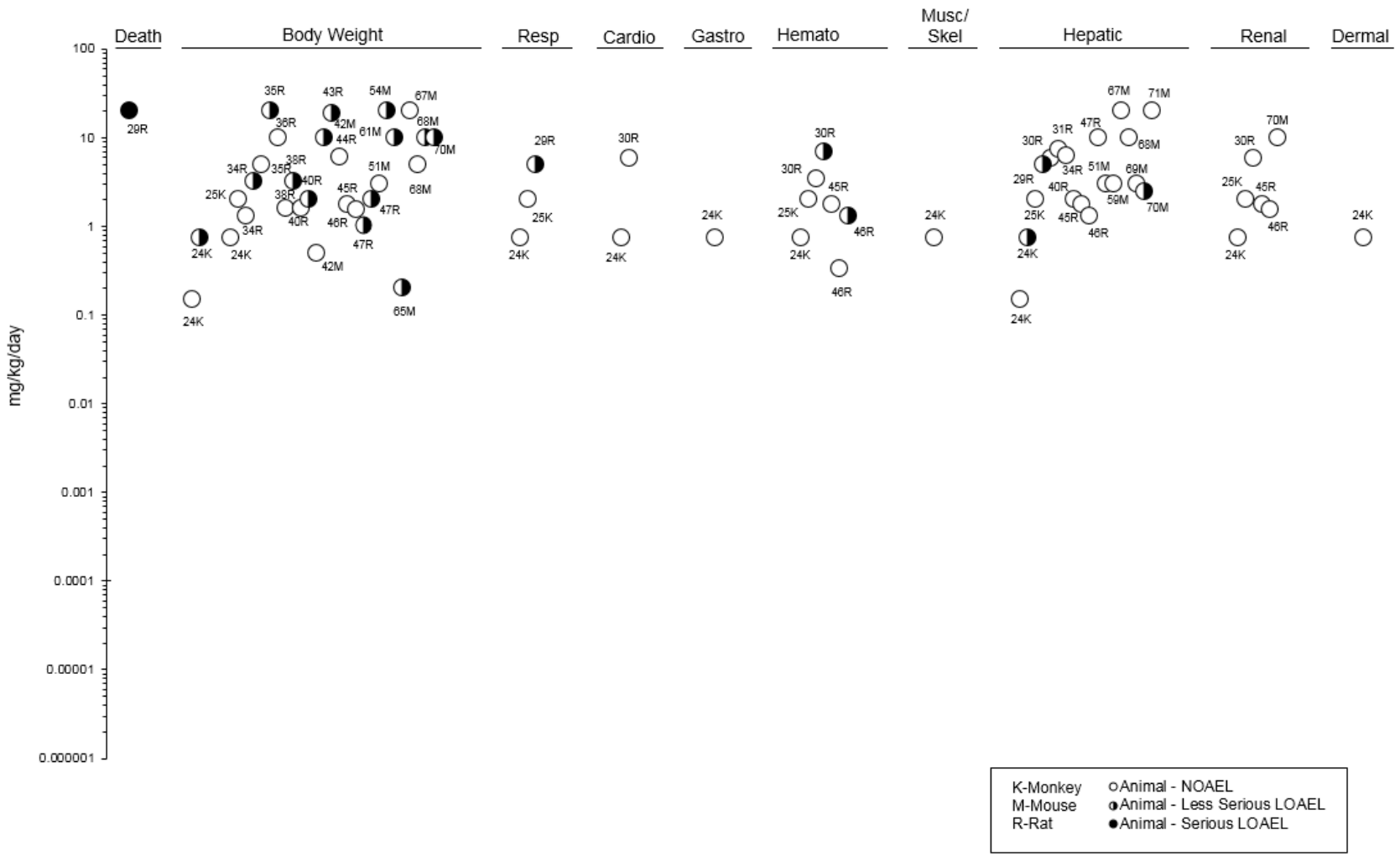


Figure 2-9. Levels of Significant Exposure to PFOS – Oral
Intermediate (15–364 days)



KD0103

Figure 2-9. Levels of Significant Exposure to PFOS – Oral
Intermediate (15–364 days)

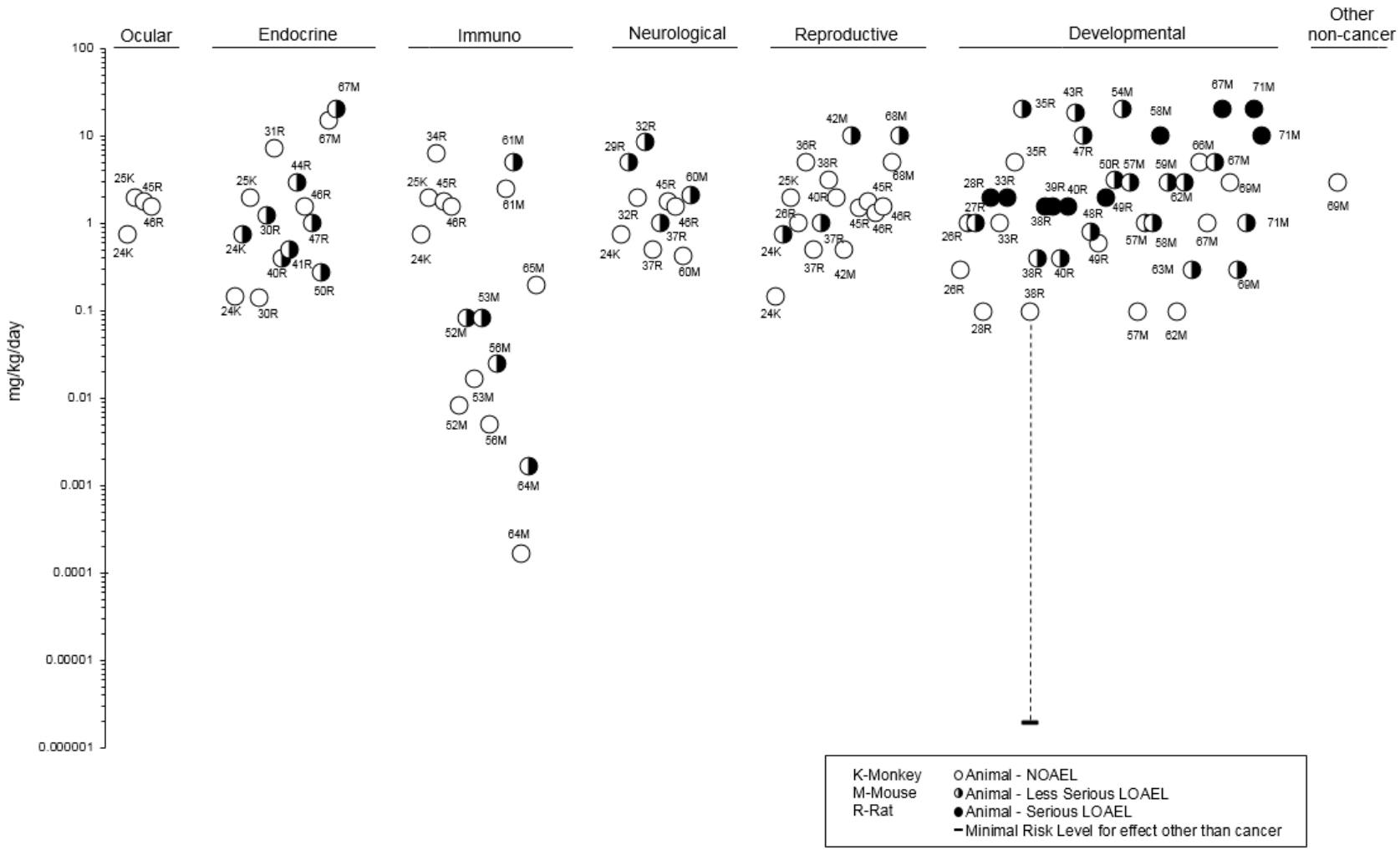


Figure 2-9. Levels of Significant Exposure to PFOS – Oral
Chronic (≥365 days)

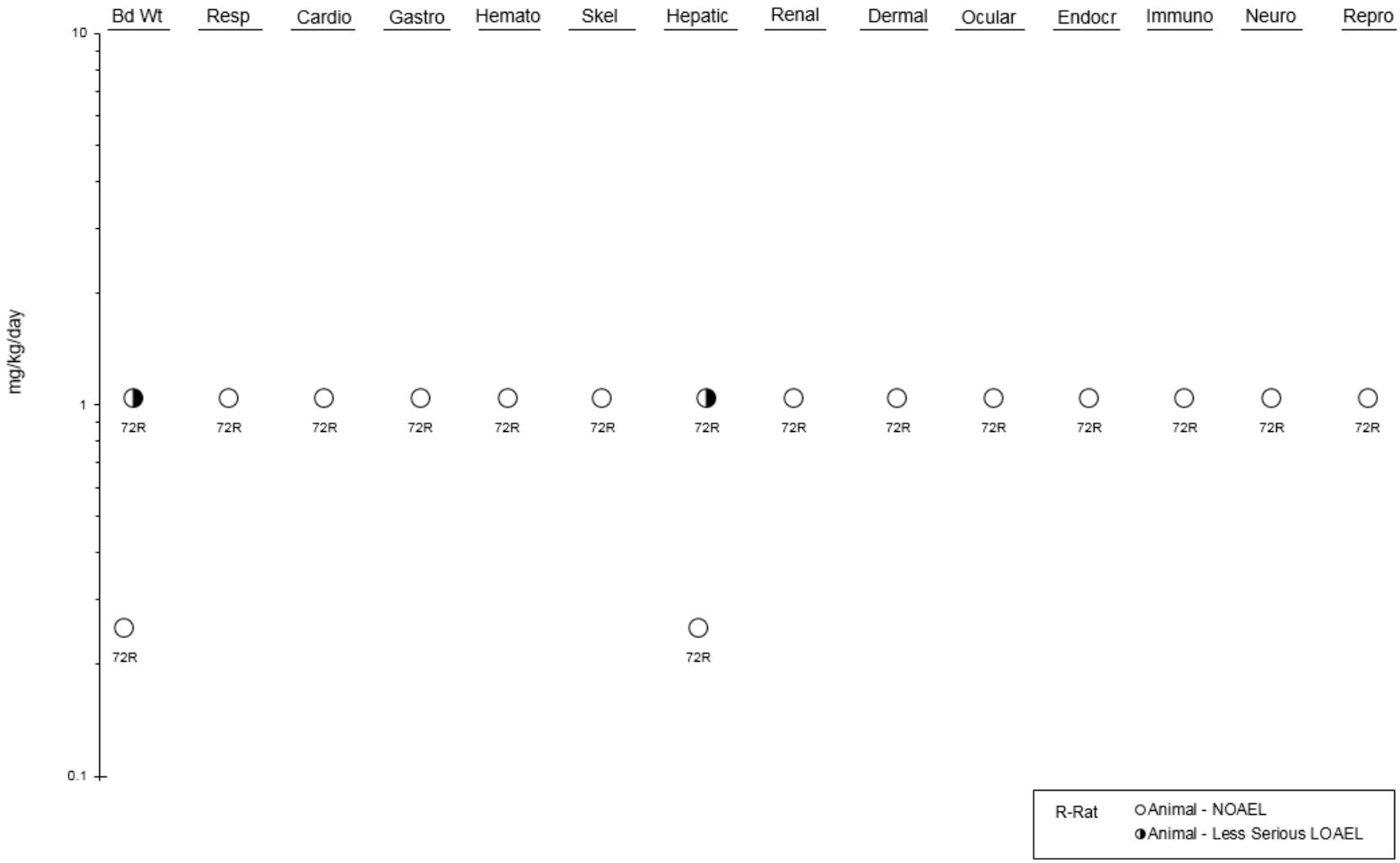


Table 2-5. Levels of Significant Exposure to Other Perfluoroalkyls – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
ACUTE EXPOSURE									
PFHxS									
1	Mouse (SV129 WT and PPARα null) 4 M	7 days (G)	0, 10 PFHxS	BW, HP	Bd wt	10			
					Hepatic	10			Hepatocellular hypertrophy, steatosis, and increased hepatic triglyceride levels
Das et al. 2017									
2	Mouse (NMRI) 14–18 M,F	PND 10 Once (GO)	0, 0.61, 6.1, 9.2 PFHxS		Develop	6.1	9.2		Altered spontaneous behavior and habituation in adults exposed as neonates
Viberg et al. 2013									
PFNA									
3	Rat (Sprague-Dawley) 10 M	14 days (GW)	0, 1, 3, 5 PFNA	BC, BW, OW	Bd wt	1	3		Decreases in body weight at 3 and 5 mg/kg/day (18 and 39%)
					Immuno		1		24% increase in relative thymus weight at 1 mg/kg/day; 20% decrease in thymus weight at 3 or 5 mg/kg/day, increases in thymic cortex:medulla ratios, alterations in cytokine levels at ≥3 mg/kg/day
Fang et al. 2009									
4	Rat (Sprague-Dawley) 10 M	14 days (GW)	0, 1, 3, 5 PFNA	BC, OW	Immuno	3	5		Decreased relative spleen weight and increases in cytokine levels
Fang et al. 2010									
5	Rat (Sprague-Dawley) 6 M	14 days (GW)	0, 0.2, 1, 5 PFNA	BC	Hepatic	5			Decreased HDL levels at ≥1 mg/kg/day
					Other noncancer (glucose)	0.2	1		Increased serum glucose levels (1.11-fold at 1 mg/kg/day and 1.16-fold at 5 mg/kg/day)
Fang et al. 2012a									
6	Rat (Sprague-Dawley) 6 M	14 days (GW)	0, 0.2, 1, 5 PFNA	HP	Hepatic	5			Hepatocellular vacuolation and lipid accumulation at 5 mg/kg/day
Fang et al. 2012b									

Table 2-5. Levels of Significant Exposure to Other Perfluoroalkyls – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
7	Rat (Sprague-Dawley) 6 M	14 days (GW)	0, 1, 3, 5 PFNA	BC, HP	Repro	3	5		85.4% decrease in serum testosterone and 105% increase in estradiol levels at 5 mg/kg/day; atrophy of seminiferous tubule epithelium
Feng et al. 2009									
8	Rat (Sprague-Dawley) 6 M	14 days (GW)	0, 1, 3, 5 PFNA	HP	Repro	3	5		Large vacuoles between testicular Sertoli cells and spermatogonia
Feng et al. 2010									
9	Rat (Wistar)	14 days (GO) 8 or 10M	0, 0.0125, 0.25, 5 PFNA	BI, BW, OW, GN, HP	Bd wt		5		Decreased body weight; magnitude of effect was not reported
					Endocr		5		Decreased androstenedione and testosterone concentrations (data not shown)
Hadrup et al. 2016									
10	Mouse (SV129 WT and PPARα null) 4 M	7 days (G)	0, 10 PFNA	BW, HP	Bd wt	10			
					Hepatic	10			Hepatocellular hypertrophy, steatosis, and increased hepatic triglyceride levels
Das et al. 2017									
11	Mouse (BALB/c) 6 M	14 days (G)	0, 1, 3, 5 PFNA	FX	Immuno		1		Decreases in the percentages of F4/80+ and CD49b+ cells in the spleen; no alteration in the response of splenic lymphocytes to ConA at ≤5 mg/kg/day
Fang et al. 2008									
12	Mouse (CD-1) 5 M,F	14 days (F)	0, 0.5, 1.8, 5.3, 54, 537 PFNA	LE, OW	Death			54	100% mortality before day 14
					Hepatic	5.3			50–70% increase in absolute liver weight at ≥0.5 mg/kg/day
Kennedy 1987									

Table 2-5. Levels of Significant Exposure to Other Perfluoroalkyls – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
13	Mouse (BALB/c) 8 M	14 days (G)	0, 0.2, 1, 5 PFNA	BW, OW, BC	Bd wt	1	5		~25% decrease in body weight
					Hepatic	1	5		Increases in liver weight (~160%) and increases in hepatic triglyceride (~66%) and cholesterol levels (~26%) at ≥0.2 mg/kg/day; decreases in serum triglyceride (~67%) and cholesterol levels (~32%) and increases in serum ALT (~900%) and AST (~280%) levels at 5 mg/kg/day
Wang et al. 2015a									
PFDA									
14	Rat (Wistar) 5–12 M	Once (GO)	0, 50 PFDA	BW, FX	BW			50	8% weight loss was observed 10-days post-exposure
					Neuro	50			No alteration in performance on novel object recognition test
Kawabata et al. 2017									
15	Rat (Wistar) 25 M	1 week (F)	0, 1.2, 2.4, 4.7, 9.5 PFDA	BE, OW, EA	Bd wt	4.7		9.5	~32% weight loss
					Hepatic	9.5			Increases in liver weight at ≥2.4 mg/kg/day; increases in hepatic cholesterol at 9.5 mg/kg/day
Kawashima et al. 1995									
16	Mouse (C57BL/6N) 4 F	Once (GO)	0, 40, 80, 100, 120, 160 PFDA	OW, EA	Hepatic	160			Increase in hepatic lipids and liver weight 2 days post-exposure at 40 mg/kg/day
Brewster and Birnbaum 1989									
17	Mouse (C57BL/6N) 12–14 F	GDs 6–15 (GO)	0, 0.03, 0.1, 0.3, 1, 3, 6.4, 12.8 PFDA	MX, DX, BW, OW	Bd wt	3	6.4	12.8	No weight gain at 6.4 mg/kg/day and weight loss at 12.8 mg/kg/day (net change of -2.4)
					Develop	0.3	1	12.8	18–22% decreases in fetal weight per litter at ≥1 mg/kg/day; decreases in live fetuses per litter at 12.8 mg/kg/day (4.6 versus 7.2 in controls)
Harris and Birnbaum 1989									

Table 2-5. Levels of Significant Exposure to Other Perfluoroalkyls – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
18	Mouse (C57BL/6N) 10 F	Once (GO)	0, 20, 40, 80, 160, 320 PFDA	BW, OW, GN, HP	Death			120	LD_{50} in 30-day observation period
					Bd wt	40	80		12% decreased body weight 30 days post-exposure
					Cardio	80			No histological alterations in the heart 30 days post-exposure; decreased relative heart weight at 80 mg/kg/day
					Hepatic	80			Increases in liver weight and pancellular hypertrophy at ≥20 mg/kg/day 30 days post-exposure
					Renal	80			No histological alterations 30 days post-exposure
					Endocr	40	80		2-fold increase in T3 and 4-fold increase in T4 levels 30 days post-exposure
					Immuno	40	80	160	28% decrease in relative spleen weight at 80 mg/kg/day; atrophy and lymphoid depletion in thymus and spleen at 160 mg/kg/day
Harris et al. 1989									
19	Mouse (CD-1) 10 M	Once on PND 10 (G)	0, 0.72, 10.8 PFDA	CS, DX	Develop	10.8			No alteration in spontaneous activity or habituation at 2–4 months of age
Johansson et al. 2008									
20	Mouse (C57BL/6N) 4 M	10 days (F)	0, 78 PFDA	BW, OW, EA	Bd wt			78	33% weight loss
					Hepatic	78			36% increase in liver weight
Permadi et al. 1992, 1993									
PFBA									
21	Rat (Sprague-Dawley) 3 M,F	5 days 1 time/day (GW)	0, 18, 58, 184 PFBA	CS, BW, OW, HE, BI, GN, HP	Bd wt	184			
					Resp	184			
					Cardio	184			
					Gastro	184			
					Hemato	184			
					Musc/skel	184			
					Hepatic	184			
					Renal	184			

Table 2-5. Levels of Significant Exposure to Other Perfluoroalkyls – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
					Endocr	184			
					Immuno	184			No histological alterations
					Neuro	184			No histological alterations
					Repro	184			No histological alterations
3M 2007a									
22	Rat (Sprague-Dawley) 3 M	14 days ad lib (F)	0, 20 PFBA	OW, EA	Hepatic	20			Biochemical and ultrastructural evidence of peroxisome proliferation
Ikeda et al. 1985									
23	Mouse (C57BL/6N) 4 M	10 days (F)	0, 78 PFBA	BW, OW, EA	Bd wt	78			
					Hepatic	78			63% increase in absolute liver weight
Permadi et al. 1992, 1993									
PFDoDA									
24	Rat (Wistar) 5–12 M	Once (GO)	0, 5, 20, 50 PFDoDA	BW, FX	BW		50		44% decrease in body weight gain (measured 10-days post-exposure)
					Neuro		50		Impaired performance on novel object recognition test
Kawabata et al. 2017									
25	Rat (Wistar) 10 M	Once (GO)	0, 50 PFDoDA	FX	Neuro	50			No alterations in open field activity
Kawabata et al. 2017									
26	Rat (Wistar) 8 M	Once (GO)	0, 50 PFDoDA	FX	Neuro	50			No alterations in tests of working memory or depressive behavior
Kawabata et al. 2017									

Table 2-5. Levels of Significant Exposure to Other Perfluoroalkyls – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
27	Rat (Sprague-Dawley) 10 M	14 days 1 time/day (GW)	0, 1, 5, 10 PFDoDA	BW, OW, BI	Bd wt	1	5		25% reduction in final body weight
					Hepatic	10			35% increase in total serum cholesterol at 10 mg/kg/day
					Repro	1	5		Decreased serum testosterone (38%) and estradiol (~38%), and ultrastructural alterations in testes at ≥5 mg/kg/day; decreased testicular weight at 10 mg/kg/day (22%)
Shi et al. 2007									
28	Rat (Sprague-Dawley) 10 M	14 days 1 time/day (GW)	0, 1, 5, 10 PFDoDA	OW, BI, HP	Hepatic	10			Increased liver weight, increased hepatic triglyceride and cholesterol levels at ≥5 mg/kg/day; increased serum triglyceride levels at 10 mg/kg/day
Zhang et al. 2008									
FOSA									
29	Rat (Sprague-Dawley) 15 M	Once (G)	0, 5 FOSA	BW, OW	Bd wt	5			
					Hepatic	5			No alterations in liver weight
Seacat and Luebker 2000									
PFHxA									
30	Mouse (CD1) 20 F	GDs 6–18 (GW)	0, 100, 350, 500 PFHxA	CS, DX	Develop	100	350		12.5% decrease in birth weight and delayed eye opening at ≥350 mg/kg/day; increased pup mortality (PND 0–3) and decreased pup survival at 500 mg/kg/day
Iwai and Hoberman 2014									
31	Mouse (CD1) 20 F	GDs 6–18 (GW)	0, 7, 35, 175 PFHxA	CS, DX	Develop	35	175		Increase in stillborn pups and 12.5% decrease in birth weight
Iwai and Hoberman 2014									
32	Rat (Crl:CD (SD)) NS F	Once (GW)	175, 550, 1,550, 5,000 NaPFHx	LE, CS, BW	Death			1,750	1/4 died at 1,750 mg/kg/day; 3/3 died at 5,000 mg/kg/day
					Neuro			175	Abnormal gait, salivation, ataxia, lethargy
Loveless et al. 2009									

Table 2-5. Levels of Significant Exposure to Other Perfluoroalkyls – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
INTERMEDIATE EXPOSURE									
PFHxS									
33	Rat (Sprague-Dawley) 15 M,F	42–56 days 1 time/day (GW)	0, 0.3, 1, 3, 10 PFHxS	CS, BW, MX, DX, OW, OF, BI, HE, GN, HP, FI	Bd wt	10			
					Resp	10			
					Cardio	10			
					Gastro	10			
					Hemato	10 F	0.3 M		6% increase in prothrombin time in males at ≥0.3 mg/kg/day; decreases in hemoglobin (4%), hematocrit (3%), and RBC levels (2.4%) in males at ≥3 mg/kg/day
					Hepatic	10			Increased liver weight; centrilobular hepatocellular hypertrophy in males at ≥3 mg/kg/day
					Renal	3 M	10 M		31% increase in BUN in males
					Endocr	1[b] M 10 F	3 M		Hypertrophy and hyperplasia of thyroid follicular cells in males
					Immuno	10			No histological alterations
					Neuro	10			No histological alterations
					Repro	10			No histological alterations or effects on fertility
					Develop	10 F			
Butenhoff et al. 2009a (results also reported in Hoberman and York 2003)									
34	Rat (Wistar) 8 F	GDs 7–22 (GO)	0, 25, 45 PFHxS	CS, BW, BC, DX, OW	Bd wt	45			
					Endocr		25		60% decreases in serum T4 levels in dams
					Develop		25		40% decrease in serum T4 levels in pups on PND 16
Ramhøj et al. 2018									

Table 2-5. Levels of Significant Exposure to Other Perfluoroalkyls – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
35	Rat (Wistar) 16–20F	GDs 7–22 (GO)	0, 0.05, 5, 25 PFHxS	CS, BW, BC, DX, OW	Bd wt	25			
					Endocr	0.05	5		20–30% decreases in serum T4 levels in dams
					Develop	0.05	5		20% decrease in serum T4 levels in pups on PND 16/17
Ramhøj et al. 2018									
36	Mouse (E3L CETP) 6–8 M	4–6 weeks (F)	0, 6 PFHxS	BW, FI, BC, OW	Bd wt	6			
					Hepatic	6			Decreased plasma triglyceride, cholesterol, non-HDL cholesterol, and HDL cholesterol levels; increased hepatic triglyceride levels, increased liver weight
Bijland et al. 2011									
37	Mouse (CD-1) 30 M, 30 F	M: 42 days F: 14 days premating, mating, gestation, lactation (G)	0, 0.3, 1, 3 PFHxS	CS, BW, FI, HE, BC, GN, HP, FX, RX, DX	Bd wt	3			
					Resp	3			
					Cardio	3			
					Gastro	3			
					Hemato	3			
					Musc/skel	3			
					Hepatic	1	3		Single cell necrosis and microvascular fatty changes at 3 mg/kg/day; centrilobular hepatocellular hypertrophy at ≥0.3 mg/kg/day in F0 mice and at 3 mg/kg/day in F1 mice
					Renal	3			
					Endocr	3			
					Neuro	**3**			
					Repro	3			
					Develop	0.3	1		Decrease in number of pups per litter with no change in pup to implantation site ratio
Chang et al. 2018									

Table 2-5. Levels of Significant Exposure to Other Perfluoroalkyls – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
PFNA									
38	Rat (Sprague-Dawley) 18 dams; 10–12 M,F pups/litter	GDs 1–20 (GW)	5 PFNA	BW, OF, OW	Bd wt		5		Reduced maternal body weight; approximately 33% on GD 7 and 10% on GD 20
					Develop		5		Decreased birth weight in female pups only (approximately 11%); increased systolic blood pressure in 10-week-old male and female offspring; reduced nephron endowment
Rogers et al. 2014									
39	Mouse (CD-1) 8–10 F	GDs 1–17 (GW)	0, 1, 3, 5, 10 PFNA	BC, DX, FX, MX, OW	Bd wt			10	43% maternal weight loss at GD 13
					Hepatic	10			Increases in absolute and relative liver weights in dams on GD 17 and on post-weaning day 28 at ≥1 mg/kg/day
					Develop	1[c]	3	5	Delayed postnatal development [eye opening, preputial separation and vaginal opening] and decreased body weight gain persisting in males up to PND 287 at ≥3 mg/kg/day; decreased postnatal survival between PND 2 and 10 with 80% mortality at ≥5 mg/kg/day; full litter resorptions at 10 mg/kg/day
Das et al. 2015									
40	Mouse (Parkes) 14 M	90 days (GW)	0, 0.2, 0.5 PFNA	BW, OW, BC HP, RX	Bd wt	0.5			
					Hepatic	0.5			33% decrease in serum cholesterol
					Repro	0.2	0.5		Decreased sperm motility, viability, and number; degenerative changes in seminiferous tubules; decreased litter size
Singh and Singh 2018									

Table 2-5. Levels of Significant Exposure to Other Perfluoroalkyls – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
41	Mouse (129S1/ Svlm) 8 F	GDs 1–18 (G)	0, 0.83, 1.1, 1.5, 2.0 PFNA	BW, OW, DX	Bd wt	2.0			No alterations in maternal body weight or gestational weight gain
					Hepatic	2.0			Increase in dam liver weight at ≥0.83 mg/kg/day
					Develop	0.83	1.1		Decreased number of live births (36 and 31%) and pup survival at 1.1 and 2.0 mg/kg/day, but not 1.5 mg/kg/day; decreased number of live pups per litter and decreased pup body weight gain in females at 2 mg/kg/day; increased pup liver weight at ≥0.83 mg/kg/day
Wolf et al. 2010									
42	Mouse (PPARα knockout) 8 F	GDs 1–18 (G)	0, 0.83, 1.1, 1.5, 2.0 PFNA	BW, OW, DX	Bd wt	2.0			No alterations in maternal body weight or gestational weight gain
					Hepatic	2.0			Increases in liver weight at ≥1.5 mg/kg/day non-pregnant adults, but not in the dams
					Develop	2.0			No alterations in the number of live pups per litter, birth weight, pup survival, day of eye opening, or pup body weight gain; no increases in pup liver weight were observed
Wolf et al. 2010									

Table 2-5. Levels of Significant Exposure to Other Perfluoroalkyls – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
PFDA									
43	Rat (Sprague-Dawley) 8 F	28 days	0, 0.125, 0.25, 0.5, 1, 2 PFDA	BW, HE, OW, HP, IX	Bd wt	0.5	1		Decreased body weight gain (21%)
					Resp	0.5			
					Gastro	0.5			
					Hemato	0.125	0.25		Decreased MCH and MCHC
					Hepatic	0.25	0.5		Single cell necrosis
					Hepatic	0.5			
					Renal	0.5			
					Endocr	0.5			
					Immuno	0.125	0.25		Decreased phagocytosis by fixed tissue macrophages in the liver
Frawley et al. 2018									
44	Mouse (B6C3F1/N) 8 F	1 time/week 4 weeks	0, 0.325, 0.625, 1.25, 2.5, 5 PFDA	BW, HE, OW, HP, IX	Bd wt	5			
					Resp	5			
					Gastro	5			
					Hemato	5			
					Hepatic	5			
					Renal	5			
					Endocr	5			
					Immuno	0.625	1.25		Decreases in splenic T-cells, T-cell subsets, and macrophages
Frawley et al. 2018									
PFUnA									
45	Rat (Crl:CD[SD]) 12 M,F (main); 5 M,F (other)	41–46 days (GO)	0, 0.1, 0.3, 1.0 PFUnA	BH, BW, CS, HP, OF, OW, UR	Bd wt	0.3	1.0		Decreased body weight (~10%) in males during exposure and recovery and in satellite females during dosing (~23% on day 40) and recovery (~10%)
					Hemato	0.3	1.0		Main study males: decreased MCV (5%), MCH (5%), APTT (25%), and fibrinogen (33%) and increased platelet counts (7%); satellite males: increased WBC (52%) and decreased APTT (16%) and fibrinogen (19%); main study females: increased MCV (10%) and MCH (10%) and decreased fibrinogen (32%)

Table 2-5. Levels of Significant Exposure to Other Perfluoroalkyls – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
					Hepatic	1.0			Increased absolute and relative liver weight and centrilobular hypertrophy at 1.0 mg/kg/day
					Renal	0.3	1.0		Increased BUN (61%) and ALP (140%); decreased total protein (11%) and albumin (7%) in main group males
					Develop	0.3 F	1.0 F		Decreased body weight in pups on PNDs 0 and 4 (13–19% in males and 12–16% in females)
Takahashi et al. 2014									
PFBS									
46	Rat (Sprague-Dawley)	28 days 1 time/day (GO)	0, 100, 300, 900 PFBS	CS, BW, FI, HE, BI, GN, HP, OF	Bd wt	900			
					Resp	900			
					Cardio	900			
					Gastro	900			
					Hemato	900			
					Musc/Skel	900			
					Hepatic	900			Increased absolute and relative liver weight at 900 mg/kg/day
					Renal	900			
					Ocular	900			
					Endocr	900			
					Immuno	900			No histological alterations
					Neuro	900			No histological alterations
					Repro	900			No histological alterations
3M 2001									
47	Rat (Sprague-Dawley) 10 NS	90 days (G)	0, 60, 200, 600 PFBS	LT, BW, OW, GN, HP, BC, CS, BI, BH, HE	Resp	600			
					Cardio	600			
					Gastro	200	600		Necrosis of individual squamous cells in forestomach and hyperplasia and hyperkeratosis of limiting ridge
					Hemato	60 M	200 M		Decreased hemoglobin (4.9%) and hematocrit (5.2%)
					Musc/skel	600			

Table 2-5. Levels of Significant Exposure to Other Perfluoroalkyls – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
					Hepatic	600			
					Renal	200	600		Hyperplasia of medullary and papillary tubules and medullary ducts and focal papillary edema
					Endocr	600			
					Neuro	600			
Lieder et al. 2009a (data also reported in York 2003b)									
48	Rat (Sprague-Dawley)	P0: starting 70 days prior to mating; F1: starting at weaning (G)	0, 30, 100, 300, 1,000 PFBS	BW, OW, FI, GN, HP, FX, MX, DX, TG	Bd wt	1,000			
					Hepatic	1,000			Increased liver weight in males at ≥300 mg/kg/day
					Renal	100	300		Medullary/papillary tubular and ductal hyperplasia in P0 and F1
					Repro	1,000			
					Develop	1,000			
Lieder et al. 2009b (data also reported in York 2003c)									
49	Mouse (E3L CETP) 6–8 M	4–6 weeks (F)	0, 30 PFBS	BW, FI, BC, OW	Bd wt	30			
					Hepatic	30			Decreased plasma triglyceride levels
Bijland et al. 2011									
50	Mouse (ICR) 30 F	GDs 1–20 (G)	0, 50, 200, 500 PFBS	BW, BC, OW, DX	Bd wt	500			
					Endocr	50	200		Decreased maternal total thyroxine, free thyroxine, and total triiodothyronine and increased TSH
					Develop	50	200		Decreases in pup body weight; delays in eye opening, vaginal opening, and first estrous; decreases in ovarian follicles; decreases in uterine endometrial and myometrial thickness; alterations in reproductive hormone levels; decreases in total T4 and total T3 and increases in TSH
Feng et al. 2017									

Table 2-5. Levels of Significant Exposure to Other Perfluoroalkyls – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
51	Rat (Crl:CD(SD) 25 F	GDs 6–20 (GW)	0, 100, 300, 1,000 PFBS	BW FI DX	Bd wt	300	1,000		31% decrease in maternal body weight gain
					Develop	300	1,000		Decreases in fetal body weight (9%) and delays in hindlimb ossification
York 2002									
52	Rat (Crl:CD(SD) 8 F	GDs 6–20 (GW)	0, 100, 300, 1,000, 2,000 PFBS	BW FI DX	Bd wt	1,000	2,000		12% decrease in maternal body weight
					Develop	1,000	2,000		12–13% decrease in fetal body weights
York 2003a									
PFBA									
53	Rat (Sprague-Dawley) 10 M,F	28 days 1 time/day	0, 6, 30, 150 PFBA	CS, BW, OW, FI, BI, HE, GN, HP, OF	Bd wt	150			
					Resp	150			
					Cardio	150			
					Gastro	150			
					Hemato	150			
					Musc/skel	150			
					Hepatic	150			Increased absolute and relative liver weight and decreased serum cholesterol in males at ≥30 mg/kg/day; hepatocellular hypertrophy in males at 150 mg/kg/day
					Renal	150			
					Dermal	150			
					Ocular	150			
					Endocr	6 M	30 M		Hyperplasia/hypertrophy of follicular epithelium of the thyroid
					Immuno	150			No histological alterations
					Neuro	30 M	150 M		Delayed pupillary reflex
					Repro	150			No histological alterations
Butenhoff et al. 2012a; van Otterdijk 2007a									

Table 2-5. Levels of Significant Exposure to Other Perfluoroalkyls – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
54	Rat (Sprague-Dawley) 10 M,F	90 days 1 time/day	0, 1.2, 6, 30 PFBA	CS, BW, FI, OW, BI, HE, GN, HP	Bd wt	30			
					Resp	30			
					Cardio	30			
					Gastro	30			
					Hemato	6	30 M		Reduced erythrocyte counts (3.8%), hemoglobin (5.7%), and hematocrit (4.5%)
					Musc/skel	30			
					Hepatic	30			Diffuse panlobular hepatocyte hypertrophy at 30 mg/kg/day in males
					Renal	30			
					Dermal	30			
					Ocular	30			
					Endocr	6 M	30 M		Hypertrophy/hyperplasia of follicular epithelium of the thyroid gland
					Immuno	30			No histological alterations
					Neuro	30			No histological alterations
					Repro	30			No histological alterations
Butenhoff et al. 2012a; van Otterdijk 2007b									
55	Mouse (CD-1)	18 days GDs 1–17 1 time/day (GW)	0, 35, 175, 350 PFBA	BW, MX, DX	Hepatic	350			Significant increase in absolute and relative liver weight at ≥175 mg/kg/day
					Develop		35		Eye opening delayed approximately 1 day
Das et al. 2008									

Table 2-5. Levels of Significant Exposure to Other Perfluoroalkyls – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
PFDoDA									
56	Rat (Crl:CD (SD)) 12 M, 17 F	42–47 days (G)	0, 0.1, 0.5, 2.5 PFDoDA	CS, BW, BC, HE, OW, HP, NX, RX, DX	Death			2.5 F	58% mortality
					Bd wt	0.5	2.5		Decreased body weight gain (20–30%)
					Cardio	2.5			
					Gastro	2.5			
					Hemato	0.5	2.5		Decreased mean corpuscular volume and reticulocytes and increased mean corpuscular hemoglobin concentration in males
					Hepatic	0.5 F	2.5 F		Single cell necrosis in females; hepatocellular hypertrophy in males and females at 2.5 mg/kg/day; bile duct proliferation at 2.5 mg/kg/day in females in recovery group
					Renal	2.5			
					Endocr	0.5	2.5		Pancreas: edema in the interstitium in females and decrease in zymogen granules in males; thymus: atrophy of the cortex in females; adrenals: atrophy of the cortex in males
					Neuro	0.5	2.5		Decreased forelimb grip in males and motor activity in females during recovery period
					Repro	0.5	2.5 F		Hemorrhage at the implantation site; continuous diestrus in nonmated rats
					Develop	0.5	2.5		Decreases in pup body weight (only one litter had live pups)
Kato et al. 2015									
57	Rat (Sprague-Dawley) 8 F	28 days PNDs 24–72 (GW)	0, 0.5, 1.5, 3 PFDoDA		Endocr	1	3		40% reduced serum estradiol in pubertal females
Shi et al. 2009b									

Table 2-5. Levels of Significant Exposure to Other Perfluoroalkyls – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
58	Rat (Sprague-Dawley) 6 M	110 days GW	0, 0.02. 0.05, 0.2, 0.5 PFDoDA	BW, BI, OW, HP	Bd wt	0.5			
					Repro	0.05	0.2		Decreased serum testosterone (44%)
Shi et al. 2009a									
PFHxA									
59	Rat (Crl:CD (SD)) 10 M, 10 F	90 days (GW)	0, 10, 50, 200 PFHxA	CS, BW, FI, HE, BC, UR, OP, NX, OW, GN, HP	Bd wt	200			
					Resp	200			
					Cardio	200			
					Gastro	200			
					Hemato	50	200		Slight decreases in RBC, hemoglobin, and hematocrit and increases in reticulocytes
					Musc/skel	200			
					Hepatic	200			Centrilobular hepatocellular hypertrophy at 200 mg/kg/day
					Renal	200			
					Ocular	200			
					Endo	200			
					Neuro	200			
					Repro	200			
Chengelis et al. 2009b									

Table 2-5. Levels of Significant Exposure to Other Perfluoroalkyls – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
60	Rat (CRL:CD(SD)) 10–15 M, 10–15 F	32–44 days (GW)	0, 50, 150, 315 (TWA dose), 450 (4 days) **PFHxA**	CS, BW, FI, HE, BC, RX, DX, OW, GN, HP	Death			450	8/30 deaths (includes rats sacrificed *in extremis*) during first 4 days of exposure
					Bd wt	315			
					Gastro	315			Stomach erosions/ulceration in dying or sacrificed *in extremis* rats administered 450 mg/kg/day for 4 days
					Hemato	50 M 315 F	150 M		Decreased hemoglobin levels; increased reticulocytes at 315 mg/kg/day
					Hepatic	315			Increased relative liver weight at ≥150 mg/kg/day; hepatocellular hyperplasia and decreased serum cholesterol observed at 315 mg/kg/day
					Renal	315			
					Immuno	150 F 315 M	315 F		Thymic atrophy in 3/9 females
					Repro	315			
					Develop	315			
Kirkpatrick 2005									
61	Rat (CRL:CD (SD)) 30 M, 30 F	92–93 days (GW)	0, 20, 100, 500 NaPFHx	CS, BW, FI, OP, BC, HE, UR, OW, GN, HP, NX	Bd wt	200 M	500 M		19% decrease in body weight gain
					Resp	20	100		Degeneration/atrophy of nasal olfactory epithelium at ≥100 mg/kg/day and respiratory metaplasia at 500 mg/kg/day
					Hemato	100	500		Decreased RBC, hemoglobin, and hematocrit; increased reticulocytes; erythroid hypertrophy in bone marrow and extramedullary hematopoiesis in spleen
					Hepatic	500			Hepatocellular hypertrophy in males at ≥100 mg/kg/day and females at 500 mg/kg/day
					Ocular	500			
					Endocr	100 F	500 F		Thyroid follicular epithelial hypertrophy
					Neuro	500			
Loveless et al. 2009									

Table 2-5. Levels of Significant Exposure to Other Perfluoroalkyls – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
62	Rat (CRL:CD (SD) 20M, 20F	110–126 days; 70 days prior to mating and during mating gestation, lactation (GW)	0, 20, 100, 500 NaPFHx	CS, BW, FI, RX, DX	Bd wt	20 M 100 F	100 M 500 F		Decreased weight gain (12%); decreased maternal weight gain during GDs 0–7 and increased maternal weight gain during lactation
					Repro	500			
					Develop	100	500		Decreased pup body weight (17–18%) during lactation period
Loveless et al. 2009									
63	Rat (CRL:CD (SD) 22 F	GDs 1–20 (GW)	0, 20, 100, 500 NaPFHx	BW, FI, DX	Bd wt	100	500		Decreased maternal weight gain (19%)
					Develop	100	500		Decreased fetal weight (10%)
Loveless et al. 2009									
CHRONIC EXPOSURE									
PFHxA									
64	Rat (Sprague-Dawley) 60 or 70 M,F	104 weeks (GW)	M: 0, 2.5, 15, 100 F: 0, 5, 30, 200 PFHxA	BC, BW, CS, GN, HP, LE, OP, OW, UR	Death			200 F	36, 43, 33, and 22% survival rate in females at 0, 5, 30, and 200 mg/kg/day, respectively
					Bd wt	100 M 200 F			
					Hemato	100 M	200 F		8.1% reduction in mean RBC count and 5.2% reduction in hemoglobin at 51 weeks; 23.6 and 53.6% increase in reticulocyte counts at weeks 25 and 51, respectively
					Hepatic	100 M 30F	200 F		Males: 42% decrease in triglycerides, 19% decrease in free fatty acids in males at 100 mg/kg/day; hepatocellular necrosis; 66% increase in triglycerides, 44% decrease in non-HDL cholesterol in females at 200 mg/kg/day

Table 2-5. Levels of Significant Exposure to Other Perfluoroalkyls – Oral

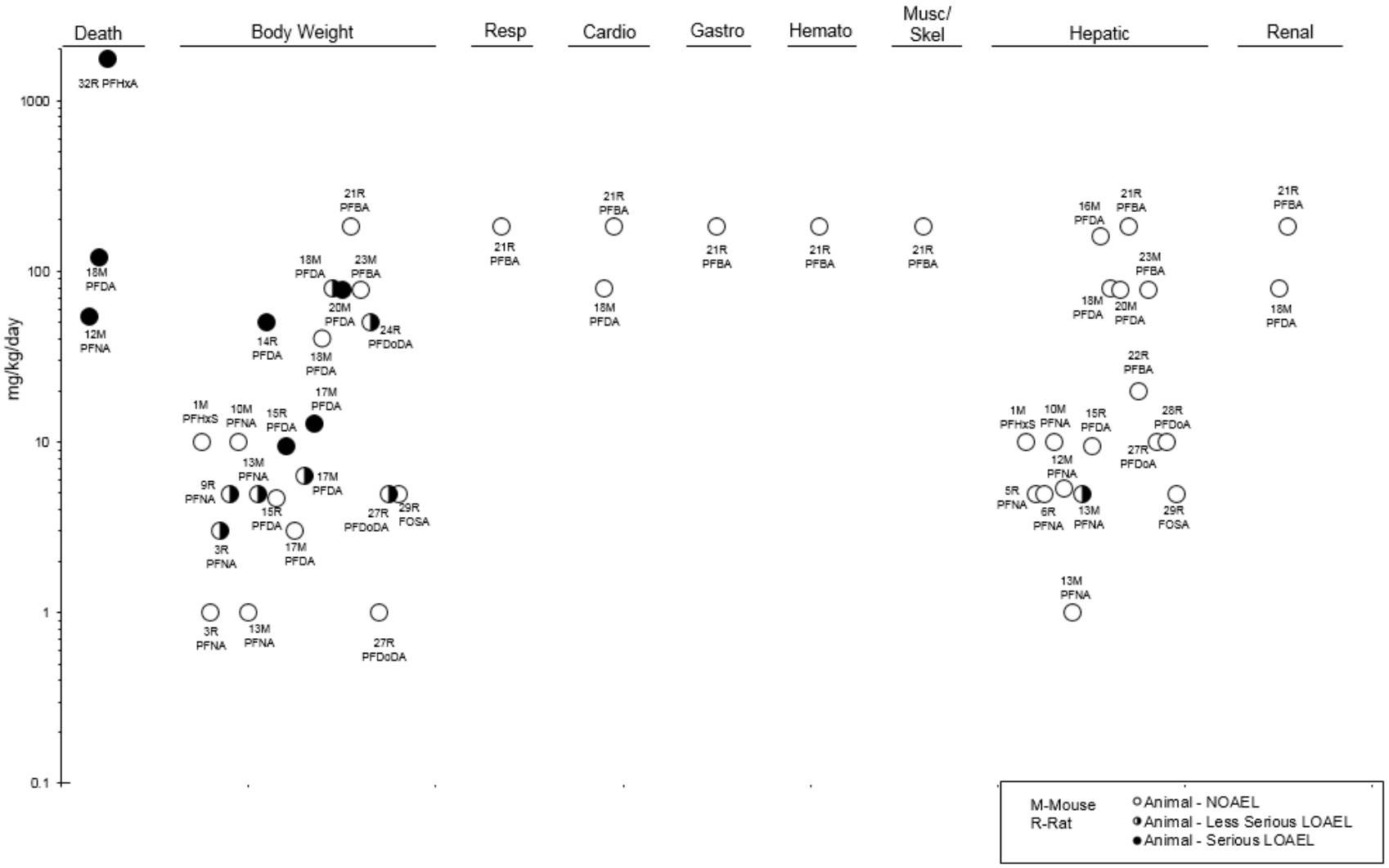
Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
					Renal	100 M	200 F		Mild renal tubular degeneration and mild to severe papillary necrosis; increased mean urine volume (109%) and reduced specific gravity (0.96%)
					Neuro	100 M 200 F			
Klaunig et al. 2015									

[a]The number corresponds to entries in Figure 2-10.
[b]Used to derive an intermediate-duration oral MRL of $2x10^{-5}$ mg/kg/day for PFHxS based on a measured serum PFHxS level of 89.12 µg/mL at the NOAEL dose and an empirical clearance model to estimate a HED. The $NOAEL_{HED}$ of 0.0047 mg/kg/day was divided by an uncertainty factor of 30 (3 for extrapolation from animals to humans with dosimetric adjustment and 10 for human variability) and a modifying factor of 10 for database deficiencies.
[c]Used to derive an intermediate-duration oral MRL of $3x10^{-6}$ mg/kg/day for PFNA based on a measured serum PFNA level of 8.91 µg/mL at the NOAEL dose and an empirical clearance model to estimate a HED. The $NOAEL_{HED}$ of 0.001 mg/kg/day was divided by an uncertainty factor of 30 (3 for extrapolation from animals to humans with dosimetric adjustment and 10 for human variability) and a modifying factor of 10 for database deficiencies.

ad lib = *ad libitum*; ALT = alanine aminotransferase; APTT = activated partial thromboplastin time; AST = aspartate aminotransferase; BC = biochemistry; BH = behavioral; BI = biochemical changes; BUN = blood urea nitrogen; BW or Bd wt = body weight; Cardio = cardiovascular; CI = confidence interval; CS = clinical signs; Develop = developmental; DX = developmental toxicity; EA = enzyme activity; Endocr = endocrine; (F) = feed; F = female(s); FI = food intake; FOSA = perfluorooctane sulfonamide; FX = fetal toxicity; G = gavage; Gastro = gastrointestinal; GD = gestation day; GN = gross necropsy; GO = gavage in oil vehicle; GW = gavage in water vehicle; HDL = high-density lipoprotein; HE or Hemato = hematological; HP = histopathology; Immuno = immunotoxicological; IX = immunotoxicity; LD_{50} = lethal dose, 50% kill; LE = lethality; LOAEL = lowest-observed-adverse-effect level; M = male(s); MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration; MCV = mean corpuscular volume; Musc/skel = musculoskeletal; MX = maternal toxicity; NaPFHx = sodium perfluorohexanoate; Neuro = neurological; NOAEL = no observed-adverse-effect level; NS = not specified; NS = neurotoxicity; OF = organ function; OP = ophthalmology; OW = organ weight; PFBA = perfluorobutanoic acid; PFBS = perfluorobutane sulfonic acid; PFDA = perfluorodecanoic acid; PFDoDA = perfluorododecanoic acid; PFHxA = perfluorohexanoic acid; PFHxS = perfluorohexane sulfonic acid; PFNA = perfluorononanoic acid; PFUnA = perfluoroundecanoic acid; PPARα = peroxisome proliferator-activated receptor-α; RBC = red blood cell; Repro = reproductive; Resp = respiratory; RX = reproductive toxicity; T3 = triiodothyronine; T4 = thyroxine; TG = teratogenicity; TSH =thyroid stimulating hormone; TWA = time-weighted average; UR = urinalysis

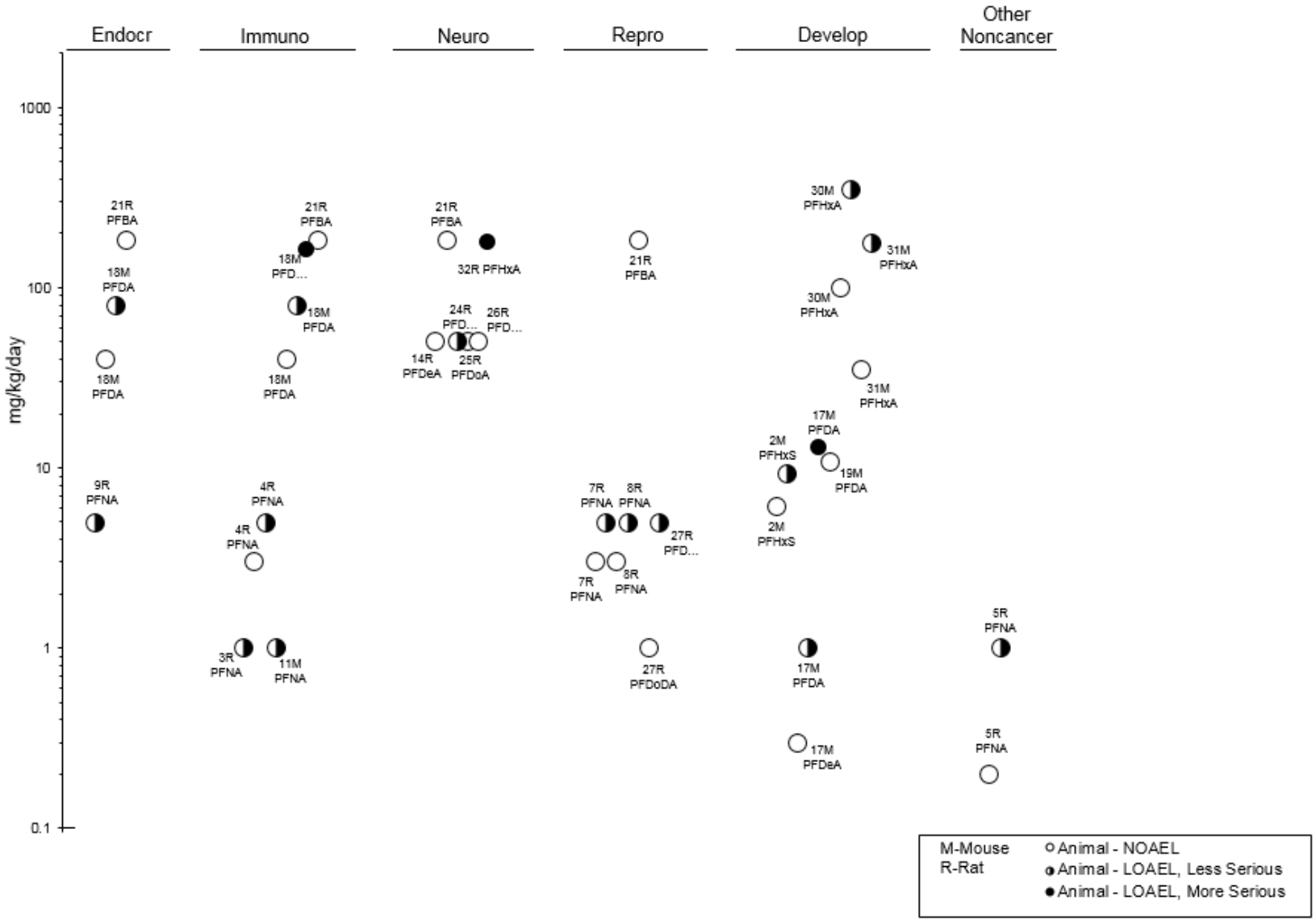
Figure 2-10. Levels of Significant Exposure to Other Perfluoroalkyls – Oral

Acute (≤14 days)



KD0126

Figure 2-10. Levels of Significant Exposure to Other Perfluoroalkyls – Oral
Acute (≤14 days)



KD0127

Figure 2-10. Levels of Significant Exposure to Other Perfluoroalkyls – Oral
Intermediate (15–364 days)

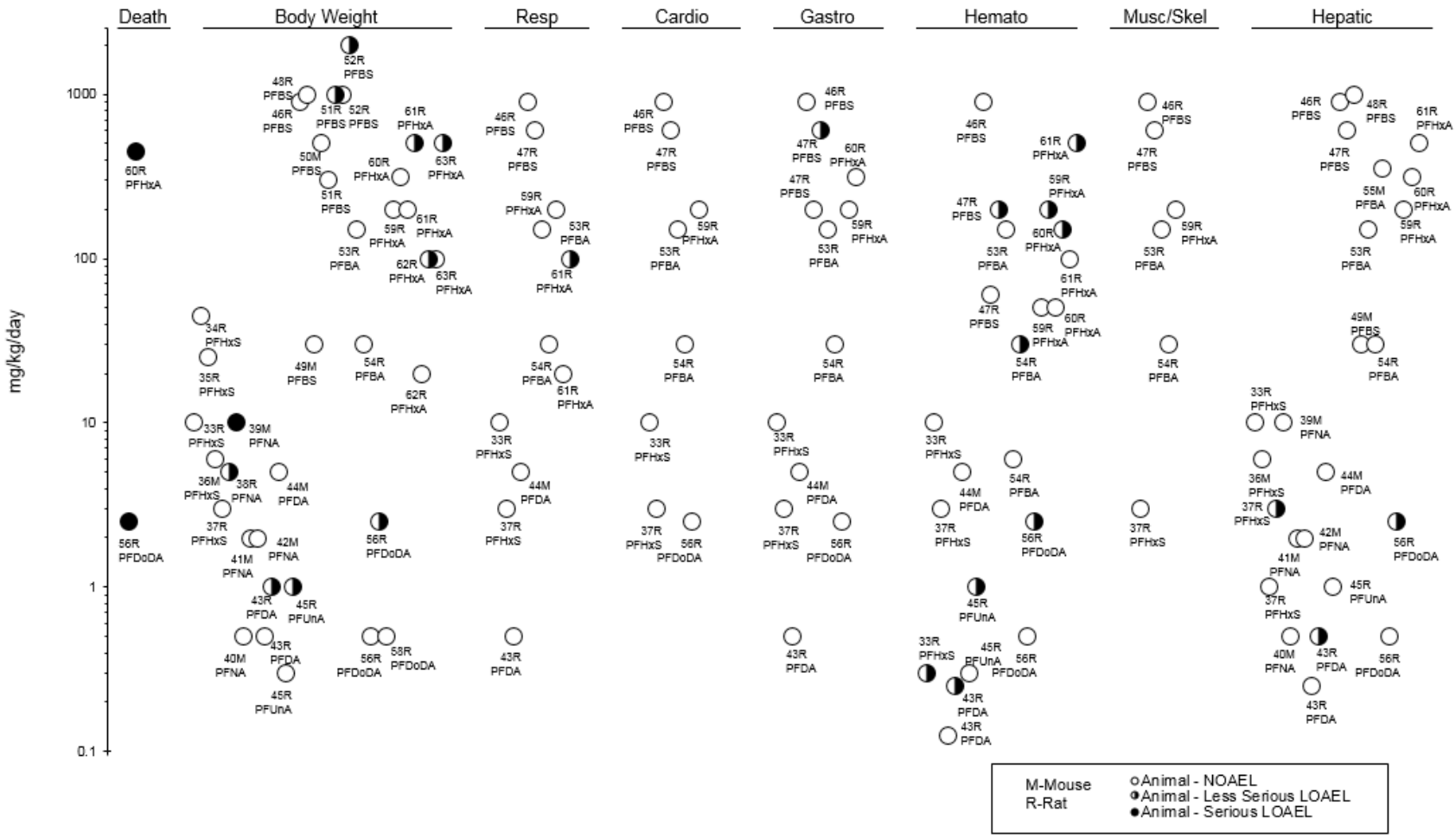


Figure 2-10. Levels of Significant Exposure to Other Perfluoroalkyls – Oral
Intermediate (15–364 days)

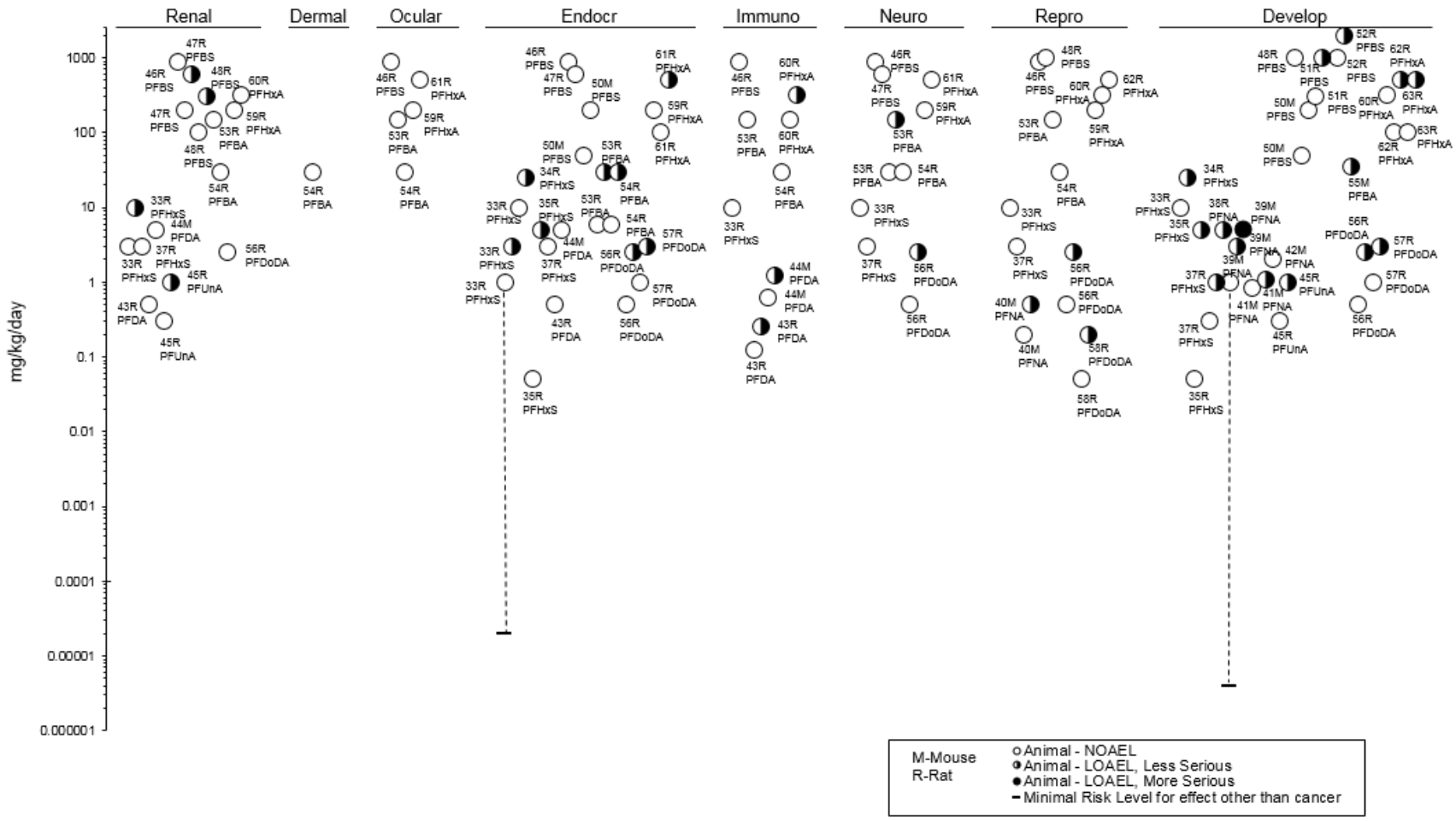
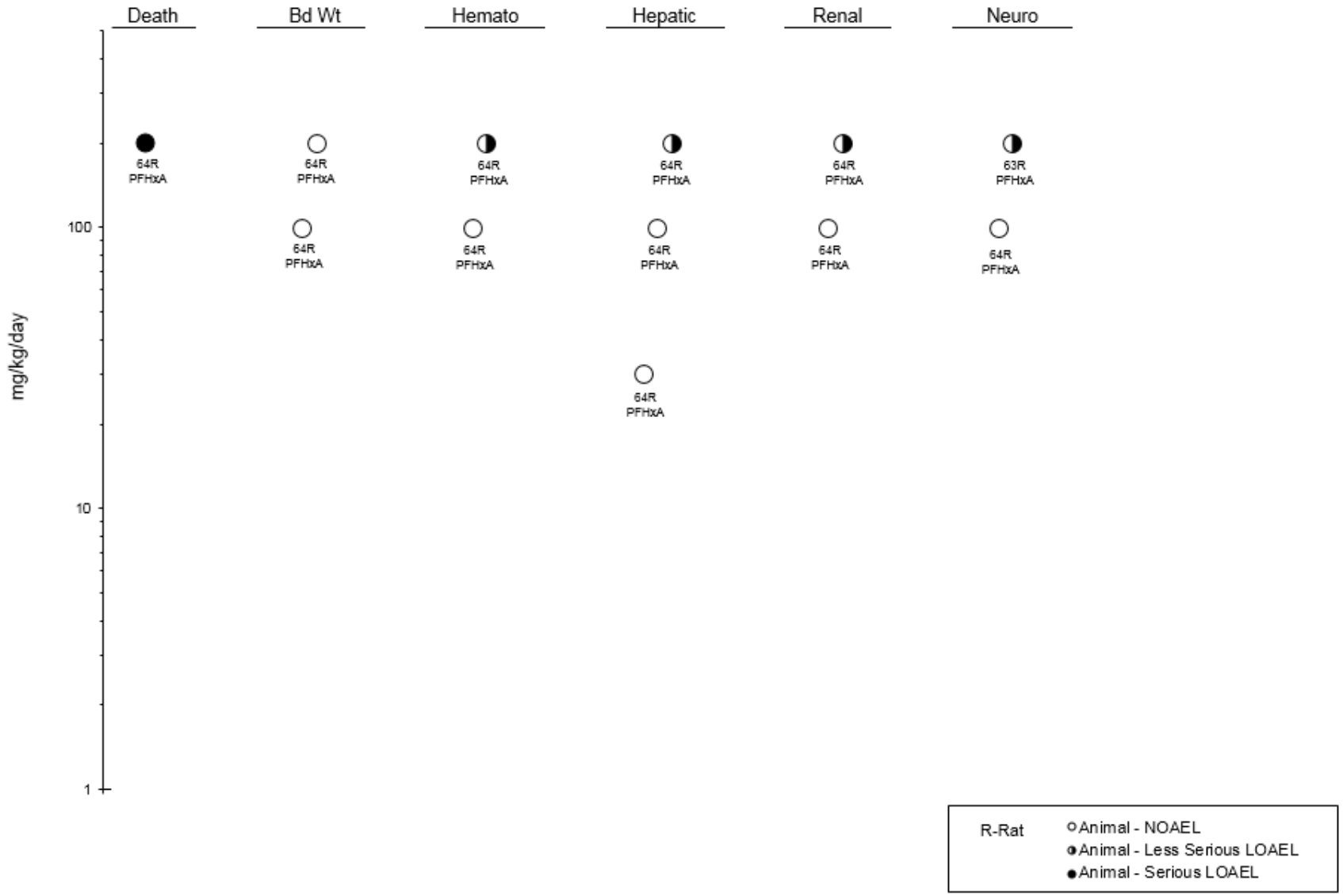


Figure 2-10. Levels of Significant Exposure to Other Perfluoroalkyls – Oral
Chronic (≥365 days)



KD0130

Table 2-6. Levels of Significant Exposure to PFOA – Dermal

Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effect
ACUTE EXPOSURE								
Rat (CD) 15 M,F	Once	3,000, 5,000, 7,500 mg/kg	CS, LE	Death			7,000 M	14-day LD_{50}
				Bd wt			3,000 M F	5–7% transient weight loss
				Dermal	3,000 M F	5,000 M F		Mild skin irritation
Kennedy 1985								
APFO								
LD_{50} in females was >7,500 mg/kg								
Rat (CD) 60 M	2 weeks 6 hours/day 5 days/week	0, 20, 200, 2,000 mg/kg/day	CS, BW, HE, BI, GN, HP	Bd wt	20 M		200 M	14% weight loss
				Resp	2,000 M			
				Cardio	2,000 M			
				Gastro	2,000 M			
				Hemato	2,000 M			
				Hepatic		20 M		Foci of coagulative necrosis
				Renal	2,000 M			
				Dermal	20 M	200 M	2,000 M	Skin irritation; acute necrotizing dermatitis
				Ocular	2,000 M			
				Endocr	2,000 M			
				Immuno	2,000 M			
				Neuro	2,000 M			
				Repro	2,000 M			
Kennedy 1985								
APFO								
The immunological NOAEL is for histopathology of the spleen, thymus, and lymph nodes. The neurological NOAEL is for histopathology of the brain. The reproductive NOAEL is for histopathology of the testes.								
Mouse (BALB/c) 35 F	4 days 1 time/day	0, 0.25, 2.5, 6.25, 12.5, 25, 50 mg/kg/day	BW, OW, OF	Bd wt	50 F			
				Hepatic	2.5 F	6.25 F		52% increase in absolute liver weight
Fairley et al. 2007								
PFOA								

Table 2-6. Levels of Significant Exposure to PFOA – Dermal

Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effect
Mouse (BALB/c) 35 F	4 days 1 time/day	0, 12.5, 18.8, 25, 50 mg/kg/day	BW, OW, OF	Immuno	12.5	18.8		Increased serum IgE following ovalbumin challenge
Fairley et al. 2007								
PFOA								
Rabbit (albino) 6 NS	Once (NS)	100 mg	CS	Ocular		100		Moderate eye irritation
Griffith and Long 1980								
APFO								
Rabbit (albino) 6 NS	24 hours (NS)	500 mg	CS	Dermal	500			
Griffith and Long 1980								
APFO								
Rabbit (New Zealand) 17 M	Once	1,500, 3,000, 5,000, 7,500 mg/kg	CS, LE	Death		4,300		14-day LD_{50}
Kennedy 1985								
APFO								

APFO = ammonium perfluorooctanoate; BI = biochemical changes; BW or Bd wt = body weight; Cardio = cardiovascular; CS = clinical signs; Endocr = endocrine; F = female(s); Gastro = gastrointestinal; GN = gross necropsy; HE or Hemato = hematological; HP = histopathology; Immuno = immunotoxicological; LD_{50} = lethal dose, 50% kill; LE = lethality; LOAEL = lowest-observed-adverse-effect level; M = male(s); Neuro = neurological; NOAEL = no-observed-adverse-effect level; NS = not specified; OF = organ function; OW = organ weight; PFOA = perfluorooctanoic acid; Repro = reproductive; Resp = respiratory

2.2 DEATH

Overview. There are limited data regarding the lethality of perfluoroalkyls in humans; the available data primarily come from cohort mortality studies in workers; data were only available for PFOA and PFOS. These studies did not find increases in deaths from all causes associated with PFOA and PFOS, although some increases in disease-specific mortalities were observed. Laboratory animal studies have measured LC_{50} and LD_{50} values and reported deaths following inhalation, oral, or dermal exposure to perfluoroalkyls. Increases in mortality have also been observed in repeated-exposure studies. These data are presented in Tables 2-1, 2-2, 2-3, 2-4, 2-5, and 2-6 and Figures 2-6, 2-7, 2-8, 2-9, and 2-10. No laboratory animal data were available for PFHxS, PFUnA, PFHpA, PFBS, PFBA, or FOSA.

PFOA

Epidemiological Studies. Five occupational exposure studies at two PFOA manufacturing facilities have examined the possible associations between PFOA exposure and increases in mortality from all causes and have not found associations (Gilliland and Mandel 1993; Leonard 2006; Leonard et al. 2008; Lundin et al. 2009; Raleigh et al. 2014; Steenland and Woskie 2012). Some increases in disease-specific mortality have been observed; these data are discussed in subsequent sections of this chapter (Sections 2.5, 2.8, 2.10, 2.18, and 2.19).

Laboratory Animal Studies. Limited data are available regarding death in animals following inhalation exposure to perfluoroalkyls. Exposure of male and female rats to 18,600 mg/m^3 ammonium perfluorooctanoate (APFO) dusts for 1 hour did not result in deaths during exposure or during a 14-day observation period (Griffith and Long 1980); APFO is the ammonium salt of PFOA. An LC_{50} of 980 mg/m^3 was reported in male CD rats exposed head-only to APFO dusts for 4 hours (Kennedy et al. 1986). Deaths occurred at all exposure levels (380–5,700 mg/m^3) and all deaths occurred within 48 hours of exposure. Rats dying during exposure had hyperinflated lungs. A similar LC_{50} value of 820 mg/m^3 was calculated for male CD rats exposed nose-only to APFO dusts for 4 hours (Kinney et al. 1989). Unlike the Kennedy et al. (1986) study, one death was observed at 590 mg/m^3 and no deaths occurred at 620 mg/m^3. In a developmental study with APFO, whole-body exposure of 12 pregnant rats to 25 mg/m^3, 6 hours/day during GDs 6–15 resulted in three deaths on GDs 12, 13, and 17 compared with no deaths in groups exposed to $\leq$10 mg/m^3 (Staples et al. 1984). The cause of death was not reported.

Oral LD_{50} values of 680 and 430 mg/kg were reported for male and female albino rats, respectively, administered single gavage doses of APFO and observed for 14 days (Griffith and Long 1980); all animals at the highest dose of 2,150 mg/kg died on day 1. Nonlethal signs observed included ptosis, piloerection, hypoactivity, decreased limb tone, ataxia, and corneal opacity. All signs were intermittent and there was no apparent dose-response relationship. In a 28-day dietary study with APFO in rats, all rats (males and females) in groups receiving approximately 1,000–1,130 mg/kg/day APFO died before the end of the first week (Griffith and Long 1980). In a similar study in mice, all mice receiving doses of approximately 180–195 mg/kg/day died before the second week of the study (Griffith and Long 1980). In this study, doses of approximately 54–58 mg/kg/day APFO were lethal to 4/5 male and 5/5 female mice before the 4^{th} week of the study.

In a 90-day gavage study, treatment of Rhesus monkeys with 100 mg/kg/day APFO by gavage resulted in the death of an unspecified number of animals (group size was 10/sex) on week 2 (Griffith and Long 1980). Doses of approximately 30 mg/kg/day were lethal to one male and two females during weeks 7–12. All animals that died in the 30 and 100 mg/kg/day groups had anorexia, emesis, black stool, pale face and gums, swollen face and eyes, hypoactivity, and prostration. Microscopic examination of tissues showed marked diffuse lipid depletion in the adrenals, slight to moderate hypocellularity of the bone marrow, moderate atrophy of the lymphoid follicles of the spleen, and moderate atrophy of the lymphoid follicles of the lymph nodes. No deaths occurred at 10 mg/kg/day. Deaths were also reported in intermediate-duration studies in Cynomolgus monkeys (Butenhoff et al. 2002). One monkey exposed to 30/20 mg/kg/day PFOA (12 days of exposure to 30 mg/kg/day, 10 days with no exposure, 23 weeks of exposure to 20 mg/kg/day) was sacrificed in moribund condition; the animal had a body weight loss of 12.5%, was notably hypoactive, and was cold to the touch (Butenhoff et al. 2002). The investigators noted that the death was likely due to the high toxicity of the 30 mg/kg/day dose. It is unclear if these deaths were compound-related; one monkey had pulmonary necrosis with a severe acute recurrence of pulmonary inflammation and the cause of morbidity for the second monkey was likely hyperkalemia. Neither effect was observed in the surviving animals.

The dermal LD_{50} values for APFO were 7,000 mg/kg in male CD rats and >7,500 mg/kg in female rats (Kennedy 1985). The protocol consisted of application of PFOA (as an aqueous paste) to a clipped area of the skin, which immediately was covered with gauze pads and wrapped with rubber sheeting around the trunk. The contact period was 24 hours, at which time the application site was washed with water and the rats were observed for clinical signs for 14 days. Using the same protocol, the dermal LD_{50} in male rabbits was 4,300 mg/kg (Kennedy 1985). Rabbits treated with 1,500 mg/kg showed skin irritation with

formation of a large crusty area at the application site. No deaths occurred at 1,500 mg/kg. Rabbits treated with 3,000 mg/kg were lethargic and a single death occurred 7 days after treatment. At 5,000 mg/kg, deaths occurred in 3–4 days. These rabbits also showed nasal discharge, pallor, diarrhea, weakness, severe weight loss, and severe skin irritation along with areas of necrosis.

PFOS

Epidemiological Studies. One occupational exposure study evaluated the potential of PFOS to increase lethality; the study did not find increases in deaths from all causes in workers at a PFOS manufacturing facility (Alexander et al. 2003). Alterations in disease-specific mortality are discussed in subsequent sections of this chapter.

Laboratory Animal Studies. Unpublished information summarized by the Organization for Economic Co-operation and Development (OECD) (2002) indicates that an LC_{50} of 5,200 mg/m^3 was calculated for PFOS in male and female Sprague-Dawley rats exposed to airborne concentrations of PFOS dusts from 1,890 to 45,970 mg/m^3 for 1 hour. All rats exposed to 24,090 mg/m^3 died by day 6.

Unpublished information summarized by OECD (2002) indicate that LD_{50} values of 233 and 271 mg/kg were calculated for male and female CD rats, respectively, following administration by gavage of single doses of up to 1,000 mg/kg of powdered PFOS suspended in an acetone/oil mixture and observed for 14 days. All rats (5/sex/dose group) dosed with ≥464 mg/kg PFOS died before the end of the study. The signs most frequently observed were hypoactivity, decreased limb tone, and ataxia. Gross necropsy showed stomach distension and signs of irritation of the glandular mucosa, and lung congestion. OECD (2002) also reported that a different study estimated that the acute oral LD_{50} for PFOS by gavage in water in Sherman-Wistar albino rats was >50 and <1,500 mg/kg. An oral LD_{50} value of 579 mg/kg/day was reported for male C57/BL/6 mice administered single gavage doses of PFOS and observed for 14 days (Xing et al. 2016). Mortality occurred within 3 hours of dosing, and moribund mice displayed signs of neurotoxicity (abdominal breathing, hind limb spasticity, tics, and urinary incontinence).

In a 26-week study, 2/6 male Cynomolgus monkeys administered 0.75 mg/kg/day PFOS via a capsule died or were sacrificed due to morbidity (Seacat et al. 2002). The cause of death in one monkey was pulmonary inflammation; the cause of morbidity in the second monkey was not determined, but the animal did have hyperkalemia.

PFNA

Laboratory Animal Studies. A LC_{50} of 820 mg/m^3 was identified in rats exposed to airborne PFNA for 4 hours (Kinney et al. 1989). In a 14-day dietary exposure study, all mice administered approximately 54 mg/kg/day PFNA died before the study period ended; no deaths occurred at 5.3 mg/kg/day (Kennedy 1987).

PFDA

Laboratory Animal Studies. An LD_{50} of 120 mg/kg was estimated for PFDA in female C57BL/6N mice administered single doses between 20 and 320 mg/kg/day PFDA by gavage in corn oil and observed for 30 days (Harris et al. 1989). All mice receiving 160 or 320 mg/kg were dead by 14 days; no mice died at ≤80 mg/kg PFDA. Early death was associated with mural thrombosis in the left ventricle of the heart. Without providing any details, George and Andersen (1986) reported that the 30-day oral LD_{50} for PFDA in male Fischer-344 rats was 57 mg/kg.

PFDoDA

Laboratory Animal Studies. Increases in mortality were observed in pregnant rats administered 2.5 mg/kg/day for 14 days prior to mating and throughout gestation; 4/12 dams between GD 18 and 22 and another 3 dams were sacrificed during the period due to morbidity (Kato et al. 2015). No deaths were observed in males or nonpregnant females exposed to 2.5 mg/kg/day (Kato et al. 2015).

PFHxA

Laboratory Animal Studies. In a single exposure gavage study, deaths occurred in rats administered 1,750 or 5,000 mg/kg sodium perfluorohexanoate (NaPFHx) (Loveless et al. 2009). Decreased survival was observed in female Sprague-Dawley rats administered 200 mg/kg/day PFHxA via gavage in a 104-week study (Klaunig et al. 2015). There was no significant effect on survival rates of males. Mortality and morbidity were observed in male and female rats administered 450 mg/kg/day PFHxA via gavage for 4 days (Kirkpatrick 2005). The cause of death was determined to be renal papillary necrosis and/or stomach erosion/ulceration.

2.3 BODY WEIGHT

Overview. Epidemiological studies have examined the possible associations between *in utero* and/or early life exposure to perfluoroalkyls and body weight, body mass index (BMI; measure of body fat based on body weight and height), etc. Other studies have examined possible associations between serum perfluoroalkyl levels in older children or adults and body weight, adiposity markers, and the risk of being overweight or obese. The results of the epidemiological studies are summarized in Table 2-7, with more detailed descriptions presented in the *Supporting Document for Epidemiological Studies for Perfluoroalkyls*, Table 1. No epidemiological data were available for PFHpA, PFBS, PFBA, PFDoDA, or PFHxA. Animal studies have evaluated changes in body weight, including maternal body weight, in response to inhalation, oral, or dermal exposure to perfluoroalkyls; these data are summarized in Tables 2-1, 2-2, 2-3, 2-4, 2-5, and 2-6 and Figures 2-6, 2-7, 2-8, 2-9, and 2-10. No laboratory animal studies examining body weight were identified for PFHpA.

Overall, the evidence from epidemiological studies does not suggest an association between *in utero* and/or early life exposure to perfluoroalkyls and alterations in growth (body weight or length), body composition (e.g., BMI), or the risk of being overweight or obese in children for PFOA, PFOS, PFHxS, or PFNA. Conclusions cannot be drawn for PFDA, PFUnA, PFDoDA, or FOSA because of the small number of studies (less than 5 studies for each compound) examining potential body weight endpoints. A small number of studies examined potential associations between PFOA and body weight effects in adults and only one study examined PFOS, PFHxS, PFNA, and PFDA associations; these data were considered inadequate for assessing potential associations in adults.

Studies in laboratory animals exposed to PFOA, PFOS, PFNA, PFDA, PFUnA, PFDoDA, or PFHxA have consistently shown decreases in body weight or decreases in body weight gain. Studies with PFOA suggest that the decrease in body weight gain does not appear to be associated with alterations in food consumption and the mechanism may involve PPARα as studies in PPARα null have not found decreases in body weight gain. The small number of studies examining PFHxS, PFBS, PFBA, and FOSA have not reported decreases in body weight; although decreases in maternal body weight gain were observed for PFBS.

Table 2-7. Body Weight Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFOA			
Barry et al. 2014 Community (C8) (n=8,764 20–40-year-olds)	164.6 and 194.3 ng/mL (estimated early life [first 3 years] median PFOA)	Overweight or obesity at age 20–40 years	OR 0.9 (0.7–1.1), males OR 0.9 (0.7–1.1), females
Alkhalawi et al. 2016 General population (n=156 mother-child pairs)	2.43 ng/mL (maternal geometric mean serum PFOA)	Body weight at 1, 4, 6, and 12 months of age	NS (p>0.05)
		Body length at 1, 4, 6, and 12 months of age	NS (p>0.05)
Andersen et al. 2010 General population (n=1,010 infants)	5.21 ng/mL (maternal median PFOA)	Body weight (age 5 and 12 months)	**Inverse association (p<0.05)*, boys NS (p>0.05), girls**
		BMI (age 5 and 12 months)	**Inverse association (p<0.05)*, boys NS (p>0.05), girls**
		Height (age 5 and 12 months)	NS (p>0.05), boys NS (p>0.05), girls
Andersen et al. 2013 General population (n=811 children aged 7 years)	5.25 ng/mL (maternal median PFOA)	BMI	NS (p>0.05)
		Waist circumference	NS (p>0.05)
Braun et al. 2016a, 2016b General population (n=204 children)	5.3 ng/mL (maternal median PFOA)	Changes in BMI scores between 2 and 8 years of age	**Association (p=0.03)***
		Overweight/obesity risk	RR 1.54 (0.77–3.07), 3rd tertile
Cao et al. 2018 General population (n=337 infants)	1.59 ng/mL (mean cord serum PFOA)	Body weight at 19 months	NS (p=0.57)
		Length at 19 months	NS (p=0.16)
		Head circumference at 19 months	NS (p=0.94)
de Cock et al. 2014 General population (n=89 infants aged 1–11 months)	0.9402 ng/mL (cord blood mean PFOA)	Weight	NS (p=0.350)
		Height	NS (p=0.045)
		BMI	NS (p=0.813)
		Head circumference	NS (p=0.774)

Table 2-7. Body Weight Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Halldorsson et al. 2012 General population (n=665 20-year-olds)	3.7 and 5.8 ng/mL (maternal median PFOA and 4th quartile median)	BMI	**Association (p=0.001)*, females**
		Waist circumference	**Association (p=0.006)*, females**
		Overweight risk	**RR 3.1 (1.4–6.9)*, females 4th quartile**
		High waist circumference	**RR 3.0 (1.3–6.8)*, females 4th quartile**
Hartman et al. 2017 General population (n=359 girls aged 9 years)	3.7 ng/mL (maternal median PFOA)	Total body fatness	NS (p=0.20)
		Trunk fatness	NS (p=0.05)
		BMI	NS (p=0.05)
Høyer et al. 2015b General population (n=1,122 children aged 5–9 years; n=531 for Greenland cohort and n=491 for Ukraine cohort)	2.2–5.1 and 1.1–9.8 ng/mL (maternal 3rd tertile PFOA for Greenland and Ukraine cohorts)	Overweight Greenland cohort Ukraine cohort	 RR 1.23 (0.87–1.74), 3rd tertile RR 0.78 (0.47–1.29), 3rd tertile
		Waist-to-height ratio >0.5 Greenland cohort Ukraine cohort	 RR 1.18 (0.80–1.74), 3rd tertile RR 1.11 (0.48–2.57), 3rd tertile
Karlsen et al. 2017 General population (n=444 children)	1.37 ng/mL (maternal geometric mean serum PFOA)	BMI score, 18 months	NS (p>0.05)
		BMI score, 5 years	NS (p>0.05)
		Risk of being overweight 18 months	RR 1.14 (0.92–1.4)
		Risk of being overweight 5 years	**RR 1.50 (1.01–2.24, p<0.05)***
Karlsen et al. 2017 General population (n=444 children aged 5 years)	2.22 ng/mL (child geometric mean serum PFOA)	BMI score	**Inverse association (p<0.05)***
		Risk of being overweight	RR 0.68 (0.38–1.22)
Koshy et al. 2017 General population (WTCHR, n=180 children; n=222 children in comparison group)	1.81 and 1.39 ng/mL (median serum PFOA in WTCHR group and comparison group)	Risk of being overweight	OR 0.98 (0.90–1.13)

Table 2-7. Body Weight Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Liu et al. 2018a General population (n=621 adults in weight loss clinical trial)	4.5 ng/mL (median serum PFOA)	Weight loss	NS (p=0.73, trend)
		Weight regain Males Females	NS (p=0.16, trend) NS (p=0.78, trend) **Association (p=0.007, trend)***
		Resting metabolic rate Weight loss period Weight regain period	 NS (p=0.48, trend) **Association (p=0.03, trend)***
Manzano-Salgado et al. 2017b General population (n=1,230 children)	2.32 ng/mL (maternal geometric mean serum PFOA)	Weight gain until 6 months of age	β 0.04 (-0.04–0.12)
		BMI at 4 years of age	β 0.04 (-0.04–0.13)
		BMI at 7 years of age	β 0.03 (-0.08–0.13)
		Waist circumference at 4 years of age	β 0.00 (-0.09–0.10)
		Waist circumference at 7 years of age	β -0.02 (-0.11–0.06)
Mora et al. 2017 General population (n=1,006 at median age of 3.2 years and n=876 at median age of 7.7 years)	5.6 ng/mL (maternal median plasma PFOA in early childhood group)	BMI	β 0.09 (-0.02–0.19),
		Waist circumference	**β 0.31 (0.04–0.57)*, boys and girls** **β 0.50 (0.06–0.93)*, boys only** β 0.14 (-0.18–0.47), girls only
		Risk of being overweight	RRR 1.05 (0.87–1.26)
		Risk of being obese	RRR 1.03 (0.80–1.32)
Mora et al. 2017 General population (n=1,006 at median age of 3.2 years and n=876 at median age of 7.7 years)	5.6 ng/mL (maternal median plasma PFOA in late childhood group)	BMI	β 0.13 (-0.10–0.35)
		Total fat mass index	β 0.13 (0.02–0.29)
		Waist circumference	β 0.20 (-0.39–0.80)
		Risk of being overweight	RRR 1.02 (0.88–1.29)
		Risk of being obese	RRR 1.10 (0.88–1.37)

Table 2-7. Body Weight Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Timmermann et al. 2014 General population (n=499 8–10-year-old children)	9.3 ng/mL (mean PFOA)	Adiposity markers	NS (p>0.05), per 10 ng/mL PFOA increase
Wang et al. 2016 General population (n=117 boys and 106 girls examined at 2, 5, 8, and 11 years of age)	2.37 and 2.34 ng/mL (median maternal PFOA for boys and girls)	Growth during childhood	NS (p>0.05)
PFOS			
Alkhalawi et al. 2016 General population (n=156 mother-child pairs)	9.04 ng/mL (maternal geometric mean serum PFOS)	Body weight at 1, 4, 6, and 12 months of age	NS (p>0.05)
		Body length at 1, 4, 6, and 12 months of age	NS (p>0.05)
Andersen et al. 2010 General population (n=1,010 infants)	33.8 ng/mL (maternal median PFOS)	Body weight (age 5 months)	NS (p>0.05), boys NS (p>0.05), girls
		Body weight (age 12 months)	**Inverse association (p<0.05)*, boys** NS (p>0.05), girls
		BMI (age 5 months)	NS, boys NS (p>0.05), girls
		BMI (age 12 months)	**Inverse association (p<0.05)*, boys** NS (p>0.05), girls
		Height (age 5 and 12 months)	NS (p>0.05), boys NS (p>0.05), girls
Andersen et al. 2013 General population (n=811 children aged 7 years)	33.8 ng/mL (maternal median PFOS)	BMI	NS (p>0.05)
		Waist circumference	NS (p>0.05)
Braun et al. 2016a, 2016b General population (n=204 children)	13 ng/mL (maternal median PFOS)	Changes in BMI scores between 2 and 8 years of age	NS (p>0.23)
		Overweight/obesity risk	RR 1.08 (0.59–1.95), 3rd tertile

Table 2-7. Body Weight Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Cao et al. 2018 General population (n=337 infants)	1.43 ng/mL (mean cord serum PFOS)	Body weight at 19 months	NS (p=0.72)
		Length at 19 months	NS (p=0.91)
		Head circumference at 19 months	NS (p=0.63)
Halldorsson et al. 2012 General population (n=665 20-year-olds)	21.5 and 5.8 ng/mL (maternal median PFOS)	BMI	NS (p>0.56)
		Waist circumference	NS (p>0.56)
Høyer et al. 2015b General population (n=1,122 children aged 5–9 years; n=531 for Greenland cohort and n=491 for Ukraine cohort)	23.9–87.3 and 5.9–18.1 ng/mL (maternal 3rd tertile PFOS for Greenland and Ukraine cohorts)	Overweight Greenland cohort Ukraine cohort	 RR 0.84 (0.61–1.14), 3rd tertile RR 0.89 (0.57–1.37), 3rd tertile
		Waist-to-height ratio >0.5 Greenland cohort Ukraine cohort	 RR 1.22 (0.86–1.74), 3rd tertile RR 1.44 (0.62–3.31), 3rd tertile
Hartman et al. 2017 General population (n=359 girls aged 9 years)	19.7 ng/mL (maternal median PFOS)	Total body fatness	NS (p=0.12)
		Trunk fatness	**Inverse association (p=0.02)**
		BMI	**Inverse association (p=0.03)***
Karlsen et al. 2017 General population (n=444 children)	8.04 ng/mL (maternal geometric mean serum PFOS)	BMI score, 18 months	**Association (p<0.05)**
		BMI score, 5 years	NS (p>0.05)
		Risk of being overweight 18 months	**RR 1.29 (1.01–1.64)***
		Risk of being overweight 5 years	RR 1.01 (0.58–1.75)
Karlsen et al. 2017 General population (n=444 children aged 5 years)	4.68 ng/mL (child geometric mean serum PFOS)	BMI score	NS (p>0.05)
		Risk of being overweight	RR 0.68 (0.36–1.29)
Koshy et al. 2017 General population (WTCHR, n=180 children; n=222 children in comparison group)	3.72 and 2.78 ng/mL (median serum PFOS in WTCHR group and comparison group)	Risk of being overweight	OR 1.00 (0.90–1.07)

Table 2-7. Body Weight Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Liu et al. 2018a General population (n=621 adults in weight loss clinical trial)	24.5 ng/mL (median serum PFOS)	Weight loss	NS (p=0.27, trend).
		Weight regain Males Females	**Association (p=0.009, trend)*** NS (p=0.34, trend) **Association (p=0.001, trend)***
		Resting metabolic rate Weight loss period Weight regain period	 **Association (p<0.001, trend)*** **Association (p<0.001, trend)***
Maisonet et al. 2012 General population (n=447 girls)	19.6 ng/mL (median maternal PFOS)	Body weight at 20 months (adjusted for birth weight)	**Significant trend (p<0.0001) when adjusted for birth weight and height**
Manzano-Salgado et al. 2017b General population (n=1,230 children)	5.80 ng/mL (maternal geometric mean serum PFOS)	Weight gain until 6 months of age	β -0.02 (-0.11–0.07)
		BMI at 4 years of age	β 0.04 (-0.05–0.13)
		BMI at 7 years of age	β 0.03 (-0.08–0.14)
		Waist circumference at 4 years of age	β -0.03 (-0.13–0.07)
		Waist circumference at 7 years of age	β 0.00 (-0.09–0.09)
Mora et al. 2017 General population (n=1,006 at median age of 3.2 years and n=876 at median age of 7.7 years)	24.8 ng/mL (maternal median plasma PFOS in early childhood group)	BMI	**β 0.04 (0.05–0.12)*, boys and girls** β 0.02 (-0.11–0.15), boys only β 0.04 (-0.08–0.16), girls only
		Waist circumference	β 0.05 (-0.17–0.27)
		Risk of being overweight	RRR 1.07 (0.92–1.24)
		Risk of being obese	RRR 0.97 (0.76–1.23)
Mora et al. 2017 General population (n=1,006 at median age of 3.2 years and n=876 at median age of 7.7 years)	24.7 ng/mL (maternal median plasma PFOS in late childhood group)	BMI	β 0.16 (-0.04–0.36)
		Total fat mass index	β 0.11 (-0.03–0.25)
		Waist circumference	β 0.34 (-0.19–0.87),
		Risk of being overweight	RRR 1.15 (0.95–1.40)
		Risk of being obese	RRR 1.12 (0.99–1.47)

Table 2-7. Body Weight Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Timmermann et al. 2014 General population (n=499 8–10-year-old children)	41.5 ng/mL (mean PFOS)	Adiposity markers	NS (p>0.05), per 10 ng/mL PFOS increase
PFHxS			
Alkhalawi et al. 2016 General population (n=156 mother-child pairs)	0.62 ng/mL (maternal geometric mean serum PFHxS)	Body weight at 1, 4, 6, and 12 months of age	NS (p>0.05)
		Body weight (longitudinal analysis)	**β -5.270 (-9.591 to -0.950)***
		Body length at 1, 4, 6, and 12 months of age	NS (p>0.05)
		Body length (longitudinal analysis)	**β 4.516 (1.368–7.664)***
Braun et al. 2016a, 2016b General population (n=204 children)	1.4 ng/mL (maternal median PFHxS)	Changes in BMI scores between 2 and 8 years of age	NS (p>0.23)
		Overweight/obesity risk	RR 1.48 (0.75–2.96), 3rd tertile
Cao et al. 2018 General population (n=337 infants)	0.16 ng/mL (mean cord serum PFHxS)	Body weight at 19 months	NS (p=0.96)
		Length at 19 months	NS (p=0.31)
Hartman et al. 2017 General population (n=359 girls aged 9 years)	1.6 ng/mL (maternal median PFHxS)	Total body fatness	NS (p=0.47)
		Trunk fatness	NS (p=0.77)
		BMI	NS (p=0.37)
Karlsen et al. 2017 General population (n=444 children)	0.19 ng/mL (maternal geometric mean serum PFHxS)	BMI score, 18 months	NS (p>0.05)
		BMI score, 5 years	NS (p>0.05)
		Risk of being overweight 18 months	RR 1.12 (0.97–1.30)
		Risk of being overweight 5 years	RR 1.11 (0.77–1.59)

Table 2-7. Body Weight Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Karlsen et al. 2017 General population (n=444 children aged 5 years)	0.34 ng/mL (child geometric mean serum PFHxS)	BMI score	NS (p>0.05)
		Risk of being overweight	RR 0.73 (0.44–1.23)
Koshy et al. 2017 General population (WTCHR, n=180 children; n=222 children in comparison group)	0.67 and 0.53 ng/mL (median serum PFHxS in WTCHR group and comparison group)	Risk of being overweight	OR 1.04 (0.97–1.11)
Liu et al. 2018a General population (n=621 adults in weight loss clinical trial)	3.6 ng/mL (median serum PFHxS)	Weight loss	NS (p=0.45, trend).
		Weight regain Males Females	NS (p=0.49, trend**)** NS (p=0.17 trend) **Association (p=0.009, trend)***
		Resting metabolic rate Weight loss period Weight regain period	 **Association (p=0.04, trend)*** **Association (p=0.02, trend)***
Maisonet et al. 2012 General population (n=447 girls)	1.6 ng/mL (maternal median PFHxS)	Body weight at 20 months	NS (p=0.4375 for trend)
Manzano-Salgado et al. 2017b General population (n=1,230 children)	0.61 ng/mL (maternal geometric mean serum PFHxS)	Weight gain until 6 months of age	β -0.06 (-0.15–0.02)
		BMI at 4 years of age	β -0.02 (-0.10–0.07)
		BMI at 7 years of age	β -0.04 (-0.14–0.06)
		Waist circumference at 4 years of age	B -0.04 (0.14–0.15)
		Waist circumference at 7 years of age	NS β -0.04 (-0.12–0.04)

Table 2-7. Body Weight Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Mora et al. 2017 General population (n=1,006 at median age of 3.2 years and n=876 at median age of 7.7 years)	2.4 ng/mL (maternal median plasma PFHxS in early childhood group)	BMI	β 0.01 (-0.05–0.06)
		Waist circumference	β 0.03 (-0.10–0.16)
		Subscapular and triceps skinfold thickness	**β 0.16 (0.01–0.31)*, boys and girls** β 0.15 (-0.09–0.38), boys only β 0.18 (-0.03–0.38, girls only
		Risk of being overweight	RRR 1.03 (0.94–1.13)
		Risk of being obese	RRR 1.02 (0.89–1.17)
Mora et al. 2017 General population (n=1,006 at median age of 3.2 years and n=876 at median age of 7.7 years)	2.3 ng/mL (maternal median plasma PFHxS in late childhood group)	BMI	β 0.04 (-0.08–0.17)
		Total fat mass index	β 0.11 (-0.03–0.25)
		Waist circumference	β 0.11 (-0.22–0.43)
		Subscapular to triceps skinfold thickness ratio	β 0.02 (-0.02–0.06), boys and girls β -0.50 (-1.70–0.71), boys only **β 1.61 (0.58–2.65)*, girls only**
		Risk of being overweight	RRR 1.04 (0.92–1.17)
		Risk of being obese	RRR 1.07 (0.94–1.22)
PFNA			
Braun et al. 2016a, 2016b General population (n=204 children)	0.9 ng/mL (maternal median PFNA)	Changes in BMI scores between 2 and 8 years of age	NS (p>0.23)
		Overweight/obesity risk	RR 1.26 (0.64–2.48), 3rd tertile
Cao et al. 2018 General population (n=337 infants)	0.13 ng/mL (mean cord serum PFNA)	Body weight at 19 months	NS (p=0.88)
		Length at 19 months	NS (p=0.15)
		Head circumference at 19 months	NS (p=0.62)
Halldorsson et al. 2012 General population (n=665 20-year-olds)	0.3 ng/mL (maternal median PFNA)	BMI	NS (p>0.56)
		Waist circumference	NS (p>0.56)
Hartman et al. 2017 General population (n=359 girls aged 9 years)	0.5 ng/mL (maternal median PFNA)	Total body fatness	NS (p=0.26)
		Trunk fatness	NS (p=0.97)
		BMI	NS (p=0.68)

Table 2-7. Body Weight Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Karlsen et al. 2017 General population (n=444 children)	0.67 ng/mL (maternal geometric mean serum PFNA)	BMI score, 18 months	NS (p>0.05)
		BMI score, 5 years	NS (p>0.05)
		Risk of being overweight 18 months	RR 1.02 (0.79–1.31)
		Risk of being overweight 5 years	RR 1.15 (0.67–1.98)
Karlsen et al. 2017 General population (n=444 children aged 5 years)	1.12 ng/mL (child geometric mean serum PFNA)	BMI score	**Inverse association (p<0.05)***
		Risk of being overweight	RR 0.67 (0.45–1.00)
Koshy et al. 2017 General population (WTCHR, n=180 children; n=222 children in comparison group)	0.61 and 0.49 ng/mL (median serum PFNA in WTCHR group and comparison group)	Risk of being overweight	OR 1.01 (0.92–1.13)
Liu et al. 2018a General population (n=621 adults in weight loss clinical trial)	1.5 ng/mL (median serum PFNA)	Weight loss	NS (p=0.28, trend).
		Weight regain Males Females	**Association (p=0.01, trend)*** NS (p=0.48 trend) **Association (p=0.006, trend)***
		Resting metabolic rate Weight loss period Weight regain period	 **Association (p<0.001, trend)*** **Association (p=0.03, trend)***
Manzano-Salgado et al. 2017b General population (n=1,230 children)	0.61 ng/mL (maternal geometric mean serum PFNA)	Weight gain until 6 months of age	β 0.0 (-0.07–0.09)
		BMI at 4 years of age	β 0.05 (-0.03–0.13)
		BMI at 7 years of age	β 0.06 (-0.04–0.16)
		Waist circumference at 4 years of age	β 0.02 (-0.07–0.10)
		Waist circumference at 7 years of age	β -0.02 (-0.07–0.10)

Table 2-7. Body Weight Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Mora et al. 2017 General population (n=1,006 at median age of 3.2 years and n=876 at median age of 7.7 years)	0.6 ng/mL (maternal median plasma PFNA in early childhood group)	BMI	β 0.02 (-0.07–0.12)
		Waist circumference	β 0.01 (-0.23–0.22),
		Risk of being overweight	RRR 1.12 (0.96–1.30)
		Risk of being obese	RRR 0.97 (0.75–1.27)
Mora et al. 2017 General population (n=1,006 at median age of 3.2 years and n=876 at median age of 7.7 years)	0.6 ng/mL (maternal median plasma PFNA in late childhood group)	BMI	β 0.17 (-0.03–0.36)
		Total fat mass index	β 0.08 (-0.07–0.23)
		Waist circumference	β 0.31 (-0.19–0.82)
		Subscapular and triceps skinfold thickness	**β 0.62 (0.01–1.22)*, boys and girls** β 0.13 (0.74–1.01), boys only **β 1.01 (0.16–1.86)*, girls only**
		Subscapular to triceps skinfold thickness ratio	**β 1.78 (0.57–2.98)*, boys and girls** β 1.23 (-0.58–3.03), boys only **β 2.17 (0.52–3.83)*, girls only**
		Risk of being overweight	RRR 1.06 (0.85–1.32)
		Risk of being obese	RRR 1.21 (0.99–1.47)
Wang et al. 2016 General population (n=117 boys and 106 girls examined at 2, 5, 8, and 11 years of age)	1.55 and 1.58 ng/mL (median maternal PFNA for boys and girls)	Growth during childhood	NS (p>0.05)
PFDA			
Cao et al. 2018 General population (n=337 infants)	0.12 ng/mL (mean cord serum PFDA)	Body weight at 19 months	NS (p=0.57)
		Length at 19 months	NS (p=0.18)
		Head circumference at 19 months	NS (p=0.94)
Karlsen et al. 2017 General population (n=444 children)	0.26 ng/mL (maternal geometric mean serum PFDA)	BMI score, 18 months	NS (p>0.05)
		BMI score, 5 years	NS (p>0.05)
		Risk of being overweight 18 months	RR 1.14 (0.91–1.43)
		Risk of being overweight 5 years	RR 1.02 (0.61–1.70)

Table 2-7. Body Weight Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Karlsen et al. 2017 General population (n=444 children aged 5 years)	0.33 ng/mL (child geometric mean serum PFDA)	BMI score	**Inverse association (p<0.05)***
		Risk of being overweight	**RR 0.64 (0.46–0.90)***
Koshy et al. 2017 General population (WTCHR, n=180 children; n=222 children in comparison group)	0.14 and 0.11 ng/mL (median serum PFDA in WTCHR group and comparison group)	Risk of being overweight	OR 0.98 (0.93–1.03)
Liu et al. 2018a General population (n=621 adults in weight loss clinical trial)	0.37 ng/mL (median serum PFDA)	Weight loss	NS (p=0.45, trend).
		Weight regain Males Females	NS (p=0.16, trend) NS (p=0.75 trend) **Association (p=0.03, trend)***
		Resting metabolic rate Weight loss period Weight regain period	 **Association (p=0.01, trend)*** **Association (p=0.05, trend)***
Wang et al. 2016 General population (n=117 boys and 106 girls examined at 2, 5, 8, and 11 years of age)	0.46 and 0.43 ng/mL (median maternal PFDA for boys and girls)	Growth during childhood Weight Height	 **Inverse association (p<0.05)*, girls** **Inverse association (p<0.05)*, girls**
PFUnA			
Cao et al. 2018 General population (n=337 infants)	0.06–0.11 ng/mL (2nd quartile cord serum PFUnA)	Body weight at 19 months	NS (p=0.88)
		Length at 19 months	**β 1.19 (-0.68–3.07, p<0.05)*, 2nd quartile**
		Head circumference at 19 months	NS (p=0.60)
Koshy et al. 2017 General population (World Trade Center Health Registry, n=180 children; n=222 children in comparison group)	0.12 and 0.04 ng/mL (median serum PFUnA in WTCH group and comparison group)	Risk of being overweight	**OR 0.95 (0.91–0.99)***

Table 2-7. Body Weight Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Wang et al. 2016 General population (n=117 boys and 106 girls examined at 2, 5, 8, and 11 years of age)	3.52 and 3.31 ng/mL (median maternal PFUnA for boys and girls)	Growth during childhood Weight Height	 **Inverse association (p<0.05)*, girls** **Inverse association (p<0.05)*, girls**
PFDoDA			
Cao et al. 2018 General population (n=337 infants)	0.04 ng/mL (mean cord serum PFDoDA)	Body weight at 19 months	NS (p=0.74)
		Head circumference at 19 months	NS (p=0.97)
Wang et al. 2016 General population (n=117 boys and 106 girls examined at 2, 5, 8, and 11 years of age)	0.37 and 0.37 ng/mL (median maternal PFDoDA for boys and girls)	Growth during childhood Weight Height	 **Inverse association (p<0.05)*, girls** **Inverse association (p<0.05)*, girls**
FOSA			
Halldorsson et al. 2012 General population (n=665 20-year-olds)	1.1 ng/mL (maternal median FOSA)	BMI	NS (p>0.56)
		Waist circumference	NS (p>0.56)

[a]See the *Supporting Document for Epidemiological Studies for Perfluoroalkyls,* Table 1 for more detailed descriptions of studies.
[b]Participants in occupational exposure may have also lived near the site and received residential exposure; community exposure studies involved subjects living near PFOA facilities with known exposure to high levels of PFOA.
[c]Asterisk indicates association with perfluoroalkyl; unless otherwise specified, values in parenthesis are 95% confidence intervals.

BMI = body mass index; FOSA = perfluorooctane sulfonamide; HR = hazard ratio; NR = not reported; NS = not significant; OR = odds ratio; PFDA = perfluorodecanoic acid; PFDoDA = perfluorododecanoic acid; PFHxS = perfluorohexane sulfonic acid; PFNA = perfluorononanoic acid; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid; PFUnA = perfluoroundecanoic acid; RR = relative risk; WTCHR = World Trade Center Health Registry

1

PFOA

Epidemiological Studies. Mixed results were found in studies of monitoring infant growth from 1 to 12 months of age. Andersen et al. (2010) found an inverse association between maternal serum PFOA and body weight and BMI in male infants at 5 and 12 months of age; no associations were found in girls. Other studies of infants less than 19 months of age did not find associations between maternal serum PFOA (Alkhalawi et al. 2016; Manzano-Salgado et al. 2017b) or cord blood PFOA (Cao et al. 2018; de Cock et al. 2014) levels and weight, length, head circumference, or BMI. One study of children (Braun et al. 2016a) found an association between changes in BMI scores between ages 2 and 8 years and maternal PFOA levels; however, there was no increase in the risk of being overweight or obese. Another study of young children (median age 3.2 years) found an association between maternal PFOA and waist circumference (Mora et al. 2017); when the children were segregated by sex, the association was only found in boys. This study did not find associations between maternal PFOA and waist circumference when the children were older (median age 7.7 years). Other studies in children (2–11 years of age) found no associations between maternal PFOA or cord blood PFOA and growth during childhood (Wang et al. 2016), risk of being overweight or obese (Andersen et al. 2013; Braun et al. 2016a; Høyer et al. 2015b; Mora et al. 2017), waist circumference (Manzano-Salgado et al. 2017b), BMI (Hartman et al. 2017; Karlsen et al. 2017; Manzano-Salgado et al. 2017b; Mora et al. 2017), body fatness (Hartman et al. 2017), or risk of having a waist-to-height ratio of >0.5 (Høyer et al. 2015b). In a study of children aged 8–10 years, no associations were found between plasma PFOA levels and markers of adiposity (BMI, skinfold thickness, waist circumference, adiponectin levels, and leptin levels) (Timmermann et al. 2014). Similarly, in a study of children in the World Trade Center Health Registry, no association was found between serum PFOA and risk of being overweight (Koshy et al. 2017). In contrast, a study of 5-year-old children found an inverse association between the child's serum PFOA levels and BMI score, but no association with the risk of being overweight (Karlsen et al. 2017). Overall, the available epidemiological data do not suggest a connection between serum PFOA levels and body weight or risk of being overweight/obese in children.

Two studies in adults have not found associations between PFOA and body weight gain. A general population study of 20-year-old females found associations between maternal PFOA levels and BMI and waist circumferences, and increases in the risk of being overweight and having a high waist circumference (Halldorsson et al. 2012); these associations were not observed in males. No increases in the risk of being overweight or obese were observed in male or female C8 participants (20–40 years of age) when estimated early life PFOA exposure was used as the exposure metric (Barry et al. 2014). In a

study of participants in a weight loss study, no association between weight loss and PFOA levels was found; however, PFOA was associated with weight gain in females, but not males (Liu et al. 2018a). PFOA was also associated with a lower increase in resting metabolic rate in all participants during the weight regain period of the study.

Laboratory Animal Studies. Male rats that survived a 4-hour inhalation exposure to 380 mg/m^3 APFO dusts lost weight for 1–2 days after exposure, but resumed normal weight gain thereafter (Kennedy et al. 1986). Male rats exposed via inhalation intermittently to 84 mg/m^3 APFO dusts for 2 weeks lost approximately 7% of their body weight by day 5 of exposure (250 g at start of study, 237 g on day 5) (Kennedy et al. 1986), but recovered by day 16 after exposure ceased. Nose-only exposure of male CD rats to 590 mg/m^3 ammonium perfluorononanoate dusts for 4 hours resulted in 18 and 36% reductions in body weight 5 and 12 days after exposure, respectively (Kinney et al. 1989). Inhalation exposure to 67 mg/m^3 had no significant effect on body weight. In a developmental study, inhalation exposure of pregnant rats to 25 mg/m^3 APFO dusts during GDs 6–15 induced a 37% reduction in maternal body weight gain relative to controls during the exposure period (Staples et al. 1984); in a pair-fed group, the reduction of weight gain during the same period was 61% relative to *ad libitum* controls.

Reductions in body weight or body weight gain are typical, although not particularly sensitive, responses of rodents to oral exposure to perfluoroalkyls. In many cases, this effect is not associated with reduced food intake, and in some cases, exposed animals have shown an increase in relative food consumption (grams of food/grams of body weight) relative to controls. For example, administration of 50 mg/kg/day APFO for 7 days resulted in 17% weight loss; a similar decrease was observed in a pair-fed group (Pastoor et al. 1987). In mice, doses of approximately 25–30 mg/kg/day PFOA in the food for 7 days reduced terminal body weight by >10% relative to controls without a significant reduction in food intake (Xie et al. 2003; Yang et al. 2000, 2002a, 2002b). However, administration of the same dose to PPARα-null mice did not cause a reduction in weight gain, suggesting that the effect on body weight is a specific effect of peroxisome proliferators possibly due to increased fat utilization (Yang et al. 2002b). In general, body weight recovered once treatment ceased.

Intermediate-duration oral studies in rats have also reported reduced body weight gain with doses ≥10 mg/kg/day APFO (Butenhoff et al. 2004b; Griffith and Long 1980). In the former study, mean absolute food consumption was decreased, but mean relative food consumption was increased. In a 2-year bioassay, body weight gain in rats dosed with 15 mg/kg/day PFOA was reduced >10% relative to controls at the 1-year mark and at termination (Biegel et al. 2001). Similar observations have been made

in mice dosed with approximately ≥18 mg/kg/day APFO for 28 days (Griffith and Long 1980), or 10 mg/kg 5 days/week for 4 weeks (Yang et al. 2009), and in pregnant mice dosed with ≥10 mg/kg/day APFO during GDs 1–17 (Lau et al. 2006). A study comparing wild-type mice and PPARα knockout mice (DeWitt et al. 2016) found a decrease in body weight gain in the wild-type mice, but not in the knockout mice. A 90-day and a 26-week study in monkeys also reported significant reductions in body weight gain or weight loss associated with decreased food consumption at dose levels in the range of 20–30 mg/kg/day APFO (Butenhoff et al. 2002; Griffith and Long 1980), but a 4-week study in monkeys dosed with 20 mg/kg/day PFOA did not (Thomford 2001).

Transient weight loss was reported in rats applied 3,000 mg/kg APFO to the shaven skin for 24 hours (Kennedy 1985). In the 2-week study, rats in the 200 and 2,000 mg/kg/day groups lost weight during the treatment period (14 and 24%, respectively, on test day 10), but body weights were comparable to controls after 42 days of recovery. No changes in body weight were reported in mice applied up to 50 mg/kg/day PFOA daily for 4 days on the dorsal surface of the ears (Fairley et al. 2007).

PFOS

Epidemiological Studies. General population studies have evaluated body weight, height, and BMI in infants, children, and adults to assess whether there were associations between growth and maternal serum PFOS levels. Andersen et al. (2010) found that maternal PFOS levels were inversely related to body weight and BMI in 12-month-old male infants; no associations were found in females at 12 months of age or in males and females at 5 months of age. The magnitude of the effect on body weight in the boys was small, 9 g per 1 ng/mL increase in maternal serum PFOS level. Other studies have not found associations between maternal PFOS or cord blood PFOS and body weight, length, or head circumference in infants <2 years of age (Alkhalawi et al. 2016; Cao et al. 2018; Manzano-Salgado et al. 2017b). Hartman et al. (2017) also found an inverse association between maternal serum PFOS and trunk body fatness in 9-year-old girls, but no associations with total body fatness or BMI. Karlsen et al. (2017) found associations between maternal PFOS levels and BMI and risk of being overweight at 18 months of age, but not at 5 years of age. Maisonet et al. (2012) found that at 20 months of age, girls whose mothers had serum PFOS levels in the 3rd tertile weighed 438 g more than those in the first tertile. Studies in children (Andersen et al. 2013; Braun et al. 2016a; Høyer et al. 2015b; Koshy et al. 2017; Manzano-Salgado et al. 2017b; Mora et al. 2017) or young adults (Halldorsson et al. 2012) did not find associations between maternal PFOS levels and BMI, waist circumference, and/or risk of being overweight. No associations between plasma PFOS and markers of adiposity (BMI, skinfold thickness, waist circumference,

adiponectin levels, and leptin levels) were found in a study of children aged 8–10 years (Timmermann et al. 2014). Similarly, a study of 5-year-old children found no association between child serum PFOS levels and BMI score or risk of being overweight (Karlsen et al. 2017). Overall, the epidemiological studies do not suggest a connection between serum PFOS and body weight or the risk of being overweight/obese.

In a study of weight loss programs, PFOS did not influence weight loss, but was associated with greater weight regain in women (Liu et al. 2018a). PFOS was also associated with greater declines in resting metabolic rate in all participants during the weight loss period of the study and lower increases in resting metabolic rate during the weight regain period.

Laboratory Animal Studies. Dietary treatment of rats with 15 mg/kg/day PFOS (only dose level tested) for 7 days did not significantly alter body weight (Haughom and Spydevold 1992). Oral treatment of pregnant rats with 25 mg/kg/day PFOS on GDs 2–5 or 6–9 resulted in maternal weight loss during treatment, whereas treatment on GDs 10–13, 14–17, or 17–20 resulted in significant reductions in maternal weight gain (Grasty et al. 2003). In pregnant mice, oral dosing with up to 6 mg/kg/day PFOS on GDs 6–18 or 12–18 did not significantly affect body weight (Fuentes et al. 2006, 2007b). Decreases in maternal body weight were observed in rats administered 20 mg/kg/day on GDs 12–18 (Li et al. 2016). Pregnant rabbits appeared to be more sensitive as oral doses of 1 mg/kg/day on GDs 6–20 caused a 21% reduction in weight gain during treatment without altering food consumption (Case et al. 2001).

Alterations in body weight have also been observed following intermediate- or chronic-duration exposure. Reductions in body weight gain of >10% have been reported in intermediate-duration studies in rats dosed with ≥2 mg/kg/day PFOS associated with reductions in mean absolute and relative food consumption (Luebker et al. 2005a, 2005b). In a developmental toxicity study, treatment of pregnant rats with ≥2 mg/kg/day PFOS on GDs 2–20 resulted in significant reductions in body weight gain, which were associated with significant reductions in mean absolute food and water consumption (Thibodeaux et al. 2003). In a 4-week study, treatment of Cynomolgus monkeys with up to 2 mg/kg/day, administered via a capsule, did not affect body weight gain (Thomford 2002a). In a 26-week study in Cynomolgus monkeys, the highest dose of PFOS tested, 0.75 mg/kg/day, produced a 13.5% reduction in final body weight, at which time the mean concentration of PFOS in serum was 172 µg/mL (Seacat et al. 2002). In a 2-year dietary study in rats, final mean body weight of females that received doses of approximately 1.04 mg/kg/day PFOS was 14% lower than controls; this could have been due, in part, to a tendency of decreased food consumption during weeks 28 through 104 of the study (Butenhoff et al. 2012b;

Thomford 2002b). No significant effect (<10% difference with controls) was seen in females dosed with ≤0.25 mg/kg/day PFOS.

PFHxS

Epidemiological Studies. Nine studies have evaluated the influence of *in utero* PFHxS exposure on childhood growth and found no associations between maternal PFHxS levels and body weight in infants <2 years of age (Alkhalawi et al. 2016; Cao et al. 2018; Maisonet et al. 2012; Manzano-Salgado et al. 2017b), body fatness or BMI at 9 years of age (Hartman et al. 2017), BMI or waist circumference at 3 or 7 years of age (Mora et al. 2017), changes in BMI scores between 2 and 8 years of age (Braun et al. 2016a), BMI at 18 months or 5 years of age (Karlsen et al. 2017), BMI at 4 or 7 years of age (Manzano-Salgado et al. 2017b), or the risk of childhood overweight/obesity (Braun et al. 2016a; Karlsen et al. 2017; Mora et al. 2017). Similarly, no associations were found between serum PFHxS levels in 5-year-old children and their BMI score or risk of being overweight (Karlsen et al. 2017) or between serum PFHxS and risk of being overweight in children in the World Trade Center Healthy Registry (Koshy et al. 2017). Alkhalawi et al. (2016) found no associations between maternal PFHxS levels and infant body weight or length at 1, 4, 6, or 12 months of age; however, longitudinal analysis of growth during this period showed an inverse association for body weight and an association for length.

In a clinical trial of weight loss programs, PFHxS was not associated with weight loss during the first 6 months of the study, but was associated with weight regain in females during the last 18 months of the study (Liu et al. 2018a). PFHxS was also associated with greater declines in resting metabolic rate in all participants during the weight loss period and lower increases in resting metabolic rate during the weight regain period.

Laboratory Animal Studies. Administration of PFHxS by gavage for 40–60 days did not significantly affect body weight in rats at ≤10 mg/kg/day PFHxS (Butenhoff et al. 2009a) or mice at ≤3 mg/kg/day (Chang et al. 2018); the mean terminal body weights were within 10% of the body weight of the control group (Butenhoff et al. 2009a).

PFNA

Epidemiological Studies. Several studies have examined the influence of maternal serum PFNA levels on childhood growth. These studies did not find associations between maternal PFNA levels and growth

during childhood (Cao et al. 2018; Wang et al. 2016), BMI (Braun et al. 2016a; Halldorsson et al. 2012; Hartman et al. 2017; Karlsen et al. 2017; Manzano-Salgado et al. 2017b; Mora et al. 2017), body fatness (Hartman et al. 2017), or overweight/obesity risk (Braun et al. 2016a; Karlsen et al. 2017; Mora et al. 2017). However, when the child's serum PFNA levels at age 5 years were used as the exposure biometric, an inverse association was found for BMI, but not for the risk of being overweight (Karlsen et al. 2017). Koshy et al. (2017) found no associations between serum PFNA and the risk of being overweight in children enrolled in the World Trade Center Health Registry.

PFNA was associated with greater weight regains in a study of participants in a 2-year weight loss clinical trial, but was not associated with weight loss during the first 6 months of the study (Liu et al. 2018b). PFNA also affected resting metabolic rate in all participants; it was associated with a greater decline during the weight loss period of the study and a lower increase during the weight regain period.

Laboratory Animal Studies. Decreases in body weight gain have been observed in rats administered ≥3 mg/kg/day for 14 days (Fang et al. 2009, 2010; Hadrup et al. 2016) and in mice administered 5 mg/kg/day for 14 days (Wang et al. 2015a). The NOAEL for body weight effects was 1 mg/kg/day for both species. In intermediate-duration developmental toxicity studies, decreases in body weight were observed at 5 mg/kg/day in rats (Rogers et al. 2014) and weight loss was observed in mice at 10 mg/kg/day (Das et al. 2015). No alterations in maternal weight gain were observed in mice at 2.0 mg/kg/day (Wolf et al. 2010).

PFDA

Epidemiological Studies. Four studies examined the effect of PFDA levels on childhood growth. Cao et al. (2018) did not find associations between cord blood PFDA and body weight, length, or head circumference in 19-month-old infants. Wang et al. (2016) reported decreases in weight and height in girls associated with increasing maternal serum PFDA levels. Inverse associations between serum PFDA levels in 5-year-old children and BMI and the risk of being overweight were reported by Karlsen et al. (2017). When using maternal serum PFDA levels (measured 2 weeks after childbirth) as the biomarker of exposure, no associations were found with BMI or the risk of being overweight in children aged 18 months or 5 years (Karlsen et al. 2017). In a study of children in the World Trade Center Health Registry, no association between serum PFDA and risk of being overweight was found (Koshy et al. 2017).

In a study of adult participants in a 2-year weight loss clinical trial, PFDA was not associated with weight loss but was associated with weight regain in females during the last 18 months of the study (Liu et al. 2018a). PFDA also was associated with greater declines in resting metabolic rate during the weight loss period and lower increases in resting metabolic rate during the weight regain period of the study.

Laboratory Animal Studies. Ten days following administration of a single gavage dose of 50 mg/kg, weight loss was observed in rats (Kawabata et al. 2017). In a 1-week study, exposure to 9.5 mg/kg/day PFDA in the diet resulted in a 32% weight loss in rats (Kawashima et al. 1995); the NOAEL was 4.7 mg/kg/day. Rats administered 1 mg/kg/day PFDA for 28 days exhibited a 21% decrease in body weight gain (Frawley et al. 2018).

Body weight of female C57BL/6N mice administered a single gavage dose of 80 mg/kg PFDA was reduced 12% relative to controls 30 days post dosing (Harris et al. 1989); no significant effect was seen at 40 mg/kg PFDA. In a developmental study, pregnant mice dosed with 6.4 mg/kg/day PFDA on GDs 6–15 gained 92% less weight (adjusted for the weight of the gravid uterus) on GDs 6–18 than controls; mice dosed with 12.8 mg/kg/day lost weight (Harris and Birnbaum 1989). Weight loss was also observed in C57BL/6N mice exposed to 78 mg/kg/day PFDA in the diet for 10 days (Permadi et al. 1992, 1993).

PFUnA

Epidemiological Studies. Cao et al. (2018) found an association between cord blood PFUnA levels and length at 19 months of age, but found no associations for body weight or head circumference. Wang et al. (2016) found an inverse association between maternal serum PFUnA levels and weight and height in girls. Koshy et al. (2017) also found an inverse association between the serum PFUnA levels and the risk of being overweight in children enrolled in the World Trade Center Health Registry.

Laboratory Animal Studies. Decreases in body weight gain (10% in males and 23% in females) were observed in rats exposed to 1.0 mg/kg/day in a 41–46-day developmental toxicity study (Takahashi et al. 2014).

PFBS

Laboratory Animal Studies. No significant alterations in body weight gain were observed in Sprague-Dawley rats administered ≤900 mg/kg/day PFBS via gavage for 28 days (3M 2001) or in Sprague-

Dawley rats administered ≤1,000 mg/kg/day PFBS via gavage for at least 70 days (Lieder et al. 2009b). Two studies did report decreases in maternal body weight gain in rats administered 1,000 or 2,000 mg/kg/day (York 2002, 2003a).

PFBA

Laboratory Animal Studies. Alterations in body weight do not appear to be a sensitive outcome of PFBA exposure in rats or mice. No alterations in body weight gain were observed in Sprague-Dawley rats administered 184 mg/kg/day PFBA via gavage for 5 days (3M 2007a), C57BL/6 mice exposed to 78 mg/kg/day PFBA in the diet for 10 days (Permadi et al. 1992, 1993), Sprague-Dawley rats administered 150 mg/kg/day PFBA via gavage for 28 days (Butenhoff et al. 2012a; van Otterdijk 2007a), or Sprague-Dawley rats administered 30 mg/kg/day PFBA via gavage for 90 days (Butenhoff et al. 2012a; van Otterdijk 2007b).

PFDoDA

Epidemiological Studies. Cao et al. (2018) found no association between cord blood PFDoDA and body weight or head circumference at 19 months of age. In contrast, Wang et al. (2016) found an inverse association between maternal serum PFDoDA levels and growth (weight and height) in girls.

Laboratory Animal Studies. Dosing of Sprague-Dawley rats with 5 mg/kg/day PFDoDA by gavage for 14 days resulted in a 25% reduction in final body weight relative to a control group or 7% loss of body weight compared with the starting body weight (Shi et al. 2007). Decreases in body weight gain (measured 10 days postexposure) were also observed in rats administered a single gavage dose of 50 mg/kg PFDoDA (Kawabata et al. 2017). Gavage administration of 2.5 mg/kg/day for 42 days resulted in approximately 30% decreases in male rats; the decreases in body weight gain persisted during a 14-day recovery period (Kato et al. 2015). An approximately 20% decrease in body weight gain was also observed in pregnant and nonpregnant females similarly exposed to 2.5 mg/kg/day (Kato et al. 2015). The decreases in body weight gain were accompanied by decreases in food intake in males and females. In a longer duration study (110 days), no alterations in body weight gain were observed in rats administered 0.5 mg/kg/day (Shi et al. 2009a).

PFHxA

Laboratory Animal Studies. Gavage administration of up to 315 mg/kg/day did not result in alterations in body weight gain in rats exposed for 32–44 days (Kirkpatrick 2005), 90 days (Chengelis et al. 2009b), 92–93 days (Loveless et al. 2009), or 2 years (Klaunig et al. 2015). A 19% decrease in body weight gain was observed in rats administered 500 mg/kg/day NaPFHx for 92–93 days (Loveless et al. 2009) and a 19% decrease in maternal body weight gain was observed in rats administered 500 mg/kg/day on GDs 1–20 (Loveless et al. 2009). In contrast to these findings, a 110–126-day study found a 12% decrease in male rats administered 100 mg/kg/day NaPFHx (Loveless et al. 2009).

FOSA

Epidemiological Studies. Halldorsson et al. (2012) did not find associations between maternal serum FOSA levels and BMI or waist circumference in 20-year-olds.

Laboratory Animal Studies. No alterations in body weight were observed in Sprague-Dawley rats following a single gavage dose of 5 mg/kg FOSA in 2% Tween 80 vehicle (Seacat and Luebker 2000).

2.4 RESPIRATORY

Overview. A small number of epidemiological studies have examined the potential of PFOA to damage the respiratory tract; detailed descriptions of these studies are presented in Table 2 in the *Supporting Document for Epidemiological Studies for Perfluoroalkyls*. Epidemiological studies examining respiratory endpoints were not identified for the other perfluoroalkyls. These studies were primarily conducted in PFOA workers or in residents of nearby communities. The possible associations between perfluoroalkyl exposure and asthma are discussed along with other immune effects in Section 2.14. Studies in laboratory animals have examined the potential for perfluoroalkyls to induce histological lesions in the lungs following inhalation (see Tables 2-1 and 2-2) or oral exposure (see Tables 2-3, 2-4, and 2-5). No laboratory animal studies examining potential respiratory tract effects were identified for PFUnA, PFHpA, PFDoDA, or FOSA.

Epidemiological studies examining respiratory effects are only available for PFOA. No alterations in lung function were observed in workers at a PFOA facility but increases in respiratory illnesses were observed in residents living near the PFOA facility.

Inhalation exposure to PFOA, PFOS, or PFNA dusts have resulted in nasal discharge, rales, and/or labored breathing in laboratory animals. Oral exposure studies in laboratory animals have not found consistent evidence of histological alterations for PFOA, PFOS, PFHxS, PFBS, or PFBA. An oral study with PFHxA reported nasal lesions in rats, however, a second study did not find these effects at higher doses.

PFOA

Epidemiological Studies. There are limited data on the potential of PFOA to damage the respiratory tract. Pulmonary function tests and chest roentgenograms conducted on workers potentially exposed to PFOA at the Washington Works fluoropolymers production facility were within normal limits (Sakr et al. 2007b); the serum PFOA levels ranged from 5 to 9,550 ng/mL. Another study of workers at this facility did not find an association between estimated cumulative serum PFOA levels and the risk of chronic obstructive pulmonary disease (Steenland et al. 2015). In contrast, a study of residents living near this facility found an increase in the risk of chronic bronchitis (standard prevalence ratio [SPR] of 3.60, 95% confidence interval [CI] 2.92–4.44) and shortness of breath (SPR 2.05, 95% CI 1.70–2.46) (Anderson-Mahoney et al. 2008); it is noted that results were based on health surveys, and some of the subjects also worked at the facility. Summaries of these studies are presented in the *Supporting Document for Epidemiological Studies for Perfluoroalkyls*, Table 2.

Laboratory Animal Studies. Inhalation exposure of male and female rats to 18,600 mg/m^3 APFO dusts for 1 hour induced a red nasal discharge and dry rales (Griffith and Long 1980). Necropsy conducted 14 days after exposure showed bilateral mottling of the lungs in 8 out of 10 rats. Head-only exposure for 4 hours to 380 mg/m^3 APFO dusts, a concentration that was lethal to some rats, produced pulmonary edema, which disappeared within 1 week of exposure (Kennedy et al. 1986). Examination of the lungs and trachea from rats exposed head-only to up to 84 mg/m^3 APFO dusts 6 hours/day, 5 days/week for 2 weeks showed no significant gross or microscopic alterations (Kennedy et al. 1986). Male CD rats exposed nose-only to ≥590 mg/m^3 ammonium perfluorononanoate dusts for 4 hours exhibited lung noise and labored breathing during exposure and throughout a 12-day recovery period (Kinney et al. 1989).

Oral dosing of male and female CD rats with ≤110 mg/kg/day APFO did not induce gross or microscopic changes in the lungs (Griffith and Long 1980; Perkins et al. 2004). Dosing for 2 years with 15 mg/kg/day APFO increased the incidence of lung hemorrhage in males (3M 1983; Butenhoff et al. 2012c). The

incidences were 10/50, 14/50, and 22/50 for groups receiving doses of 0, 1.5, and 15 mg/kg/day, respectively. Pair-wise comparison between controls and high-dose groups revealed a statistically significant difference ($p<0.05$). The investigators suggested that pulmonary lesions were not related to PFOA based on lower incidence of interstitial pneumonia in the 15 mg/kg/day males. In a study in monkeys administered up to 20 mg/kg/day APFO, administered via a capsule, for 26 weeks, no signs of respiratory problems were observed during the study and no gross or microscopic alterations in the lungs and trachea were observed at termination (Butenhoff et al. 2002).

No gross or microscopic alterations were found in the lung and trachea from male CD rats following application of up to 2,000 mg/kg/day APFO as an aqueous paste to an area of the shaven back (approximately 15% of the total body surface) 6 hours/day, 5 days/week for 2 weeks (Kennedy 1985).

PFOS

Laboratory Animal Studies. Unpublished data summarized by OECD (2002) indicate that inhalation exposure of rats to concentrations of PFOS dust between 1,890 and 45,970 mg/m^3 for 1 hour induced dry rales and other breathing disturbances.

Dosing of Cynomolgus monkeys with up to 2 mg/kg/day PFOS, administered in a capsule, for 4 weeks had no effect on the gross or microscopic morphology of the lungs (Thomford 2002a). Administration of doses of up to 0.75 mg/kg/day of PFOS (potassium salt) administered via a capsule to Cynomolgus monkeys for 26 weeks did not produce any gross or microscopic alterations in the lungs or the trachea (Seacat et al. 2002). Dosing rats with up to 1.04 mg PFOS/kg/day in the diet for 104 weeks did not induce significant gross or microscopic alterations in the lungs or trachea (Butenhoff et al. 2012b; Thomford 2002b).

PFHxS

Laboratory Animal Studies. Examination of the respiratory tract of rats administered ≤10 mg/kg/day PFHxS or mice administered ≤3 mg/kg/day by gavage in a reproductive study (40–60 days of dosing) showed no treatment-related effects (Butenhoff et al. 2009a; Chang et al. 2018).

PFNA

Laboratory Animal Studies. Labored breathing during and after a 4-hour nose-only exposure to 590 mg/m^3 PFNA dust was reported in rats (Kinney et al. 1989).

PFDA

Laboratory Animal Studies. No histological alterations were observed in the respiratory tract of rats administered 0.5 mg/kg/day for 28 days or mice administered 5 mg/kg once a week for 4 weeks (Frawley et al. 2018).

PFBS

Laboratory Animal Studies. Administration of PFBS at gavage doses of ≤900 mg/kg/day for 28 days (3M 2001) or 600 mg/kg/day for 90 days (Lieder et al. 2009a) had no significant effect on the gross or microscopic morphology of the lungs or trachea in rats; no increases in nasal lesions were observed in the 90-day study (Lieder et al. 2009a).

PFBA

Laboratory Animal Studies. Administration of PFBA to rats by gavage in doses ≤184 mg/kg/day for 5 days (3M 2007a), ≤150 mg/kg/day for 28 days (Butenhoff et al. 2012a; van Otterdijk 2007a), or ≤30 mg/kg/day for 90 days (Butenhoff et al. 2012a; van Otterdijk 2007b) did not cause morphological alterations in the respiratory tract.

PFHxA

Laboratory Animal Studies. Degeneration/atrophy of the nasal olfactory epithelium was observed in rats administered via gavage 100 mg/kg/day NaPFHx for 92–93 days (Loveless et al. 2009); at 500 mg/kg/day, respiratory metaplasia was observed in the nasal cavity. A second study did not report histological alterations in the nasal cavity of rats administered up to 200 mg/kg/day NaPFHx for 90 days (Chengelis et al. 2009b).

2.5 CARDIOVASCULAR

Overview. Epidemiological and laboratory animal studies have evaluated the toxicity of perfluoroalkyls to the cardiovascular system. The epidemiological studies evaluated several cardiovascular outcomes including ischemic heart disease, cerebrovascular disease, stroke, cardiovascular disease, myocardial infarction, hypertension, and pregnancy-induced hypertension. The results of these studies are summarized in Table 2-8, with more detailed descriptions presented in the *Supporting Document for Epidemiological Studies for Perfluoroalkyls*, Table 3. The available occupational, community, and general population studies have not consistently found increases in the risk of heart disease or stroke that were associated with serum PFOA levels. Considerably less epidemiological data are available for other perfluoroalkyls; general population studies for PFOS, PFHxS, PFNA, PFDA, PFHpA, and PFDoDA have not consistently found increases in the risk of cardiovascular disease, although single studies for PFUnA, PFBS, PFHxA, and FOSA have found associations. Most of the available epidemiological studies did not find an association between serum PFOA and hypertension. A small number of studies (three or less for each compound) have examined potential associations with hypertension for other perfluoroalkyls. These studies found associations (PFBA), no associations (PFHxS, PFDA, PFUnA, PFHpA, PFBS, PFDoDA), or mixed results (PFOS, PFNA).

Several studies have evaluated the possible associations between serum perfluoroalkyls and pregnancy-induced hypertension and pre-eclampsia. Pregnancy-induced hypertension, also referred to as gestational hypertension, is the onset of hypertension after the 20th week of pregnancy. Pre-eclampsia is pregnancy-induced hypertension accompanied by signs of damage to another organ system, such as the kidney or liver; elevated levels of protein in the urine are often present. While the two diseases are distinct, they can be inaccurately reported in studies that relied on self-reporting or use of birth certificates (birth certificates often only have an option for pregnancy-induced hypertension; thus, pre-eclampsia may be reported as pregnancy-induced hypertension). Due to possibility of misreporting, ATSDR has opted to group these two outcomes together. Although mixed results were found in studies of highly exposed community residents, the strongest methodological study (Darrow et al. 2013) found an increased risk of pregnancy-induced hypertension that was associated with serum PFOA levels. Increases in the risk of pregnancy-induced hypertension associated with serum PFOS levels were also found in two community studies. General population studies have not found associations between serum PFHxS or PFDA and pre-eclampsia; one study on PFUnA found an inverse association.

Table 2-8. Summary of Cardiovascular Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFOA			
Leonard 2006 Occupational (n=6,027)	5–9,550 ng/mL (PFOA range)	Heart disease deaths	SMR 110 (98–123)
		Cerebrovascular disease deaths	SMR 86 (60–120)
		Ischemic heart disease deaths	SMR 109 (96–124)
Lundin et al. 2009 Occupational (n=3,993)	2,600–5,200 ng/mL (range of definite exposure group)	Heart disease deaths	SMR 0.7 (0.5–1.3)
		Cerebrovascular disease deaths	SMR 1.6 (0.5–3.7)
		Ischemic heart disease deaths	SMR 0.8 (0.5–1.4)
		Cerebrovascular disease risk	**HR 4.6 (1.3–17.0)* workers with definite exposure of ≥6 months HR 2.1 (1.0–4.6)* workers with definite exposure ≥5 years**
Raleigh et al. 2014 Occupational (n=9,027)	Cumulative PFOA exposure	Ischemic heart disease deaths	**SMR 0.84 (0.74–0.95)***
		Cerebrovascular disease	SMR 0.81 (0.61–1.05)
		Ischemic heart disease risk	HR 0.89 (0.66–1.21), 4th quartile
		Cerebrovascular disease risk	HR 0.98 (0.53–1.81), 4th quartile
Sakr et al. 2009 Occupational (n=4,747)	NR	Ischemic heart disease risk	NS (p=0.16 for trend)

Table 2-8. Summary of Cardiovascular Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Steenland et al. 2015 Occupational (n=3,713)	Estimated cumulative PFOA exposure	Coronary artery disease risk	NS (p=0.78 for trend), no lag NS (p=0.75 for trend), 10-year lag
		Hypertension	NS (p=0.95 for trend), no lag NS (p=0.54 for trend), 10-year lag
		Stroke	NS (p=0.35 for trend), no lag NS (p=0.64 for trend), 10-year lag
Steenland and Woskie 2012 Occupational (n=1,084)	7,800 ng/mL-years (mean estimated cumulative PFOA exposure)	Ischemic heart disease deaths	SMR 0.93 (0.72–1.19), no lag
Anderson-Mahoney et al. 2008 Community (n=566)	NR	Cardiovascular disease (self-reported)	**SPR 4.29 (3.47–5.29)***
		Angina (self-reported)	**SPR 8.07 (6.54–9.95)***
		Myocardial infarction	**SPR (1.91 (1.40–2.62)***
		Stroke	**SPR 2.17 (1.47–3.21)***
		Hypertension	SPR 1.18 (0.97–1.43)
Darrow et al. 2013 Community (C8) (n=1,330)	6.9–<11.1 ng/mL (2nd PFOA quintile)	Pregnancy-induced hypertension	**OR 2.39 (1.05–5.46)* (2nd quintile)**
Nolan et al. 2009 Community (C8) (n=1,555 women)	NR	Pregnancy-induced hypertension LHWA residents Partial LHWA residents	 OR 1.2 (0.7–2.0), unadjusted OR 0.8 (0.5–1.4), unadjusted
Savitz et al. 2012a Community (C8) (n=11,737 pregnant women)	6.8–<16.6 ng/mL (2nd PFOA quartile, estimated)	Pre-eclampsia	**OR 1.2 (1.0–1.5)***
Savitz et al. 2012b Community (C8) (n=224 cases of pregnancy-induced hypertension)	21.0–717.6 ng/mL (5th PFOA quintile, estimated)	Pregnancy induced hypertension	OR 1.0 (0.7–1.3), 5th quintile

Table 2-8. Summary of Cardiovascular Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Savitz et al. 2012b Community (C8) (n=4,547 pregnant women)	21.0–717.6 ng/mL (5th PFOA quintile, estimated)	Pregnancy induced hypertension	OR 1.1 (0.8–1.5)
Simpson et al. 2013 Community (C8) (n=28,541; 11% also had occupational exposure)	>178–319 ng/mL (cumulative, estimated 2nd PFOA quintile)	Stroke	**OR 1.39 (1.11–1.76)*, 2nd quintile**
Stein et al. 2009 Community (C8) (n=5.262 pregnant women)	120.6–894.4 ng/mL (4th PFOA quartile)	Pre-eclampsia	OR 0.9 (0.5–1.8)
Winquist and Steenland 2014a Community (C8) (n=28,541; 11% also had occupational exposure)	≥3,579 ng/mL (cumulative, estimated 5th PFOA quintile)	Hypertension	HR 0.98 (0.91–1.06), 5th quintile
		Coronary artery disease	HR 1.07 (0.93–1.23), 5th quintile
Bao et al. 2017 General population (n=1,612 adults)	6.19 ng/mL (median serum PFOA in males and females); 6.59 and 5.08 ng/mL (median serum PFOA in males and females, respectively)	Risk of hypertension	OR 1.12 (0.97–1.30)
		Systolic blood pressure	β-0.06 mm Hg (-1.70–1.59), males **β 2.91 mm Hg (0.10–5.72)*, females** **β 1.69 mm Hg (0.25–3.13)*, combined**
		Diastolic blood pressure	**β 1.48 mm Hg (0.60–2.35)*, males** β 1.34 mm Hg (-0.14–3.05), females **β 2.12 mm Hg (1.33–2.90)*, combined**
Geiger et al. 2014a General population (NHANES) (n=1,655 adolescents)	>5.4 ng/mL (4th quartile PFOA)	Hypertension	OR 0.69 (0.41–1.17), 4th quartile
Huang et al. 2018 General population (NHANES, n=10,859 adults)	3.17 ng/mL (median serum PFOA)	Risk of cardiovascular disease	OR 1.25 (0.91–1.70), 4th quartile

Table 2-8. Summary of Cardiovascular Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Koshy et al. 2017 General population (n=180 children enrolled in the WTCHR; n=222 children in comparison group)	1.81 and 1.39 ng/mL (median serum PFOA in WTCHR group and comparison group, respectively)	Arterial wall stiffness	Association (p=0.03)*
		Arterial pulse wave velocity	NS (p=0.39)
Lin et al. 2013a, 2013b General population (n=644)	3.49 ng/mL (median PFOA)	Carotid intima media thickness	NS (p=0.285 for trend)
Lind et al. 2017b General population (n=1,016, 70-year-old adults)	NR	Intima media thickness in common carotid artery	NS (p=0.58)
		Echogenicity of intima media complex	NS (p=0.80), males NS (p=0.25), females
Manzano-Salgado et al. 2017b General population (n=1,230 children)	2.32 ng/mL (maternal geometric mean PFOA)	Blood pressure at 4 years of age	β -0.06 (-0.16–0.04)
		Blood pressure at 7 years of age	β -0.02 (-0.11–0.07)
Mattsson et al. 2015 General population (n=231 cases with CHD, 231 controls)	4.2 and 4.0 ng/mL (median PFOA in cases and controls)	Coronary artery disease	OR 0.88 (0.50–1.55), 4th quartile
Melzer et al. 2010 General population (NHANES) (n=3,966 adults)	10.39 and 9.47 ng/mL (mean 4th quartile PFOA)	Coronary artery disease, angina, and/or heart attack	OR 1.08 (0.70–1.69, p=0.715), 4th quartile
Min et al. 2012 General population (NHANES) (n=2,208)	4.00 ng/mL (geometric mean PFOA)	Systolic blood pressure	**Association (p=0.0004)***
		Hypertension risk	**OR 1.71 (1.23–2.36)*, 4th quartile**
Shankar et al. 2012 General population (NHANES) (n=1,216)	4.0–5.6 and 4.4–6.1 ng/mL (females and males, 3rd PFOA quartile) >5.6 and >6.1 ng/mL (females and males, 4th PFOA quartile)	Cardiovascular disease	**OR 1.77 (1.04–3.02)*, 3rd quartile**
		Peripheral arterial disease	**OR 1.78 (1.03–3.08)*, 4th quartile**
		Coronary heart disease	**OR 2.24 (1.02–4.94)*, 4th quartile**
		Stroke	**OR 4.26 (1.84–9.89)*, 4th quartile**

Table 2-8. Summary of Cardiovascular Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Starling et al. 2014b General population (n=976 pregnant women)	2.78 ng/mL (median PFOA)	Pre-eclampsia	HR 0.89 (0.65–1.22), per ln unit
PFOS			
Darrow et al. 2013 Community (C8) (n=1,330)	12.1–<15.9 ng/mL (3rd PFOS quintile)	Pregnancy-induced hypertension	**OR 2.71 (1.33–5.52)* (3rd quintile)**
Stein et al. 2009 Community (C8) (n=5,262 pregnant women)	23.2–83.4 ng/mL (4th PFOS quartile)	Pre-eclampsia	**OR 1.6 (1.2–2.3)***
Bao et al. 2017 General population (n=1,612 adults)	24.22 ng/mL (median serum PFOS in males and females); 27.39 and 14.05 ng/mL (median serum PFOS in males and females, respectively)	Risk of hypertension	OR 1.08 (0.90–1.29), males **OR 1.63 (1.24–2.13)*, females** **OR 1.24 (1.08–1.44)*, combined**
		Systolic blood pressure	B 1.50 mm Hg (-0.17–3.18), males **β 6.65 mm Hg (4.32–8.99)*, females** **β 4.84 mm Hg (3.55–6.12)*, combined**
		Diastolic blood pressure	β 0.45 mm Hg (-0.47–4.36), males **β 2.86 mm Hg (1.51–4.20)*, females** **β 2.70 mm Hg (1.98–3.42)*, combined**
Geiger et al. 2014a General population (NHANES) (n=1,655 adolescents)	>25.5 ng/mL (4th PFOS quartile)	Hypertension	OR 0.77 (0.37–1.61), 4th quartile
Huang et al. 2018 General population (NHANES, n=10,859 adults)	12.40 ng/mL (median serum PFOS)	Risk of cardiovascular disease	OR 1.25 (0.92–1.69), 4th quartile
Koshy et al. 2017 General population (n=180 children enrolled in the WTCHR; n=222 children in comparison group)	3.72 and 2.78 ng/mL (median serum PFOS in WTCHR group and comparison group, respectively)	Arterial wall stiffness	NS (p=0.06)
		Arterial pulse wave velocity	NS (p=0.51)

Table 2-8. Summary of Cardiovascular Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Lin et al. 2013a, 2013b General population (n=644)	8.65 ng/mL (median PFOS)	Carotid intima media thickness	**Association (p<0.001 for trend)***
Lind et al. 2017b General population (n=1,016, 70-year-old adults)	NR	Intima media thickness in common carotid artery	NS (p=0.72)
		Echogenicity of intima media complex	NS (p=0.40), males NS (p=0.56), females
Manzano-Salgado et al. 2017b General population (n=1,230 children)	5.80 ng/mL (maternal geometric mean PFOS)	Blood pressure at 4 years of age	β 0.00 (-0.09–0.10)
		Blood pressure at 7 years of age	β -0.05 (-0.15–0.06)
Mattsson et al. 2015 General population (n=231 cases with CHD, 231 controls)	22.8 and 22.0 ng/mL (median PFOS in cases and controls)	Coronary artery disease	OR 1.07 (0.60–1.92), 4th quartile
Melzer et al. 2010 General population (NHANES) (n=3,966 adults)	57.73 and 50.96 ng/mL (mean 4th quartile PFOS)	Coronary artery disease, angina, and/or heart attack	OR 0.91 (0.570–1.64, p=0.745), 4th quartile
Starling et al. 2014b General population (n=976 pregnant women)	12.87 ng/mL (median PFOS)	Pre-eclampsia	HR 1.13 (0.84–1.52), per ln unit
PFHxS			
Bao et al. 2017 General population (n=1,612 adults)	0.71 ng/mL (median serum PFHxS in males and females)	Risk of hypertension	OR 0.99 (0.95–1.03)
		Systolic blood pressure	β 0.10 mm Hg (-0.30–0.51)
		Diastolic blood pressure	β 0.12 mm Hg (-0.11–0.35)
Huang et al. 2018 General population (NHANES, n=10,859 adults)	1.60 ng/mL (median serum PFHxS)	Risk of cardiovascular disease	OR 0.96 (0.68–1.37), 4th quartile

Table 2-8. Summary of Cardiovascular Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Koshy et al. 2017 General population (n=180 children enrolled in the WTCHR; n=222 children in comparison group)	0.67 and 0.53 ng/mL (median serum PFHxS in WTCHR group and comparison group, respectively)	Arterial wall stiffness	NS (p=0.69)
		Arterial pulse wave velocity	NS (p=0.89)
Lind et al. 2017b General population (n=1,016, 70-year-old adults)	NR	Intima media thickness in common carotid artery	NS (p=0.90)
		Echogenicity of intima media complex	NS (p=0.40), males NS (p=0.95), females
Manzano-Salgado et al. 2017b General population (n=1,230 children)	0.61 ng/mL (maternal geometric mean PFHxS)	Blood pressure at 4 years of age	β -0.01 (-0.10–0.09)
		Blood pressure at 7 years of age	β 0.04 (-0.04–0.13)
Mattsson et al. 2015 General population (n=231 cases with CHD, 231 controls)	1.6 ng/mL (median PFHxS in cases and controls)	Coronary artery disease	OR 0.95 (0.54–1.67), 4th quartile
Starling et al. 2014b General population (n=976 pregnant women)	0.69 ng/mL (median PFHxS)	Pre-eclampsia	HR 0.91 (0.72–1.14), per ln unit
PFNA			
Bao et al. 2017 General population (n=1,612 adults)	1.96 ng/mL (median serum PFNA in males and females); 2.19 and 1.31 ng/mL (median serum PFNA in males and females, respectively)	Risk of hypertension	OR 1.08 (0.92–1.26), males **OR 1.49 (1.16–1.92)*, females** **OR 1.19 (1.04–1.36)*, combined**
		Systolic blood pressure	B -0.12 mm Hg (-1.62–1.39), males **β 5.70 mm Hg (3.55–7.85)*, females** **β** 3.01 mm Hg (1.79–4.23)***, combined**
		Diastolic blood pressure	**β 0.94 mm Hg (0.12–1.76)*, males** **β 2.74 mm Hg (1.51–3.97)*, females** **β 2.48 mm Hg (1.80–3.16)*, combined**

Table 2-8. Summary of Cardiovascular Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Huang et al. 2018 General population (NHANES, n=10,859 adults)	0.98 ng/mL (median serum PFNA)	Risk of cardiovascular disease	OR 1.30 (0.99–1.72), 4th quartile **OR 1.42 (1.07–1.88)*, 4th quartile with adjustment for serum total proteins and eGFR**
		Risk of coronary heart disease	**OR 1.89 (1.29–2.76)*, 4th quartile**
		Risk of heart attack	**OR 1.51 (1.02–2.23)*, 3rd quartile**
Koshy et al. 2017 General population (n=180 children enrolled in the WTCHR; n=222 children in comparison group)	0.61 and 0.49 ng/mL (median serum PFNA in WTCHR group and comparison group, respectively)	Arterial wall stiffness	**Association (p=0.04)***
		Arterial pulse wave velocity	NS (p=0.14)
Lin et al. 2013a, 2013b General population (n=644)	0.38 ng/mL (median PFNA)	Carotid intima media thickness	**Inverse association (p=0.014 for trend)***
Lind et al. 2017b General population (n=1,016, 70-year-old adults)	NR	Intima media thickness in common carotid artery	NS (p=0.76)
		Echogenicity of intima media complex	NS (p=0.66), males **Association (p=0.01)*, females**
Manzano-Salgado et al. 2017b General population (n=1,230 children)	0.66 ng/mL (maternal geometric mean PFNA)	Blood pressure at 4 years of age	β -0.01 (-0.10–0.08)
		Blood pressure at 7 years of age	β 0.00 (-0.08–0.09)
Mattsson et al. 2015 General population (n=231 cases with CHD, 231 controls)	0.5 ng/mL (median PFNA in cases and controls)	Coronary artery disease	OR 0.68 (0.39–1.20), 4th quartile
Starling et al. 2014b General population (n=976 pregnant women)	0.54 ng/mL (median PFNA)	Pre-eclampsia	HR 0.90 (0.70–1.16), per ln unit

Table 2-8. Summary of Cardiovascular Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFDA			
Bao et al. 2017 General population (n=1,612 adults)	0.86 ng/mL (median serum PFNA in males and females)	Risk of hypertension	OR 0.96 (0.85–1.09)
		Systolic blood pressure	β -0.19 mm Hg (-1.39–1.02)
		Diastolic blood pressure	**β 0.81 mm Hg (0.08–1.54)*, males** β 0.61 mm Hg (-0.81–2.04), females **β 1.19 mm Hg (0.52–1.37)*, combined**
Huang et al. 2018 General population (NHANES, n=10,859 adults)	0.20 ng/mL (median serum PFDA)	Risk of cardiovascular disease	OR 1.32 (0.99–1.78), 4th quartile **OR 1.43 (1.06–1.92)*, 4th quartile with adjustment for serum total proteins and eGFR**
Koshy et al. 2017 General population (n=180 children enrolled in the WTCHR; n=222 children in comparison group)	0.14 and 0.11 ng/mL (median serum PFDA in WTCHR group and comparison group, respectively)	Arterial wall stiffness	NS (p=0.10)
		Arterial pulse wave velocity	NS (p=0.39)
Lind et al. 2017b General population (n=1,016, 70-year-old adults)	NR	Intima media thickness in common carotid artery	NS (p=0.85)
		Echogenicity of intima media complex	NS (p=0.84), males NS (p=014), females
Mattsson et al. 2015 General population (n=231 cases with CHD, 231 controls)	0.2 ng/mL (median PFDA in cases and controls)	Coronary artery disease	OR 0.92 (0.53–1.60), 4th quartile
Starling et al. 2014b General population (n=976 pregnant women)	0.10 ng/mL (median PFDA)	Pre-eclampsia	HR 0.88 (0.75–1.04), per ln unit
PFUnA			
Bao et al. 2017 General population (n=1,612 adults)	0.5 ng/mL (median serum PFUnA in males and females)	Risk of hypertension	OR 0.95 (0.90–1.01)
		Systolic blood pressure	β -0.49 mm Hg (-1.04–0.05)
		Diastolic blood pressure	β -0.11 mm Hg (-0.41–0.20)

Table 2-8. Summary of Cardiovascular Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Huang et al. 2018 General population (NHANES, n=10,859 adults)	0.20 ng/mL (median serum PFUnA)	Risk of cardiovascular disease	**OR 1.58 (1.17–2.12)*, 2nd quartile**
		Risk of coronary heart disease	**OR 1.57 (1.00–2.46)*, 2nd quartile**
		Risk of angina pectoris	**OR 1.97 (1.09–3.55)*, 3rd quartile**
Koshy et al. 2017 General population (n=180 children enrolled in the WTCHR; n=222 children in comparison group)	0.12 and 0.04 ng/mL (median serum PFUnA in WTCHR group and comparison group, respectively)	Arterial wall stiffness	NS (p=0.97)
		Arterial pulse wave velocity	NS (p=0.41)
Lin et al. 2013a, 2013b General population (n=644)	6.59 ng/mL (median PFUnA)	Carotid intima media thickness	NS (p=0.953 for trend)
Lind et al. 2017b General population (n=1,016, 70-year-old adults)	NR	Intima media thickness in common carotid artery	NS (p=0.96)
		Echogenicity of intima media complex	NS (p=0.09), males NS (p=0.14), females
Mattsson et al. 2015 General population (n=231 cases with CHD, 231 controls)	0.2 ng/mL (median PFUnA in cases and controls)	Coronary artery disease	OR 0.88 (0.51–1.51), 4th quartile
Starling et al. 2014b General population (n=976 pregnant women)	0.17 ng/mL (median PFUnA)	Pre-eclampsia	**HR 0.78 (0.66–0.92)*, per ln unit**

Table 2-8. Summary of Cardiovascular Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFHpA			
Bao et al. 2017 General population (n=1,612 adults)	0.01 ng/mL (median serum PFHpA in males and females); 0.01 and 0.01 ng/mL (median serum PFHpA in males and females, respectively)	Risk of hypertension	OR 1.02 (0.89–1.16)
		Systolic blood pressure	**β 1.84 mm Hg (0.43–3.25)*, males** β 0.34 mm Hg (-2.47–3.15), females **β 1.50 mm Hg (0.21–2.80)*, combined**
		Diastolic blood pressure	**β 0.79 mm Hg (0.02–1.55)*, males** β 0.14 mm Hg (-1.45–1.73), females β 0.66 mm Hg (0.05–1.40), combined
Huang et al. 2018 General population (NHANES, n=10,859 adults)	0.20 ng/mL (median serum PFHpA)	Risk of cardiovascular disease	OR 1.16 (0.71–1.91), 4th quartile
Lind et al. 2017b General population (n=1,016, 70-year-old adults)	NR	Intima media thickness in common carotid artery	NS (p=0.78)
		Echogenicity of intima media complex	NS (p=0.53), males NS (p=0.13), females
Mattsson et al. 2015 General population (n=231 cases with CHD, 231 controls)	0.06 and 0.04 ng/mL (median PFHpA in cases and controls)	Coronary artery disease	**OR 2.58 (1.39–4.78)*, 3rd quartile** OR 1.73 (0.94–3.16), 4th quartile
PFBS			
Bao et al. 2017 General population (n=1,612 adults)	0.01 ng/mL (median serum PFBS in males and females)	Risk of hypertension	OR 0.94 (0.78–1.12)
		Systolic blood pressure	β -0.69 mm Hg (-2.49–1.11)
		Diastolic blood pressure	β -0.41 mm Hg (-1.42–0.60)
Huang et al. 2018 General population (NHANES, n=10,859 adults)	0.07 ng/mL (median serum PFBS)	Risk of cardiovascular disease	**OR 1.34 (1.05–1.723)*, 2nd quartile**

Table 2-8. Summary of Cardiovascular Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFBA			
Bao et al. 2017 General population (n=1,612 adults)	0.15 ng/mL (median serum PFBA in males and females); 0.17 and 0.12 ng/mL (median serum PFBA in males and females, respectively)	Risk of hypertension	**OR 1.09 (1.02–1.16)*, males** **OR 1.16 (1.04–1.29)*, females** **OR 1.10 (1.04–1.17)*, combined**
		Systolic blood pressure	**β 0.66 mm Hg (0.03–1.28)*, males** β 0.77 mm Hg (-0.27–1.80), females **β 0.80 mm Hg (0.25–1.34)*, combined**
		Diastolic blood pressure	β 0.09 mm Hg (-0.22–0.40), combined
PFDoDA			
Bao et al. 2017 General population (n=1,612 adults)	0.12 ng/mL (median serum PFDoDA in males and females); 0.17 and 0.12 ng/mL (median serum PFDoDA in males and females, respectively)	Risk of hypertension	OR 1.02 (0.93–1.11)
		Systolic blood pressure	β -0.74 (-1.71–0.22), males **β 1.89 mm Hg (0.21–3.56*, females** β 0.30 mm Hg (-0.56–1.16), combined
		Diastolic blood pressure	β 0.13 mm Hg (-0.40–0.66), males **β 1.02 mm Hg (0.07–1.97)*, females** **β 0.59 mm Hg (0.12–1.07)*, combined**
Huang et al. 2018 General population (NHANES, n=10,859 adults)	0.14 ng/mL (median serum PFDoDA)	Risk of cardiovascular disease	**OR 1.53 (1.14–2.04)*, 4th quartile**
		Risk of congestive heart failure	**OR 1.55 (1.07–2.25)*, 3rd quartile**
		Risk of angina pectoris	**OR 1.64 (1.06–2.54)*, 4th quartile**
Mattsson et al. 2015 General population (n=231 cases with CHD, 231 controls)	0.02 ng/mL (median PFDoDA in cases and controls)	Coronary artery disease	OR 0.63 (0.35–1.11), 4th quartile

Table 2-8. Summary of Cardiovascular Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
FOSA			
Huang et al. 2018 General population (NHANES, n=10,859 adults)	0.07 ng/mL (median serum FOSA)	Risk of cardiovascular disease	**OR 1.29 (1.01–1.65)*, 2nd quartile**
Lind et al. 2017b General population (n=1,016, 70-year-old adults)	NR	Intima media thickness in common carotid artery	NS (p=0.35), males **Association (p=0.004)*, females** **Association (p=0.01)*, combined**
		Echogenicity of intima media complex	NS (p=0.84), males NS (p=0.78, females

[a]See the *Supporting Document for Epidemiological Studies for Perfluoroalkyls,* Table 3 for more detailed descriptions of studies.
[b]Participants in occupational exposure may have also lived near the site and received residential exposure; community exposure studies involved subjects living near PFOA facilities with known exposure to high levels of PFOA.
[c]Asterisk indicates association with perfluoroalkyl; unless otherwise specified, values in parenthesis are 95% confidence intervals.

CHD = coronary heart disease; FOSA = perfluorooctane sulfonamide; HR = hazard ratio; LHWA = Little Hocking Water Authority; NHANES = National Health and Nutrition Examination Survey; NR = not reported; NS = not significant; OR = odds ratio; PFBA = perfluorobutanoic acid; PFBS = perfluorobutane sulfonic acid; PFDA = perfluorodecanoic acid; PFDoDA = perfluorododecanoic acid; PFHpA = perfluoroheptanoic acid; PFHxS = perfluorohexane sulfonic acid; PFNA = perfluorononanoic acid; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid; PFUnA = perfluoroundecanoic acid; SMR = standardized mortality ratio; SPR = standard prevalence ratio; WTCHR = World Trade Center Health Registry

Examination of the cardiovascular system in laboratory animals primarily consists of inhalation, oral, and dermal studies examining the heart for morphological alterations (see Tables 2-1, 2-3, 2-4, 2-5, and 2-6). No studies in laboratory animals were identified for PFNA, PFUnA, PFHpA, or FOSA.

The laboratory animal studies did not find increases in the incidence of histological alterations in the heart following exposure to PFOA, PFOS, PFHxS, PFDA, PFBS, PFBA, PFDoDA, or PFHxA.

PFOA

Epidemiological Studies—Heart Disease. Possible associations between PFOA exposure and increased risk of heart disease have been examined in cohort mortality studies of workers, community members living near a PFOA facility, and the general population. Occupational exposure studies have not found increases in deaths from all heart disease, cerebrovascular disease, or ischemic heart disease when compared to U.S. general populations, state populations, and/or a population of workers at other company facilities (Leonard 2006; Lundin et al. 2009; Raleigh et al. 2014; Steenland and Woskie 2012). One occupational exposure study found an increase in the risk of cerebrovascular disease in workers with definite exposure for at least 6 months compared to an internal referent group (Lundin et al. 2009). However, other studies have not found increased risks of ischemic heart disease (Raleigh et al. 2014; Sakr et al. 2009), cerebrovascular disease (Raleigh et al. 2014), or coronary artery disease (Steenland et al. 2015). In another occupational exposure study, the investigators noted that electrocardiograms (EKGs) were within normal limits (Sakr et al. 2007b).

Studies of residents living near the Washington Works facility in West Virginia reported increased risks of self-reported cardiovascular disease (Anderson-Mahoney et al. 2008), angina (Anderson-Mahoney et al. 2008), myocardial infarction (Anderson-Mahoney et al. 2008), and stroke (Anderson-Mahoney et al. 2008; Simpson et al. 2013). It is noted that the Anderson-Mahoney et al. (2008) study did not measure serum PFOA levels; the incidences of self-reported diseases were compared to NHANES rates. Another community study of residents in this area did not find an increased risk of coronary artery disease (Winquist and Steenland 2014a). Seven general population studies have examined possible associations between serum PFOA and heart disease risks. A case-control study did not find increases in the risk of coronary artery disease in subjects with median serum PFOA levels of 4.2 ng/mL (cases) or 4.0 ng/mL (controls) (Mattsson et al. 2015). Utilizing the NHANES data set, Shankar et al. (2012) found increases in the risk of peripheral arterial disease, coronary heart disease, or stroke in participants with serum PFOA levels in the 4th quartile (>5.6 and >6.1 ng/mL in females and males, respectively) and for cardiovascular

disease in participants with serum PFOA levels in the 3rd and 4th quartiles (>4.0 and >4.4 ng/L for females and males, respectively). In contrast, two other NHANES studies did not find associations between serum PFOA and physician-diagnosed coronary artery disease, angina, and/or heart attack (Melzer et al. 2010) or total cardiovascular heart disease (Huang et al. 2018). Two general population studies did not find associations between serum PFOA levels and carotid intima media thickness (Lin et al. 2013a; Lind et al. 2017b). Another study did not find associations with arterial wall stiffness or arterial pulse wave velocity (Koshy et al. 2017).

Epidemiological Studies—Hypertension. Occupational, community, and general population exposure studies have investigated the possible association between PFOA and blood pressure, the risk of hypertension, and the risk of pregnancy-induced hypertension and/or pre-eclampsia. A study by Min et al. (2012) utilizing NHANES data found an increase in hypertension risk among participants with serum PFOA levels in the 4th quartile. Another general population study did not find an association between serum PFOA and the risk of hypertension, but did find associations between serum PFOA and systolic and diastolic blood pressure (Bao et al. 2017). In contrast, no increases in the risk of hypertension were observed in workers at the Washington Works facility (Steenland et al. 2015), adult community members living near this facility (Winquist and Steenland 2014a), or adolescent NHANES participants (Geiger et al. 2014a). Additionally, Manzano-Salgado et al. (2017b) did not find associations between maternal serum PFOA levels and blood pressure in children at ages 4 or 7 years. There is some epidemiological evidence suggesting that an elevated uric acid level is a risk factor for hypertension (Johnson et al. 2003; Sündstrom et al. 2005). Several occupational, community, and general population studies have found increases in uric acid levels and increased risks of hyperuricemia; these data are discussed in Section 2.10. Overall, the results of these studies are suggestive of a connection between serum PFOA and increased risk of hyperuricemia.

Several studies have examined the possible associations between PFOA and pregnancy-induced hypertension/pre-eclampsia. Four studies have evaluated the community living near the Washington Works facility using different approaches to assess PFOA exposure. Savitz et al. (2012a, 2012b) used residential history and environmental dispersion of PFOA to estimate serum PFOA levels over time. Stein et al. (2009) used serum PFOA levels measured in 2005–2006 to assess the risk of pre-eclampsia occurring prior to the blood sampling. Darrow et al. (2013) primarily used serum PFOA levels measured in 2005–2006 to assess the association with pregnancy-induced hypertension occurring after the blood samples were collected. Savitz et al. (2012a) found an increased risk of self-reported pre-eclampsia in C8 Health Project participants with elevated PFOA levels and Darrow et al. (2013) found significant

increases in the odds ratios (ORs) for self-reported pregnancy-induced hypertension in women with higher PFOA (≥6.9 ng/mL) levels. A third study of highly exposed residents reported a weak association between serum PFOA and self-reported pre-eclampsia in subjects whose serum PFOA levels were above the median (Stein et al. 2009); however, there was no dose-response gradient. Using birth record data and serum PFOA levels predicted from addresses, Savitz et al. (2012b) found no consistent associations between serum PFOA and the occurrence of pregnancy-induced hypertension in participants in the C8 Health Project. Similarly, Stein et al. (2009) did not find increases in the odds of self-reported pre-eclampsia among C8 Health Project participants categorized by serum PFOA levels. Another study of residents of this area did not find increases in the risk of pregnancy-induced hypertension among residents living in an area where PFOA-contaminated water was supplied by the Little Hocking Water Authority (Nolan et al. 2010). A general population study did not find an association between plasma PFOA and the risk of pre-eclampsia (Starling et al. 2014a).

Laboratory Animal Studies. A small number of laboratory animal studies have evaluated the cardiovascular toxicity of PFOA. These studies focused on potential histological alterations in the heart; none of the available studies evaluated endpoints related to hypertension. No histopathological alterations were seen in the heart from rats exposed intermittently head-only to up to 84 mg/m^3 APFO dusts for 2 weeks (Kennedy et al. 1986). Administration of APFO in the diet at doses up to approximately 100–110 mg/kg/day to male and female CD rats or 10 mg/kg/day by gavage to Rhesus monkeys did not cause gross or microscopic alterations in the heart or aorta (Griffith and Long 1980). Similar negative findings were reported in Cynomolgus monkeys administered up to 20 mg/kg/day APFO by capsule for 26 weeks (Butenhoff et al. 2002) and in male and female Sprague-Dawley rats that received doses of up to 15 mg/kg/day APFO for 2 years (3M 1983; Butenhoff et al. 2012c). No morphological alterations were seen in the heart from male rats dermally exposed to ≤2,000 mg/kg APFO for 2 weeks (Kennedy 1985).

Summary. Cardiovascular toxicity as assessed by deaths from heart disease, risk of heart disease, and risk of hypertension has been evaluated in workers, community members living near a PFOA facility, and the general population. In general, occupational exposure studies have not found increases in the risks of deaths from heart disease or in the risks of ischemic heart disease, cerebrovascular disease, or coronary disease. Inconsistent results have been found in a small number of studies examining residents living in areas with high PFOA drinking water contamination or the general population. Studies of hypertension have also not found associations between serum PFOA and hypertension risk. However, studies of highly exposed residents provide some suggestive evidence of an association between serum PFOA and

increased risks of pregnancy-induced hypertension/pre-eclampsia. Studies in laboratory animals did not find histological alterations in the heart following acute-, intermediate-, or chronic-duration oral exposure.

PFOS

Epidemiological Studies—Heart Disease. Three studies have evaluated the possible association between PFOS and heart disease. Melzer et al. (2010) did not find an association between serum PFOS and the risk of physician-diagnosed coronary artery disease, angina, and/or heart attack among NHANES participants; Huang et al. (2018) did not find increases in the risk of cardiovascular disease among NHANES participants. In a case-control study (Mattsson et al. 2015), no alterations in the risk of coronary artery disease were observed. Lin et al. (2013a) found an association between serum PFOS levels and carotid intima media thickness in a general population study. When the subjects were divided into subpopulations, associations between PFOS and carotid intima media thickness were found for females, nonsmokers, subjects 12–19 years of age, BMI <24, and those with an apolipoprotein E genotype of E2 carrier or E3/E3. A second study of 70-year-old subjects did not find associations between serum PFOS and the intima media thickness of the common carotid artery (Lind et al. 2017b). Similarly, no alterations in arterial wall stiffness or pulse wave velocity were found in children enrolled in the World Trade Center Health Registry (Koshy et al. 2017).

Epidemiological Studies—Hypertension. An increased risk of hypertension associated with serum PFOS levels were observed in adults; when categorized by sex, the association was only found in females (Bao et al. 2017). The study also found associations for systolic and diastolic blood pressure in males and females combined and in females only. No increases in the risk of hypertension associated with serum PFOS levels were observed in adolescent NHANES participants (Geiger et al. 2014a). Similarly, no associations between maternal serum PFOS levels and blood pressure were found in children at ages 4 and 7 years (Manzano-Salgado 2017b). Two studies found increases in the risk of self-reported pregnancy-induced hypertension (Darrow et al. 2013) or self-reported pre-eclampsia (Stein et al. 2009) associated with serum PFOS levels among C8 participants. No increase in the risk of pre-eclampsia was observed in a general population study (Starling et al. 2014b).

Laboratory Animal Studies. Studies in laboratory animal studies have evaluated the cardiovascular toxicity of PFOS but have not evaluated endpoints related to hypertension. Administration of doses of up to 0.75 mg/kg/day PFOS (potassium salt) via capsule to Cynomolgus monkeys for 26 weeks did not cause any significant gross or microscopic alterations in the heart or aorta (Seacat et al. 2002). Rats that

received up to approximately 1.04 mg/kg/day of PFOS in the diet for 2 years had no significant gross or microscopic changes in the heart (Butenhoff et al. 2012b; Thomford 2002b).

PFHxS

Epidemiological Studies. Eight general population studies examined possible cardiovascular outcomes associated with PFHxS exposure. No increases in the risk of coronary artery disease (Mattsson et al. 2015) or cardiovascular disease (Huang et al. 2018) were found. Serum PFHxS levels were not associated with arterial wall stiffness (Koshy et al. 2017) or carotid artery intima media thickness (Lind et al. 2017b). Studies examining blood pressure have not found associations in adults (Bao et al. 2017) or children (Manzano-Salgado et al. 2017b). Additionally, no association between serum PFHxS and pre-eclampsia were found (Starling et al. 2014b).

Laboratory Animal Studies. Dosing of rats with≤10 mg/kg/day PFHxS or mice with ≤3 mg/kg/day by gavage for 40–60 days did not cause morphological alterations in the heart (Butenhoff et al. 2009a; Chang et al. 2018).

PFNA

Epidemiological Studies. In a general population study, an inverse association between serum PFNA levels and carotid intima media thickness was observed (Lin et al. 2013a). The investigators suggested that this finding may be secondary to an interaction between higher serum PFOS levels and lower serum PFNA levels in the study population. Associations were only found in subjects with serum PFOS higher than the 50th percentile regardless of whether the serum PFNA was higher or lower than the 60th percentile. A second study did not find an association between serum PFNA and intima media thickness (Lind et al. 2017b), but did find an association with the echogenicity of the intima media complex, an indicator of early changes in the carotid artery. Koshy et al. (2017) also found an association between serum PFNA and arterial wall stiffness in children enrolled in the World Trade Center Health Registry. Increased risks of cardiovascular disease, coronary heart disease, and heart attack were found in NHANES participants (Huang et al. 2018). In contrast, another general population study did not find increases in the risk of coronary heart disease (Mattsson et al. 2015). An association between serum PFNA and hypertension risk and systolic and diastolic blood pressure was found in a general population study (Bao et al. 2017). Manzano-Salgado et al. (2017b) did not find associations between maternal

serum PFNA and blood pressure in children aged 4 or 7 years, and Starling et al. (2014b) did not find associations between serum PFNA and pre-eclampsia (Starling et al. 2014b).

PFDA

Epidemiological Studies. In a study of NHANES participants, Huang et al. (2018) found an increased risk of any type of cardiovascular disease among participants with the highest serum PFDA levels when the statistical analyses adjusted for serum total protein levels and estimated glomerular filtration rate; however, no associations were found for specific types of cardiovascular disease. In another general population study, Mattsson et al. (2015) found no association between serum PFDA and the risk of coronary artery disease. Studies examining carotid artery intima media thickness or arterial wall stiffness of the brachial artery did not find associations with serum PFDA levels (Koshy et al. 2017; Lind et al. 2017b). Although Bao et al. (2017) did not find an association between serum PFDA levels and the risk of hypertension or systolic blood pressure levels, associations were found in diastolic blood pressure levels in males only and in males and females combined. No association was found between serum PFDA and pre-eclampsia (Starling et al. 2014b).

Laboratory Animal Studies. Death in female C57BL/6N mice following administration of a single lethal dose of 160 or 320 mg/kg PFDA by gavage was associated with mural thrombosis of the left ventricle of the heart (Harris et al. 1989). Doses ≤80 mg/kg did not cause gross or microscopic alterations in the heart, assessed 30 days after dosing, but 80 mg/kg significantly decreased relative heart weight (Harris et al. 1989).

PFUnA

Epidemiological Studies. Serum PFUnA levels were associated with increased risks of any type of cardiovascular disease, coronary heart disease, and angina pectoris in NHANES participants (Huang et al. 2018). No associations between serum PFUnA levels and the risk of hypertension of systolic or diastolic blood pressure were observed (Bao et al. 2017). Starling et al. (2014b) found an inverse association between serum PFUnA levels and the risk of pre-eclampsia in pregnant women. No associations between serum PFUnA levels and carotid intima artery thickness (Lin et al. 2013a; Lind et al. 2017b) or brachial artery wall stiffness (Koshy et al. 2017) were observed in general population studies. Another general population study (Mattsson et al. 2015) did not find an increase in the risk of coronary artery disease associated with serum PFUnA levels.

PFHpA

Epidemiological Studies. Mattsson et al. (2015) found an increase in the risk of coronary artery disease in individuals with serum PFHpA levels in the 3rd quartile; however, the risk was not increased for those with serum levels in the 4th quartile. A study of NHANES participants did not find an association between the serum PFHpA levels and any type of cardiovascular disease or a specific type of heart disease (Huang et al. 2018). No associations between serum PFHpA and the thickness of the intima media of the common carotid artery were observed in a general population study of 70-year-old adults (Lind et al. 2017b). Bao et al. (2017) did not find an association between serum PFHpA levels and the risk of hypertension; the study did find associations for systolic and diastolic blood pressure levels in males only.

PFBS

Epidemiological Studies. Two general population studies have evaluated the potential associations between serum PFBS and cardiovascular effects. Huang et al. (2018) found increased risks of cardiovascular disease (all types combined) in NHANES participants with serum PFBS levels in the 2nd quartile and higher; however, no associations were found for specific disease types. Bao et al. (2017) did not find associations between serum PFBS levels and the risk of hypertension or systolic or diastolic blood pressure levels among adults.

Laboratory Animal Studies. No morphological alterations were reported in the heart or aorta from rats dosed with ≤900 mg/kg/day PFBS by gavage for 28 days (3M 2001) or ≤600 mg/kg/day PFBS for 90 days (Lieder et al. 2009a).

PFBA

Epidemiological Studies. Only one epidemiological study examined potential cardiovascular health outcomes. Bao et al. (2017) found increases in the risk of hypertension in male and female adults, which was associated with serum PFBA levels. Systolic blood pressure levels were also associated with serum PFBA levels in males and females combined or in males only; no associations were found for diastolic blood pressure.

Laboratory Animal Studies. PFBA administered to rats by gavage in doses of up to 184 mg/kg/day for 5 days, 150 mg/kg/day for 28 days, or 30 mg/kg/day for 90 days did not induce gross or microscopic alterations in the heart (3M 2007a; Butenhoff et al. 2012a; van Otterdijk 2007a, 2007b).

PFDoDA

Epidemiological Studies. No increase in the risk of coronary heart disease associated with serum PFDoDA levels was found in a general population study (Mattsson et al. 2015). In contrast, Huang et al. (2018) found increased risks of cardiovascular disease (any type), congestive heart failure, or angina pectoris in NHANES participants with higher serum PFDoDA levels. Bao et al. (2017) reported associations between serum PFDoDA levels in systolic and diastolic blood pressure levels among women, but there was no association with the risk of hypertension.

Laboratory Animal Studies. No histological alterations were observed in male rats administered 2.5 mg/kg/day for 42 days (Kato et al. 2015).

PFHxA

Epidemiological Studies. An increased risk of cardiovascular disease (any type) was found in NHANES participants with higher serum PFHxA levels (Huang et al. 2018). A study of 70-year-old adults reported increases in the intima media thickness in the common carotid artery that was associated with serum PFHxA levels (Lind et al. 2017b).

Laboratory Animal Studies. No histological alterations were observed in the heart of rats administered up to 500 mg/kg/day NaPFHx for 90–93 days (Chengelis et al. 2009b; Loveless et al. 2009).

FOSA

Epidemiological Studies. Serum FOSA levels were associated with an increased risk of cardiovascular disease (any type) in a study of NHANES participants (Huang et al. 2018). Increases in the intima media thickness in the common carotid artery was associated with serum FOSA levels in a study of 70-year-old men and women (Lind et al. 2017b).

2.6 GASTROINTESTINAL

Overview. Available epidemiological data on the potential of perfluoroalkyls to induce gastrointestinal effects are limited to two studies of workers at a PFOS facility that found mixed results on the possible association between PFOS and colon polyps; summaries of these studies are presented in the *Supporting Document for Epidemiological Studies for Perfluoroalkyls*, Table 4. Epidemiological studies examining potential gastrointestinal effects were not identified for the other perfluoroalkyls. Studies examining ulcerative colitis are discussed in Section 2.14, Immunological. Laboratory animal studies have examined the gastrointestinal tract for morphological alterations following inhalation, oral, or dermal exposure to PFOA (Tables 2-1, 2-3, and 2-6), oral exposure to PFOS (Table 2-4), and oral exposure to other perfluoroalkyls (Table 2-5); the NOAELs and LOAELs are presented in Figures 2-6, 2-8, and 2-9. No laboratory animal studies were identified for PFNA, PFUnA, PFHpA, or FOSA. Studies on PFOA and PFBS have reported some signs of gastrointestinal irritation following gavage administration. Most studies did not report histological alterations in the gastrointestinal tract following exposure to PFOA, PFOS, PFHxS, PFDA, PFBA, PFDoDA, or PFHxA.

PFOA

Laboratory Animal Studies. The available data in rats and monkeys do not suggest that the gastrointestinal tract is a sensitive target of toxicity, although two studies did report some signs of irritation. Stomach irritation was reported in male rats exposed head-only to ≥380 mg/m^3 APFO dusts for 4 hours (Kennedy et al. 1986). No histopathological alterations were seen in the stomach, small intestine, or large intestine from male rats exposed intermittently nose-only to up to 84 mg/m^3 APFO dusts for 2 weeks (Kennedy et al. 1986).

No significant gross or microscopic alterations of the gastrointestinal tract were observed in male or female rats exposed to approximately 100–110 mg/kg/day APFO through the diet for 90 days (Griffith and Long 1980). Similar observations were reported in male and female rats exposed to 15 mg/kg/day APFO via the diet for 2 years (3M 1983; Butenhoff et al. 2012c). The same investigators also reported that emesis occurred in Rhesus monkeys exposed to lethal doses (30 and 100 mg/kg/day) of APFO by gavage for 90 days (Griffith and Long 1980). In another intermediate-duration study in which Cynomolgus monkeys were exposed to up to 20 mg/kg/day APFO administered via a capsule for

26 weeks, no treatment-related alterations in the gastrointestinal tract were observed at termination (Butenhoff et al. 2002).

Intermittent application of up to 2,000 mg/kg/day APFO to the skin of male rats for up to 2 weeks did not result in gross or microscopic alterations in the gastrointestinal tract (Kennedy 1985).

PFOS

Epidemiological Studies. There are limited data available on the potential of PFOS to induce gastrointestinal damage. A study of current, retired, or former workers employed for at least 1 year at a PFOS-based fluorochemical manufacturing facility in Decatur, Alabama found no association between self-reported incidence of gastric ulcer or colon polyps and having worked in a job with either low (estimated serum PFOS levels of 390–890 ng/mL) or high (estimated PFOS serum levels of 1,300–1,970 ng/mL) exposure to PFOS, as compared to workers with no direct workplace exposure (estimated serum PFOS levels of 110–290 ng/mL) (Grice et al. 2007). A second study of workers at the Decatur facility found an increase in the risk ratio episodes of care for benign colonic polyps in workers with high potential exposure to PFOS (Olsen et al. 2004a).

Laboratory Animal Studies. Unpublished data summarized by OECD (2002) indicate that distension of the small intestine was observed in rats exposed to lethal concentrations of airborne PFOS dusts (1,890–45,970 mg/m^3) for 1 hour. Treatment of rats with up to approximately 1.04 mg/kg/day PFOS via the diet for 2 years did not induce morphological alterations in the gastrointestinal tract (Butenhoff et al. 2012b; Thomford 2002b).

PFHxS

Laboratory Animal Studies. No morphological alterations were observed in the gastrointestinal tract of rats administered ≤10 mg/kg/day or mice administered ≤3 mg/kg/day PFHxS via gavage for 40–60 days (Butenhoff et al. 2009a; Chang et al. 2018).

PFDA

Laboratory Animal Studies. Administration of 0.5 mg/kg/day PFDA to rats for 28 days or 5 mg/kg to mice for 4 weeks (once/week) did not result in histological alterations in the gastrointestinal tract (Frawley et al. 2018).

PFBS

Laboratory Animal Studies. Necrosis of individual squamous cells and hyperplasia and hyperkeratosis were observed in the limiting ridge of the forestomach of male and female rats administered 600 mg/kg/day PFBS via gavage for 90 days (Lieder et al. 2009a); these lesions were likely due to irritation from the repeated gavage administration with PFBS. In another study, no morphological alterations were observed in the gastrointestinal tract of rats administered ≤900 mg/kg/day PFBS via gavage for 28 days (3M 2001).

PFBA

Laboratory Animal Studies. Administration of PFBA to rats by gavage in doses of up to 184 mg/kg/day for 5 days, 150 mg/kg/day for 28 days, or 30 mg/kg/day for 90 days did not cause morphological alterations in the gastrointestinal tract (3M 2007a; Butenhoff et al. 2012a; van Otterdijk 2007a, 2007b).

PFDoDA

Laboratory Animal Studies. No histological alterations were observed in the gastrointestinal tract of male rats receiving gavage administration of 2.5 mg/kg/day for 42 days or in male and female rats administered 42 mg/kg/day for 42 days and allowed to recover for 14 days (Kato et al. 2015).

PFHxA

Laboratory Animal Studies. Rat administered 200 mg/kg/day NaPFHx for 90 days did not exhibit histological alterations in the gastrointestinal tract (Chengelis et al. 2009b). Erosions/ulcerations were observed in the glandular or nonglandular stomach of rats receiving gavage doses of 450 mg/kg/day PFHxA for 4 days; all animals exhibiting these lesions died early or were sacrificed *in extremis*

(Kirkpatrick 2005). No gastrointestinal lesions were observed in rats administered a time-weighted average (TWA) dose of 315 mg/kg/day for 32–44 days (Kirkpatrick 2005).

2.7 HEMATOLOGICAL

Overview. A small number of epidemiological studies have evaluated hematological endpoints in workers exposed to PFOA or PFOS and in a community exposure study; these studies did not find alterations in hematological indices; epidemiological data were not identified for the other perfluoroalkyls. Details of these studies are presented in the *Supporting Document for Epidemiological Studies for Perfluoroalkyls,* Table 5. Laboratory animal studies have evaluated potential alterations in hematological endpoints for a variety of perfluoroalkyls (Tables 2-1, 2-3, 2-4, 2-5, and 2-6). No studies examining hematological endpoints were identified for PFNA, PFHpA, or FOSA. Some laboratory animal studies have reported alterations in hematological indices following exposure to higher doses of PFOA, PFOS, PFHxS, PFDA, PFUnA, PFBS, PFBA, PFDoDA, or PFHxA.

PFOA

Epidemiological Studies. Information on effects on hematological parameters is available from a study of residents in the Little Hocking water district in southeastern Ohio where there was significant environmental exposure to PFOA via the water supply (Emmett et al. 2006b). No significant correlations between any of the hematology parameters evaluated (including hemoglobin, hematocrit, red blood cell indices, white cell count, and platelet count) and serum PFOA were observed, whether the analysis included all of the individuals as a group or separate analyses were done for adults or children. In an occupational study, the investigators reported no alterations in blood counts in workers, with a range of serum PFOA levels of 5–9,550 ng/mL (Sakr et al. 2007b). A second occupational exposure study found an inverse association between serum fluorine (used as a measure of PFOA exposure) and hemoglobin levels (Gilliland 1992); no alterations in mean corpuscular hemoglobin or volume were found. Although no associations were found for total leukocyte counts, an inverse association with lymphocyte count and association with monocyte counts was found.

Laboratory Animal Studies. No treatment-related hematological alterations were reported in male rats exposed intermittently nose-only to up to 84 mg/m^3 APFO dusts for 2 weeks (Kennedy et al. 1986). The specific parameters evaluated included erythrocyte counts, hemoglobin concentration, hematocrit, and differential leukocyte counts.

No significant hematological alterations were reported in male and female rats orally dosed with approximately 100–110 mg/kg/day APFO in diet for 90 days (Griffith and Long 1980). Similar results were reported in Cynomolgus monkeys treated daily with up to 20 mg/kg/day APFO administered via a capsule (Butenhoff et al. 2002; Thomford 2001) or in Rhesus monkeys dosed daily by gavage with up to 30 mg/kg/day (Griffith and Long 1980). In a 2-year dietary study in rats dosed with 1.5 or 15 mg/kg/day APFO, hematology tests performed at various times during the study showed changes in treated groups consisting of decreases in red blood cell counts, hemoglobin concentration, and hematocrit that were not always dose-related or consistent among sexes and were within acceptable ranges for the rat (3M 1983; Butenhoff et al. 2012c).

Hematology tests (erythrocyte count, hemoglobin concentration, hematocrit, total and differential leukocyte count, and red cell indices) conducted in blood from rats following intermittent dermal exposure to ≤2,000 mg/kg/day APFO for 2 weeks showed inconsistent alterations or changes of unlikely biological significance (Kennedy 1985).

PFOS

Epidemiological Studies. Two occupational exposure studies (Olsen et al. 1998a, 2003a) have examined the potential association between serum PFOS and hematological parameters (including hematocrit, hemoglobin, red blood cells, white blood cells, and platelets) in workers at 3M facilities in Decatur, Alabama and Antwerp, Belgium; mean measured levels of serum PFOS ranged from 800 to 2,440 ng/mL. No consistent alterations in hematological parameters were observed at either facility or at the different measuring time points.

Laboratory Animal Studies. Treatment of male and female rats with approximately 1.5–1.8 mg/kg/day PFOS (potassium salt) in the diet for 4 weeks did not result in significant alterations in hematological parameters (Seacat et al. 2003). Oral dosing with 1.3–1.6 mg/kg/day for 14 weeks resulted in a significant increase (45%) in non-segmented neutrophils (Seacat et al. 2003). The biological significance of this finding was not discussed by the investigators. In a 4-week study, oral administration of up to 2 mg/kg/day PFOS to Cynomolgus monkeys had no effect on hematological parameters (Thomford 2002a). In Cynomolgus monkeys dosed with 0, 0.03, 0.15, or 0.75 mg/kg/day PFOS (potassium salt) administered via a capsule for 26 weeks and subjected to comprehensive hematological tests during the study, the only significant effect was a 9% decrease in hemoglobin in 0.75 mg/kg/day males at

termination (Seacat et al. 2002). The investigators considered this a treatment-related effect, but not biologically significant given that the value was within the published range and there was no evidence of blood in the stools. No significant hematological effects were reported in a 2-year study in rats dosed with approximately 1.04 mg/kg/day PFOS in the diet (Butenhoff et al. 2012b; Thomford 2002b).

PFHxS

Laboratory Animal Studies. Treatment of male rats with doses ≥0.3 mg/kg/day PFHxS by gavage for at least 42 days significantly increased prothrombin time (Butenhoff et al. 2009a). Doses ≥1 mg/kg/day significantly decreased hemoglobin concentration, whereas ≥3 mg/kg/day decreased erythrocyte count and hematocrit; the decrease in hemoglobin (<5%) was not considered adverse at 1 mg/kg/day. Oral treatment of female rats with up to 10 mg/kg/day PFHxS did not significantly alter hematological parameters (Butenhoff et al. 2009a). No alterations in hematological parameters were observed in mice administered up to 3 mg/kg/day prior to mating and during mating, gestation, and lactation (Chang et al. 2018).

PFDA

Laboratory Animal Studies. Significant decrease in mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration were observed in rats administered 0.25 or 0.5 mg/kg/day for 28 days (Frawley et al. 2018). No other alterations in hematological parameters were observed. Hematological alterations were also not observed in mice receiving once weekly doses of 5 mg/kg PFDA for 4 weeks (Frawley et al. 2018).

PFUnA

Laboratory Animal Studies. Treatment of rats with 1.0 mg/kg/day PFUnA via gavage for 41–46 days resulted in significant hematological changes (Takahashi et al. 2014). Effects in males included decreased mean corpuscular volume (MCV) (5%), mean corpuscular hemoglobin (MCH) (5%), activated partial thromboplastin time (APTT) (16–25%), and fibrinogen (19–33%), and increased platelet counts (13%) and white blood cells (7%). In females, there were increases in MCV (10%) and MCH (10%) and a decrease in fibrinogen (32%). The NOAEL was 0.3 mg/kg/day.

PFBS

Laboratory Animal Studies. A 90-day exposure to PFBS resulted in significant decreases in hemoglobin and hematocrit levels in males orally administered 200 or 600 mg/kg/day, and a decrease in erythrocyte levels was observed in males administered 600 mg/kg/day; the NOAEL was 60 mg/kg/day (Lieder et al. 2009a). In contrast, no hematological alterations were observed in rats administered 900 mg/kg/day PFBS for 28 days (3M 2001).

PFBA

Laboratory Animal Studies. Administration of PFBA by gavage to rats in doses of up to 184 mg/kg/day for 5 days (3M 2007a) or up to 150 mg/kg/day for 28 days (Butenhoff et al. 2012a; van Otterdijk 2007a) did not result in significant alterations in hematological parameters. Oral doses of 30 mg/kg/day, but not 6 mg/kg/day, for 90 days resulted in significant reductions in red blood cell counts, hemoglobin, and hematocrit, and an increase in red cell distribution width in male rats (Butenhoff et al. 2012a; van Otterdijk 2007b). This dose level also caused a reduction in MCH and reduced MCH concentration in male rats. The lower hemoglobin and hematocrit observed in males were still detected at the end of a 3-week recovery period. These hematological effects were considered minor and not evidence of an adverse effect on red blood cell turnover by the investigator based on lack of alterations in bone marrow or the spleen.

PFDoDA

Laboratory Animal Studies. Gavage administration of 2.5 mg/kg/day for 42 days resulted in decreases in mean corpuscular volume and reticulocytes and increases in mean corpuscular hemoglobin concentration in male rats (Kato et al. 2015). In animals allowed to recover for 14 days, decreases in red blood cells, hemoglobin, hematocrit, and leukocyte levels and increases in reticulocytes were observed. In females administered 2.5 mg/kg/day for 42 days and allowed to recover for 14 days, decreases in hemoglobin, hematocrit, and mean corpuscular hemoglobin and increases in neutrophil levels were observed (Kato et al. 2015).

PFHxA

Laboratory Animal Studies. Several studies in rats have identified the hematological system as a target of PFHxA toxicity. Decreases in red blood cell counts, hemoglobin levels, and/or hematocrit levels and increases in reticulocyte levels have been observed in rats administered 315 mg/kg/day PFHxA for 32–44 days (Kirkpatrick 2005), 200 mg/kg/day NaPFHx for 90 days (Chengelis et al. 2009b), 500 mg/kg/day NaPFHx for 92–93 days (Loveless et al. 2009), or 200 mg/kg/day PFHxA for 104 weeks (Klaunig et al. 2015). A decrease in hemoglobin levels was also observed in rats administered 150 mg/kg/day PFHxA for 32–44 days (Kirkpatrick 2005). Hematological alterations were not observed at doses ≤100 mg/kg/day. Hematological alterations were only observed in female rats in the Klaunig et al. (2015) study and only in males in the Kirkpatrick (2005) study; sex-specific differences were not observed in the Chengelis et al. (2009b) or Loveless et al. (2009) intermediate-duration studies.

2.8 MUSCULOSKELETAL

Overview. Several epidemiological studies have evaluated possible associations between perfluoroalkyls and bone mineral density, risk of bone fractures, and risk of osteoarthritis; the results of these studies are summarized in Table 2-9, with more detailed descriptions presented in the *Supporting Document for Epidemiological Studies for Perfluoroalkyls*, Table 6. Several cross-sectional community and general population studies have found associations between serum PFOA and the risk of osteoarthritis, particularly in participants under the age of 55 years. However, associations were not found in a study of mostly male workers. Mixed results were found in studies of PFOS, with studies finding a decreased risk of osteoarthritis, increased risk in women under 50 years of age, or no association. One general population study found increased risks of osteoarthritis associated with serum PFHxS and PFNA. The data provide some suggestive evidence of a relationship between serum perfluoroalkyls and osteoarthritis. Assessing whether there is an association between perfluoroalkyl exposure and osteoarthritis is complicated by the lack of mechanistic data to support this association and it is noted that there are a number of factors that contribute to the osteoarthritis risk, and that some of these factors may be affected by perfluoroalkyls, including elevations in uric acid levels. Epidemiological information on bone mineral density is limited to a study of women and a study of children both examining PFOA, PFOS, PFHxS, and PFNA; the database was not considered adequate for assessing possible associations. No epidemiological studies evaluating musculoskeletal outcomes were identified for PFDA, PFUnA, PFHpA, PFBS, PFBA, PFDoDA, PFHxA, or FOSA. No morphological alterations were noted in bone or skeletal muscle in

Table 2-9. Summary of Skeletal Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFOA			
Steenland et al. 2015 Occupational (n=3,713 workers)	Estimated cumulative exposure	Osteoarthritis risk	NS (p=0.92 for trend), no lag NS (p=0.13 for trend), 10-year lag
Innes et al. 2011 Community (C8) (n=49,432 adults)	13.6–28.0 ng/mL (2nd PFOA quartile)	Osteoarthritis risk (physician diagnosed)	**OR 1.16 (1.03–1.31)*, 2nd quartile** **OR 1.22 (1.02–1.45)*, 2nd quartile participants <55 years of age**
Khalil et al. 2016 General population (NHANES) (n=1,914 participants)	3.7 ng/mL (mean PFOA)	Total femur neck mineral density	**β -0.017 (-0.033 to -0.001)*, women** β 0.001 (-0.025–0.022), men
		Osteoporosis risk (women)	**OR 1.84 (1.17–2.90; p=0.008)*, per ln-PFOA increase**
Khalil et al. 2018 General population (n=48 obese 8–12-year-old children)	0.99 ng/mL (mean serum PFOA)	Bone mineral density	NS (p>0.05)
Lin et al. 2014 General population (NHANES) (n=2,339 participants)	4.70 and 3.31 ng/mL (geometric mean PFOA in males and females)	Total lumbar spine bone mineral density	NS (p>0.01), premenopausal women, postmenopausal women, men
		Total hip bone mineral density	NS (p>0.01), premenopausal women, postmenopausal women, men
		All fracture types	OR 0.98 (0.75–1.28), premenopausal women OR 1.53 (0.63–3.74), postmenopausal women OR 0.84 (0.67–1.07), men
		Hip fracture	OR 1.59 (0.57–4.46), premenopausal women OR 0.48 (0.06–4.16), postmenopausal women OR 0.64 (0.39–1.06), men

Table 2-9. Summary of Skeletal Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
		Wrist fracture	OR 1.07 (0.65–1.77), premenopausal women OR 1.21 (0.46–3.13), postmenopausal women OR 1.12 (0.75–1.70), men
		Spine fracture	OR 1.83 (0.59–5.61), premenopausal women OR 0.84 (0.46–1.53), postmenopausal women OR 1.54 (0.85–2.79), men
Uhl et al. 2013 General population (NHANES) (n=1,888 male and 1,921 female adults)	>5.89 ng/mL (4th PFOA quartile)	Osteoarthritis risk (self-reported)	**OR 1.98 (1.24–3.19)*, 4th quartiles females** OR 0.82 (0.40–1.70), 4th quartile males **OR 4.95 (1.27–19.4)*, 4th quartile women 20–49 years of age** OR 1.33 (0.82–1.16), 4th quartile women 50–84 years of age
PFOS			
Innes et al. 2011 Community (C8) (n=49,432 adults)	≥29.4 ng/mL (4th PFOS quartile)	Osteoarthritis risk (physician diagnosed)	**OR 0.76 (0.68–0.85)*, 4th quartile**
Khalil et al. 2016 General population (NHANES) (n=1,914 participants)	12.7 ng/mL (mean PFOS)	Total femur neck mineral density	**β -0.016 (-0.029 to -0.002)*, women** **β -0.013 (-0.024 to -0.002)*, men**
		Osteoporosis risk (women)	OR 1.14 (0.68–1.94; p=0.619), per ln-PFOS increase
Khalil et al. 2018 General population (n=48 obese 8–12-year-old children)	2.79 ng/mL (mean serum PFOS)	Bone mineral density	NS (p>0.05)
Lin et al. 2014 General population (NHANES) (n=2,339 participants)	19.23 and 12.09 ng/mL (geometric mean PFOS in males and females)	Total lumbar spine bone mineral density	**β -0.022 (-0.038 to -0.007)*, premenopausal women** NS (p>0.01), postmenopausal women NS (p>0.01), men

Table 2-9. Summary of Skeletal Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
		Total hip bone mineral density	NS (p>0.01), premenopausal women, postmenopausal women, men
		All fracture types	OR 0.97 (0.75–1.24), premenopausal women OR 1.59 (0.88–2.86), postmenopausal women OR 0.92 (0.73–1.16), men
		Hip fracture	OR 1.12 (0.62–2.03), premenopausal women OR 0.83 (0.23–3.00), postmenopausal women OR 1.07 (0.76–1.52), men
		Wrist fracture	OR 1.04 (0.63–1.72), premenopausal women OR 1.22 (0.61–2.45), postmenopausal women OR 1.09 (0.72–1.66), men
		Spine fracture	OR 0.52 (0.15–1.86), premenopausal women OR 1.12 (0.26–4.78), postmenopausal women OR 1.27 (0.67–2.42), men
PFHxS			
Khalil et al. 2016 General population (NHANES) (n=1,914 participants)	2.5 ng/mL (mean PFHxS)	Total femur bone mineral density	**β -0.014 (-0.074 to -0.014)*, women** β -0.026 (-0.065–0.013), men
		Osteoporosis risk (women)	**OR 1.64 (1.14–2.38; p=0.008)*, per ln-PFHxS increase**
Khalil et al. 2018 General population (n=48 obese 8–12-year-old children)	1.09 ng/mL (mean serum PFHxS)	Bone mineral density	NS (p>0.05)

Table 2-9. Summary of Skeletal Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFNA			
Khalil et al. 2016 General population (NHANES) (n=1,914 participants)	1.9 ng/mL (mean PFNA)	Total femur bone mineral density	**β -0.040 (-0.077 to -0.003)*, women** β 0.007 (-0.031–0.045), men
		Osteoporosis risk (women)	**OR 1.45 (1.02–2.05; p=0.001)*, per ln-PFNA increase.**
Khalil et al. 2018 General population (n=48 obese 8–12-year-old children)	0.24 ng/mL (mean serum PFNA)	Bone mineral density	**Inverse association (p<0.05)*** NS (p>0.05) after adjustment for multiple testing

[a]See the *Supporting Document for Epidemiological Studies for Perfluoroalkyls,* Table 6 for more detailed descriptions of studies.
[b]Participants in occupational exposure may have also lived near the site and received residential exposure; community exposure studies involved subjects living near PFOA facilities with known exposure to high levels of PFOA.
[c]Asterisk indicates association with perfluoroalkyl; unless otherwise specified, values in parenthesis are 95% confidence intervals.

NHANES = National Health and Nutrition Examination Survey; NS = not significant; OR = odds ratio; PFHxS = perfluorohexane sulfonic acid; PFNA = perfluoro-nonanoic acid; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid

laboratory animals following exposure to PFOA, PFOS, PFHxS, PFBS, PFBA, or PFHxA; these data are summarized in Tables 2-1, 2-3, 2-4, and 2-5 and Figures 2-6, 2-8, 2-9, and 2-10. No laboratory animal data were available for PFNA, PFDA, PFUnA, PFHpA, PFDoDA, or FOSA.

PFOA

Epidemiological Studies. Several studies have examined the possible association between serum PFOA levels and the risk of osteoarthritis; the possible mechanisms associated with these findings have not been elucidated. In an occupational study (80% male), no association between estimated cumulative serum PFOA levels and the risk of osteoarthritis was found (Steenland et al. 2015). Innes et al. (2011) examined adult participants in the C8 Health Project and found that the odds of reporting osteoarthritis were higher in participants with serum PFOA levels in the 2nd, 3rd, and 4th quartiles compared to participants in the 1st quartile. When segregated by age and BMI, the strongest associations between serum PFOA levels and osteoarthritis were found in subjects under 55 years of age and in nonobese (BMI <30) subjects. Increases in the risk of osteoarthritis associated with serum PFOA levels were observed in female NHANES participants (Uhl et al. 2013); there were no associations in men. When stratified by age, the associations were found in women 20–49 years of age, but not in older women (50–84 years old) (Uhl et al. 2013). An association between increases in risk of osteoporosis and serum PFOA levels was found in another study of female NHANES participants (Khalil et al. 2016). Two studies of adult NHANES participants found no associations between serum PFOA and bone mineral density of the total femur (Khalil et al. 2016), hip (Lin et al. 2014), or lumbar spine (Khalil et al. 2016; Lin et al. 2014); however, an inverse association was found in the neck portion of the femur in the Khalil et al. (2016) study. A study in obese children did not find an association between serum PFOA levels and measures of bone mineral density (Khalil et al. 2018). Additionally, Lin et al. (2014) did not find associations between serum PFOA levels and the risk of bone fractures (total fractures, hip fractures, wrist fractures, or spine fractures) in premenopausal women, postmenopausal women, or men.

Laboratory Animal Studies. In male rats exposed head-only to up to 84 mg/m^3 APFO dusts for up to 2 weeks, examinations of the sternebrae were unremarkable (Kennedy et al. 1986). Similarly, no gross or microscopic alterations were reported in the sternum from rats following dietary exposure to 100–110 mg/kg/day APFO for 90 days (Griffith and Long 1980) or in the femur, sternum, or thigh skeletal muscle from Cynomolgus monkeys dosed with up to 20 mg/kg/day APFO administered via a capsule for 26 weeks (Butenhoff et al. 2002). *In utero* exposure to 0.3 mg/kg/day PFOA resulted in morphometrical alterations in the femur (increases in the periosteal area) and decreases in bone mineral density in the tibia

of 13- or 17-month-old mice (Koskela et al. 2016). No alterations in biomechanical properties were found.

PFOS

Epidemiological Studies. Several epidemiological studies have evaluated the potential of PFOS to induce skeletal damage. In the participants of the C8 Health Study, a decreased risk of osteoarthritis was found in participants with serum PFOS levels in the 2nd, 3rd, and 4th quartiles (Innes et al. 2011). In contrast, Uhl et al. (2013) found an increased risk of osteoarthritis in NHANES participants with serum levels of >20.97 ng/mL. When categorized by sex and age, the osteoarthritis risk was approximately 5 times higher in women aged 20–49 years with serum PFOS levels in the 4th quartile. Another study of NHANES participants (Khalil et al. 2016) did not find an increased risk of osteoporosis in women. However, the study did find an inverse association between serum PFOS and femur neck bone mineral density, but no associations with total femur or lumbar spine bone mineral density. No associations between serum PFOS levels and measures of bone mineral density were observed in a study of obese children (Khalil et al. 2018).

Laboratory Animal Studies. Treatment of monkeys with up to 0.75 mg/kg/day PFOS (potassium salt) administered via a capsule for 26 weeks had no significant effect on the gross or microscopic appearance of the femur, sternum, or thigh skeletal muscle (Seacat et al. 2002). Similar observations were made in rats treated with up to 1.04 mg/kg/day PFOS in the diet for 2 years (Butenhoff et al. 2012b; Thomford 2002b).

PFHxS

Epidemiological Studies. A study of NHANES participants found an increase in the risk of osteoporosis among women that was associated with serum PFHxS levels (Khalil et al. 2016). An inverse association between serum PFHxS (fourth quartile) and total femur bone mineral density was also found in women. There were no associations between serum PFHxS and femur neck or lumbar spine bone mineral density (Khalil et al. 2016). In contrast, no association between serum PFHxS levels and bone mineral density were observed in obese children (Khalil et al. 2018).

Laboratory Animal Studies. No histological alterations were observed in bone or muscle of mice administered up to 3 mg/kg/day prior to mating and during mating, gestation, and lactation periods (Chang et al. 2018).

PFNA

Epidemiological Studies. Khalil et al. (2016) found an increase in the risk of osteoporosis in women NHANES participants that was associated with serum PFNA levels. Increasing serum PFNA levels did not result in alterations in bone mineral density of the lumbar spine or femur neck, but was inversely associated with total femur bone mineral density in women with serum PFNA levels in the fourth quartile. A study of 48 obese children found an inverse association between serum PFNA levels and bone mineral density; however, the association was no longer significant after adjusting for multiple testing (Khalil et al. 2018).

PFBS

Laboratory Animal Studies. Treatment of rats with up to 900 mg/kg/day PFBS by gavage for 28 days (3M 2001) or 90 days (Lieder et al. 2009a) did not induce morphological alterations in skeletal muscle.

PFBA

Laboratory Animal Studies. PFBA administered to rats by gavage in doses of up to 184 mg/kg/day for 5 days did not induce morphological alterations in skeletal muscle (3M 2007a). Administration of 150 mg/kg/day PFBA for 28 days or 30 mg/kg/day for 90 days did not induce gross or microscopic alterations in bone (femur and sternum) or skeletal muscle (Butenhoff et al. 2012a; van Otterdijk 2007a, 2007b).

PFHxA

Laboratory Animal Studies. An intermediate-duration gavage study did not find histological alterations in the bone or muscle of rats administered up to 200 mg/kg/day NaPFHx for 90 days (Chengelis et al. 2009b).

2.9 HEPATIC

Overview. Epidemiological studies on perfluoroalkyls have examined three potential hepatic outcomes: liver disease, alterations in serum enzyme and bilirubin levels, and alterations in serum lipid levels. Summaries of the epidemiological studies examining these outcomes are presented in Tables 2-10, 2-11, and 2-12, with more detailed descriptions presented in the *Supporting Document for Epidemiological Studies for Perfluoroalkyls*, Table 7. There are limited epidemiological data on potential associations between serum perfluoroalkyls and risk of liver disease. Occupational exposure and community studies did not find increased risk of liver disease associated with PFOA or PFOS. As assessed by serum enzyme and bilirubin levels, the epidemiological studies provide suggestive evidence of liver damage. Increases in aspartate aminotransferase (AST), alanine aminotransferase (ALT), and gamma-glutamyl transferase (GGT) levels and decreases in serum bilirubin levels have been reported in occupational, community, and/or general population studies. These increases in serum enzyme levels, particularly ALT, are associated with increasing levels of PFOA, PFOS, and PFHxS; it is noted that there is considerable variability across studies and not all of the studies adjusted for potential confounders. No consistent results were found for PFNA. The results of available epidemiological studies suggest associations between increases in serum lipids, particularly total cholesterol and LDL cholesterol, and serum PFOA, PFOS, PFNA, and PFDA. For PFHxS, PFUnA, PFHpA, PFBS, PFBA, and PFDoDA, there are too few studies or the results are too inconsistent to determine if they also would affect serum lipid levels at environmental exposure levels. No epidemiological studies examining hepatic endpoints were identified for PFHxA or FOSA.

Numerous animal studies have evaluated the hepatotoxicity of perfluoroalkyls following inhalation, oral, and dermal exposure; summaries of these studies are presented in Tables 2-1, 2-2, 2-3, 2-4, 2-5, and 2-6 and the NOAEL and LOAEL values are graphically presented in Figures 2-6, 2-7, 2-8, 2-9, and 2-10. No laboratory animal studies were identified for PFHpA.

The results of these studies provide strong evidence that the liver is a sensitive target of PFOA, PFOS, PFHxS, PFNA, PFDA, PFUnA, PFBS, PFBA, PFDoDA, and PFHxA toxicity. Observed effects in rodents include increases in liver weight; hepatocellular hypertrophy, hyperplasia, and necrosis; and decreases in serum cholesterol and triglyceride levels. As discussed in greater detail in Section 2.20, these effects are believed to be initiated by PPARα; however, studies in PPARα-null mice suggest that other mechanisms are also involved. Increases in liver weight have also been observed in monkey studies for PFOA and PFOS; these studies have also found alterations in serum lipid levels and hepatocellular hypertrophy (PFOS only).

Table 2-10. Summary of Liver Disease in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFOA			
Anderson-Mahoney et al. 2008 Community (n=566)	NR	Liver problems (self-reported)	SPR 1.01 (0.64–1.59)
Darrow et al. 2016 Community (C8) (n=28,831)	Estimated cumulative 16.5 ng/mL (median 2005/2006)	Liver disease	HR 0.97 (0.92–1.03), no lag per ln increase in PFOA HR 0.98 (0.93–1.04), 10-year lag
		Enlarged liver, fatty liver, or cirrhosis	HR 0.97 (0.91–1.04), no lag per ln increase in PFOA HR 1.00 (0.94–1.07), 10-year lag
Steenland et al. 2015 Occupational (n=3,713)	Estimated cumulative	Non-hepatitis liver disease risk	NS (p=0.86), no lag NS (p=0.40), 10-year lag
PFOS			
Alexander et al. 2003 Occupational (n=2,083)	NR	Liver cirrhosis deaths	SMR 0.81 (0.10–2.94)
Grice et al. 2007 Occupational (n=1,400)	1,300–1,970 ng/mL (high potential workers)	Liver disease	OR 1.21 (0.56–2.60)
		Cholelithiasis	OR 0.91 (0.57–1.46)
		Cholecystitis	OR 1.15 (0.65–2.06)

Table 2-10. Summary of Liver Disease in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Olsen et al. 2004a Occupational (n=652 exposed, n=659 for non-exposed)	NR	Cholelithiasis or acute cholecystitis	**RRE_pC 8.6 (1.1–>100)*** **RRE_pC 25 (2.1–>100)*, workers with ≥10 years high exposure potential**
		Liver disease	RRE_pC 1.2 (0.2–8.6)
		Biliary duct disorders	RRE_pC 1.6 (0.8–2.9) **RRE_pC 2.6 (1.2–5.5)*, workers with ≥10 years high exposure potential**

[a]See the *Supporting Document for Epidemiological Studies for Perfluoroalkyls,* Table 7 for more detailed descriptions of studies.
[b]Participants in occupational exposure may have also lived near the site and received residential exposure; community exposure studies involved subjects living near PFOA facilities with known exposure to high levels of PFOA.
[c]Asterisk indicates association with perfluoroalkyl; unless otherwise specified, values in parenthesis are 95% confidence intervals.

HR = hazard ratio; NR = not reported; NS = not significant; OR = odds ratio; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid; RRE_pC = risk ratio episode of care; SMR = standardized mortality ratio; SPR = standard prevalence ratio

Table 2-11. Summary of Alterations in Serum Hepatic Enzymes and Bilirubin Levels in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFOA			
Costa et al. 2009 Occupational (n=37 current workers; n=16 former workers; n=107 non-exposed workers)	12,930 ng/mL (mean PFOA current workers) 6,810 ng/mL (mean former workers)	AST	NS (p>0.05) (34 current workers)
		ALT	NS (p>0.05) (34 current workers) **Association (p<0.01)* (56 current, former, non-exposed workers)**
		GGT	NS (p>0.05) (34 current workers) **Association (p<0.01)* (56 current, former, non-exposed workers)**
		Total bilirubin	**Inverse association (p<0.01)* (56 current, former, non-exposed workers)**
Gilliland 1992; Gilliland and Mandel 1996 Occupational (n=115)	NR (serum fluorine levels used as surrogate for serum PFOA)	ALT	NS (p=0.32)
		AST	NS (p=0.80)
		GGT	NS (p=0.81)
Olsen et al. 2000 Occupational (n=111, 80, and 74 in 1993, 1995, and 1997)	5,000, 6,400, and 6,400 ng/mL (mean PFOA in 1993, 1995, and 1997) Workers divided into three groups: 0–<1,000, 1,000–<10,000, and ≥10,000 ng/mL	ALT	NS (p=0.82, 0.30, 0.73) differences between exposure groups for each measurement period
		AST	NS (p=0.33, 0.45, 0.83) differences between exposure groups for each measurement period
		GGT	NS (p=0.24, 0.41, 0.78) differences between exposure groups for each measurement period
		Total bilirubin	NS (p=0.48, 0.11, 0.58) differences between exposure groups for each measurement period
		Direct bilirubin	NS (p=0.82, 0.05, 0.74) differences between exposure groups for each measurement period

Table 2-11. Summary of Alterations in Serum Hepatic Enzymes and Bilirubin Levels in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Olsen and Zobel 2007 Occupational (n=552)	2170 ng/mL (mean 8th PFOA decile) 12,150 ng/mL (mean 10th PFOA decile)	GGT Elevated GGT	**Association (p=0.05)*** OR 1.0 (0.3–2.9), 10th decile
		Total bilirubin	**Inverse association (p=0.001)***
		ALT Elevated ALT	NS (p=0.06) OR 1.2 (0.5–3.4), 10th decile
		AST	NS (p=0.55)
Sakr et al. 2007a Occupational (n=454)	1,130 ng/mL (mean PFOA)	Total bilirubin	**Association (p=0.006)***
		AST	**Association (p=0.009)***
		ALT	NS (p>0.05)
		GGT	NS (p>0.05)
Sakr et al. 2007b Occupational (n=1,025)	428 ng/mL (mean PFOA)	GGT	**Association (p=0.016)***
		AST	NS (p=0.317)
		ALT	NS (p=0.124)
		Bilirubin	NS (p=0.590)
Wang et al. 2012 Occupational (n=55)	2,157.74 ng/mL (mean PFOA)	AST	**Association (p=0.02)***
		ALT	NS (p=0.38)
Darrow et al. 2016 Community (C8) (n=28,831)	Estimated cumulative 16.5 ng/mL (median PFOA in 2005/2006)	ALT	**Association (p<0.0001 for trend)*, estimated cumulative levels** **Association (p<0.0001 for trend)*, 2005/2006 levels**
		GGT	NS (p=0.1021), cumulative levels NS (p=0.1552), 2005/2006 levels
		Bilirubin	**Inverse association (p=0.0029 for trend)*, estimated cumulative levels** **Inverse association (p=0.0036 for trend)*, 2005/2006 levels**

Table 2-11. Summary of Alterations in Serum Hepatic Enzymes and Bilirubin Levels in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Emmett et al. 2006b Community (n=371)	354 ng/mL (median PFOA)	ALT Abnormal ALT	NS (p>0.05) NS (p>0.05)
		AST Abnormal AST	NS (p>0.05) **Inverse association (p=0.03)***
		GGT Abnormal GGT	NS (p>0.05) NS (p>0.05)
Gallo et al. 2012 Community (C8) (n=46,452)	NR	ALT Abnormal ALT	**Correlation (p<0.001)*** **OR 1.19 (1.03–1.37)*, 3rd decile**
		GGT Abnormal GGT	**Correlation (p<0.001)*** NS (p=0.213 for trend)
		Direct bilirubin Abnormal bilirubin	NS (p>0.05) NS (p=0.496 for trend)
Wang et al. 2012 Community (n=132)	378.30 ng/mL (mean PFOA)	ALT	NS (p=0.05)
		AST	NS (p=0.22)
Gleason et al. 2015 General population (NHANES) (n=4,333)	3.7 ng/mL (median PFOA)	ALT	**Association (p<0.001)***
		Elevated ALT	**Association (p=0.007 for trend)***
		AST	**Association (p<0.01)***
		Elevated AST	NS (p=0.058 for trend).
		GGT	**Association (p<0.01)***
		Elevated GGT	**Association (p=0.042 for trend)***
		Total bilirubin	**Association (p<0.01)***
		Elevated bilirubin	**Association (p<0.001 for trend)***
Lin et al. 2010 General population (NHANES) (n=2,216)	5.05 and 4.06 ng/mL (geometric mean PFOA in males and females)	ALT	**Association (p=0.005)***
		GGT	**Association (p=0.019)***
		Total bilirubin	NS (p=0.645)
Yamaguchi et al. 2013 General population (n=608)	2.1 ng/mL (mean PFOA)	ALT	**Association (p=0.02)***
		AST	**Association (p=0.001)***
		GGT	**Association (p=0.03)***

Table 2-11. Summary of Alterations in Serum Hepatic Enzymes and Bilirubin Levels in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFOS			
Grice et al. 2007 Occupational (n=1,400)	1,300–1,970 ng/mL (high potential workers)	Cholelithiasis	OR 0.91 (0.57–1.46)
		Cholecystitis	OR 1.15 (0.65–2.06)
Olsen et al. 1999 Occupational (n=178 in 1995; n=149 in 1997)	2,440 and 1,930 ng/mL (mean PFOS in 1995 in Decatur and Antwerp) 1,960 and 1,480 ng/mL (mean PFOS in 1997 in Decatur and Antwerp)	AST	NS (p=0.14 for trend), 1995 NS (p=0.67 for trend), 1997
		ALT	NS (p=0.38 for trend), 1995 NS (p=0.46 for trend), 1997
		GGT	NS (p=0.71 for trend), 1995 NS (p=0.34 for trend), 1997
Olsen et al. 2003a Occupational (n=518)	2460 ng/mL (median 4th PFOS quartile)	AST	NS (p>0.05), no adjustments
		ALT Risk of abnormal ALT	**Higher levels (p<0.05)*, males only with no adjustments** OR 2.1 (0.6–7.3)
		GGT Risk of abnormal GGT	**Difference (p<0.05)*, females only with no adjustments** OR 2.0 (0.7–5.8)
Gallo et al. 2012 Community (C8) (n=46,452)	NR	ALT Abnormal ALT	**Correlation (p<0.001)*** **OR 1.19 (1.04–1.37)*, 5th decile**
		GGT Abnormal GGT	NS (p>0.05) **Association (p=0.047 for trend)***
		Direct bilirubin Abnormal bilirubin	**Correlation (p<0.001)*** **Association (p=0.015 for trend)***

Table 2-11. Summary of Alterations in Serum Hepatic Enzymes and Bilirubin Levels in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Gleason et al. 2015 General population (NHANES) (n=4,333)	11.3 ng/mL (median PFOS)	ALT	NS (p>0.01)
		Elevated ALT	NS (p=0.370 for trend)
		AST	NS (p>0.01)
		Elevated AST	NS (p=0.438 for trend)
		GGT	NS (p>0.01)
		Elevated GGT	NS (p=0.654 for trend)
		Total bilirubin	NS (p>0.01)
		Elevated bilirubin	**Association (p=0.028 for trend)***
Lin et al. 2010 General population (NHANES) (n=2,216)	27.39 and 22.20 ng/mL (geometric mean PFOS in males and females)	ALT	NS (p=0.066)
		GGT	NS (p=0.808)
		Total bilirubin	NS (p=0.223)
Yamaguchi et al. 2013 General population (n=608)	5.8 ng/mL (mean PFOS)	ALT	**Association (p=0.03)***
		AST	**Association (p=0.01)***
		GGT	**Association (p=0.03)***
PFHxS			
Gleason et al. 2015 General population (NHANES) (n=4,333)	1.8 ng/mL (median PFHxS)	ALT	**Association (p<0.01)***
		Elevated ALT	NS (p=0.484 for trend)
		AST	**Association (p<0.001)***
		Elevated AST	NS (p=0.230 for trend)
		GGT	NS (p>0.01)
		Elevated GGT	NS (p=0.415 for trend)
		Total bilirubin	**Association (p<0.01)***
		Elevated bilirubin	**Association (p=0.041 for trend)***
Lin et al. 2010 General population (NHANES) (n=2,216)	2.29 and 1.72 ng/mL (geometric mean PFHxS in males and females)	ALT	NS (p=0.691)
		GGT	NS (p=0.898)
		Total bilirubin	NS (p=0.063)

Table 2-11. Summary of Alterations in Serum Hepatic Enzymes and Bilirubin Levels in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFNA			
Mundt et al. 2007 Occupational (n=592)	NR	ALT	NS, longitudinal analysis
		AST	NS, longitudinal analysis
		GGT	NS, longitudinal analysis
		Bilirubin	NS, longitudinal analysis
Gleason et al. 2015 General population (NHANES) (n=4,333)	1.4 ng/mL (median PFNA)	ALT	**Association (p<0.001)***
		Elevated ALT	NS (p=0.042 for trend)
		AST	NS (p>0.01)
		Elevated AST	NS (p=0.516 for trend)
		GGT	**Association (p<0.01)***
		Elevated GGT	NS (p=0.126 for trend)
		Total bilirubin	NS (p>0.01)
		Elevated bilirubin	NS (p=0.614 for trend)
Lin et al. 2010 General population (NHANES) (n=2,216)	0.89 and 0.72 ng/mL (geometric mean PFNA in males and females)	ALT	NS (p=0.131)
		GGT	NS (p=0.857)
		Total bilirubin	NS (p=0.053)

[a]See the *Supporting Document for Epidemiological Studies for Perfluoroalkyls,* Table 7 for more detailed descriptions of studies.
[b]Participants in occupational exposure may have also lived near the site and received residential exposure; community exposure studies involved subjects living near PFOA facilities with known exposure to high levels of PFOA.
[c]Asterisk indicates association with perfluoroalkyl; unless otherwise specified, values in parenthesis are 95% confidence intervals.

ALT = alanine aminotransferase; AST = aspartate aminotransferase; GGT = gamma-glutamyl transferase; OR = odds ratio; NHANES = National Health and Nutrition Examination Survey; NR = not reported; NS = not significant; PFHxS = perfluorohexane sulfonic acid; PFNA = perfluorononanoic acid; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid

Table 2-12. Summary of Serum Lipid Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFOA			
Costa 2004 Occupational (n=35)	NR	Total cholesterol	**Association (p=0.03)***
		Non-HDL cholesterol	**Association (p=0.03)***
		HDL cholesterol	NS (p>0.05)
		LDL cholesterol	NS (p>0.05)
		Total triglycerides	NS (p>0.05)
Costa et al. 2009 Occupational (n=37 current workers; n=16 former workers; n=107 non-exposed workers)	12,930 ng/mL (mean PFOA in current workers) 6,810 ng/mL (mean PFOA in former workers)	Total cholesterol	**Association (p=0.005)* (current workers)** **Association (p<0.05)* (56 current, former, non-exposed workers)**
		HDL cholesterol	NS (p>0.05) (34 current workers)
		Triglycerides	NS (p>0.05)
Gilliland 1992; Gilliland and Mandel 1996 Occupational (n=115)	NR (serum fluorine levels used as surrogate for serum PFOA)	Total cholesterol	NS (p=0.62)
		Total LDL	NS (p=0.87)
		Total HDL	NS (p=0.66)
Olsen et al. 2000 Occupational (n=111, 80, and 74 in 1993, 1995, and 1997)	5,000, 6,400, and 6,400 ng/mL (mean PFOA in 1993, 1995, and 1997) Workers divided into three groups: 0–<1,000, 1,000–<10,000, and ≥10,000 ng/mL	Total cholesterol	NS (p=0.45, 0.48, 0.08) differences between exposure groups for each measurement period
		LDL cholesterol	NS (p=0.84, 0.96, 0.11) differences between exposure groups for each measurement period
		HDL cholesterol	NS (p=0.32, 0.70, 0.40) differences between exposure groups for each measurement period
		Triglycerides	NS (p=0.77, 0.07, 0.13) differences between exposure groups for each measurement period

Table 2-12. Summary of Serum Lipid Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Olsen and Zobel 2007 Occupational (n=552)	2,170 ng/mL (mean of 8th PFOA decile) 12,150 ng/mL (mean of 10th PFOA decile)	Total cholesterol	NS (p=0.20)
		Elevated total cholesterol	OR 1.1 (0.5–2.6), 10th decile
		LDL cholesterol	NS (p=0.81)
		Elevated LDL cholesterol	OR 1.2 (0.5–2.8), 10th decile
		HDL cholesterol	**Association (p=0.01)***
		Decreased HDL cholesterol	OR 1.8 (0.7–4.8), 10th decile
		Triglycerides	**Association (p=0.0001)***
		Elevated triglycerides	OR 1.8 (0.8–4.4), 10th decile
Sakr et al. 2007a Occupational (n=454)	1,130 ng/mL (mean PFOA)	Total cholesterol	**Association (p=0.011)***
		LDL cholesterol	NS (p>0.05)
		HDL cholesterol	NS (p>0.05)
		Triglycerides	NS (p>0.05)
		Total bilirubin	**Association (p=0.006)***
Sakr et al. 2007b Occupational (n=1,025)	428 ng/mL (mean PFOA)	Total cholesterol	**Association (p=0.002)***
		LDL cholesterol	**Association (p=0.008)***
		VLDL cholesterol	**Association (p=0.031)***
		HDL cholesterol	NS (p=0.680)
		Triglycerides	NS (p=0.384)
Steenland et al. 2015 Occupational (n=3,713)	Estimated cumulative PFOA	Elevated cholesterol	NS (p=0.56), no lag NS (p=0.62), 10-year lag
Wang et al. 2012 Occupational (n=55)	2,157.74 ng/mL (mean PFOA)	Total cholesterol	NS (p=0.36)
		LDL cholesterol	NS (p=0.43)
		HDL cholesterol	**Inverse association (p=0.01)***
		Triglycerides	NS (p=0.37)
Emmett et al. 2006b Community (n=371)	354 ng/mL (median PFOA)	Total cholesterol Abnormal cholesterol	NS (p>0.05) NS (p>0.05)

Table 2-12. Summary of Serum Lipid Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Fitz-Simon et al. 2013 Community (C8) (n=560 adults)	140.1 and 68.2 ng/mL (mean PFOA at first and second examinations)	Total cholesterol	**-1.65% (-0.32 to -2.97)*, 50% decrease in PFOA**
		LDL cholesterol	**-3.58% (-1.47 to -5.66)*, 50% decrease in PFOA**
		HDL cholesterol	-1.33% (0.21 to -2.85), 50% decrease in PFOA
		Triglycerides	0.78% (5.34 to -3.58), 50% decrease in PFOA
Frisbee et al. 2010 Community (C8) (n=12,476 children and adolescents)	77.7 ng/mL (mean PFOA in children) 61.8 ng/mL (mean PFOA in adolescents)	Total cholesterol	**Association (p<0.001)*, children 5th quintile** **Association (p<0.001)*, adolescents 5th quintile**
		Abnormal cholesterol	OR 1.1 (1.0–1.3), 2nd quintile
		LDL cholesterol	**Association (p=0.001)*, children 5th quintile** **Association (p=0.004)*, adolescents 5th quintile**
		Abnormal LDL levels	OR 1.2 (1.0–1.5), 2nd quintile
		HDL cholesterol	NS (p=0.88), children 5th quintile NS (p=0.20), adolescents 5th quintile
		Triglycerides	NS (p=0.1), children 5th quintile NS (p=0.1), adolescents 5th quintile
Steenland et al. 2009b Community (C8) (n=46,294)	80.3 ng/mL (mean PFOA) 13.2–26.5 ng/mL (2nd PFOA quartile)	Total cholesterol	**Association (p<0.001 for trend)***
		Abnormal cholesterol	**OR 1.21 (1.12–1.31)*, 2nd quartile**
		LDL cholesterol	**Association (p<0.05 for trend)***
		HDL cholesterol	NS (p>0.05)
		Triglycerides	**Association (p<0.05 for trend)***

Table 2-12. Summary of Serum Lipid Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Wang et al. 2012 Community (n=132)	378.30 ng/mL (mean PFOA)	Total cholesterol	NS (p=0.85)
		LDL cholesterol	NS (p=0.97)
		Triglycerides	NS (p=0.73)
		HDL cholesterol	NS (p=0.39)
Winquist and Steenland 2014a Community (C8) (n=28,541)	142–<234 ng/mL (estimated 2nd quintile for cumulative PFOA)	Hypercholesterolemia	**HR 1.24 (1.15–1.33)*, 2nd quintile for estimated cumulative exposure**
Eriksen et al. 2013 General population (n=753)	7.1 ng/mL (mean PFOA)	Total cholesterol	**Association (p=0.01)***
Fisher et al. 2013 General population (n=2,368)	2.46 ng/mL (mean PFOA)	Total cholesterol	NS (p=0.22)
		High cholesterol levels	OR 1.5 (0.86–2.62), 4th quartile
		Non HDL cholesterol	NS (p=0.13)
		LDL cholesterol	NS (p=0.63)
		HDL cholesterol	NS (p=0.96)
Fu et al. 2014a General population (n=133)	1.43 ng/mL (median PFOA)	Total cholesterol	**Association (p=0.015)***
		Elevated cholesterol	OR 0.55 (0.09–3.31)
		LDL cholesterol	**Association (p=0.022)***
		Elevated LDL	OR 0.71 (0.14–3.49)
		HDL cholesterol	NS (p=0.260)
		Elevated HDL	OR 0.67 (0.13–3.51)
		Triglycerides	NS (p=0.298)
		Elevated triglyceride	OR 1.97 (0.59–6.55)

Table 2-12. Summary of Serum Lipid Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Geiger et al. 2014b General population (NHANES) (n=815 12–18-year-old adolescents)	4.2 ng/mL (mean PFOA)	Total cholesterol	**Association (p=0.0170 for trend)***
		Elevated cholesterol	**OR 1.44 (1.11–1.88, p=0.0253 for trend)*, log transformed PFOA**
		LDL cholesterol	**Association (p=0.0027 for trend)***
		Elevated LDL	NS (p=0.0539 for trend)
		HDL cholesterol	NS (p=0.1769 for trend)
		Decreased HDL	NS (p=0.1493 for trend)
		Triglycerides	NS (p=0.9943 for trend)
		Elevated triglycerides	NS (p=0.5975 for trend)
Kang et al. 2018 General population (n=150 children ages 3–18 years)	1.88 ng/mL (median serum PFOA)	Total cholesterol	β -2.256 (-11.490–6.978, p=0.630)
		LDL cholesterol	β 3.899 (-4.810–12.608, p=0.377)
		Triglycerides	β 0.020 (-0.134–0.175, p=0.796)
Koshy et al. 2017 General population (WTCHR, n=180 children; n=222 children in comparison group)	1.81 and 1.39 ng/mL (median serum PFOA in WTCHR group and comparison group)	Total cholesterol	**β 0.09 mg/dL (0.04–0.14, p<0.001)***
		LDL cholesterol	**β 0.11 mg/dL (0.03–0.19, p=0.006)***
		HDL cholesterol	β 0.04 mg/dL (-0.04–0.12, p=0.34)
		Triglycerides	**β 0.14 mg/dL (0.02 to 0.27, p=0.03)***
Liu et al. 2018b General population (NHANES, n=1,871 adults)	1.86 ng/mL (geometric mean serum PFOA)	Total cholesterol	Association (p<0.05)
		LDL cholesterol	NS (p>0.05)
		HDL cholesterol	Association (p<0.01)
		Triglycerides	NS (p>0.05)
Maisonet et al. 2015a General population (n=111 for 7-year-old and n=88 for 15-year-old girls	1.1–3.1, 3.2–4.4, and 4.5–16.4 ng/mL (maternal PFOA for 1st, 2nd, and 3rd tertiles)	Total cholesterol in 7-year-olds	**Association (β 13.75, 0.05–27.45)*, 1st tertile** NS, 2nd and 3rd tertiles
		Total cholesterol in 15-year-olds	**Association (β 17.19, 0.405–33.93)*, 1st tertile** NS, 2nd and 3rd tertiles
		LDL cholesterol in 7-year-olds	**Association (β 14.01, 3.26–24.76)*, 1st tertile** NS, 2nd and 3rd tertiles

Table 2-12. Summary of Serum Lipid Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
		LDL cholesterol in 15-year-olds	**Association (β 14.261, 0.25–28.26)*, 1st tertile** NS, 2nd and 3rd tertiles
		HDL cholesterol	NS (β -0.40, -1.82–1.01), 3rd tertile 7-year-olds NS (β -0.520, -2.10–1.06), 3rd tertile 15-year-olds
		Triglycerides	NS (β -0.020, -0.068–0.029), 3rd tertile, 7-year-olds NS (β -0.013, -0.051–0.025), 3rd tertile, 15-year-olds
Manzano-Salgado et al. 2017b General population (n=1,230 children; evaluated at 4 years of age)	2.32 ng/mL (maternal geometric mean PFOA)	Total cholesterol	β -0.02 (-0.10–0.15)
		LDL cholesterol	β 0.03 (-0.12–0.21)
		HDL cholesterol	β -0.04 (-0.15–0.08)
		Triglycerides	β -0.01 (-0.17–0.16)
Nelson et al. 2010 General population (NHANES) (n=860)	4.6 ng/mL (mean PFOA)	Total cholesterol	NS (p=0.07)
		LDL cholesterol	NS (p=0.84)
		Non-HDL cholesterol	**Association (p=0.05)* β 1.38 (0.12–2.65), per ng/mL increase in PFOA**
		HDL cholesterol	NS (p=0.34)
Skuladottir et al. 2015 General population (n=854 pregnant women)	4.1 ng/mL (mean PFOA)	Total cholesterol	**Association (p=0.01 for trend)***

Table 2-12. Summary of Serum Lipid Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Starling et al. 2014a General population (n=854 pregnant women)	2.25 ng/mL (50th PFOA percentile)	Total cholesterol	NS (β 2.58; -4.32–9.47), per ln-unit increase in PFOA
		LDL cholesterol	NS (β 0.35 -3.97–8.48), per ln-unit increase in PFOA
		HDL cholesterol	**Association (β 3.42 0.56–6.28)*, 4th quartile**
		Triglycerides	NS (β 0.00 (-0.07–0.06), per ln-unit increase in PFOA
Timmermann et al. 2014 General population (n=499 children, 8–10 years old)	9.3 ng/mL (median PFOA)	Triglycerides	NS (p=0.91), normal weight children **Association (p=0.002)*, obese children**
Yang et al. 2018 General population (n=148 men; 81 diagnosed with metabolic syndrome)	1.90 ng/mL (median serum PFOA)	HDL cholesterol	β 0.15 (-0.17–0.46)
		Triglycerides	**β 2.3 (0.77–8.38)***
Zeng et al. 2015 General population (n=225 adolescents, 12–15 years old)	1.1 and 0.92 ng/mL (mean PFOA in boys and girls)	Total cholesterol	**Association (p=0.001)***
		LDL cholesterol	**Association (p=0.002)***
		HDL cholesterol	NS (p=0.06)
		Triglycerides	**Association (p<0.001)***
PFOS			
Olsen et al. 1999 Occupational (n=178 in 1995; n=149 in 1997)	2,440 and 1,930 ng/mL (mean PFOS in 1995 in Decatur and Antwerp) 1,960 and 1,480 ng/mL (mean in 1997 in Decatur and Antwerp)	Total cholesterol	NS (p=0.96 for trend), 1995 **Association (p=0.006 for trend)*, 1997**
		LDL cholesterol	NS (p=0.87 for trend), 1995 **Association (p=0.01 for trend)*, 1997**
		HDL cholesterol	**Inverse association (p=0.04 for trend)*, 1995** NS (p=0.34) 1997
		Triglycerides	NS (p=0.35 for trend), 1995 NS (p=0.67 for trend), 1997

Table 2-12. Summary of Serum Lipid Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Olsen et al. 2003a Occupational (n=518)	2,460 ng/mL (median 4th PFOS quartile)	Total cholesterol	NS (p>0.05), no adjustments **Association (p=0.04)*, with adjustments**
		HDL cholesterol	NS (p>0.05), no adjustments
		Triglycerides	**Higher levels (p<0.05)*, males only with no adjustments** **Association (p=0.01)*, with adjustments**
Frisbee et al. 2010 Community (C8) (n=12,476 children and adolescents)	23.6 ng/mL (mean PFOS in children) 21.9 ng/mL (mean PFOS in adolescents)	Total cholesterol	**Association (p<0.001)*, children 5th quintile** **Association (p<0.001)*, adolescents 5th quintile**
		Abnormal cholesterol	**OR 1.3 (1.1–1.4)*, 2nd quintile**
		LDL cholesterol	**Association (p=0.002)*, children 5th quintile** **Association (p<0.001)*, adolescents 5th quintile**
		Abnormal LDL levels	**OR 1.2 (1.0–1.5)*, 2nd quintile**
		HDL cholesterol	**Association (p=0.007)*, children 5th quintile** **Association (p=0.001)*, adolescents 5th quintile**
		Triglycerides	NS (p=0.1), children 5th quintile NS (p=0.1), adolescents 5th quintile
Steenland et al. 2009b Community (C8) (n=46,294)	22.4 ng/mL (mean PFOS) 13.3–19.5 ng/mL (2nd quartile)	Total cholesterol	**Association (p<0.001 for trend)***
		Abnormal cholesterol	**OR 1.14 (1.05–1.23)*, 2nd quartile**
		LDL cholesterol	**Association (p<0.05 for trend)***
		HDL cholesterol	NS (p>0.05)
		Triglycerides	**Association (p<0.05 for trend)***

Table 2-12. Summary of Serum Lipid Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Château-Degat et al. 2010 General population (n=723)	25.7 ng/mL (mean PFOS)	Total cholesterol	NS (p=0.086)
		LDL cholesterol	NS (p=0.242)
		HDL cholesterol	**Association (p<0.001)*, men** **Association (p=0.001)*, women**
		Triglycerides	**NS (p=0.162), men** **Inverse association (p=0.040)*, women**
Eriksen et al. 2013 General population (n=753)	36.1 ng/mL (mean PFOS)	Total cholesterol	**Association (p=0.02)***
Fisher et al. 2013 General population (n=2,368)	8.04 ng/mL (mean PFOS)	Total cholesterol	NS (p=0.35)
		High cholesterol levels	OR 1.36 (0.87–2.12), 4th quartile
		Non HDL cholesterol	NS (p=0.14)
		LDL cholesterol	NS (p=0.42)
		HDL cholesterol	NS (p=0.33)
Fu et al. 2014a General population (n=133)	1.47 ng/mL (median PFOS)	Total cholesterol	NS (p=0.287)
		Elevated cholesterol	OR 2.27 (0.47–10.92)
		LDL cholesterol	NS (p=0.357)
		Elevated LDL	OR 2.27 (0.50–10.37)
		HDL cholesterol	NS (p=0.260)
		Elevated HDL	OR 0.29 (0.06–1.50)
		Triglycerides	NS (p=0.711)
		Elevated triglycerides	OR 1.26 (0.41–3.90)
Geiger et al. 2014b General population (NHANES) (n=815 12–18-year-old adolescents)	17.7 ng/mL (mean PFOS)	Total cholesterol	NS (p=0.0512 for trend)
		Elevated cholesterol	**OR 1.35 (1.11–1.64, p=0.0183 for trend)*, log transformed PFOS**
		LDL cholesterol	**Association (p=0.0081 for trend)***
		Elevated LDL	**OR 1.48 (1.15–1.90, p=0.0178 for trend)*, log transformed PFOS**

Table 2-12. Summary of Serum Lipid Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
		HDL cholesterol	NS (p=0.9703)
		Decreased HDL	NS (p=0.9873 for trend)
		Triglycerides	NS (p=0.1104 for trend)
		Elevated triglycerides	NS (p=0.2418 for trend)
Kang et al. 2018 General population (n=150 children ages 3–18 years)	5.68 ng/mL (median serum PFOS)	Total cholesterol	β -0.450 (-10.667–9.768, p=0.931)
		LDL cholesterol	β 2.507 (-6.879–11.893, p=0.598)
		Triglycerides	β -0.020 (-0.186–0.146, p=0.809)
Koshy et al. 2017 General population (WTCHR, n=180 children; n=222 children in comparison group)	3.72 and 2.78 ng/mL (median serum PFOS in WTCHR group and comparison group)	Total cholesterol	**β 0.08 mg/dL (0.05–0.12, p<0.001)***
		LDL cholesterol	**β 0.10 mg/dL (0.05–0.16, p<0.001)***
		HDL cholesterol	**β 0.06 mg/dL (0.003–0.13, p=0.04)***
		Triglycerides	β 0.04 mg/dL (0.05–0.13, p=0.36)
Liu et al. 2018b General population (NHANES, n=1,871 adults)	5.28 ng/mL (geometric mean serum PFOS)	Total cholesterol	NS (p>0.05)
		LDL cholesterol	NS (p>0.05)
		HDL cholesterol	NS (p>0.05)
		Triglycerides	NS (p>0.05)
Maisonet et al. 2015b General population (n=111 for 7-year-old and n=88 for 15-year-old girls)	23.5–94.5 ng/mL (3rd tertile maternal PFOS)	Total cholesterol	NS (β -0.10, -0.73–0.54), 7-year-olds **Association (β -0.77, -1.40 to -0.13)*, 15-year-olds**
		LDL cholesterol	NS (β 0.02, -0.48–0.53), 7-year-olds **Association (β -0.54, -1.08 to -0.003)*, 15-year-olds**
		HDL cholesterol	NS (β -0.04, -0.33–0.25), 7-year-olds NS (β -0.18, -0.47–0.12), 15-year-olds
		Triglycerides	NS (β -0.004, -0.015–0.006), 7-year-olds NS (β -0.004, -0.011–0.004), 15-year-olds

Table 2-12. Summary of Serum Lipid Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Manzano-Salgado et al. 2017b General population (n=1,230 children; evaluated at 4 years of age)	5.80 ng/mL (maternal geometric mean PFOS)	Total cholesterol	β -0.02 (-0.10–0.15)
		LDL cholesterol	β 0.02 (-0.12–0.15)
		HDL cholesterol	β -0.03 (-0.14–0.09),
		Triglycerides	β 0.05 (-0.06–0.17)
Nelson et al. 2010 General population (NHANES) (n=860)	25.3 ng/mL (mean PFOS)	Total cholesterol	**Association (p=0.01)***
		LDL cholesterol	NS (p=0.27)
		Non-HDL cholesterol	**Association (p=0.02)***
		HDL cholesterol	NS (p=0.78)
Skuladottir et al. 2015 General population (n=854 pregnant women)	22.3 ng/mL (mean PFOS)	Total cholesterol	**Association (p=0.01 for trend)***
Starling et al. 2014a General population (n=854 pregnant women)	13.03 ng/mL (50th PFOS percentile)	Total cholesterol	**Association (p<0.05)***
		LDL cholesterol	NS (β 6.48, -0.07–13.03), per ln-unit increase in PFOS
		HDL cholesterol	**Association (β 4.39, 2.37–6.42)*, per ln-unit increase in PFOS**
		Triglycerides	NS (β -0.02, -0.09–0.04), per ln-unit increase in PFOS
Timmermann et al. 2014 General population (n=499 children, 8–10 years old)	41.5 ng/mL (median PFOS)	Triglycerides	NS (p=0.78), normal weight children **Association (p=0.002)*, obese children**
Yang et al. 2018 General population (n=148 men; 81 diagnosed with metabolic syndrome)	3.00 ng/mL (median serum PFOS)	HDL cholesterol	β 0.02 (-0.17–0.2)
		Triglycerides	β 0.3 (-0.63–1.22)
Zeng et al. 2015 General population (n=225 children, 12–15 years old)	32.4 and 34.2 ng/mL (mean PFOS in boys and girls)	Total cholesterol	**Association (p<0.001)***
		LDL cholesterol	**Association (p<0.001)***
		HDL cholesterol	NS (p=0.72)
		Triglycerides	**Association (p=0.05)***

Table 2-12. Summary of Serum Lipid Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFHxS			
Fisher et al. 2013 General population (n=2,368)	2.18 ng/mL (mean PFHxS)	Total cholesterol	**Association (p=0.005)***
		High cholesterol levels	**OR 1.27 (1.11–1.45)*, 4th quartile**
		Non HDL cholesterol	**Association (p=0.002)***
		LDL cholesterol	**Association (p=0.02)***
		HDL cholesterol	NS (p=0.67)
Kang et al. 2018 General population (n=150 children ages 3–18 years)	0.793 ng/mL (median serum PFHxS)	Total cholesterol	β 0.989 (-9.526–11.503, p=0.853)
		LDL cholesterol	β -4.222 (-13.979–5.534, p=0.393)
		Triglycerides	β 0.081 (-0.092–0.253, p=0.355)
Koshy et al. 2017 General population (WTCHR, n=180 children; n=222 children in comparison group)	0.67 and 0.53 ng/mL (median serum PFHxS in WTCHR group and comparison group)	Total cholesterol	**β 0.04 mg/dL (0.04–0.06, p=0.01)***
		LDL cholesterol	**β 0.05 mg/dL (0.01–0.09, p=0.02)***
		HDL cholesterol	β 0.03 mg/dL (-0.02–0.07, p=0.26)
		Triglycerides	β 0.04 mg/dL (-0.02–0.11, p=0.20)
Manzano-Salgado et al. 2017b General population (n=1,230 children; evaluated at 4 years of age)	0.61 ng/mL (maternal geometric mean PFHxS)	Total cholesterol	β 0.02 (-0.09–0.12)
		LDL cholesterol	β -0.01 (-0.12–0.09)
		HDL cholesterol	β -0.01 (-0.11 to 0.10)
		Triglycerides	**β 0.11 (0.01–0.21)***
Nelson et al. 2010 General population (NHANES) (n=860)	2.6 ng/mL (mean PFHxS)	Total cholesterol	NS (p=0.07)
		LDL cholesterol	NS (p=0.10)
		Non-HDL cholesterol	**Association (p=0.04)***
		HDL cholesterol	NS (p=0.11)

Table 2-12. Summary of Serum Lipid Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Starling et al. 2014a General population (n=854 pregnant women)	0.60 ng/mL (50th PFHxS percentile)	Total cholesterol	NS (β 3.00, -1.75–7.76), per ln-unit increase in PFHxS
		LDL cholesterol	NS (β 1.92, -2.50–6.33), per ln-unit increase in PFHxS
		HDL cholesterol	**Association (β 1.46; 0.19–2.73)*, per ln-unit increase in PFHxS**
		Triglycerides	NS (β -0.01, -0.05–0.03), per ln-unit increase in PFHxS
Yang et al. 2018 General population (n=148 men; 81 diagnosed with metabolic syndrome)	3.80 ng/mL (median serum PFHxS)	HDL cholesterol	**β 0.22 (0 to 0.43, p<0.05)***
		Triglycerides	**β 1.18 (0.12–2.25, p<0.05)***
Zeng et al. 2015 General population (n=225 children, 12–15 years old)	2.1 and 2.1 ng/mL (mean PFHxS in boys and girls)	Total cholesterol	NS (p=0.23)
		LDL cholesterol	NS (p=0.17)
		HDL cholesterol	NS (p=0.54)
		Triglycerides	NS (p=0.15)
PFNA			
Mundt et al. 2007 Occupational (n=592)	NR	Total cholesterol	NS, longitudinal analysis
		Triglycerides	NS, longitudinal analysis
Fu et al. 2014a General population (n=133)	0.37 ng/mL (median PFNA)	Total cholesterol	**Association (p=0.002)***
		Elevated cholesterol	OR 1.03 (0.24–4.46)
		LDL cholesterol	**Association (p=0.004)**
		Elevated LDL	OR 2.51 (0.59–10.74)
		HDL cholesterol	NS (p=0.191)
		Lowered HDL	OR 1.06 (0.20–5.57)
		Triglycerides	NS (p=0.460)
		Elevated triglycerides	OR 0.80 (0.26–2.49)

Table 2-12. Summary of Serum Lipid Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Kang et al. 2018 General population (n=150 children ages 3–18 years)	0.938 ng/mL (median serum PFNA)	Total cholesterol	β -1.624 (-10.218–6.970, p=0.709)
		LDL cholesterol	β 2.304 (-6.558–11.167, p=0.607)
		Triglycerides	β 0.065 (-0.092–0.221, p=0.820)
Koshy et al. 2017 General population (WTCHR, n=180 children; n=222 children in comparison group)	0.61 and 0.49 ng/mL (median serum PFNA in WTCHR group and comparison group)	Total cholesterol	**β 0.05 mg/dL (0.01–0.09, p=0.01)***
		LDL cholesterol	**β 0.07 mg/dL (0.01–0.14, p=0.01)***
		HDL cholesterol	β 0.05 mg/dL (0.02–0.12, p=0.13)
		Triglycerides	β -0.07 mg/dL (0.11–0.01, p=0.89)
Manzano-Salgado et al. 2017b General population (n=1,230 children; evaluated at 4 years of age)	0.66 ng/mL (maternal geometric mean PFNA)	Total cholesterol	β-0.00 (-0.11–0.12)
		LDL cholesterol	β 0.01 (-0.10–0.12)
		HDL cholesterol	β -0.03 (-0.14–0.08),
		Triglycerides	β 0.03 (-0.07–0.14)
Nelson et al. 2010 General population (NHANES) (n=860)	1.3 ng/mL (mean PFNA)	Total cholesterol	**Association (p=0.04)***
		LDL cholesterol	NS (p=0.08)
		Non-HDL cholesterol	**Association (p=0.04)***
		HDL cholesterol	NS (p=0.31)
Starling et al. 2014a General population (n=854 pregnant women)	0.39 ng/mL (50th PFNA percentile)	Total cholesterol	NS (β 0.01, -5.98–6.00), per ln-unit increase in PFNA
		LDL cholesterol	NS (β -2.15, -7.31–3.02), per ln-unit increase in PFNA
		HDL cholesterol	**Association (β 2.84; 0.97–4.71)*, per ln-unit increase in PFNA**
		Triglycerides	NS (β -0.02, -0.07–0.03), per ln-unit increase in PFNA
Yang et al. 2018 General population (n=148 men; 81 diagnosed with metabolic syndrome)	0.50 ng/mL (median serum PFNA)	HDL cholesterol	**β 0.3 (0.05–0.56)***
		Triglycerides	**β 1.54 (0.27–2.8)***

Table 2-12. Summary of Serum Lipid Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Zeng et al. 2015 General population (n=225 children, 12–15 years old)	0.8 and 0.9 ng/mL (mean PFNA in boys and girls)	Total cholesterol	**Association (p=0.04)***
		LDL cholesterol	**Association (p=0.05)***
		HDL cholesterol	NS (p=0.37)
		Triglycerides	**Association (p=0.007)***
PFDA			
Fu et al. 2014a General population (n=133)	0.19 ng/mL (median PFDA)	Total cholesterol	**Association (p=0.048)***
		Elevated cholesterol	OR 3.84 (0.87–16.95)
		LDL cholesterol	NS (p=0.251)
		Elevated LDL	OR 2.17 (0.52–9.04)
		HDL cholesterol	**Association (p=0.007)***
		Elevated HDL	OR 2.21 (0.49–10.07)
		Triglycerides	NS (p=0.317)
		Elevated triglycerides	OR 0.51 (0.17–1.58)
Kang et al. 2018 General population (n=150 children ages 3–18 years)	0.0592 ng/mL (median serum PFDA)	Total cholesterol	β -3.330 (-7.484–0.824, p=0.115)
		LDL cholesterol	β -1.858 (-5.694–1.979, p=0.339)
		Triglycerides	β -0.036 (-0.103–0.032, p=0.302)
Koshy et al. 2017 General population (WTCHR, n=180 children; n=222 children in comparison group)	0.14 and 0.11 ng/mL (median serum PFDA in WTCHR group and comparison group)	Total cholesterol	**β 0.04 mg/dL (0.02–0.06, p<0.001)***
		LDL cholesterol	**β 0.04 mg/dL (0.02–0.06, p=0.03)***
		HDL cholesterol	**β 0.05 mg/dL (0.02–0.09, p=0.003)***
		Triglycerides	β -0.01 mg/dL (-0.047–0.057,p=0.85)
Starling et al. 2014a General population (n=854 pregnant women)	0.09 ng/mL (50th PFDA percentile)	Total cholesterol	NS (β 1.84, -2.12–5.79), per ln-unit increase in PFDA
		LDL cholesterol	NS (β 0.19, -3.30–3.69), per ln-unit increase in PFDA
		HDL cholesterol	**Association (β 2.54, 1.22–3.87)*, per ln-unit increase in PFDA**
		Triglycerides	NS (β -0.03, -0.07–0.01), per ln-unit increase in PFDA

Table 2-12. Summary of Serum Lipid Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Yang et al. 2018 General population (n=148 men; 81 diagnosed with metabolic syndrome)	0.40 ng/mL (median serum PFDA)	HDL cholesterol	β 0.24 (0.04–0.52)
		Triglycerides	β 0.64 (-0.77–2.05)
Zeng et al. 2015 General population (n=225 children, 12–15 years old)	1.0 and 1.0 ng/mL (mean PFDA in boys and girls)	Total cholesterol	NS (p=0.74)
		LDL cholesterol	NS (p=0.85)
		HDL cholesterol	NS (p=0.47)
		Triglycerides	NS (p=0.92)
PFUnA			
Fu et al. 2014a General population (n=133)	0.26 ng/mL (median PFUnA)	Total cholesterol	NS (p=0.184)
		Elevated cholesterol	OR 3.70 (0.76–18.03)
		LDL cholesterol	NS (p=0.270)
		Elevated LDL	OR 4.16 (0.96–18.00)
		HDL cholesterol	NS (p=0.279)
		Elevated HDL	OR 0.54 (0.11–2.57)
		Triglycerides	NS (p=0.755)
		Elevated triglycerides	OR 0.74 (0.25–2.21)
Kang et al. 2018 General population (n=150 children ages 3–18 years)	0.652 ng/mL (median serum PFUnA)	Total cholesterol	**β 7.906 (2.681–13.131, p=0.003)***
		LDL cholesterol	**β 7.101 (2.448–11.754, p=0.003)***
		Triglycerides	β 0.043 (-0.042–0.129, p=0.317)
Koshy et al. 2017 General population (WTCHR, n=180 children; n=222 children in comparison group)	0.12 and 0.04 ng/mL (median serum PFUnA in WTCHR group and comparison group)	Total cholesterol	β 0.02 mg/dL (0–0.04, p=0.06)
		LDL cholesterol	β 0.01 mg/dL (-0.02–0.04, p=0.49)
		HDL cholesterol	**β 0.04 mg/dL (0.01–0.07, p=0.01)***
		Triglycerides	β -0.04 mg/dL (-0.09–0.003, p=0.07)

Table 2-12. Summary of Serum Lipid Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Starling et al. 2014a General population (n=854 pregnant women)	0.22 ng/mL (50th PFUnA percentile)	Total cholesterol	NS (β 0.89, -3.28–5.06), per ln-unit increase in PFUnA
		LDL cholesterol	NS (β -2.36, -5.97–1.25), per ln-unit increase in PFUnA
		HDL cholesterol	**Association (β 4.05, 2.75–5.35)*, per ln-unit increase in PFUnA**
		Triglycerides	NS (β -0.04, -0.08–0.00), per ln-unit increase in PFUnA
Yang et al. 2018 General population (n=148 men; 81 diagnosed with metabolic syndrome)	0.30 ng/mL (median serum PFUnA)	HDL cholesterol	β 0.11 (-0.11–0.34)
		Triglycerides	β 0.61 (-0.48–1.7)
PFHpA			
Fu et al. 2014a General population (n=133)	0.04 ng/mL (median PFHpA)	Total cholesterol	NS (p>0.05)
		LDL cholesterol	NS (p>0.05)
		HDL cholesterol	NS (p>0.05)
		Triglycerides	NS (p>0.05)
Yang et al. 2018 General population (n=148 men; 81 diagnosed with metabolic syndrome)	0.20 ng/mL (median serum PFHpA)	HDL cholesterol	β -0.33 (-0.77–0.11)
		Triglycerides	β -0.92 (-3.12–1.28)
PFBS			
Zeng et al. 2015 General population (n=225 children, 12–15 years old)	0.5 and 0.4 ng/mL (mean PFBS in boys and girls)	Total cholesterol	**Association (p=0.04)***
		LDL cholesterol	NS (p=0.14)
		HDL cholesterol	NS (p=0.15)
		Triglycerides	NS (p=0.81)

Table 2-12. Summary of Serum Lipid Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFBA			
Fu et al. 2014a General population (n=133)	0.11 ng/mL (median PFBA)	Total cholesterol	NS (p>0.05)
		LDL cholesterol	NS (p>0.05)
		HDL cholesterol	NS (p>0.05)
		Triglycerides	NS (p>0.05)
PFDoDA			
Zeng et al. 2015 General population (n=225 children, 12–15 years old)	4.5 and 4.4 ng/mL (mean PFDoDA in boys and girls)	Total cholesterol	NS (p=0.37)
		LDL cholesterol	NS (p=0.44)
		HDL cholesterol	NS (p=0.68)
		Triglycerides	NS (p=0.40)

[a]See the *Supporting Document for Epidemiological Studies for Perfluoroalkyls,* Table 7 for more detailed descriptions of studies.
[b]Participants in occupational exposure may have also lived near the site and received residential exposure; community exposure studies involved subjects living near PFOA facilities with known exposure to high levels of PFOA.
[c]Asterisk indicates association with perfluoroalkyl; unless otherwise specified, values in parenthesis are 95% confidence intervals.

HDL = high density lipoprotein; LDL = low density lipoprotein; NHANES = National Health and Nutrition Examination Survey; NR = not reported; NS = not significant; OR = odds ratio; PFBA = perfluorobutanoic acid; PFBS = perfluorobutane sulfonic acid; PFDA = perfluorodecanoic acid; PFDoDA = perfluorododecanoic acid; PFHpA = perfluoroheptanoic acid; PFHxS = perfluorohexane sulfonic acid; PFNA = perfluorononanoic acid; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid; PFUnA = perfluoroundecanoic acid; VLDL = very low-density lipoprotein; WTCHR = World Trade Center Health Registry

To address concern over the relevance of liver enlargement in rodents to human health risk, the European Society of Toxicologic Pathology (ESTP) convened an expert panel to define what constitutes an adverse hepatic effect and whether hepatic effects induced by nuclear hormone receptors such as PPARα, constitutive androstane receptor (CAR), or pregnane X receptor (PXR) are rodent-specific adaptive reactions; the findings of the panel are summarized by Hall et al. (2012). As discussed by Hall et al. (2012), criteria were established for determining whether increases in liver organ weight and liver cell hypertrophy observed in studies of rodents exposed to agents inducing enzyme induction can be considered adaptive responses and of little relevance to humans. According to the ESTP criteria, increases in liver weight without histological evidence, such as (1) degenerative or necrotic changes including hepatocyte necrosis, inflammation, and steatotic vascular degeneration; (2) biliary/oval cell proliferation, degeneration, fibrosis, and cholestasis; or (3) necrosis and degeneration of other resident cells within the liver, are not considered adverse or relevant for human risk assessment. In the absence of histological changes, increases in liver organ weight are not considered relevant for human risk assessment unless at least two of the following three parameters are present: (1) at least 2–3 times increase in ALT levels; (2) biologically significant change in other biomarkers of hepatobiliary damage (alkaline phosphatase, AST, GGT, etc.); or (3) biologically significant change in another clinical pathology marker indicating liver dysfunction (albumin, bilirubin, bile acids, coagulation factors, cholesterol, triglycerides, etc.). ATSDR has adopted the criteria from Hall et al. (2012) for determining the adversity of the liver effects reported in the rodent perfluoroalkyl studies. Doses associated with increases in liver weight and hepatocellular hypertrophy were not considered adverse effect levels unless hepatocellular degenerative or necrotic changes or evidence of biliary or other liver cell damage were also present. The lowest doses associated with the liver weight increases and hepatocellular hypertrophy are noted in the LSE tables even though the dose levels are considered NOAELs.

PFOA

Epidemiological Studies—Liver Disease. Three studies of highly exposed populations have examined possible associations between PFOA and increased risk of liver disease. In workers, no association between estimated cumulative serum PFOA levels and the risk of non-hepatitis liver disease was observed (Steenland et al. 2015). Similarly, two studies of residents living near the Washington Works PFOA facility reported no increases in liver disease. In a study by Anderson-Mahoney et al. (2008), no significant increases in self-reported liver problems were found in residents primarily served by the Lubeck Public Water Service District or Little Hocking Water District; the study did not measure serum PFOA levels. In a C8 Health Project study that included workers at the Washington Works facility,

estimated cumulative serum PFOA levels were not associated with any liver disease or enlarged liver, fatty liver, or cirrhosis (Darrow et al. 2016).

Epidemiological Studies—Hepatic Serum Enzymes and Bilirubin Levels. The possible association between PFOA exposure and hepatic enzymes has been examined in seven occupational exposure studies that have found inconsistent results. A small study of Italian perfluoroalkyl workers did not find associations between serum PFOA and ALT, AST, or GGT activities when only current workers were examined (Costa et al. 2009). In analysis of all workers (current, former, and non-exposed workers), associations between serum PFOA levels and ALT and GGT activities were found; total bilirubin was also inversely associated with serum PFOA. Another small study of workers at a fluorochemical facility in China found an association between serum PFOA and AST activity, but not ALT activity (Wang et al. 2012). Gilliland and Mandel (1996; data also reported in Gilliland 1992) did not find associations between serum fluorine levels (used as a surrogate for serum PFOA) and ALT, AST, or GGT levels in workers. In a follow-up study of this facility, there were no differences between AST, ALT, GGT, or total bilirubin levels between workers in three exposure groups (Olsen et al. 2000); the mean serum PFOA levels in this study ranged from 5,000 to 6,400 ng/mL at three time points and the serum PFOA levels in the lowest exposure group ranged from 0 to <1,000 ng/mL. Increases in GGT and decreases in total bilirubin levels associated with increases in serum PFOA were observed in a study of workers exposed to high levels of PFOA and PFOS (Olsen and Zobel 2007); ALT activity was not affected. In a cross-sectional study of active workers at a PFOA facility, a modest but statistically significant positive association between serum PFOA and GGT activity was found (Sakr et al. 2007b). No associations were found for bilirubin levels or ALT and AST activities.

The possible associations between serum PFOA and serum enzyme and bilirubin levels were examined in two longitudinal occupational exposure studies. Sakr et al. (2007a) examined the relationship between serum PFOA and liver enzymes in a longitudinal study of 454 workers who had two or more measurements of serum PFOA from 1979 until the study was conducted. The average length of employment among workers with multiple PFOA measurements was 11 years, and, on average, 10.8 years elapsed between their first and last serum PFOA measurement. The means of the first and last PFOA measurement were 1,040 and 1,160 ng/mL, respectively. After adjustment for potential confounders, serum PFOA was associated with AST activity, but not ALT, GGT, or total bilirubin. The second study included 179 workers involved in the demolition of 3M perfluoroalkyl manufacturing facilities examined over a mean period of 164 days (Olsen et al. 2012). In workers with prior exposure to

PFOA who had a decrease in serum PFOA levels during the study period, there was a significant increase in ALT levels. An increase in serum PFOA levels did not significantly alter AST or total bilirubin levels.

Community and general population exposure studies have also examined possible associations between serum PFOA levels and alterations in serum hepatic enzyme and bilirubin levels. As with the occupational exposure studies, several studies of populations living near PFOA facilities have found inconsistent results. Darrow et al. (2016) found associations between ALT and bilirubin (inverse association) and estimated cumulative and 2005/2006 serum PFOA levels in participants of the C8 Health Project (6.5% of the participants also worked at the facility); there were no associations with GGT activity. Gallo et al. (2012) also reported a significant correlation between serum PFOA levels and ALT activity in C8 Health Project participants. Unlike the Darrow et al. (2016) study, a significant correlation between serum PFOA levels and GGT activity, but no correlation with direct bilirubin levels, was found. An earlier study of residents in the same area, as well as a study of residents near a facility in China, did not find associations between serum PFOA and ALT, AST, or GGT (Emmett et al. 2006b; Wang et al. 2012).

More consistent results were found in three general population studies. In studies utilizing data from NHANES, Gleason et al. (2015) and Lin et al. (2010) reported associations between serum PFOA levels and ALT, AST, and GGT activities; total bilirubin was also found to be associated with serum PFOA in the Gleason et al. (2015) study, but not in the Lin et al. (2010) study. A general population study conducted in Japan (Yamaguchi et al. 2013) also found associations between serum PFOA levels and AST, ALT, and GGT activities.

Although a number of epidemiological studies have found associations between serum PFOA and serum hepatic enzyme and bilirubin levels, many of the investigators noted that liver biomarker levels were typically within the normal range. Four studies examining the risk of having biomarker levels outside of the normal range provide useful information for evaluating the health impact of the enzyme level alterations. For ALT, Gallo et al. (2012) and Gleason et al. (2015) found increased risks of abnormal levels in C8 and NHANES participants, respectively. In contrast, Olsen and Zobel (2007) and Emmett et al. (2006b) did not find increased risks of abnormal ALT levels in workers and C8 participants, respectively. No alterations in the risk of abnormal AST levels associated with elevated serum PFOA levels were observed in NHANES participants (Gleason et al. 2015). Emmett et al. (2006b) found a decrease in the risk of abnormal AST levels with increasing serum PFOA levels in community members. Associations between the risk of elevated GGT and serum PFOA were found in the study conducted by

Gleason et al. (2015), but not in the Olsen and Zobel (2007), Gallo et al. (2012), or Emmett et al. (2006b) studies. Similarly, Gleason et al. (2015) reported an association between serum PFOA and the risk of elevated bilirubin levels, whereas Gallo et al. (2012) did not find this association in the higher exposed population.

One limitation to the interpretation of the serum hepatic enzyme data is confounding factors that should be considered in analyses; these include age, body mass index (BMI), serum lipid levels (triglycerides and total cholesterol), alcohol consumption, smoking, physical activity, and glucose levels (Deb et al. 2018; Kim et al. 2008). Although many of the studies accounted for age, BMI, smoking, and alcohol consumption, none of the studies adjusted for all of these potential confounders.

Epidemiological Studies—Serum Lipids. Occupational, community, and general population studies have examined the possible associations between serum PFOA levels and serum lipid levels; the results of these studies are presented in Table 2-12. Summaries of the changes in serum total cholesterol and LDL cholesterol levels, as well as the risk associated with elevated serum cholesterol and LDL cholesterol levels, are presented in Figures 2-11, 2-12, 2-13, and 2-14.

A study of workers at a manufacturing facility in Italy found higher total cholesterol and non-high-density lipoprotein (HDL)-cholesterol levels (non-HDL cholesterol was estimated by subtracting HDL cholesterol from total cholesterol) in the PFOA-exposed workers, as compared to levels in workers who were not exposed to PFOA (Costa 2004). A second study at this facility (Costa et al. 2009) also found an association between serum PFOA levels and total cholesterol levels, but no association with HDL cholesterol levels. No associations were found for HDL cholesterol or triglyceride levels. In another small study of workers at a fluorochemical facility in China (Wang et al. 2012), no associations between serum PFOA and total cholesterol, LDL cholesterol, or triglyceride levels were observed; the study did find an inverse association between serum PFOA and HDL cholesterol levels.

Several studies have examined workers at 3M facilities in Cottage Grove, Minnesota, Decatur, Alabama, and/or Antwerp, Belgium; workers at these facilities were also exposed to high levels of PFOS. Gilliland and Mandel (1996; data also reported in Gilliland 1992) examined workers at the Cottage Grove facility in 1990 and found no associations between serum fluorine levels (used as a surrogate for PFOA) and total cholesterol, LDL cholesterol, or HDL cholesterol. In a follow-up to this study, Olsen et al. (2000) examined workers in 1993, 1995, and 1997; only 17 workers were examined at all three time periods,

Figure 2-11. Serum Total Cholesterol Levels Relative to Serum PFOA Levels
(Presented as percent change in cholesterol levels)

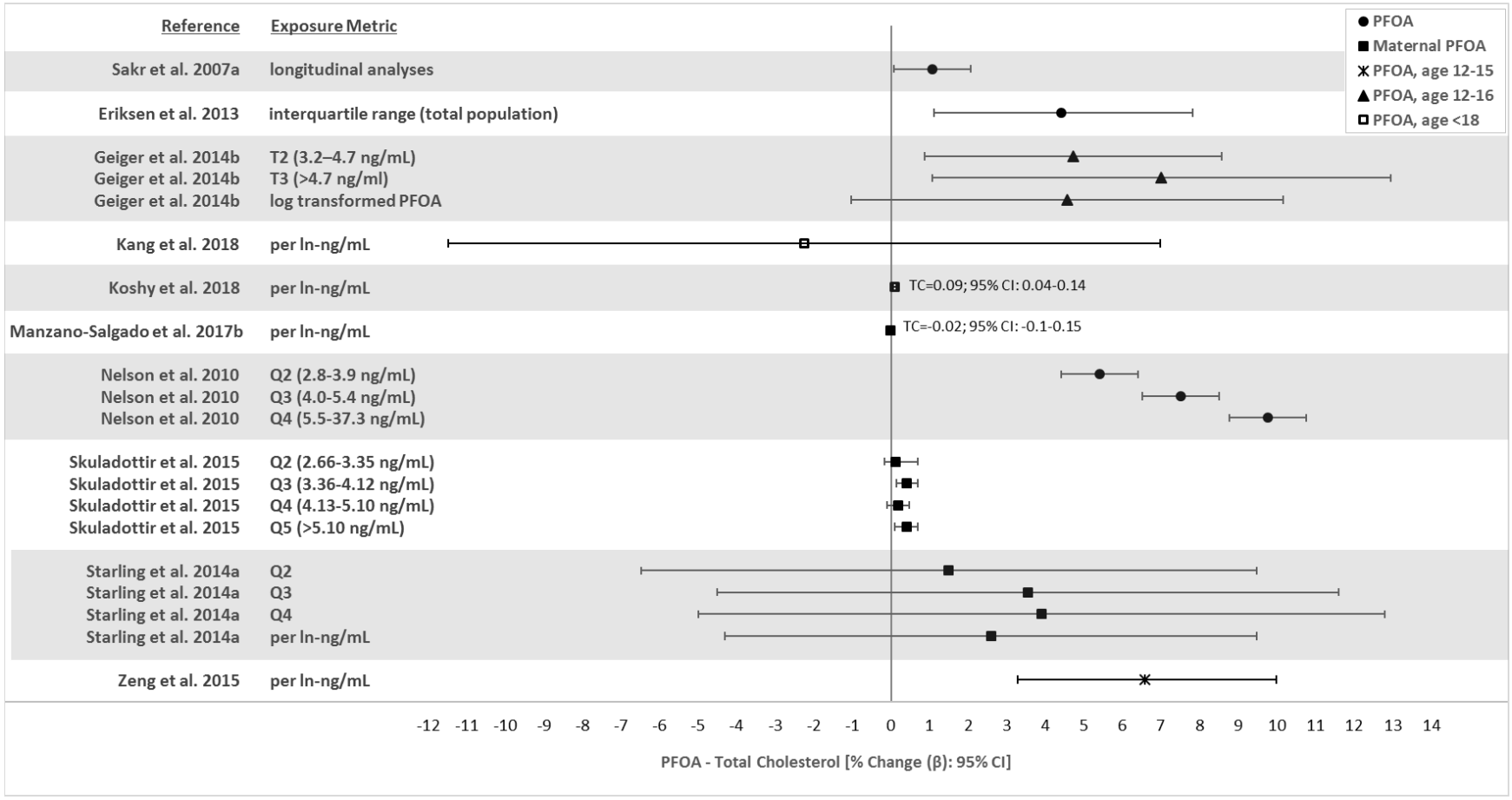
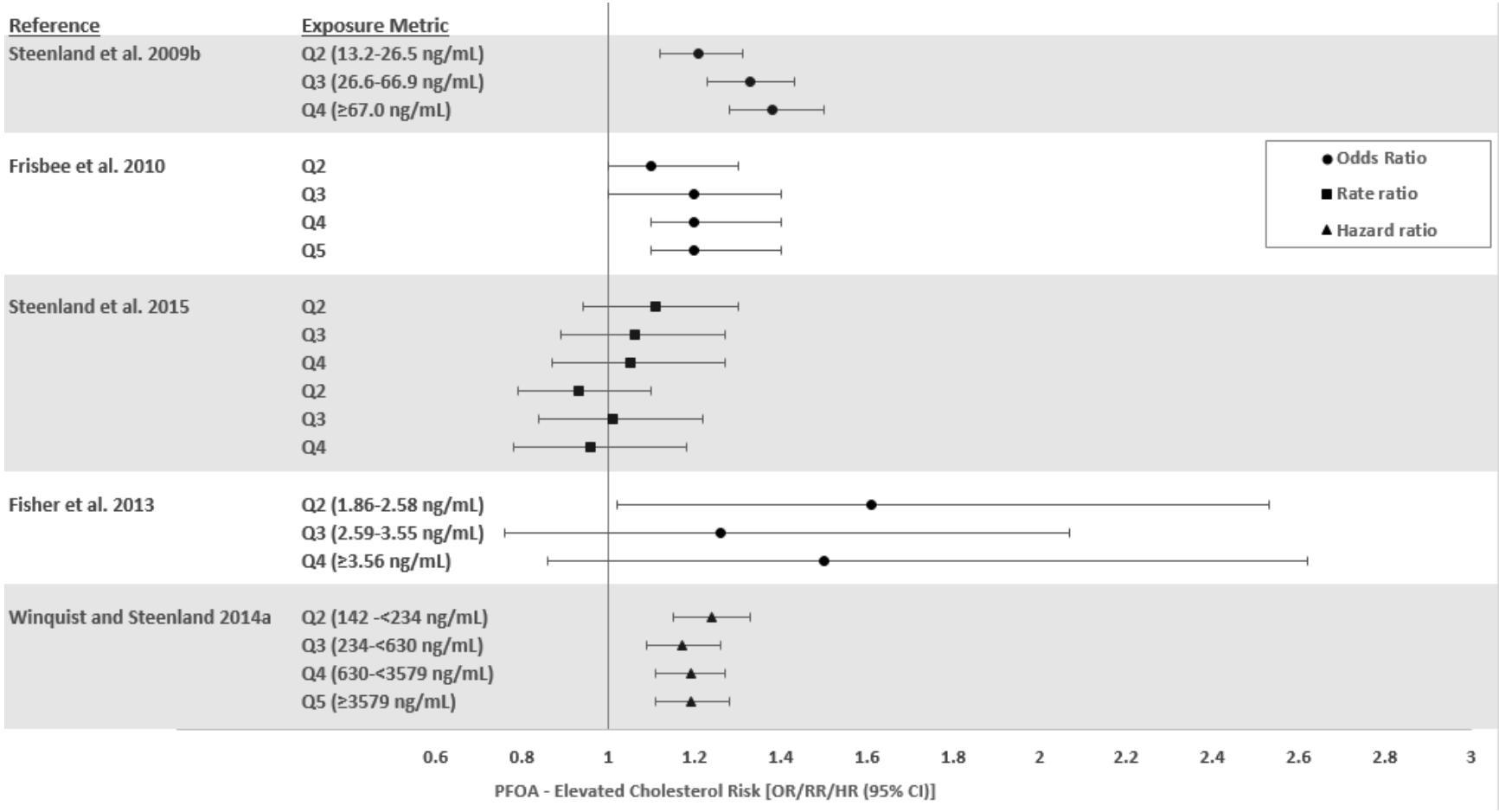


Figure 2-12. Risk of Abnormal Cholesterol Levels Relative to PFOA Levels (Presented as Adjusted Ratios)



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Figure 2-13. Serum LDL Cholesterol Levels Relative to Serum PFOA Levels
(Presented as percent change in LDL cholesterol levels)

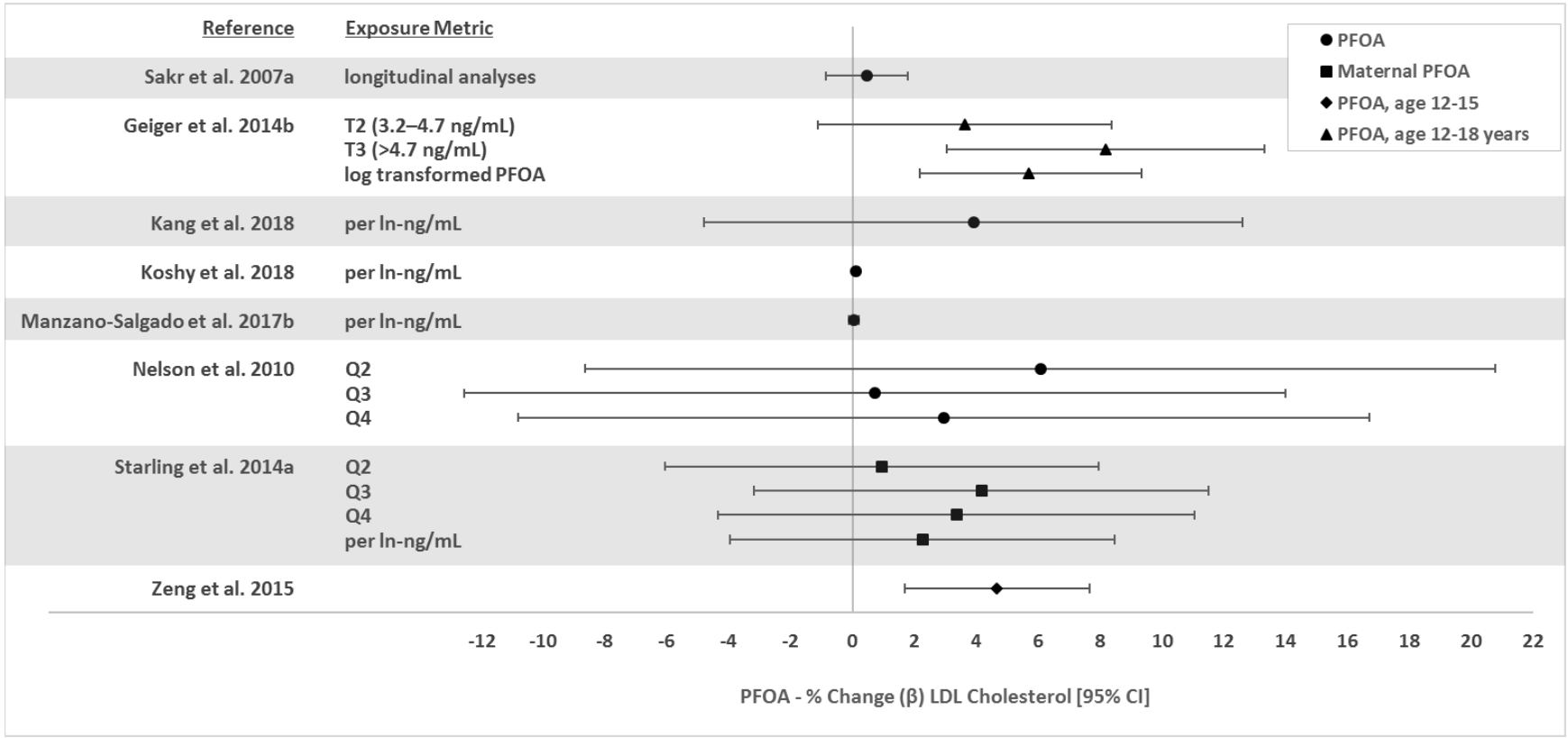
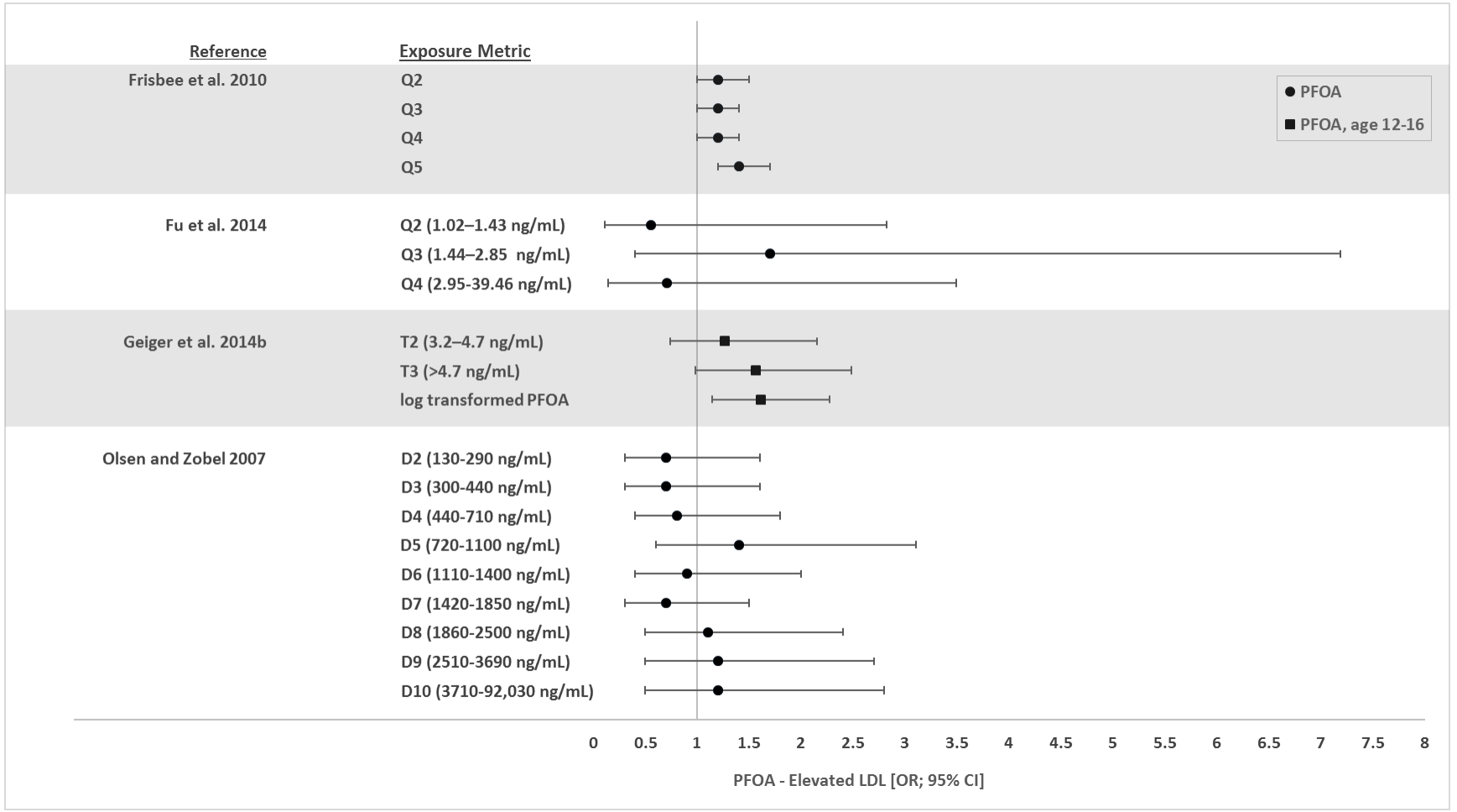


Figure 2-14. Risk of Abnormal LDL Cholesterol Levels Relative to PFOA Levels (Presented as Adjusted Ratios)



21 workers were examined in 1995 and 1997, and 68 workers were examined in 1993 and 1995. The study did not adjust for the use of cholesterol-lowering medication. When workers were categorized by blood PFOA levels (0–<1,000, 1,000–<10,000, and >10,000 ng/mL), no significant differences in serum cholesterol, LDL cholesterol, HDL cholesterol, or triglyceride levels were found at any of the monitoring periods. A study in workers at the three 3M facilities, most of whom were not taking cholesterol-lowering medications, did not find associations between serum PFOA levels and total cholesterol or LDL cholesterol levels; however, serum PFOA levels were associated with elevated triglyceride levels and inversely associated with HDL cholesterol levels (Olsen and Zobel 2007). The study did not find increases in the risk of elevated total cholesterol (≥200 mg/dL), elevated LDL cholesterol (≥130 mg/dL), elevated triglyceride (≥150 mg/dL), or decreased HDL cholesterol (≤40 mg/dL) levels in workers with serum PFOA levels in the highest deciles. In addition to these cross-sectional studies, two longitudinal studies were conducted at these facilities. Using data for 174 workers with medical surveillance data in 2000 and 1997 and/or 1995, Olsen et al. (2003a) found that serum PFOA was a significant predictor of cholesterol and triglyceride levels, which was primarily due to 21 workers at the Antwerp facility (mean serum level 8,400 ng/mL) whose serum PFOA levels increased over time. In a longitudinal study, Olsen et al. (2012) examined workers (none of the subjects reported using cholesterol-lowering medication) involved in the demolition of 3M perfluoroalkyl manufacturing facilities; serum PFOA and lipid levels were measured prior to the demolition and after demolition (mean time interval of 164 days). The mean baseline serum PFOA levels were 881 ng/mL in 14 3M workers with prior PFOA or PFOS exposure and 28.9 ng/mL in the remaining 165 workers. Among the 119 workers whose serum PFOA/PFOS levels (mean increase 50.9 ng/mL) increased during the observation period, there was a significant increase in HDL cholesterol levels, but no change in total cholesterol or non-HDL cholesterol levels. No significant alterations in serum lipid levels were observed in the 55 workers whose serum PFOA/PFOS levels decreased during the observation period. In workers whose baseline levels of PFOA and PFOS were <15 and <50 ng/mL, respectively, there were no significant differences between pre- and post-exposure serum lipid levels.

Investigators have also examined workers at the DuPont Washington Works facility in West Virginia. In a cross-sectional study, Sakr et al. (2007b) found associations between serum PFOA levels and total cholesterol, LDL cholesterol, and very-low-density lipoprotein (VLDL) cholesterol levels in all subjects and in a subset of subjects not taking cholesterol-lowering medication. The study did not find any association between serum PFOA and HDL cholesterol or triglyceride levels. In a second study, Steenland et al. (2015) did not find an association between estimated serum PFOA levels and the occurrence of elevated cholesterol levels that required medication. In a longitudinal study of workers

who had at least two serum PFOA measurements between 1979 and 2004, Sakr et al. (2007a) found a positive association between serum PFOA and total cholesterol levels; no associations with triglycerides, LDL cholesterol, or HDL cholesterol were found. Total cholesterol levels increased 1.06 mg/dL for each 1,000 ng/mL increase in serum PFOA.

Several studies have been conducted of residents living near the Washington Works facility. A study by Emmett et al. (2006b) of adults and children living in a community serviced by the Little Hocking Water Authority did not find an association between serum PFOA levels and total cholesterol levels; the study included an adjustment for the use of cholesterol-lowering medication. Four larger-scale studies of participants in the C8 Science Panel studies found associations between serum PFOA levels and serum lipid levels (Fitz-Simon et al. 2013; Frisbee et al. 2010; Steenland et al. 2009b; Winquist and Steenland 2014a). Positive associations between serum PFOA levels and total cholesterol and LDL cholesterol were found in a study of over 12,000 children and adolescents, with mean serum PFOA levels of 32.6 ng/mL in children (aged 1.0–11.9 years) and 26.3 ng/mL in adolescents (aged 12.0–17.9 years) (Frisbee et al. 2010). Serum PFOA was also positively associated with triglyceride levels. Additionally, there was an increased risk of elevated cholesterol (≥170 mg/dL) in subjects with serum PFOA levels in the 4th or 5th quintiles. Increased odds of high LDL cholesterol (≥110 mg/dL) were also observed for the 5th PFOA quintile (OR 1.4, 95% CI 1.2–1.7). The investigators noted that the dose-response relationship between serum PFOA and serum lipids was nonlinear, with greater increases in lipids observed at the lower serum PFOA levels. Similar findings were reported in a study of >46,000 adults with a median serum PFOA level of 26.6 ng/mL; the study excluded subjects who reported taking cholesterol-lowering medication (Steenland et al. 2009b). Associations were found between serum PFOA levels and total cholesterol, LDL cholesterol, and non-HDL cholesterol; a positive association between serum PFOA and triglycerides was also found. No associations between serum PFOA levels and HDL cholesterol levels were found. Increased risks of having high total cholesterol (≥240 mg/dL) were found in subjects with serum PFOA levels in the 2nd, 3rd, and 4th quartiles. The investigators noted that the odds of high total cholesterol from the 1st to the 5th quartile were approximately 40% for PFOA, which may be important given that the Framingham study found that the risk of coronary heart disease was about 1.8 times higher in subjects with total cholesterol levels >240 mg/dL as compared to subjects with levels <200 mg/dL. Steenland et al. (2009b) also found an association between serum PFOA levels and total cholesterol levels in a study of 10,746 adults taking cholesterol-lowering medication. Using both groups of subjects (taking or not taking cholesterol-lowering medication), the investigators analyzed whether taking cholesterol-lowering medication was associated with lower serum PFOA levels, which may be indicative of reverse causality. Although serum PFOA levels were significantly lower in subjects taking cholesterol-lowering

medication, the difference between the groups was low (4%). Using estimated cumulative serum PFOA levels as the exposure metric, Winquist and Steenland (2014a) found increased risks of hypercholesterolemia at estimated cumulative exposure levels ≥142 ng/mL. In a longitudinal study by Fitz-Simon et al. (2013), adults participating in the C8 Health Project and not taking cholesterol-lowering medication were examined twice, with an average of 4.4 years between examinations. Mean serum PFOA levels were 74.8 ng/mL at the first examination and 30.8 ng/mL at the second examination. In subjects whose serum PFOA levels halved between examinations, there was a 3.6% decrease in LDL cholesterol levels and 1.7% decrease in total cholesterol levels. However, there were very small changes in LDL cholesterol and total cholesterol levels in subjects whose serum PFOA levels decreased by >64% and there were slight increases in LDL cholesterol and total cholesterol levels in subjects whose serum PFOA levels fell by <50%. Changes in PFOA levels were not associated with changes in HDL cholesterol or triglyceride levels. Similarly, Wang et al. (2012) found no associations between serum PFOA levels and total cholesterol, HDL cholesterol, LDL cholesterol, or triglycerides in a study of adults living near a PFOA manufacturing facility in China; the mean serum PFOA level was 378.30 ng/mL and did not include an adjustment for the use of cholesterol-lowering medication.

General population studies were conducted in the United States, Canada, Denmark, Norway, Spain, Japan, Korea, China, and Taiwan; these studies have examined possible associations between serum PFOA levels and serum lipid levels in children, adolescents, pregnant women, and adults. In a study of 8–10-year-old children (median serum PFOA of 9.3 ng/mL), Timmermann et al. (2014) found an association between serum PFOA and triglyceride levels among obese children; this association was not found among normal weight children. In a study of adolescents (12–18 years of age) participating in NHANES (mean serum PFOA level of 4.2 ng/mL), Geiger et al. (2014b) found associations between serum PFOA and total cholesterol and LDL cholesterol levels; no associations were found for HDL cholesterol or triglycerides. The study also found increased risks of elevated total cholesterol levels (>170 mg/dL) associated with serum PFOA levels. No alterations in the risk of elevated LDL cholesterol or triglycerides or decreased HDL cholesterol were found. Associations between serum total cholesterol, LDL cholesterol, and triglycerides have also been observed in a study of Taiwanese adolescents (12–15 years of age, median PFOA level of 9.3 ng/mL) (Zeng et al. 2015); no association was found for HDL cholesterol. A fourth study found associations between maternal PFOA levels and total cholesterol and LDL cholesterol in 7- and 15-year-old girls, but no associations for girls whose maternal PFOA levels were in the 2nd or 3rd tertiles (Maisonet et al. 2015b). No associations were found for HDL cholesterol or triglyceride levels. A study of children enrolled in the World Trade Center Health Registry found associations between serum PFOA levels and elevated serum cholesterol, LDL cholesterol, and

triglyceride levels, but no association with HDL cholesterol (Koshy et al. 2017). Another study of children aged 3–18 years found no associations between serum PFOA levels and total cholesterol, LDL cholesterol, or triglycerides (Kang et al. 2018). Manzano-Salgado et al. (2017b) found no association between maternal serum PFOA levels and serum lipid levels in 4-year-old children.

Studies in adults have found mixed results for serum lipids. Using NHANES data for adults not taking cholesterol-lowering medication (mean serum PFOA level of 4.6 ng/mL), Nelson et al. (2010) found an association between serum PFOA levels and non-HDL cholesterol levels; no associations were found for total cholesterol, LDL cholesterol, or HDL cholesterol. Another study of NHANES participants that statistically adjusted for use of cholesterol-lowering medication found no associations between serum PFOA and total cholesterol, LDL cholesterol, HDL cholesterol, or triglyceride levels (Liu et al. 2018b). Associations between serum PFOA levels and total cholesterol levels were also found in a study of Danish adults not taking cholesterol-lowering medication (mean serum PFOA level of 7.1 ng/mL) (Eriksen et al. 2013). A study in Chinese adults (median PFOA level of 1.43 ng/mL) also found associations between serum PFOA and total cholesterol and LDL cholesterol, with no associations for HDL cholesterol or triglycerides (Fu et al. 2014a). This study did not find increased risks of elevated total cholesterol, LDL cholesterol, or triglycerides or decreased HDL cholesterol associated with serum PFOA. A second study of Chinese men found an association between serum PFOA and triglyceride levels, but no association with HDL cholesterol levels (Yang et al. 2018). A study of pregnant women in Denmark also found an association between serum PFOA (mean serum PFOA level of 4.1 ng/mL at gestation week 30) and total cholesterol levels (Skuladottir et al. 2015). No associations between serum PFOA levels and total cholesterol, LDL cholesterol, or non-HDL cholesterol levels were found in Canadian adults not taking cholesterol-lowering medication with a geometric mean serum PFOA level of 2.46 ng/mL (Fisher et al. 2013). In a second study of pregnant women (median PFOA level of 2.25 ng/mL at gestation week 18), no associations between plasma PFOA and total cholesterol, LDL cholesterol, or triglycerides were found (Starling et al. 2014a). The study did find an association between plasma PFOA and HDL cholesterol.

A number of epidemiological studies have reported associations between serum PFOA levels and serum lipid levels; the most consistently found alteration was for increased serum total cholesterol levels. Associations between serum PFOA and serum cholesterol levels have been observed in occupational (Costa 2004; Costa et al. 2009; Sakr et al. 2007a, 2007b), community (Fitz-Simon et al. 2013; Frisbee et al. 2010; Steenland et al. 2009b; Winquist and Steenland 2014a), and general population (Eriksen et al. 2013; Fu et al. 2014a; Geiger et al. 2014b; Skuladottir et al. 2015; Zeng et al. 2015) studies, whereas

other investigators have not found associations in worker populations (Gilliland and Mandel 1996; Olsen et al. 2000; Olsen and Zobel 2007; Steenland et al. 2015; Wang et al. 2012), community populations (Emmett et al. 2006b; Wang et al. 2012), or general populations (Fisher et al. 2013; Nelson et al. 2010; Starling et al. 2014a). Longitudinal studies conducted in workers and highly exposed residents strengthen the interpretation of this association between serum PFOA and serum lipid levels. Serum PFOA levels were found to be a significant predictor of serum cholesterol levels in workers examined at least twice in a ≥5-year period (Olsen et al. 2003a; Sakr et al. 2007a). Similarly, a study of highly-exposed residents examined twice with approximately 4 years between examinations found that there was a 1.7% decrease in serum total cholesterol levels in subjects whose serum PFOA levels decreased by 50% between examinations (Fitz-Simon et al. 2013). As noted in Steenland et al. (2010a), there is considerable variation in the strength of the association between PFOA and serum cholesterol, with the greatest changes in serum cholesterol occurring at lower PFOA levels. The change in cholesterol levels per ng/mL change in serum PFOA ranged from 0.0007, calculated from data from the Olsen et al. (2000) occupational exposure study, to 2.0 calculated from data from the Nelson et al. (2010) general population study; the mean serum PFOA levels in these studies were ~22,000 and 4 ng/mL respectively. In a clinical trial, administration of APFO to patients with advanced solid tumors at doses of 50–1,200 mg weekly for 6 weeks resulted in decreases in serum cholesterol levels; the marked decreases in serum cholesterol levels were observed at serum PFOA concentrations of 175,000–230,000 ng/mL (Convertino et al. 2018). These results are similar to those observed in laboratory animals, suggesting that the dose-response curve may be biphasic. Steenland et al. (2010a) and Frisbee et al. (2010) suggested that this may be due to a steep dose-response curve at low PFOA levels, which flattens out at higher PFOA levels and may be indicative of saturation. A similar pattern was also observed in the risks of elevated cholesterol per increases in serum PFOA levels (Figure 2-14). Several investigators have explored whether PFOA and cholesterol could be jointly affected or whether the associations were due to reverse causality (i.e., increased cholesterol resulted in increased serum PFOA levels). Butenhoff et al. (2012c) explored the issues of whether PFOA distributes into serum lipoprotein fractions, and whether increases in serum lipoproteins would result in increases in serum PFOA. They concluded that there was limited distribution to plasma lipoproteins, and did not consider it a non-causal factor. The Steenland et al. (2009b) study found slightly lower serum PFOA levels (4%) among individuals taking cholesterol medication, as compared to those not taking medication and noted that this was primarily a function of the large sample size. This finding does not support reverse causality.

Laboratory Animal Studies. Information from inhalation studies in animals is limited. Head-only exposure of male rats to 810 mg/m^3 APFO dusts for 4 hours caused liver enlargement, but

microscopically, the liver tissue appeared normal (Kennedy et al. 1986). Exposure head-only of male rats to 0, 1, 7.6, or 84 mg/m^3 APFO dusts 6 hours/day, 5 days/week for 2 weeks resulted in significant increases in absolute and relative liver weight at 7.6 and 84 mg/m^3 on exposure day 10; in rats from the 84 mg/m^3 group, absolute and relative liver weights were still significantly increased 28 days after exposure ceased (Kennedy et al. 1986). The activities of serum enzymes markers of liver function were unremarkable except for alkaline phosphatase, which was significantly increased in the 7.6 and 84 mg/m^3 groups immediately after exposure on day 10 and remained elevated in the 84 mg/m^3 group on day 14 of recovery. Histopathological changes were restricted to the 7.6 and 84 mg/m^3 groups and consisted of panlobular and centrilobular hepatocellular hypertrophy and necrosis. Panlobular hepatocellular hypertrophy was seen only after the 10th exposure, but was limited to the centrilobular hepatocytes 14 or 28 days after exposure terminated, and was absent 42 days following cessation of exposure. Inhalation exposure of pregnant rats to 25 mg/m^3 APFO dusts 6 hours/day during GDs 6–15 induced an 18% increase in absolute liver weight (Staples et al. 1984); no significant effect was reported in rats exposed to ≤10 mg/m^3.

Nose-only exposure of male CD rats to 67 mg/m^3 ammonium perfluorononanoate dusts for 4 hours induced significant increases (28–37%) in absolute and relative liver weight, assessed 5 and 12 days after exposure (Kinney et al. 1989). Histopathological examinations were not conducted in this study.

The liver is the main target organ for perfluoroalkyls in animals following short- or long-term oral exposures. The hepatic response to exposure to many perfluoroalkyls, particularly in rodents, is initiated by the activation of the nuclear hormone receptor, PPARα, which triggers a characteristic sequence of morphological and biochemical events characterized by liver hypertrophy and alteration of a wide range of enzymes, particularly those involved in lipid metabolism. It appears that PFOA can also damage the liver via a method independent of PPARα resulting in increases in liver weight, hepatocellular hypertrophy, microvesicular steatosis, and cholangiopathy (Abbott et al. 2007; Das et al. 2017; Minata et al. 2010; Wolf et al. 2008a; Yang e al. 2002b).

The most sensitive liver effect observed in rats and mice after acute oral exposure to PFOA is an increase in liver weight (Cook et al. 1992; Das et al. 2017; Eldasher et al. 2013; Haughom and Spydevold 1992; Ikeda et al. 1985; Iwai and Yamashita 2006; Kawashima et al. 1995; Kennedy 1987; Liu et al. 1996; Loveless et al. 2006; Pastoor et al. 1987; Permadi et al. 1992, 1993; Qazi et al. 2012; White et al. 2009; Wolf et al. 2007, 2008a; Xie et al. 2003; Yahia et al. 2010; Yang et al. 2001, 2002b). In rats orally administered 50 mg/kg/day PFOA for 1, 3, or 7 days, a 10% increase in liver weight was observed after

the first dose; however, the relative liver weight was not significantly different from controls (Pastoor et al. 1987). After 3 days of exposure, the relative liver weight was significantly higher (36%) than controls. Similarly, in mice, exposure to 390 mg/kg/day PFOA in the diet resulted in a significant increase in liver weight after 5 days of exposure, but not after 2 days of exposure (Permadi et al. 1992). The lowest LOAELs for increased relative liver weight in rats were 4.7 mg/kg/day in a 7-day study (Kawashima et al. 1995) and 2 mg/kg/day in a 14-day study (Liu et al. 1996); these studies also identified NOAELs of 2.4 and 0.2 mg/kg/day, respectively. In mice, the lowest LOAEL for increases in liver weight was 1 mg/kg/day PFOA administered in the diet for 10 days (Yang et al. 2001) or administered via gavage for 7 days (Eldasher et al. 2013; Wolf et al. 2008a). Pastoor et al. (1987) noted that oral administration of 50 mg/kg/day PFOA to rats for 7 days resulted in a 2-fold increase in absolute and relative liver weight, but no significant change in total deoxyribonucleic acid (DNA), indicating that the hepatomegaly represented hypertrophy rather than hyperplasia. Few acute-duration studies included histological examinations of the liver. Centrilobular and midzonal hypertrophy was observed in mice administered 1 or 3 mg/kg/day PFOA via gavage for 7 days; panlobular hypertrophy with cytoplasmic vacuolation was observed at 10 mg/kg/day (Wolf et al. 2008a). Qazi et al. (2010a) reported hepatocellular hypertrophy in mice exposed to 3.5 mg/kg/day PFOA in the diet for 10 days. Elcombe et al. (2010) reported hepatocellular hypertrophy in rats orally exposed to 18 mg/kg/day for 7 days, but not after 1 day of exposure. Increases in steatosis and triglyceride levels were observed in the livers of mice administered 10 mg/kg/day for 7 days (Das et al. 2017). A related liver effect was the finding of reduced serum cholesterol and triacylglycerol levels in rats administered 16 mg/kg/day PFOA in the diet for 7 days (Haughom and Spydevold 1992) and decreases in serum cholesterol and triglyceride levels in rats administered 18 mg/kg/day PFOA via gavage for 7 days (Elcombe et al. 2010).

Similar to the acute-duration studies, intermediate-duration oral exposure to PFOA resulted in increases in absolute and relative liver weights in rats (Biegel et al. 2001; Butenhoff et al. 2004b; Griffith and Long 1980; Perkins et al. 2004) and mice (Abbott et al. 2007; Ahmed and Abd Ellah 2012; Albrecht et al. 2013; Griffith and Long 1980; Kennedy 1987; Lau et al. 2006; Son et al. 2008; Wolf et al. 2007; Yang et al. 2009). The lowest dose resulting in increases in liver weight in rats was 0.96 mg/kg/day, observed following gavage administration of APFO for 28 days (Loveless et al. 2008); the lowest dose in mice was 0.5 mg/kg/day, observed in two 28-day studies using APFO (Kennedy 1987; Son et al. 2008). No significant alterations in liver weight were observed in rats administered 0.29 mg/kg/day for 28 days (Loveless et al. 2008) or in mice exposed to 0.2 mg/kg/day for 21 days (Kennedy 1987). Hepatocellular hypertrophy was the predominant histopathological alteration in rats (Cui et al. 2009; Griffith and Long 1980; Loveless et al. 2008; Perkins et al. 2004) and mice (Albrecht et al. 2013; Filgo et al. 2015a; Griffith

and Long 1980; Loveless et al. 2008; Tan et al. 2013); the severity of the hypertrophy was dose-related (Filgo et al. 2015a; Loveless et al. 2008). At higher doses, focal necrosis was observed (LOAEL of 29 mg/kg/day in rats and 0.96 mg/kg/day in mice exposed for 28 days) (Loveless et al. 2008). Fatty changes were observed in rats administered 20 mg/kg/day for 28 days (Cui et al. 2009) and mice administered 9.6 mg/kg/day (Loveless et al. 2008). No significant alterations in liver weight or histopathology were observed in rats allowed to recover for 8 weeks following a 13-week exposure to 0.6–6.5 mg/kg/day (Perkins et al. 2004). Intermediate-duration exposure to PFOA also resulted in decreases in serum HDL cholesterol levels in rats and mice administered ≥0.29 or 0.96 mg/kg/day, respectively, for 28 days (Loveless et al. 2008). Serum cholesterol levels were decreased in rats administered 0.29 or 0.96 mg/kg/day (no changes were observed at higher doses) and in mice administered 9.6 or 29 mg/kg/day (Loveless et al. 2008). Similarly, serum triglyceride levels were decreased in rats administered 0.29–9.6 mg/kg/day and in mice administered 9.6 or 29 mg/kg/day (Loveless et al. 2008). In a study of mice fed a western-type diet, increases in plasma cholesterol levels were observed after 6 weeks of dietary exposure to 0.55 mg/kg/day in BALB/c or C57BL/6 mice (Rebholz et al. 2016). The results of this study suggest that diet (fat intake and/or cholesterol levels) may influence the response to PFOA and may account for some of the differences observed in humans and rats fed a standard diet, which is typically low in fat.

Chronic exposure of rats to PFOA resulted in hepatocellular hypertrophy, hepatocellular necrosis, and portal mononuclear cell infiltration after a 1-year exposure to a LOAEL of 15 mg/kg/day in the diet (3M 1983; Butenhoff et al. 2012c). A 2-year exposure to 15 mg/kg/day resulted in hepatocellular hypertrophy, cystoid degeneration, and portal mononuclear cell infiltration (3M 1983; Butenhoff et al. 2012c). The study also found significant increases in ALT and AST levels in male rats exposed to 1.5 mg/kg/day. A second chronic exposure study found significant increases in relative liver weight in rats exposed to 13.6 mg/kg/day in the diet for 2 years; no non-neoplastic lesions were noted in the liver (Biegel et al. 2001).

Studies in monkeys suggest that longer-term exposure may also result in liver effects. Significant increases in absolute and relative liver weight were observed in Cynomolgus monkeys exposed to 20/30 mg/kg/day administered via capsules for 26 weeks (Butenhoff et al. 2002). A significant increase in absolute, but not relative, liver weight was also observed in monkeys administered 3 or 10 mg/kg/day. However, no histological alterations were observed in the livers at the doses tested. Similarly, no histological alterations were observed in the livers of Cynomolgus monkeys administered 2 or 20 mg/kg/day via capsules for 30 days (Thomford 2001) or Rhesus monkeys administered 3 or

10 mg/kg/day via gavage for 90 days (Griffith and Long 1980). Significant increases in serum triglyceride levels were observed in the 10 and 20/30 mg/kg/day groups; the increases were statistically significant at only some of the time points (Butenhoff et al. 2002). At 10 mg/kg/day, increases in serum triglyceride levels at 4, 10, and 14 weeks of exposure were significantly higher than pre-treatment levels. Increases in cholesterol levels were only observed in the 20/30 mg/kg/day group after 13 weeks of exposure, but not after 26 weeks. No alterations in serum cholesterol or triglyceride levels were observed in the Thomford (2001) study.

Several studies have examined PPARα-null mice to assess whether PFOA-induced liver effects can also occur via a mechanism independent of PPARα-receptor activation. Similar to wild-type mice, exposure to PFOA resulted in significant increases in liver weight (Abbott et al. 2007; Das et al. 2017; Minata et al. 2010; Wolf et al. 2008a; Yang e al. 2002b). Abbott et al. (2007) found that the effect level was slightly higher in PPARα-null mice than wild-type mice (3 versus 1 mg/kg/day) following oral exposure on GDs 1–17 (liver weights measured at weaning). Wolf et al. (2008a) and Minata et al. (2010) reported the same effect level (1 or 5 mg/kg/day, respectively) in PPARα-null mice and wild-type mice administered PFOA via gavage for 7 days or 4 weeks. Wolf et al. (2008a) found dose-related increases in hepatocellular cytoplasmic vacuoles at ≥1 mg/kg/day and suggested that the increase in liver weight was due to the accumulation of PFOA in the hepatocytes rather than a toxic response. Hepatocyte proliferation was also observed at 10 mg/kg/day. Unlike the Wolf et al. (2008a) study, the Minata et al. (2010) 4-week study reported hepatocellular hypertrophy and microvesicular steatosis in the PPARα-null mice (no incidence data were provided and it is unclear at what dose levels these effects were found); cytoplasmic vacuolation was also reported in the hepatocytes. Filgo et al. (2015a) also reported hepatocellular hypertrophy in PPARα-null mice; the LOAEL was 3 mg/kg/day, which was higher than the LOAEL of 0.3 mg/kg/day found in wild-type mice. Minata et al. (2010) also reported cholangiopathy in both the wild-type and PPARα-null mice, but noted that the effect was more intensive in the PPARα-null mice. No significant alterations in steatosis or triglyceride accumulation were observed in PPARα-null mice administered 10 mg/kg/day for 7 days, but were observed in wild-type mice (Das et al. 2017). Additionally, significant decreases in serum total cholesterol levels at 5.2 and 10.2 mg/kg/day and increases at 20.7 mg/kg/day were observed in the PPARα-null mice; significant decreases in total cholesterol were observed in the wild-type mice at 10.2 and 20.7 mg/kg/day doses. Serum triglyceride levels were increased in both strains at 5.2 and 10.2 mg/kg/day doses and in the PPARα-null mice at 20.7 mg/kg/day (Minata et al. 2010).

Intermittent application of 20, 200, or 2,000 mg/kg APFO to the skin of rats for 2 weeks resulted in the presence of one or more foci of coagulative necrosis in the livers from all treated groups (Kennedy 1985). The Kupffer cells within the foci of hepatocellular necrosis contained large vesicular nuclei and were markedly increased in number. At 2,000 mg/kg/day, these changes were seen in three out of five rats killed on the 10th day of exposure, in three out of five rats killed on recovery day 14, and in one out of five rats killed on recovery day 42. This lesion occurred in two out of five rats from the 20 mg/kg/day dose group killed on day 10 of exposure. Serum ALT activity appeared elevated at termination of exposure in a dose-related manner, but without achieving statistical significance. A similar trend was seen for AST activity, but achieving statistical significance in the high-dose group. The blood concentrations of organofluorine on the 10th day of exposure were 10.2, 52.4, 79.2, and 117.8 µg/mL in the control, low-, mid-, and high-dose groups, respectively. A study in mice reported that application of 6.25 mg/kg/day PFOA on the dorsal surface of each ear for 4 days resulted in a 52% increase in absolute liver weight (Fairley et al. 2007); no significant effect occurred after application of 2.5 mg/kg/day.

Summary. Epidemiological studies examining the hepatotoxicity of PFOA have examined three outcomes—risk of liver disease, evidence of hepatocellular damage (as measured by alterations in serum hepatic enzymes and bilirubin levels), and alterations in serum lipid levels (total cholesterol, LDL cholesterol, HDL cholesterol, and triglycerides)—among workers, residents living near a PFOA manufacturing facility with high levels of drinking water contamination, and the general population. Exposure to PFOA does not appear to be associated with increased risks of liver disease in workers or highly exposed community members. The epidemiological studies have found associations between serum PFOA levels and increases in serum ALT, AST, and GGT enzyme levels and decreases in serum bilirubin levels. However, the results have not been consistently found, and serum enzyme levels were typically within the normal range. Four studies examined the risk of serum enzyme levels outside of the normal range; the results were mixed for the risk of elevated ALT, with two studies finding an increased risk and two studies finding no association. A number of occupational, community, and general population studies have found associations between serum PFOA levels and serum total cholesterol levels; several studies have also found no associations. Studies examining the change in cholesterol levels per change in serum PFOA levels have found greater increases in serum cholesterol levels associated with serum PFOA levels at the lower range of PFOA levels and the dose-response curve suggests a biphasic relationship. Positive associations have also been observed for LDL cholesterol, although associations have not been consistently found. In general, no consistent associations were found between serum PFOA and HDL cholesterol or triglyceride levels.

Studies in laboratory animals have found strong associations between PFOA exposure and hepatotoxicity. Liver effects have been observed in rats exposed to airborne APFO dusts; in rats, mice, and monkeys following oral exposure for acute-, intermediate-, or chronic-durations; and in rats following dermal exposure. The observed effects typically include increases in liver weight, hepatocellular hypertrophy, and decreases in serum cholesterol and triglyceride levels. Other effects that have been observed include hyperplasia, necrosis, and fatty degeneration. Available evidence suggests that the increased liver weight, hypertrophy, and serum lipid alterations are likely due to PPARα initiation and therefore, may not be relevant to humans. However, other mechanisms of liver toxicity are also involved, as evidenced by liver effects observed in PPARα-null mice (Das et al. 2017; Minata et al. 2010; Wolf et al. 2008a). In contrast to the results observed in epidemiological studies, a clinical trial study in humans with advanced solid tumors exposed to very large doses of PFOA (Convertino et al. 2018) and human exposure to other PPARα agonists, such as fibrates (Staels et al. 1998), suggest that hypolipidemic effects, similar to those observed in rodents, may occur in humans exposed to PFOA, although humans may not be as sensitive as rodents.

PFOS

Epidemiological Studies—Liver Disease. Several studies have examined the possible association between PFOS exposure and liver diseases. No increases in deaths from cirrhosis of the liver were found in workers at the 3M facility in Decatur, Alabama (Alexander et al. 2003). Another study of workers at this facility found no significant alterations in the episodes of care for all liver disorders or all biliary duct disorders (Olsen et al. 2004a). However, among workers with at least 10 years of high potential exposure to PFOS, there were significant increases in episodes of care for cholelithiasis or acute cholecystitis and for all biliary tract disorders. A third study of workers at a PFOS facility in Decatur, Alabama did not find increases in cholelithiasis, cholecystitis, or liver disease (including cirrhosis and hepatitis) (Grice et al. 2007).

Epidemiological Studies—Hepatic Serum Enzymes and Bilirubin Levels. A series of studies conducted by Olsen and associates evaluated liver function (as assessed by serum liver enzymes) in workers at several 3M facilities involved in PFOS production. Using health data collected in 1995 and 1997, Olsen et al. (1999) did not find associations between serum PFOS and serum ALT, AST, or GGT enzymes at PFOS levels <6,000 ng/mL; a positive association with total bilirubin levels was found. No conclusions were drawn from the few workers with serum PFOS ≥6,000 ng/mL due to their small number (seven in 1995 and five in 1997 data). Similarly, no association of ALT, AST, or GGT and serum PFOA levels

were observed in groups of workers at these facilities examined in 1993 (111 subjects), 1995 (80 subjects), and/or 1997 (74 subjects) (Olsen et al. 2000). A subsequent evaluation of workers from the same plants, but that included women and a longitudinal analysis of the workers, reported that, after adjusting for potential confounding factors, there were no substantial changes in hepatic parameters (Olsen et al. 2003a). GGT levels in females and ALT levels in males with PFOS levels in the 4th quartile were significantly elevated in comparisons between individuals with serum PFOS levels in the 4th quartile to those with levels in the 1st quartile; however, there were no statistical adjustments for potential confounders. In contrast to these findings in workers, Gallo et al. (2012) reported significant increases in the risks of elevated ALT, GGT, and bilirubin levels in a study of C8 participants. Conflicting results have been found in general populations studies. Studies using the NHANES data set (Gleason et al. 2015; Lin et al. 2010) did not find associations between serum PFOS and ALT, AST, GGT, or total bilirubin levels. No increases in the risk of elevated levels of ALT, AST, or GGT were found (Gleason et al. 2015), although there was an increased risk of elevated total bilirubin levels. In a study of adults in Japan (Yamaguchi et al. 2013), significant correlations between serum PFOS and ALT, AST, and GGT levels were found.

Epidemiological Studies—Serum Lipids. Occupational, community, and general population studies have examined possible associations between serum PFOS levels and serum lipids; these data are summarized in Table 2-12. A graphical presentation of differences in total cholesterol and LDL cholesterol levels relative to serum PFOS levels and the risks of elevated total cholesterol and LDL cholesterol are presented in Figures 2-15, 2-16, 2-17, and 2-18.

In the Olsen occupational studies, significantly higher serum total cholesterol levels were found in workers with serum PFOS levels between 3,000 and 6,000 ng/mL (Olsen et al. 1999, 2003a). However, the studies found mixed results for associations between serum PFOS and other serum lipids, with one study finding an association with LDL cholesterol (Olsen et al. 1999) and the other finding an association with triglycerides (Olsen et al. 2003a). Longitudinal analysis was conducted using data for 174 workers with medical surveillance data in 2000 and 1997 and/or 1995 (Olsen et al. 2003a). No significant differences in serum PFOS levels were observed across the three time periods, and serum PFOS levels were not a significant predictor of cholesterol or triglyceride levels.

Two large-scale studies of participants in the C8 Science Panel studies found associations between serum PFOS levels and serum lipid levels (Frisbee et al. 2010; Steenland et al. 2009b). Associations between serum PFOS levels and total cholesterol, LDL cholesterol, and HDL cholesterol were found in a study of

Figure 2-15. Serum Total Cholesterol Levels Relative to Serum PFOS Levels
(Presented as percent change in cholesterol levels)

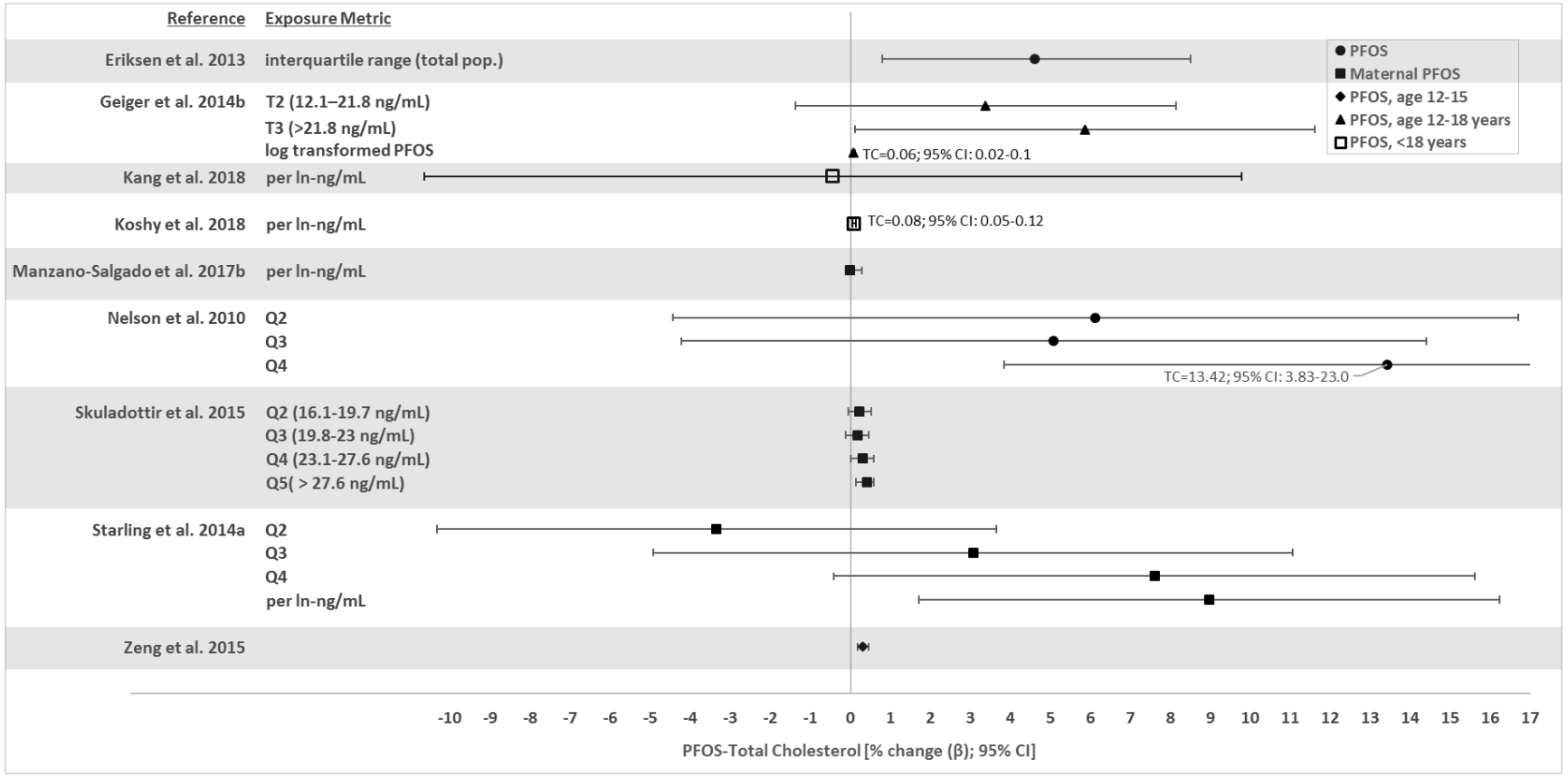


Figure 2-16. Risk of Abnormal Cholesterol Levels Relative to PFOS Levels (Presented as Adjusted Ratios)

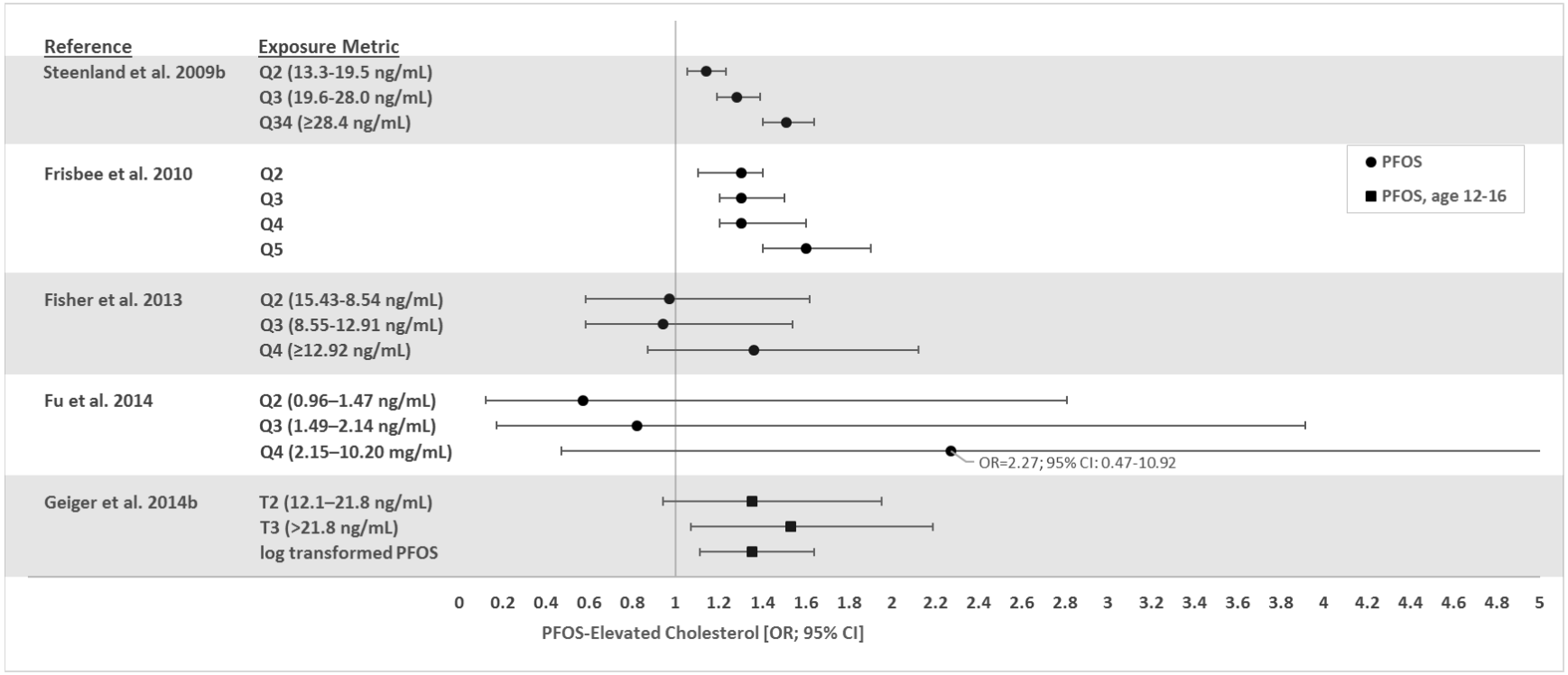


Figure 2-17. Serum LDL Cholesterol Levels Relative to Serum PFOS Levels
(Presented as percent change in LDL cholesterol levels)

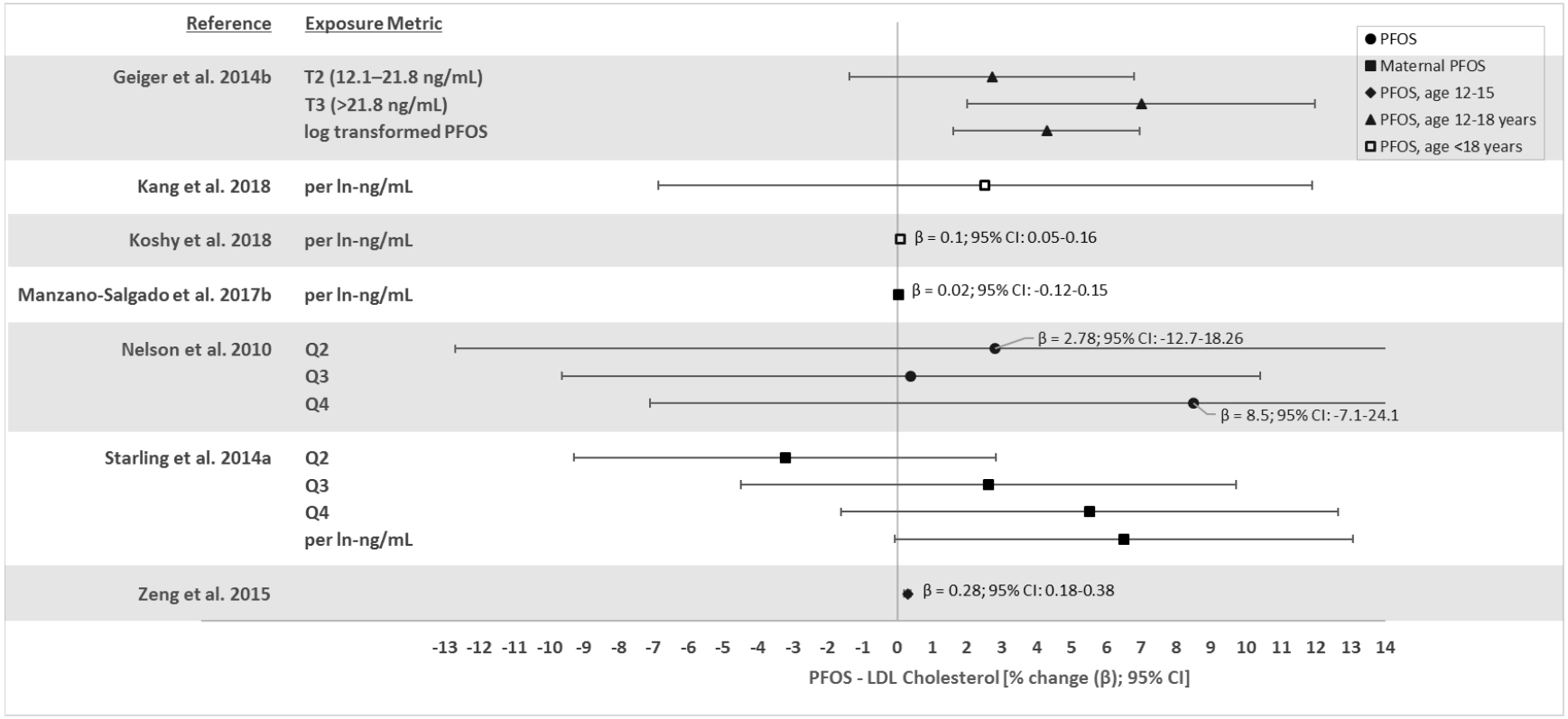
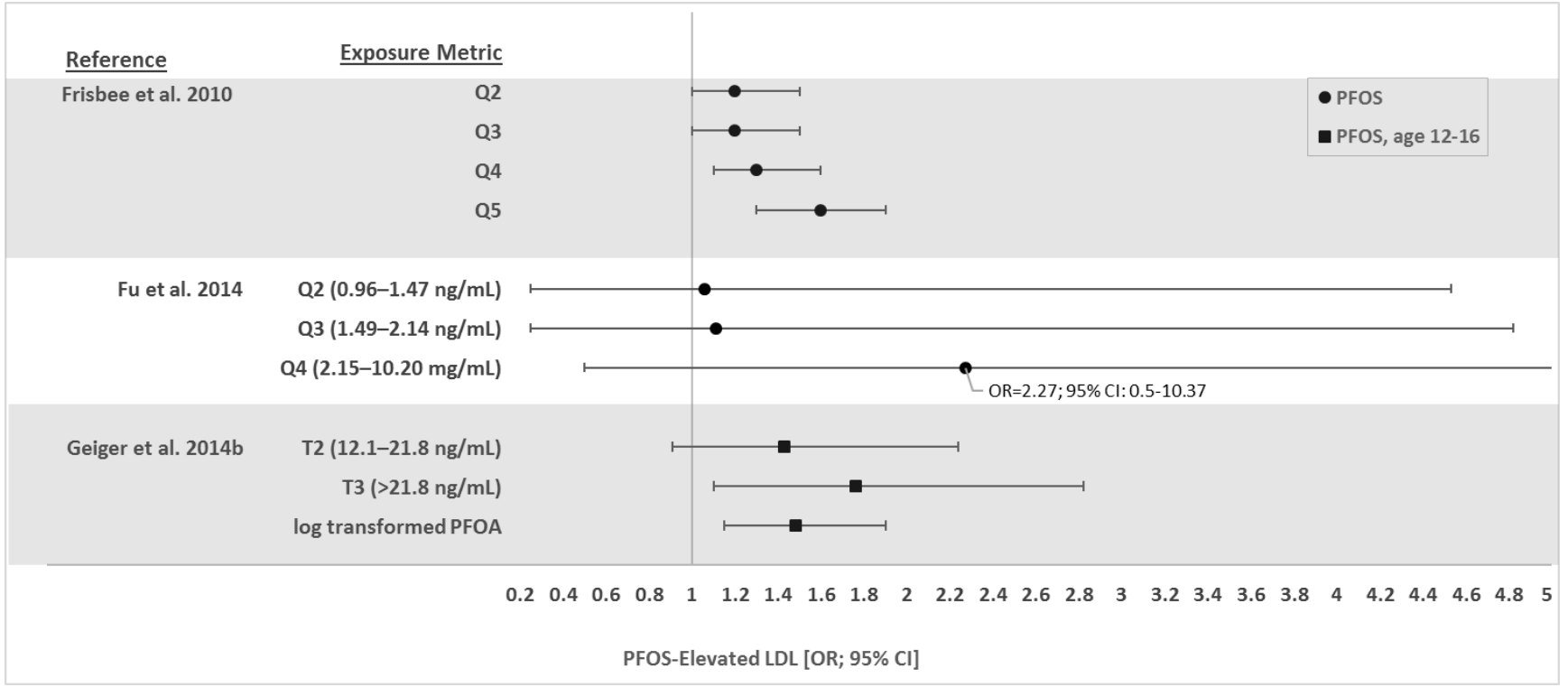


Figure 2-18. Risk of Abnormal LDL Cholesterol Levels Relative to PFOS Levels (Presented as Adjusted Ratios)



over 12,000 children and adolescents; the mean serum PFOS levels were 20.7 ng/mL in children (aged 1.0–11.9 years) and 19.3 ng/mL in adolescents (aged 12.0–17.9 years) (Frisbee et al. 2010). Similar findings were reported in a study of adults with a median serum PFOS level of 19.6 ng/mL; the study excluded subjects who reported taking cholesterol-lowering medication (Steenland et al. 2009b).

Associations were found between serum PFOS and total cholesterol, LDL cholesterol, and triglyceride levels, but not with HDL cholesterol. Participants with serum PFOS levels in the 2nd, 3rd, and 4th quartiles also had elevated risks of high cholesterol levels. Steenland et al. (2009b) noted that the odds of high cholesterol from the 1st to the 5th quintile was approximately 50% for PFOS, which may be important given that the Framingham study found that the risk of coronary heart disease was about 1.8 times higher in subjects with total cholesterol levels >240 mg/dL as compared to subjects with levels <200 mg/dL.

Steenland et al. (2009b) also examined over 10,000 participants who were taking cholesterol-lowering medication; an association between serum PFOS and total cholesterol levels was found in this group. Using both groups of subjects (taking or not taking cholesterol-lowering medication), the investigators analyzed whether taking cholesterol medication was associated with lower serum PFOA or PFOS levels, which may be indicative of reverse causality; no differences in serum PFOS levels were found between the two groups.

General population studies were conducted in the United States, Canada, and several European and Asian countries; these studies have found mixed results for associations between serum PFOS levels and serum lipids. Some studies have found associations between serum PFOS levels and serum total cholesterol (Nelson et al. 2010; Skuladottir et al. 2015; Starling et al. 2014a) and HDL cholesterol (Château-Degat et al. 2010); inverse associations between serum PFOS and HDL cholesterol (Starling et al. 2014a) and triglycerides (Château-Degat et al. 2010) were also found. However, other studies in adults have not found associations between serum PFOS and total cholesterol (Château-Degat et al. 2010; Eriksen et al. 2013; Fisher et al. 2013; Fu et al. 2014a; Liu et al. 2018b), non-HDL cholesterol (Fisher et al. 2013), LDL cholesterol (Château-Degat et al. 2010; Fisher et al. 2013; Fu et al. 2014a; Liu et al. 2018b; Starling et al. 2014a), HDL cholesterol (Fisher et al. 2013; Fu et al. 2014a; Liu et al. 2018b; Yang et al. 2018), or triglycerides (Fu et al. 2014a; Starling et al. 2014a; Liu et al. 2018b; Yang et al. 2018). Additionally, two studies did not find increased risks of elevated cholesterol levels (Fisher et al. 2013; Fu et al. 2014a). Several of these studies controlled for use of cholesterol-lowering medication (Château-Degat et al. 2010; Eriksen et al. 2013; Fisher et al. 2013; Nelson et al. 2010; Liu et al. 2018b). Overall, studies of children and adolescents have found associations for serum lipid levels. Geiger et al. (2014b) found increases in

the risk of elevated cholesterol and LDL cholesterol in children and adolescents aged 12–18 years; an association between serum PFOS and LDL cholesterol levels was also found. Zeng et al. (2015) found associations between serum PFOS and serum total cholesterol, LDL cholesterol, and triglyceride levels in children aged 12–15 years. Koshy et al. (2017) found an association between serum PFOS levels and serum total cholesterol, LDL cholesterol, and HDL cholesterol in children enrolled in the World Trade Center Health Registry. Timmermann et al. (2014) also found an association between serum PFOS and triglycerides only in obese Danish children (8–10 years of age), but not in normal weight children. In contrast, Maisonet et al. (2015b) found an inverse association between maternal serum PFOS and total cholesterol and LDL cholesterol in 15-year-old girls; no association was found when the girls were 7 years of age. Kang et al. (2018) did not find an association between serum PFOS and cholesterol, LDL cholesterol, or triglyceride levels in children aged 3–18 years, and Manzano-Salgado et al. (2017b) did not find associations between maternal serum PFOS and cholesterol, LDL cholesterol, HDL cholesterol, or triglyceride levels in 4-year-old children.

Laboratory Animal Studies. Unpublished data summarized by OECD (2002) indicate that inhalation exposure of rats to lethal concentrations (1,890–45,970 mg/m^3) of PFOS dusts for 1 hour resulted in varying discoloration of the liver.

Consistent with the results for PFOA, acute-duration oral exposure of rats to PFOS resulted in increases in liver weight (Elcombe et al. 2012b; Era et al. 2009; Haughom and Spydevold 1992), hepatocellular hypertrophy (Elcombe et al. 2012b), and decreases in serum cholesterol and/or triglyceride levels (Elcombe et al. 2012a, 2012b; Haughom and Spydevold 1992). The lowest adverse effect level for increased liver weight, hypertrophy, and decreased serum cholesterol was 1.79 mg/kg/day in rats exposed to PFOS in the diet for 7 days (Elcombe et al. 2012b); however, a similar study by this group did not find significant alterations in liver weight or ALT, AST, or serum cholesterol levels after 7 days of exposure to 1.72 mg/kg/day (Elcombe et al. 2012a). Likewise, in mice, increases in liver weight (Fuentes et al. 2006; Qazi et al. 2009b, 2010a; Wan et al. 2011), hepatocellular hypertrophy (Qazi et al. 2010a), and decreases in serum cholesterol levels (Qazi et al. 2010a) were observed following acute exposure to PFOS. The lowest LOAEL for liver weight was 3 mg/kg/day in mice administered PFOS via gavage on GDs 6–18 (Fuentes et al. 2006); no effects were observed at 1.5 mg/kg/day. The only acute-duration mouse study that included histopathological examination of the liver and measurement of serum cholesterol levels identified a LOAEL of 8.5 mg/kg/day in mice exposed to PFOS in the diet for 10 days (Qazi et al. 2010a).

Intermediate-duration exposure to PFOS resulted in increased liver weight in rats (Cui et al. 2009; Curran et al. 2008; Elcombe et al. 2012a; Seacat et al. 2003; Thibodeaux et al. 2003) and mice (Bijland et al. 2011; Thibodeaux et al. 2003; Wan et al. 2011, 2014b; Xing et al. 2016; Yahia et al. 2008), hepatocellular hypertrophy in rats (Cui et al. 2009; Curran et al. 2008; Elcombe et al. 2012a; Seacat et al. 2003), decreased serum cholesterol levels in rats (Curran et al. 2008; Elcombe et al. 2012a; Luebker et al. 2005b; Seacat et al. 2003), decreased total cholesterol, triglyceride, non-HDL cholesterol, and HDL cholesterol levels in mice (Bijland et al. 2011), and increased serum AST and GGT levels in mice (Xing et al. 2016). A mouse study (Bijland et al. 2011) also showed dramatic decreases in the hepatic production of VLDL and HDL (Bijland et al. 2011). Another mouse study (Lee et al. 2015b) did not find increases in hepatic lipid levels in dams, although there were alterations in fetal livers. Only one of the intermediate-duration mouse studies included histopathological examination of the liver. Xing et al. (2016) reported cytoplasmic vacuolization, focal necrosis, and hepatocellular hypertrophy in mice exposed to PFOS via gavage for 30 days; however, the study did not report incidence; the lowest dose tested was 2.5 mg/kg/day. The lowest adverse effect level for liver effects in rats was 0.14 mg/kg/day for a significant increase in relative liver weight in female rats, but not male rats, exposed to PFOS in the diet for 28 days (Curran et al. 2008). This study also found significant decreases in serum cholesterol levels and increases in absolute and relative liver weights in males and females at 2.98 mg/kg/day and hepatocellular hypertrophy at 5.89 mg/kg/day. Seacat et al. (2003) reported increases in liver weight, hepatocellular hypertrophy, and decreased serum cholesterol levels in rats following a 14-week dietary exposure to 1.33 mg/kg/day; however, no significant alterations in liver weight or liver histopathology were observed in rats exposed to 1.77 mg/kg/day PFOS in the diet for 4 weeks (Seacat et al. 2003). In contrast, Elcombe et al. (2012a) reported increases in liver weight, hepatocellular hypertrophy, and decreased serum cholesterol in rats exposed to 1.54 mg/kg/day PFOS in the diet for 28 days.

Data on the chronic toxicity of PFOS to the liver in rodents are limited to a study in rats (Butenhoff et al. 2012b; Thomford 2002b). Hepatotoxicity characterized by centrilobular hypertrophy, centrilobular eosinophilic hepatocytic granules, and centrilobular hepatocytic vacuolation was noted in rats exposed to PFOS in the diet for 2 years. Among rats sacrificed at the end of the study, significant increases in the incidence of centrilobular hepatocellular hypertrophy were observed in male and female rats exposed to ≥0.25 mg/kg/day (Thomford 2002b). When animals sacrificed at interim periods (14 or 52 weeks) and unscheduled deaths were included with animals sacrificed at exposure termination, the incidence of centrilobular hepatocellular hypertrophy was also increased in males exposed to 0.1 mg/kg/day. At ≥0.1 mg/kg/day, significant increases in the incidences of eosinophilic clear cell altered foci and cystic hepatocellular degeneration were observed in male rats. An increase in cystic degeneration was observed

in male rats exposed to ≥0.025 mg/kg/day. However, this was mainly due to a high incidence in unscheduled deaths; among animals sacrificed at exposure termination, the incidence was only increased in males exposed to 1.04 mg/kg/day. An increased incidence of single cell necrosis was observed in males and females at 1.04 mg/kg/day (all groups combined). Observations made in a group of rats exposed to 1.17 mg/kg/day PFOS for 52 weeks and allowed to continue on the control diet for an additional year showed that hepatotoxicity was not a persistent response, as hepatotoxicity was generally absent at the end of the recovery period. At termination, electron microscopy showed mild to moderate smooth endoplasmic reticulum hyperplasia and minimal to mild hepatocellular hypertrophy primarily in rats dosed with 1.5 mg/kg/day PFOS, the highest dose tested.

In a study of Cynomolgus monkeys administered via gavage three doses of PFOS over 315 days, decreases in HDL cholesterol levels were found; the investigators noted that the levels were still within the normal variation (Chang et al. 2017). No alterations in other serum clinical chemistry parameters were found. Treatment of Cynomolgus monkeys with up to 2 mg/kg/day PFOS administered via a capsule for 4 weeks did not induce gross or microscopic morphological alterations in the liver and did not increase cell proliferation (Thomford 2002a). In a 26-week study in Cynomolgus monkeys, exposure to 0.75 mg/kg/day PFOS, administered via a capsule resulted in increased absolute liver weight after 183 days of treatment (Seacat et al. 2002). Significant decreases in serum total cholesterol were also observed at 0.75 mg/kg/day after 91, 153, and 182 days of exposure. On day 182, total cholesterol decreased to 35 and 53% of predosing values in males and females, respectively. The HDL cholesterol levels were significantly lower in males at 0.03 and 0.75 mg/kg/day on days 153 and 182 and in females at 0.15 and 0.75 mg/kg/day on days 153 and 182; the lack of pre-treatment HDL cholesterol measurements precludes within-group comparisons. Serum bilirubin was significantly lower in males at 0.75 mg/kg/day on days 91, 153, and 182. Light microscopy of liver sections showed centrilobular vacuolation, hypertrophy, and mild bile stasis in some monkeys exposed to 0.75 mg/kg/day. Electron microscopy showed lipid-droplet accumulation in some males and females exposed to 0.75 mg/kg/day. Increased glycogen content was also noted at this dose level. No histological alterations were observed in the livers of monkeys exposed to 0.75 mg/kg/day for 26 weeks and allowed to recover for 7 months or 1 year. Similarly, serum cholesterol returned to pretreatment levels 36 days post exposure and HDL cholesterol levels returned to pretreatment levels after 61 days of recovery.

Summary. Epidemiological studies have examined the possible associations between PFOS exposure and liver disease in workers and hepatocellular damage and alterations in serum lipid levels in workers and the general population. The available occupational exposure studies or general population studies do not

consistently suggest an association between PFOS exposure and increases in the risk of liver disease or biliary tract disorders. A small number of occupational exposure studies have not found associations between serum PFOS levels and increases in ALT, AST, or GGT levels. Overall, the epidemiological studies suggest an association between serum PFOS levels and increases in serum total cholesterol levels and possibly serum LDL cholesterol levels. Studies of workers at a PFOS manufacturing facility found elevated serum total cholesterol levels in workers with high serum PFOS levels; however, a longitudinal analysis at the same facility did not find that serum PFOS was a significant predictor of cholesterol levels. Studies of residents living in an area with very high PFOA water levels found increases in serum total cholesterol levels associated with elevated serum PFOS levels in children, adolescents, and adults. Mixed results have been found for associations between serum PFOS and increases in serum total cholesterol levels in general population studies. Associations have been found between serum PFOS levels and serum LDL-cholesterol levels among non-occupational populations.

In laboratory animals, oral exposure to PFOS results in increases in liver weight, hepatocellular hypertrophy, and decreases in serum lipid levels. A small number of studies also reported focal necrosis and centrilobular hepatocytic vacuolization. The proposed mechanism of action for the increased liver weight, hepatocellular hypertrophy, and decreased serum lipid levels involves PPARα receptor activation. Due to species differences for this mechanism, these effects observed in rodents are not considered relevant to humans. The applicability of the hepatic hypertrophy and serum lipid alterations observed in rodent studies to humans has been questioned due to species differences in the presumed mechanism of action for these effects in rodents.

PFHxS

Epidemiological Studies—Hepatic Serum Enzymes and Bilirubin Levels. Lin et al. (2010) did not find associations between serum ALT and GGT levels with serum PFHxS levels in a general population study using the NHANES data set.

Epidemiological Studies—Serum Lipids. Eight studies have evaluated the potential association between serum PFHxS levels and serum lipids in the general population. A study utilizing the NHANES data set for adults not taking cholesterol-lowering medication reported an association between serum PFHxS and non-HDL cholesterol, but no associations with total cholesterol, LDL cholesterol, or HDL cholesterol (Nelson et al. 2010). In a study of Canadian adults not taking cholesterol-lowering medication with a geometric mean serum PFHxS level of 2.16 ng/mL, associations were found for total cholesterol, LDL

cholesterol, and non-HDL cholesterol (Fisher et al. 2013). The study also found increased odds of having a high cholesterol level with increasing PFHxS levels. Associations between serum PFHxS levels and HDL cholesterol and triglyceride levels were found in a study of Chinese men (Yang et al. 2018). In pregnant women in Norway with median serum PFHxS levels of 0.60 ng/mL, serum PFHxS levels were associated with serum HDL cholesterol, but not with total cholesterol, LDL cholesterol, or triglycerides (Starling et al. 2014a). No associations between serum PFHxS and total cholesterol, LDL cholesterol, HDL cholesterol, or triglyceride levels were found in a study of Taiwanese children aged 12–15 years (mean serum PFHxS of 2.1 ng/mL) (Zeng et al. 2015) or Korean children aged 3–18 years (mean serum PFHxS of 0.793 ng/mL) (Kang et al. 2018). A study of Spanish children aged 4 years found an association between maternal serum PFHxS and triglyceride levels, but not with cholesterol, LDL cholesterol, or HDL cholesterol (Manzano-Salgado et al. 2017b). A fourth study in children reported associations between serum PFHxS levels and serum cholesterol and LDL cholesterol, but not HDL cholesterol or triglycerides, in World Trade Center Health Registry enrollees (Koshy et al. 2017).

Laboratory Animal Studies. Acute-duration gavage administration of PFHxS resulted in increases in liver weight, steatosis, and increases in hepatic triglyceride levels in mice; increases in liver weight and steatosis were also observed in similarly exposed PPARα-null mice (Das et al. 2017). An intermediate-duration study with PFHxS in rats reported that gavage doses of ≥3 mg/kg/day induced a significant increase in absolute and relative liver weight in males (Butenhoff et al. 2009a). Light microscopy revealed minimal to moderate enlargement of centrilobular hepatocytes. Clinical chemistry tests showed a significant decrease in serum cholesterol at ≥0.3 mg/kg/day and decreased serum triglycerides at 10 mg/kg/day. None of these alterations were observed in female rats. Centrilobular hepatocellular hypertrophy was observed in mice administered ≥0.3 mg/kg/day PFHxS for 42–60 days (Chang et al. 2018); at 3 mg/kg/day single cell necrosis and microvascular fatty changes were also observed. In male mice, dietary exposure to PFHxS in a western-type diet resulted in >50% decreases in plasma triglyceride, total cholesterol, non-HDL cholesterol, and HDL cholesterol levels and approximately 75% decreases in the hepatic production of VLDL (Bijland et al. 2011). Increases in liver weight and hepatic triglyceride levels were also observed.

PFNA

Epidemiological Studies—Hepatic Serum Enzymes and Bilirubin Levels. A health evaluation of workers at a U.S. polymer production facility using PFNA did not find alterations in ALT, AST, GGT, or bilirubin levels related to increases in exposure intensity score in a longitudinal analysis (Mundt et al.

2007). Associations between serum PFNA and ALT and GGT levels were observed in a NHANES data study (Gleason et al. 2015); however, another study (Lin et al. 2010) utilizing the NHANES data did not find associations between serum PFNA and these enzymes. Neither study found associations for AST or total bilirubin.

Epidemiological Studies—Serum Lipids. Longitudinal analysis of serum lipid levels in the occupational exposure study (Mundt et al. 2007) did not find significant differences in serum total cholesterol or triglycerides over time. In general population studies, associations have been observed between serum PFNA levels and total cholesterol levels in adults (Fu et al. 2014a; Nelson et al. 2010) and children (Koshy et al. 2017; Zeng et al. 2015). No associations with cholesterol were found in a study in pregnant women (Starling et al. 2014a) or studies in children (Kang et al. 2018; Manzano-Salgado et al. 2017b). Several studies have also found associations with LDL cholesterol (Fu et al. 2014a; Koshy et al. 2017; Zeng et al. 2015) or non-HDL cholesterol (Nelson et al. 2010), but others did not find associations for LDL cholesterol (Nelson et al. 2010; Kang et al. 2018; Starling et al. 2014a). Most studies did not find an association between serum PFNA and HDL cholesterol (Fu et al. 2014a; Nelson et al. 2010; Koshy et al. 2017; Manzano-Salgado et al. 2017b; Zeng et al. 2015) or triglycerides (Fu et al. 2014a; Kang et al. 2018; Koshy et al. 2017; Manzano-Salgado et al. 2017b;Starling et al. 2014a). Exceptions were the Starling et al. (2014a) study of pregnant women, which found a positive association for HDL cholesterol, Yang et al. (2018) study of men, which found associations for HDL cholesterol and triglycerides, and Zeng et al. (2015), which found an association with triglycerides in children. Fu et al. (2014a) did not find increased risks of elevated cholesterol, LDL cholesterol, or triglyceride levels or lowered HDL cholesterol levels in adults.

Laboratory Animal Studies. Ten studies have evaluated the hepatic toxicity of PFNA. The observed effects are consistent with effects observed for other perfluoroalkyls. Alterations in serum lipid levels consisted of decreases in serum HDL cholesterol levels in rats administered via gavage ≥1 mg/kg/day PFNA for 14 days (Fang et al. 2012a), decreases in serum triglyceride and cholesterol levels in mice receiving gavage doses of ≥1 mg/kg/day PFNA (Wang et al. 2015a), and decreases in serum cholesterol levels in mice administered 0.5 mg/kg/day PFNA (Singh and Singh 2018). Increases in liver weight were observed in rats nose-only exposed to ≥67 mg/m^3 PFNA (Kinney et al. 1989), in mice administered via gavage 10 mg/kg/day PFNA for 7 days (Das et al. 2017), in mice exposed to 0.5 mg/kg/day PFNA in the diet for 14 days (Kennedy 1987), in mice administered ≥0.2 mg/kg/day PFNA via gavage for 14 days (Wang et al. 2015a), and in the offspring of mice administered via gavage ≥0.83 mg/kg/day PFNA on GDs 1–17 or 1–18 (Das et al. 2015; Wolf et al. 2010). The increases in liver weight, hepatocellular

hypertrophy, and deceases in serum lipid levels are considered adaptive and not relevant to humans (Hall et al. 2012). Hepatocellular vacuolation was observed in mice administered via gavage 5 mg/kg/day PFNA for 14 days (Fang et al. 2012b). In PPARα-null mice, increases in liver weight were observed in non-pregnant mice administered via gavage ≥1.5 mg/kg/day PFNA for 18 days, but were not found in pregnant animals (Wolf et al. 2010). Das et al. (2017) found increases in liver weight, steatosis, and increases in liver triglyceride levels in PPARα-null mice administered 10 mg/kg/day PFNA for 10 days.

PFDA

Epidemiological Studies—Serum Lipids. Six general population studies have evaluated the potential relationships between serum PFDA and serum lipids and reported inconsistent results. Fu et al. (2014a) found an association between serum PFDA and total cholesterol in adults and Koshy et al. (2017) found associations between serum PFDA and total cholesterol in children. A study of men did not find associations between serum PFDA and HDL cholesterol or triglycerides (Yang et al. 2018). Studies in pregnant women (Starling et al. 2014a) and other studies in children (Kang et al. 2018; Zeng et al. 2015) did not find associations. Fu et al. (2014a), Starling et al. (2014a), and Koshy et al. (2017) found positive associations with HDL cholesterol; this was not found in the Zeng et al. (2015) study. Koshy et al. (2017) also found an association with LDL cholesterol. The other studies did not find associations between serum PFDA and LDL cholesterol (Fu et al. 2014a; Starling et al. 2014a; Zeng et al. 2015), and none found association with triglycerides (Fu et al. 2014a; Kang et al. 2018; Koshy et al. 2017; Starling et al. 2014a; Zeng et al. 2015). Only the Fu et al. (2014a) study looked for alterations in the risk of elevated cholesterol, LDL cholesterol, or triglyceride levels or decreased HDL cholesterol levels, but the study did not find significant increases in the risk.

Laboratory Animal Studies. Hepatic effects observed in laboratory animals exposed to PFDA include alterations in liver weight and morphology. Increases in liver weight have been observed in mice following a single gavage dose of PFDA; the alterations were observed 2 days after exposure to 40 mg/kg/day (Brewster and Birnbaum 1989) or 30 days after exposure to ≥20 mg/kg/day (Harris et al. 1989). Repeated dietary exposure to 2.4 mg/kg/day PFDA for 1 week (Kawashima et al. 1995) or 78 mg/kg/day for 10 days (Permadi et al. 1992, 1993) also resulted in increases in liver weight. Oral doses ≥9.5 mg/kg/day also resulted in increases in hepatic cholesterol levels in rats (Kawashima et al. 1995) and hepatic lipids in mice (Brewster and Birnbaum 1989). These acute doses were also associated with hepatocellular hypertrophy and evidence of peroxisome proliferation. Thirty days after a single gavage dose of ≥20 mg/kg/day PFDA, effects included periportal to panlobular hepatocellular

hypertrophy characterized by swollen hepatocytes with abundant granular eosinophilic cytoplasm and enlarged and hyperchromatic nuclei (Harris et al. 1989).

In intermediate-duration exposure studies, an increased incidence of minimal single cell hepatocellular necrosis were observed in rats administered 0.5 mg/kg/day PFDA for 28 days (Frawley et al. 2018).

PFUnA

Epidemiological Studies—Serum Lipids. Of the five studies evaluating potential associations between serum PFUnA and serum lipids, only a study by Kang et al. (2018) in children found an association between serum PFUnA and total cholesterol and LDL cholesterol. The other studies did not find associations between serum PFUnA and total cholesterol or LDL cholesterol (Fu et al. 2014a; Koshy et al. 2107; Starling et al. 2014a) or with HDL cholesterol or triglycerides (Yang et al. 2018). None of the studies found associations with triglyceride levels. Starling et al. (2014a) and Koshy et al. (2017) found associations of serum PFUnA levels with HDL cholesterol levels; Fu et al. (2014a) did not find an association for this parameter. No alterations in the risk of abnormal serum lipid levels were found in the adults examined by Fu et al. (2014a).

Laboratory Animal Studies. Only one animal study was identified that examined the liver following oral exposure to PFUnA. In an intermediate-duration study of rats administered PFUnA via gavage, increases in relative liver weight were observed in males at 0.3 mg/kg/day and in females at 1.0 mg/kg/day, and mild to moderate centrilobular hepatocellular hypertrophy was observed in males and females at 1.0 mg/kg/day (Takahashi et al. 2014).

PFHpA

Epidemiological Studies—Serum Lipids. Epidemiological data on PFHpA are limited to a study in adults conducted by Fu et al. (2014a), which found no associations between serum PFHpA and total cholesterol, LDL cholesterol, HDL cholesterol, or triglyceride levels and a study in men conducted by Yang et al. (2018), which found no associations between serum PFHpA and HDL cholesterol or triglycerides.

PFBS

Epidemiological Studies—Serum Lipids. In the only epidemiological study examining serum lipids and possible associations with serum PFBS, Zeng et al. (2015) found an association with total cholesterol levels in children. No associations were found between serum PFBS and LDL cholesterol, HDL cholesterol, or triglycerides.

Laboratory Animal Studies. Treatment of male rats with 900 mg/kg/day PFBS by gavage for 28 days induced a significant increase in absolute and relative liver weight (25–30%) relative to controls, which was no longer detected following a 14-day recovery period (3M 2001). Clinical chemistry tests of liver function were unremarkable and there were no chemical-related microscopic alterations. No alterations in liver weight, serum chemistry parameters (ALT, AST, cholesterol), or liver morphology were observed in rats administered gavage doses as high as 600 mg/kg/day PFBS for 90 days (Lieder et al. 2009a). Significant increases in liver weight were observed at 300 and 1,000 mg/kg/day in a 2-generation study (Lieder et al. 2009b); the alterations were only observed in male rats. An increase in hepatocellular hypertrophy was also observed in the male P0 and F1 rats administered via gavage 1,000 mg/kg/day. Dietary exposure to mice resulted in decreases in plasma triglyceride levels and hepatic cholesterol levels, but no alterations in liver weight or plasma cholesterol, HDL cholesterol, or non-HDL cholesterol (Bijland et al. 2011).

PFBA

Epidemiological Studies—Serum Lipids. Only one epidemiological study examined hepatic outcomes; this study (Fu et al. 2014a) did not find any associations between serum PFBA levels and total cholesterol, LDL cholesterol, HDL cholesterol, or triglycerides in adults.

Laboratory Animal Studies. Treatment of rats with up to 184 mg/kg/day PFBA by gavage for 5 days did not affect liver weight, nor did it cause gross or microscopic morphological alterations in the liver (3M 2007a). In addition, clinical chemistry tests did not indicate altered liver function. Similarly, administration of approximately 20 mg/kg/day PFBA in the diet to male rats for 2 weeks did not significantly affect relative liver weight, but the same dose of PFOA induced a 45% increase in liver weight (Ikeda et al. 1985). Dietary administration of doses of approximately 78 mg/kg/day PFBA to male mice for 10 days induced a 63% increase in absolute liver weight (Permadi et al. 1992, 1993).

PFBA intermediate-duration studies have consistently found increases in liver weight and histological alterations. Dosing rats with PFBA by gavage for 28 days resulted in significant increases in absolute and relative liver weight and decreases in serum cholesterol at ≥30 mg/kg/day and hepatocellular hypertrophy at 150 mg/kg/day (Butenhoff et al. 2012a; van Otterdijk 2007a). Administration of 150 mg/kg/day PFBA induced hepatocyte hypertrophy. These liver effects were no longer detected after a 21-day recovery period. In a similar 90-day study, administration of 30 mg/kg/day PFBA resulted in increased absolute liver weight and panlobular hepatocyte hypertrophy (Butenhoff et al. 2012a; van Otterdijk 2007b); no liver effects were observed at 6 mg/kg/day. None of the liver alterations were observed after a 21-day recovery period.

PFDoDA

Epidemiological Studies—Serum Lipids. A general population study of adolescents (Zeng et al. 2015) did not find any associations between serum PFDoDA and total cholesterol, LDL cholesterol, HDL cholesterol, or triglyceride levels.

Laboratory Animal Studies. Dosing of male Sprague-Dawley rats with 10 mg/kg/day PFDoDA by gavage for 14 days induced a 35% increase in total serum cholesterol; doses of 1 or 5 mg/kg/day had no significant effect (Shi et al. 2007). In a subsequent study, the same group of investigators reported that in rats dosed via gavage with 1 or 5 mg/kg/day PFDoDA, there was a trend for decreased serum triglycerides, but the differences with controls were not statistically significant (Zhang et al. 2008); at 10 mg/kg/day, serum triglyceride levels were significantly increased. Liver triglyceride and liver cholesterol levels were increased at ≥5 mg/kg/day. Absolute liver weight was significantly reduced in the 5 mg/kg/day group (19%) relative to controls, but this may have been due to a marked reduction in body weight (shown in Shi et al. [2007], but not in Zhang et al. [2008]).

In a 42-day PFDoDA gavage administration study, increases in relative liver weight were observed in males at ≥0.5 mg/kg/day and hepatocellular hypertrophy was observed at 2.5 mg/kg/day (Kato et al. 2015). The study also found decreases in serum cholesterol at 0.1 and 0.5 mg/kg/day, but not at 2.5 mg/kg/day. In pregnant females (most dying before the end of the study), single cell hepatocyte necrosis was observed at 2.5 mg/kg/day (Kato et al. 2015). Prebiliary infiltration of inflammatory cells (males), disposition of bilirubin (females), and hepatocellular hypertrophy (females) were observed in males and nonpregnant females administered 2.5 mg/kg/day PFDoDA for 42 days followed by a 42-day recovery period (Kato et al. 2015).

PFHxA

Laboratory Animal Studies. Increases in liver weight, decreases in serum cholesterol levels, and centrilobular hepatocellular hypertrophy have been observed in rats administered 315 mg/kg/day PFHxA for 32–44 days (Kirkpatrick 2005) or ≥100 mg/kg/day NaPFHx for 90–93 days (Chengelis et al. 2009b; Loveless et al. 2009). In a chronic-duration study, gavage administration of 200 mg/kg/day for 2 years resulted in increases in the incidence of hepatocellular necrosis in female rats (Klaunig et al. 2015). At 100 mg/kg/day, decreases in triglyceride levels were observed in male rats.

FOSA

Laboratory Animal Studies. In the only study examining hepatic effects, Seacat and Luebker (2000) reported no alterations in liver weight in rats receiving a single gavage dose of 5 mg/kg FOSA.

2.10 RENAL

Overview. Epidemiological and laboratory animal studies have evaluated the potential of perfluoroalkyls to be renal toxicants. Human studies have evaluated the risk of kidney disease, alterations in renal function, damage to the kidney, and alterations in uric acid levels. The results of epidemiological studies evaluating kidney disease and renal function are summarized in Table 2-13; Table 2-14 contains the studies evaluating alterations in uric acid levels. More detailed descriptions of these studies can be found in the *Supporting Document for Epidemiological Studies for Perfluoroalkyls*, Table 8. Although there are a couple of studies finding associations between PFOA exposure and kidney disease, the results are not consistent across study populations. However, there is some indication that perfluoroalkyls may affect renal function. Decreases in estimated glomerular filtration rate and increases in uric acid levels associated with serum PFOA or PFOS have been reported in a number of epidemiological studies. However, these alterations may be due to reverse causality (i.e., increases in serum perfluoroalkyl levels could be due to a decrease in glomerular filtration and shared renal transporters for perfluoroalkyls and uric acid). Based on the small number of epidemiological studies or the inconsistency of the results, possible associations between other perfluoroalkyls (PFHxS, PFNA, PFDA, PFBS, PFDoDA, or PFHxA) and renal functions cannot be assessed. No studies were available for PFUnA, PFHpA, PFBA, or FOSA.

Table 2-13. Summary of Renal Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFOA			
Costa et al. 2009 Occupational (n =53)	12,930 ng/mL (mean PFOA in current workers)	Serum urea	NS (p>0.05)
		Serum creatinine	NS (p>0.05)
		Total proteins	NS (p>0.05)
		α1 globulins, α2 globulins, β globulins, or γ globulins	NS (p>0.05)
		α2 globulins	**Association (p<0.01), current, former and non-exposed workers.**
Lundin et al. 2009 Occupational (n=3,992)	NR	Nephritis and nephrosis deaths	SMR 5.2 (0.6–18.9)
Raleigh et al. 2014 Occupational (n=9,027)	NR	Chronic kidney disease deaths	HR 0.73 (0.21–2.48), 4th quartile
Steenland et al. 2015 Occupational (n=3,713)	Estimated cumulative PFOA	Chronic kidney disease risk	NS (p=0.92), no lag NS (p=0.99), 10-year lag
Steenland and Woskie 2012 Occupational (n=1,084)	7,800 ng/mL-year (mean PFOA)	Chronic kidney disease deaths	**SMR 3.79 (1.03–9.71)*, 2nd quartile**
Anderson-Mahoney et al. 2008 Community (n=566)	NR	Kidney disease (self-reported)	**SPR 2.26 (1.45–3.51)***
Dhingra et al. 2016b Community (C8) (n=28,541)	Estimated cumulative PFOA	Chronic kidney disease	NS (p=0.80 for trend), no lag NS (p=0.81 for trend), 5-year lag NS (p=0.88 for trend), 10-year lag NS (p=0.30 for trend), 20-year lag
Emmett et al. 2006b Community (n=371)	354 ng/mL (median PFOA)	Serum creatinine	NS (p>0.05)
		BUN	NS (p>0.05)
		Total serum proteins	NS (p>0.05)

Table 2-13. Summary of Renal Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Watkins et al. 2013 Community (C8) (9,660 children)	28.3 ng/mL (median PFOA)	GFR	**Inverse association (p=0.02)***
Kataria et al. 2015 General population (NHANES) (n=1,960 adolescents)	≥4.7 ng/mL (4th PFOA quartile)	GFR	**Inverse association (p<0.01)*, 4th quartile**
		Serum uric acid	**Association (p<0.01)***
Shankar et al. 2011a General population (NHANES) (n=4,587)	>5.9 ng/mL (4th PFOA quartile)	GFR	**Inverse association (p<0.001 for trend)***
		Chronic kidney disease	**OR 1.73 (1.04–2.88)*, 4th quartile**
PFOS			
Olsen et al. 1998a Occupational (n=178 in 1995; n=149 in 1997)	2,440 and 1,930 ng/mL (mean PFOS in 1995 in Decatur and Antwerp) 1,960 and 1,480 ng/mL (mean in 1997 in Decatur and Antwerp)	Serum creatinine	**Association (p<0.06)*, 1997 only**
		BUN	NS (p>0.1)
Watkins et al. 2013 Community (C8) (9,660 children)	20.0 ng/mL (median PFOS)	GFR	**Inverse association (p=0.0001)***
Kataria et al. 2015 General population (NHANES) (n=1,960 adolescents)	7.9–12.8 ng/mL (2nd PFOS quartile) ≥19.4 ng/mL (4th PFOS quartile)	GFR	**Inverse association (p<0.05)*, 2nd quartile**
Shankar et al. 2011a General population (NHANES) (n=4,587)	>29.5 ng/mL (4th PFOS quartile) 11.2–17.8 ng/mL (2nd PFOS quartile)	GFR	**Inverse association (p<0.001 for trend)***
		Chronic kidney disease	**OR 1.82 (1.02–3.27)*, 4th quartile**
PFHxS			
Watkins et al. 2013 Community (C8) (9,660 children)	5.2 ng/mL (median PFHxS)	GFR	**Inverse association (p=0.003)***

Table 2-13. Summary of Renal Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Kataria et al. 2015 General population (NHANES) (n=1,960 adolescents)	≥4 ng/mL (4th PFHxS quartile)	GFR	NS (p>0.05)
PFNA			
Mundt et al. 2007 Occupational (n=592)	NR	BUN Creatinine	Small, but not clinically significant
Watkins et al. 2013 Community (C8) (9,660 children)	1.5 ng/mL (median PFNA)	GFR	**Inverse association (p=0.002)***
Kataria et al. 2015 General population (NHANES) (n=1,960 adolescents)	≥1.5 ng/mL (4th PFNA quartile)	GFR	NS (p>0.05)

[a]See the *Supporting Document for Epidemiological Studies for Perfluoroalkyls,* Table 8 for more detailed descriptions of studies.
[b]Participants in occupational exposure may have also lived near the site and received residential exposure; community exposure studies involved subjects living near PFOA facilities with known exposure to high levels of PFOA.
[c]Asterisk indicates association with perfluoroalkyl; unless otherwise specified, values in parenthesis are 95% confidence intervals.

BUN = blood urea nitrogen; GFR = glomerular filtration rate; HR = hazard ratio; NHANES = National Health and Nutrition Examination Survey; NR = not reported; NS = not significant; OR = odds ratio; PFHxS = perfluorohexane sulfonic acid; PFNA = perfluorononanoic acid; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid; SMR = standardized mortality ratio

Table 2-14. Summary of Uric Acid Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFOA			
Costa et al. 2009 Occupational (n=53)	12,930, ng/mL (mean PFOA in current workers)	Serum uric acid	**Association (p=0.039)***
Sakr et al. 2007b Occupational (n=1,025)	490 ng/mL (median PFOA)	Serum uric acid	**Association (reported by investigator)***
Steenland et al. 2010b Community (n=54,591)	11.5–20.6 ng/mL (2nd quintile PFOA)	Hyperuricemia risk	**OR 1.33 (1.24–1.43)* (2nd quintile)**
Gleason et al. 2015 General population (NHANES) (n=4,333)	3.7 ng/mL (median PFOA)	Serum uric acid	**Association (p<0.001)***
		Hyperuricemia risk	**Association (p<0.001)***
Geiger et al. 2013 General population (NHANES) (n=1,772 adolescents and adults)	4.3 ng/mL (mean PFOA), >5.4 ng/mL (4th PFOA quartile)	Serum uric acid	**Association (p=0.0001)***
		Hyperuricemia risk	**OR 1.62 (1.10–2.37)* (4th quartile)**
Kataria et al. 2015 General population (NHANES) (n=1,960 adolescents)	≥4.7 ng/mL (4th PFOA quartile)	Serum uric acid	**Association (p<0.01)***
Qin et al. 2016 General population (n=225 adolescents)	0.5 ng/mL (median PFOA)	Serum uric acid	**Association (p<0.05)***
		Hyperuricemia risk	**OR 2.16 (1.29–3.61)* (full cohort)** **OR 2.76 (1.37–5.56)* (boys only)**
Shankar et al. 2011b General population (NHANES) (n=3,883 adults)	3.5–5.1 ng/mL (3rd PFOA quartile)	Serum uric acid	**Association (p<0.0001)***
		Hyperuricemia risk	**OR 1.90 (1.35–2.69)*, 3rd quartile**
PFOS			
Steenland et al. 2010b Community (n=54,591)	17.5–23,26 ng/mL (3rd PFOS quintile)	Hyperuricemia risk	**OR 1.11 (1.04–1.20)* (3rd quintile)**

Table 2-14. Summary of Uric Acid Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Gleason et al. 2015 General population (NHANES, n=4,333)	11.3 ng/mL (median PFOS)	Serum uric acid	**Association (p<0.01)***
		Hyperuricemia risk	NS (p=0.502)
Geiger et al. 2013 General population (NHANES) (n=1,772 adolescents and adults)	18.4 ng/mL (mean PFOS), >25.5 ng/mL (4th PFOS quartile)	Serum uric acid	NS (p=0.0575)
		Hyperuricemia risk	**OR 1.65 (1.10–2.49)* (4th quartile)**
Kataria et al. 2015 General population (NHANES) (n=1,960 adolescents)	≥19.4 ng/mL (4th PFOS quartile)	Serum uric acid	**Association (p<0.05)***
Qin et al. 2016 General population (n=225 adolescents)	28.9 ng/mL (median PFOS)	Serum uric acid	NS (p>0.05)
		Hyperuricemia risk	OR 1.35 (0.95–1.93) (full cohort)
Shankar et al. 2011b General population (NHANES) (n=3,883 adults)	11.2–17.8 ng/mL (2nd PFOS quartile)	Serum uric acid	**Association (p=0.0018)***
		Hyperuricemia risk	**OR 1.46 (1.11–1.91)*, 2nd quartile**
PFHxS			
Gleason et al. 2015 General population (NHANES) (n=4,333)	1.8 ng/mL (median PFHxS)	Serum uric acid	NS (p>0.01)
		Hyperuricemia risk	NS (p=0.110 for trend)
Kataria et al. 2015 General population (NHANES) (n=1,960 adolescents)	≥4 ng/mL (4th PFHxS quartile)	Serum uric acid	NS (p>0.05)
Qin et al. 2016 General population (n=225 adolescents)	1.3 ng/mL (median PFHxS)	Serum uric acid	**Association (p<0.05)***
		Hyperuricemia risk	OR 1.39 (0.93–2.07)
PFNA			
Mundt et al. 2007 Occupational (n=592)	NR	Serum uric acid	Small, but not clinically significant

Table 2-14. Summary of Uric Acid Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Gleason et al. 2015 General population (NHANES) (n=4,333)	1.4 ng/mL (median PFNA)	Serum uric acid	**Association (p<0.001)***
		Hyperuricemia risk	NS (p=0.42 for trend)
Kataria et al. 2015 General population (NHANES) (n=1,960 adolescents)	≥1.5 ng/mL (4th PFNA quartile)	Serum uric acid	NS (p>0.05)
Qin et al. 2016 General population (n=225 adolescents)	0.8 ng/mL (median PFNA)	Serum uric acid	NS (p>0.05)
		Hyperuricemia risk	OR 1.28 (0.83–1.96)
PFDA			
Qin et al. 2016 General population (n=225 adolescents)	0.9 ng/mL (median PFDA)	Serum uric acid	NS (p>0.05)
		Hyperuricemia risk	OR 1.26 (0.82–1.92)
PFBS			
Qin et al. 2016 General population (n=225 adolescents)	0.5 ng/mL (median PFBS)	Serum uric acid	NS (p>0.05)
		Hyperuricemia risk	OR 1.23 (0.86–1.75)
PFDoDA			
Qin et al. 2016 General population (n=225 adolescents)	2.7 ng/mL (median PFDoDA)	Serum uric acid	NS (p>0.05)
		Hyperuricemia risk	OR 0.93 (0.65–1.34)

Table 2-14. Summary of Uric Acid Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFHxA			
Qin et al. 2016 General population (n=225 adolescents)	0.2 ng/mL (median PFHxA)	Serum uric acid	NS (p>0.05)
		Hyperuricemia risk	OR 1.08 (0.77–1.61)

[a]See the *Supporting Document for Epidemiological Studies for Perfluoroalkyls,* Table 8 for more detailed descriptions of studies.
[b]Participants in occupational exposure may have also lived near the site and received residential exposure; community exposure studies involved subjects living near PFOA facilities with known exposure to high levels of PFOA.
[c]Asterisk indicates association with perfluoroalkyl; unless otherwise specified, values in parenthesis are 95% confidence intervals.

NHANES = National Health and Nutrition Examination Survey; NR = not reported; NS = not significant; OR = odds ratio; PFBS = perfluorobutane sulfonic acid; PFDoDA = perfluorododecanoic acid; PFHxS = perfluorohexane sulfonic acid; PFNA = perfluorononanoic acid; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid

Laboratory animal studies have primarily evaluated kidney morphology; these studies are summarized in Tables 2-1, 2-3, 2-4, 2-5, and 2-6. The NOAEL and LOAEL values for these studies are illustrated in Figures 2-6, 2-8, 2-9, and 2-10. In general, the laboratory animal studies have not found evidence of impaired renal function or morphological damage following exposure to PFOA, PFOS, PFHxS, PFDA, PFUnA, PFBS, PFBA, PFDoDA, or PFHxA. No laboratory animal studies examining renal endpoints were available for PFNA, PFHpA, or FOSA.

PFOA

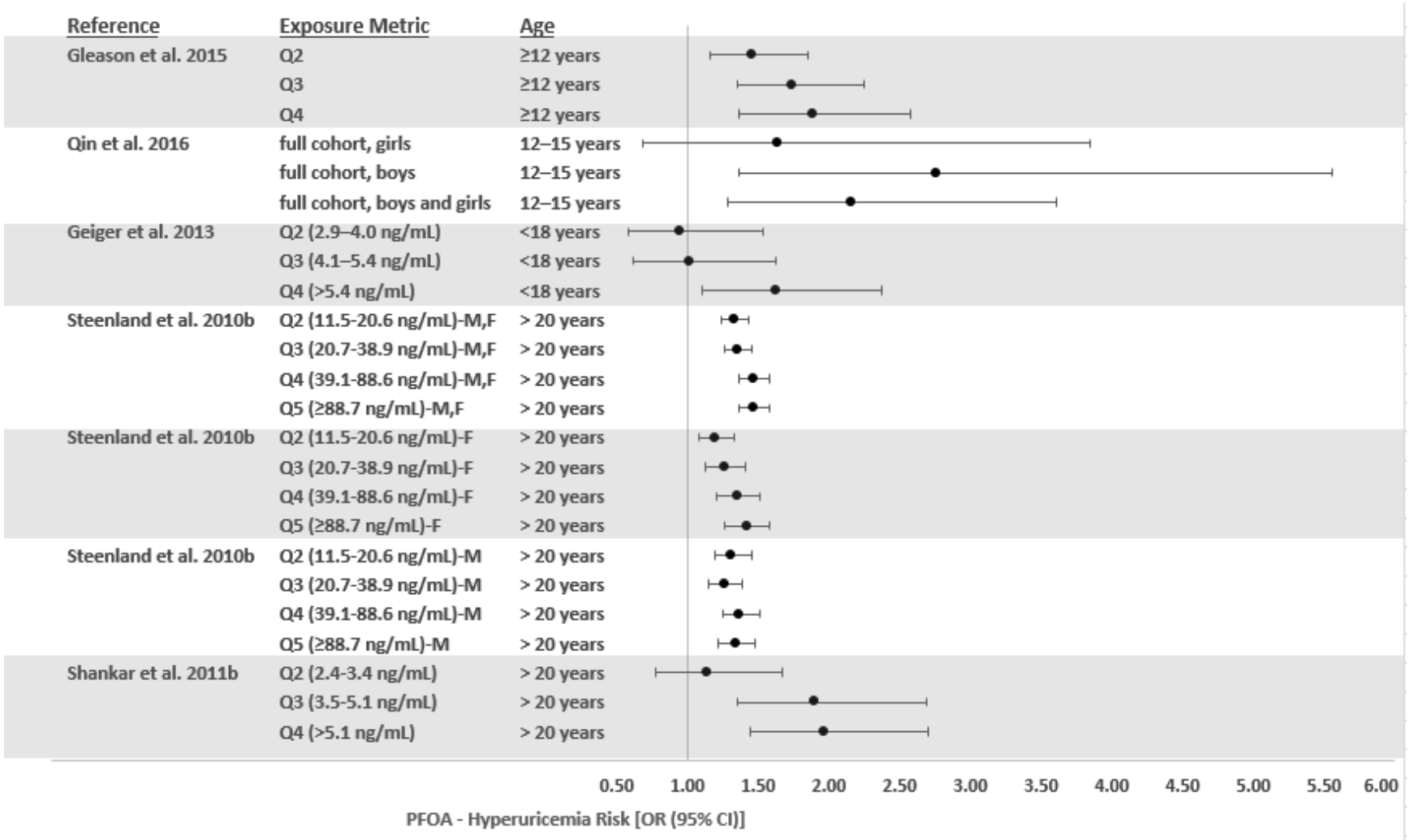
Epidemiological Studies—Kidney Disease. Several epidemiological studies have examined the possible association between PFOA exposure and increased risk of kidney disease. In a cohort mortality study of workers at the DuPont PFOA facility in West Virginia, Steenland and Woskie (2012) found an increase in deaths from chronic renal disease when compared to DuPont workers at other regional facilities. When estimated cumulative PFOA exposure was estimated based on the worker's job history and data from a biomonitoring survey conducted from 1979 to 2004, there was a significant positive trend for nonmalignant kidney disease when the workers were divided in estimated cumulative exposure quartiles. Two studies of workers at the 3M APFO facility in Cottage Grove, Minnesota did not find increases in deaths from chronic kidney disease (Raleigh et al. 2014) or nephritis and nephrosis (Lundin et al. 2009) as compared to mortality rates for the state of Minnesota. Similar results were found when chronic kidney disease deaths were compared to those in a cohort of workers in St. Paul Minnesota who worked at a non-APFO facility (Raleigh et al. 2014). An occupational exposure study (Steenland et al. 2015) and C8 community study (Dhingra et al. 2016b) found no associations between estimated cumulative PFOA exposure and the risk of chronic kidney disease. Another study of the community living near the Washington Works facility found a higher prevalence of self-reported kidney disease as compared to rates reported in NHANES (Anderson-Mahoney et al. 2008).

Epidemiological Studies—Biomarkers of Renal Function. Several biomarkers of renal function have been evaluated in epidemiological studies; these include BUN, serum creatinine, glomerular filtration rate, and uric acid levels (discussed in the following section). Kidney function, assessed by levels of BUN and serum creatinine, was not associated with exposure to PFOA in the occupational exposure studies by Olsen et al. (2003a) or Costa et al. (2009) or a community exposure study by Emmett et al. (2006b).

Three studies have found inverse associations between serum PFOA and glomerular filtration rate. Using the NHANES data for the 1999–2008 cycles, Shankar et al. (2011a) found an inverse association between serum PFOA levels and estimated glomerular filtration rate in adults. The likelihood of chronic kidney disease, defined as a glomerular filtration rate of <60 mL/minute/1.73 m^2, was significantly higher in adults with the highest serum PFOA (>5.9 ng/mL, OR 1.73, 95% CI 1.04–2.88) levels than in adults with serum PFOA levels in the lowest quartile. The study also investigated whether the association between serum PFOA levels and chronic kidney disease was due to reverse causality (i.e., decreased glomerular filtration leads to a decrease in perfluoroalkyl filtration) and found a stronger negative correlation between estimated glomerular filtration rate and serum PFOA levels in subjects without chronic kidney disease, suggesting that it was not due to reverse causality. In another study utilizing NHANES data, an inverse association was found in adolescents with serum PFOA levels in the 4th quartile (Kataria et al. 2015). Similarly, an inverse association between serum PFOA and glomerular filtration rate was found in children participating in the C8 Health Project (Watkins et al. 2013). Unlike Shankar et al. (2011a), Watkins et al. (2013) suggested that the association between serum perfluoroalkyl levels and estimated glomerular filtration rates may be a consequence of reverse causation because no associations were found between estimated serum PFOA levels 3 or 10 years prior to enrollment in the study or at the time of study enrollment and estimated glomerular filtration rates; predicted serum PFOA levels were based on environmental PFOA levels, self-reported residential history, and PBPK modeling.

Epidemiological Studies—Alterations in Uric Acid Levels. Associations between serum PFOA levels and serum uric acid levels have been found in several occupational, community, and general population studies. Costa et al. (2009) and Sakr et al. (2007b) reported associations between serum PFOA levels and serum uric acid levels in workers with high serum PFOA levels. In adult participants of the C8 Health Project, positive linear trends between serum uric acid levels and serum PFOA levels were found (Steenland et al. 2010b). When the subjects were categorized by PFOA levels, significantly increased risks of hyperuricemia (>6.0 mg/dL for women, >6.8 mg/dL for men) were observed for subjects with serum PFOA levels in the 2nd, 3rd, 4th, and 5th quintiles (≥11.5 ng/mL). Four studies utilizing NHANES data have found associations between serum PFOA and serum uric acid levels in adults (Gleason et al. 2015; Shankar et al. 2011b) and adolescents (Geiger et al. 2013; Kataria et al. 2015). A study in Taiwanese adolescents also found this association between PFOA and uric acid (Qin et al. 2016). Several studies have also found increases in the risk of hyperuricemia in a highly exposed population (Steenland et al. 2010b) and the general population (Gleason et al. 2015; Geiger et al. 2013; Qin et al. 2016; Shankar et al. 2011b). The ORs for the risk of hyperuricemia in these studies are summarized in Figure 2-19.

Figure 2-19. Risk of Hyperuricemia Relative to PFOA Levels (Presented as Adjusted Odds Ratios)



KD0272

Laboratory Animal Studies. No gross or microscopic alterations were observed in the kidneys from male rats following head-only inhalation exposure to up to 84 mg/m^3 APFO dusts for 2 weeks (Kennedy et al. 1986). Significantly elevated absolute and relative kidney weight was reported in male rats dosed with ≥3 mg/kg/day PFOA by gavage in water for 70 days (Butenhoff et al. 2004b), but histological evaluation of the kidney was not conducted in this study. Rats that received much higher doses (100–110 mg/kg/day) of APFO for 90 days in the diet showed no significant morphological alterations in the kidneys, and BUN and the urinalysis were unremarkable (Griffith and Long 1980). Also, male mice dosed with up to 47 mg/kg/day APFO in the drinking water for 21 days showed no morphological alterations in the kidneys, and BUN and serum creatinine levels were not significantly affected (Son et al. 2008). Treatment of Cynomolgus monkeys with daily doses of up to 20 mg/kg/day APFO, administered via a capsule, for 26 weeks (Butenhoff et al. 2002) or Rhesus monkeys dosed with up to 10 mg/kg/day by gavage for 90 days (Griffith and Long 1980) did not cause morphological alterations in the kidneys, and blood chemistries and urinalyses provided no evidence of alterations in kidney function. In a 2-year dietary study in rats, relative kidney weight from males dosed with 15 mg/kg/day APFO was significantly elevated (14%) at the 1-year interim evaluation relative to controls, but gross and microscopic appearance (at 1 year and at termination), BUN, and urinalyses (several times during the study) were not significantly affected (3M 1983; Butenhoff et al. 2012c). No gross or microscopic alterations were seen in the kidneys from rats that received dermal applications of up to 2,000 mg/kg/day APFO to the shaven skin for 2 weeks (Kennedy 1985).

Summary. Epidemiological studies have examined possible associations between exposure to PFOA and increases in the risk of kidney disease and alterations in renal function. Mixed results for associations between serum PFOA and risks of kidney disease have been reported in occupational exposure studies and studies of highly exposed residents with more studies not finding associations. Several general population and community studies have found inverse associations between serum PFOA and glomerular filtration rate; however, there is suggestive evidence that this association may be due to reverse causation rather than a direct effect. Associations between serum PFOA levels and serum uric acid levels have been consistently observed in occupational, community, and general populations. Laboratory animal studies have not found evidence of alterations in renal function or histological alterations.

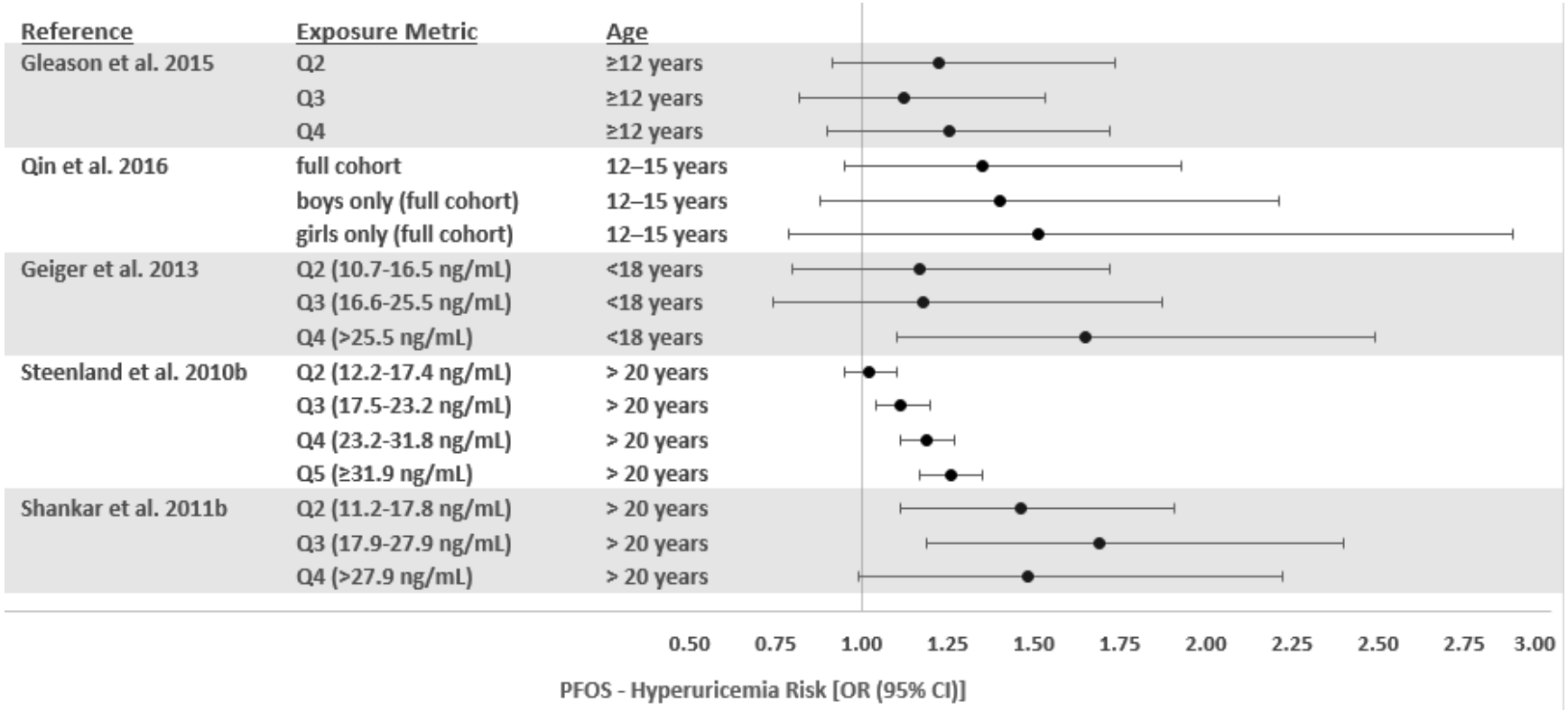
PFOS

Epidemiological Studies—Biomarkers of Renal Function. Three studies have found inverse associations between serum PFOS levels and glomerular filtration rate in adults (Shankar et al. 2011a), adolescents (Kataria et al. 2015), and children (Watkins et al. 2013). In the Watkins et al. (2013) study of C8 Health Project participants, a concentration-related linear trend between decreasing estimated glomerular filtration rates and increases in serum PFOS levels was observed in children and adolescents 1–<18 years old. In adolescents 12–19 years of age participating in NHANES, the estimated glomerular filtration rate was lower in participants with serum PFOA levels in the 2nd, 3rd, and 4th quartiles than those with levels in the 1st quartile (Kataria et al. 2015). In addition to the inverse association between serum PFOS and estimated glomerular filtration rate observed in adult NHANES participants, Shankar et al. (2011a) also found increased risks of chronic kidney disease (defined as a glomerular filtration rate of <60 mL/minute/1.73 m^2) in participants with serum PFOS levels in the 4th quartile.

Epidemiological Studies—Alterations in Uric Acid Levels. In a study of C8 Health Project participants, a linear trend between serum uric acid levels and serum PFOS levels was found (Steenland et al. 2010b). When the subjects were categorized by serum PFOS levels, increased risks of hyperuricemia (>6.0 mg/dL for women, >6.8 mg/dL for men) were observed for subjects with serum PFOS levels in the 3rd, 4th, and 5th quintiles. Similar findings were found in NHANES adult participants (Shankar et al. 2011b). A study of adolescent NHANES participants found associations between serum PFOS and serum uric acid levels (Kataria et al. 2015); a second study did not find an association (Geiger et al. 2013). The Geiger et al. (2013) study did find an increased risk of hyperuricemia for adolescents with serum PFOS levels in the 4th quartile. A study of Taiwanese adolescents did not find associations between serum PFOS and uric acid or an increased risk of hyperuricemia (Qin et al. 2016). The ORs for the risk of hyperuricemia in these studies are summarized in Figure 2-20.

Laboratory Animal Studies. No significant morphological alterations or clinical evidence of impaired kidney function was reported in male and female rats dosed with up to 1.77 mg/kg/day PFOS (potassium salt) (Seacat et al. 2003) or 5.89 mg/kg/day (Curran et al. 2008) for 4 weeks. Extending the treatment to 14 weeks resulted in an increase in BUN in male (23% increase) and female rats (41% increase), but histopathology of the kidneys and urinalyses were unremarkable (Seacat et al. 2003). The NOAEL values were 0.34 and 0.4 mg/kg/day in males and females, respectively. Gavage administration of three doses of PFOS to Cynomolgus monkeys over 315 days did not result in alterations in BUN or serum creatinine or

Figure 2-20. Risk of Hyperuricemia Relative to PFOS Levels (Presented as Adjusted Odds Ratios)



total protein levels (Chang et al. 2017). Treatment of Cynomolgus monkeys with up to 0.75 mg/kg/day PFOS (potassium salt) administered via a capsule for 26 weeks did not cause morphological alterations in the kidneys, nor did it affect BUN, serum creatinine, or urinary parameters (Seacat et al. 2002). Similar results were reported in a 4-week study in monkeys dosed with up to 2 mg/kg/day PFOS (Thomford 2002a). A mild increase in BUN was reported in rats treated with approximately 0.25 or 1.04 mg/kg/day PFOS in the diet for 53 weeks in a 2-year study (Butenhoff et al. 2012b; Thomford 2002b). However, there were no significant gross or microscopic alterations in the kidneys at week 53 or at termination.

PFHxS

Epidemiological Studies—Biomarkers of Renal Function. A small number of epidemiological studies have evaluated biomarkers of renal function. In a study of C8 Health Project child participants (aged 1–<18 years), an inverse association between serum PFHxS and estimated glomerular filtration rate was observed (Watkins et al. 2013). A study of adolescent participants in NHANES did not find this association (Kataria et al. 2015). It is noted that the reported median PFHxS level in the Watkins et al. (2013) study (5.2 ng/mL) exceeded the lower end of the 4th quartile serum PFHxS level in the Kataria et al. (2015) study (≥4 ng/mL).

Epidemiological Studies—Alterations in Uric Acid Levels. In NHANES participants ≥12 years of age (Gleason et al. 2015) and adolescent NHANES participants (Kataria et al. 2015), no associations between serum PFHxS levels and serum uric acid levels or risk of hyperuricemia (Gleason et al. 2015) were found. A study of Taiwanese adolescents found an association between serum PFHxS levels and serum uric acid levels, but did not find increased risks of hyperuricemia (Qin et al. 2016).

Laboratory Animal Studies. Male rats treated by gavage with 10 mg/kg/day PFHxS for at least 42 days showed a significant increase in BUN levels, but there were no significant gross or microscopic alterations in the kidneys (Butenhoff et al. 2009a); the NOAEL was 3 mg/kg/day. No significant effect on BUN was reported in female rats. No histological alterations were observed in the kidneys of mice following intermediate-duration administration of ≤3 mg/kg/day PFHxS (Chang et al. 2018).

PFNA

Epidemiological Studies—Biomarkers of Renal Function. Two epidemiological studies have evaluated the possible associations between serum PFNA and alterations in renal function biomarkers. In a study of

children participating in the C8 Health Project, an inverse association between serum PFNA and estimated glomerular filtration rate was observed, but not in adolescents participating in NHANES (Watkins et al. 2013). Mundt et al. (2007) noted that there were small, but not clinically significant, alterations in BUN, creatinine, and serum uric acid levels in workers exposed to PFNA.

Epidemiological Studies—Alterations in Uric Acid Levels. Gleason et al. (2015) found an association between serum PFNA and serum uric acid levels in NHANES participants; this association was not found in studies of adolescents (Kataria et al. 2015; Qin et al. 2016). Studies by Gleason et al. (2015) and Qin et al. (2016) did not find increases in the risk of hyperuricemia associated with serum PFNA levels.

PFDA

Epidemiological Studies—Alterations in Uric Acid Levels. Epidemiological studies examining renal outcomes are limited to a study of Taiwanese adolescents that found no association between serum PFDA levels and serum uric acid levels and did not find increased risks of hyperuricemia (Qin et al. 2016).

Laboratory Animal Studies. Administration of a single dose of up to 80 mg/kg PFDA to female C57BL/6N mice by gavage did not induce gross or microscopic changes in the kidneys (Harris et al. 1989). However, 2 out of 10 mice that died following administration of a dose of 320 mg/kg showed mild acute necrosis of the proximal convoluted tubules. No histological alterations were observed in the kidneys of rats administered 0.5 mg/kg/day PFDA for 28 days or mice receiving weekly gavage doses of 5 mg/kg for 4 weeks (Frawley et al. 2018).

PFUnA

Laboratory Animal Studies. Treatment of male and female rats with 1.0 mg/kg/day PFUnA via gavage for 41–46 days resulted in significant increases in BUN levels (35–61% in males, 19–45% in females) and alkaline phosphatase activity (86–140% in males, 83% in females) and significant decreases in total protein (11% in males, 10–13% in females) and albumin (7% in males) levels (Takahashi et al. 2014); the NOAEL was 0.3 mg/kg/day.

PFBS

Epidemiological Studies—Alterations in Uric Acid Levels. Serum PFBS levels were not associated with serum uric acid levels or increases in the risk of hyperuricemia in a study of adolescents in Taiwan (Qin et al. 2016).

Laboratory Animal Studies. Treatment of female rats with 900 mg/kg/day PFBS by gavage for 28 days caused a significant increase (9–11%) in absolute and relative kidney weight, but caused no significant alterations in the microscopic appearance of the kidneys (3M 2001). The weight of the kidneys returned to control levels following a recovery period of approximately 14 days; the NOAEL for kidney weight effects was 900 mg/kg/day PFBS. In a 90-day rat study, PFBS did not result in alterations in kidney weights, but did result in hyperplasia of the medullary and papillary tubular and ductal epithelial cells in the inner medullary region at 600 mg/kg/day, but not at 200 mg/kg/day (Lieder et al. 2009a). Minimal to moderate papillary epithelial tubular/acinar hyperplasia was also observed in a 2-generation rat study at 300 mg/kg/day; the study identified a NOAEL of 100 mg/kg/day (Lieder et al. 2009b).

PFBA

Laboratory Animal Studies. No alterations in renal morphology or clinical indications of impaired renal function were reported in rats treated with PFBA in doses of up to 184 mg/kg/day for 5 days (3M 2007a), 150 mg/kg/day for 28 days (Butenhoff et al. 2012a; van Otterdijk 2007a), or 30 mg/kg/day by gavage for 90 days (Butenhoff et al. 2012a; van Otterdijk 2007b).

PFDoDA

Epidemiological Studies—Alterations in Uric Acid Levels. In adolescents, no associations between serum PFDoDA levels and serum uric acid levels or the risk of hyperuricemia were observed (Qin et al. 2016).

Laboratory Animal Studies. No histopathological alterations were observed in rats administered up to 2.5 mg/kg/day PFDoDA for 42–47 days (Kato et al. 2015).

PFHxA

Epidemiological Studies—Alterations in Uric Acid Levels. In adolescents, no associations between serum PFHxA levels and serum uric acid levels or the risk of hyperuricemia were observed (Qin et al. 2016).

Laboratory Animal Studies. Renal papillary necrosis was determined to be one of the causes of death in rats administered 450 mg/kg/day PFHxA for 4 days (Kirkpatrick 2005). No increases in renal lesions were observed in surviving rats administered a TWA dose of 315 mg/kg/day for 32–44 days (Kirkpatrick 2005). No histological alterations were observed in the kidneys of rats administered up to 200 mg/kg/day NaPFHx for 90 days (Chengelis et al. 2009b). In a 2-year gavage study, treatment of female rats with 200 mg/kg/day PFHxA resulted in mild renal tubular degeneration and mild to severe papillary necrosis (Klaunig et al. 2015); the NOAEL was 100 mg/kg/day. In addition, urinalysis revealed an increased mean urine volume and reduced specific gravity. There were no histological alternations in the kidneys of males.

2.11 DERMAL

Overview. No studies were located regarding dermal effects in humans. Studies in laboratory animals have not found dermal effects following head-only inhalation exposure to PFOA (see Table 2-1) or oral exposure to PFOA, PFOS, or PFBA (see Tables 2-3, 2-4, and 2-5). Dermal exposure to PFOA has resulted in skin damage (see Table 2-6).

PFOA

In an inhalation head-only exposure study, no histopathological alterations were observed in the abdominal skin of male rats exposed to ≤84 mg/m^3 APFO dusts for 2 weeks (Kennedy et al. 1986).

No microscopic alterations were observed in the skin following oral exposure of rats to ≤100–110 mg/kg/day APFO via the diet for 90 days (Griffith and Long 1980) or monkeys exposed to up to 20 mg/kg/day PFOA or 0.75 mg/kg/day PFOS for 26 weeks (Butenhoff et al. 2002; Seacat et al. 2002).

Application of a single dose of 5,000 mg/kg of an aqueous paste of APFO to a clipped area of the skin of rats, and left in place covered for 24 hours produced mild skin irritation (Kennedy 1985); no irritation was

apparent with a dose of 3,000 mg/kg. In a 2-week dermal exposure study, skin irritation was observed in rats exposed to 200 mg/kg/day (Kennedy 1985). Acute necrotizing dermatitis was observed in two out of five rats exposed to 2,000 mg/kg/day; this lesion was observed after the 10th treatment. Application of 500 mg/kg APFO to the intact or abraded skin of young rabbits and left covered for 24 hours was non-irritating, as scored according to the Draize procedure immediately after removal of the cover and 48 hours later (Griffith and Long 1980).

PFOS

Administration of up to approximately 1.04 mg/kg/day PFOS to rats in the diet for 2 years did not induce morphological alterations in the skin (Butenhoff et al. 2012b; Thomford 2002b).

PFBA

There were no significant gross or microscopic alterations in the skin of rats receiving gavage doses of ≤150 mg/kg/day PFBA for 28 days or ≤30 mg/kg/day PFBA for 90 days (Butenhoff et al. 2012a; van Otterdijk 2007a, 2007b).

2.12 OCULAR

Overview. No information was located regarding ocular effects in humans. Ocular irritation has been observed in laboratory animals following exposure to airborne APFO dust or instillation of PFOA into the eye (see Tables 2-1 and 2-6). However, ocular effects have not been found following oral exposure to PFOA, PFOS, PFBS, PFBA, or PFHxA (see Tables 2-3, 2-4, and 2-5).

PFOA

Rats exposed to 18,600 mg/m^3 APFO dusts for 1 hour exhibited a red material around the eyes and lacrimation during exposure (Griffith and Long 1980). Male rats exposed to ≥810 mg/m^3 APFO dusts for 4 hours showed corneal opacity and corrosion, which was confirmed by fluorescein staining (Kennedy et al. 1986). Examination of the eyes of male rats exposed intermittently to up to 84 mg/m^3 APFO for 2 weeks using a bright light and a slit-lamp biomicroscope on days 5 and 9 of exposure did not reveal any significant exposure-related alterations (Kennedy et al. 1986). Microscopic examination of the eyes from these rats at termination and following a recovery period of up to 42 days was unremarkable.

In oral exposure studies, examination of the eyes from rats exposed to approximately 100–110 mg/kg/day APFO in the diet for 90 days did not reveal any significant gross or microscopic alterations (Griffith and Long 1980). Similar results were reported in rats that received dietary doses up to 15 mg/kg/day APFO for 2 years (3M 1983; Butenhoff et al. 2012c) and in monkeys dosed with up to 20 mg/kg/day APFO for 26 weeks (Butenhoff et al. 2002).

No significant gross alterations were observed in the eyes of rats following repeated dermal exposure to APFO (Kennedy 1985). Microscopic examination of the eyes also did not reveal treatment-related changes. In a study in rabbits, 0.1 g APFO was instilled once in the conjunctival sac of the right eye and examinations were conducted after 1, 24, 48, and 72 hours and 5 and 7 days after the application (Griffith and Long 1980). APFO produced moderate irritation of the eye characterized by iridal and conjunctival effects. The effects were most pronounced 1 hour after instillation. The irritation was persistent, but by day 7, it had subsided. In a different experiment in which 0.1 g APFO was instilled for 5 or 30 seconds before washing with 200 mL of water, there was limited conjunctival irritation, but the effects were immediate and persistent.

PFOS

No gross or microscopic alterations were observed in the eyes from rats exposed to ≤1.77 mg/kg/day PFOS in the diet for 4 weeks or ≤1.56 mg/kg/day for 14 weeks (Seacat et al. 2003). Similar findings were reported in monkeys dosed daily with up to 2 mg/kg/day PFOS administered via a capsule for 4 weeks (Thomford 2002a) or up to 0.75 mg/kg/day PFOS administered via a capsule for 26 weeks (Seacat et al. 2002), and in rats dosed with up to 1.04 mg/kg/day in the diet for 2 years (Butenhoff et al. 2012b; Thomford 2002b).

PFBS

No gross or microscopic alterations were observed in the eyes of rats administered ≤900 mg/kg/day PFBS via gavage for 28 days (3M 2001).

PFBA

Examination of the eyes of rats orally exposed to ≤150 mg/kg/day PFBA for 28 days or ≤30 mg/kg/day for 90 days did not reveal any significant alterations in the eyes (Butenhoff et al. 2012a; van Otterdijk 2007a, 2007b).

PFHxA

No ophthalmological alterations were observed in rats administered up to 500 mg/kg/day NaPFHx for 90–93 days (Chengelis et al. 2009b; Loveless et al. 2009).

2.13 ENDOCRINE

Overview. Epidemiological studies have examined a number of endocrine targets including thyroid gland and hormones, reproductive hormones, and insulin levels. A discussion of the thyroid effects is included in this section; the reproductive hormone effects are discussed in Section 2.16, Reproductive, and the insulin effects (as well as other effects associated with glucose metabolism and utilization) are discussed in Section 2.18, Other Noncancer. Summaries of results of epidemiological studies evaluating thyroid outcomes are presented in Table 2-15; more in-depth summaries of the studies are presented in the *Supporting Document for Epidemiological Studies for Perfluoroalkyls,* Table 9. Although some associations between serum PFOA, PFOS, PFHxS, PFNA, PFDA, and PFUnA and thyroid stimulating hormone (TSH), triiodothyronine (T3), or thyroxine (T4) levels or thyroid disease have been found, the results are not consistent across studies and a larger number of studies have not found associations. A small number of studies have evaluated PFDoDA and most studies have not found consistent associations between serum perfluoroalkyl levels and thyroid hormone levels. No epidemiological studies examining endocrine health outcomes were identified for PFHpA, PFBS, PFBA, PFHxA, or FOSA.

Laboratory animal studies have primarily evaluated potential morphological alterations in endocrine tissues following oral exposure; these studies are summarized in Tables 2-3, 2-4, and 2-5. Some alterations in thyroid hormone levels have been observed in laboratory animals exposed to PFOA, PFOS, PFHxS, or PFDA. Histopathological alterations have been observed in the thyroid of some laboratory animal studies for PFHxS, PFBA, and PFHxA; the investigators noted that these effects were likely secondary to the hepatocellular hypertrophy, although the mechanism has not been established for these compounds. In general, the pituitary, parathyroid, thyroid, and adrenal glands do not appear to be

Table 2-15. Summary of Thyroid Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFOA			
Gilliland 1992 Occupational (n=115)	NR (serum fluorine levels used as surrogate for serum PFOA)	TSH	**Association (p=0.004)***
Olsen et al. 1998b Occupational (n=111 in 1993 and n=80 in 1995)	10,000–<30,000 ng/mL (PFOA range)	TSH	NS (p=0.09 for trend), 1993 group Association (p=0.002), 1995 group
Sakr et al. 2007b Occupational (n=1,025)	428 ng/mL (mean PFOA)	TSH	The investigators noted that the levels were within the reference range
		T4	
		T3	
Steenland et al. 2015 Occupational (n=3,713)	Estimated cumulative PFOA	Thyroid disease risk	NS (p=0.98 for trend) no lag, males NS (p=0.55 for trend) 10-year lag, males NS (p=0.97 for trend) no lag, females NS (p=0.27 for trend) 10-year lag, females
Olsen and Zobel 2007 Occupational (n=552)	2,210 ng/mL (mean PFOA)	Free T4	**Association (p=0.01)***
		T4	NS (p=0.29)
		T3	**Association (p=0.05)***
		TSH	NS (p=0.08)
Anderson-Mahoney et al. 2008 Community (n=566)	NR	Self-reported thyroid problems	**SPR 1.56 (1.22–1.98)***
Emmett et al. 2006b Community (n=371)	354 ng/mL (median PFOA)	TSH	NS (p>0.05)

Table 2-15. Summary of Thyroid Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Knox et al. 2011a Community (n=50,113 adults ≥20 years of age)	52.6, 91.0, 98.6, and 124.3 ng/mL (mean PFOA in women ≤50 years, men ≤50 years, women >50 years, men >50 years, respectively)	T4	**Association (p≤0.0001)*, women ≤50 years** **Association (p<0.001), men and women >50 years**
		T3 uptake	**Inverse association (p=0.0001)* women ≤50 years** **Inverse association (p=0.005)*, women >50 years** **Inverse association (p=0.037)*, men >50 years**
Lopez-Espinosa et al. 2012 Community (n=10,725 children aged 1–17 years)	29.3 and 67.7–2,071 ng/mL (median and 4th quartile PFOA)	Thyroid disease	**OR 1.44 (1.02–2.03)*, per interquartile shift**
		Hypothyroidism	**OR 1.54 (1.00–2.37)*, per interquartile shift**
		Subclinical hypothyroidism	OR 0.98 (0.86–1.15), per interquartile shift
		Subclinical hyperthyroidism	OR 0.81 (0.58–1.15), per interquartile shift
		TSH	β -1.1 (-5.3–3.4), 4th quartile
		Total T4	β -0.1 (-1.7–1.4), 4th quartile
Winquist and Steenland 2014b Community (C8 and occupational) (n=28,541)	114.7–<202.2 ng/mL-year (2nd quintile estimated cumulative PFOA)	Functional thyroid disease	**HR 1.24 (1.02–1.51;p=0.031)* (women), retrospective analysis** HR 1.01 (0.94–1.07 per log linear increase in PFOA, p=0.853) (men), retrospective analysis NS (p=0.549) (women), prospective analysis NS (p=0.087) (men), prospective analysis

Table 2-15. Summary of Thyroid Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
		Hyperthyroidism	NS (p=0.074) (women), retrospective analysis NS (p=0.858) (men), retrospective analysis NS (p=0.268) (women), prospective analysis NS (p=0.760) (men), prospective analysis
		Hypothyroidism	NS (p=0.076) (women), retrospective analysis NS (p=0.684) (men), retrospective analysis NS (p=0.247) (women), prospective analysis **HR 1.24 (1.03–1.49)* (men), prospective analysis**
Berg et al. 2017 General population (n=370 pregnant women)	1.53 ng/mL (median maternal serum PFOA)	Total T4	NS (p>0.05)
		Free T4	NS (p>0.05)
		Total T3	NS (p>0.05)
		Free T3	NS (p>0.05)
		TSH	NS (p>0.05)
		Thyroxine binding capacity	NS (p>0.05)
Bloom et al. 2010 General population (n=31)	1.33 ng/mL (geometric mean PFOA)	TSH	NS (p=0.871)
		Free T4	NS (p=0.896)
Chan et al. 2011 General population (n=94 women with hypothyroxinemia and 175 matched controls)	1.28 and 1.37 ng/mL (geometric mean PFOA in cases and controls)	Hypothyroxinemia risk	OR 0.94 (0.74–1.18)

Table 2-15. Summary of Thyroid Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Crawford et al. 2017 General population (n=99 30–44-year-old women)	2.79 ng/mL (geometric mean serum PFOA)	Total T4	NS (p=0.07)
		Free T4	NS (p=0.11)
		T3	**Association (β 6.05, p=0.03)***
		TSH	NS (p=0.37)
Dufour et al. 2018 General population (n=214 pregnant women)	0.80 ng/mL (cord blood mean PFOA); 0.44–0.68 ng/mL (2nd quartile cord blood PFOA)	Hypothyroidism	**OR 4.42 (1.23–21.14)*, 4th quartile**
		TSH in infants	NS (p=0.196)
Jain 2013 General population (NHANES) (n=1,525)	NR	Total T3	**Association (p=0.013)***
		TSH	NS (p>0.05)
		Free T3	NS (p>0.05)
		Free T4	NS (p>0.05)
		Total T4	NS (p>0.05)
		Thyroglobulin	NS (p>0.05)
Ji et al. 2012 General population (n=633)	2.74 ng/mL (median PFOA)	TSH	NS (p=0.4055)
		T4	NS (p=0.2221)
Kang et al. 2018 General population (150 children, 3–18 years)	1.88 ng/mL (median serum PFOA)	Free T4	NS (p=0.075)
		TSH	NS (p=0.565)
Lewis et al. 2015 General population (NHANES) (n=1,682)	1.42–2.55 ng/mL (range of median PFOA for different age groups)	TSH	**Association (p<0.05)*, 12–20-year-old females**
		Free T4	**Association (p<0.05)*, 20–<40-year-old females**
		Total T4	NS (p>0.05)
		Free T3	**Association (p<0.05)*, 60–80-year-old females**
		Total T3	**Association (p<0.05)*, 60–80-year-old females**

Table 2-15. Summary of Thyroid Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Melzer et al. 2010 General population (NHANES) (n=3,966)	9.47 and 10.39 ng/mL (4th PFOA quartile mean in women and men)	Thyroid disease risk	**OR 1.64 (1.09–2.46)*, females** OR 1.58 (0.74–3.39), males
Preston et al. 2018 General population (n=732 mothers and 480 infants)	5.6 ng/mL (maternal median serum PFOA)	Total T4	β 0.09 (-0.08–0.27)
		Free T4	**β -1.87 (-3.40 to -0.31)***
		TSH	β 0.28 (-9.26–10.8)
		Neonatal T4	**β -1.1 (-2.1 to -0.1)*, 4th quartile**
Raymer et al. 2012 General population (n=256)	10.4 ng/mL (mean PFOA)	TSH	NS (p>0.05)
		T3	NS (p>0.05)
		T4	NS (p>0.05)
Shah-Kulkarni et al. 2016 General population (n=279 pregnant women)	0.91 ng/mL (cord blood median PFOA)	Cord blood T4	NS (p=0.99)
		Cord blood T3	NS (p=0.99)
		Cord blood TSH	NS (p=0.24)
Shrestha et al. 2015 General population (n=87 with thyroid disease)	9.17 ng/mL (geometric mean PFOA)	TSH	NS (p=0.176)
		Free T4	NS (p=0.536)
		T4	NS (p=0.097)
		T3	NS (p=0.208)
Tsai et al. 2017 General population (n=118 mother-infant pairs)	3.14 ng/mL (mean cord blood PFOA)	Cord blood T4	β -0.031 (-0.414–0.342)
		Cord blood T3	β 0.025 (-0.054–0.103)
		Cord blood TSH	β 0.059 (-0.136–0.254)
Wang et al. 2013a General population (n=903 pregnant women)	2.13 ng/mL (geometric mean PFOA)	TSH	NS (p>0.05)
		Elevated TSH risk	NS (p>0.05)
Wang et al. 2014 General population (n=285 pregnant women)	2.39 ng/mL (median PFOA)	TSH	NS (p>0.05)
		Free T4	NS (p>0.05)
		Total T4	NS (p>0.05)
		Total T3	NS (p>0.05)

Table 2-15. Summary of Thyroid Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Webster et al. 2016 General population (NHANES) (n=1,525)	4.2 ng/mL (geometric mean PFOA)	TSH	NS (p>0.05)
		Free T4	NS (p>0.05)
		Total T4	NS (p>0.05)
		Free T3	**Association (p<0.05)***
		Total T3	NS (p<0.05)
Wen et al. 2013 General population (NHANES) (n=1,181)	4.15 ng/mL (geometric mean PFOA)	TSH	NS (p=0.916), men NS (p=0.732), women
		Total T4	NS (p=1.0), men NS (p=0.705), women
		Total T3	NS (p=0.673), men **Association (p=0.035)*, women**
		Thyroglobulin	NS (p=0.226), men NS (p=0.341), women
		Subclinical hypothyroidism risk	OR1.29 (0.40–4.10), men **OR 7.42 (1.14–48.12, p<0.05), women**
		Subclinical hyperthyroidism risk	**OR 0.38 (0.16–0.95, p<0.05)*, men** OR 0.99 (0.13–7.59), women
Yang et al. 2016a General population (n=157 pregnant women)	1.95 ng/mL (mean PFOA)	TSH	NS (p>0.05)
		Free T4	NS (p>0.05)
		Total T4	NS (p>0.05)
		Free T3	NS (p>0.05)
		Total T3	NS (p>0.05)
PFOS			
Olsen et al. 1998a Occupational (n=327)	1,480–2,440 ng/mL (range of mean PFOS)	TSH	NS (p=0.95)
		Cortisol	NS (p=0.45)
Olsen et al. 2003a Occupational (n=518)	1,320 and 800 ng/mL (mean PFOS at the Decatur and Antwerp facilities, respectively)	T3	**Association (p=0.04)***

Table 2-15. Summary of Thyroid Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Knox et al. 2011a Community (n=50,113 adults ≥20 years of age)	17.3, 24.8, 25.7, and 29.1 ng/mL (mean PFOA in women ≤50 years, men ≤50 years, women >50 years, men >50 years, respectively)	T4	**Association (p<0.0001)*, women ≤50 or >50 years** **Association (p=0.0001)*, men ≤50 or >50 years**
		T3 uptake	**Inverse association (p<0.0001)* women ≤50 years** **Inverse association (p=0.0001)*, women >50 years** **Inverse association (p=0.009)*, men ≤50 years** **Inverse association (p=0.0001)*, men >50 years**
Lopez-Espinosa et al. 2012 Community (n=10,725 children aged 1–17 years)	20.0 ng/mL (median PFOS)	Thyroid disease	OR 0.8 (0.62–1.08), per interquartile shift
		Hypothyroidism	OR 0.91 (0.63–1.31), per interquartile shift
		Subclinical hypothyroidism	OR 0.99 (0.86–1.13), per interquartile shift
		Subclinical hyperthyroidism	OR 0.80 (0.62–1.02), per interquartile shift
		TSH	β -1.0 (-0.3–2.3), per interquartile shift
		Total T4	β 1.1 (0.6–1.5), per interquartile shift
Berg et al. 2015 General population (n=391)	8.1–11.0 ng/mL (3rd PFOS quartile)	TSH	**Association (p=0.03)*, 3rd quartile**

Table 2-15. Summary of Thyroid Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Berg et al. 2017 General population (n=370 pregnant women)	8.03 ng/mL (median maternal serum PFOS)	Total T4	NS (p>0.05)
		Free T4	NS (p>0.05)
		Total T3	NS (p>0.05)
		Free T3	NS (p>0.05)
		TSH	**Association (p<0.05)*** NS (p<0.05) after adjustment for other perfluoroalkyls or persistent organic pollutants
		Thyroxine binding capacity	NS (p>0.05)
Bloom et al. 2010 General population (n=31)	19.57 ng/mL (geometric mean PFOS)	TSH	NS (p=0.896)
		Free T4	NS (p=0.623)
Chan et al. 2011 General population (n=94 women with hypothyroxinemia and 175 matched controls)	7.59 and 7.08 ng/mL (geometric mean PFOS in cases and controls)	Hypothyroxinemia risk	OR 0.88 (0.63–1.24)
Crawford et al. 2017 General population (n= 99 30–44-year-old women)	9.29 ng/mL (geometric mean serum PFOS)	Total T4	NS (p=0.28)
		Free T4	NS (p=0.42)
		T3	NS (p=0.19)
		TSH	NS (p=0.98)
Dallaire et al. 2009 General population (n=623)	18.28 ng/mL (geometric mean PFOS)	TSH	**Inverse association (p≤0.05)***
		T3	**Inverse association (p≤0.05)***
		T4-binding globulin	**Inverse association (p≤0.01)***
		Free T4	**Association (p≤0.05)***
Dufour et al. 2018 General population (n=214 pregnant women)	0.88 ng/mL (cord blood mean PFOS); 0.73–1.01 ng/mL (3rd quartile cord blood PFOS)	Hypothyroidism	**OR 3.22 (1.08–10.92)*, 3rd quartile** OR 2.95 (0.98–10.07), 4th quartile
		TSH in infants	NS (p=0.679)

Table 2-15. Summary of Thyroid Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Jain 2013 General population (NHANES) (n=1,525)	NR	TSH	NS (p>0.05)
		Free T3	NS (p>0.05)
		Total T3	NS (p>0.05)
		Free T4	NS (p>0.05)
		Total T4	NS (p>0.05)
		Thyroglobulin	NS (p>0.05)
Ji et al. 2012 General population (n=633)	7.96 ng/mL (median PFOS)	TSH	NS (p=0.3537)
		T4	NS (p=0.1134)
Kang et al. 2018 General population (150 children, 3–18 years)	5.68 ng/mL (median serum PFOS)	Free T4	NS (p=0.987)
		TSH	NS (p=0.628)
Lewis et al. 2015 General population (NHANES) (n=1,682)	3.76–11.1 ng/mL (range of median PFOS for different age groups)	TSH	NS (p>0.05)
		Free T4	**Association (p<0.05)*, 20–<40-year-old females**
		Total T4	NS (p>0.05)
		Free T3	NS (p>0.05)
		Total T3	NS (p>0.05)
Melzer et al. 2010 General population (NHANES) (n=3,966)	57.73 and 50.96 ng/mL (4th PFOS quartile mean in women and men)	Thyroid disease risk	OR 1.15 (0.7–1.91, p=0.568), females OR 1.58 (0.72–3.47, p=0.251), males **OR 2.68 (1.03–6.98, p=0.043)*, males 4th quartile versus combined 1st and 2nd quartiles**
Preston et al. 2018 General population (n=732 mothers and 480 infants)	24.0 ng/mL (maternal median serum PFOS)	Total T4	β 0.01 (-0.14–0.16)
		Free T4	β -1.04 (-2.36–0.29)
		TSH	β 0.90 (-7.27–9.80) **β-16.4 (-29.8 to -0.38)*, TPOAb positive mothers**
		Neonatal T4	**β -1.1 (-2.1 to -0.1)*, 4th quartile.**

Table 2-15. Summary of Thyroid Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Raymer et al. 2012 General population (n=256)	37.4 ng/mL (mean PFOS)	TSH	NS (p>0.05)
		T3	NS (p>0.05)
		T4	NS (p>0.05)
Shah-Kulkarni et al. 2016 General population (n=279 pregnant women)	0.66 ng/mL (cord blood median PFOS)	Cord blood T4	NS (p=0.10)
		Cord blood T3	NS (p=0.37)
		Cord blood TSH	NS (p=0.73)
Shrestha et al. 2015 General population (n=87 with thyroid disease)	31.6 ng/mL (geometric mean PFOS)	TSH	NS (p=0.094)
		Free T4	**Association (p=0.044)***
		T4	**Association (p=0.001)***
		T3	NS (p=0.287)
Tsai et al. 2017 General population (n=118 mother-infant pairs)	7.24 ng/mL (mean cord blood PFOS)	Cord blood T4	**β -0.458 (-0.916 to -0.001, p<0.05)***
		Cord blood T3	β 0.027 (-0.072–0.125)
		Cord blood TSH	**β 0.346 (0.101–0.591, p<0.05)***
Wang et al. 2013a General population (903 pregnant women)	12.77 ng/mL (geometric mean PFOS)	TSH	**Association (p=0.03)***
		Elevated TSH risk	NS (p>0.05)
Wang et al. 2014 General population (285 pregnant women)	12.73 ng/mL (median PFOS)	TSH	NS (p>0.05)
		Free T4	NS (p>0.05)
		Total T4	NS (p>0.05)
		Total T3	NS (p>0.05)
Webster et al. 2016 General population (NHANES) (n=1,525)	13.9 ng/mL (geometric mean PFOS)	TSH	NS (p>0.05)
		Free T4	NS (p>0.05)
		Total T4	NS (p>0.05)
		Free T3	NS (p>0.05)
		Total T3	NS (p<0.05)

Table 2-15. Summary of Thyroid Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Wen et al. 2013 General population (NHANES) (n=1,181)	14.2 ng/mL (geometric mean PFOS)	TSH	NS (p=0.931), men NS (p=0.358), women
		Total T4	NS (p=0.840), men NS (p=0.433), women
		Total T3	NS (p=0.404), men NS (p=0.384), women
		Thyroglobulin	NS (p=0.342), men NS (p=0.061), women
		Subclinical hypothyroidism risk	**OR 1.98 (1.19–3.28, p<0.05)*, men** **OR 3.03 (1.14–8.07, p<0.05)*, women**
		Subclinical hyperthyroidism risk	OR 0.92 (0.19–4.46), men OR 1.90 (0.33–6.80), women
Yang et al. 2016a General population (n=157 pregnant women)	5.08 ng/mL (mean PFOS)	TSH	**Inverse association (p<0.01)***
		Free T4	NS (p>0.05)
		Total T4	NS (p>0.05)
		Free T3	NS (p>0.05)
		Total T3	NS (p>0.05)
PFHxS			
Berg et al. 2017 General population (n=370 pregnant women)	0.44 ng/mL (median maternal serum PFHxS)	Total T4	NS (p>0.05)
		Free T4	NS (p>0.05)
		Total T3	NS (p>0.05)
		Free T3	NS (p>0.05)
		TSH	NS (p>0.05)
		Thyroxine binding capacity	NS (p>0.05)

Table 2-15. Summary of Thyroid Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Bloom et al. 2010 General population (n=31)	0.75 ng/mL (geometric mean PFHxS)	TSH	NS (p=0.956)
		Free T4	NS (p=0.567)
Chan et al. 2011 General population (n=94 women with hypothyroxinemia and 175 matched controls)	1.28 and 1.37 ng/mL (geometric mean PFHxS in cases and controls)	Hypothyroxinemia risk	OR 1.12 (0.89–1.41)
Crawford et al. 2017 General population (n= 99 30–-44-year-old women)	1.59 ng/mL (geometric mean serum PFHxS)	Total T4	NS (p=0.50)
		Free T4	NS (p=0.84)
		T3	NS (p=0.22)
		TSH	NS (p=0.71)
Dufour et al. 2018 General population (n=214 pregnant women)	0.18 ng/mL (cord blood mean PFHxS)	Hypothyroidism	OR 1.92 (95% CI 0.87–4.25),detected versus non-detected
		TSH in infants	NS (p=0.894)
Jain 2013 General population (NHANES) (n=1,525)	NR	TSH	NS (p>0.05)
		Free T3	NS (p>0.05)
		Total T3	NS (p>0.05)
		Free T4	NS (p>0.05)
		Total T4	NS (p>0.05)
		Thyroglobulin	NS (p>0.05)
Ji et al. 2012 General population (n=633)	1.51 ng/mL (median PFHxS)	TSH	NS (p=0.8144)
		T4	NS (p=0.5147)
Kang et al. 2018 General population (150 children, 3–18 years)	0.793 ng/mL (median serum PFHxS)	Free T4	NS (p=0.308)
		TSH	NS (p=0.901)

Table 2-15. Summary of Thyroid Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Lewis et al. 2015 General population (NHANES) (n=1,682)	0.69–1.81 ng/mL (range of median PFHxS for different age groups)	TSH	NS (p>0.05)
		Free T4	NS (p>0.05)
		Total T4	NS (p>0.05)
		Free T3	NS (p>0.05)
		Total T3	NS (p>0.05)
Preston et al. 2018 General population (n=732 mothers and 480 infants)	2.4 ng/mL (maternal median serum PFHxS)	Total T4	β -0.05 (-0.14–0.04)
		Free T4	β -0.60 (-1.39–0.19)
		TSH	β 2.89 (-2.12–8.17)
		Neonatal T4	**β -1.1 (-2.1 to -0.1)*, 4th quartile**
Shah-Kulkarni et al. 2016 General population (n=279 pregnant women)	0.38 ng/mL (cord blood median PFHxS)	Cord blood T4	NS (p=0.83)
		Cord blood T3	NS (p=0.15)
		Cord blood TSH	NS (p=0.15)
Wang et al. 2013a General population (n=903 pregnant women)	0.62 ng/mL (geometric mean PFHxS)	TSH	NS (p>0.05)
		Elevated TSH risk	NS (p>0.05)
Wang et al. 2014 General population (n=285 pregnant women)	0.81 ng/mL (median PFHxS)	TSH	**Association (p<0.05)**
		Free T4	NS (p>0.05)
		Total T4	NS (p>0.05)
		Total T3	NS (p>0.05)
Webster et al. 2016 General population (NHANES) (n=1,525)	1.9 ng/mL (geometric mean PFHxS)	TSH	NS (p>0.05)
		Free T4	NS (p>0.05)
		Total T4	NS (p>0.05)
		Free T3	NS (p>0.05)
		Total T3	NS (p>0.05)

Table 2-15. Summary of Thyroid Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Wen et al. 2013 General population (NHANES) (n=1,181)	2.0 ng/mL (geometric mean PFHxS)	TSH	NS (p=0.608), men NS (p=0.720), women
		Total T4	NS (p=0.641), men **Association (p=0.022)*, women**
		Total T3	NS (p=0.917), men **Association (p<0.001)*, women**
		Thyroglobulin	NS (p=0.455), men NS (p=0.725), women
		Subclinical hypothyroidism risk	OR 1.57 (0.76–3.25), men **OR 3.10 (1.22–7.86, p<0.05)*, women**
		Subclinical hyperthyroidism risk	OR 0.56 (0.24–1.20.92), men **OR 12.27 (1.07–4.80.90)*,women**
Yang et al. 2016a General population (n=157 pregnant women)	0.63 ng/mL (mean PFHxS)	TSH	NS (p>0.05)
		Free T4	NS (p>0.05)
		Total T4	NS (p>0.05)
		Free T3	NS (p>0.05)
		Total T3	NS (p>0.05)
PFNA			
Mundt et al. 2007 Occupational (n=592)	NR	TSH	Investigators noted differences between groups was small and not clinically relevant
		T4	
		T3	
Lopez-Espinosa et al. 2012 Community (n=10,725 children aged 1–17 years)	1.5 ng/mL (median PFNA)	Thyroid disease	OR 1.05 (0.78–1.41), per interquartile shift
		Hypothyroidism	OR 1.11 (0.77–1.60), per interquartile shift
		Subclinical hypothyroidism	OR 0.99 (0.88–1.12), per interquartile shift
		Subclinical hyperthyroidism	OR 0.78 (0.61–1.01), per interquartile shift

Table 2-15. Summary of Thyroid Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
		TSH	β -1.1 (CI 0.7–1.5), per interquartile shift
		Total T4	B 0.8 (-0.4–2.0), per interquartile shift
Berg et al. 2017 General population (n=370 pregnant women)	0.56 ng/mL (median maternal serum PFNA)	Total T4	NS (p>0.05)
		Free T4	NS (p>0.05)
		Total T3	NS (p>0.05)
		Free T3	NS (p>0.05)
		TSH	NS (p>0.05)
		Thyroxine binding capacity	NS (p>0.05)
Bloom et al. 2010 General population (n=31)	0.79 ng/mL (geometric mean PFNA)	TSH	NS (p=0.789)
		Free T4	NS (p=0.424)
Crawford et al. 2017 General population (n= 99 30–44-year-old women)	0.84 ng/mL (geometric mean serum PFNA)	Total T4	NS (p=0.34)
		Free T4	**Association (β 0.08, p<0.01)***
		T3	**Association (β 5.65, p=0.02)***
		TSH	NS (p=0.91)
Dufour et al. 2018 General population (n=214 pregnant women)	0.18 ng/mL (cord blood mean PFNA), 0.23–0.68 ng/mL (4th quartile cord blood PFNA)	Hypothyroidism	OR 1.17 (0.37–3.92), 4th quartile
		TSH in infants	NS (p=0.064)
Jain 2013 General population (NHANES) (n=1,525)	NR	TSH	NS (p>0.05)
		Free T3	NS (p>0.05)
		Total T3	NS (p>0.05)
		Free T4	NS (p>0.05)
		Total T4	NS (p>0.05)
		Thyroglobulin	NS (p>0.05)

Table 2-15. Summary of Thyroid Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Ji et al. 2012 General population (n=633)	2.09 ng/mL (median PFNA)	TSH	NS (p=0.1354)
		T4	NS (p=0.7436)
Kang et al. 2018 General population (150 children, 3–18 years)	0.938 ng/mL (median serum PFNA)	Free T4	**β 0.052 (0.007–0.097, p=0.025)***
		TSH	NS (p=0.840)
Preston et al. 2018 General population (n=732 mothers and 480 infants)	0.6 ng/mL (maternal median serum PFNA)	Total T4	β -0.05 (-0.16–0.05)
		Free T4	β -0.57 (-1.52–0.40)
		TSH	β -0.27 (-6.19–6.03)
		Neonatal T4	β 0.05 (-0.29–0.39)
Shah-Kulkarni et al. 2016 General population (n=279 pregnant women)	0.2 ng/mL (cord blood median PFNA)	Cord blood T4	NS (p=0.70)
		Cord blood T3	NS (p=0.93)
		Cord blood TSH	NS (p=0.14)
Tsai et al. 2017 General population (n=118 mother-infant pairs)	7.55 ng/mL (mean cord blood PFNA)	Cord blood T4	β -0.067 (-0.252–0.009)
		Cord blood T3	β -0.03 (0.069–0.103)
		Cord blood TSH	β 0.045 (-0.051–0.142)
Wang et al. 2013a General population (n=903 pregnant women)	0.37 ng/mL (geometric mean PFNA)	TSH	NS (p>0.05)
		Elevated TSH risk	NS (p>0.05)
Wang et al. 2014 General population (n=285 pregnant women)	1.51 ng/mL (median PFNA)	TSH	NS (p>0.05)
		Free T4	**Inverse association (p<0.001)***
		Total T4	**Inverse association (p<0.001)***
		Total T3	NS (p>0.05)
Webster et al. 2016 General population (NHANES) (n=1,525)	1.5 ng/mL (geometric mean PFNS)	TSH	NS (p>0.05)
		Free T4	NS (p>0.05)
		Total T4	NS (p>0.05)
		Free T3	NS (p>0.05)
		Total T3	NS (p>0.05)

Table 2-15. Summary of Thyroid Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Wen et al. 2013 General population (NHANES) (n=1,181)	1.54 ng/mL (geometric mean PFNA)	TSH	NS (p=0.973), men NS (p=0.407), women
		Total T4	NS (p=0.097), men NS (p=0.632), women
		Total T3	NS (p=0.063), men NS (p=0.258), women
		Thyroglobulin	NS (p=0.537), men NS (p=0.395), women
		Subclinical hypothyroidism risk	OR 1.30 (0.65–2.60), men OR 2.54 (0.40–16.05), women
		Subclinical hyperthyroidism risk	OR 2.41 (0.48–12.04), men OR 1.91 (0.83–4.38), women
Yang et al. 2016a General population (n=157 pregnant women)	0.52 ng/mL (mean)	TSH	**Inverse association (p<0.05)***
		Free T4	NS (p>0.05)
		Total T4	NS (p>0.05)
		Free T3	NS (p>0.05)
		Total T3	NS (p>0.05)
PFDA			
Berg et al. 2015 General population (n=391 pregnant women)	0.31–2.34 ng/mL (4th PFDA quartile)	T3	**Association (p=0.03) (4th quartile)**
Berg et al. 2017 General population (n=370 pregnant women)	0.23 ng/mL (median maternal serum PFNA) and 0.32–2.34 ng/mL (4th quartile serum PFNA)	Total T4	NS (p>0.05)
		Free T4	NS (p>0.05)
		Total T3	**β -0.02 (-0.044 to -0.005, p<0.05)***
		Free T3	NS (p>0.05)
		TSH	NS (p>0.05)
		Thyroxine binding capacity	NS (p>0.05)

Table 2-15. Summary of Thyroid Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Bloom et al. 2010 General population (n=31)	0.21 ng/mL (geometric mean PFDA)	TSH	NS (p=0.365)
		Free T4	NS (p=0.107)
Jain 2013 General population (NHANES) (n=1,525)	NR	TSH	NS (p>0.05)
		Free T3	NS (p>0.05)
		Total T3	NS (p>0.05)
		Free T4	NS (p>0.05)
		Total T4	NS (p>0.05)
		Thyroglobulin	NS (p>0.05)
Ji et al. 2012 General population (n=633)	0.91 ng/mL (median PFDA)	TSH	NS (p=0.2721)
		T4	NS (p=0.2176)
Kang et al. 2018 General population (150 children, 3–18 years)	0.0592 ng/mL (median serum PFDA)	Free T4	NS (p=0.153)
		TSH	NS (p=0.420)
Shah-Kulkarni et al. 2016 General population (n=279 pregnant women)	0.1 ng/mL (cord blood median PFDA)	Cord blood T4	NS (p=0.40)
		Cord blood T3	NS (p=0.07)
		Cord blood TSH	NS (p=0.22)
Wang et al. 2013a General population (903 pregnant women)	0.09 ng/mL (geometric mean PFDA)	TSH	NS (p>0.05)
		Elevated TSH risk	NS (p>0.05)
Wang et al. 2014 General population (285 pregnant women)	0.46 ng/mL (median PFDA)	TSH	NS (p>0.05)
		Free T4	NS (p>0.05)
		Total T4	NS (p>0.05)
		Total T3	**Association (p<0.01)***
Yang et al. 2016a General population (n=157 pregnant women)	0.45 ng/mL (mean PFDA)	TSH	**Inverse association (p<0.01)***
		Free T4	NS (p>0.05)
		Total T4	NS (p>0.05)
		Free T3	NS (p>0.05)
		Total T3	NS (p>0.05)

Table 2-15. Summary of Thyroid Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFUnA			
Berg et al. 2015 General population (n=391)	0.4–0.96 ng/mL (4th PFUnA quartile)	Free T3	**Association (p=0.00)*, 4th quartile**
Berg et al. 2017 General population (n=370 pregnant women)	0.26 ng/mL (median maternal serum PFUnA) and 0.39–1.46 (4th quartile serum PFUnA)	Total T4	NS (p>0.05)
		Free T4	NS (p>0.05)
		Total T3	NS (p>0.05)
		Free T3	**β -0.02 (-0.033 to -0.003, p<0.05)**
		TSH	NS (p>0.05)
		Thyroxine binding capacity	NS (p>0.05)
Bloom et al. 2010 General population (n=31)	0.20 ng/mL (geometric mean PFUnA)	TSH	NS (p=0.527)
		Free T4	NS (p=0.204)
Ji et al. 2012 General population (n=633)	1.75 ng/mL (median PFUnA)	TSH	NS (p=0.5368)
		T4	NS (p=0.0642)
Kang et al. 2018 General population (150 children, 3–18 years)	0.0652 ng/mL (median serum PFUnA)	Free T4	NS (p=0.581)
		TSH	NS (p=0.510)
Shah-Kulkarni et al. 2016 General population (n=279 pregnant women)	0.26 ng/mL (cord blood median PFUnA)	Cord blood T4	NS (p=0.86)
		Cord blood T3	NS (p=0.35)
		Cord blood TSH	NS (p=0.37)
Tsai et al. 2017 General population (n=118 mother-infant pairs)	15.94 ng/mL (mean cord blood PFNA)	Cord blood T4	β 0.045 (-0.223–0.313)
		Cord blood T3	β 0.048 (-0.008–0.104)
		Cord blood TSH	β 0.077 (0.063–0.216)
Wang et al. 2013a General population (n=903 pregnant women)	0.20 ng/mL (geometric mean PFUnA)	TSH	NS (p>0.05)
		Elevated TSH risk	NS (p>0.05)

Table 2-15. Summary of Thyroid Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Wang et al. 2014 General population (n=285 pregnant women)	3.26 ng/mL (median PFUnA)	TSH	NS (p>0.05)
		Free T4	**Inverse association (p<0.001)***
		Total T4	**Inverse association (p<0.001)***
		Total T3	NS (p>0.05)
Yang et al. 2016a General population (n=157 pregnant women)	0.45 ng/mL (mean PFUnA)	TSH	**Inverse association (p<0.05)***
		Free T4	NS (p>0.05)
		Total T4	NS (p>0.05)
		Free T3	NS (p>0.05)
		Total T3	NS (p>0.05)
PFDoDA			
Ji et al. 2012 General population (n=633)	0.92 ng/mL (median PFDoDA)	TSH	NS (p=0.6925)
		T4	NS (p=0.7153)
Shah-Kulkarni et al. 2016 General population (n=279 pregnant women)	0.08 ng/mL (cord blood median PFDoDA)	Cord blood T4	NS (p=0.69)
		Cord blood T3	NS (p=0.30)
		Cord blood TSH	NS (p=0.20)
Wang et al. 2014 General population (285 pregnant women)	0.36 ng/mL (median PFDoDA)	TSH	NS (p>0.05)
		Free T4	**Inverse association (p<0.001)***
		Total T4	**Inverse association (p<0.01)***
		Total T3	NS (p>0.05)
Yang et al. 2016a General population (n=157 pregnant women)	0.046 ng/mL (mean PFDoDA)	TSH	**Inverse association (p<0.01)***
		Free T4	**Inverse association (p<0.05)***
		Total T4	**Inverse association (p<0.05)***
		Free T3	**Inverse association (p<0.01)***
		Total T3	**Inverse association (p<0.01)***

Table 2-15. Summary of Thyroid Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Me-FOSA-AcOH			
Jain 2013 General population (NHANES) (n=1,525)	NR	TSH	NS (p>0.05)
		Free T3	NS (p>0.05)
		Total T3	NS (p>0.05)
		Free T4	NS (p>0.05)
		Total T4	NS (p>0.05)
		Thyroglobulin	NS (p>0.05)

[a]See the *Supporting Document for Epidemiological Studies for Perfluoroalkyls,* Table 9 for more detailed descriptions of studies.
[b]Participants in occupational exposure may have also lived near the site and received residential exposure; community exposure studies involved subjects living near PFOA facilities with known exposure to high levels of PFOA.
[c]Asterisk indicates association with perfluoroalkyl; unless otherwise specified, values in parenthesis are 95% confidence intervals.

HR = hazard ratio; NHANES = National Health and Nutrition Examination Survey; NR = not reported; NS = not significant; OR = odds ratio; PFDA = perfluorodecanoic acid; PFDoDA = perfluorododecanoic acid; PFHxS = perfluorohexane sulfonic acid; PFNA = perfluorononanoic acid; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid; PFUnA = perfluoroundecanoic acid; T3 = triiodothyronine; T4 = thyroxine; TSH = thyroid stimulating hormone

sensitive targets following exposure to PFOA, PFOS, PFDA, PFBS, or PFBA. Endocrine effects have not been examined in laboratory animal studies on PFNA, PFUnA, PFHpA, or FOSA.

PFOA

Epidemiological Studies. A number of epidemiological studies have examined the potential of PFOA to damage the thyroid. Steenland et al. (2015) did not find an association between serum PFOA and the risk of thyroid disease in male or female workers at the Washington Works facility. The occupational exposure studies do not suggest an association between serum PFOA and alterations in thyroid hormone levels. One study (Olsen and Zobel 2007) reported associations between serum PFOA levels and free T4 and T3 levels in workers at 3M facilities; it is noted that the investigators did not consider the results clinically relevant since the levels were within the normal range. A study reported an association between serum PFOA and TSH, but this was only observed at one time point (Olsen et al. 1998b); another study of the 3M Cottage Grove facility, reported an association between serum fluorine levels and TSH levels (Gilliland 1992). A fifth occupational study reported that TSH, T4, and T3 levels were within the reference range (Sakr et al. 2007b).

Three studies of the community affected by the Washington Works facility reported increases in self-reported thyroid disease (Anderson-Mahoney et al. 2008), any type of functional thyroid disease (Lopez-Espinosa et al. 2012; Winquist and Steenland 2014b), or hypothyroidism (Lopez-Espinosa et al. 2012). No associations between estimated cumulative serum PFOA and hyperthyroidism or hypothyroidism were found in retrospective analysis (Winquist and Steenland 2014b). However, in prospective analysis, an association between estimated cumulative serum PFOA and hypothyroidism was found in men (Winquist and Steenland 2014b). Consistent with the occupational exposure data, no association between serum PFOA and TSH levels was found (Emmett et al. 2006b; Knox et al. 2011a; Lopez-Espinosa et al. 2012). Increases in serum PFOA were also associated with increases in T4 levels and decreases in T3 uptake in adults (Knox et al. 2011a).

A number of studies have examined the thyroid outcomes associated with serum PFOA levels in the general population. An association between serum PFOA and thyroid disease risk was found in female NHANES participants, but not in males (Melzer et al. 2010). Another study utilizing NHANES data (Wen et al. 2013) found an increased risk of subclinical hypothyroidism among women, but not men, and a decreased risk of subclinical hyperthyroidism among men, but not women. An increased risk of hypothyroidism was also observed in a study of pregnant women (DuFour et al. 2018). A case-control

study of women did not find that serum PFOA levels were associated with the risk of hypothyroxinemia (Chan et al. 2011). Although five studies found associations between serum PFOA and T3 levels (Crawford et al. 2017; Jain 2013; Lewis et al. 2015; Webster et al. 2016; Wen et al. 2013), five other studies did not find these associations (Berg et al. 2017; Raymer et al. 2012; Shrestha et al. 2015; Wang et al. 2014; Yang et al. 2016a). No associations between serum PFOA and TSH or T4 levels were found in the general population studies (Berg et al. 2017; Bloom et al. 2010; Crawford et al. 2017; Jain 2013; Ji et al. 2012; Kang et al. 2018; Raymer et al. 2012; Shrestha et al. 2015; Wang et al. 2013a, 2014; Webster et al. 2016; Wen et al. 2013; Yang et al. 2016a), with the exception of two studies which found an association for TSH and T4 levels (Lewis et al. 2015) or free T4 (Preston et al. 2018).

Studies examining possible relationships between cord blood PFOA and cord blood thyroid hormone levels have not found associations for T4, T3, or TSH (Dufour et al. 2018; Shah-Kulkarni et al. 2016; Tsai et al. 2017). Preston et al. (2018) found an inverse association between maternal serum PFOA and neonatal T4 levels.

In a clinical trial of patients with advanced solid tumors administered 50–1,200 mg APFO (approximately 0.10–2.4 mg/kg/day) for 6 weeks, increases in free T4 levels were observed with no apparent alterations in TSH (Convertino et al. 2018).

Laboratory Animal Studies. Repeated intermittent head-only exposure of male rats to up to 84 mg/m^3 APFO dusts for 2 weeks did not result in significant gross or microscopic alterations in the thyroid or adrenal gland (Kennedy et al. 1986).

In a 2-generation study in rats, daily treatment of the parental generation with 0, 1, 3, 10, or 30 mg/kg/day APFO by gavage in water for 70–90 days produced an increased incidence of hypertrophy and/or vacuolation of the zona glomerulosa of the adrenal gland from high-dose males (Butenhoff et al. 2004b). The respective incidences were 0/10, 0/10, 0/10, 2/10, and 7/10. This effect was also observed in F1 generation males treated with the same dose level. No explanation was apparent for this finding. In rats dosed with up to 15 mg/kg/day APFO in the diet for 2 years, there were no significant morphological alterations in the adrenals (3M 1983; Butenhoff et al. 2012c). A study in monkeys treated with APFO also reported effects on the adrenal glands. Griffith and Long (1980) reported diffuse lipid depletion in the adrenals from Rhesus monkeys dosed daily for 90 days with 30 mg/kg/day APFO by gavage. This dose level was lethal to some monkeys; no such effect was seen in monkeys dosed with 10 mg/kg/day.

For the most part, morphological evaluations of other endocrine glands in animals treated with PFOA have been negative. For example, male and female rats dosed via the diet with approximately 100–110 mg/kg/day APFO for 90 days showed no gross or microscopic alterations in the pituitary or thyroid glands (Griffith and Long 1980). Similar observations were reported in the pituitary, thyroid, and parathyroid glands from male and female rats dosed with up to 15 mg/kg/day APFO in the diet for 2 years (Butenhoff et al. 2012c; 3M 1983).

Administration of up to 20 mg/kg/day PFOA administered via a capsule to Cynomolgus monkeys for 4 weeks did not significantly alter free T4, total T4, free T3, total T3, or TSH (Thomford 2001). Serum T4 and total T4 were significantly reduced in Cynomolgus monkeys dosed with 10 mg/kg/day APFO administered via a capsule for up to 6 months, but were still within the normal range (Butenhoff et al. 2002). No significant changes were seen on serum free T3, total T3, or TSH, or thyroid histology.

The only relevant dermal information is that no morphological alterations were observed in the thyroid of rats following dermal application of up to 2,000 mg/kg/day APFO for 2 weeks in the Kennedy (1985) study.

PFOS

Epidemiological Studies. A number of epidemiological studies have examined the risk of thyroid disease and alterations in thyroid hormone levels to evaluate whether the thyroid gland is a target of PFOS toxicity. In studies of NHANES participants, no increases in the risk of thyroid disease were observed in men or women (Lewis et al. 2015; Melzer et al. 2010). Melzer et al. (2010) did find an increase in the risk of having thyroid disease and currently taking thyroid medication among men, and Wen et al. (2013) found increased risks of subclinical hypothyroidism among men and women. Dufour et al. (2018) also found an association between cord blood PFOS and risk of maternal hypothyroidism. Although some studies have found alterations in thyroid hormone levels, the results are not consistent across studies. Associations between serum PFOS and TSH levels were observed in three general population studies (Berg et al. 2015, 2017; Wang et al. 2014); however, one of the studies (Berg et al. 2017) found that the association was no longer significant after adjustments for exposure to other perfluoroalkyls and persistent organic compounds. In contrast, two other studies found inverse associations for TSH (Dallaire et al. 2009; Yang et al. 2016a). A third study also found an inverse association with TSH but only among pregnant women who were positive for thyroid peroxides antibodies (Preston et al. 2018). An occupational exposure study (Olsen et al. 1998a) and ten general population studies (Bloom et al. 2010;

Crawford et al. 2017; Jain 2013; Ji et al. 2012; Kang et al. 2018; Lewis et al. 2015; Raymer et al. 2012; Shrestha et al. 2015; Wang et al. 2014; Wen et al. 2013) did not find associations between serum PFOS and TSH levels. Conflicting results were also reported for T3 levels, with some studies reporting associations (Olsen et al. 2003a), inverse associations (Dallaire et al. 2009), or no association (Berg et al. 2017; Crawford et al. 2017; Jain 2013; Lewis et al. 2015; Raymer et al. 2012; Shrestha et al. 2015; Wang et al. 2014; Webster et al. 2016; Wen et al. 2013; Yang et al. 2016a). Most studies did not find an association with T4 levels (Berg et al. 2017; Crawford et al. 2017; Jain 2013; Ji et al. 2012; Kang et al. 2018; Lewis et al. 2015; Raymer et al. 2012; Preston et al. 2018; Wang et al. 2014; Webster et al. 2016; Wen et al. 2013; Yang et al. 2016a), but three studies did find associations between T4 levels and serum PFOS (Dallaire et al. 2009; Lewis et al. 2015; Shrestha et al. 2015). In NHANES participants with two indicators of thyroid stress (low iodine levels and high thyroid peroxidase antibody), serum PFOS levels were significantly ($p<0.05$) associated with increases in free and total T3, decreases in free T4, and increases in TSH levels (Webster et al. 2016).

Conflicting results were also found in studies using cord blood PFOS as the biomarker of exposure. Tsai et al. (2017) found an inverse association with cord blood T4 and a positive association with cord blood TSH; no association was found for T3. Shah-Kulkarni et al. (2016) found no associations for cord blood T4, T3, or TSH. It is noted that cord blood serum PFOS levels were much higher in the Tsai et al. (2017) study compared to the Shah-Kulkarni et al. (2016) study.

Laboratory Animal Studies. Chang et al. (2008b) conducted a study of thyroid function in rats exposed to PFOS (potassium salt). Administration of a single dose of 15 mg/kg by gavage in water (only dose level tested) reduced serum total T4 significantly at 2, 6, and 24 hours after dosing. This effect was attributed to a PFOS-induced transient increase in tissue availability of thyroid hormones and turnover of T4 with a resulting reduction in serum total T4. Chang et al. (2008b) concluded that PFOS did not induce a classical hypothyroid state or alter the hypothalamic-pituitary-thyroid axis. In another acute-duration study, dosing of pregnant mice with 6 mg/kg/day PFOS (potassium salt) on GDs 6–18 did not affect maternal serum levels of free or total T3 or T4 (Fuentes et al. 2006).

Changes in thyroid hormones have also been reported following intermediate-duration exposure to PFOS. For example, in a 2-generation gavage study in which dosing of rats started before mating and continued through gestation, doses ≥0.4 mg/kg/day (the lowest dose tested) caused a significant and dose-related reduction in total T4 in maternal serum on postpartum day 5 (Luebker et al. 2005b). Free T4 and TSH were not significantly affected. Exposure of pregnant rats to ≥1 mg/kg/day PFOS on GDs 2–20 induced

significant reductions in total T4 and free T4 and less marked reductions in T3 during pregnancy, particularly on GD 7 (Thibodeaux et al. 2003); however, serum TSH values were not significantly altered. A similar study in pregnant mice reported a decrease in total T4 on GD 6 in mice dosed with 20 mg/kg/day PFOS on GDs 1–17 (Thibodeaux et al. 2003). No alterations in total T4 were reported in mice dosed with 15 mg/kg/day. No information was provided regarding other thyroid hormones. Decreases in T4 levels were observed in male and female rats exposed to PFOS in the diet for 28 days (Curran et al. 2008); T3 levels were decreased in female rats exposed to 50 or 100 mg/kg/day and in male rats at 100 mg/kg/day. No histological alterations were observed in the thyroid. Another study with PFOS found no thyroid histological effects in rats exposed to 10.3 mg/kg/day for 1 day, 8.17 mg/kg/day for 7 days, or 7.34 mg/kg/day for 28 days (Elcombe et al. 2012a). Exposure of rats to ≥0.27 mg/kg/day PFOS in drinking water for 91 days resulted in decreases in total T4 levels (Yu et al. 2009a), but no changes in T3 or TSH levels (highest dose tested was 2.37 mg/kg/day). Curran et al. (2008) suggested that the apparent decreases in T4 levels, in the absence of TSH alterations and histological alterations in the thyroid, may be a result of measurement error when analog assays (chemiluminometric immunoassay and radioimmunoassay) are used due to binding interference. A decrease in serum total T4 levels was observed in Cynomolgus monkeys administered three doses of PFOS (average dose of 13.3 mg/kg in males and 14 mg/kg in females) over 315 days (Chang et al. 2017). The investigators did not consider this an adverse effect because the values were within the normal variation and there were not changes in free T4 levels or TSH levels. In another study in Cynomolgus monkeys, T3 was numerically lower than controls in one female and one male monkey dosed with 2 mg/kg/day PFOS by capsule for 4 weeks (Thomford 2002a). However, it is difficult to determine whether the effect was treatment-related based on only two animals. In a 26-week study in Cynomolgus monkeys, the highest dose of PFOS tested, 0.75 mg/kg/day, induced a significant increase in serum TSH (approximately twice control value, but still within the reference range) and a decrease in total T3 at termination, but not at earlier time points; variations in other thyroid hormones, including T4, were inconsistent regarding dose and over time (Seacat et al. 2002). The clinical relevance of the lowered total T3 values was not apparent since there was no indication of a clinical hypothyroid response, and thyroid histology was not altered by treatment with PFOS.

Examination of the adrenal glands from rats dosed with up to 1.77 mg/kg/day PFOS via the diet for 4 or 14 weeks did not show any significant gross or microscopic alterations (Seacat et al. 2003). No significant gross or microscopic lesions were reported in the adrenals, thyroid and parathyroid, or pituitary gland from rats dosed with up to 1.04 mg/kg/day PFOS in the diet for 2 years (Butenhoff et al. 2012b; Thomford 2002b).

PFHxS

Epidemiological Studies. Fifteen general population studies have evaluated possible associations between serum PFHxS levels and alterations in thyroid hormone levels. With the exception of a study of pregnant women, which found an association between serum PFHxS levels and TSH levels (Wang et al. 2014), and a study of NHANES participants, which found associations between serum PFHxS and total T4 and T3 in women (Wen et al. 2013), the epidemiological studies did not find associations for TSH, T3, or T4 (Berg et al. 2017; Bloom et al. 2010; Crawford et al. 2017; Jain 2013; Ji et al. 2012; Kang et al. 2018; Lewis et al. 2015; Preston et al. 2018; Wang et al. 2013a, 2014; Webster et al. 2016; Yang et al. 2016a). No associations were also found between cord blood PFHxS levels and cord blood T4, T3, or TSH (Shah-Kulkarni et al. 2016). Chan et al. (2011) did not find an increase in the risk of hypothyroxinemia associated with serum PFHxS levels. Wen et al. (2013) found increases in the risk of subclinical hypothyroidism and subclinical hyperthyroidism among women, but not men and Dufour et al. (2018) did not find an association between cord blood PFHxS levels and risk of hypothyroidism in pregnant women.

Laboratory Animal Studies. Hypertrophy and hyperplasia of the follicular cells were observed in the thyroids of male rats treated with ≥3 mg/kg/day PFHxS for at least 42 days (Butenhoff et al. 2009a). The NOAEL was 1 mg/kg/day. The investigators noted that the observed changes in rats are consistent with the known effects of inducers of microsomal enzymes where the hepatocellular hypertrophy results in a compensatory hypertrophy and hyperplasia of the thyroid due to an increase in plasma turnover of T4 and associated stimulation of TSH. Neither thyroid hormones nor TSH were measured in the study. In studies of pregnant rats, 20–30 and 60% decreases in serum thyroxine were observed in the dams administered 5 mg/kg/day or 25 mg/kg/day PFHxS on GD 7–22 (Ramhøj et al. 2018). In mice administered up to 3 mg/kg/day PFHxS prior to mating and during mating, gestation, and lactation, no alterations in TSH were observed in the parental males or females (Chang et al. 2018); this study also found no histological alterations in the thyroid gland.

PFNA

Epidemiological Studies. Inverse associations between serum PFNA levels and T4 levels (Wang et al. 2014) and TSH levels (Yang et al. 2016a) have been reported in general population studies. However, several other studies have not found alterations in TSH, T4, or T3 levels associated with serum PFNA

levels (Berg et al. 2017; Bloom et al. 2010; Jain 2013; Ji et al. 2012; Lopez-Espinosa et al. 2012; Preston et al. 2018; Wang et al. 2013a; Webster et al. 2016; Wen et al. 2013; Yang et al. 2016a). The investigators for an occupational exposure study reported that differences in TSH, T4, and T3 levels were small and clinically insignificant in groups of workers exposed to low levels, high levels, or no PFNA (Mundt et al. 2007). Preston et al. (2018) found an inverse association between serum PFNA levels and TSH levels, but only in pregnant women who were positive for maternal thyroid peroxides antibodies. Crawford et al. (2017) found associations between serum PFNA and free T4 and T3 levels in women, but no associations with total T4 or TSH. No associations between cord blood PFNA levels and cord blood T4, T3, or TSH were found in two studies (Shah-Kulkarni et al. 2016; Tsai et al. 2017). No associations were found for thyroid disease, hypothyroidism, or subclinical hypo- or hyperthyroidism among residents living near the Washington Works PFOA facility (Lopez-Espinosa et al. 2012), in NHANES participants (Wen et al. 2013), or in pregnant women (Dufour et al. 2018).

PFDA

Epidemiological Studies. Most general population studies did not find associations between serum PFDA levels and TSH, T3, or T4 levels (Berg et al. 2017; Bloom et al. 2010; Ji et al. 2012; Kang et al. 2018; Wang et al. 2013a, 2014; Yang et al. 2016a). The exceptions were studies in pregnant women that found positive associations (Berg et al. 2015; Wang et al. 2014) or inverse associations with T3 (Berg et al. 2017), or an inverse association with TSH levels (Yang et al. 2016a). No associations between cord blood PFDA and cord blood T4, T3, or TSH were found in a study by Shah-Kulkarni et al. (2016).

Laboratory Animal Studies. Administration of a single dose of 80 mg/kg PFDA to female C57BL/6N mice by gavage resulted in 2- and 4-fold increases in serum T3 and T4, respectively, relative to controls 30 days after dosing (Harris et al. 1989). No alterations were observed in the adrenal glands of rats administered 0.5 mg/kg/day PFDA for 28 days or mice receiving weekly gavage doses of 5 mg/kg for 4 weeks (Frawley et al. 2018).

PFUnA

Epidemiological Studies. Inverse associations between serum PFUnA and serum TSH (Yang et al. 2016a) T4 (Wang et al. 2014), or T3 (Berg et al. 2015, 2017) have been reported in pregnant women. However, other general population studies have not found association between PFUnA and TSH, T4, or T3 levels (Bloom et al. 2010; Ji et al. 2012; Kang et al. 2018; Wang et al. 2013a, 2014; Yang et al. 2016a)

or between cord blood PFUnA and cord blood T4, T3, or TSH (Shah-Kulkarni et al. 2016; Tsai et al. 2017).

PFBS

Laboratory Animal Studies. Treatment of rats with up to 900 mg/kg/day PFBS by gavage for 28 days did not alter the gross or microscopic appearance of the adrenal, pituitary, or thyroid/parathyroid glands (3M 2001). Levels of thyroid hormones in serum were not available in this study. A study in pregnant mice administered ≥200 mg/kg/day PFBS on GDs 1–20 found decreases in maternal levels of total T4, free T4, and total T3 and increases in TSH levels (Feng et al. 2017).

PFBA

Laboratory Animal Studies. Treatment of rats with up to 184 mg/kg/day PFBA by gavage for 5 days did not affect the gross or microscopic morphology of the adrenal, thyroid, or pituitary glands (3M 2007a). Treatment with ≥30 mg/kg/day for 28 or 90 days significantly increased the incidence of hyperplasia/ hypertrophy of the follicular epithelium of the thyroid gland (Butenhoff et al. 2012a; van Otterdijk 2007a, 2007b). These changes were not observed following a 3-week recovery period. Van Otterdijk (2007a, 2007b; Butenhoff et al. 2012a) suggested that the thyroid lesion likely reflected an increase in T4 producing follicular cells in response to feedback mechanisms from the increased turnover of T4 by the hypertrophic hepatocytes. None of these studies measured thyroid hormones or TSH in serum.

PFDoDA

Epidemiological Studies. Four general population studies have evaluated the effect of PFDoDA on thyroid hormone levels. Wang et al. (2014) reported inverse associations between serum PFDoDA and free T4 and total T4 in pregnant women; no associations were found for TSH or total T3. In another study of pregnant women (Yang et al. 2016a), inverse associations were found for TSH, free T4, total T4, free T3, and total T3. The third study (Ji et al. 2012) found no associations between serum PFDoDA and TSH or T4. Shah-Kulkarni et al. (2016) did not find associations between cord blood PFDoDA levels and cord blood T4, T3, or TSH levels.

Laboratory Animal Studies. Histological alterations were observed in the pancreas, adrenal gland, and/or thymus of rats administered 2.5 mg/kg/day PFDoDA for 42–47 days (Kato et al. 2015). Decreases in

zymogen granules were observed in the pancreas of male rats and edema of the pancreas interstitium was observed in females (most female rats died before the end of the study). Atrophy of the adrenal cortex was observed in males and in females exposed for 42 days and allowed to recover for 14 days. Atrophy of the thymic cortex was observed in females (most dying before the end of the study). A 28-day study found a 40% reduction in serum estradiol levels in pubertal female rats administered 3 mg/kg/day for 28 days (Shi et al. 2009b).

PFHxA

Laboratory Animal Studies. An increased incidence of thyroid follicular epithelial hypertrophy was observed in female rats administered 500 mg/kg/day NaPFHx for 93 days (Loveless et al. 2009). No alterations were observed in male rats in this study or in a second 90-day study in which male and female rats were administered doses as high as 200 mg/kg/day NaPFHx.

2.14 IMMUNOLOGICAL

Overview. Epidemiological studies have evaluated three categories of altered immune response related to exposure to perfluoroalkyls: immunosuppression (altered antibody response, infectious disease resistance), hypersensitivity (asthma, wheezing, eczema, atopic dermatitis, allergies), and autoimmunity. A summary of epidemiological studies evaluating immunological endpoints is presented in Table 2-16; more detailed descriptions of individual studies are presented in the *Supporting Document for Epidemiological Studies for Perfluoroalkyls*, Table 10. Epidemiological data evaluating potential immunological effects are available for all perfluoroalkyls except PFBA. In general, the epidemiological studies identify the immune system as a target of perfluoroalkyl toxicity. The strongest evidence of the immunotoxicity of perfluoroalkyls in humans comes from epidemiological studies finding associations evaluating the antibody response to vaccines. Associations have been found for PFOA, PFOS, PFHxS, and PFDA. There is also some limited evidence for decreased antibody response for PFNA, PFUnA, and PFDoDA, although many of the studies did not find associations for these compounds. In general, decreases in disease resistance have not been found for PFOA, PFOS, PFHxS, or PFNA. There is marginal evidence for associations between PFOA, PFOS, PFHxS, PFNA, PFDA, PFBS, and PFDoDA and increased risk of asthma; the evidence was considered marginal due to the small number of studies evaluating the outcome and/or conflicting study results. There are limited data of effects on

Table 2-16. Summary of Immunological Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFOA			
Anderson-Mahoney et al. 2008 Community (n=566 adults)	NR	Asthma	**SPR 1.82 (1.47–2.25)***
Ashley-Martin et al. 2015 General population (1,258 women)	NR	IL-33/TSLP (cord blood)	OR 1.1 (0.6–1.8)
		IgE (cord blood)	OR 1.1 (0.6–1.9)
Buser and Scinicariello 2016 General population (NHANES) (n=637 and 701 adolescents in 2005–2006 and 2007–2010)	3.59 and 3.27 ng/mL (geometric mean 2005–2006 and 2007–2010) >4.47 ng/mL (4th quartile)	Food allergies	**OR 9.09 (3.52–24.90)*, 4th quartile**
		Food sensitization	NS (p=0.74 for trend)
Dalsager et al. 2016 General population (n=359 1–4-year-old children)	2.04–10.12 ng/mL (maternal 3rd tertile PFOA)	Risk of number of days above the median	
		Fever	**OR 1.97 (1.07–3.62)*, 3rd tertile**
		Cough	NS (p>0.05)
		Nasal discharge	NS (p>0.05)
		Diarrhea	NS (p>0.05)
		Vomiting	NS (p>0.05)
		Risk of number of days	
		Fever	OR 1.12 (0.82–1.54), 3rd tertile
		Cough	NS (p>0.05)
		Nasal discharge	NS (p>0.05)
		Diarrhea	NS (p>0.05)
		Vomiting	NS (p>0.05)
Dong et al. 2013 General population (n=231 asthmatic and 225 non-asthmatic children) This is the same group of children evaluated by Zhu et al. (2016)	1.5 and 1.0 ng/mL (mean serum PFOA levels in the asthmatic and non-asthmatic children, respectively; serum levels were not reported for full cohort)	Asthma diagnosis	**OR 2.67 (1.49–4.79)*, 3rd quartile**
		IgE	**Association (p<0.05)*, asthmatics** NS (p>0.05), non-asthmatics
		Absolute eosinophil counts	**Association (p<0.05)*, asthmatics** NS (p>0.05), non-asthmatics
		Eosinophil cationic protein	**Association (p<0.05)*, asthmatics** NS (p>0.05), non-asthmatics

Table 2-16. Summary of Immunological Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Fei et al. 2010 General population. (n=1,400 pregnant women and young children)	5.6 ng/mL (maternal PFOA)	Risk of hospitalization for infectious disease in young children	IRR 0.96 (0.87–1.06) for trend **IRR 1.21 (1.04–1.42)* for trend, girls** **IRR 0.83 (0.73–0.95)* for trend, boys**
Goudarzi et al. 2016a General population (n=1,558 4-year-old children)	2.713 ng/mL (mean maternal plasma PFOA)	Prevalence of allergic disease	OR 0.830 (0.591–1.16), 4th quartile
		Prevalence of wheezing	OR 1.09 (0.729–1.65), 4th quartile
Goudarzi et al. 2017 General population (n=1,558 mother-child pairs); children examined up to 4 years of age	2.713 ng/mL (mean maternal serum PFOA)	Risk of total infectious diseases	OR 1.11 (0.806–1.54), 4th quartile, p=0.393 for trend.
Grandjean et al. 2012; Mogensen et al. 2015a General population (n=456 and n=464 children 5 and 7 years of age)	4.1 and 4.4 ng/mL (median PFOA at age 5 and 7 years) 3.20 ng/mL (geometric mean maternal PFOA)	Tetanus antibody levels at age 5	NS, maternal PFOA NS, PFOA at age 5
		Tetanus antibody levels at age 7	NS, maternal PFOA **β -35.8% (-51.9 to -14.2)*, per 2-fold increase in PFOA levels at age 5** NS, PFOA at age 7
		Diphtheria antibody levels at age 5	NS, maternal PFOA NS (p=0.69), PFOA at age 5
		Diphtheria antibody levels at age 7	NS, maternal PFOA **β -25.2% (-42.9 to -2.0)*, per 2-fold increase in PFOA levels at age 5** **β -25.4% (-40.9 to -5.8)*, per 2-fold increase in PFOA levels, PFOA at age 7**

Table 2-16. Summary of Immunological Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Grandjean et al. 2017 General population (n=516 children examined at age 7 and 13 years)	4.4 and 2.0 ng/mL (median PFOA at age 7 and 13 years)	Tetanus antibody levels at age 13	NS (p=0.637), PFOA at age 7 NS (p=0.856), PFOA at age 13
		Diphtheria antibody levels at age 13	Full cohort NS (p=0.742), PFOA at age 7 NS (p=0.129), PFOA at age 13 Cohort restricted to children without possible unscheduled booster vaccines NS (p=0.480), PFOA at age 7 **Association (p=0.029)*, PFOA at age 13**
Granum et al. 2013 General population (n=56 children age 3 years)	1.1 ng/mL (mean maternal PFOA)	Rubella antibody levels	**Inverse association (p=0.001)***
		Hemophilus influenza type B antibody levels	NS (p>0.05)
		Tetanus antibody levels	NS (p>0.05)
		Asthma diagnosis	NS (p>0.05)
		Atopic eczema	NS (p>0.05)
		Eczema and itchiness	NS (p>0.05)
		Number of episodes of otitis media	NS (p>0.05)
		Number of episodes of common cold	**Association (p<0.001)***
		Number of episodes of gastroenteritis	**Association (p=0.048)***
Humblet et al. 2014 General population (NHANES) (n=1,877 adolescents)	4.3 and 4.0 ng/mL (median PFOA in asthmatics and nonasthmatics)	Asthma episode in last 12 months	**OR 1.18 (1.01–1.39)*, per doubling PFOA**
		Current asthma	NS (p=0.26)
		Wheezing	NS (p=0.98)

Table 2-16. Summary of Immunological Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Impinen et al. 2018 General population (n=641 infants followed through age 10)	**Exposure:** level 1.8 ng/mL (mean cord PFOA)	Number of common colds (0–2 years of age)	NS (p=0.089)
		Number of lower respiratory infections (0–10 years of age)	**β 0.28 (0.22–0.35; p=<0.0001)***
		Rhinitis	NS
		Rhinoconjunctivitis	NS
		Asthma diagnosis	NS
		Current asthma	NS
		Asthma ever	NS
		Allergic sensitization	NS
Kielsen et al. 2016 General pop. (n=12 adults)	1.69 ng/mL (median PFOA)	Diphtheria antibody levels	NS (p=0.250), unadjusted
		Tetanus antibody levels	NS (p=0.970), unadjusted.
Looker et al. 2014 Community (C8) (n=411)	33.74 ng/mL (geometric mean) 13.8–31.5 ng/mL (2nd quartile)	Seroprotection from influenza A H3N2 virus	**OR 0.34 (0.14–0.83)*, 2nd quartile**
		Seroprotection from influenza A H1N1 virus	NS (p=0.02)
		Seroprotection from influenza type B virus	NS (p=0.68)
		Cold or flu infection	NS (p>0.05)
		Frequency of colds	NS (p>0.05)
Okada et al. 2012 General population (n=343 pregnant women)	1.3 ng/mL (maternal median PFOA)	Cord IgE levels Males Females	 NS (p>0.05) **Inverse association (p<0.05)***
		Infant food allergy	OR 1.67 (0.52–5.37)
		Eczema	OR 0.96 (0.23–4.02)
		Wheezing	OR 1.27 (0.27–6.05)
		Otitis media	OR 1.51 (0.45–5.12)

Table 2-16. Summary of Immunological Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Okada et al. 2014 General population (n=2,603 infants)	2.67 ng/mL (maternal mean PFOA)	Risk of allergic diseases Males Females	 OR 0.63 (0.63–1.37), 4th quartile **OR 0.64 (0.42–0.97)*, 4th quartile**
		Eczema Males Females	 OR 0.75 (0.48–1.18), 4th quartile OR 0.65 (0.39–1.09), 4th quartile
Qin et al. 2017 General population (n=132 children aged 10–15 years and 168 matched controls)	1.02 and 0.50 ng/mL (median serum PFOA in cases and controls)	Asthma	**OR 2.76 (1.82–4.17)***
Smit et al. 2015 General population (n=1,024 children)	0.97 and 1.79 ng/mL (maternal mean PFOA in Ukraine and Greenland cohorts)	Ever having asthma	OR 0.80 (0.62–1.04), whole cohort
		Ever having eczema	OR 0.97 (0.81–1.17), whole cohort
		Current eczema	OR 1.01 (0.79–1.29), whole cohort
		Ever having wheezing	OR 0.91 (0.76–1.10), whole cohort
		Current wheezing	OR 0.97 (0.71–1.33), whole cohort
Steenland et al. 2013 Community (C8) (28,441)	Estimated cumulative	Ulcerative colitis	**OR 1.76 (1.04–2.99)*, 2nd quartile**
		Rheumatoid arthritis	NS (p>0.05)
		Crohn's disease	NS (p>0.05)
		Type I diabetes	NS (p>0.05)
		Lupus	NS (p>0.05)
		Multiple sclerosis	NS (p>0.05)
Steenland et al. 2015 Occupational (n=3,713)	Estimated cumulative	Asthma	NS (p=0.27), with no lag NS (p=0.53), with 10-year lag **Positive categorical trend (p=0.05)*, with no lag**
		Ulcerative colitis	**RR 6.57 (1.47–29.40)*, with 10-year lag**
		Rheumatoid arthritis	**Positive categorical trend (p=0.04)*, with no lag**

Table 2-16. Summary of Immunological Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Stein et al. 2016a General population (NHANES) (n=1,191 adolescents)	4.13 ng/mL (geometric mean)	Measles antibody titers	NS (95% CI included unity)
		Mumps antibody titers	NS (95% CI included unity), whole cohort **β -6.6% (-11.7 to -1.5)*, per 2-fold increase in PFOA levels, seropositive subcohort**
		Rubella antibody titers	NS (95% CI included unity), whole cohort **β -8.9% (-14.6 to -2.9)*, per 2-fold increase in PFOA levels, seropositive subcohort**
Stein et al. 2016a General population (NHANES) (n=640 adolescents)	3.59 ng/mL (geometric mean)	Rhinitis	**OR 1.35 (1.10–1.66)***
		Current asthma	OR 1.28 (0.81–2.04)
		Wheeze	OR 0.94 (0.51–1.73)
		Allergy	OR 1.12 (0.85–1.47)
		Allergic sensitization	
		Plants	OR 0.88 (0.67–1.15)
		Dust mites	OR 0.93 (0.75–1.16)
		Pets	OR 1.17 0.81–1.68)
		Cockroach or shrimp	OR 0.79 (0.55–1.13)
		Rodents	OR 1.65 (0.59–4.60
		Mold	OR 1.21 (0.85–1.72)
		Food	OR 1.02 (0.60–1.73)
Stein et al. 2016b General population (n=78 adults receiving influenza vaccine)	2.28 ng/mL (geometric mean)	Seroconversion	
		Hemagglutinin	NS (p=0.07 for trend)
		Immunohistochem.	NS (p=0.27 for trend)
		Serum cytokine levels	NS (p>0.05 for trend)
		Serum chemokine levels	NS (p>0.05 for trend)
		Nasal cytokine levels	NS (p>0.05 for trend)
		Nasal chemokine levels	NS (p>0.05 for trend)
		Serum IgA levels	NS (p>0.05 for trend)

Table 2-16. Summary of Immunological Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Wang et al. 2011 General population (n=244 children aged 2 years)	1.71 ng/mL (median cord PFOA)	Serum IgE levels	NS (p=0.870)
		Cord blood IgE levels	Association (p=0.047)*
		Atopic dermatitis	NS (p>0.05)
Zhu et al. 2016 General population (n=231 asthmatic and 225 non-asthmatic children) This is the same group of children evaluated by Dong et al. (2013)	1.51 and 1.00 ng/mL (mean in asthmatics and non-asthmatics)	Asthma diagnosis	**OR 4.24 (1.91–9.42)*, males 4th quartile** **OR 3.68 (1.43–9.48)*, females 4th quartile**
		T-helper cytokines	
		IL-4	**Association (p=0.001 for trend)***
		IL-5	**Association (p=0.004 for trend)***
		IFN-γ	NS (p>0.05 for trend)
		IL-2	NS (p>0.05 for trend)
		Serum IgE	NS (p>0.05 for trend)
PFOS			
Ashley-Martin et al. 2015 General population (1,258 women)	NR	IL-33/TSLP (cord blood)	1.1 (0.6–1.9)
		IgE (cord blood)	OR 1.1 (0.6–1.9)
Buser and Scinicariello 2016 General population (NHANES) (n=637 and 701 adolescents in 2005–2006 and 2007–2010)	14.98 and 8.74 ng/mL (geometric mean PFOS 2005–2006 and 2007–2010) 9.17–13.75 ng/mL (3rd quartile)	Food allergies	**OR 2.43 (1.05–5.59)*, 3rd quartile (trend not significant, p=0.27)**
		Food sensitization	NS (p=0.49 for trend)
Dalsager et al. 2016 General population (n=359 1–4-year-old children)	10.19–25.10 ng/mL (maternal 3rd tertile PFOS)	Risk of number of days above the median	
		Fever	**OR 2.35 (1.34–4.11)*, 3rd tertile**
		Cough	NS (p>0.05)
		Nasal discharge	NS (p>0.05)
		Diarrhea	NS (p>0.05)
		Vomiting	NS (p>0.05)

Table 2-16. Summary of Immunological Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
		Risk of number of days	
		Fever	**OR 1.65 (1.24–2.18)*, 3rd tertile**
		Cough	NS (p>0.05)
		Nasal discharge	NS (p>0.05)
		Diarrhea	NS (p>0.05)
		Vomiting	NS (p>0.05)
Dong et al. 2013 General population (n=231 asthmatic and 225 non-asthmatic children) This is the same group of children evaluated by Zhu et al. (2016)	45.5 and 33.4 ng/mL (mean serum PFOS levels in the asthmatic and non-asthmatic children, respectively; serum levels were not reported for full cohort)	Asthma diagnosis	**OR 2.63 (1.48–4.69)*, 4th quartile**
		Asthma severity	**Association (p=0.045 for trend)***
		IgE	**Association (p<0.05)*, asthmatics** NS (p>0.05), non-asthmatics
		Absolute eosinophil counts	**Association (p<0.05)*, asthmatics** NS (p>0.05), non-asthmatics
		Eosinophil cationic protein	**Association (p<0.05)*, asthmatics** NS (p>0.05), non-asthmatics
Fei et al. 2010 General population (n=1,400 pregnant women and young children)	35.3 ng/mL (maternal PFOS)	Risk of hospitalization for infectious disease in young children	IRR 1.00 (0.91–1.09) for trend **IRR 1.18 (1.03–1.36)* for trend, girls** IRR 0.90 (0.80–1.12) for trend, boys
Goudarzi et al. 2016a General population (n=1,558 4-year-old children)	5.456 ng/mL (mean maternal plasma PFOS)	Prevalence of allergic disease	OR 0.815 (0.596–1.11), 4th quartile
		Prevalence of wheezing	OR 0.770 (0.526–1.12), 4th quartile
Goudarzi et al. 2017 General population (n=1,558 mother-child pairs); children examined up to 4 years of age	5.456 ng/mL (mean maternal serum PFOS)	Risk of total infectious diseases	**OR 1.44 (1.06–1.96)*, 2nd quartile; p=0.008 for trend**
Grandjean et al. 2012; Mogensen et al. 2015a General population (n=456 and n=464 children 5 and 7 years of age)	17.3 and 15.5 ng/mL (median PFOS at age 5 and 7 years) 27.3 ng/mL (geometric mean maternal PFOS)	Tetanus antibody levels at age 5	NS, maternal PFOS **β -28.5% (-45.5 to -6.1)*, per 2-fold increase in PFOS levels at age 5**
		Tetanus antibody levels at age 7	NS, maternal PFOS NS, PFOS at ages 5 and 7

Table 2-16. Summary of Immunological Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
		Diphtheria antibody levels at age 5	NS, maternal PFOS NS, PFOS at age 5
		Diphtheria antibody levels at age 7	**β -27.6% (-45.8 to -3.3)*, per 2-fold increase in PFOS levels at age 5** **β -30.3% (47.3 to -7.8)*, per 2-fold increase in PFOS levels at age 7**
Grandjean et al. 2017 General population (n=516 children examined at age 7 and 13 years)	15.3 and 6.7 ng/mL (median at age 7 and 13 years)	Tetanus antibody levels at age 13	Full cohort NS (p=0.240), PFOS at age 7 NS (p=0.237), PFOS at age 13 Cohort restricted to children without possible unscheduled booster vaccines **Association (p=0.043)*, PFOS at age 7** NS (p=0.144), PFOS at age 13
		Diphtheria antibody levels at age 13	NS (p=0.07), age 7 NS (p=0.454), age 13
Granum et al. 2013 General population (n=56 children age 3 years)	5.6 ng/mL (mean maternal PFOS)	Rubella antibody levels	**Inverse association (p=0.007)***
		Hemophilus influenza type B antibody levels	NS (p>0.05)
		Tetanus antibody levels	NS (p>0.05)
		Asthma diagnosis	NS (p>0.05)
		Atopic eczema	NS (p>0.05)
		Eczema and itchiness	NS (p>0.05)
		Number of episodes of otitis media	NS (p>0.05)
		Number of episodes of common cold	NS (p=0.501)
		Number of episodes of gastroenteritis	NS (p=0.367)

Table 2-16. Summary of Immunological Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Humblet et al. 2014 General population (NHANES) (n=1,877 adolescents)	17.0 and 16.8 ng/mL (median PFOS in asthmatics and non-asthmatics)	Asthma episode in last 12 months	NS (p=0.13), per doubling PFOS
		Current asthma	NS (p=0.24)
		Wheezing	NS (p=0.08)
Impinen et al. 2018 General population (n=641 infants followed through age 10)	5.6 ng/mL (mean cord PFOS)	Number of common colds (0–2 years of age)	NS (p=0.173)
		Number of lower respiratory infections (0–10 years of age)	**β 0.50 (0.42–0.57; p=<0.0001)***
		Rhinitis	NS
		Rhinoconjunctivitis	NS
		Asthma diagnosis	NS
		Current asthma	NS
		Asthma ever	NS
		Allergic sensitization	NS
Kielsen et al. 2016 General population (n=12 adults)	9.52 ng/mL (median PFOS)	Diphtheria antibody levels	**Inverse association (p=0.044)*, unadjusted**
		Tetanus antibody levels	NS (p=0.420), unadjusted
Looker et al. 2014 Community (C8) (n=411)	8.32 ng/mL (geometric mean PFOS)	Response to influenza A H3N2 virus vaccine	NS (p>0.05)
		Response to influenza A H1N1 virus vaccine	NS (p>0.05)
		Response to influenza type B virus vaccine	NS (p>0.05)
		Cold or flu infection	NS (p>0.05)
		Frequency of colds	NS (p>0.05)

Table 2-16. Summary of Immunological Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Okada et al. 2012 General population (n=343 infants)	5.2 ng/mL (maternal median PFOS)	Cord IgE levels	NS (p>0.05)
		Infant food allergy	OR 3.72 (0.81–17.10)
		Eczema	OR 0.87 (0.15–5.08)
		Wheezing	OR 2.68 (0.39–18.30)
		Otitis media	OR 1.40 (0.33–6.00)
Okada et al. 2014 General population (n=2,603 infants)	5.56 ng/mL (maternal mean PFOS)	Risk of allergic diseases Males Females	 OR 0.95 (0.65–1.37), 4th quartile OR 0.79 (0.53–1.17), 4th quartile
		Eczema Males Females	 OR 0.98 (0.63–1.53), 4th quartile OR 0.84 (0.51–1.38), 4th quartile
Qin et al. 2017 General population (n=132 children aged 10–15 years and 168 matched controls)	31.51 and 28.83 ng/mL (median serum PFOS in cases and controls)	Asthma	**OR 1.30 (1.00–1.69)***
Smit et al. 2015 General population (n=1,024 children)	4.88 and 20.6 ng/mL (maternal mean PFOS in Ukraine and Greenland cohorts)	Ever having asthma	OR 0.86 (0.67–1.10), whole cohort
		Ever having eczema	OR 0.98 (0.88–1.18), whole cohort
		Current eczema	OR 1.05 (0.82–1.33), whole cohort
		Ever having wheezing	OR 0.83 (0.69–1.00), whole cohort
		Current wheezing	**OR 0.60 (0.38–0.92)*, Ukraine cohort** OR 0.91 (0.62–1.36) Greenland OR 0.76 (0.56–1.01), whole cohort
Stein et al. 2016a General population (NHANES) (n=1,191 adolescents)	20.8 ng/mL (geometric mean PFOS)	Measles antibody titers	NS (95% CI included unity)
		Mumps antibody titers	**β -7.4% (-12.8 to -1.7)*, per 2-fold increase in PFOS levels, whole cohort** **β -5.9% (-9.9 to -1.6)*, per 2-fold increase in PFOS levels, seropositive subcohort**
		Rubella antibody titers	NS (95% CI included unity), whole cohort **β -13.3% (-19.9–6.2)*, per 2-fold increase in PFOS levels, seropositive subcohort**

Table 2-16. Summary of Immunological Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Stein et al. 2016a General population (NHANES) (n=640 adolescents)	15.0 ng/mL (geometric mean)	Rhinitis	OR 1.16 (0.90–1.50)
		Current asthma	OR 1.20 (0.88–1.63)
		Wheeze	OR 0.76 (0.45–1.19)
		Allergy	OR 1.05 (0.80–1.37)
		Allergic sensitization	
		Plants	**OR 0.17 (0.53–0.97)***
		Dust mites	OR 1.00 (0.73–1.38)
		Pets	OR 0.83 (0.56–1.22)
		Cockroach or shrimp	**OR 0.67 (0.48–0.93)***
		Rodents	OR 0.85 (0.29–2.45)
		Mold	**OR 1.33 (1.06–1.69)***
		Food	OR 0.74 (0.39–1.40)
Stein et al. 2016b General population (n=78 adults receiving influenza vaccine)	5.22 ng/mL (geometric mean)	Seroconversion	
		Hemagglutinin	NS (p=0.81 for trend)
		Immunohistochemistry	NS (p=0.12 for trend)
		Serum cytokine levels	NS (p>0.05 for trend)
		Serum chemokine levels	NS (p>0.05 for trend)
		Nasal cytokine levels	NS (p>0.05 for trend)
		Nasal chemokine levels	NS (p>0.05 for trend)
		Serum IgA levels	NS (p>0.05 for trend)
Wang et al. 2011 General population (n=244 children aged 2 years)	5.50 ng/mL (median cord PFOS)	Serum IgE levels	NS (p=0.179)
		Cord blood IgE levels	Association (p=0.017)*
		Atopic dermatitis	NS (p>0.05)
Zhu et al. 2016 General population (n=231 asthmatic and 225 non-asthmatic children) This is the same group of children evaluated by Dong et al. (2013)	45.86 and 33.9 ng/mL (mean in asthmatics and non-asthmatics)	Asthma diagnosis	**OR 4.38 (2.02–9.50)*, males 4th quartile** NS (p=0.899 for trend), females
		T-helper cytokines	
		IL-4	NS (p>0.05 for trend)
		IL-5	NS (p>0.05 for trend)
		IFN-γ	NS (p>0.05 for trend)
		IL-2	NS (p>0.05 for trend)
		Serum IgE	NS (p>0.05 for trend)

Table 2-16. Summary of Immunological Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFHxS			
Ashley-Martin et al. 2015 General population (1,258 women)	NR	IL-33/TSLP (cord blood)	1.0 (0.7–1.4)
		IgE (cord blood)	OR 1.0 (0.7–1.4)
Buser and Scinicariello 2016 General population (NHANES) (n=637 and 701 adolescents in 2005–2006 and 2007–2010)	2.09 and 2.19 ng/mL (geometric mean 2005–2006 and 2007–2010) >4.00 ng/mL (4th quartile)	Food allergies	**OR 3.06 (1.35–6.93)*, 4th quartile** (trend not significant, p=0.11)
		Food sensitization	NS (p=0.72 for trend)
Dalsager et al. 2016 General population (n=359 1–4-year-old children)	0.32 ng/mL (maternal median PFHxS)	Risk of number of days above the median	
		Fever	NS (p>0.05)
		Cough	NS (p>0.05)
		Nasal discharge	NS (p>0.05)
		Diarrhea	NS (p>0.05)
		Vomiting	NS (p>0.05)
		Risk of number of days	
		Fever	NS (p>0.05)
		Cough	NS (p>0.05)
		Nasal discharge	NS (p>0.05)
		Diarrhea	NS (p>0.05)
		Vomiting	NS (p>0.05)
Dong et al. 2013 General population (n=231 asthmatic and 225 non-asthmatic children) This is the same group of children evaluated by Zhu et al. (2016)	3.9 and 2.1 ng/mL (mean serum PFHxS levels in the asthmatic and non-asthmatic children, respectively; serum levels were not reported for full cohort)	Asthma diagnosis	**OR 2.94 (1.65–5.25)*, 3rd quartile**
		Asthma severity	NS (p=0.722 for trend)
		IgE	NS (p>0.05), asthmatics NS (p>0.05), non-asthmatics
		Absolute eosinophil counts	**Association (p<0.05)*, asthmatics** NS (p>0.05), non-asthmatics
		Eosinophil cationic protein	**Association (p<0.05)*, asthmatics** NS (p>0.05), non-asthmatics

Table 2-16. Summary of Immunological Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Goudarzi et al. 2016a General population (n=1,558 4-year-old children)	0.322 ng/mL (mean maternal plasma PFHxS)	Prevalence of allergic disease	OR 0.841 (0.615–1.151), 4th quartile
		Prevalence of wheezing	OR 0.728 (0.497–1.06), 4th quartile
Goudarzi et al. 2017 General population (n=1,558 mother-child pairs); children examined up to 4 years of age	0.322 ng/mL (mean maternal serum PFHxS)	Risk of total infectious diseases	OR 0.957 (0.7.3–1.41), 4th quartile, p=0.928 for trend Females only: **OR 1.81 (1.14–2.88)*, 3rd quartile; p=0.045 for trend** Males only: p=0223 for trend
Grandjean et al. 2012; Mogensen et al. 2015a General population (n=456 and n=464 children 5 and 7 years of age)	0.6 and 0.5 ng/mL (median PFHxS at age 5 and 7 years) 4.41 ng/mL (geometric mean maternal PFHxS)	Tetanus antibody levels at age 5	NS, maternal PFHxS **-19.0% (-29.8 to -6.6)*, per 2-fold increase in PFHxS levels at age 5**
		Tetanus antibody levels at age 7	NS, maternal PFHxS **β -19.7% (-31.6 to -5.7)*, per 2-fold increase PFHxS levels at age 5** **β -22.3% (-36.3 to -5.2)*, per 2-fold increase PFHxS levels at age 7**
		Diphtheria antibody levels at age 5	NS, maternal PFHxS NS, PFHxS at age 5
		Diphtheria antibody levels at age 7	NS, maternal PFHxS NS, PFHxS at age 5 or 7
Grandjean et al. 2017 General population (n=516 children examined at age 7 and 13 years)	0.5 and 0.4 ng/mL (median PFHxS at age 7 and 13 years)	Tetanus antibody levels at age 13	NS (p=0.334), PFHxS at age 7 NS (p=0.568), PFHxS at age 13
		Diphtheria antibody levels at age 13	NS (p=0.264), PFHxS at age 7 NS (p=0.583), PFHxS at age 13

Table 2-16. Summary of Immunological Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Granum et al. 2013 General population (n=56 children age 3 years)	0.3 ng/mL (mean maternal PFHxS levels)	Rubella antibody levels	**Inverse association (p=0.008)***
		Hemophilus influenza type B antibody levels	NS (p>0.05)
		Tetanus antibody levels	NS (p>0.05)
		Asthma diagnosis	NS (p>0.05)
		Atopic eczema	NS (p>0.05)
		Eczema and itchiness	NS (p>0.05)
		Number of episodes of otitis media	NS (p>0.05)
		Number of episodes of common cold	NS (p=0.078)
		Number of episodes of gastroenteritis	**Association (p=0.007)***
Humblet et al. 2014 General population (NHANES) (n=1,877 adolescents)	2.2 and 2.0 ng/mL (median PFHxS in asthmatics and nonasthmatics)	Asthma episode in last 12 months	NS (p=0.66), per doubling PFHxS
		Current asthma	NS (p=0.99)
		Wheezing	NS (p=0.92)
Impinen et al. 2018 General population (n=641 infants followed through age 10)	0.3 ng/mL (mean cord PFHxS)	Number of common colds (0–2 years of age)	NS (p=0.530)
		Number of lower respiratory infections (0–10 years of age)	NS (p=0.119)
		Rhinitis	NS
		Rhinoconjunctivitis	NS
		Asthma diagnosis	NS
		Current asthma	NS
		Asthma ever	NS
		Allergic sensitization	NS

Table 2-16. Summary of Immunological Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Kielsen et al. 2016 General population (n=12 adults)	0.37 ng/mL (median PFHxS)	Diphtheria antibody levels	NS (p=0.055), unadjusted
		Tetanus antibody levels	NS (p=0.390), unadjusted
Okada et al. 2014 General population (n=2,603 infants)	0.324 ng/mL (maternal mean PFHxS)	Risk of allergic diseases Males Females	 OR 0.81 (0.56–1.16), 4th quartile OR 1.13 (0.75–1.69), 4th quartile
		Eczema Males Females	 OR 0.78 (0.51–1.19), 4th quartile OR 0.82 (0.49–1.36), 4th quartile
Qin et al. 2017 General population (n=132 children aged 10–15 years and 168 matched controls)	2.38 and 1.07 ng/mL (median serum PFHxS in cases and controls)	Asthma	**OR 2.14 (1.48–3.11)***
Smit et al. 2015 General population (n=1,024 children)	1.53 and 2.14 ng/mL (maternal mean PFHxS in Ukraine and Greenland cohorts)	Ever having asthma	OR 0.91 (0.69–1.18), whole cohort
		Ever having eczema	OR 1.03 (0.86–1.24), whole cohort
		Current eczema	OR 0.93 (0.73–1.20), whole cohort
		Ever having wheezing	OR 0.96 (0.79–1.17), whole cohort
		Current wheezing	OR 0.93 (0.68–1.27), whole cohort
Stein et al. 2016a General population (NHANES) (n=1,191 adolescents)	2.47 ng/mL (geometric mean PFHxS)	Measles antibody titers	NS (95% CI included unity)
		Mumps antibody titers	NS (95% CI included unity)
		Rubella antibody titers	NS (95% CI included unity), whole cohort **β -6.0% (-9.6 to -2.2)*, per 2-fold increase PFHxS levels, seropositive subcohort**

Table 2-16. Summary of Immunological Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Stein et al. 2016a General population (NHANES) (n=640 adolescents)	2.09 ng/mL (geometric mean PFHxS)	Rhinitis	OR 0.81 (0.57–1.16)
		Current asthma	OR 0.98 (0.51–1.87)
		Wheeze	OR 0.99 (0.68–1.44)
		Allergy	OR 0.83 (0.59–1.17)
		Allergic sensitization	
		Plants	OR 0.93 (0.62–1.39)
		Dust mites	OR 1.01 (0.84–1.22)
		Pets	OR 0.96 (0.71–1.30)
		Cockroach or shrimp	OR 0.72 (0.56–0.93)
		Rodents	OR 0.81 (0.54–1.21)
		Mold	OR 0.98 (0.65–1.47)
		Food	OR 1.03 (0.74–1.42)
Stein et al. 2016b General population (n=78 adults receiving influenza vaccine)	1.1 ng/mL (geometric mean PFHxS)	Seroconversion	
		Hemagglutinin	NS (p=0.22 for trend)
		Immunohistochem.	NS (p=0.34 for trend)
		Serum cytokine levels	
		IFN-γ	**Association (p=0.05 for trend)***
		IFN-α2	NS (p=0.09 for trend)
		TNF-α	**Association (p=0.04 for trend)***
		IP-10	NS (p=0.59 for trend)
		Serum chemokine levels	NS (p>0.05 for trend)
		Nasal cytokine levels	NS (p>0.05 for trend)
		Nasal chemokine levels	NS (p>0.05 for trend)
		Serum IgA levels	NS (p>0.05 for trend)

Table 2-16. Summary of Immunological Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Zhu et al. 2016 General population (n=231 asthmatic and 225 non-asthmatic children) This is the same group of children evaluated by Dong et al. (2013)	3.86 and 2.10 ng/mL (mean PFHxS in asthmatics and non-asthmatics)	Asthma diagnosis	**OR 2.97 (1.33–6.64)*, males 4th quartile** **OR 5.02 (2.05–12.30)*, females 4th quartile**
		T-helper cytokines	
		IL-4	NS (p>0.05 for trend)
		IL-5	NS (p>0.05 for trend)
		IFN-γ	NS (p>0.05 for trend)
		IL-2	NS (p>0.05 for trend)
		Serum IgE	NS (p>0.05 for trend)
PFNA			
Buser and Scinicariello 2016 General population (NHANES) (n=637 and 701 adolescents in 2005–2006 and 2007–2010)	0.93 and 1.13 ng/mL (geometric mean PFNA 2005–2006 and 2007–2010) >1.36 ng/mL (4th quartile)	Food allergies	NS (p=0.28 for trend)
		Food sensitization	**OR 0.51 (0.28–0.92)*, 4th quartile (trend not significant, p=0.15)**
Dalsager et al. 2016 General population (n=359 1–4-year-old children)	0.56–0.81 and 0.82–3.64 ng/mL (maternal 2nd and 3rd tertile PFNA)	Risk of number of days above the median	
		Fever	NS (p>0.05)
		Cough	NS (p>0.05)
		Nasal discharge	**OR 0.53 (0.31–0.92)*, 2nd tertile**
		Diarrhea	NS (p>0.05)
		Vomiting	NS (p>0.05)
		Risk of number of days	
		Fever	NS (p>0.05)
		Cough	NS (p>0.05)
		Nasal discharge	OR 1.12 (0.84–1.49), 3rd tertile
		Diarrhea	NS (p>0.05)
		Vomiting	NS (p>0.05)

Table 2-16. Summary of Immunological Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Dong et al. 2013 General population (n=231 asthmatic and 225 non-asthmatic children) This is the same group of children evaluated by Zhu et al. (2016)	1.1 and 0.9 ng/mL (mean serum PFNA levels in the asthmatic and non-asthmatic children, respectively; serum levels were not reported for full cohort)	Asthma diagnosis	**OR 2.56 (1.41–4.65)*, 4th quartile**
		Asthma severity	NS (p=0.217 for trend)
		IgE	**Association (p<0.05)*, asthmatics** NS (p>0.05), non-asthmatics
		Absolute eosinophil counts	**Association (p<0.05)*, asthmatics** NS (p>0.05), non-asthmatics
		Eosinophil cationic protein	**Association (p<0.05)*, asthmatics** NS (p>0.05), non-asthmatics
Goudarzi et al. 2016a General population (n=1,558 4-year-old children)	1.402 ng/mL (mean maternal plasma PFNA)	Prevalence of allergic disease	OR 0.873 (0.562–1.35), 4th quartile
		Prevalence of wheezing	OR 1.11 (0.760–1.63), 4th quartile
Goudarzi et al. 2017 General population (n=1,558 mother-child pairs); children examined up to 4 years of age	1.402 ng/mL (mean maternal serum PFNA)	Risk of total infectious diseases	OR 0.918 (0.672–1.25), 4th quartile, p=0.748 for trend
Grandjean et al. 2012 General population (n=456 and n=464 children 5 and 7 years of age)	1.00 ng/mL (geometric mean PFNA at age 5 years) 0.60 ng/mL (geometric mean PFNA at age 7 years)	Tetanus antibody levels at age 5	NS, maternal PFNA NS, PFNA at age 5
		Tetanus antibody levels at age 7	NS, maternal PFNA NS, PFNA at age 5
		Diphtheria antibody levels at age 5	NS, maternal PFNA **β -16.1% (-28.8 to -1.0)*, per 2-fold increase PFNA levels at age 5**
		Diphtheria antibody levels at age 7	NS, maternal PFNA NS, PFNA at age 5
Grandjean et al. 2017 General population (n=516 children examined at age 7 and 13 years)	1.1 and 0.7 ng/mL (median PFNA at age 7 and 13 years)	Tetanus antibody levels at age 13	NS (p=0.075), PFNA at age 7 NS (p=0.394), PFNA at age 13
		Diphtheria antibody levels at age 13	NS (p=0.243), PFNA at age 7 NS (p=0.693), PFNA at age 13

Table 2-16. Summary of Immunological Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Granum et al. 2013 General population (n=56 children age 3 years)	0.3 ng/mL (mean maternal PFNA levels)	Rubella antibody levels	**Inverse association (p=0.007)***
		Hemophilus influenza type B antibody levels	NS (p>0.05)
		Tetanus antibody levels	NS (p>0.05)
		Asthma diagnosis	NS (p>0.05)
		Atopic eczema	NS (p>0.05)
		Eczema and itchiness	NS (p>0.05)
		Number of episodes of otitis media	NS (p>0.05)
		Number of episodes of common cold	**Association (p=0.035)***
		Number of episodes of gastroenteritis	NS (p=0.883)
Humblet et al. 2014 General population (NHANES) (n=1,877 adolescents)	0.9 and 0.8 ng/mL (median PFNA in asthmatics and nonasthmatics)	Asthma episode in last 12 months	NS (p=0.92), per doubling PFNA
		Current asthma	NS (p=0.97)
		Wheezing	NS (p=0.94)
Impinen et al. 2018 General population (n=641 infants followed through age 10)	0.2 ng/mL (mean cord PFNA)	Number of common colds (0–2 years of age)	NS (p=0.983)
		Number of lower respiratory infections (0–10 years of age)	**β 0.0.09 (0.03–0.14;p=0.001)***
		Rhinitis	NS
		Rhinoconjuctivitis	NS
		Asthma diagnosis	NS
		Current asthma	NS
		Asthma ever	NS
		Allergic sensitization	NS

Table 2-16. Summary of Immunological Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Kielsen et al. 2016 General population (n=12 adults)	0.66 ng/mL (median PFNA)	Diphtheria antibody levels	**Inverse association (p=0.004)*, unadjusted**
		Tetanus antibody levels	NS (p=0.250), unadjusted
Okada et al. 2014 General population (n=2,603 infants)	1.36 ng/mL (maternal mean PFNA)	Risk of allergic diseases Males Females	 OR 0.95 (0.66–1.38), 4th quartile **OR 0.55 (0.36–0.82)*, 4th quartile**
		Eczema Males Females	 OR 0.96 (0.61–1.52), 4th quartile OR 0.63 (0.38–1.02), 4th quartile
Qin et al. 2017 General population (n=132 children aged 10–15 years and 168 matched controls)	2.00 and 0.80 ng/mL (median serum PFNA in cases and controls)	Asthma	**OR 1.61 (1.12–2.31)***
Smit et al. 2015 General population (n=1,024 children)	0.62 and 0.73 ng/mL (maternal mean PFNA in Ukraine and Greenland cohorts)	Ever having asthma	OR 0.90 (0.70–1.14), whole cohort
		Ever having eczema	OR 0.94 (0.78–1.14), whole cohort
		Current eczema	OR 1.03 (0.82–1.30), whole cohort
		Ever having wheezing	OR 0.91 (0.75–1.09), whole cohort
		Current wheezing	OR 0.90 (0.66–1.23), whole cohort
Stein et al. 2016a General population (NHANES) (n=1,191 adolescents)	0.765 ng/mL (geometric mean)	Measles antibody titers	NS (95% CI included unity)
		Mumps antibody titers	NS (95% CI included unity)
		Rubella antibody titers	NS (95% CI included unity)

Table 2-16. Summary of Immunological Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Stein et al. 2016a General population (NHANES) (n=640 adolescents)	0.929 ng/mL (geometric mean)	Rhinitis	OR 1.24 (0.97–1.60)
		Current asthma	OR 1.26 (0.79–2.01)
		Wheeze	OR 0.99 (0.58–1.68)
		Allergy	OR 1.12 (0.85–1.47)
		Allergic sensitization	
		Plants	OR 0.96 (0.74–1.23)
		Dust mites	OR 1.05 (0.78–1.41)
		Pets	OR 1.26 (0.85–1.87)
		Cockroach or shrimp	OR 0.86 (0.60–1.24)
		Rodents	OR 2.25 (0.83–6.10)
		Mold	OR 1.31 (0.83–2.06)
		Food	OR 0.91 (0.55–1.50)
Stein et al. 2016b General pop. (n=78 adults receiving influenza vaccine)	0.77 ng/mL (geometric mean PFNA)	Seroconversion	
		Hemagglutinin	NS (p=0.33 for trend)
		Immunohistochem.	NS (p=0.40 for trend)
		Serum cytokine levels	NS (p>0.05 for trend)
		Serum chemokine levels	NS (p>0.05 for trend)
		Nasal cytokine levels	NS (p>0.05 for trend)
		Nasal chemokine levels	NS (p>0.05 for trend)
		Serum IgA levels	NS (p>0.05 for trend)
Wang et al. 2011 General population (n=244 children aged 2 years)	2.30 ng/mL (median cord PFNA)	Serum IgE levels	NS (p=0.837)
		Cord blood IgE levels	NS (p=0.908)
		Atopic dermatitis	NS (p>0.05)
Zhu et al. 2016 General population (n=231 asthmatic and 225 non-asthmatic children) This is the same group of children evaluated by Dong et al. (2013)	1.07 and 0.87 ng/mL (mean PFNA in asthmatics and non-asthmatics)	Asthma diagnosis	**OR 3.33 (1.46–7.58)*, males 4th quartile** NS (p=0.142 for trend), females
		T-helper cytokines	
		IL-4	**Association (p=0.031 for trend)***
		IL-5	**Association (p=0.011 for trend)***
		IFN-γ	NS (p>0.05 for trend)
		IL-2	NS (p>0.05 for trend)
		Serum IgE	**Association (p=0.008 for trend)***

Table 2-16. Summary of Immunological Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFDA			
Dalsager et al. 2016 General population (n=359 children aged 1–4 years)	0.27 ng/mL (median maternal serum PFDA level)	Symptoms of infection	NS (p>0.05)
Dong et al. 2013 General population (n=231 asthmatic and 225 non-asthmatic children) This is the same group of children evaluated by Zhu et al. (2016)	1.2 and 1.0 ng/mL (mean serum PFDA levels in the asthmatic and non-asthmatic children, respectively; serum levels were not reported for full cohort)	Asthma diagnosis	**OR 3.22 (1.75–5.94)*, 4th quartile**
		Asthma severity	**Association (p=0.005 for trend)***
		IgE	**Association (p<0.05)*, asthmatics** NS (p>0.05), non-asthmatics
		Absolute eosinophil counts	**Association (p<0.05)*, asthmatics** NS (p>0.05), non-asthmatics
		Eosinophil cationic protein	**Association (p<0.05)*, asthmatics** **Association (p<0.05)*, non-asthmatics**
Goudarzi et al. 2016a General population (n=1,558 4-year-old children)	0.575 ng/mL (mean maternal plasma PFDA)	Prevalence of allergic disease	OR 0.906 (0.663–1.23), 4th quartile
		Prevalence of wheezing	OR 0.879 (0.602–1.28), 4th quartile
Goudarzi et al. 2017 General population (n=1,558 mother-child pairs); children examined up to 4 years of age	0.575 ng/mL (mean maternal serum PFDA)	Risk of total infectious diseases	OR 0.799 (0.588–1.08), 4th quartile, p=0.114 for trend
Grandjean et al. 2012 General population (n=456 and n=464 children 5 and 7 years of age)	0.28 ng/mL (geometric mean PFDA at age 5 years) 0.28 ng/mL (geometric mean maternal PFDA)	Tetanus antibody levels at age 5	NS, maternal PFDA **β -19.9% (-33.1 to -3.9), per 2-fold increase PFDA levels at age 5**
		Tetanus antibody levels at age 7	NS, maternal PFDA **β -22.3 (-35.8 to -5.8), per 2-fold increase PFDA levels at age 5**
		Diphtheria antibody levels at age 5	NS, maternal PFDA NS, PFDA at age 5
		Diphtheria antibody levels at age 7	NS, maternal PFDA NS, PFDA at age 5

Table 2-16. Summary of Immunological Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Grandjean et al. 2017 General population (n=516 children examined at age 7 and 13 years)	0.4 and 0.3 ng/mL (median at age 7 and 13 years)	Tetanus antibody levels at age 13	**Association (p=0.022)*, PFDA at age 7** NS (p=0.258), PFDA at age 13
		Diphtheria antibody levels at age 13	**Association (p=0.008)*, PFDA at age 7** NS (p=0.726), PFDA at age 13
Kielsen et al. 2016 General population (n=12 adults)	0.30 ng/mL (median PFDA)	Diphtheria antibody levels	**Inverse association (p=0.009)*, unadjusted**
		Tetanus antibody levels	NS (p=0.130), unadjusted
Okada et al. 2014 General population (n=2,603 infants)	0.563 ng/mL (maternal mean PFDA)	Risk of allergic diseases Males Females	 OR 1.13 (0.78–1.64), 4th quartile OR 0.70 (0.47–1.04), 4th quartile
		Eczema Males Females	 OR 0.93 (0.60–1.44), 4th quartile OR 0.78 (0.49–1.25), 4th quartile
Qin et al. 2017 General population (n=132 children aged 10–15 years and 168 matched controls)	1.13 and 0.93 ng/mL (median serum PFDA in cases and controls)	Asthma	OR 1.24 (0.97–1.58)
Smit et al. 2015 General population (n=1,024 children)	0.16 and 0.42 ng/mL (maternal mean PFDA in Ukraine and Greenland cohorts)	Ever having asthma	OR 0.92 (0.73–1.16), whole cohort
		Ever having eczema	OR 0.88 (0.73–1.06), whole cohort
		Current eczema	OR 0.95 (0.75–1.20), whole cohort
		Ever having wheezing	OR 0.85 (0.70–1.01), whole cohort
		Current wheezing	OR 0.76 (0.56–1.04), whole cohort
Zhu et al. 2016 General population (n=231 asthmatic and 225 non-asthmatic children) This is the same group of children evaluated by Dong et al. (2013)	1.24 and 1.02 ng/mL (mean in asthmatics and non-asthmatics)	Asthma diagnosis	**OR 3.45 (1.51–7.88)*, males 4th quartile** **OR 3.68 (1.43–9.48)*, females 4th quartile**
		T-helper cytokines IL-4 IL-5 IFN-γ IL-2	 NS (p>0.05 for trend) NS (p>0.05 for trend) NS (p>0.05 for trend) NS (p>0.05 for trend)
		Serum IgE	**Association (p=0.002 for trend)***

Table 2-16. Summary of Immunological Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFUnA			
Goudarzi et al. 2016a General population (n=1,558 4-year-old children)	1.534 ng/mL (mean maternal plasma PFUnA)	Prevalence of allergic disease	OR 0.736 (0.538–1.00), 4th quartile
		Prevalence of wheezing	OR 1.04 (0.714–1.51), 4th quartile
Goudarzi et al. 2017 General population (n=1,558 mother-child pairs); children examined up to 4 years of age	1.534 ng/mL (mean maternal serum PFUnA)	Risk of total infectious diseases	OR 1.03 (0.764–1.40), 4th quartile, p=0.786 for trend
Impinen et al. 2018 General population (n=641 infants followed through age 10)	0.1 ng/mL (mean cord PFUnNA)	Number of common colds (0–2 years of age)	**β 0.11 (0.08–0.14; p=<0.0001)***
		Number of lower respiratory infections (0–10 years of age)	**β 0.18 (0.13–0.23; p=<0.0001)***
		Rhinitis	NS
		Rhinoconjunctivitis	NS
		Asthma diagnosis	NS
		Current asthma	NS
		Asthma ever	NS
		Allergic sensitization	NS
Kielsen et al. 2016 General population (n=12 adults)	0.21 ng/mL (median PFUnA)	Diphtheria antibody levels	**Inverse association (p=0.036)*, unadjusted**
		Tetanus antibody levels	**Inverse association (p=0.039)*, unadjusted**
Okada et al. 2014 General population (n=2,603 infants)	1.50 ng/mL (maternal mean PFUnA)	Risk of allergic diseases Males Females	 OR 1.13 (0.79–1.63), 4th quartile **OR 0.58 (0.39–0.86)*, 4th quartile**
		Eczema Males Females	 OR 1.16 (0.75–10.81), 4th quartile **OR 0.50 (0.30–0.81)*, 4th quartile**

Table 2-16. Summary of Immunological Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Smit et al. 2015 General population (n=1,024 children)	0.16 and 0.68 ng/mL (maternal mean PFUnA in Ukraine and Greenland cohorts)	Ever having asthma	OR 0.96 (0.77–1.21), whole cohort
		Ever having eczema	OR 0.95 (0.79–1.15), whole cohort
		Current eczema	OR 1.07 (0.85–1.34), whole cohort
		Ever having wheezing	OR 0.84 (0.70–1.00), whole cohort
		Current wheezing	OR 0.87 (0.65–1.17), whole cohort
PFHpA			
Kielsen et al. 2016 General population (n=12 adults)	0.12 ng/mL (median PFHpA)	Diphtheria antibody levels	NS (p=0.750), unadjusted
		Tetanus antibody levels	NS (p=0.280), unadjusted
Smit et al. 2015 General population (n=1,024 children)	0.03 and 0.05 ng/mL (maternal mean PFHpA in Ukraine and Greenland cohorts)	Ever having asthma	OR 0.93 (0.71–1.22), whole cohort
		Ever having eczema	OR 0.93 (0.78–1.11), whole cohort
		Current eczema	OR 0.90 (0.70–1.15), whole cohort
		Ever having wheezing	OR 1.03 (0.84–1.25), whole cohort
		Current wheezing	**OR 0.62 (0.40–0.97)*, Ukraine cohort** OR 1.24 (0.79–1.93), Greenland cohort OR 0.88 (0.64–1.20), whole cohort
PFBS			
Dong et al. 2013 General population (n=231 asthmatic and 225 non-asthmatic children) This is the same group of children evaluated by Zhu et al. (2016)	0.5 and 0.5 ng/mL (mean serum PFBS levels in the asthmatic and non-asthmatic children, respectively; serum levels were not reported for full cohort)	Asthma diagnosis	**OR 1.90 (1.08–3.37)*, 4th quartile**
		Asthma severity	NS (p=0.092 for trend)
		IgE	NS (p>0.05), asthmatics NS (p>0.05), non-asthmatics
		Absolute eosinophil counts	**Association (p<0.05)*, asthmatics** NS (p>0.05), non-asthmatics
		Eosinophil cationic protein	NS (p>0.05), asthmatics NS (p>0.05), non-asthmatics
Qin et al. 2017 General population (n=132 children aged 10–15 years and 168 matched controls)	0.48 and 0.48 ng/mL (median serum PFBS in cases and controls)	Asthma	OR 1.06 (0.93–1.20)

Table 2-16. Summary of Immunological Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Zhu et al. 2016 General population (n=231 asthmatic and 225 non-asthmatic children) This is the same group of children evaluated by Dong et al. (2013)	0.53 and 0.48 ng/mL (mean serum PFBS in asthmatics and non-asthmatics)	Asthma diagnosis	**OR 2.59 (1.14–5.87)*, males 4th quartile** NS (p=0.505 for trend), females
		T-helper cytokines IL-4 IL-5 IFN-γ IL-2	 NS (p>0.05 for trend) **Association (p=0.023 for trend)*** NS (p>0.05 for trend) NS (p>0.05 for trend)
		Serum IgE	NS (p>0.05 for trend)
PFDoDA			
Dong et al. 2013 General population (n=231 asthmatic and 225 non-asthmatic children)	5.8 and 4.5 ng/mL (mean serum PFDoDA levels in the asthmatic and non-asthmatic children, respectively; serum levels were not reported for full cohort)	Asthma diagnosis	**OR 1.81 (1.02–3.23)*, 4th quartile**
		Asthma severity	**Association (p=0.024 for trend)***
		IgE	**Association (p<0.05)*, asthmatics** NS (p>0.05), non-asthmatics
		Absolute eosinophil counts	**Association (p<0.05)*, asthmatics** NS (p>0.05), non-asthmatics
		Eosinophil cationic protein	**Association (p<0.05)*, asthmatics** Association (p<0.05)*, non-asthmatics
Goudarzi et al. 2016a General population (n=1,558 4-year-old children)	0.191 ng/mL (mean maternal plasma PFDoDA)	Prevalence of allergic disease	**OR 0.621 (0.454–0.847)*, 4th quartile**
		Prevalence of wheezing	OR 0.999 (0.684–1.45), 4th quartile
Goudarzi et al. 2017 General population (n=1,558 mother-child pairs); children examined up to 4 years of age	0.191 ng/mL (mean maternal serum PFDoDA)	Risk of total infectious diseases	OR 1.07 (0.790–1.46), 4th quartile, p=0.502 for trend
Kielsen et al. 2016 General population (n=12 adults)	0.039 ng/mL (median PFDoDA)	Diphtheria antibody levels	**Inverse association (p=0.038)*, unadjusted**
		Tetanus antibody levels	**Inverse association (p=0.038)*, unadjusted**

Table 2-16. Summary of Immunological Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Okada et al. 2014 General population (n=2,603 infants)	0.188 ng/mL (maternal mean PFDoDA)	Risk of allergic diseases Males Females	 OR 0.93 (0.65–1.34), 4th quartile OR 0.58 (0.39–0.85)*, 4th quartile
		Eczema Males Females	 OR 1.00 (0.64–1.55), 4th quartile OR 0.73 (0.45–1.18), 4th quartile
Smit et al. 2015 General population (n=1,024 children)	0.04 and 0.13 ng/mL (maternal mean PFDoDA in Ukraine and Greenland cohorts)	Ever having asthma	OR 1.03 (0.81–1.30), whole cohort
		Ever having eczema	OR 0.90 (0.75–1.08), whole cohort
		Current eczema	OR 0.88 (0.70–1.14), whole cohort
		Ever having wheezing	OR 0.97 (0.80–1.16), whole cohort
		Current wheezing	OR 0.87 (0.64–1.18), whole cohort
PFHxA			
Dong et al. 2013 General population (n=231 asthmatic and 225 non-asthmatic children)	0.3 and 0.2 ng/mL (mean serum PFHxA levels in the asthmatic and non-asthmatic children, respectively; serum levels were not reported for full cohort)	Asthma diagnosis	OR 1.60 (0.90–2.86), 4th quartile
		Asthma severity	NS (p=0.854)
		IgE	NS (p>0.05), asthmatics NS (p>0.05), non-asthmatics
		Absolute eosinophil counts	NS (p>0.05), asthmatics NS (p>0.05), non-asthmatics
		Eosinophil cationic protein	NS (p>0.05), asthmatics NS (p>0.05), non-asthmatics
Qin et al. 2017 General population (n=132 children aged 10–15 years and 168 matched controls)	0.20 and 0.18 ng/mL (median serum PFHxA in cases and controls)	Asthma	OR 0.99 (0.80–1.21)

Table 2-16. Summary of Immunological Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
FOSA			
Impinen et al. 2018 General population (n=641 infants followed through age 10)	0.4 ng/mL (mean cord FOSA)	Number of common colds (0–2 years of age)	NS (p=0.477)
		Number of lower respiratory infections (0–10 years of age)	**β 0.10 (0.06–0.14; p=<0.0001)***
		Rhinitis	NS
		Rhinoconjunctivitis	NS
		Asthma diagnosis	NS
		Current asthma	NS
		Asthma ever	NS
		Allergic sensitization	NS

[a]See the *Supporting Document for Epidemiological Studies for Perfluoroalkyls,* Table 10 for more detailed descriptions of studies.
[b]Participants in occupational exposure may have also lived near the site and received residential exposure; community exposure studies involved subjects living near PFOA facilities with known exposure to high levels of PFOA.
[c]Asterisk and bold indicates association with perfluoroalkyl; unless otherwise specified, values in parenthesis are 95% confidence intervals.

CI = confidence interval; IFN-α-2 = interferon-α2; IFN-γ = interferon-γ; IgA = immunoglobulin A; IgE = immunoglobulin E; IP-10 = interferon-γ-inducible protein 10; IRR= incidence risk ratio; NHANES = National Health and Nutrition Examination Survey; NR = not reported; NS = not significant; OR = odds ratio; PFBS = perfluorobutane sulfonic acid; PFDA = perfluorodecanoic acid; PFDoDA = perfluorododecanoic acid; PFHpA = perfluoroheptanoic acid; PFHxS = perfluorohexane sulfonic acid; PFNA = perfluorononanoic acid; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid; PFUnA = perfluoroundecanoic acid; RR= relative risk; SPR = standard prevalence ratio; TNF-α =tumor necrosis factor-α

autoimmunity; epidemiological studies provide suggestive evidence of an association between serum PFOA and the risk of ulcerative colitis. The small number of studies investigating immunotoxicity following exposure to PFHpA and PFHxA did not find associations.

Laboratory animal studies have also evaluated immunosuppression (disease resistance, antibody response, NK cell activity, delayed-type hypersensitivity response, monocyte phagocytosis), hypersensitivity (airway resistance, local lymph node assay), and autoimmunity. In addition, laboratory animal studies have examined secondary outcomes (lymphoid organ weights, lymphocyte counts or subpopulations, lymphocyte proliferation, cytokine levels, serum antibody levels, histological alterations in immune organs). Summaries of the laboratory animal studies are presented in the LSE tables for PFOA, PFOS, and other perfluoroalkyls (Tables 2-3, 2-4, 2-5, and 2-6); the NOAEL and LOAEL values are presented in Figures 2-8, 2-9, and 2-10. No laboratory animal studies were identified for PFUnA, PFHpA, PFDoDA, or FOSA. Studies in laboratory animals identify the immune system as a sensitive target of toxicity following exposure to PFOA and PFOS. The observed effects include impaired responses to T-dependent antigens, impaired response to infectious disease, and secondary outcomes (decreases in spleen and thymus weights and in the number of thymic and splenic lymphocytes). A small number of studies evaluated the immunotoxicity of other perfluoroalkyls and most did not evaluate immune function. No alterations in spleen or thymus organ weights or morphology were observed in studies on PFHxS, PFBA, and PFDA. A study on PFNA found decreases in spleen and thymus weights and alterations in splenic lymphocyte phenotypes.

The National Toxicology Program (NTP 2016b) concluded that exposure to PFOA or PFOS is presumed to be an immune hazard to humans based on a high level of evidence that PFOA and PFOS suppressed the antibody response from animals and a moderate level of evidence from studies in humans. It was noted that the strongest evidence is for suppression of the antibody response and increased hypersensitivity (PFOA only).

PFOA

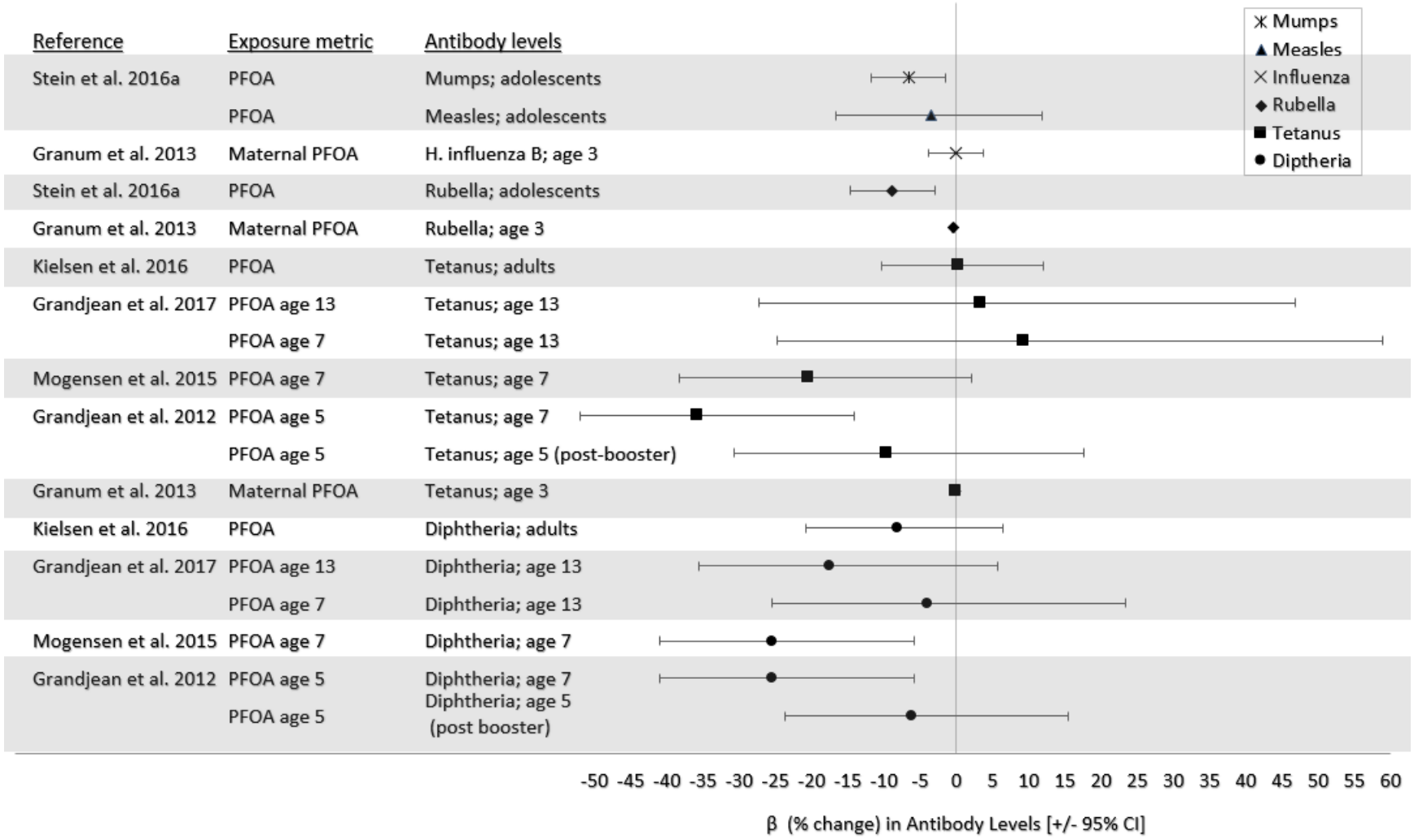
Epidemiological Studies—Immunosuppression Outcomes. Studies evaluating the immunosuppressive effects of PFOA have examined disease resistance and antibody responses. One study found associations between maternal serum PFOA and the number of episodes of the common cold and other respiratory tract infections and the number of episodes of gastroenteritis with vomiting or diarrhea in 3-year-old children (Granum et al. 2013). Another study found an association between maternal PFOA and the risk

of having a greater number of days with a fever greater than the median (Dalsager et al. 2016), although there was no increase in the number of days with a fever. A third study found an increased risk of lower respiratory tract infections associated cord PFOA from birth to 10 years of age (Impinen et al. 2018). However, other studies have not found associations between PFOA levels and the frequency of the common cold or flu in adults (Looker et al. 2014), between maternal PFOA levels and otitis media in 1.5–3-year-old children (Granum et al. 2013; Okada et al. 2012), between maternal PFOA and the risk of hospitalization for infectious diseases in young children (Fei et al. 2010), between maternal PFOA and the risk of number of days with cough, nasal discharge, diarrhea, or vomiting (Dalsager et al. 2016), between cord PFOA and number of common colds (Impinen et al. 2018), or between maternal serum PFOA and total number of infectious diseases between birth and 2 years of age (Goudarzi et al. 2017).

Several studies have evaluated the antibody response to vaccination in adults and children; the changes in the response to antibody levels relative to serum PFOA levels are graphically presented in Figure 2-21; the figure does not include data from other studies that used different statistical methods. In adults, decreases in antibody response against influenza A H3N2 virus were associated with increasing serum PFOA levels; however, there were no associations with two other strains of influenza virus (influenza A H1N1 and influenza B) (Looker et al. 2014). Another study of adults also did not find an altered immune response to influenza A H1N1 virus (Stein et al. 2016b). A small-scale study of 12 adults did not find significant alterations in the response to diphtheria or tetanus booster vaccines associated with serum PFOA levels (Kielsen et al. 2016). Increasing current serum PFOA levels were associated with lower antibody levels for mumps and rubella, but not for measles, in a cross-sectional study of adolescents (Stein et al. 2016a). A series of prospective studies by Grandjean and associates (Grandjean et al. 2012, 2017; Mogensen et al. 2015a) evaluated tetanus and diphtheria antibody levels in children at 5, 7, and 13 years of age. Diphtheria antibody levels at age 7 and 13 were inversely associated with serum PFOA levels at age 5 and 7 (Grandjean et al. 2012; Mogensen et al. 2015a) and with serum PFOA at age 13 (Grandjean et al. 2017), respectively. Decreases in tetanus antibody levels at age 7 were associated with increases in serum PFOA levels at age 5, but not at age 7 (Grandjean et al. 2012; Mogensen et al. 2015a) and tetanus antibody levels were not associated with serum PFOA at age 7 or 13 (Grandjean et al. 2017). In studies comparing maternal serum PFOA with antibody levels in children, no associations were found for tetanus antibodies at age 3 (Granum et al. 2013), age 5 (Grandjean et al. 2012), or age 7 (Grandjean et al. 2012) or for diphtheria at age 5 or 7 (Grandjean et al. 2012). It is noted that Grandjean and associates also found an inverse association between serum polychlorinated biphenyls (PCBs) and serum antibody concentrations against tetanus and diphtheria in children living in the Faroe

Figure 2-21. Antibody Responses Relative to Serum PFOA Levels in Epidemiological Studies

(Presented as percent difference in antibody concentration per 2-fold increase in serum PFOA)



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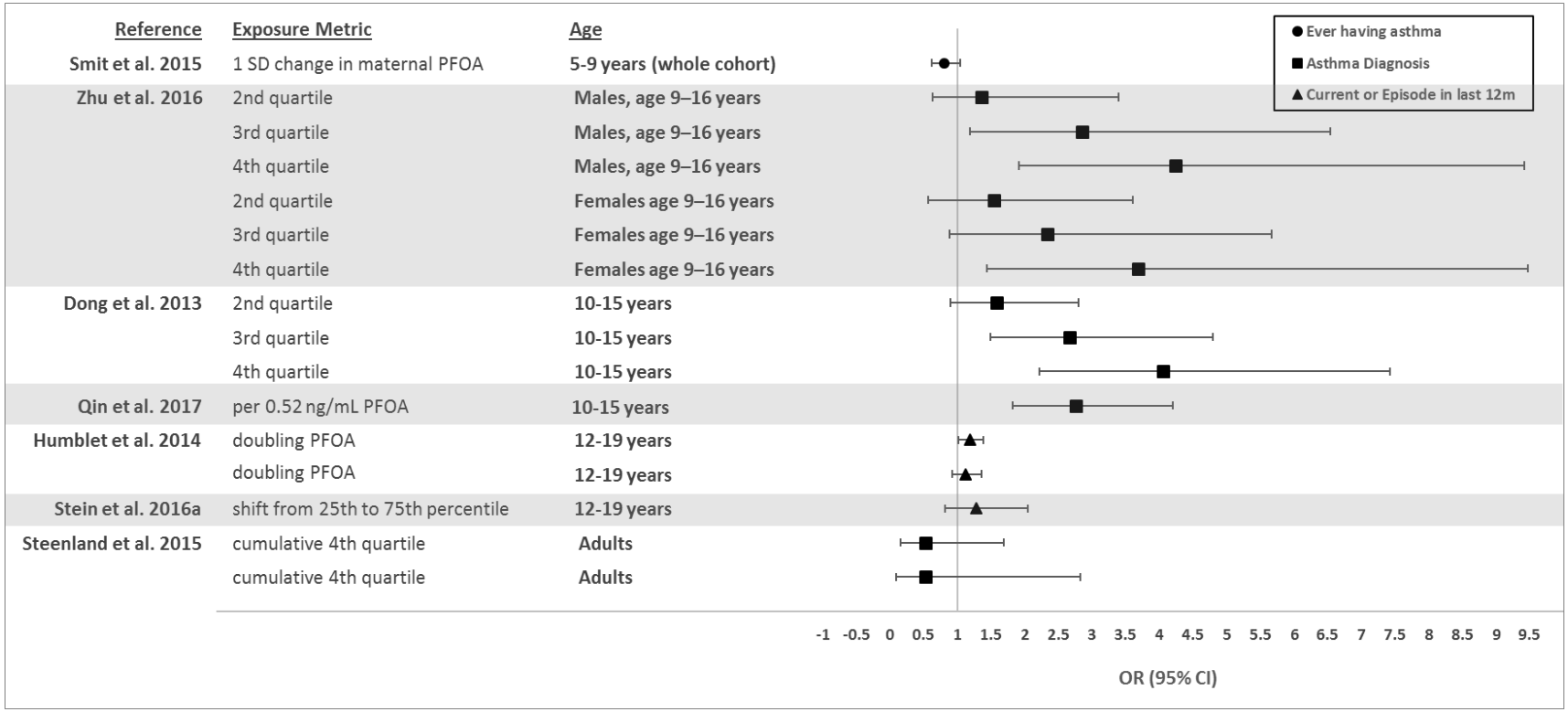
Islands (Heilmann et al. 2010). Statistically adjusting for PCB exposure (milk and serum PCB levels) did not alter the results (Grandjean et al. 2012). Lower levels of rubella antibodies at age 3 were associated with increasing maternal PFOA (Granum et al. 2013).

NTP (2016b) concluded that there is moderate confidence that exposure to PFOA is associated with suppression of the antibody response based on the available human studies. NTP (2016b) also concluded that there is low confidence that exposure to PFOA is associated with increased incidence of infectious disease (or lower ability to resist or respond to infectious disease).

Epidemiological Studies—Hypersensitivity Outcomes. Of the different types of hypersensitivity effects, the most widely studied endpoint is asthma; the possible association between exposure to PFOA and asthma has been studied in occupational, community, and general population studies. Several studies have found associations between current serum PFOA levels and diagnosis of asthma in children (Dong et al. 2013; Humblet et al. 2014; Qin et al. 2017) and adults (Anderson-Mahoney et al. 2008; Zhu et al. 2016). A case-control study found significantly higher serum PFOA levels in asthmatic adolescents as compared to adolescents without asthma (Zhou et al. 2017).

However, other studies have found no association between estimated cumulative serum PFOA levels and incidence of asthma being treated with medication in workers (Steenland et al. 2015) or asthma in the general population (Stein et al. 2016a). In children, no associations between maternal serum PFOA levels and asthma-related health outcomes were observed in 3-year-old children (Granum et al. 2013), 5–9-year-old children (Smit et al. 2015) or 1–10-year-old children (Impinen et al. 2018), or between current PFOA levels and current asthma in adolescents (Stein et al. 2016a). However, the Stein et al. (2016a) study did find an association with rhinitis in adolescents. No associations between maternal PFOA and wheezing were found in infants up to 18 months of age (Okada et al. 2012), infants 12 or 24 months of age (Okada et al. 2014), children 3 years of age (Granum et al. 2013), children 5–9 years of age (Smit et al. 2015), children 2–10 years old (Impinen et al. 2018), or between current serum PFOA levels and wheezing in adults (Stein et al. 2016a). The ORs for asthma diagnosis relative to serum PFOA levels are graphically presented in Figure 2-22; studies using different statistical methods are not included. No associations between maternal PFOA and prevalence of allergic diseases or wheezing were found in 4-year-old children (Goudarzi et al. 2016a). No associations between maternal PFOA and eczema were found in infants up to 18 months of age (Okada et al. 2012), children 3 years of age (Granum et al. 2013), or children 5–9 years of age (Smit et al. 2015). Similarly, no association was found between cord blood PFOA and atopic dermatitis in children 2 years of age (Wang et al. 2011).

Figure 2-22. Risk of Asthma Diagnosis Relative to PFOA Levels (Presented as Adjusted Odds Ratios)



No associations were found between risks of allergy or allergic sensitization and current serum PFOA levels in adults (Stein et al. 2016a) or between cord PFOA in 2–10-year-old children (Impinen et al. 2018). Two studies examining the possible association between current serum PFOA levels in adults and food allergies have found mixed results, with one study finding an association (Buser and Scinicariello 2016) and one not finding an association (Stein et al. 2016a); a study in infants did not find an association between the risk of food allergy and maternal serum PFOA levels (Okada et al. 2012). It is noted that IgE levels, which were used to assess food allergies, is not a sensitive measure of clinical food allergy. No association was found for food sensitization (Buser and Scinicariello 2016).

Associations between serum PFOA and IgE, eosinophil counts, and eosinophil cationic protein levels were observed in asthmatic children (9–16 years of age), but not in non-asthmatic children (Dong et al. 2013; Zhu et al. 2016). Significantly higher IL-4 and IL-5 levels were observed in male children with asthma with the highest PFOA levels (Zhu et al. 2016). Two studies found associations between PFOA and IgE levels in infants. An inverse association was found between maternal PFOA and IgE levels in female infants but not in male infants (Okada et al. 2012), whereas Wang et al. (2011) found a correlation between cord blood PFOA and child IgE levels in males only or in males and females combined. A third study did not find an association between cord blood PFOA and IgE levels in infants (Ashley-Martin et al. 2015). NTP (2016b) concluded that there is low confidence that exposure to PFOA during childhood is associated with increased hypersensitivity responses.

Epidemiological Studies—Autoimmune Outcomes. There are limited data that can be used to evaluate the possible association between PFOA exposure and the risk of autoimmune diseases. Significant increases in the risk of ulcerative colitis were observed in an occupational exposure study (Steenland et al. 2015) and a C8 Science Panel study (Steenland et al. 2013). Although both studies found consistent results, it should be noted that the community study also included participants with occupational exposure to PFOA. The occupational study also found an association between PFOA exposure and rheumatoid arthritis; this was not observed in the community study. The community study (Steenland et al. 2013) also found no associations for other autoimmune diseases (Crohn's disease, Type I diabetes, lupus, and multiple sclerosis). A third study examined neural- and non-neural-specific antibodies and found no associations with cord blood PFOA or current serum PFOA in 7-year-old children (Osuna et al. 2014).

NTP (2016b) concluded that there is low confidence that exposure to PFOA is associated with ulcerative colitis.

Laboratory Animal Studies. The results of several mouse studies support the epidemiological data suggesting that exposure to PFOA can result in immunosuppression. Significant alterations in IgM levels in response to T-dependent antigens, such as sheep red blood cells (sRBCs) or horse red blood cells were observed in acute and intermediate oral mouse studies (DeWitt et al. 2008, 2009, 2016; Loveless et al. 2008; Yang et al. 2002a); the lowest-adverse-effect level was 3.75 mg/kg/day in mice exposed to PFOA in the drinking water for 15 days (DeWitt et al. 2008). Rats appear to be less sensitive than mice; no alterations in IgM levels were observed in rats administered PFOA via gavage for 28 days (Loveless et al. 2008). In a mouse developmental toxicity study, exposure to PFOA on GDs 6–17 was not associated with alterations in IgM or IgG levels in the offspring (Hu et al. 2010). Limited data suggest that alterations in NK cells or delayed type hypersensitivity are not sensitive endpoints for PFOA in laboratory animals. Exposure of male rats to 50 mg/kg/day PFOA by gavage for 14 days did not significantly affect the numbers of T cells, NK cells, or helper T cells (Iwai and Yamashita 2006), and tests for delayed-type hypersensitivity response in mice challenged with bovine serum albumin following exposure to 30 mg/kg/day PFOA via drinking water for 15 days were negative (DeWitt et al. 2008).

Two studies have evaluated hypersensitivity in mice. Application of ≥18.8 mg/kg/day PFOA to the dorsal surface of the ears of mice and subsequently injected with ovalbumin resulted in a significant increase in serum total IgE compared to mice exposed only to ovalbumin (Fairley et al. 2007). Ovalbumin-specific airway hyperreactivity also increased in mice co-exposed to ovalbumin and 25 mg/kg PFOA relative to mice exposed to ovalbumin alone. The investigators suggested that PFOA exposure may increase the IgE response to environmental allergens (Fairley et al. 2007). In contrast to the results of the dermal study, no increases in airway hyperresponsiveness were observed in ovalbumin-sensitized mice exposed *in utero* and post-weaning to PFOA in the diet (Ryu et al. 2014). In nonsensitized mice, PFOA did induce airway hyperresponsiveness in 12-week-old pups.

Numerous studies have evaluated secondary outcomes in monkeys, rats, and mice. In the spleen and thymus, exposure to PFOA resulted in decreases in organ weight, decreases in the number of cells, and/or atrophy (DeWitt et al. 2008; Loveless et al. 2008; Qazi et al. 2009a, 2012; Son et al. 2009; Yang et al. 2000, 2001, 2002b). Acute exposure resulted in decreases in absolute thymus weight at 11.5 mg/kg/day (Yang et al. 2001), decreases in spleen weight at 30 mg/kg/day (Qazi et al. 2012; Yang et al. 2000), and severe thymic atrophy at 30 mg/kg/day (Qazi et al. 2012; Yang et al. 2000). Exposure of male rats to 50 mg/kg/day PFOA by gavage for 14 days did not significantly affect the absolute or relative spleen weight nor did it alter lymphocyte subsets (Iwai and Yamashita 2006).

Decreases in relative spleen weight were observed at ≥0.96 mg/kg/day PFOA, and absolute spleen weight and absolute and relative thymus weights were decreased at 9.6 and 29 mg/kg/day (Loveless et al. 2008). The lowest-adverse-effect levels for spleen and thymus weight changes identified in mouse intermediate studies were 3.75 mg/kg/day PFOA for decreases in absolute spleen weight (DeWitt et al. 2008) and 9.6 mg/kg/day for decreases in absolute and relative thymus weight (Loveless et al. 2008). In rats, no alterations in spleen weight were observed following chronic exposure to 15 mg/kg/day in the diet (3M 1983; Butenhoff et al. 2012c).

Decreases in the number of splenic and thymic lymphocytes were observed in mice administered via gavage ≥9.6 mg/kg/day PFOA for 28 days (Loveless et al. 2008). In contrast, administration of 29 mg/kg/day PFOA by gavage for 28 days did not result in alterations in the number of splenic or thymic lymphocytes in rats (Loveless et al. 2008). A 10-day exposure of mice to 3.0 mg/kg/day PFOA resulted in decreases in the number of bone marrow B-lymphoid cells (Qazi et al. 2012); a decrease in bone marrow myeloid cells was also observed at 30 mg/kg/day. Examination of the B-lymphoid cell subpopulations showed decreases in pro/pre B cells, immature B cells, and early mature B cells, with the greatest reductions observed for pro/pre B cells. When mice were allowed to recover for 10 days following a 10-day exposure to 30 mg/kg/day PFOA in the diet, only a partial recovery of B-lymphoid cells was observed. Significant increases in CD4-CD8- and CD4-CD8+ thymic lymphocytes were observed in mice exposed to 47.21 mg/kg/day for 21 days; increases in CD4+CD8+ lymphocytes were observed at 17.63 and 47.21 mg/kg/day (Son et al. 2009). Similarly, there were decreases in splenic CD4-CD8- lymphocytes at 47.21 mg/kg/day and CD4-CD8+ lymphocytes at ≥0.49 mg/kg/day and increases in splenic CD4+CD8- lymphocytes at 17.63 and 47.21 mg/kg/day.

Two studies examined the immune response to mitogens in mice exposed to PFOA. Marked decreases in total leukocytes, lymphocytes, and neutrophils levels and increases in tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) were observed in the peritoneal cavity, bone marrow, and spleen cells in response to lipopolysaccharide (LPS) stimulation in mice exposed to approximately 40 mg/kg/day PFOA for 10 days (Qazi et al. 2009a). Exposure of splenic lymphocytes isolated from PFOA-exposed mice to concavalin A (ConA) or LPS resulted in decreases in lymphocyte proliferation (Yang et al. 2002a).

A number of studies have evaluated the potential of PFOA to induce histological alterations in immune organs. In monkeys, administration of approximately 20 mg/kg/day PFOA administered via a capsule to Cynomolgus monkeys for 4 or 26 weeks did not affect the gross or microscopic morphology of the spleen (Butenhoff et al. 2002; Thomford 2001). Administration via gavage of 30 mg/kg/day PFOA to Rhesus

monkeys for 90 days induced atrophy of lymphoid follicles in the spleen and lymph nodes and slight to moderate hypocellularity of the bone marrow (Griffith and Long 1980). No histological alterations were observed in the spleen or thymus of rats exposed intermittently to ≤84 mg/m^3 APFO dusts for 2 weeks (Kennedy et al. 1986), ≤29 mg/kg/day administered via gavage for 28 days (Loveless et al. 2008), or dermal doses of ≤2000 mg/kg/day for 2 weeks (Kennedy 1985) or in the spleen and mesenteric lymph nodes of rats exposed to ≤110 mg/kg/day PFOA in the diet for 90 days (Griffith and Long 1980) or ≤15 mg/kg/day PFOA in the diet for 2 years (3M 1983; Butenhoff et al. 2012c).

Studies in wild-type mice and PPARα-null mice demonstrate that PFOA-induced immunomodulation results from PPARα-dependent and -independent mechanisms (DeWitt et al. 2016; Yang et al. 2002b). Exposure to 30 or 33 mg/kg/day PFOA resulted in decreases in spleen weight, thymus weight, number of splenic lymphocytes, number of thymic lymphocytes, and CD4+ and CD8+ splenic and thymic lymphocytes in wild-type mice. Similar exposures of PPARα knockout mice did not result in alterations in spleen weight, number of splenic lymphocytes, or their phenotypes. Although decreases in thymus weight, number of thymic lymphocytes, and their phenotypes were observed in the knockout mice, the magnitudes of the changes were lower in the knockout mice than in the wild-type mice. However, similar responses were observed in T-cell-dependent antibody responses. Exposure to 30 mg/kg/day PFOA resulted in 16 and 14% decreases in the response to sRBCs in wild-type and knockout mice, respectively (DeWitt et al. 2016).

In a systematic review of the available laboratory animal data, NTP (2016b) concluded that there is high confidence that exposure to PFOA is associated with suppression of the antibody response, very low confidence that PFOA is associated with the ability to respond to infectious disease, and moderate confidence that PFOA is associated with increased hypersensitivity.

Summary. Epidemiological studies have evaluated several aspects of immunotoxicity including immunosuppression, hypersensitivity, and autoimmunity. A number of general population studies have found significant inverse associations between serum PFOA levels and antibody responses to vaccines. However, no consistent associations were found between serum PFOA and disease resistance, as measured by episodes of the common cold, cough, fever, or hospitalization for infectious disease. In tests of hypersensitivity, there is some evidence of an association between serum PFOA and asthma diagnosis in children and adults, although this finding was not consistent across studies; increased risk of allergy or allergic sensitization does not appear to be associated with serum PFOA. Based on the findings of an occupational exposure and community exposure study, there is some suggestive association between

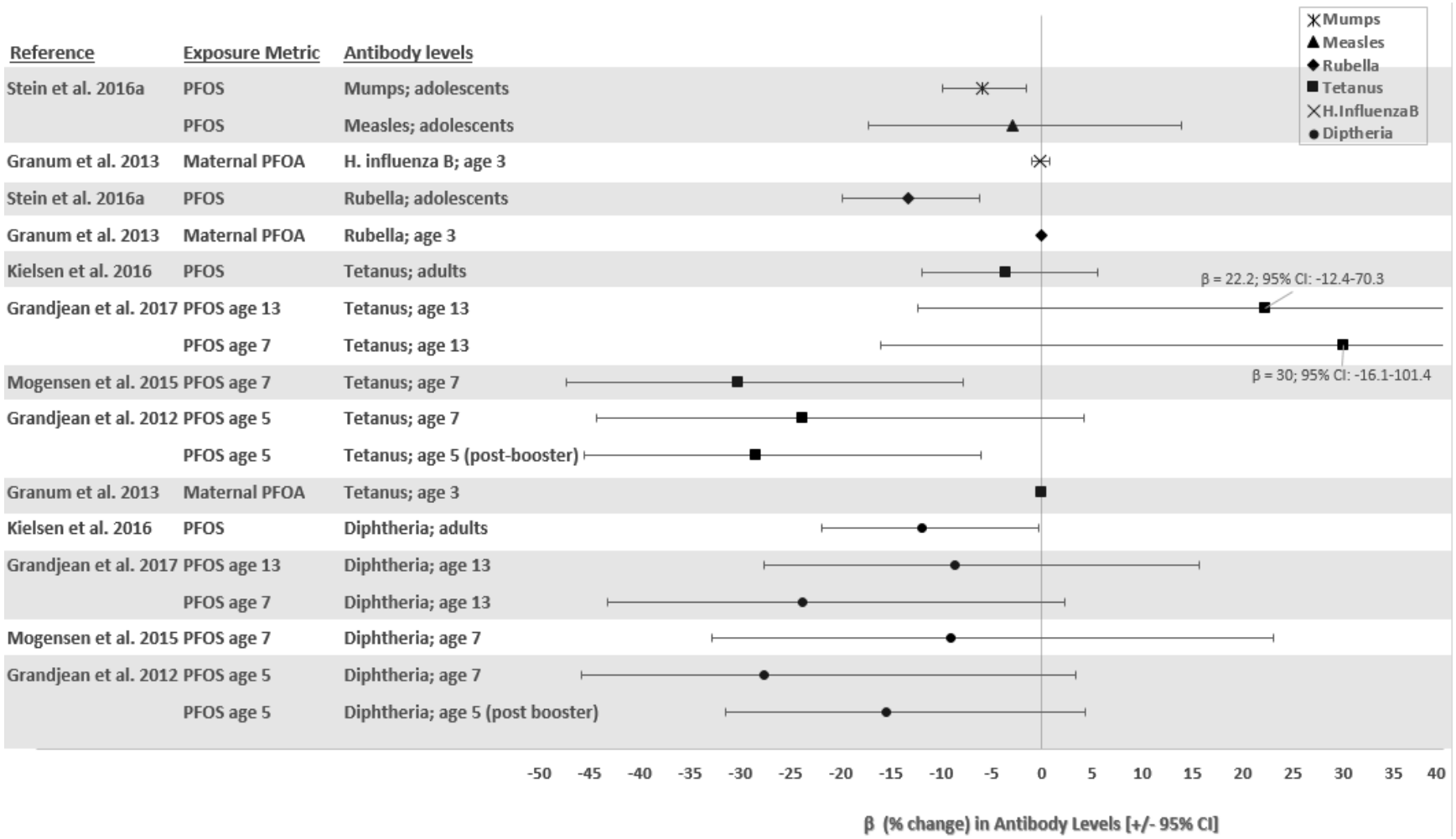
serum PFOA and an increased risk of ulcerative colitis, but not for other autoimmune diseases. Animal studies suggest that the immune system is a sensitive target of PFOA toxicity. A number of studies in mice have demonstrated evidence of immunosuppression and increased hypersensitivity. Laboratory animal studies have also found secondary immune outcomes in the spleen and thymus, which included decreases in organ weight and decreases in the number of lymphocytes.

PFOS

Epidemiological Studies—Immunosuppression Outcomes. Several epidemiological studies have evaluated the potential of PFOS to cause immunosuppression. In studies that evaluated infectious disease resistance, no alterations in the risk of otitis media were observed in infants monitored through 18 months or 3 years of age (Granum et al. 2013; Okada et al. 2012), common cold or other upper respiratory infections (Granum et al. 2013), gastroenteritis with vomiting or diarrhea (Granum et al. 2013), hospitalizations due to infectious diseases in children (Fei et al. 2010), or symptoms of infection such as nasal discharge, cough, diarrhea, or vomiting in children (Dalsager et al. 2016). In contrast, other studies have found associations between PFOS and infectious diseases. Associations between the number of days with symptoms of infection and maternal PFOS levels were observed in children (Dalsager et al. 2016) and between maternal serum PFOS and the risk of total infectious disease in early life (age 4 years) (Goudarzi et al. 2017). Associations were also found between cord PFOS levels and the number of common colds from 0 to 2 years of age and the number of lower respiratory tract infections between 0 and 10 years of age (Impinen et al. 2018).

Other studies evaluating immunosuppression found significant alterations in the response to vaccines; the changes in the response to antibody levels relative to serum PFOS levels are graphically presented in Figure 2-23; studies utilizing different statistical methods are not included in this figure. In children receiving a tetanus vaccination at age 5, there were associations between serum PFOS levels at age 5 and tetanus antibody levels at age 5 (Grandjean et al. 2012) and between serum PFOS levels at age 7 and tetanus antibody levels at age 7 when the analysis was restricted to children who were not likely to have had a booster vaccine after age 5 (Grandjean and Budtz-Jorgensen 2013). However, no associations were found between tetanus antibody levels at age 5 and maternal PFOS or child PFOS levels (Grandjean et al. 2012), between tetanus antibody levels at age 7 and maternal PFOS or child PFOS levels at age 5 or 7 years (Grandjean et al. 2012; Mogensen et al. 2015a), or between tetanus antibody levels at age 14 and child PFOS levels at age 13 (Grandjean et al. 2017). Similarly, diphtheria antibody levels at age 7 were

Figure 2-23. Antibody Responses Relative to Serum PFOS Levels in Epidemiological Studies
(Presented as percent difference in antibody concentration per 2-fold increase in serum PFOS)

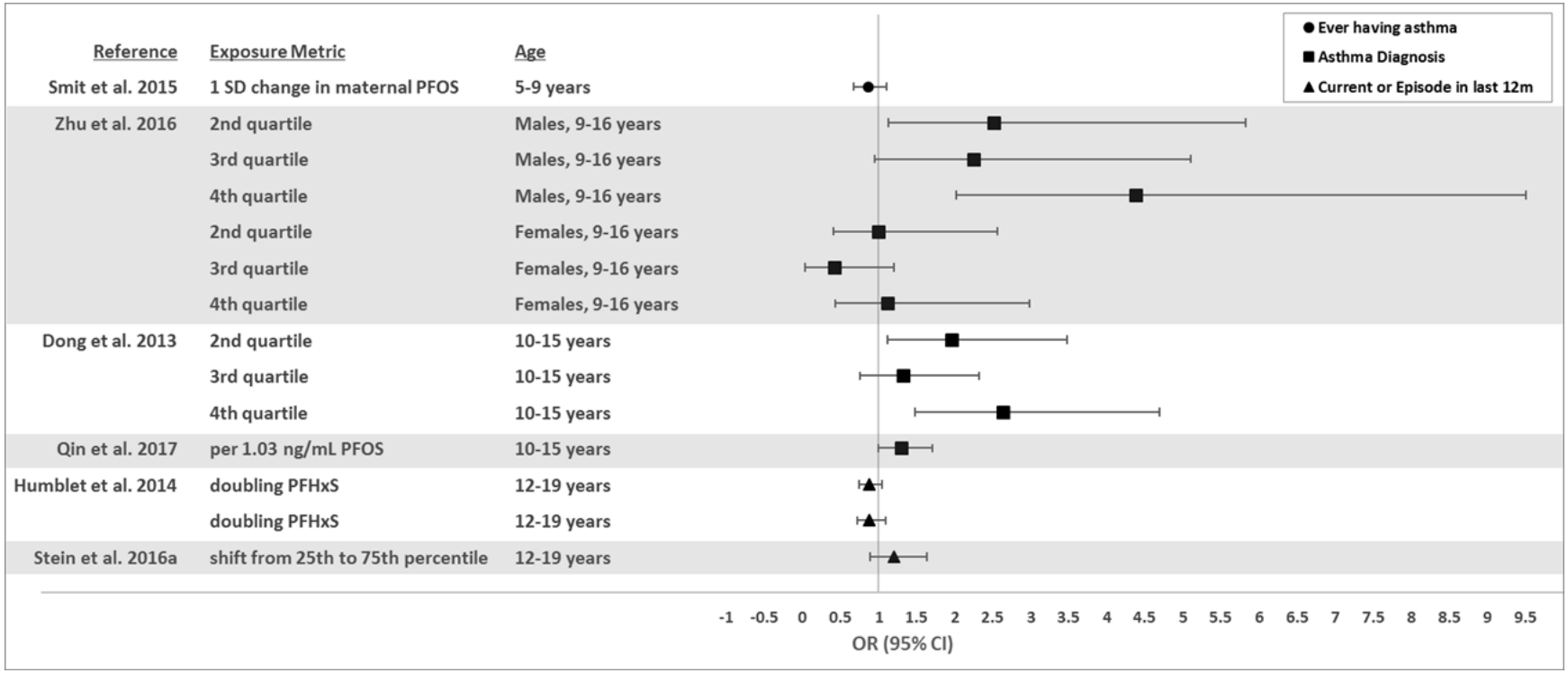


significantly associated with serum PFOS levels at age 5 and 7 (Grandjean et al. 2012; Mogensen et al. 2015a), but antibody levels at age 5 were not associated with maternal PFOS or child PFOS at age 5 years (Grandjean et al. 2012) and antibody levels at age 13 were not associated with child PFOS levels at age 7 or 13 years (Grandjean et al. 2017). In another study of children (Granum et al. 2013), decreased rubella antibody levels were associated with higher maternal PFOS levels, but no associations were found for tetanus or Haemophilus influenza type B antibodies. In adolescents, recent serum PFOS levels were inversely associated with mumps and rubella antibody levels, but not with measles antibody levels (Stein et al. 2016a). In studies in adults, recent PFOS levels were inversely associated with diphtheria antibody levels 30 days after booster administration (Kielsen et al. 2016), but not with tetanus antibody levels 30 days after booster administration (Kielsen et al. 2016) or influenza types A H3N2, A H1N1, or B antibody levels 21 days post-vaccination (Looker et al. 2014).

NTP (2016b) concluded that there is moderate confidence that exposure to PFOS is associated with suppression of the antibody response and that there is low confidence that exposure to PFOS is associated with increased incidence of infectious disease (or lower ability to resist or respond to infectious disease).

Epidemiological Studies—Hypersensitivity Outcomes. Several studies examined the risk of hypersensitivity associated with serum PFOS in children and adolescents; however, the results are inconsistent. In three case-control studies, increased risks of asthma were observed. Qin et al. (2017) reported increased risk of asthma in children associated with serum PFOS levels. Dong et al. (2013) reported an increased risk of asthma diagnosis and increased severity of asthma episodes in children with PFOS levels in the 4th quartile. Zhu et al. (2016) also reported an association between asthma diagnosis and serum PFOS levels in the 4th quartile; however, the association was only significant in males. A third case-control study found significantly elevated serum PFOS levels in asthmatic adolescents (Zhu et al. 2016). Prospective and cross-sectional studies in children (Granum et al. 2013) did not find an association between maternal PFOS levels and the risk of asthma diagnosis in 3-year-old children; between cord PFOS and asthma diagnosis, current asthma, or ever having asthma in 2–10-year-old children (Impinen et al. 2018); or between maternal PFOS and asthma diagnosis in adolescents (Humblet et al. 2014; Stein et al. 2016a). Data evaluating associations between serum PFOS and the risk of asthma diagnosis are presented in Figure 2-24.

Figure 2-24. Risk of Asthma Diagnosis Relative to PFOS Levels (Presented as Adjusted Odds Ratios)



No associations between maternal PFOS or cord PFOS and eczema, atopic dermatitis, or wheezing or total allergic diseases have been found in children (Goudarzi et al. 2016a; Granum et al. 2013; Impinen et al. 2018; Okada et al. 2012, 2014; Smit et al. 2015; Wang et al. 2011). Similarly, no associations between recent serum PFOS levels in adolescents and food allergies or sensitizations (Buser and Scinicariello 2016; Stein et al. 2016a) or maternal PFOS levels and food allergies in infants (Okada et al. 2012) were observed. However, in a cross-sectional study of adolescents, recent PFOS levels were associated with mold allergies and inversely associated with the risk of plant or cockroach or shrimp allergies (Stein et al. 2016a). In related studies, cord blood PFOS levels were associated with an increase in cord IgE levels, but not in infant serum IgE levels (Wang et al. 2011). Two other studies did not find associations between maternal PFOS levels and cord IgE levels (Ashley-Martin et al. 2015; Okada et al. 2012).

NTP (2016b) concluded that there is very low confidence that exposure to PFOS is associated with changes in the hypersensitivity response in children.

Laboratory Animal Studies. A limited number of laboratory animal studies examined PFOS-induced immunosuppression. Guruge et al. (2009) reported an impaired response to an influenza A virus challenge in mice administered 0.025 mg/kg/day PFOS via gavage for 21 days (Guruge et al. 2009). Several studies have found an impaired response to sRBCs (Dong et al. 2009, 2011; Peden-Adams et al. 2008); however, decreases in NK cell activity were observed at higher doses (0.83–2.08 mg/kg/day) (Dong et al. 2009). Qazi et al. (2009a) reported several alterations in parameters associated with the innate immune system in mice exposed to approximately 40 mg/kg/day PFOS in the diet for 10 days. These alterations included marked decreases in total leukocyte and lymphocyte levels and increases in TNF-α and IL-6 levels in the peritoneal cavity and bone marrow in response to LPS stimulation; no alterations were observed in mice exposed to a 20-fold lower dose. As discussed in Section 2.17, a developmental toxicity study (Keil et al. 2008) found an altered response to sRBCs in mice exposed to PFOS *in utero*.

No alterations in spleen or thymus weights were observed in mice exposed to 0.025 mg/kg/day PFOS (Guruge et al. 2009); at a higher dose (0.42 mg/kg/day), significant decreases in relative spleen and thymus weights were observed (Dong et al. 2009; Zheng et al. 2009). Decreases in splenic and thymic cellularity were also observed at ≥0.42 mg/kg/day PFOS (Dong et al. 2009; Qazi et al. 2009b, 2012; Zheng et al. 2009). Bone marrow cells (B-lymphoid and myeloid cells) were also significantly decreased in mice exposed to 30 mg/kg/day PFOS for 10 days (Qazi et al. 2012). Within the B-lymphoid cell population, there were decreases in the number of pro/pre B cells and immature cells (Qazi et al. 2012).

Significant alterations in all splenic T cell CD4 and CD8 subpopulations were observed at ≥0.00331 mg/kg/day PFOS (Peden-Adams et al. 2008) and thymic lymphocyte phenotypes were altered at 0.42 mg/kg/day PFOS (Dong et al. 2009).

Rats treated with 1.77 mg/kg/day PFOS for 4 weeks, 6.34 mg/kg/day for 28 days, 1.56 mg/kg/day for 14 weeks, or 1.04 mg/kg/day for 2 years did not show significant morphological alterations in the spleen, thymus, or mesenteric lymph nodes (Butenhoff et al. 2012b; Lefebvre et al. 2008; Seacat et al. 2003; Thomford 2002b). Similar findings were reported in Cynomolgus monkeys dosed with up to 2 mg/kg/day for 4 weeks or up to 0.75 mg/kg/day PFOS for 26 weeks (Seacat et al. 2002; Thomford 2002a).

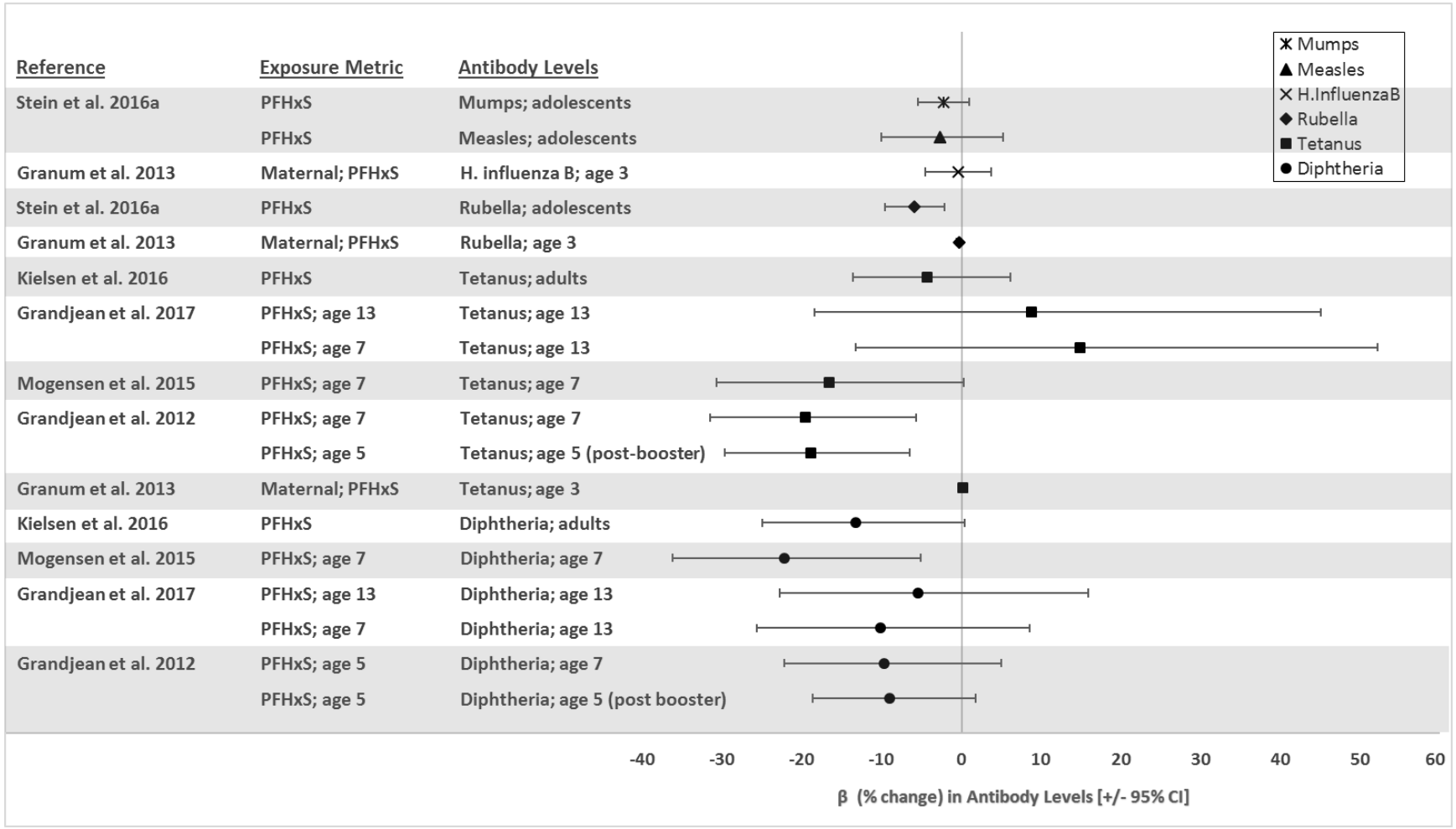
In a systematic review of the available laboratory animal data, NTP (2016b) concluded that there is high confidence that exposure to PFOS is associated with suppression of the antibody response, moderate confidence that PFOS is associated with the ability to respond to infectious disease, and low confidence that PFOS is associated with increased hypersensitivity.

Summary. A number of epidemiological studies have examined the potential immunotoxicity of PFOS. The database provides convincing evidence of an association between serum PFOS levels and immunosuppression, particularly impaired antibody responses to vaccines in adults and children. Mixed results have been observed in studies evaluating infectious disease resistance. Similarly, inconsistent results have been examined in studies evaluating associations between serum PFOS and hypersensitivity outcomes, such as asthma; no associations were found for eczema, dermatitis, food allergies/ sensitizations. Laboratory animal studies, particularly studies in mice, provide strong evidence of the immunotoxicity of PFOS. The strongest evidence comes from studies reporting impaired antibody responses resulting from oral exposure to relatively low doses of PFOS. Other immune effects include decreased response to infectious disease, decreases in spleen and thymus weights, and decreases in splenic and thymic cellularity and bone marrow cells.

PFHxS

Epidemiological Studies—Immunosuppression Outcomes. Several epidemiological studies have examined the potential of PFHxS to suppress the immune system. Altered antibody responses relative to serum PFHxS levels are graphically presented in Figure 2-25. Inverse associations were observed between tetanus antibody levels in 5- and 7-year-old children and serum PFHxS levels at age 5 or 7 years

Figure 2-25. Antibody Responses Relative to Serum PFHxS Levels in Epidemiological Studies
(Presented as percent difference in antibody concentration per 2-fold increase in serum PFHxS)

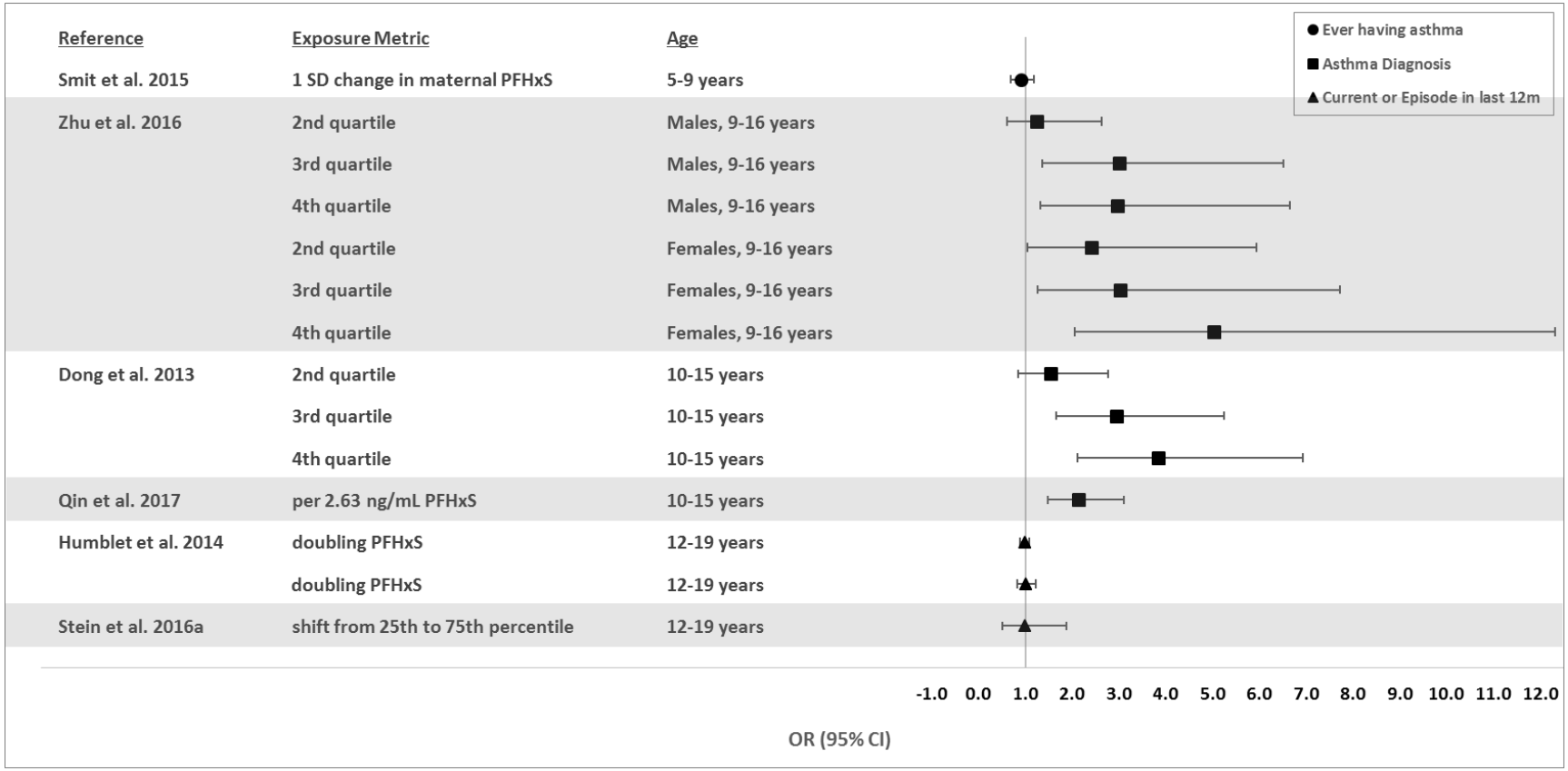


(Grandjean et al. 2012; Mogensen et al. 2015a); but there were no associations between serum PFHxS levels at age 7 or 13 and tetanus antibody levels at age 13 (Grandjean et al. 2017). No associations were found between maternal PFHxS levels and tetanus antibody levels in the children. These studies found no associations between diphtheria antibody levels at ages 5, 7, or 13 and serum PFHxS levels in the mother or in the children. A study in 3-year-old children found an inverse association between maternal PFHxS levels and rubella antibody levels, but no association with influenza type B or tetanus antibody levels (Granum et al. 2013). In adolescents, serum PFHxS levels were also inversely associated with rubella antibody titers in a seropositive subcohort (Stein et al. 2016a); no associations were found for measles or mumps antibody titers. Another study in adolescents did not find associations between recent serum PFHxS levels and tetanus or diphtheria antibody levels (Kielsen et al. 2016). A study in adults did not find associations between PFHxS levels and response to influenza vaccine; some alterations in serum cytokine levels were observed, but chemokine and IgA levels were not altered (Stein et al. 2016b).

In general, the available studies do not suggest an association between serum PFHxS and decreased infectious disease resistance. No alterations in the frequency of fever, cough, nasal discharge, otitis media, diarrhea, or vomiting were observed in children (Dalsager et al. 2016; Granum et al. 2013). Cord PFHxS levels were not associated with increased prevalence of common colds in children 0–2 years of age or lower respiratory tract infections in children 0–10 years of age (Impinen et al. 2018). No association between maternal PFHxS levels and total infectious disease prevalence was found in children up to the age of 4 years (Goudarzi et al. 2017); however, when boys and girls were analyzed separately, an association was found in girls. An association between maternal PFHxS levels and the number of episodes of gastroenteritis was found in children (Granum et al. 2013).

Epidemiological Studies—Hypersensitivity Outcomes. Data evaluating associations between serum PFHxS and the risk of asthma diagnosis are presented in Figure 2-26. No associations were observed between asthma diagnosis, wheezing, and/or eczema or total allergic diseases in children and maternal serum PFHxS levels (Goudarzi et al. 2016a; Granum et al. 2013; Smit et al. 2015) or with recent PFHxS levels in adolescents (Humblet et al. 2014; Okada et al. 2014). In contrast, case-control studies in asthmatic children did find associations between recent PFHxS serum levels and asthma diagnosis (Dong et al. 2013; Qin et al. 2017; Zhu et al. 2016), but no association with asthma severity (Dong et al. 2013). Another case-control study found significantly elevated serum PFHxS levels in adolescents with asthma (Zhu et al. 2016). Dong et al. (2013) also reported associations between serum PFHxS levels and

Figure 2-26. Risk of Asthma Diagnosis Relative to PFHxS Levels (Presented as Adjusted Odds Ratios)



eosinophil counts and eosinophil cationic protein levels in asthmatic children, but not in non-asthmatics. No associations were found with IgE levels in either case-control study (Dong et al. 2013; Zhu et al. 2016) or in a study measuring cord blood IgE (Ashley-Martin et al. 2015).

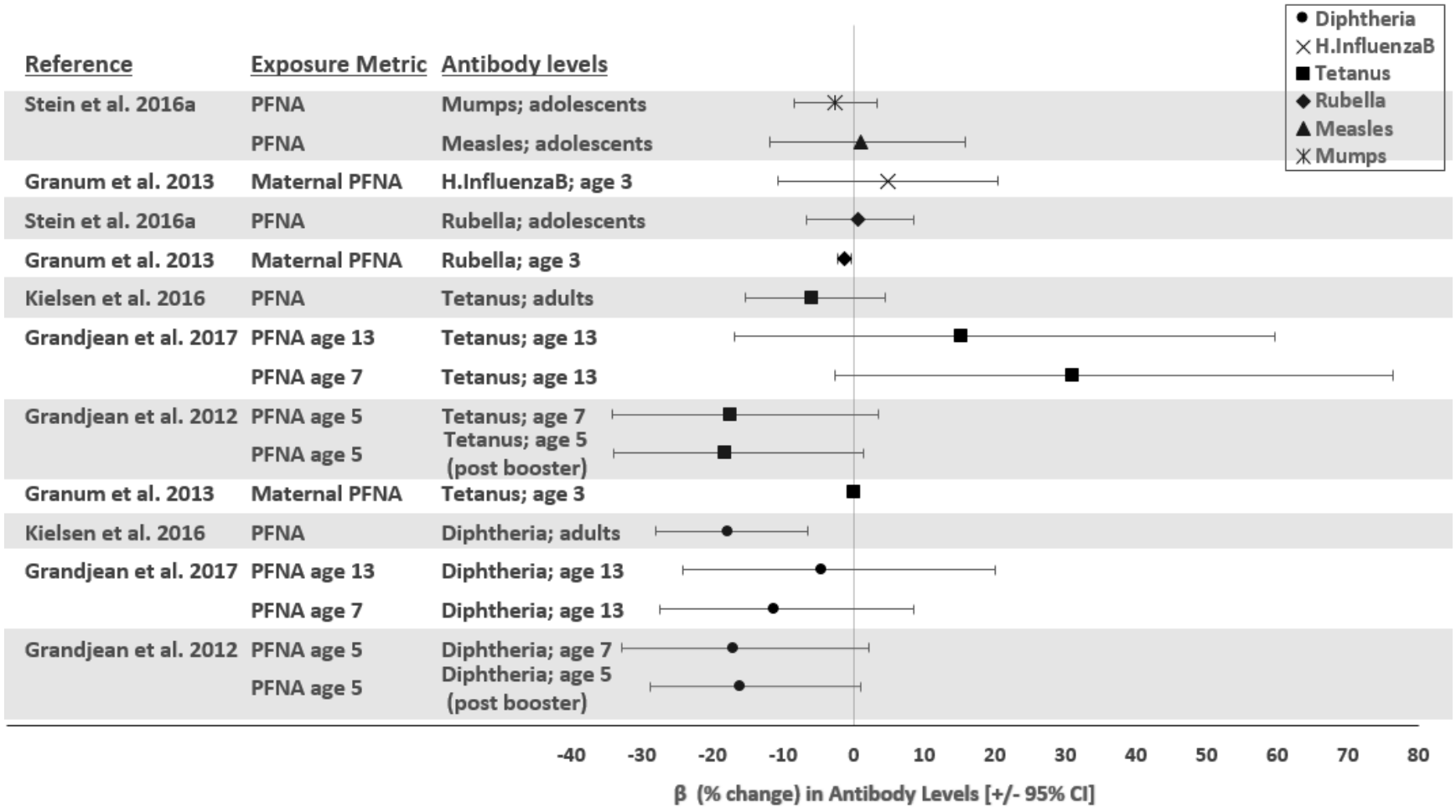
An increased risk of food allergies associated with serum PFHxS levels, but not increased sensitivity to foods, was found in adolescents (Buser and Scinicariello 2016). Another study found no associations between serum PFHxS levels and allergic sensitization to plants, dust mites, pets, cockroaches/shrimp, rodents, mold, or food in adolescents (Stein et al. 2016a).

Laboratory Animal Studies. In the only available study evaluating immunotoxicity for PFHxS, Butenhoff et al. (2009a) did not find histological alterations in the spleen, thymus, or lymph nodes of rats administered 10 mg/kg/day PFHxS via gavage for 42–56 days.

PFNA

Epidemiological Studies—Immunosuppression Outcomes. Most studies examining a possible association between serum PFNA levels and immunosuppression have not found associations. No associations were found between maternal or child PFNA levels and tetanus antibody levels at ages 3, 5, 7, or 13 (Grandjean et al. 2012, 2017; Granum et al. 2013) or in adults (Kielsen et al. 2016). Some studies have found associations between serum PFNA and diphtheria antibody levels, but the results were not consistent. Grandjean and associates found a significant inverse association between diphtheria antibodies levels at age 5 (Grandjean et al. 2012) and serum PFNA levels at age 5, but not for antibody levels at age 13 and PFNA levels at age 7 or 13 (Grandjean et al. 2017). Kielsen et al. (2016) also reported an inverse association (unadjusted for potential confounders) between serum PFNA and diphtheria antibody levels in a small study of adults. An inverse association between maternal serum PFNA and rubella antibody levels was observed in children (Granum et al. 2013), but there was no association for influenza type B antibody levels. Similarly, no associations were found between recent PFNA serum levels and measles, mumps, or rubella antibody titers in adolescents (Stein et al. 2016a). Data evaluating associations between serum PFNA and altered antibody response are presented in Figure 2-27.

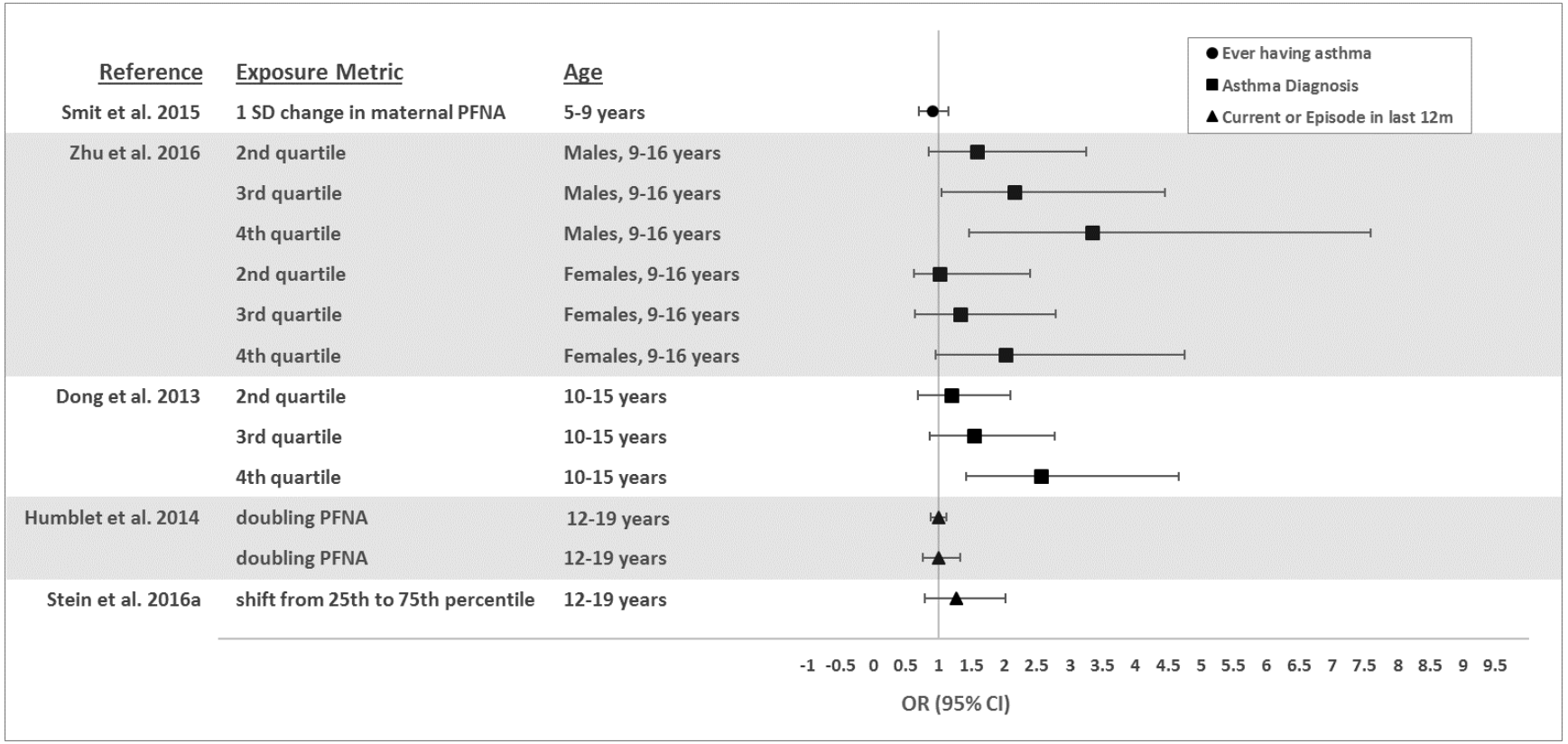
Figure 2-27. Antibody Responses Relative to Serum PFNA Levels in Epidemiological Studies
(Presented as percent difference in antibody concentration per 2-fold increase in serum PFNA)



The epidemiological data provide mixed results on whether there are associations between decreased infectious disease resistance and PFNA levels. No alterations in the risk of increased number of days with fever, cough, nasal discharge, diarrhea, or vomiting were observed in children (Dalsager et al. 2016), although the study did find a significant increase in the number of days above the median for nasal discharge. In a prospective study of children to the age of 4 years, no associations between maternal PFNA levels and prevalence of total infectious diseases were found (Goudarzi et al. 2017). Another study found that the number of episodes of the common cold in children was associated with maternal serum PFNA; no associations were found for otitis media or gastroenteritis (Granum et al. 2013). No associations between cord PFNA levels and the prevalence of common colds were found in children up to 2 years of age (Impinen et al. 2018), but cord PFNA levels were positively associated with the prevalence of lower respiratory infections in children up to the age of 10 years (Impinen et al. 2018).

Epidemiological Studies—Hypersensitivity Outcomes. Case-control studies of asthmatic children have reported associations between serum PFNA and asthma diagnosis (Dong et al. 2013; Qin et al. 2017; Zhu et al. 2016), but no association with asthma severity (Dong et al. 2013); another study found significantly higher serum PFNA levels in adolescents with asthma (Zhu et al. 2016). However, cross-sectional or retrospective studies (Humblet et al. 2014; Smit et al. 2015; Stein et al. 2016a) have not found associations. A prospective study of children to the age of 10, did not find associations between cord PFNA levels and current asthma, ever having asthma, asthma diagnosis, or wheezing (Impinen et al. 2018). Data evaluating associations between serum PFNA and the risk of asthma diagnosis are presented in Figure 2-28. Another study found no associations between maternal PFNA levels and prevalence of total allergic diseases or wheezing (Goudarzi et al. 2016a). No associations were found in adolescents between PFNA and food allergies (Buser and Scinicariello 2016), allergies (Stein et al. 2016a), or allergic sensitizations to plants, dust mites, pets, cockroach/shrimp, rodents, mold, or food (Stein et al. 2016a). However, inverse associations between serum PFNA and food sensitizations were observed in adolescents (Buser and Scinicariello 2016) and between maternal serum PFNA and allergic diseases in infants (Okada et al. 2014). No increases in the risk of other hypersensitivity effects (wheezing, eczema, or atopic dermatitis) were observed (Humblet et al. 2014; Okada et al. 2014; Smit et al. 2015; Stein et al. 2016a; Wang et al. 2011).

Figure 2-28. Risk of Asthma Diagnosis Relative to PFNA Levels (Presented as Adjusted Odds Ratios)



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Laboratory Animal Studies. Administration of PFNA for 14 days resulted in decreases in thymus and/or spleen weights at ≥3 mg/kg/day in rats and mice (Fang et al. 2008, 2009, 2010); at 1 mg/kg/day, an increase in thymus weight was observed in rats (Fang et al. 2009). Fang et al. (2009) reported increases in the ratio of thymic cortex to medulla in rats presumably administered ≥3 mg/kg/day PFNA. In the spleen, there were decreases in the percentage of F4/80+ and CD49b+ cells at ≥1 mg/kg/day and in CD11c+ cells at ≥3 mg/kg/day (Fang et al. 2008). Increases in pro-inflammatory cytokines were observed in the serum at ≥3 mg/kg/day (Fang et al. 2009) and spleen at 5 mg/kg/day (Fang et al. 2010).

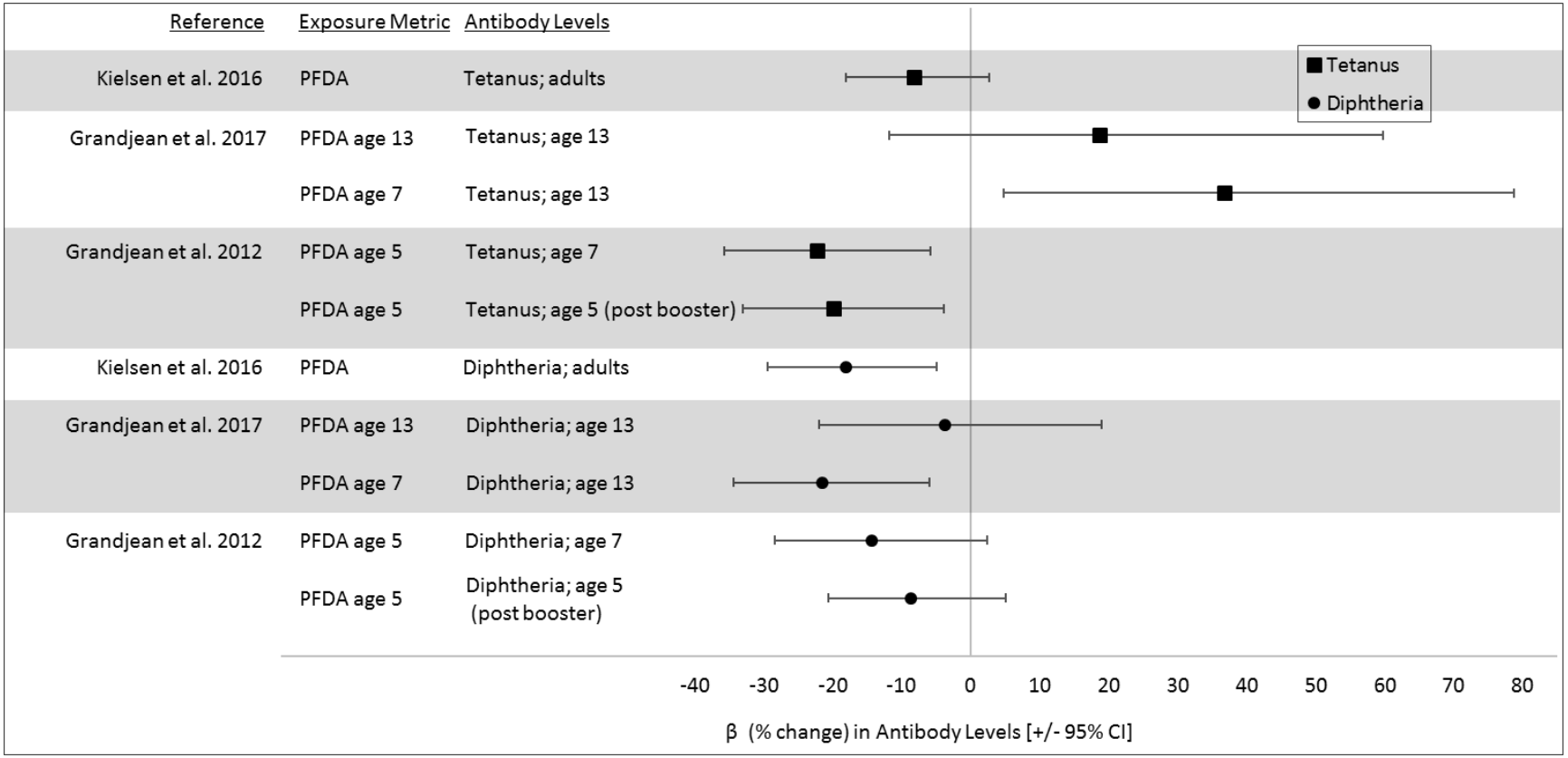
No alterations were observed in the response of splenic T lymphocytes to ConA at 5 mg/kg/day (Fang et al. 2008).

Two weeks after a single intraperitoneal administration of 46 mg/kg PFNA to male and female B57BL/6J mice, a number of immunological alterations included significant decreases in relative spleen weight and splenic leukocyte counts, alterations in splenic T-lymphocyte phenotypes (increased ratios of CD4+ and CD8+ cells), a decrease in viable thymic cells, a marked decrease in CD4+CD8+ thymic lymphocytes, and an increase in CD4+ and CD8+ thymic lymphocytes, and increased levels of tumor necrosis factor-α in response to exposure to the LPS (Rockwell et al. 2013). Similar effects were observed 4 weeks post-exposure (Rockwell et al. 2017). Comparison of the results 2 weeks post-exposure to 4 weeks post-exposure showed a partial recovery in spleen weight and specific thymic lymphocyte subpopulations, but no recovery of the ratio of specific splenic lymphocytes, thymocyte viability, or response to LPS (Rockwell et al. 2017). Some sex-related differences were noted, with females appearing to be more sensitive than males (Rockwell et al. 2017).

PFDA

Epidemiological Studies—Immunosuppression Outcomes. Studies examining possible associations between serum PFDA levels and response to vaccines have reported mixed results; see Figure 2-29 for a graphical presentation of the antibody response relative to PFDA levels. Inverse associations were observed between serum PFDA levels at age 5 and tetanus antibody levels at ages 5 and 7 (Grandjean et al. 2012) and serum PFDA levels at age 7 and antibody levels at age 13 (Grandjean et al. 2017).

Figure 2-29. Antibody Responses Relative to Serum PFDA Levels in Epidemiological Studies
(Presented as percent difference in antibody concentration per 2-fold increase in serum PFDA)

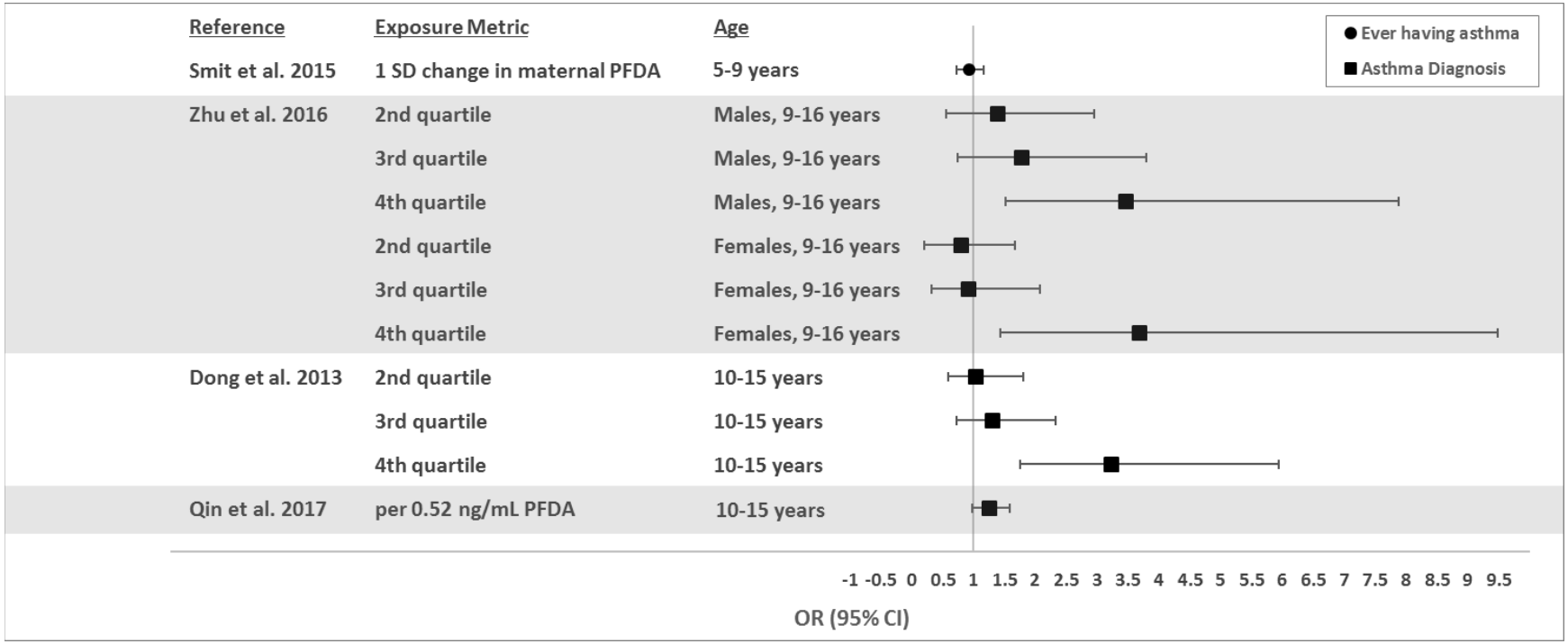


Similarly, diphtheria antibody levels at age 13 were inversely associated with serum PFDA levels at age 7 years (Grandjean et al. 2017), but no associations were observed at other time periods (Grandjean et al. 2012). In adults, diphtheria antibody levels were inversely associated with serum PFDA levels, but there was no association for tetanus antibody levels (Kielsen et al. 2016); this study did not adjust for potential confounders. Two studies examined the possible association between serum PFDA levels and infectious disease resistance, no association was found between maternal serum PFDA levels and symptoms of infection in children aged 1–4 years (Dalsager et al. 2016) and the prevalence of total infectious disease in children 0–4 years of age (Goudarzi et al. 2017).

Epidemiological Studies—Hypersensitivity Outcomes. In case-control studies, associations between asthma diagnosis and asthma severity were observed in children (Dong et al. 2013; Zhu et al. 2016); associations with serum IgE levels, absolute eosinophil counts, and eosinophil cationic protein levels were also observed. A case-control study in adolescents found significantly higher serum PFDA levels among the asthmatic cases (Zhu et al. 2016). A fourth case-control study did not find an association between serum PFDA and asthma risk in children (Qin et al. 2017). A cross-sectional study of children did not find associations between maternal PFDA levels and asthma, eczema, or wheezing in children (Smit et al. 2015). Another cross-sectional study found no association between allergic diseases or eczema in infant and maternal PFDA levels (Okada et al. 2014). In a prospective study, the prevalences of total allergic diseases or wheezing in 4-year-old children were not associated with maternal PFDA levels (Goudarzi et al. 2016a). Data evaluating associations between serum PFDA and the risk of asthma diagnosis are presented in Figure 2-30.

Laboratory Animal Studies. A single gavage dose of 80 mg/kg PFDA did not significantly alter relative thymus weight in female C57BL/6N mice, but it caused a 28% decrease in relative spleen weight 30 days after dosing (Harris et al. 1989). Lethal doses (160 and 320 mg/kg) induced atrophy and lymphoid depletion in both the thymus and spleen. No significant alterations in tests of humoral- or cell-mediated immunity, or alterations in the number of total splenic cells or splenic B-cells, T-cells, T-cell subsets, natural killer cells or macrophages were observed in rats administered up to 0.5 mg/kg/day for 28 days (Frawley et al. 2018). In tests of innate immunity, the study found decreases in the specific activity of fixed tissue macrophages in the liver in rats administered 0.25 or 0.5 mg/kg/day; the investigators suggested that interpretation of this finding may be confounded by the increased number of hepatocytes.

Figure 2-30. Risk of Asthma Diagnosis Relative to PFDA Levels (Presented as Adjusted Odds Ratios)



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In mice receiving weekly doses of PFDA for 4 weeks, decreases in the number of splenic T cells, T-cell subsets, and macrophages were observed at ≥1.25 mg/kg (Frawley et al. 2018). No alterations in humoral-mediated or cell-mediated immune tests or host-resistance to the influenza virus were found.

PFUnA

Epidemiological Studies. Six epidemiological studies have evaluated the potential immunotoxicity of PFUnA in humans. Kielsen et al. (2016) reported inverse associations between serum PFUnA (unadjusted for potential confounders) and diphtheria and tetanus antibody levels in adults. Goudarzi et al. (2017) found no association between maternal PFUnA levels and the risk of total infectious diseases in children up to the age of 4 years. However, Impinen et al. (2018) found cord PFUnA levels were associated with increases in the prevalence of common colds in children up to 2 years of age and the prevalence of lower respiratory tract infections in children up to the age of 10 years.

No significant associations between maternal PFUnA levels and the risk of asthma diagnosis, eczema, or wheezing were observed in children (Smit et al. 2015). Similarly, no associations were found between cord PFUnA levels and risk of current asthma, ever having asthma, asthma diagnosis, or wheezing in children up to the age of 10 years (Impinen et al. 2018). Maternal PFUnA levels were not associated with the prevalences of total allergic diseases or wheezing in 4-year-old children (Goudarzi et al. 2016a). Okada et al. (2012) found inverse associations between maternal serum PFUnA and risk of allergies or eczema in female infants, but not in males, and Impinen et al. (2018) found no association between serum PFUnA and allergic sensitization.

PFHpA

Epidemiological Studies. In general, the two available human immunotoxicity studies did not find associations between serum PFHpA levels and diphtheria or tetanus antibody levels in adults (Kielsen et al. 2016) or risk of asthma diagnosis, eczema, or wheezing in children (Smit et al. 2015). The Smit et al. (2015) study did find an inverse association between maternal PFHpA levels and current wheezing in one subcohort; however, this was not observed in the other subcohort with higher mean maternal PFHpA levels.

PFBS

Epidemiological Studies. The epidemiological database for PFBS consists of three case-control studies in asthmatic children (Dong et al. 2013; Qin et al. 2017; Zhu et al. 2016). Two studies reported increases in asthma diagnosis, but no association with serum IgE levels (Dong et al. 2013; Zhu et al. 2016); the third study (Qin et al. 2017) did not find an association between serum PFBS and asthma risk.

Laboratory Animal Studies. No significant histological alterations were observed in spleen, thymus, or lymph nodes of rats administered via gavage 900 mg/kg/day PFBS for 28 days (3M 2001).

PFBA

Laboratory Animal Studies. No significant gross or microscopic alterations were reported in the spleen, thymus, or mesenteric lymph nodes from rats dosed with PFBA by gavage in doses of up to 184 mg/kg/day for 5 days, 150 mg/kg/day for 28 days, or 30 mg/kg/day for 90 days (3M 2007a; Butenhoff et al. 2012a; van Otterdijk 2007a, 2007b).

PFDoDA

Epidemiological Studies. Six epidemiological studies examining potential immunotoxic endpoints were identified. Kielsen et al. (2016) found inverse associations between recent serum PFDoDA levels (not adjusted for potential confounders) and diphtheria and tetanus antibody levels in adults. No associations between maternal PFDoDA levels and the risk of total infectious diseases were found in children up to the age of 4 years (Goudarzi et al. 2017). Associations between serum PFDoDA levels and the risk of asthma diagnosis, severity of asthma, serum IgE levels, absolute eosinophil counts, and eosinophil cationic protein levels were observed in a case-control study of asthmatic children (Dong et al. 2013). A cross-sectional study of children did not find associations between maternal serum PFDoDA levels and risk of asthma diagnosis, eczema, or wheezing (Smit et al. 2015). Another study did not find associations between maternal serum PFDoDA levels and the risk of allergic disease or eczema in infants (Okada et al. 2014). In contrast, a prospective study of 4-year-old children found an inverse association between maternal PFDoDA levels and the prevalence of mother-reported total allergic diseases, but no association with the prevalence of wheezing (Goudarzi et al. 2016a).

PFHxA

Epidemiological Studies. Two epidemiological studies examined potential immunotoxic endpoints. Dong et al. (2013) found no associations between serum PFHxA levels in asthmatic and nonasthmatic children and asthma diagnosis, asthma severity, or IgE levels. Qin et al. (2017) did not find an association between serum PFHxA levels and asthma risk in children.

Laboratory Animal Studies. Thymic atrophy was observed in 3/9 female rats administered a TWA dose of 315 mg/kg/day PFHxA for 32–44 days (Kirkpatrick 2005). Thymic atrophy and necrosis was also observed in most male and female rats administered 450 mg/kg/day PFHxA for 4 days; all animals died early or were sacrificed *in extremis* (Kirkpatrick 2005).

FOSA

Epidemiological Studies. The only available epidemiological study found an association between cord FOSA levels and an increased prevalence of lower respiratory tract infections in children up to the age of 10 years (Impinen et al. 2018); no association was found for common colds in children up to the age of 2 years. This study also found no associations between cord FOSA and current asthma, ever having asthma, asthma diagnosis, wheezing, or allergic sensitization (Impinen et al. 2018).

2.15 NEUROLOGICAL

Overview. There are limited data on the neurotoxicity of perfluoroalkyls in humans or laboratory animals; epidemiological data come from three studies examining memory and animal studies primarily evaluated for morphological alterations; the results of these human studies are summarized in Table 2-17 with more detailed descriptions in the *Supporting Document for Epidemiological Studies for Perfluoroalkyls*, Table 11. The epidemiological studies found decreases in the risk of memory loss associated with serum PFOA, PFOS, PFHxS, and PFNA. The potential to induce neurodevelopmental effects (including the risk of attention deficit hyperactivity disorder [ADHD]) has been more widely studied; these data are discussed in Section 2.17, Developmental. No epidemiological studies examining potential neurological effects were found for PFDA, PFUnA, PFHpA, PFBS, PFBA, PFDoDA, PFHxA, or FOSA.

Table 2-17. Summary of Neurological Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFOA			
Gallo et al. 2013 Community (C8) (n=21,024 older adults; >50 years of age)	14.1–27.0 ng/mL (2nd PFOA quintile)	Memory loss (self-reported)	**OR 0.88 (0.79–0.97)*, 2nd quintile**
Power et al. 2013 General population (NHANES) (n=1,766 older adults aged 60–<85 years)	4.08 ng/mL (median PFOA)	Difficulty remembering or periods of confusion (self-reported)	OR 0.92 (0.78–1.09)
Shrestha et al. 2017 General population (n=126 older adults, aged 55–74 years)	8.1 ng/mL (median serum PFOA)	Memory and learning scores	**Association (p=0.03)***
		Executive function scores	**Inverse association (p=0.04, p=0.03)***
		Visual and spatial function scores	NS (p>0.05)
		Reaction time	NS (p>0.05)
		Motor function	NS (p>0.05)
PFOS			
Gallo et al. 2013 Community (C8) (n=21,024 older adults; >50 years of age)	20.5–27.1 ng/mL (3rd PFOS quintile)	Memory loss (self-reported)	**OR 0.86 (0.78–0.96)*, 3rd quintile**
Power et al. 2013 General population (NHANES) (n=1,766 older adults aged 60–<85 years)	22.63 ng/mL (median PFOS)	Difficulty remembering or periods of confusion (self-reported)	OR 0.90 (0.78–1.03)

Table 2-17. Summary of Neurological Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Shrestha et al. 2017 General population (n=126 older adults, aged 55–74 years)	33.7 ng/mL (median serum PFOS)	Memory and learning scores	**Association (p=0.04)***
		Executive function scores	NS (p>0.05)
		Visual and spatial function scores	**Association (p=0.05)***
		Reaction time	NS (p>0.05)
		Motor function	NS (p>0.05)
PFHxS			
Gallo et al. 2013 Community (C8) (n=21,024 older adults; >50 years of age)	5.7–232.6 ng/mL (5th PFHxS quintile)	Memory loss (self-reported)	**OR 0.89 (0.79–0.99)*, 5th quintile**
Power et al. 2013 General population (NHANES) (n=1,766 older adults aged 60–<85 years)	2.05 ng/mL (median PFHxS)	Difficulty remembering or periods of confusion (self-reported)	OR 0.93 (0.82–1.06)
PFNA			
Gallo et al. 2013 Community (C8) (n=21,024 older adults; >50 years of age)	1.0–1.2 ng/mL (2nd PFNA quintile)	Memory loss (self-reported)	**OR 0.86 (0.78–0.96)*, 2nd quintile**
Power et al. 2013 General population (NHANES) (n=1,766 older adults aged 60–<85 years)	1.01 ng/mL (median PFNA)	Difficulty remembering or periods of confusion (self-reported)	OR 0.91 (0.79–1.04)

[a]See the *Supporting Document for Epidemiological Studies for Perfluoroalkyls,* Table 11 for more detailed descriptions of studies.
[b]Participants in occupational exposure may have also lived near the site and received residential exposure; community exposure studies involved subjects living near PFOA facilities with known exposure to high levels of PFOA.
[c]Asterisk indicates association with perfluoroalkyl; unless otherwise specified, values in parenthesis are 95% confidence intervals.

OR = odds ratio; NHANES = National Health and Nutrition Examination Survey; PFHxS = perfluorohexane sulfonic acid; PFNA = perfluorononanoic acid; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid

The results of the laboratory animal studies are presented in Tables 2-1, 2-3, 2-4, 2-5, and 2-6 and in Figures 2-6, 2-8, 2-9, and 2-10. No morphological alterations in the brain and nerves were observed in studies of PFOA, PFOS, PFBS, or PFBA. No alterations in neurological function tests were observed in studies of PFOA, PFHxS, PFHxA, PFBS, PFBA, or PFDoDA. Impaired learning and memory were observed in a study of PFOS and decreases in grip strength were observed in a study of PFUnA. Potential neurological effects were not examined in animals exposed to PFNA, PFHpA, or FOSA.

PFOA

Epidemiological Studies. Gallo et al. (2013) found a decreased risk of self-reported memory loss in older adult (>50 years of age) C8 participants with serum PFOA levels in the 2nd, 3rd, 4th, or 5th quintiles. When the participants were categorized by diabetic status, the risk of memory loss was higher among the diabetics than nondiabetics (p=0.014). In sensitivity analyses, the association between serum PFOA levels and memory impairment was compared within and across water districts. Within a water district, the association between serum PFOA and memory impairment was significant, but there was no association between the geometric mean concentration of PFOA in a district and memory impairment. A general population study conducted by Shrestha et al. (2017) of 55–74-year-old participants also found higher memory and learning scores (6% increase) and 16–18% decreases in perseverative errors and responses. In a third study, no association between serum PFOA and self-reported difficulty remembering or periods of confusion was found in NHANES participants aged 60–<85 years (Power et al. 2013).

Laboratory Animal Studies. Exposure of rats to 18,600 mg/m^3 APFO dusts for 1 hour induced excessive salivation. Intermittent, head-only exposure of male rats exposed to up to 84 mg/m^3 APFO dusts for 2 weeks did not reveal gross or microscopic alterations in the brain (Kennedy et al. 1986).

A small number of studies have examined the potential toxicity of perfluoroalkyls to the nervous system in animals, but comprehensive testing has not been conducted. No alterations in performance on a novel recognition test were observed in rats administered a single 50 mg/kg dose of PFOA (Kawabata et al. 2017). No overt signs of neurotoxicity or altered response to stimuli were observed in rats and mice administered up to 1,000 mg/kg PFOA via gavage and observed for 14 days (Sato et al. 2009). Exposure of rats to up to approximately 110 mg/kg/day PFOA via the diet for 90 days did not induce gross or microscopic alterations in the brain, spinal cord, or peripheral nerves (Griffith and Long 1980). Similar results were reported in rats fed a diet that provided approximately 15 mg/kg/day PFOA for 2 years (3M

1983; Butenhoff et al. 2012c). Rhesus monkeys exposed to doses of PFOA that caused lethality (≥30 mg/kg/day by gavage) showed signs of hypoactivity and prostration, but examination of the brain did not reveal treatment-related alterations (Griffith and Long 1980). Treatment of Cynomolgus monkeys with doses of up to 20 mg/kg/day PFOA administered via a capsule did not induce morphological alterations in the brain or sciatic nerve (Butenhoff et al. 2002).

Similarly, no gross or microscopic alterations were reported in the brain from rats dermally exposed to APFO in the Kennedy (1985) study.

PFOS

Epidemiological Studies. Three studies have examined the influence of serum PFOS levels on self-reported memory in older adults. Gallo et al. (2013) found an inverse association between serum PFOS levels and the risk of memory loss in C8 Health Study participants. No association for difficulty remembering or periods of confusion was found in the second study of NHANES participants (Power et al. 2013). A second general population study of older adults found associations between serum PFOS levels and 11% higher scores on tests of visual reproduction delayed recall and 8% higher scores on tests of visual and spatial function (Shrestha et al. 2017), but found no associations on tests of executive function, reaction time, affective state, or motor function.

Laboratory Animal Studies. No histological alterations were observed in the brain, spinal cord, and/or sciatic nerve of rats administered a single gavage dose of up to 500 mg/kg PFOS (Sato et al. 2009), rats treated with up to 1.6–1.8 mg/kg/day PFOS for 4 or 14 weeks (Seacat et al. 2003), rats exposed to 8.5 mg/kg/day PFOS in the diet for 13 weeks (Kawamoto et al. 2011), rats exposed to 1.04 mg/kg/day PFOS in the diet for 2 years (Butenhoff et al. 2012b; Thomford 2002b), or Cynomolgus monkeys dosed with up to 0.75 mg/kg/day PFOS for 26 weeks (Seacat et al. 2002). However, ultrasonic stimulation resulted in bursts of locomotion immediately followed by tonic convulsions in mice administered 125 mg/kg PFOS and rats administered 250 mg/kg PFOS (Sato et al. 2009); the effect was observed 1–7 days postexposure and frequently resulted in death. Similarly, tonic convulsions following ultrasonic stimulation were observed in rats exposed to 8.5 mg/kg/day PFOS in the diet for 6 weeks (Kawamoto et al. 2011); this effect was not observed at ≤2.0 mg/kg/day. Impaired spatial learning and memory, assessed using the Morris water maze test, was observed in mice administered 2.15 or 10.75 mg/kg/day PFOS, but not 0.43 mg/kg/day, for 3 months (Long et al. 2013). Similarly, impaired performance on retention tasks, as assessed by the water maze test, was observed in mice administered 3 or 6 mg/kg/day

PFOS for 4 weeks (Fuentes et al. 2007c). Histopathological examination of the hypothalamus in male Sprague-Dawley rats administered PFOS via gavage for 28 days revealed degeneration of gonadotropic cells of the pituitary gland at ≥1.0 mg/kg/day and dense chromatin, condensed ribosomes, and loss of morphology in the hypothalamus at ≥3.0 mg/kg/day (López-Doval et al. 2014).

PFHxS

Epidemiological Studies. A decrease in the risk of self-reported memory loss was observed in older adult participants of the C8 Health Study who had serum PFHxS levels in the 5th quintile (Gallo et al. 2013). No association between serum PFHxS levels and self-reported difficulty remembering or periods of confusion was reported in a study of NHANES participants (Power et al. 2013).

Laboratory Animal Studies. In a reproductive study in rats dosed with PFHxS, a functional observational battery (FOB) and motor activity tests were conducted in males on exposure days 36 and 39 and in females on postpartum day 17 (Butenhoff et al. 2009a). The battery assessed autonomic functions, reactivity and sensitivity to stimuli, excitability, gait and sensorimotor coordination, limb grip strength, and abnormal clinical signs. No significant alterations were reported in males or females dosed with up to 10 mg/kg/day PFHxS.

PFNA

Epidemiological Studies. Self-reported memory loss was shown to be inversely associated with serum PFNA levels in a study of older C8 Health Study participants (Gallo et al. 2013). Another study of NHANES participants did not find an association with self-reported difficulty remembering or periods of confusion (Power et al. 2013).

PFUnA

Laboratory Animal Studies. In the only study located for PFUnA, a decrease in grip strength was observed in male and female rats administered 1.0 mg/kg/day PFUnA for 41–46 days and allowed to recover for 14 days (Takahashi et al. 2014). No other alterations in performance on FOB tests were found.

PFBS

Laboratory Animal Studies. A significant decrease in tail flick latency to a thermal stimulus was observed in all groups of male rats administered via gavage PFBS for 28 days. However, other tests of sensory reactivity to stimuli, grip strength, and motor activity were not affected (3M 2001), and the significance of this isolated finding is difficult to ascertain. Gross and microscopic examination of the brain, spinal cord, and sciatic nerve did not show any significant alterations. In a 90-day study, no significant alterations in motor activity or performance on functional observation tests were observed in rats at PFBS doses as high as 600 mg/kg/day (Lieder et al. 2009a).

PFBA

Laboratory Animal Studies. Administration of up to 184 mg/kg/day PFBA by gavage for 5 consecutive days to rats had no significant effect on the gross or microscopic morphology of the brain or spinal cord (3M 2007a). In a 28-day gavage study, male rats dosed with 150 mg/kg/day, but not 30 mg/kg/day, showed a delay in bilateral pupillary reflex at the end of the treatment period (Butenhoff et al. 2012a; van Otterdijk 2007a). Results from other tests, including hearing ability, static righting reflex, grip strength, and motor activity, were comparable between groups, and histological examinations of the brain (including the optic nerve), spinal cord, and sciatic nerve were unremarkable. In a 90-day study, pupillary reflex tests conducted in weeks 8 and 12 showed delayed dilation under dark conditions in rats dosed with 30 mg/kg/day (2/40 in controls versus 7/39 in high-dose rats; p=0.071 according to the Fisher Exact Test) (Butenhoff et al. 2012a; van Otterdijk 2007b). Since no abnormalities were recorded during a 3-week recovery period, and there were no histopathological alterations in the eyes, the effect was not considered biologically significant by the investigators. Tests for hearing ability, static righting reflex, grip strength, and motor activity showed no associations with treatment with PFBA. In addition, there were no significant gross or microscopic alterations in the brain, spinal cord, or sciatic nerve.

PFDoDA

Laboratory Animal Studies. Single-dose administration of 50 mg/kg resulted in impaired performance on a novel object recognition test, but did not result in alterations in other tests of memory, anxiety, or open field activity (Kawabata et al. 2017). A second study conducted functional observation tests in rats administered PFDoDA for 42 days (Kato et al. 2015). No alterations in sensorimotor reactivity, grip strength, or spontaneous motor activity were observed at 2.5 mg/kg/day. However, in rats allowed to

recover for 14 days, decreases in forelimb grip strength were observed in males and females at 2.5 mg/kg/day; a decrease in motor activity was also observed in females at 2.5 mg/kg/day but this was only observed during the first week of recovery (Kato et al. 2015).

PFHxA

Laboratory Animal Studies. Administration of up to 500 mg/kg/day NaPFHx for 92–93 days (Loveless et al. 2009) or 200 mg/kg/day PFHxA for 104 weeks (Klaunig et al. 2015) had no effect on locomotion or performance in the FOB test.

2.16 REPRODUCTIVE

Overview. A number of epidemiological studies have evaluated the reproductive toxicity of perfluoroalkyls; summaries of these studies are presented in the *Supporting Document for Epidemiological Studies for Perfluoroalkyls,* Table 12. These studies have evaluated the following categories of reproductive outcomes: alterations in reproductive hormone levels; effects on sperm; effects on menopause onset, menstrual cycle length, endometriosis, and breastfeeding duration; and effects on fertility. Overviews of the studies examining these specific endpoints are presented in Tables 2-18, 2-19, 2-20, and 2-21, respectively. In addition to these reproductive outcomes, several epidemiological studies have evaluated the influence of perfluoroalkyls on sexual maturation; these data are discussed in Section 2.17, Developmental. Although some studies examining reproductive hormone levels have found associations with PFOA, PFOS, PFHxS, PFNA, PFUnA, PFDoDA, or PFHxA levels, the findings are not consistent across studies or there are too few studies to interpret the results. Alterations in reproductive hormone levels have not been found in studies of FOSA. Some associations between serum perfluoroalkyls (PFOA, PFOS, PFHxS, PFNA, PFDA) levels and sperm parameters have been found; often, only one sperm parameter was altered and it is difficult to assess the adversity of this alteration. There is some suggestive evidence of an association between serum PFOA, PFOS, PFHxS, or PFNA levels and an increased risk of early menopause; however, this may be due to reverse causation since an earlier onset of menopause would result in a decrease in the removal of perfluoroalkyls in menstrual blood. Epidemiological studies provide mixed evidence of impaired fertility (increased risks of longer time to pregnancy and infertility); there is also some evidence for PFOA, PFOS, PFHxS PFNA, PFHpA, and PFBS but the results are not consistent across studies or were only based on a single study. The small number of studies evaluating fertility for PFDA, PFUnA, PFDoDA, and FOSA did not find associations. Reproductive outcomes have not been evaluated in epidemiological studies on PFBA.

Table 2-18. Summary of Alterations in Reproductive Hormone Levels in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFOA			
Gilliland 1992 Occupational (n=115)	NR (serum fluorine levels used as surrogate for serum PFOA)	Bound testosterone	**Inverse association (p=0.05)***
		Free testosterone	**Inverse association (p=0.03)***
		Estradiol	**Association (p=0.03)***
		LH	NS (p=0.93)
		FSH	NS (p=0.91)
		Prolactin	**Association (p=0.0002)***
Olsen et al. 1998b Occupational (n=111 males in 1993 and 80 males in 1995)	0–80,000 ng/mL (PFOA range)	Prolactin	**Association (p=0.01 for trend)*, 1993** NS (p=0.58 for trend), 1995
		Estradiol	NS (p=0.66 and 0.56 for trend), 1993 and 1995
		17α-Hydroxy-progesterone	NS (p=0.21 and 0.18 for trend), 1993 and 1995
		Bound testosterone	NS (p=0.07 and 0.85 for trend), 1993 and 1995
		Free testosterone.	NS (p=0.15 or 0.82 for trend), 1993 and 1995
Sakr et al. 2007b Occupational (n=1,025)	428 ng/mL (mean PFOA)	Estradiol	**Association (p=0.017)*, males**
		Testosterone	**Association (p=0.034)*, males**
Knox et al. 2011 Community (C8) (n=25,957 women)	11.3–19.8 ng/mL (2nd PFOA quintile)	Estradiol concentration	NS (p>0.05), menopausal and perimenopausal subgroups
Barrett et al. 2015 General population (n=178 women)	3.61 and 2.31 ng/mL (mean PFOA in nulliparous and parous women)	Follicular estradiol	NS (95% CI included unity), whole cohort and parous and nulliparous subcohorts
		Luteal progesterone	NS (95% CI included unity), whole cohort and parous and nulliparous subcohorts

Table 2-18. Summary of Alterations in Reproductive Hormone Levels in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Joensen et al. 2013 General population (n=247 young men; mean age 19.6 years)	3.46 ng/mL (mean PFOA)	Total testosterone	NS (p>0.05)
		Free testosterone	NS (p>0.05)
		Free androgen index	NS (p>0.05)
		LH	NS (p>0.05)
		Estradiol	NS (p>0.05)
		SHBG	NS (p>0.05)
		FSH	NS (p>0.05)
Raymer et al. 2012 General population (n=256 men)	10.4 ng/mL (mean PFOA)	Estradiol	NS (p=0.751)
		Prolactin	NS (p=0.349)
		FSH	NS (p=0.581)
		LH	**Correlation (p=0.011)***
		Free testosterone	**Correlation (p=0.015)***
		Total testosterone	NS (p=0.440)
Specht et al. 2012 General population (n=604 men)	1.3–4.8 (range of PFOA means of different sites)	SHBG	NS (p=0.39 for trend)
Tsai et al. 2015 General population (n=540 adolescents and young adults aged 12–30 years)	2.74 ng/mL (geometric mean PFOA)	SHBG	**Association (p<0.05)*, females 12–17 years old**
		FSH	NS (p>0.05)
		Testosterone	NS (p>0.05)
Vested et al. 2013 General population (n=169 males aged 19–21 years)	3.8 ng/mL (median maternal PFOA)	Testosterone	NS (p>0.05)
		Estradiol	NS (p>0.05)
		Inhibin B	NS (p>0.05)
		SHBG	NS (p>0.05)
		Free antigen index	NS (p>0.05)
		LH	**Association (p=0.03)***
		FSH	**Association (p=0.01)***

Table 2-18. Summary of Alterations in Reproductive Hormone Levels in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Zhou et al. 2016 General population (n=225 adolescents, 13–15 years of age)	0.5 and 0.5 ng/mL (median serum PFOA in boys and girls)	Testosterone	β -0.0549 (-0.1186–0.0088), boys β -0.1627 (-0.1627–0.0233), girls
		Estradiol	**β 0.0921 (0.0186–0.1656)*, boys** β 0.1015 (-0.0023–0.0033), girls
PFOS			
Olsen et al. 1998a Occupational (n=327)	1,480–2,440 ng/mL (range of PFOS means at different time periods)	DHEAS	NS (p=0.60)
		FSH	NS (p=0.91)
		17-HP	NS (p=0.99)
		LH	NS (p=0.69)
		Prolactin	NS (p=0.25)
		SHBG	NS (p=0.77)
		Free testosterone	NS (p=0.90)
		Bound testosterone	NS (p=035)
		Estradiol	NS (p=0.14), after removal of 1 outlier
Knox et al. 2011 Community (C8) (n=25,957 women)	11.9–17.0 and 17.1–22.4 ng/mL (2nd and 3rd PFOS quintiles)	Estradiol concentration Perimenopausal subgroup Menopausal subgroup	 **Inverse association (p=0.0001)*** **Inverse association (p=0.007)***
Barrett et al. 2015 General population (n=178 women)	16.44 and 14.18 ng/mL (mean PFOS in nulliparous and parous women)	Follicular estradiol	**Inverse association (β -0.013, 95% CI -0.023 to -0.001)*, whole cohort** **Inverse association (β -0.025, 95% CI -0.043 to -0.007)*, nulliparous subcohort**
		Luteal progesterone	NS (95% CI included unity), whole cohort and parous and nulliparous subcohorts

Table 2-18. Summary of Alterations in Reproductive Hormone Levels in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Joensen et al. 2013 General population (n=247 young men; mean age 19.6 years)	8.46 ng/mL (mean PFOS)	Total testosterone	**Inverse association (p<0.05)***
		Free testosterone	**Inverse association (p<0.05)***
		Free androgen index	**Inverse association (p<0.05)***
		LH	NS (p>0.05)
		Estradiol	NS (p>0.05)
		SHBG	NS (p>0.05)
		FSH	NS (p>0.05)
Raymer et al. 2012 General population (n=256 men)	37.4 ng/mL (mean PFOS)	Estradiol	NS (p>0.05)
		Prolactin	NS (p>0.05)
		FSH	NS (p>0.05)
		LH	NS (p>0.05)
		Free testosterone	NS (p>0.05)
		Total testosterone	NS (p>0.05)
Tsai et al. 2015 General population (n=540 male and female adolescents and young adults aged 12–30 years)	7.78 ng/mL (geometric mean PFOS)	SHBG	NS (p>0.05)
		FSH	**Inverse association (p<0.05)*, males 12–17 years old**
		Testosterone	**Inverse association (p<0.05)*, females 12–17 years old**
Vested et al. 2013 General population (n=169 males aged 19–21 years)	21.2 ng/mL (median maternal PFOS)	Testosterone	NS (p>0.05)
		Estradiol	NS (p>0.05)
		Inhibin B	NS (p>0.05)
		SHBG	NS (p>0.05)
		Free antigen index	NS (p>0.05)
		LH	NS (p>0.05)
		FSH	NS (p>0.05)
Zhou et al. 2016 General population (n=225 adolescents, 13–15 years of age)	29.9 and 28.8 ng/mL (median serum PFOS in boys and girls)	Testosterone	**β -0.0029 (-0.0055 to -0.0003)*, boys** β 0.0005 (-0.0018–0.0028), girls
		Estradiol	β 0.0024 (-0.0007–0.0055), boys β 0.0005 (-0.0023–0.0033), girls

Table 2-18. Summary of Alterations in Reproductive Hormone Levels in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFHxS			
Barrett et al. 2015 General population (n=178 women)	1.22 and 1.65 ng/mL (mean PFHxS in nulliparous and parous women)	Follicular estradiol	NS (95% CI included unity), whole cohort and parous and nulliparous subcohorts
		Luteal progesterone	NS (95% CI included unity), whole cohort and parous and nulliparous subcohorts
Joensen et al. 2013 General population (n=247 young men; mean age 19.6 years)	0.81 ng/mL (mean PFHxS)	Total testosterone	NS (p>0.05)
		Free testosterone	NS (p>0.05)
		Free androgen index	NS (p>0.05)
		LH	NS (p>0.05)
		Estradiol	NS (p>0.05)
		SHBG	NS (p>0.05)
		FSH	NS (p>0.05)
Zhou et al. 2016 General population (n=225 adolescents, 13–15 years of age)	1.4 and 1.2 ng/mL (median serum PFHxS in boys and girls)	Testosterone	β 0.0173 (-0.0211–0.0588), boys β -0.0182 (-0.0451–0.0087), girls
		Estradiol	**β 0.0462 (0.0020–0.0925)*, boys** β 0.0017 (-0.0154–0.0496), girls
PFNA			
Barrett et al. 2015 General population (n=178 women)	0.67 and 0.60 ng/mL (mean PFNA in nulliparous and parous women)	Follicular estradiol	NS (95% CI included unity), whole cohort and parous and nulliparous subcohorts
		Luteal progesterone	NS (95% CI included unity), whole cohort and parous and nulliparous subcohorts

Table 2-18. Summary of Alterations in Reproductive Hormone Levels in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Joensen et al. 2013 General population (n=247 young men; mean age 19.6 years)	1.23 ng/mL (mean PFNA)	Total testosterone	NS (p>0.05)
		Free testosterone	NS (p>0.05)
		Free androgen index	NS (p>0.05)
		LH	NS (p>0.05)
		Estradiol	**Association (p<0.05)***
		SHBG	NS (p>0.05)
		FSH	NS (p>0.05)
Tsai et al. 2015 General population (n=540 male and female adolescents and young adults aged 12–30 years)	1.10 ng/mL (geometric mean PFNA)	SHBG	NS (p>0.05)
		FSH	NS (p>0.05)
		Testosterone	NS (p>0.05)
Zhou et al. 2016 General population (n=225 adolescents, 13–15 years of age)	0.8 and 0.9 ng/mL (median serum PFNA in boys and girls)	Testosterone	**β -0.4233 (-0.6998 to -0.1467)*, boys** β -0.1018 (-0.2684–0.0648), girls
		Estradiol	β 0.3204 (-0.0115–0.6522), boys β 0.1252 (-0.0758–0.3263), girls
PFDA			
Barrett et al. 2015 General population (n=178 women)	0.25 and 0.24 ng/mL (mean PFDA in nulliparous and parous women)	Follicular estradiol	NS (95% CI included unity), whole cohort and parous and nulliparous subcohorts
		Luteal progesterone	NS (95% CI included unity), whole cohort and parous and nulliparous subcohorts
Joensen et al. 2013 General population (n=247 young men; mean age 19.6 years)	0.38 ng/mL (mean PFDA)	Total testosterone	NS (p>0.05)
		Free testosterone	NS (p>0.05)
		Free androgen index	NS (p>0.05)
		LH	NS (p>0.05)
		Estradiol	NS (p>0.05)
		SHBG	NS (p>0.05)
		FSH	NS (p>0.05)

Table 2-18. Summary of Alterations in Reproductive Hormone Levels in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Zhou et al. 2016 General population (n=225 adolescents, 13–15 years of age)	0.3 and 1.0 ng/mL (median serum PFDA in boys and girls)	Testosterone	**β -0.2565 (-0.4135 to -0.0994)*, boys** β -0.0626 (-0.1730–0.0477), girls
		Estradiol	β 0.0734 (-0.1189–0.2657), boys β 0.0131 (-0.1208–0.1469), girls
PFUnA			
Barrett et al. 2015 General population (n=178 women)	0.40 and 0.42 ng/mL (mean PFUnA in nulliparous and parous women)	Follicular estradiol	NS (95% CI included unity), whole cohort and parous and nulliparous subcohorts
		Luteal progesterone	NS (95% CI included unity), whole cohort and parous and nulliparous subcohorts
Tsai et al. 2015 General population (n=540 males and females aged 12–30 years)	5.84 ng/mL (geometric mean PFUnA)	SHBG	NS (p>0.05)
		FSH	**Inverse association (p<0.05)*, females 12–17 years old**
		Testosterone	NS (p>0.05)
PFBS			
Zhou et al. 2016 General population (n=225 adolescents, 13–15 years of age)	0.5 and 0.5 ng/mL (median serum PFBS in boys and girls)	Testosterone	β -0.0387 (-0.3261–0.2487), boys β 0.1326 (-0.3576–0.6229), girls
		Estradiol	β 0.0149 (-0.3216–0.3513), boys β 0.3129 (-0.2771–0.9028), girls
PFDoDA			
Zhou et al. 2016 General population (n=225 adolescents, 13–15 years of age)	2.4 and 3.1 ng/mL (median serum PFDoDA in boys and girls)	Testosterone	β 0.0056 (-0.0056–0.0168), boys **β -0.0119 (-0.0227 to -0.0010)*, girls**
		Estradiol	β -0.0007 (-0.0139–0.0124), boys β 0.0106 (-0.0026–0.0218), girls
PFHxA			
Zhou et al. 2016 General population (n=225 adolescents, 13–15 years of age)	0.2 and 0.2 ng/mL (median serum PFHxA in boys and girls)	Testosterone	**β -0.3095 (0.5942 to -0.0248)*, boys** β -0.1896 (-0.4387–0.0595), girls
		Estradiol	β 0.0600 (-0.2803–0.4003), boys β -0.1492 (-0.4515–0.1531), girls

Table 2-18. Summary of Alterations in Reproductive Hormone Levels in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
FOSA			
Barrett et al. 2015 General population (n=178 women)	0.25 and 0.23 ng/mL (mean FOSA in nulliparous and parous women)	Follicular estradiol	NS (95% CI included unity), whole cohort and parous and nulliparous subcohorts
		Luteal progesterone	NS (95% CI included unity), whole cohort and parous and nulliparous subcohorts

[a]See the *Supporting Document for Epidemiological Studies for Perfluoroalkyls,* Table 12 for more detailed descriptions of studies.
[b]Participants in occupational exposure may have also lived near the site and received residential exposure; community exposure studies involved subjects living near PFOA facilities with known exposure to high levels of PFOA.
[c]Asterisk indicates association with perfluoroalkyl; unless otherwise specified, values in parenthesis are 95% confidence intervals.

CI = confidence interval; DHEAS = dihydroepiandrosterone sulfate; FOSA = perfluorooctane sulfonamide; FSH = follicle stimulating hormone; LH = luteinizing hormone; NS = not significant; PFHxS = perfluorohexane sulfonic acid; PFNA = perfluorononanoic acid; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid; PFUnA = perfluoroundecanoic acid; SHBG = sex hormone binding globulin

Table 2-19. Summary of Male Reproductive Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFOA			
Buck Louis et al. 2015 General population (n=96 in Michigan and 366 in Texas)	4.6 and 5.3 ng/mL (median PFOA in Michigan and Texas)	Sperm viability	NS (p>0.05)
		Sperm count	NS (p>0.05)
		Sperm motility ↑ curvilinear velocity Other parameters	 **Association (p<0.05)*** NS (p>0.05)
		Sperm morphology ↑ percentage of sperm head acrosome area ↓ percentage sperm with coiled tails Other parameters	 **Association (p<0.05)*** **Association (p<0.05)*** NS (p<0.05)
Joensen et al. 2013 General population (n=247 young men; mean age of 19.6 years)	3.46 ng/mL (mean PFOA)	Sperm volume	NS (p>0.05)
		Sperm concentration	NS (p>0.05)
		Sperm count	NS (p>0.05)
		Percentage progressive motile sperm	NS (p>0.05)
		Sperm morphology	NS (p>0.05)
Kvist et al. 2012 General population (n=588 men)	1.91–5.19 ng/mL (range of PFOA means)	Y-X chromosome ratio	NS (p>0.05)
Raymer et al. 2012 General population (n=256 men)	10.4 ng/mL (mean PFOA)	Semen volume	NS (p>0.05)
		Semen pH	NS (p>0.05)
		Sperm motility	NS (p>0.05)
		Sperm concentration	NS (p>0.05)
Toft et al. 2012 General population (n=588 males)	3.8 ng/mL (median PFOA)	↑ percent motile sperm	**Association (p<0.05)***
		Sperm concentration	NS (p>0.05)
		Sperm volume	NS (p>0.05)
		Sperm count	NS (p>0.05)
		Sperm morphology	NS (p>0.05)

Table 2-19. Summary of Male Reproductive Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Vested et al. 2013 General population (n=169 males aged 19–21 years)	3.8 ng/mL (median maternal PFOA)	Sperm concentration	**Inverse association (p=0.01)***
		Total sperm count	**Inverse association (p=0.001)***
		Semen volume	NS (p>0.05)
		Percentage progressive spermatozoa	NS (p>0.05)
		Percentage morphologically normal spermatozoa	NS (p>0.05)
		Mean testicular volume	NS (p>0.05)
PFOS			
Buck Louis et al. 2015 General population (n=96 in Michigan and 366 in Texas)	19.15 and 21.6 ng/mL (median PFOS in Michigan and Texas)	Sperm viability	NS (p>0.05)
		Sperm count	NS (p>0.05)
		Sperm motility ↑ distance travelled Other parameters	 **Association (p<0.05)*** NS (p>0.05)
		Sperm morphology	NS (p<0.05)
Joensen et al. 2013 General population (n=247 young men; mean age of 19.6 years)	8.46 ng/mL (mean PFOS)	Sperm volume	NS (p>0.05)
		Sperm concentration	NS (p>0.05)
		Sperm count	NS (p>0.05)
		Percentage progressive motile sperm	NS (p>0.05)
Kvist et al. 2012 General population (n=588 men)	8.20–51.65 ng/mL (range of mean PFOS) 51.65 ng/mL (mean for Greenland subcohort)	Y-X chromosome ratio	**Association (p<0.05)*, whole cohort Inverse association (p=0.044 for trend)*, Greenland subcohort**
Raymer et al. 2012 General population (n=256 men)	37.4 ng/mL (mean PFOS)	Semen volume	NS (p>0.05)
		Semen pH	NS (p>0.05)
		Sperm motility	NS (p>0.05)
		Sperm concentration	NS (p>0.05)

Table 2-19. Summary of Male Reproductive Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Toft et al. 2012 General population (n=588 males)	18.4 ng/mL (median PFOS)	Percent motile sperm	NS (p>0.05)
		Sperm concentration	NS (p>0.05)
		Sperm volume	NS (p>0.05)
		Sperm count	NS (p>0.05)
		Percent normal sperm	**Inverse association (p<0.05)***
Vested et al. 2013 General population (n=169 males aged 19–21 years)	21.2 ng/mL (median maternal PFOS)	Sperm concentration	NS (p>0.05)
		Total sperm count	NS (p>0.05)
		Semen volume	NS (p>0.05)
		Percentage progressive spermatozoa	NS (p>0.05)
		Percentage morphologically normal spermatozoa	NS (p>0.05)
		Mean testicular volume	NS (p>0.05)
PFHxS			
Joensen et al. 2013 General population (n=247 young men; mean age of 19.6 years)	0.81 ng/mL (mean PFHxS)	Sperm volume	NS (p>0.05)
		Sperm concentration	NS (p>0.05)
		Sperm count	NS (p>0.05)
		Percentage progressive motile sperm	NS (p>0.05)
		Sperm morphology	NS (p>0.05)
Toft et al. 2012 General population (n=588 males)	1.1 ng/mL (median PFHxS)	Percent motile sperm	NS (p>0.05)
		Sperm concentration	NS (p>0.05)
		Sperm volume	NS (p>0.05)
		Sperm count	NS (p>0.05)
		Percent normal sperm	**Inverse association (p<0.05)***

Table 2-19. Summary of Male Reproductive Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFNA			
Buck Louis et al. 2015 General population (n=96 in Michigan and 366 in Texas)	1.0 and 1.65 ng/mL (median PFNA in Michigan and Texas)	Sperm viability	NS (p>0.05)
		Sperm count	NS (p>0.05)
		Sperm motility	NS (p>0.05)
		Sperm morphology ↑ percentage of normal sperm ↓ percentage sperm with coiled tails Other parameters	 **Association (p<0.05)*** **Association (p<0.05)*** NS (p<0.05)
Joensen et al. 2013 General population (n=247 young men; mean age of 19.6 years)	1.23 ng/mL (mean PFNA)	Sperm volume	NS (p>0.05)
		Sperm concentration	NS (p>0.05)
		Sperm count	NS (p>0.05)
		Percentage progressive motile sperm	NS (p>0.05)
		Sperm morphology	NS (p>0.05)
Toft et al. 2012 General population (n=588 males)	1.2 ng/mL (median PFNA)	Percent motile sperm	NS (p>0.05)
		Sperm concentration	NS (p>0.05)
		Sperm volume	NS (p>0.05)
		Sperm count	NS (p>0.05)
		Percent normal sperm	NS (p>0.05)
PFDA			
Buck Louis et al. 2015 General population (n=96 in Michigan and 366 in Texas)	0.3 and 0.5 ng/mL (median PFDA in Michigan and Texas)	Sperm viability	NS (p>0.05)
		Sperm count	NS (p>0.05)
		Sperm motility	NS (p>0.05)
		Sperm morphology ↑ sperm head length ↓ percentage sperm with coiled tails Other parameters	 **Association (p<0.05)*** **Association (p<0.05)*** NS (p<0.05)

Table 2-19. Summary of Male Reproductive Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Joensen et al. 2013 General population (n=247 young men; mean age of 19.6 years)	0.38 ng/mL (mean PFDA)	Sperm volume	NS (p>0.05)
		Sperm concentration	NS (p>0.05)
		Sperm count	NS (p>0.05)
		Percentage progressive motile sperm	NS (p>0.05)
		Sperm morphology	NS (p>0.05)

[a]See the *Supporting Document for Epidemiological Studies for Perfluoroalkyls,* Table 12 for more detailed descriptions of studies.
[b]Participants in occupational exposure may have also lived near the site and received residential exposure; community exposure studies involved subjects living near PFOA facilities with known exposure to high levels of PFOA.
[c]Asterisk indicates association with perfluoroalkyl; unless otherwise specified, values in parenthesis are 95% confidence intervals.

NS = not significant; PFDA = perfluorodecanoic acid; PFHxS = perfluorohexane sulfonic acid; PFNA = perfluorononanoic acid; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid

Table 2-20. Summary of Female Reproductive Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFOA			
Dhingra et al. 2016a Community (C8) (n=8,759; retrospective analysis)	>2,130 ng/mL·year (estimated cumulative PFOA exposure 5th quintile)	Menopause age	HR 1.11 (0.97–1.26, p=0.14), 5th quintile
		Early menopause risk	NS (p=0.45), 5-year lag NS (p=0.58), 10-year lag NS (p=0.57), 15-year lag NS (p=0.20), 20-year lag
Dhingra et al. 2016a Community (C8) (n=3,334, prospective analysis)	>4,670 ng/mL·year (estimated cumulative PFOA exposure 5th quintile) >80.8 ng/mL (measured 5th PFOA quintile)	Menopause age (estimated cumulative)	HR 1.10 (0.84–1.43, p=0.51), 5th quintile
		Menopause age (measured)	HR 1.12 (0.86–1.45, p=0.40), 5th quintile
Knox et al. 2011b Community (C8) (n=25,957)	11.3–19.8 ng/mL (2nd PFOA quintile)	Early menopause risk (menopausal subgroup)	**OR 1.5 (1.1–2.1)*, 2nd quintile**
		Early menopause risk (perimenopausal subgroup)	**OR 1.4 (1.1–1.8)*, 2nd quintile**
Buck Louis et al. 2012 General population (n=473)	2.65 and 2.15 ng/mL (geometric mean PFOA in women with or without endometriosis)	Endometriosis	**OR 1.89 (1.17–3.06)*, without parity adjustment** OR 1.62 (0.99–2.66), with parity adjustment
		Risk of moderate to severe endometriosis	**OR 2.58 (1.18–5.64)*, without parity adjustment** OR 1.86 (0.81–4.24) with parity adjustment
Campbell et al. 2016 General population (NHANES) (n=753 women aged 20–50 years)	2.70–3.99 and 4.00–20.60 ng/mL (3rd and 4th quartile serum PFOA)	Self-reported endometriosis	**OR 5.45 (1.19–25.04)*, 3rd quartile** OR 1.33 (0.82–2.17), 4th quartile

Table 2-20. Summary of Female Reproductive Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Fei et al. 2010 General population (n=1,347 pregnant women)	3.91–5.20 ng/mL (2nd quartile for maternal PFOA)	Breastfeeding duration ≤3 months	**OR 1.98 (1.17–3.24)*, 2nd quartile**
		Breastfeeding duration ≤6 months	**OR 1.88 (1.31–2.72)*, 2nd quartile**
Lum et al. 2017 General population (n=501 women)	3.1, 3.5, and 3.1 ng/mL (median serum PFOA in women with menstrual cycles of ≤24 days, 25–31 days, or ≥32 days	Menstrual cycle length	OR 0.98 (0.96–1.00)
Lyngsø et al. 2014 General population (n=1,623 pregnant women)	1.5 ng/mL (median PFOA)	Irregular menstrual cycle	OR 1.4 (0.9–2.2)
		Long menstrual cycle	**OR 1.7 (1.1–2.6)***
		Short menstrual cycle	OR 0.7 (0.3–1.5)
Romano et al. 2016 General population (n=336 women)	5.5–7.6 ng/mL (maternal 3rd quartile PFOA)	Breastfeeding duration ≤3 months	**RR 1.63 (1.16–2.28)*, 3rd quartile**
		Breastfeeding duration ≤6 months	**RR 1.38 (1.06–1.79)*, 3rd quartile**
Taylor et al. 2014 General population (n=2,151 women)	>2.5–4.4 and >4.4 ng/mL (2nd and 3rd PFOA tertiles)	Menopause	**HR 1.36 (1.05–1.75)*, 3rd tertile**
		Hysterectomy	**HR 1.83 (1.31–2.56)*, 2nd tertile**
Timmermann et al. 2017 General population (n=1,130 woman)	2.40 ng/mL (median maternal PFOA)	Breastfeeding duration (in months)	**β -1.3 (-1.9 to -0.7)*, per doubling of serum PFOA levels**
		Exclusive breastfeeding (in months)	**β -0.5 (-0.7 to -0.3)*, per doubling of serum PFOA levels**
Vagi et al. 2014 General population (n=52 cases and 50 controls)	4.1 and 2.3 ng/mL (geometric mean PFOA for cases and controls)	Polycystic ovary syndrome risk	**OR 6.93 (1.79–29.92, p=0.003)*, 3rd tertile**

Table 2-20. Summary of Female Reproductive Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFOS			
Knox et al. 2011b Community (C8) (n=25,957)	11.9–17.0 and 17.1–22.4 ng/mL (2nd and 3rd PFOS quintiles)	Early menopause risk (menopausal subgroup)	**OR 1.5 (1.1–2.1)*, 2nd quintile**
		Early menopause risk (perimenopausal subgroup)	**OR 1.1 (1.1–1.8)*, 3rd quintile**
Buck Louis et al. 2012 General population (n=473)	7.20 and 6.11 ng/mL (geometric mean PFOS in women with or without endometriosis)	Endometriosis	OR 1.39 (0.98–1.98), without parity adjustment OR 1.25 (0.87–1.80), with parity adjustment
		Risk of moderate to severe endometriosis	**OR 1.86 (1.05–3.30)*, without parity adjustment** OR 1.50 (0.82–2.74) with parity adjustment
Campbell et al. 2016 General population (NHANES) (n=753 women aged 20–50 years)	18.20–392.00 ng/mL (4th quartile PFOS)	Self-reported endometriosis	OR 3.48 (1.00–12.00), 4th quartile
Fei et al. 2010 General population (n=1,347 pregnant women)	3.91–5.20 ng/mL (2nd quartile for maternal PFOA)	Breastfeeding duration ≤3 months	**OR 1.89 (1.19–3.01)*, 4th quartile**
		Breastfeeding duration ≤6 months	**OR 1.56 (1.10–2.22)*, 2nd quartile**
Lum et al. 2017 General population (n=501 women)	12.3, 12.6, and 11.5 ng/mL (median serum PFOS in women with menstrual cycles of ≤24 days, 25–31 days, or ≥32 days	Menstrual cycle length	OR 1.01 (0.98–1.03), 3rd tertile
Lyngsø et al. 2014 General population (n=1,623 pregnant women)	8.0 ng/mL (median PFOS)	Irregular menstrual cycle	OR 1.0 0.6–1.6)
		Long menstrual cycle	OR 0.7 (0.4–1.2)
		Short menstrual cycle	OR 0.7 (0.3–1.5)

Table 2-20. Summary of Female Reproductive Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Romano et al. 2016 General population (n=336 women)	13.9 ng/mL (maternal median PFOS)	Breastfeeding duration ≤3 months	NS (p=0.065 for trend)
		Breastfeeding duration ≤6 months	NS (p=0.111 for trend)
Taylor et al. 2014 General population (n=2,151 women)	>9–18.4 and >18.4 ng/mL (2nd and 3rd PFOS tertiles)	Menopause	HR 1.16 (0.91–1.48), 3rd tertile
		Hysterectomy	**HR 1.44 (1.12–1.85)*, 2nd tertile**
Timmermann et al. 2017 General population (n=1,130 woman)	19.47 ng/mL (median maternal PFOS)	Breastfeeding duration (in months)	**β -1.4 (-2.1 to -0.6)*, per doubling of serum PFOS levels**
		Exclusive breastfeeding (in months)	**β -0.3 (-0.6 to -0.1)*, per doubling of serum PFOS levels**
Vagi et al. 2014 General population (n=52 cases and 50 controls)	8.2 and 4.9 ng/mL (geometric mean PFOS for cases and controls)	Polycystic ovary syndrome risk	**OR 5.79 (1.58–24.12, p=0.005)*, 3rd tertile**
PFHxS			
Buck Louis et al. 2012 General population (n=473)	0.48 and 0.43 ng/mL (geometric mean PFHxS in women with or without endometriosis)	Endometriosis	OR 1.14 (0.58–2.24), without parity adjustment OR 0.85 (0.42–1.73), with parity adjustment
		Risk of moderate to severe endometriosis	OR 2.12 (0.85–5.27), without parity adjustment OR 1.24 (0.47–3.31) with parity adjustment
Campbell et al. 2016 General population (NHANES) (n=753 women aged 20–50 years)	2.20–19.40 ng/mL (4th quartile PFHxS)	Self-reported endometriosis	OR 1.47 (0.40–1.41), 4th quartile
Romano et al. 2016 General population (n=336 women)	1.5 ng/mL (maternal median PFHxS)	Breastfeeding duration ≤3 months	NS (p=0.124 for trend)
		Breastfeeding duration ≤6 months	NS (p=0.087 for trend)

Table 2-20. Summary of Female Reproductive Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Taylor et al. 2014 General population (n=2,151 women)	>0.9–1.8 and >1.8 ng/mL (2nd and 3rd PFHxS tertiles)	Menopause	**HR 1.42 (1.08–7.87)*, 2nd tertile**
		Hysterectomy	**HR 2.22 (1.66–2.98)*, 2nd tertile**
Timmermann et al. 2017 General population (n=1,130 woman)	1.45 ng/mL (median maternal PFHxS)	Breastfeeding duration (in months)	β -0.2, (-0.5–0.2), per doubling of serum PFHxS levels
		Exclusive breastfeeding (in months)	β -0.1 (-0.2–0.2), per doubling of serum PFHxS levels
Vagi et al. 2014 General population (n=52 cases and 50 controls)	1.1 and 0.7 ng/mL (geometric mean PFHxS for cases and controls)	Polycystic ovary syndrome risk	OR 1.20 (0.35–4.07), 3rd tertile
PFNA			
Buck Louis et al. 2012 General population (n=473)	0.69 and 0.58 ng/mL (geometric mean PFNA in women with or without endometriosis)	Endometriosis	**OR 2.20 (1.02–4.75)*, without parity adjustment** OR 1.99, 0.91–4.33), with parity adjustment
		Risk of moderate to severe endometriosis	OR 1.21 (0.35–4.19), without parity adjustment OR 0.99 (0.27–3.65) with parity adjustment
Campbell et al. 2016 General population (NHANES) (n=753 women aged 20–50 years)	1.20–15.40 ng/mL (4th quartile PFNA)	Self-reported endometriosis	OR 3.24 (0.81–12.91), 4th quartile
Lum et al. 2017 General population (n=501 women)	1.3, 1.2, and 1.1 ng/mL (median serum PFNA in women with menstrual cycles of ≤24 days, 25–31 days, or ≥32 days	Menstrual cycle length	OR 1.01 (0.99–1.04), 3rd tertile
Romano et al. 2016 General population (n=336 women)	0.9 ng/mL (maternal median PFNA)	Breastfeeding duration ≤3 months	NS (p=0.591 for trend)
		Breastfeeding duration ≤6 months	NS (p=0.349 for trend)

Table 2-20. Summary of Female Reproductive Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Taylor et al. 2014 General population (n=2,151 women)	>0.80–1.5 and >1.5 ng/mL (2nd and 3rd PFNA tertiles)	Menopause	**HR 1.47 (1.14–1.90)*, 3rd tertile**
		Hysterectomy	**HR 1.39 (1.08–1.80)*, 2nd tertile**
Timmermann et al. 2017 General population (n=1,130 woman)	0.62 ng/mL (median maternal PFNA)	Breastfeeding duration (in months)	**β -1.3, (-2.0 to -0.7)*, per doubling of serum PFNA levels**
		Exclusive breastfeeding (in months)	**β -0.2 (-0.5 to -0.0)*, per doubling of serum PFNA levels**
Vagi et al. 2014 General population (n=52 cases and 50 controls)	1.2 and 0.9 ng/mL (geometric mean PFNA for cases and controls)	Polycystic ovary syndrome risk	OR 2.25 (0.67–8.00), 3rd tertile
PFDA			
Buck Louis et al. 2012 General population (n=473)	0.20 and 0.18 ng/mL (geometric mean PFDA in women with or without endometriosis)	Endometriosis	OR 2.95 (0.72–12.1), without parity adjustment OR 2.60 (0.62–10.9), with parity adjustment
		Risk of moderate to severe endometriosis	OR 0.72 (0.06–8.09), without parity adjustment OR 0.58 (0.04–7.42) with parity adjustment
Lum et al. 2017 General population (n=501 women)	0.4, 0.4, and 0.4 ng/mL (median serum PFDA in women with menstrual cycles of ≤24 days, 25–31 days, or ≥32 days	Menstrual cycle length	OR 1.01 (0.99–1.04), 3rd tertile

Table 2-20. Summary of Female Reproductive Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Timmermann et al. 2017 General population (n=1,130 woman)	0.28 ng/mL (median maternal PFDA)	Breastfeeding duration (in months)	**β -0.8 (-1.4 to -0.3) *, per doubling of serum PFDA levels**
		Exclusive breastfeeding (in months)	β -0.2 (-0.4–0.0), per doubling of serum PFDA levels

[a]See the *Supporting Document for Epidemiological Studies for Perfluoroalkyls,* Table 12 for more detailed descriptions of studies.
[b]Participants in occupational exposure may have also lived near the site and received residential exposure; community exposure studies involved subjects living near PFOA facilities with known exposure to high levels of PFOA.
[c]Asterisk indicates association with perfluoroalkyl; unless otherwise specified, values in parenthesis are 95% confidence intervals.

HR = hazard ratio; OR = odds ratio; NS = not significant; PFDA = perfluorodecanoic acid; PFHxS = perfluorohexane sulfonic acid; PFNA = perfluorononanoic acid; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid; RR = risk ratio

Table 2-21. Summary of Fertility Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFOA			
Bach et al. 2015a General population (n=1,372 pregnant women)	2.0 ng/ml (median maternal PFOA)	Fecundability	FR 1.00 (0.99–1.01), per 0.1 ng/mL
		Infertility risk	OR 1.00 (0.98–1.01), per 0.1 ng/mL
Bach et al. 2015c, 2015d General population (n=440 pregnant women)	5.6–7.7 ng/mL (4th PFOA quartile)	Fecundability Parous subgroup Nulliparous subgroup	FR 0.86 (0.63–1.19), 4th quartile FR 0.74 (0.48–1.13), 4th quartile FR 0.99 (0.64–1.54), 4th quartile
		Infertility Parous subgroup Nulliparous subgroup	OR 1.67 (0.70–4.00), 4th quartile OR 1.74 (0.46–6.55), 4th quartile OR 1.56 (0.55–4.42), 4th quartile
Bach et al. 2015c, 2015d (re-analysis of Fei et al. 2009, 2012 data) General population (n=1,161 pregnant women)	4.1–5.4 ng/mL (2nd PFOA quartile) 7.2–41.5 ng/mL (4th PFOA quartile)	Fecundability Parous subgroup Nulliparous subgroup	**FR 0.78 (0.65–0.94)*, 2nd quartile** **FR 0.76 (0.59–0.96)*, 2nd quartile** **FR 0.74 (0.56–0.98)*, 4th quartile**
		Infertility Parous subgroup Nulliparous subgroup	**OR 1.91 (1.16–3.13)*, 2nd quartile** **OR 2.30 (1.09–4.87)*, 2nd quartile** OR 1.48 (0.80–2.75), 4th quartile
Buck Louis et al. 2013 General population (n=501 couples)	Couples achieving pregnancy: 3.112 and 5.016 ng/mL or withdrawing from study or not pregnant 3.101 and 4.749 ng/mL (geometric mean serum PFOA in females and males)	Fecundability Female serum PFOA Male serum PFOA	 OR 0.95 (0.82–1.11) OR 1.01 (0.88–1.17)
Crawford et al. 2017 General population (n=99 30–44-year-old women)	2.79 ng/mL (geometric mean serum PFOA)	Fecundability	FR 1.15 (0.66–2.01)
Fei et al. 2009 General population (n=1,240 pregnant women)	3.91–5.20 ng/mL (2nd PFOA quartile, maternal)	Fecundability	**FOR 0.72 (0.57–0.90)*, 2nd quartile**
		Infertility	**OR 2.06 (1.22–3.51)*, 2nd quartile**

Table 2-21. Summary of Fertility Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Fei et al. 2012 (re-analysis of Fei et al. 2009 data) General population (n=1,240 pregnant women)	3.91–5.20 and ≥6.97 ng/mL (2nd and 4th PFOA quartiles, maternal)	Fecundability Parous subgroup Nulliparous subgroup	 **FOR 0.61 (0.46–0.80)*, 2nd quartile** FOR 0.63 (0.39–1.04), 4th quartile
		Infertility Parous subgroup Nulliparous subgroup	 **OR 3.39 (1.75–6.53)*, 2nd quartile** OR 1.30 (0.52–3.21; p=0.082 for trend), 4th quartile
Jørgensen et al. 2014a, 2014b General population (n=938 pregnant women)	1.65 ng/mL (median PFOA)	Fecundability Primiparous subgroup	FR1.04 (0.87–1.25) **FR 1.31 (1.03–1.68)***
		Infertility	OR 1.11 (0.74–1.66)
Lum et al. 2017 General population (501 couples)	≥4.20 ng/mL (3rd tertile serum PFOA)	Probability of pregnancy	OR 0.7 (0.5–1.1)
Vélez et al. 2015 General population (n=1,743 pregnant women)	1.7 ng/mL (median maternal PFOA)	Fecundability	**FOR 0.89 (0.83–0.94, p<0.001)***
		Infertility	**OR 1.31 (1.11–1.53, p=0.001)***
Vestergaard et al. 2012 General population (n=222 nulliparous couples)	5.58 and 5.61 ng/mL (median PFOA in women with no pregnancy and pregnant)	Fecundability	OR 1.18 (0.78–1.78)
		Not becoming pregnant within first six cycles	OR 1.21 (0.67–2.18)
Wang et al. 2017 General population (n=157 women with endometriosis-related infertility and 178 controls)	>19.6–72.1 ng/mL (3rd tertile serum PFOA)	Endometriosis-related infertility	OR 1.05 (0.58–1.91)
Whitworth et al. 2012b General population (n=416 subfecund pregnant women and 474 controls)	1.66–2.24, 2.25–3.02, and ≥3.02 ng/mL (2nd, 3rd, and 4th PFOA quartiles)	Infertility Parous subgroup Primiparous subgroup	**OR 1.6 (1.1–2.3)*, 2nd quartile** **OR 2.4 (1.4–4.1)*, 3rd quartile** OR 0.5 (0.2–1.2), 4th quartile
Whitworth et al. 2016 General population (n=451 primiparous pregnant women)	2.8 ng/mL (maternal median serum PFOA)	Fecundability	OR 1.0 (0.90–1.2)

Table 2-21. Summary of Fertility Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFOS			
Bach et al. 2015a General population (n=1,372 pregnant women)	8.3 ng/ml (median maternal PFOS) 10.85–36.10 ng/mL (4th PFOS quartile)	Fecundability	FR 1.09 (0.92–1.30), 4th quartile
		Infertility risk	OR 0.71 (0.47–1.07), 4th quartile
Bach et al. 2015c, 2015d General population (n=440 pregnant women)	36.3–103.8 ng/mL (4th PFOS quartile)	Fecundability Parous subgroup Nulliparous subgroup	FR 0.96 (0.75–1.24), 4th quartile FR 1.04 (0.70–1.55), 4th quartile FR 0.97 (0.62–1.51), 4th quartile
		Infertility Parous subgroup Nulliparous subgroup	OR 1.03 (0.54–2.00), 4th quartile OR 0.70 (0.16–3.11), 4th quartile OR 1.23 (0.452–3.39), 4th quartile
Bach et al. 2015c, 2015d (re-analysis of Fei et al. 2009, 2012 data) General population (n=1,161)	27.0–34.2, 34.3–43.8, and 43.9–106.7 ng/mL (2nd, 3rd, and 4th PFOS quartiles)	Fecundability Parous subgroup Nulliparous subgroup	**FR 0.79 (0.66–0.95)*, 2nd quartile** FR 0.90 (0.70–1.14), 4th quartile **FR 0.68 (0.52–0.91)*, 3rd quartile**
		Infertility Parous subgroup Nulliparous subgroup	**OR 1.65 (1.01–2.68)*, 2nd quartile** OR 1.60 (0.78–3.28), 4th quartile **OR 2.71 (1.38–5.30)*, 3rd quartile**
Buck Louis et al. 2013 General population (n=501 couples)	Couples achieving pregnancy: 11.764 and 20.867 ng/mL or withdrawing from study or not pregnant 11.088 and 19.765 ng/mL (geometric mean serum PFOS in females and males)	Fecundability Female serum PFOS Male serum PFOS	 OR 0.99 (0.85–1.17) OR 0.96 (0.80–1.15)
Crawford et al. 2017 General population (n=99 30–44-year-old women)	9.29 ng/mL (geometric mean serum PFOS)	Fecundability	FR 0.89 (0.49–1.60)
Fei et al. 2009 General population (n=1,240 pregnant women)	26.1–33.3 ng/mL (2nd PFOS quartile, maternal)	Fecundability	**OR 0.70 (0.56–0.87)*, 2nd quartile**
		Infertility	**OR 1.70 (1.01–2.86)*, 2nd quartile**

Table 2-21. Summary of Fertility Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Fei et al. 2012 (re-analysis of Fei et al. 2009 data) General population (n=1,240 pregnant women)	26.1–33.3 and 33.4–43.2 ng/mL (2nd and 3rd PFOS quartiles, maternal)	Fecundability Parous subgroup Nulliparous subgroup	 NS (p=0.32 for trend) **FOR 0.63 (0.43–0.91)*, 3rd quartile**
		Infertility Parous subgroup Nulliparous subgroup	 NS (p=0.26 for trend) **OR 2.50 (1.16–5.37, p=0.36 for trend)*, 3rd quartile**
Jørgensen et al. 2014a, 2014b General population (n=938 pregnant women)	10.60 ng/mL (median PFOS)	Fecundability	FR 0.90 (0.76–1.07)
		Infertility	OR 1.39 (0.93–2.07)
Lum et al. 2017 General population (501 couples)	≥15.20 ng/mL (3rd tertile serum PFOS)	Probability of pregnancy	OR 0.9 (0.6–1.3)
Vélez et al. 2015 General population (n=1,743 pregnant women)	4.7 ng/mL (median maternal PFOS)	Fecundability	FOR 0.96 (0.91–1.02, p=0.17)
		Infertility	OR 1.14 (0.98–1.34, p=0.09)
Vestergaard et al. 2012 General population (n=222 nulliparous couples)	35.75 and 36.29 ng/mL (median PFOS in women with no pregnancy and pregnant)	Fecundability	NS (p=0.29)
		Not becoming pregnant within first six cycles	OR 0.98 (0.54–1.77)
Wang et al. 2017 General population (n=157 women with endometriosis-related infertility and 178 controls)	>9.36–138 ng/mL (3rd tertile serum PFOS)	Endometriosis-related infertility	OR 0.66 (0.36–1.21)
Whitworth et al. 2012b General population (n=416 subfecund women and 474 controls)	10.34–16.60 and ≥16.61 ng/mL (3rd and 4th PFOS quartile)	Infertility Parous subgroup Primiparous subgroup	**OR 1.4 (1.0–2.0)*, 3rd quartile** **OR 2.1 (1.2–3.8)*, 4th quartile** OR 0.7 (0.4–1.3), 4th quartile
Whitworth et al. 2016 General population (n=451 primiparous pregnant women)	14.6 ng/mL (maternal median serum PFOS)	Fecundability	OR 1.00 (0.88–1.1)

Table 2-21. Summary of Fertility Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFHxS			
Bach et al. 2015a General population (n=1,372 pregnant women)	0.5 ng/ml (median maternal PFHxS)	Fecundability	FR 1.00 (0.99–1.01), per 0.1 ng/mL
		Infertility risk	OR 0.98 (0.93–1.03), per 0.1 ng/mL
Crawford et al. 2017 General population (n=99 30–44-year-old women)	1.59 ng/mL (geometric mean serum PFHxS)	Fecundability	0.84 (0.46–1.54)
Jørgensen et al. 2014a, 2014b General population (n=938 pregnant women)	1.94 ng/mL (median PFHxS)	Fecundability	FR 0.97 (0.85–1.11)
		Infertility	OR 0.99 (0.73–1.33)
Vélez et al. 2015 General population (n=1,743 pregnant women)	1 ng/mL (median maternal PFHxS)	Fecundability	**FOR 0.91 (0.86–0.97, p=0.002)***
		Infertility	**OR 1.27 (1.09–1.48, p=0.003)***
Vestergaard et al. 2012 General population (n=222 nulliparous couples)	1.12 and 1.22 ng/mL (median PFHxS in women with no pregnancy and pregnant)	Fecundability	**OR 1.33 (1.01–1.75)***
		Not becoming pregnant within first six cycles	OR 0.67 (0.37–1.20)
Wang et al. 2017 General population (n=157 women with endometriosis-related infertility and 178 controls)	>0.39–1.69 ng/mL (3rd tertile serum PFHxS)	Endometriosis-related infertility	**OR 0.47 (0.26–0.87)***
Whitworth et al. 2016 General population (n=450 primiparous pregnant women)	7.0 ng/mL (maternal median serum PFHxS)	Fecundability	OR 0.97 (0.90–1.1)

Table 2-21. Summary of Fertility Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFNA			
Bach et al. 2015a General population (n=1,372 pregnant women)	0.8 ng/mL (median maternal)	Fecundability	FR 1.00 (0.98–1.02), per 0.1 ng/mL
		Infertility risk	OR 0.99 (0.95–1.03), per 0.1 ng/mL
Buck Louis et al. 2013 General population (n=501 couples)	Couples achieving pregnancy: 1.176 and 1.558 ng/mL or withdrawing from study or not pregnant 1.112 and 1.422 ng/mL (geometric mean serum PFNA in females and males)	Fecundability Female serum PFNA Male serum PFNA	 OR 1.00 (0.84–1.19) OR 1.09 (0.90–1.32)
Crawford et al. 2017 General population (n=99 30–44-year-old women)	0.84 ng/mL (geometric mean serum PFNA)	Fecundability	1.40 (0.79–2.49)
Jørgensen et al. 2014a, 2014b General population (n=938 pregnant women)	0.64 ng/mL (median PFNA)	Fecundability Primiparous subgroup	**FR 0.80 (0.69–0.94)*** FR 0.99 (0.88–1.22)
		Infertility	**OR 1.53 (1.08–2.15)***
Lum et al. 2017 General population (501 couples)	≥1.50 ng/mL (3rd tertile serum PFNA)	Probability of pregnancy	OR 0.8 (0.6–1.2)
Vestergaard et al. 2012 General population (n=222 nulliparous couples)	0.45 and 0.51 ng/mL (median PFNA in women with no pregnancy and pregnant)	Fecundability	OR 1.17 (0.88–1.54)
		Not becoming pregnant within first six cycles	OR 0.67 (0.37–1.25)
Wang et al. 2017 General population (n=157 women with endometriosis-related infertility and 178 controls)	>1.50–7.10 ng/mL (3rd tertile serum PFNA)	Endometriosis-related infertility	**OR 0.52 (0.28–0.95)***

Table 2-21. Summary of Fertility Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Whitworth et al. 2016 General population (n=451 primiparous pregnant women)	0.43 ng/mL (maternal median serum PFNA)	Fecundability	OR 1.1 (0.92–1.3)
PFDA			
Bach et al. 2015a General population (n=1,372 pregnant women)	0.3 ng/mL (median maternal PFDA)	Fecundability	FR 1.00 (0.97–1.03), per 0.1 ng/mL
		Infertility risk	OR 0.99 (0.92–1.07), per 0.1 ng/mL
Buck Louis et al. 2013 General population (n=501 couples)	Couples achieving pregnancy: 0.385 and 0.448 ng/mL or withdrawing from study or not pregnant 0.349 and 0.416 ng/mL (geometric mean serum PFDA in females and males)	Fecundability Female serum PFDA Male serum PFDA	 OR 1.11 (0.95–1.29) OR 1.08 (0.93–1.26)
Lum et al. 2017 General population (501 couples)	≥0.05 ng/mL (3rd tertile serum PFDA)	Probability of pregnancy	OR 0.9 (0.6–1.3)
Vestergaard et al. 2012 General population (n=222 nulliparous couples)	0.10 and 0.11 ng/mL (median PFDA in women with no pregnancy and pregnant)	Fecundability	OR 1.15 (0.89–1.49)
		Not becoming pregnant within first six cycles	OR 0.61 (0.33–1.12)
Wang et al. 2017 General population (n=157 women with endometriosis-related infertility and 178 controls)	>1.79–11.2 ng/mL ng/mL (3rd tertile serum PFDA)	Endometriosis-related infertility	OR 0.74 (0.40–1.35)
Whitworth et al. 2016 General population (n=429 primiparous pregnant women)	0.11 ng/mL (maternal median serum PFDA)	Fecundability	OR 1.00 (0.85–1.2)

Table 2-21. Summary of Fertility Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFUnA			
Bach et al. 2015a General population (n=1,372 pregnant women)	0.3 ng/mL (median maternal PFUnA)	Fecundability	FR 1.01 (0.98–1.03), per 0.1 ng/mL
		Infertility risk	OR 0.98 (0.92–1.04), per 0.1 ng/mL
Wang et al. 2017 General population (n=157 women with endometriosis-related infertility and 178 controls)	>1.42–5.34 ng/mL (3rd tertile serum PFUnA)	Endometriosis-related infertility	OR 0.61 (0.33–1.13)
Whitworth et al. 2016 General population (n=447 primiparous pregnant women)	0.23 ng/mL (maternal median serum PFUnA)	Fecundability	OR 0.93 (0.78–1.1)
PFHpA			
Wang et al. 2017 General population (n=157 women with endometriosis-related infertility and 178 controls)	>0.11–0.66 ng/mL (3rd tertile serum PFHpA)	Endometriosis-related infertility	**OR 0.48 (0.26–0.86)***
PFBS			
Wang et al. 2017 General population (n=157 women with endometriosis-related infertility and 178 controls)	>0.086–0.094 ng/mL (2nd tertile serum PFBS)	Endometriosis-related infertility	**OR 3.74 (2.04—6.84)***
PFDoDA			
Wang et al. 2017 General population (n=157 women with endometriosis-related infertility and 178 controls)	>0.27–1.02 ng/mL (2nd tertile serum PFDoDA)	Endometriosis-related infertility	OR 0.61 (0.34–1.11)

Table 2-21. Summary of Fertility Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Whitworth et al. 2016 General population (n=410 primiparous pregnant women)	0.04 ng/mL (maternal median serum PFDoDA)	Fecundability	OR 0.91 (0.77–1.1)
FOSA			
Buck Louis et al. 2013 General population (n=501 couples)	Couples achieving pregnancy: 0.110 and 0.112 ng/mL or withdrawing from study or not pregnant 0.126 and 0.129 ng/mL (geometric mean serum FOSA in females and males)	Fecundability Female serum FOSA Male serum FOSA	 **OR 0.81 (0.70–0.94)*** OR 0.89 (0.78–1.02)
Vestergaard et al. 2012 General population (n=222 nulliparous couples)	0.10 and 0.11 ng/mL (median FOSA in women with no pregnancy and pregnant)	Fecundability	OR 1.01 (0.86–1.18)
		Not becoming pregnant within first six cycles	OR 0.81 (0.45–1.46)
Whitworth et al. 2016 General population (n=226 primiparous pregnant women)	0.03 ng/mL (maternal median serum FOSA)	Fecundability	OR 0.91 (0.71–1.2)

[a]See the *Supporting Document for Epidemiological Studies for Perfluoroalkyls,* Table 12 for more detailed descriptions of studies.
[b]Participants in occupational exposure may have also lived near the site and received residential exposure; community exposure studies involved subjects living near PFOA facilities with known exposure to high levels of PFOA.
[c]Asterisk indicates association with perfluoroalkyl; unless otherwise specified, values in parenthesis are 95% confidence intervals.

FOR = fecundability odds ratio; FOSA = perfluorooctane sulfonamide; FR = fecundability ratio (probability of conceiving during a given menstrual cycle); OR = odds ratio; NS = not significant; PFDA = perfluorodecanoic acid; PFHxS = perfluorohexane sulfonic acid; PFNA = perfluorononanoic acid; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid; PFUnA = perfluoroundecanoic acid

Studies in laboratory animals have evaluated the potential histological alterations in reproductive tissues, alterations in reproductive hormones, and impaired reproductive functions. Summaries of these studies are presented in Tables 2-1, 2-3, 2-4, 2-5, and 2-6 and in Figures 2-6, 2-8, 2-9, and 2-10. Multigeneration studies on PFOA, PFOS, and PFBS have not found alterations in reproductive parameters in animals; similarly, no effect on fertility was observed for PFHxS or PFDoDA. One study found alterations in sperm parameters and decreases in fertility in mice exposed to PFNA. An increase in the incidence of Leydig cell hyperplasia (reclassified as gonadal stromal hyperplasia) has been observed in animals exposed to PFOA; one study for PFDoDA reported ultrastructural alterations in the testes. Studies on PFOS, PFHxS, PFBS, and PFBA have not found histological alterations. Delays in mammary gland development have been observed in mice exposed to PFOA; this effect has also been observed in perinatally exposed mice (see Section 2.17, Developmental). No laboratory animal studies examined reproductive endpoints for PFDA, PFUnA, PFHpA, or FOSA.

PFOA

Epidemiological Studies—Reproductive Hormone Levels. Three studies have evaluated potential effects of PFOA exposure on reproductive hormone levels in workers (Gilliland 1992; Olsen et al. 1998b; Sakr et al. 2007b). Sakr et al. (2007b) found associations between serum PFOA and estradiol and testosterone levels in male workers at the Washington Works facility. Similarly, Gilliland (1992) found associations between serum fluorine levels and estradiol and prolactin levels and inverse associations with bound and free testosterone levels in workers at the 3M Cottage Grove facility. In contrast, Olsen et al. (1998b) did not find associations between serum PFOA and estradiol or testosterone in male workers at the 3M Cottage Grove facility. The study did find an association with prolactin levels, but this was only found in workers examined in 1993, but not in those examined in 1995. In a general population study of men aged 30–66 years of age, correlations were found between serum PFOA levels and free testosterone levels and LH levels; no correlations were found for estradiol, prolactin, follicle stimulating hormone (FSH), or total testosterone levels (Raymer et al. 2012). Another study of similar aged men did not find an association between serum PFOA and sex hormone binding globulin levels (Specht et al. 2012). Studies of young men (median age 19 years) (Joensen et al. 2013) or adolescents and young men (12–30 years of age) (Tsai et al. 2015) did not find associations between serum PFOA and reproductive hormone levels. A third study (Vested et al. 2013) found an association between LH and FSH levels and maternal serum PFOA levels in young adult males; other hormones were not affected. A fourth study in adolescents (aged 13–15 years) found an association between serum PFOA and estradiol levels in boys, but not in girls, and did not find associations for testosterone levels (Zhou et al. 2016).

Two studies of women (Barrett et al. 2015; Knox et al. 2011b) did not find associations with estradiol levels or luteal progesterone levels. A third study of adolescent and young women (Tsai et al. 2015) found an association between serum PFOA and sex hormone binding globulin levels in adolescents (12–17 years), but not in young adults; no associations with FSH or testosterone were observed in either group.

Epidemiological Studies—Effects on Sperm. Six general population studies have evaluated the potential alterations in sperm parameters associated with PFOA exposure. Although some associations have been found, the results are not consistent across studies. Buck Louis et al. (2015) reported an increase in curvilinear velocity and some alterations in sperm morphology that were associated with serum PFOA levels. Toft et al. (2012) found a PFOA-related increase in the percentage of motile sperm in men with serum PFOA levels in the 3rd tertile. Vested et al. (2013) reported inverse associations between maternal serum PFOA levels and sperm concentration and total sperm count in young adults; no alterations in motility or morphology were observed. Other studies did not find alterations in sperm viability, count, concentration, motility, or morphology (Buck Louis et al. 2015; Joensen et al. 2013; Raymer et al. 2012; Toft et al. 2012) or the Y-X chromosome ratio (Kvist et al. 2012).

Epidemiological Studies—Effects on Menstrual Cycle Length, Menopause Onset, Endometriosis, and Breastfeeding Duration. Two studies examined possible associations between serum PFOA levels and alterations in menstrual cycle length. An increased risk of a long menstrual cycle (≥32 days) was observed in women with serum PFOA levels in the 3rd tertile and when serum PFOA was used as a continuous variable (Lyngsø et al. 2014). No alterations in the risk of having a short menstrual cycle (≤24 days) or irregular menstrual cycles (≥7 days difference between cycles) were observed. The second study did not find an association between serum PFOA and menstrual cycle length (Lum et al. 2017).

Four studies have evaluated the risk of early menopause. In a study of C8 Health Study participants, increases in the risk of early menopause was observed in perimenopausal (>42–≤51 years of age) and menopausal (>51–≤65 years of age) women with serum PFOA levels in the 2nd, 3rd, 4th, and 5th quintiles (Knox et al. 2011b). An increase in menopause risk was also observed in a cross-sectional study of NHANES participants with serum PFOA levels in the 3rd tertile (Taylor et al. 2014). Taylor et al. (2014) also found a higher risk of hysterectomy among women with serum PFOA levels in the 2nd and 3rd tertiles. Findings of higher levels of PFOA (and other perfluoroalkyls) among women with hysterectomies and that serum PFOA levels increased after menopause provide suggestive evidence that at least part of the

association may be due to reverse causation (Taylor et al. 2014). In contrast, no alterations in the risk of early menopause or age of menopause were associated with estimated cumulative serum PFOA levels in retrospective and prospective studies of C8 Health Study participants (Dhingra et al. 2016a); age of menopause was also not associated with measured serum PFOA levels in the prospective study (Dhingra et al. 2016a). Cross-sectional analysis also showed that early menopause was associated with measured serum PFOA levels, but not with modeled serum PFOA levels (Dhingra et al. 2017), providing support that reverse causation may contribute to the observed association.

Buck Louis et al. (2012) showed that the risk of endometriosis and the risk of moderate-to-severe endometriosis were associated with serum PFOA levels; however, adjustment for parity resulted in confidence intervals that included unity. A second study found an increased risk of self-reported endometriosis in women with serum PFOA levels in the 3rd quartile; for the 4th quartile women, the confidence intervals included unity (Campbell et al. 2016). A case-control study (Vagi et al. 2014) found an increased risk of polycystic ovary syndrome among women with serum PFOA levels in the 3rd tertile.

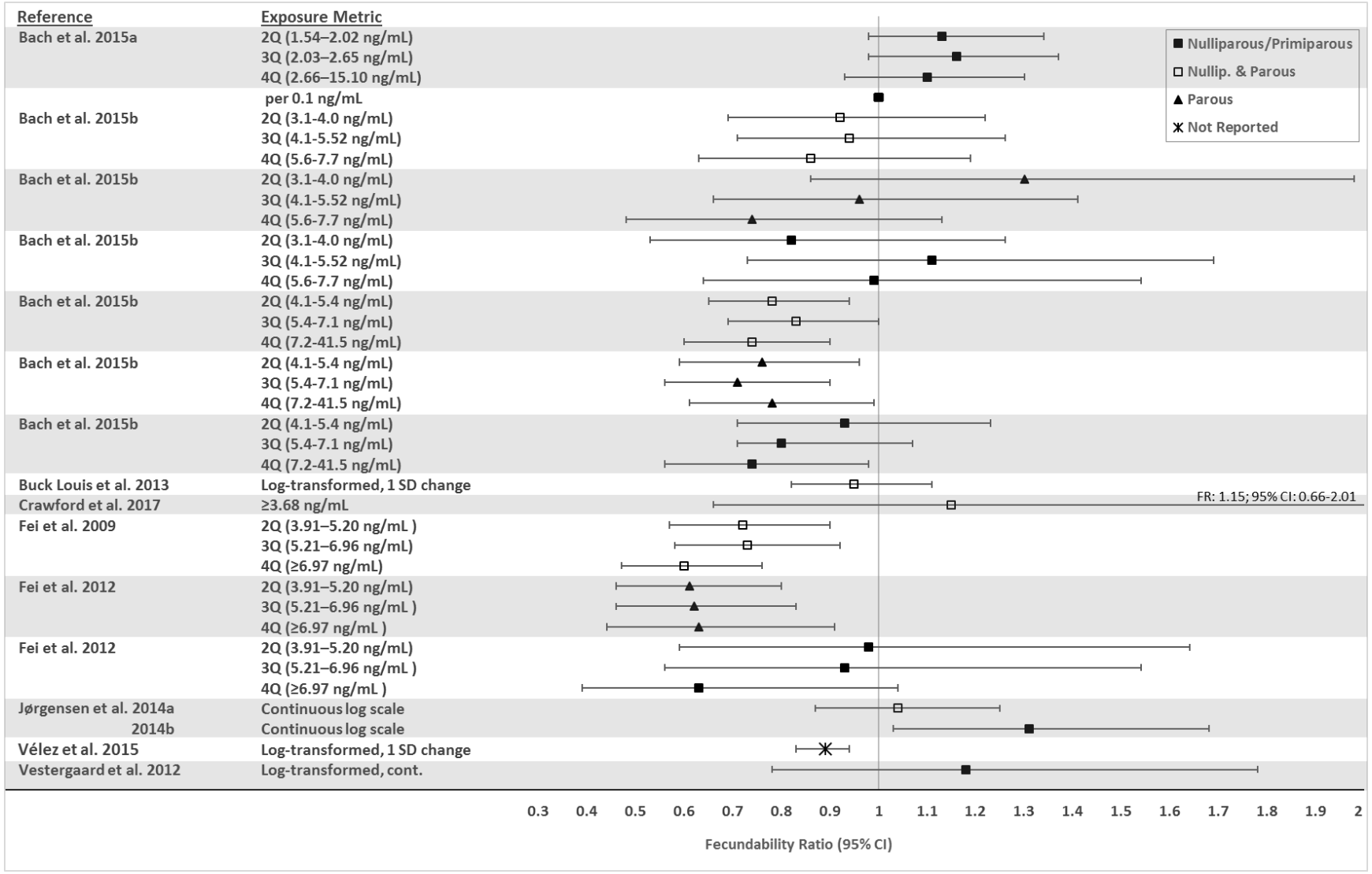
Two studies utilizing pharmacokinetic modeling have investigated whether the observed associations between PFOA exposure and early onset menopause or risk of endometriosis was due to reverse causation (Ngueta et al. 2017; Ruark et al. 2017). As discussed in Section 3.1.4, menstrual blood loss is a route of elimination of perfluoroalkyls. Therefore, variability in menstruation such as menarche, menopause, and pharmacological management of menstruation (e.g., use of oral contraceptives) could affect serum perfluoroalkyl levels, and thereby contribute to observed statistical associations between serum PFOA levels and early onset menopause (Ruark et al. 2017) or endometriosis (Ngueta et al. 2017) outcomes.

Three studies evaluated a possible association between maternal PFOA levels and breastfeeding duration. Two studies found increases in the risk of breastfeeding ≤3 or 6 months that were associated with maternal PFOA levels (Fei et al. 2010; Romano et al. 2016). Timmermann et al. (2017) found an inverse association between maternal PFOA levels and the duration of breastfeeding and the amount of time the women exclusively breastfed. Fei et al. (2010) reported that when the women were segregated by parity, the associations were only found in multiparous women. In contrast, Timmermann et al. (2017) found no differences in duration or breastfeeding exclusiveness between primiparous and multiparous women. It is noted that a number of factors can influence the duration of breastfeeding including diminished milk production, inadequate lactation support from health care providers after delivery, use of medication that is not compatible with breastfeeding, lack of spousal/family support, and individual choice. In general, these studies did not consider whether these factors may have influenced the observed associations.

Epidemiological Studies—Effects on Fertility. Several general population studies have examined the possible association between female serum PFOA levels and decreased fertility or infertility; the results are graphically presented in Figures 2-31 and 2-32, respectively. With the exception of the Buck Louis et al. (2013) and Vestergaard et al. (2012) prospective studies, all of the women were pregnant; thus, couples with unresolved infertility are underrepresented in these analyses. Maternal transfer of PFOA during pregnancy and lactation can result in lower serum PFOA levels in women (see Section 3.1.2 for additional information), as compared to nulliparous women; thus, parity should be considered when evaluating potential associations between serum PFOA and infertility. The Buck Louis et al. (2013) study is the only study that used maternal and paternal serum PFOA levels as the biomarkers of exposure. Most of the studies evaluated two aspects of fertility: fecundability, which is a measure of time to pregnancy, and risk of infertility, which is typically time to pregnancy of >12 months.

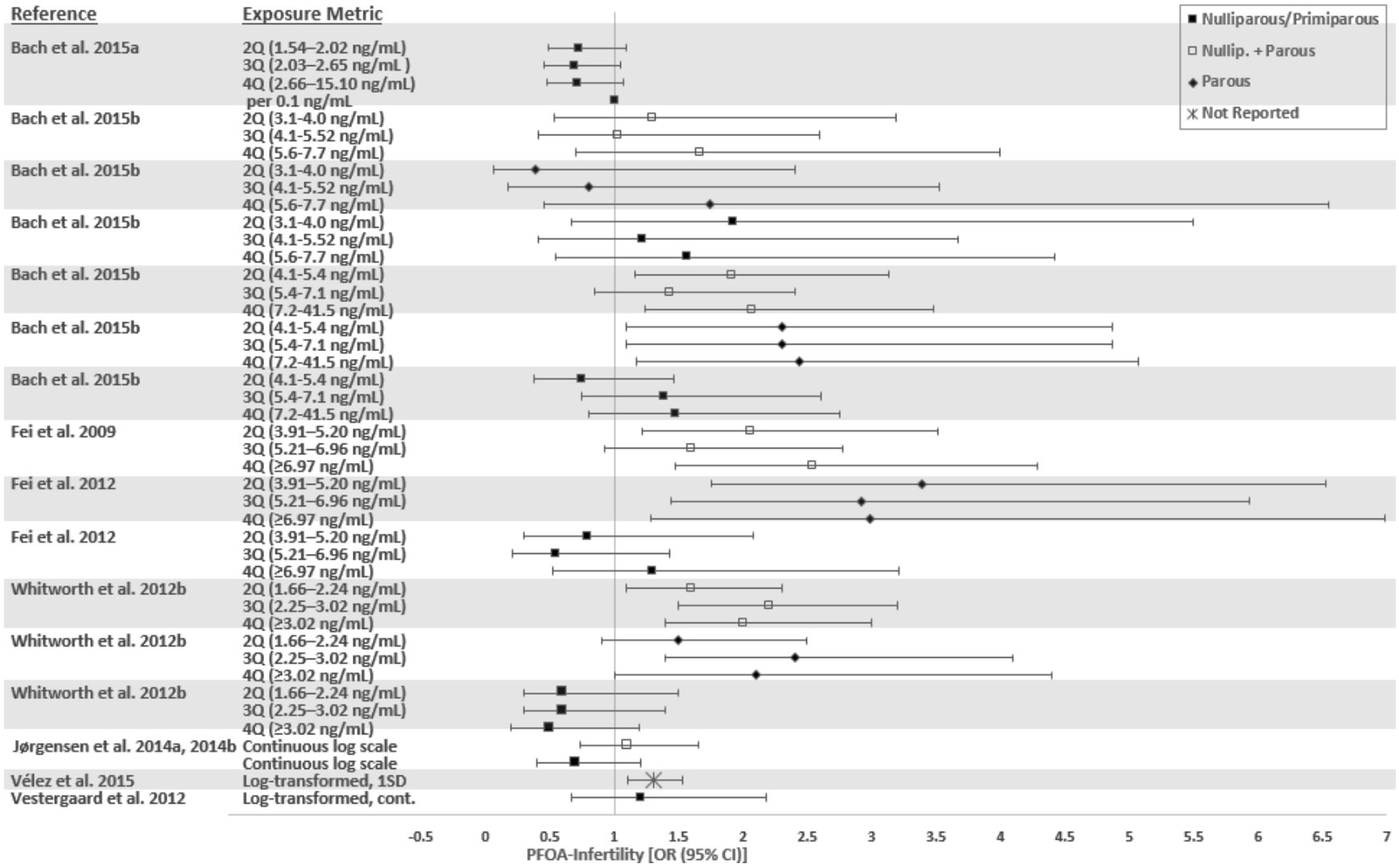
In a study of pregnant women participating in the Danish National Birth Cohort study, a decrease in fecundability and an increase in infertility were observed in women with serum PFOA (measured at gestation week 12) levels in the three highest quartiles (Fei et al. 2009). When the women were categorized by parity, decreased fecundability OR and increased infertility OR were only found in the parous group; the ORs for the nulliparous women included unity (Fei et al. 2009). A second re-analysis of these data (Bach et al. 2015a) using a different statistical approach confirmed the results of the whole group and the parous subgroup; this re-analysis also found a decrease in the fecundability risk among the nulliparous women. In another set of women participating in the Danish National Birth Cohort study (Bach et al. 2015c), no alterations in fecundability or infertility risk were observed in the whole cohort or when the women were categorized into parous and nulliparous subcohorts. It was noted that the median serum PFOA levels in this second study (4.0 ng/mL) were lower than the levels in the larger study (5.4 ng/mL). A decrease in fecundability and an increase in infertility risk were also observed in a Canadian study of pregnant women (Vélez et al. 2015). An increase in infertility risk was also found in a Norwegian study of subfecund pregnant women with serum PFOA levels in the three highest quartiles (Whitworth et al. 2012b); when the women were categorized based on parity, the infertility risk was only elevated in the parous women with serum PFOA levels in the 3rd and 4th quartiles. A multinational study also found an alteration in fecundability (Jørgensen et al. 2014a); however, this study found that higher serum PFOA levels resulted in a decrease in the time to pregnancy (fecundability ratio >1) among primiparous women.

Figure 2-31. Fecundability Relative to PFOA Levels (Presented as Adjusted Fecundability Ratios)



KD0411

Figure 2-32. Infertility Relative to PFOA Levels (Presented as Adjusted Odds Ratios)



KD0412

Other studies of pregnant women have not found alterations in fecundability, fertility, and/or infertility (Bach et al. 2015a; Crawford et al. 2017; Jørgensen et al. 2014a; Lum et al. 2017; Wang et al. 2017; Whitworth et al. 2016). The two prospective studies which followed women intending to get pregnant for 6 months (Vestergaard et al. 2012) or 12 months (Buck Louis et al. 2013) also did not find associations between serum PFOA levels in women and fecundability; Buck Louis et al. (2013) also found no association when male serum PFOA was used as the biomarker of exposure.

Laboratory Animal Studies. Examination of the testes and epididymides of rats exposed intermittently head-only to up to 84 mg/m^3 APFO dusts for 2 weeks did not reveal any gross or microscopic treatment-related alterations (Kennedy et al. 1986).

Several studies have been conducted in rats to examine whether induction of Leydig cell tumors could be due to an endocrine-related mechanism. In a 14-day gavage study in which rats were dosed with up to 50 mg/kg/day PFOA, testes weight was not significantly affected and microscopic examination did not reveal any significant alterations (Cook et al. 1992). However, the weight of the accessory sex organ unit (ventral and dorsal lateral prostate, seminal vesicles, and coagulating glands) was significantly decreased in rats dosed with 25 mg/kg/day PFOA (17% decrease) and 50 mg/kg/day PFOA (18% decrease) relative to controls and to a pair-fed group. There was also a trend for reduced serum and interstitial fluid testosterone in PFOA-treated rats; serum LH was not altered and estradiol was significantly increased (63%) at ≥10 mg/kg/day. Challenge experiments conducted with human chorionic gonadotropin, gonadotropin-releasing hormone, or naloxone suggested that the decrease in serum testosterone was due to a lesion at the level of the testes. Serum levels of progesterone and 17α-hydroxyprogesterone were not altered by 50 mg/kg/day PFOA, but androstenedione levels were reduced 2-fold. The data suggested that the decrease in serum testosterone may be due to a decrease in the conversion of 17α-hydroxy-progesterone to androstenedione, and this could be attributed to the elevated serum levels of estradiol. The decrease in weight of the accessory sex organ unit could also be attributed to the elevated estradiol serum levels. In a subsequent study from the same group of investigators, rats dosed with 25 mg/kg/day PFOA for 14 days showed a significant increase in estradiol in serum and in testicular interstitial fluid relative to controls (Biegel et al. 1995). Treatment with PFOA for 14 days significantly increased aromatase activity in the liver (aromatase converts testosterone to estradiol), but not in testes, muscle, or adipose tissue, suggesting that PFOA increases serum estradiol by inducing aromatase activity in the liver. Treatment with PFOA also increased testicular interstitial fluid transforming growth factor α (TGFα). Collectively, the results were consistent with the hypothesis that increased estradiol levels ultimately produce Leydig cell hyperplasia and adenoma by acting as a mitogen or enhancing growth

factor secretion. A study of the dose-response relationship for PFOA and serum estradiol reported a significant increase in serum estradiol in rats dosed with ≥2 mg/kg/day, which was well correlated with total hepatic aromatase activity (Liu et al. 1996). Significant increases in serum estradiol were also reported during the first year of treatment of male rats with 13.6 mg/kg/day PFOA in a 2-year dietary study (Biegel et al. 2001).

Significant increases in the incidence of Leydig cell hyperplasia were observed in rats exposed to 13.6 mg/kg/day PFOA in the diet for 2 years (Biegel et al. 2001). Another 2-year study found an increased incidence of vascular mineralization in the testes of rats exposed to 15 mg/kg/day PFOA in the diet; no effects were observed at 1.5 mg/kg/day (3M 1983; Butenhoff et al. 2012c). In female rats, increases in the incidence of tubular hyperplasia of the ovaries were observed following a 2-year exposure to 1.5 mg/kg/day (3M 1983; Butenhoff et al. 2012c). A peer review of the histological slides from this study (3M 1983; Butenhoff et al. 2012c) concluded that the more current nomenclature for the tubular hyperplasia was gonadal stromal hyperplasia (Mann and Frame 2004). Additionally, the peer reviewers substantially disagreed with the incidence of lesions in the 1.5 mg/kg/day group and slightly disagreed with the incidence in the 15 mg/kg/day group. Based on the incidence reported by the peer reviewers, no statistically significant increases in the occurrence of gonadal stromal hyperplasia were observed in either group; a significant increase in grade 3 and above lesions were observed in the 15 mg/kg/day group.

In a 2-generation reproduction study in which male and female rats were dosed with up to 30 mg/kg/day PFOA by gavage in water for 70 days before mating and until sacrifice, there were no effects on estrous cycling, sperm number and quality, mating and fertility, or histopathology of the reproductive organs assessed in the parental and F1 generations (Butenhoff et al. 2004b). Intermediate-duration studies of rats and monkeys also did not find gross or microscopic alterations in the sex organs at termination; Cynomolgus monkeys were dosed with up to 20 mg/kg/day PFOA for 4 or 26 weeks (Butenhoff et al. 2002; Thomford 2001), Rhesus monkeys with up to 100 mg/kg/day PFOA for 13 weeks (Griffith and Long 1980), and rats with up to approximately 100–110 mg/kg/day PFOA for 13 weeks (Griffith and Long). Serum levels of estradiol and estriol were not significantly altered in the 4-week study conducted by Thomford (2001), but estrone was reduced in monkeys dosed with 2 and 20 mg/kg/day PFOA; no possible explanation was discussed. In the 26-week study (Butenhoff et al. 2002), no treatment-related alterations were reported in serum estrone, estriol, estradiol, or testosterone, indicating that the reduced serum estrone levels in the 4-week study was transitory. In 2-year dietary studies in rats, doses of 13.6 mg/kg/day PFOA significantly increased the incidence of Leydig cell hyperplasia (Biegel et al. 2001), whereas 15 mg/kg/day increased the incidence of vascular mineralization in the testes and

1.5 mg/kg/day increased the incidence of tubular hyperplasia in the ovaries (3M 1983; Butenhoff et al. 2012c).

A study in pregnant mice dosed with 5 mg/kg/day PFOA (only dose level tested) reported that the mammary gland showed changes suggesting substantial delay (possibly up to 10 days) in gland differentiation on PND 20 and alterations in milk protein gene expression on PND 20 (White et al. 2007). Subsequent studies by this group support the finding of delayed mammary gland differentiation. On PND 1, the mammary glands of mice administered 5 mg/kg/day on GDs 8–17 appeared immature; the morphology was similar to that seen in late pregnancy prior to parturition and the initiation of nursing (White et al. 2009). Another study found that the normal weaning-induced mammary gland involution was compromised on PND 22 in mice exposed to 1 mg/kg/day on GDs 1–17 or 0.001 mg/kg/day administered on GD 7–PND 22 (White et al. 2011); the investigators noted that the mammary gland structure was similar to mammary gland tissue at or near the peak of lactation (PND 10). Necrosis was observed in the placenta of mice administered via gavage 10 or 25 mg/kg/day PFOA on GDs 11–16 (Suh et al. 2011); no alterations were observed at 2 mg/kg/day.

A study of pregnant mice reported increases in serum estradiol levels, with no changes in progesterone levels, at 10 mg/kg/day when PFOA was administered on GDs 1–7 (Chen et al. 2017b); however, when PFOA was administered on GD 13, there were significant decreases in serum progesterone levels at 5 and 10 mg/kg/day with no changes in estradiol levels (Chen et al. 2017b). In peripubertal female mice, administration of 5 mg/kg PFOA 5 days/week for 4 weeks resulted in significant increases in serum progesterone levels during estrus and preestrus, but no changes in estradiol levels were observed (Zhao et al. 2010).

No gross or microscopic alterations were reported in the testes from rats dermally exposed to 2,000 mg/kg/day APFO (Kennedy 1985).

Summary. Epidemiological studies have examined a several types of reproductive endpoints. Due to inconsistent results, the available data are not suitable for determining whether there are associations between serum PFOA and reproductive hormones or effects on sperm. There is some suggestive evidence that increases in serum PFOA levels can result in earlier onset of menopause; however, this is based on the findings of two studies (a third study did not find an association) and may partially be due to reverse causation. Several general population studies found associations between serum PFOA and impaired fertility (increased time to pregnancy and/or infertility), while others have not found

associations. The available epidemiological data are considered inadequate for determining whether there is an association between serum PFOA and fertility. The database limitations include inconsistency across studies, small number of studies including measurements of male serum PFOA levels, findings in parous women but not nulliparous women, and the underrepresentation of couples not becoming pregnant. The results of a multi-generational study in rats do not suggest that the reproductive system is a sensitive target of PFOA toxicity. Additionally, histological alterations have not been observed in monkeys or rats following intermediate and/or chronic oral exposure.

PFOS

Epidemiological Studies—Reproductive Hormone Levels. In an occupational exposure study of workers at 3M Decatur and Antwerp facilities (Olsen et al. 1998a) and a general population study (Raymer et al. 2012), no associations between serum PFOS and reproductive hormones were found. Studies in adolescent and young adult males have found inverse associations between serum PFOS levels and total and free testosterone levels (Joensen et al. 2013), free androgen index (Joensen et al. 2013), and FSH levels (Tsai et al. 2015). Another study of young men did not find alterations in reproductive hormone levels (Vested et al. 2013).

In a study of females participating in the C8 Health Studies, serum PFOS levels were inversely associated with estradiol levels in both perimenopausal and menopausal women (Knox et al. 2011b). An inverse association with follicular estradiol levels was also observed in a general population study (Barrett et al. 2015); when segregated by parity, the inverse association was only found in nulliparous women. An inverse association between serum PFOS levels and testosterone levels was observed in adolescent females; no association was found in older females (Tsai et al. 2015). A general population study of adolescents (aged 13–15 years) found an inverse association between serum PFOS levels and testosterone levels in boys, but not in girls; the study also found no associations with estradiol levels in boys or girls (Zhou et al. 2016).

Epidemiological Studies—Effects on Sperm. The available general population data do not provide evidence that PFOS damages sperm. One study (Buck Louis et al. 2015) found an association for one measure of sperm motility (distance travelled) but not for other measures. Another study (Toft et al. 2012) found an inverse association between serum PFOS levels and percentage of normal sperm. Other studies have not found alterations in sperm viability, count, motility, volume, or morphology (Buck Louis et al. 2015; Joensen et al. 2013; Raymer et al. 2012; Toft et al. 2012; Vested et al. 2013). A multinational

study (Kvist et al. 2012) found a nonlinear association between serum PFOS and Y-X chromosome ratio; however, when categorized by country, the only significant trend was a negative trend in the Greenland cohort. It is noted that these are studies of individuals exposed to background levels of PFOS, involved a single measurement of PFOS, and are not adequate for establishing causality.

Epidemiological Studies—Effects on Menstrual Cycle Length, Menopause Onset, Endometriosis, and Breastfeeding Duration. No alterations in the risk of irregular, short, or long menstrual cycle lengths associated with serum PFOS levels were observed in a study of pregnant women (Lyngsø et al. 2014). Similarly, no association between serum PFOS levels and menstrual cycle length was observed in another study (Lum et al. 2017). A study of C8 Health Study participants found increases in the risk of early menopause in perimenopausal and menopausal women with serum PFOS levels in the $\geq 3^{rd}$ and $\geq 2^{nd}$ quintiles, respectively (Knox et al. 2011b). In contrast, a study of NHANES participants did not find an association between serum PFOS and the risk of early menopause (Taylor et al. 2014). The risk of endometriosis was not associated with serum PFOS levels (Buck Louis et al. 2012; Campbell et al. 2016). However, there was a greater risk of having moderate to severe endometriosis; adjusting for parity decreased the risk and the CIs included unity. General population studies found increases in the risk of having a hysterectomy in women having serum PFOS levels in the 2^{nd} and 3^{rd} tertiles (Taylor et al. 2014) and the risk of having polycystic ovary syndrome in women with serum PFOS levels in the 3^{rd} tertile (Vagi et al. 2014). Most of these endpoints were only examined in one study and the evidence is inconclusive to determine whether there is an association between PFOS exposure and these female reproductive outcomes.

Utilizing pharmacokinetic modeling, Ruark et al. (2017) and Ngueta et al. (2017) have investigated whether the observed associations between PFOS exposure and early onset menopause or risk of endometriosis was due to reverse causation. Menstrual blood loss is a route of elimination of perfluoroalkyls (see Section 3.1.4) and variability in menstruation such as menarche, menopause, and pharmacological management of menstruation (e.g., use of oral contraceptives) could affect serum perfluoroalkyl levels, and thereby contribute to observed statistical associations between serum PFOS levels and early onset menopause (Ruark et al. 2017) or endometriosis (Ngueta et al. 2017) outcomes.

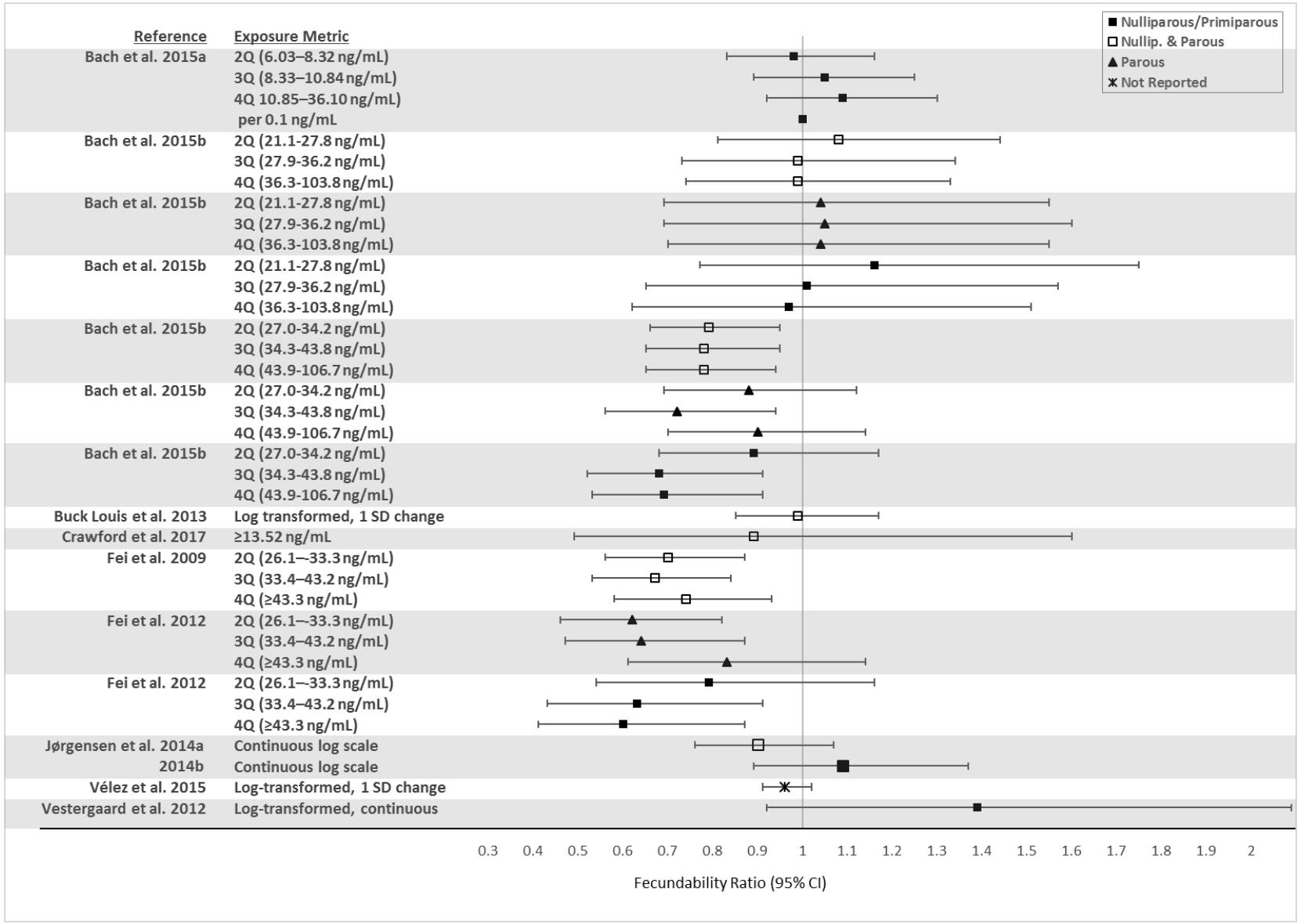
Maternal serum PFOS levels have been associated with increases in the risk of breastfeeding for ≤ 3 or 6 months (Fei et al. 2010; Romano et al. 2016) and inversely associated with the length of breastfeeding and the length of exclusive breastfeeding (Timmermann et al. 2017). When the women were segregated by parity, the associations were only found in multiparous women (Fei et al. 2010). In contrast,

Timmermann et al. (2017) found no significant alterations in breastfeeding length or exclusiveness between primiparous women and multiparous women. In general, these studies did not consider whether other factors such as the duration of breastfeeding including diminished milk production, inadequate lactation support from health care providers after delivery, use of medication that is not compatible with breastfeeding, lack of spousal/family support, and individual choice may have influenced the observed associations. Additionally, the associations between maternal PFOS and breastfeeding duration may be due to reverse causality since longer breastfeeding would likely result in lower maternal PFOS levels.

Epidemiological Studies—Effects on Fertility. Several general population studies have evaluated whether there is a possible association between serum PFOS and time-to-pregnancy (as measured using a fecundability ratio) or infertility; graphical presentations of potential associations between fecundability and infertility relative to serum PFOA levels are presented in Figures 2-33 and 2-34, respectively. A couple of studies have found associations, but most have not found associations. Fei et al. (2009) found decreases in fecundability and increases in infertility risk among pregnant women with serum PFOS levels in the top three quartiles. When the women were categorized by parity (Fei et al. 2012), the decrease in fecundability and increase in infertility risk were only observed in nulliparous women with serum PFOS levels in the 3rd and 4th quartiles; no alterations were observed among parous women. A re-analysis of these data (Bach et al. 2015c) resulted in similar associations between PFOS and fecundability and infertility. Whitworth et al. (2012b) also found an increased risk of infertility among subfecund women with serum PFOS levels in the 3rd quartile; categorizing by parity resulted in increases in only parous women with serum PFOS levels in the 4th quartile. In contrast, other studies have not found alterations in fecundability or fertility associated with maternal serum PFOS levels (Bach et al. 2015a, 2015c; Buck Louis et al. 2013; Crawford et al. 2017; Jørgensen et al. 2014a; Lum et al. 2017; Vélez et al. 2015; Vestergaard et al. 2012; Wang et al. 2016; Whitworth et al. 2016).

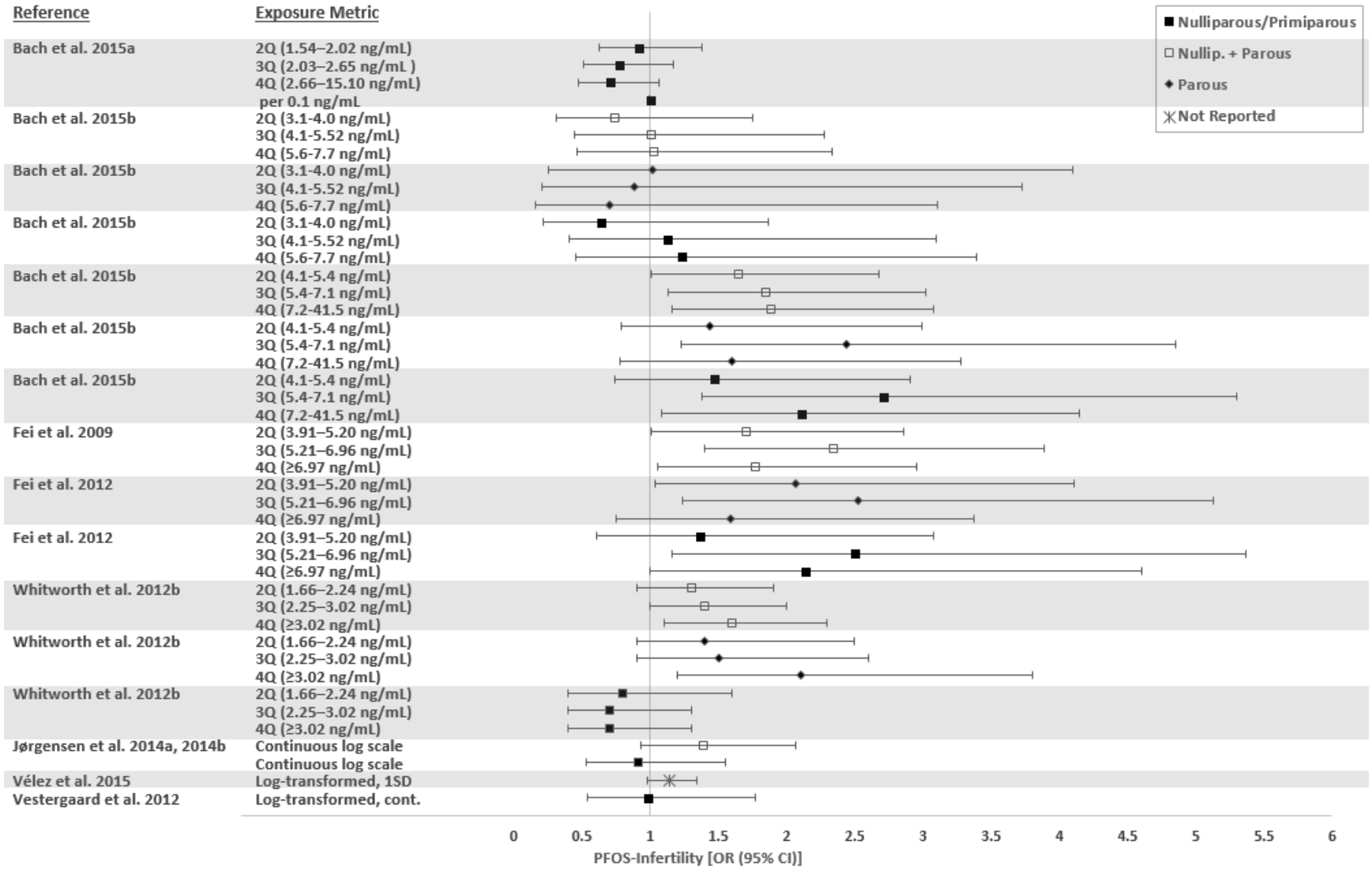
Laboratory Animal Studies. Significant decreases in serum testosterone levels and epididymal sperm count were observed in mice administered 10 mg/kg/day PFOS for 21 days (Wan et al. 2011), in rats administered 5 mg/kg/day for 21 days (Li et al. 2018), and in mice administered 10 mg/kg/day for 5 weeks (Qu et al. 2016). No alterations were observed in mice administered 5 mg/kg/day PFOS or in mice administered 5 or 10 mg/kg/day PFOS for 14 days (Wan et al. 2011). No alterations in reproductive performance (number of litters, gestation length, number of implantation sites, or potential resorptions) were observed in rats administered 1 mg/kg/day PFOS throughout gestation and lactation (Buttenoff et al. 2009b). Lee et al. (2015a) did find a decrease in placental weight and placental capacity (ratio of fetal weight to placental weight) in mice administered ≥0.5 mg/kg/day PFOS via gavage on GDs 11–16.

Figure 2-33. Fecundability Relative to PFOS Levels (Presented as Adjusted Fecundability Ratios)



KD0419

Figure 2-34. Infertility Relative to PFOS Levels (Presented as Adjusted Odds Ratios)



KD0420

Multigeneration studies with PFOS in rats did not provide indications of reproductive toxicity. Exposure of male and female rats to up to 3.2 mg/kg/day PFOS by gavage before mating and continuing during gestation did not affect mating or fertility parameters of the parental or F1 generation (Luebker et al. 2005a, 2005b). Dietary exposure of rats to 1.3–1.8 mg/kg/day PFOS for 4 or 14 weeks did not induce gross or microscopic alterations in the sex organs of males or females (Seacat et al. 2003). A similar study in Cynomolgus monkeys administered up to 0.75 mg/kg/day PFOS administered via a capsule also reported no significant morphological alterations in the sex organs, but serum estradiol was significantly decreased in males on days 62, 91, and 182 of the study (Seacat et al. 2002). In addition, treatment with PFOS had no significant effect on cell proliferation in the testes. Serum estradiol also was lower than in controls in one male and one female monkey dosed with 2 mg/kg/day PFOS for 4 weeks, but little can be concluded from results from just two animals (Thomford 2002a). In a 2-year dietary study in rats, administration of up to 1.04 mg/kg/day PFOS did not induce gross or microscopic alterations in the reproductive organs (Butenhoff et al. 2012b; Thomford 2002b). Overall, the reproductive system does not seem to be a sensitive target of PFOS toxicity, although some changes in testosterone and estradiol levels and decreases in sperm count have been observed.

PFHxS

Epidemiological Studies—Reproductive Hormone Levels. Three general population studies evaluated possible effects of PFHxS on reproductive hormone levels. In young men, no associations between serum PFHxS levels and testosterone, free androgen index, LH, estradiol, sex hormone binding globulin, or FSH levels were found (Joensen et al. 2013). Similarly, no alterations in follicular estrogen or luteal progesterone were observed in women (Barrett et al. 2015). An association between serum PFHxS levels and estradiol levels were observed in adolescent boys, but not in girls; no associations were observed for testosterone levels (Zhou et al. 2016).

Epidemiological Studies—Effects on Sperm. With the exception of the finding of an inverse association between serum PFHxS levels and percent normal sperm (Toft et al. 2012), general population studies have not found associations between PFHxS and sperm parameters (Joensen et al. 2013; Toft et al. 2012); it is noted that the Joensen et al. (2013) study of young men did not find alterations in sperm morphology.

Epidemiological Studies—Effects on Menstrual Cycle Length, Menopause Onset, Endometriosis, and Breastfeeding Duration. Five general population studies have evaluated possible associations between serum PFHxS levels and female reproductive outcomes. Taylor et al. (2014) reported increases in the risk

of earlier menopause in women with serum PFHxS levels in the 3rd tertile and the risk of hysterectomy in women with serum PFHxS levels in the 2nd and 3rd tertiles. These findings may be due to reverse causation in that early menopause may result in higher serum PFHxS levels. Other studies did not find associations with the risk and severity of endometriosis (Buck Louis et al. 2012; Campbell et al. 2016) or polycystic ovary syndrome (Vagi et al. 2014).

Romano et al. (2016) did not find associations between maternal PFHxS levels and the risk of breastfeeding ≤3 or 6 months. Similarly, Timmermann et al. (2017) did not find associations between maternal PFHxS levels and the length of breastfeeding or length of exclusive breastfeeding.

Epidemiological Studies—Effects on Fertility. Seven studies have evaluated possible effects on fertility associated with female serum PFHxS levels. Vélez et al. (2015) found increases in time to pregnancy (measured as a decreased fecundability OR) and risk of infertility, which were associated with serum PFHxS levels in pregnant women. Vestergaard et al. (2012) reported an increase in the fecundability OR, indicating a shorter time to pregnancy, when risk was calculated using continuous serum PFHxS; however, when the subjects were divided into two groups based on serum PFHxS levels above and below the median level, the fecundability ratio included unity in the above-median group (fecundability ratio 1.29, 95% CI 0.90–1.83), as compared to the below-median group. Wang et al. (2017) found a decreased risk of endometriosis-related infertility in a case-control study. Studies by Bach et al. (2015a), Crawford et al. (2017), Jørgensen et al. (2014a), and Whitworth et al. (2016) did not find alterations in time to pregnancy, fertility, or the risk of infertility.

Laboratory Animal Studies. Exposure to 10 mg/kg/day PFHxS did not result in alterations in reproductive organ weights or histopathology in male rats exposed for a minimum of 42 days beginning 14 days prior to cohabitation and female rats sacrificed on lactation day 21 or GD 25 (rats that did not deliver a litter) (exposure began 14 days prior to cohabitation) (Butenhoff et al. 2009a). Fertility was not affected by treatment with PFHxS and there were no significant effects on sperm parameters. Also, estrous cycling was not affected by dosing with PFHxS. A similarly designed study in mice also reported no alterations in reproductive toxicity parameters (Chang et al. 2018).

PFNA

Epidemiological Studies—Reproductive Hormone Levels. Reproductive hormone alterations associated with serum PFNA levels are limited to a finding for estradiol in young men (Joensen et al. 2013); no

associations with other reproductive hormones were found in this study. In another study of adolescent and young adults, no associations between serum PFNA and sex hormone binding globulin, FSH, or testosterone were found in males or females (subjects were segregated by sex and age range) (Tsai et al. 2015). Zhou et al. (2016) found an inverse association between serum PFNA and testosterone levels in boys, but not in girls, and did not find associations for estradiol levels. Another study did not find alterations in follicular estradiol or luteal progesterone levels in women (Barrett et al. 2015).

Epidemiological Studies—Effects on Sperm. Buck Louis et al. (2015) found associations between serum PFNA and increases in the percentage of normal sperm and a decrease in the percentage of sperm with coiled tails. No associations were found for other sperm parameters (Buck Louis et al. 2015; Joensen et al. 2013; Toft et al. 2012).

Epidemiological Studies—Effects on Menstrual Cycle Length, Menopause Onset, Endometriosis, and Breastfeeding Duration. No association between serum PFNA levels and menstrual cycle length was observed in a general population study (Lum et al. 2017). Increases in the risk of earlier menopause and hysterectomy were found in women with serum PFNA levels in the 3rd and ≥2nd serum PFNA tertiles (Taylor et al. 2014). The investigators examined the possibility that these effects may be due to reverse causation and found that serum PFNA levels increased post-menopause (Taylor et al. 2014). An increase in the risk of endometriosis was associated with serum PFNA levels in a general population study (Buck Louis et al. 2012); however, adjustment for parity resulted in OR CIs that included unity. A second study did not find an association between serum PFNA and self-reported endometriosis (Campbell et al. 2016). Vagi et al. (2014) did not find an increased risk of polycystic ovary syndrome that was associated with serum PFNA levels.

No associations between maternal PFNA levels and the risk of breastfeeding ≤3 or 6 months were found in a general population study (Romano et al. 2016). In contrast, Timmermann et al. (2017) found inverse associations between maternal PFNA levels and breastfeeding length and the length of exclusive breastfeeding. The study also found no differences in breastfeeding length or exclusiveness between primiparous and multiparous women.

Epidemiological Studies—Effects on Fertility. Jørgensen et al. (2014a) found increases in time to pregnancy (measured as a decrease in fecundability ratio) and an increase in infertility risk in a study of pregnant women. In sensitivity analysis, the fecundability ratio for primiparous women was 0.99 and the 95% CI range included unity (0.88–1.22). Wang et al. (2016) found an inverse association between

serum PFNA levels in women and the risk of endometriosis-induced infertility. Studies by Bach et al. (2015a), Buck Louis et al. (2013), Crawford et al. (2017), Lum et al. (2017), Vestergaard et al. (2012), and Whitworth et al. (2016) did not find associations between serum PFNA levels and fecundability ratio, fertility or risk of infertility.

Laboratory Animal Studies. Two acute-duration studies have evaluated the reproductive toxicity of PFNA in male rats (Feng et al. 2009, 2010). Gavage administration of 5 mg/kg/day for 14 days resulted in decreases in serum testosterone and increases in serum estradiol levels and atrophy of the seminiferous tubules (Feng et al. 2009). Electron microscopic examination of the testes revealed large vacuoles between the Sertoli cells and spermatogonia at 5 mg/kg/day; these changes as well as increases in serum Mullerian inhibiting substance and decreases in serum inhibin B cells were suggestive of damage to the secretory function of the Sertoli cells (Feng et al. 2010). In mice administered 0.5 mg/kg/day PFNA for 90 days, decreases in sperm motility, viability, and count and degenerative changes in the seminiferous tubules were observed (Singh and Singh 2018). When the mice were mated with unexposed females, significant decreases in litter size were observed at 0.5 mg/kg/day.

PFDA

Epidemiological Studies—Reproductive Hormone Levels. No associations were found between serum PFDA levels and testosterone, free androgen index, LH, estradiol, sex hormone binding globulin, or FSH levels in young men (Joensen et al. 2013). Similarly, no alterations in follicular estradiol or luteal progesterone levels were observed in women (Barrett et al. 2015). In adolescent boys, an inverse association between serum PFDA and testosterone was found; no association was found in girls (Zhou et al. 2016). This study also found no associations for estradiol levels in boys or girls.

Epidemiological Studies—Effects on Sperm. Two general population studies evaluated potential effects of PFDA exposure on sperm parameters. Buck Louis et al. (2015) found associations between serum PFDA levels and increases in sperm head length and decreases in the percentage of sperm with coiled tails. No alterations were found for sperm viability, count, volume, motility, or other morphological alterations (Buck Louis et al. 2015; Joensen et al. 2013).

Epidemiological Studies—Effects on Menstrual Cycle Length, Menopause Onset, Endometriosis, and Breastfeeding. Three studies examined alterations in female reproductive outcomes associated with serum PFDA levels. In two studies, no associations between serum PFDA levels and the risk or severity

of endometriosis were found (Buck Louis et al. 2012; Lum et al. 2017). In the third study, an inverse association between maternal PFDA levels and duration of breastfeeding was found (Timmermann et al. 2017). No association was found for the length of exclusive breastfeeding.

Epidemiological Studies—Effects on Fertility. Six studies examined the potential for PFDA to alter fertility. No alterations in time to pregnancy (measured as fecundability ratio) or risk of infertility were observed in pregnant women (Bach et al. 2015a). Additionally, no associations with the probability of pregnancy (Lum et al. 2017), endometriosis-related infertility (Wang et al. 2017), or fecundability (Whitworth et al. 2016) were observed in other general population studies. Two prospective studies also found no association between female serum PFDA levels (Buck Louis et al. 2013; Vestergaard et al. 2012) or male serum PFDA levels (Buck Louis et al. 2013) and time to pregnancy.

PFUnA

Epidemiological Studies—Reproductive Hormone Levels. An inverse association between serum PFUnA levels and FSH levels was observed in adolescent girls (Tsai et al. 2015). The study did not find alterations in sex hormone binding globulins or testosterone levels in adolescent and young adult males or females. Another study of women did not find alterations in follicular estradiol or luteal progesterone levels (Barrett et al. 2015).

Epidemiological Studies—Effects on Fertility. Three studies evaluated possible associations between maternal serum PFUnA levels and fertility. No alterations in time to pregnancy (measured as a fecundability ratio) or infertility risk (Bach et al. 2015a), endometriosis-related infertility risk, or fecundability (Whitworth et al. (2016) were observed.

PFHpA

Epidemiological Studies. Only one study examined potential fertility associations. Wang et al. (2017) found a decreased risk of endometriosis-related infertility in a case-control study.

PFBS

***Epidemiological Studies*—**Two studies have evaluated potential association for reproductive outcomes. Zhou et al. (2016) did not find associations between serum PFBS and testosterone or estradiol levels in adolescent boys or girls. Wang et al. (2017) found an association between serum PFBS levels and endometriosis-related infertility in a case-control study.

Laboratory Animal Studies. Administration of up to 900 mg/kg/day PFBS to rats by gavage for 28 days did not cause any significant gross or microscopic alterations in primary or secondary sex organs from males or females (3M 2001). A 2-generation study in which rats were exposed to gavage doses of potassium PFBS as high as 1,000 mg/kg/day did not result in alterations in fertility, sperm parameters, estrus cycling, or histological alterations in reproductive tissues (Lieder et al. 2009b).

PFBA

Laboratory Animal Studies. No significant gross or microscopic alterations were reported in primary and secondary reproductive organs from rats dosed with PFBA by gavage in doses of up to 184 mg/kg/day for 5 days (3M 2007a), 150 mg/kg/day for 28 days (Butenhoff et al. 2012a; van Otterdijk 2007a), or 30 mg/kg/day for 90 days (Butenhoff et al. 2012a; van Otterdijk 2007b).

PFDoDA

***Epidemiological Studies*—**A study in adolescent boys and girls found an inverse association between serum PFDoDA levels and testosterone levels in girls only; no associations were found for estradiol levels (Zhou et al. 2016). In the two studies evaluating fertility, no associations were found for endometriosis-related infertility (Wang et al. 2017) or fecundability (Whitworth et al. 2016).

Laboratory Animal Studies. Treatment of male rats with 1, 5, or 10 mg/kg/day PFDoDA by gavage for 14 days induced a dose-related decrease in testes weight, which achieved statistical significance at 10 mg/kg/day (Shi et al. 2007). Measurement of serum hormone levels showed a significant decrease in LH at 10 mg/kg/day and in testosterone at 5 and 10 mg/kg/day, no significant effect on FSH levels, and a significant decrease in serum estradiol only at 5 mg/kg/day. Alterations in the ultrastructure of the testes were seen in the 5 and 10 mg/kg/day groups and consisted of the presence of large clustered lipid droplets and enlarged mitochondria in Sertoli cells, large vacuoles, and expanded mitochondria in Leydig and

spermatogenic cells. Morphological features of apoptosis were seen in cells in the 10 mg/kg/day group. Assessment of messenger ribonucleic acid (mRNA) expression of genes involved in cholesterol transport and steroidogenesis provided evidence of altered cholesterol transport and steroid hormone synthesis, but no effects were noted for LH receptor and aromatase mRNA expression. Considering that serum total cholesterol was unaffected at 5 mg/kg/day and increased at 10 mg/kg/day and that aromatase expression was unaffected, the decrease in testosterone synthesis probably resulted from decreased steroidogenesis gene expression. In a longer-duration study (110 days) conducted by these investigators, decreased serum testosterone levels were observed at 0.2 and 0.5 mg/kg/day (Shi et al. 2009a). A third study (Kato et al. 2015) evaluated reproductive performance and found no alterations in estrous cycling during the first 14 days of exposure and no alterations in fertility, number of corpora lutea, or number of implantation sites in male and female rats administered 2.5 mg/kg/day PFDoDA for 14 days prior to mating and during gestation. In pregnant females administered 2.5 mg/kg/day, hemorrhages were observed at the implantation sites; only one female delivered live pups and 58% of the animals died or were sacrificed early. In females exposed for 42 days and not mated, continuous diestrus was observed at 2.5 mg/kg/day (Kato et al. 2015).

PFHxA

Epidemiological Studies—The only epidemiological study evaluating reproductive outcomes associated with PFHxA found an inverse association for testosterone levels in adolescent boys (Zhou et al. 2016) but did not find this association in girls and found no association with estradiol levels.

Laboratory Animal Studies. No alterations in mating, fertility, or gestation length were observed in rats administered TWA doses of 315 mg/kg/day PFHxA for 14 days prior to mating and during mating and gestation (Kirkpatrick 2005). Similarly, no alterations in mating, fertility, gestation length, number of implantation sites, estrous cycling, or sperm parameters were observed in rats administered up to 500 mg/kg/day NaPFHx for 70 days prior to mating, during the mating period, and throughout gestation and lactation (Loveless et al. 2009). A 90-day study did not find histological alterations in reproductive tissues of male or female rats administered up to 200 mg/kg/day NaPFHx (Chengelis et al. 2009b).

FOSA

Epidemiological Studies. One study examined reproductive hormone levels and did not find an association between serum FOSA and follicular estradiol or luteal progesterone levels in women (Barrett

et al. 2015). Two prospective epidemiological studies evaluated the possible association between FOSA and fertility. Vestergaard et al. (2012) did not find an increase in time to pregnancy, as measured as a fecundability ratio, or decrease in the likelihood of becoming pregnant within the first six menstrual cycles. In contrast, Buck Louis et al. (2013) found an increased time to pregnancy associated with serum FOSA levels in women, but not in men; the investigators noted that the results should be interpreted cautiously because only 10% of the blood samples had FOSA levels above the limit of detection. Another study found no association between maternal FOSA and fecundability (Whitworth et al. 2016).

2.17 DEVELOPMENTAL

Overview. A large number of epidemiological studies have examined the potential of developmental toxicity of perfluoroalkyls in the general population and in populations living in an area with high PFOA drinking water contamination. Epidemiological studies are available for 10 of the 12 perfluoroalkyls discussed in the profile; no developmental data were identified for PFHxA or PFBS. The discussion of these developmental outcomes is divided into four categories: pregnancy outcome, birth outcome, neurodevelopment, and sexual maturation. The epidemiological studies examining pregnancy outcome are summarized in Table 2-22; the pregnancy outcomes include miscarriage, stillbirth, preterm birth, and gestation age. Table 2-23 summarizes the epidemiological studies examining birth outcomes, which include birth weight, birth size, low birth weight, small for gestational age, birth defects, and sex ratio. Epidemiological studies examining neurodevelopmental endpoints, particularly risks for ADHD, are summarized in Table 2-24. Studies evaluating possible associations between serum perfluoroalkyl levels and development of the reproductive system are summarized in Table 2-25. Further details on these studies are presented in the *Supporting Document for Epidemiological Studies for Perfluoroalkyls*, Table 13. Studies examining childhood growth and examining the possible relationship between maternal serum perfluoroalkyl levels and body weight and BMI in children and adults are discussed in Section 2.3, Body Weight.

In general, the epidemiological studies did not find associations between perfluoroalkyl exposure and adverse pregnancy outcomes (miscarriage, preterm birth, or gestational age) for PFOA, PFOS, PFHxS, PFNA, PFDA, or PFUnA. Mixed results have been found for birth outcomes, particularly birth weight. Some epidemiological studies have found associations between maternal PFOA or PFOS exposure and decreases in birth weight, and meta-analyses of these data have found that increases in maternal PFOA or PFOS were associated with 11–19 g or 1–5 g decreases in birth weight, respectively; accounting for maternal glomerular filtration rates attenuated these results by about 50%. No consistent associations for

Table 2-22. Summary of Pregnancy Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFOA			
Darrow et al. 2013 Community (C8) (n=1,330 women)	≥37.2 ng/mL (5th PFOA quintile)	Preterm birth	OR 1.01 (0.55–1.86)
Darrow et al. 2014 Community (C8) (n=1,129 women)	>39.4 ng/mL (5th PFOA quintile)	Miscarriage risk Parous subgroup Nulliparous subgroup	OR 1.00 (0.63–1.58), 5th quintile OR 1.06 (0.57–1.97), 5th quintile OR 0.81 (0.38–1.71), 5th quintile
Savitz et al. 2012a Community (C8) (11,737 singleton infants)	63.1–934.3 ng/mL (4th maternal PFOA quartile)	Miscarriage	OR 0.9 (0.7–1.0)
		Stillbirth	OR 1.0 (0.5–1.8)
Savitz et al. 2012b Community (13,243 cases stillbirth, preterm birth, low birth weight or small for gestational age)	21.0–717.6 ng/mL (5th maternal PFOA quintile)	Stillbirth	OR 0.8 (0.5–1.5)
		Preterm birth (<37 weeks)	OR 1.0 (0.9–1.2)
		Preterm birth (<32 weeks)	OR 1.0 (0.7–1.3)
Savitz et al. 2012b Community (4,547 infants)	83.3–921.3 ng/mL (5th maternal PFOA quintile)	Preterm birth (<37 weeks)	OR 1.2 (0.9–1.6)
		Preterm birth (<32 weeks)	OR 1.4 (0.5–3.6)
Stein et al. 2009 Community (C8) (n=1,845 pregnancies)	48.8 ng/mL (maternal mean PFOA)	Miscarriage	OR 0.9 (0.5–1.6), >90th percentile
		Preterm birth	OR 0.9 (0.6–1.5), >90th percentile
Apelberg et al. 2007b General population (n=341 singleton births)	1.6 ng/mL (cord serum median PFOA)	Gestational age	NS (p>0.05)
Buck Louis et al. 2016 General population (n=332 couples)	3.3 ng/mL (median serum PFOA in women)	Pregnancy loss	HR 0.93 (0.75–1.16)
Chen et al. 2012a General population (n=429 infants)	1.84 ng/mL (cord blood geometric mean PFOA)	Gestational age	NS (p>0.05)
		Preterm birth	OR 0.64 (0.40–1.02)
Hamm et al. 2010 General population (n=252 pregnant women)	>2.1–18 ng/mL (maternal 3rd PFOA tertile)	Preterm birth	RR 1.31 (0.38–4.45), 3rd tertile

Table 2-22. Summary of Pregnancy Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Jensen et al. 2015 General population (n=56 cases and 336 controls)	1.58 ng/mL (maternal median PFOA)	Miscarriage before gestation week 12	OR 0.64 (0.36–1.18)
Lauritzen et al. 2017 General population (n=159 mother-infant pairs)	2.33 ng/mL (median maternal serum PFOA)	Gestational age	NS (p=0.318)
Lauritzen et al. 2017 General population (n=265 mother-infant pairs)	1.62 ng/mL (median maternal serum PFOA)	Gestational age	NS (p=0.431)
Li et al. 2017 General population (n=321 mother-infant pairs)	1.2 ng/mL (median cord serum PFOA)	Gestational age	β 0.16 (-0.02–0.33)
Lind et al. 2017a General population (n=649 pregnant women)	1.7 ng/mL (median maternal serum PFOA)	Gestational length	NS (p>0.05)
Manzano-Salgado et al. 2017a General population (n=1,202 mother-infant pairs)	2.35 ng/mL (mean maternal serum PFOA)	Preterm	OR 0.90 (0.60–1.35)
		Gestational age	β-0.05 (-0.12–0.08)
Sagiv et al. 2018 General population (n=1,645 pregnant women)	5.8 ng/mL (median maternal plasma PFOA)	Preterm	OR 1.0 (0.9–1.3)
		Gestation length	β -0.05 (-0.16–0.06)
Whitworth et al. 2012a General population (n=901 infants)	≥3.04 ng/mL (maternal 4th PFOA quartile)	Preterm birth	**OR 0.1 (0.03–0.6)*, 4th quartile**
Wu et al. 2012 General population (n=167 pregnant women at 2 hospitals)	18.32 and 9.76 ng/mL (mean maternal serum PFOA at each hospital)	Gestational age	**β -15.99 (-27.72 to -4.25, p<0.01)***

Table 2-22. Summary of Pregnancy Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFOS			
Darrow et al. 2013 Community (C8) (n=1,330 women)	15.6 ng/mL (mean PFOS)	Preterm birth	OR 1.07 (0.58–1.95)
Darrow et al. 2014 Community (C8) (n=1,129 women)	>23.3 ng/mL (5th PFOS quintile)	Miscarriage risk Parous subgroup Nulliparous subgroup	OR 1.41 (0.88–2.26), 5th quintile OR 1.12 (0.58–2.17), 5th quintile OR 2.02 (0.83–4.93), 5th quintile
Stein et al. 2009 Community (C8) (n=5,262 infants)	23.2–83.4 ng/mL (>90th PFOS percentile)	Miscarriage	OR 0.9 (0.7–1.3), >90th percentile
		Preterm birth	**OR 1.4 (1.1–1.7)*, >90th percentile**
Buck Louis et al. 2016 General population (n=332 couples)	12.2 ng/mL (median serum PFOS in women)	Pregnancy loss	HR 0.81 (0.65–1.00)
Chen et al. 2012a General population (n=429 infants)	5.94 ng/mL (cord blood geometric mean PFOS)	Preterm birth	**OR 2.45 (1.47–4.08)***
Fei et al. 2007, 2008a General population (n=1,400 pregnant women)	35.3 ng/mL (maternal median PFOS)	Gestation length	NS (p>0.01)
		Preterm birth	OR 1.43 (0.50–4.11), 4th quartile
Hamm et al. 2010 General population (n=252 pregnant women)	>10–35 ng/mL (maternal 3rd tertile PFOS)	Preterm birth	RR 1.11 (0.36–3.38), 3rd tertile
Jensen et al. 2015 General population (n=56 cases and 336 controls)	8.10 ng/mL (maternal median PFOS)	Miscarriage before gestation week 12	OR 1.16 (0.59–1.29)
Lauritzen et al. 2017 General population (n=159 mother-infant pairs)	16.4 ng/mL (median maternal serum PFOS)	Gestational age	NS (p=0.201)
Lauritzen et al. 2017 General population (n=265 mother-infant pairs)	9.74 ng/mL (median maternal serum PFOS)	Gestational age	NS (p=0.952)

Table 2-22. Summary of Pregnancy Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Li et al. 2017 General population (n=321 mother-infant pairs)	3.0 ng/mL (median cord serum PFOS)	Gestational age	β 0.11 (-0.06–0.29) **β 0.29 (0.05–0.53)*, boys only** β -0.07 (-0.35–0.20), girls only
Lind et al. 2017a General population (n=649 pregnant women)	8.1 ng/mL (median maternal serum PFOS)	Gestational length	NS (p>0.05)
Manzano-Salgado et al. 2017a General population (n=1,202 mother-infant pairs)	6.05 ng/mL (mean maternal serum PFOS)	Preterm	OR 1.10 (0.70–1.74)
		Gestational age	β-0.06 (-0.19–0.06)
Sagiv et al. 2018 General population (n=1,645 pregnant women)	25.7 ng/mL (median maternal plasma PFOS); 18.9–25.6 ng/mL (2nd quartile maternal PFOS)	Preterm	**OR 2.0 (1.1–3.7)*, 2nd quartile**
		Gestation length	β -0.08 (-0.17–0.02)
Whitworth et al. 2012a General population (n=901 infants)	13.0 and ≥16.59 ng/mL (maternal median and 4th quartile PFOS)	Preterm birth	**OR 0.3 (0.1–1.0, p=0.03)*, 4th quartile**
PFHxS			
Hamm et al. 2010 General population (n=252 pregnant women)	>1.4–43 ng/mL (maternal 3rd tertile PFHxS)	Preterm birth	**RR 0.31 (0.11–0.90)*, 3rd tertile**
Jensen et al. 2015 General population (n=56 cases and 336 controls)	0.298 ng/mL (maternal median PFHxS)	Miscarriage before gestation week 12	OR 1.53 (0.99–2.38)
Li et al. 2017 General population (n=321 mother-infant pairs)	3.9 ng/mL (median cord serum PFHxS)	Gestational age	β 0.12 (-0.03–0.27)
Lind et al. 2017a General population (n=649 pregnant women)	0.3 ng/mL (median maternal serum PFHxS)	Gestational length	NS (p>0.05)

Table 2-22. Summary of Pregnancy Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Manzano-Salgado et al. 2017a General population (n=1,202 mother-infant pairs)	0.58 ng/mL (mean maternal serum PFHxS)	Preterm	OR 0.85 (0.63–1.13)
		Gestational age	β -0.01 (-0.10–0.09)
Sagiv et al. 2018 General population (n=1,645 pregnant women)	2.4 ng/mL (median maternal plasma PFHxS)	Preterm	OR 1.0 (0.9–1.1)
		Gestation length	β 0.02 (-0.04–0.07)
PFNA			
Buck Louis et al. 2016 General population (n=332 couples)	1.2 ng/mL (median serum PFNA in women)	Pregnancy loss	HR 0.86 (0.70–1.06)
Chen et al. 2012a General population (n=429 infants)	2.36 ng/mL (cord blood geometric mean PFNA)	Preterm birth	OR 0.88 (0.71–1.11)
Jensen et al. 2015 General population (n=56 cases and 336 controls)	0.72 ng/mL (maternal median PFNA)	Miscarriage before gestation week 12	**OR 16.46 (7.39–36.62)***
Li et al. 2017 General population (n=321 mother-infant pairs)	0.2 ng/mL (median cord serum PFNA)	Gestational age	β -0.02 (-0.19–0.10)
Lind et al. 2017a General population (n=649 pregnant women)	0.7 ng/mL (median maternal serum PFNA)	Gestational length	NS (p>0.05)
Manzano-Salgado et al. 2017a General population (n=1,202 mother-infant pairs)	0.66 ng/mL (mean maternal serum PFNA)	Preterm	OR 0.87 (0.62–1.22)
		Gestational age	β -0.00 (-0.11–0.11)
Sagiv et al. 2018 General population (n=1,645 pregnant women)	0.7 ng/mL (median maternal plasma PFNA)	Preterm	OR 1.2 (1.0–1.4)
		Gestation length	β-0.07 (-0.17–0.02)

Table 2-22. Summary of Pregnancy Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFDA			
Buck Louis et al. 2016 General population (n=332 couples)	1.2 ng/mL (median serum PFDA in women)	Pregnancy loss	HR 0.83 (0.66–1.04)
Jensen et al. 2015 General population (n=56 cases and 336 controls)	0.27 ng/mL (maternal median PFDA)	Miscarriage before gestation week 12	**OR 2.30 (1.18–4.47)***
Li et al. 2017 General population (n=321 mother-infant pairs)	0.1 ng/mL (median cord serum PFDA)	Gestational age	β 0.10 (-0.09–0.29)
Lind et al. 2017a General population (n=649 pregnant women)	0.3 ng/mL (median maternal serum PFDA)	Gestational length	NS (p>0.05)
PFUnA			
Chen et al. 2012a General population (n=429 infants)	10.26 ng/mL (cord blood geometric mean PFUnA)	Preterm birth	OR 0.87 (0.64–1.16)
Li et al. 2017 General population (n=321 mother-infant pairs)	0.1 ng/mL (median cord serum PFUnA)	Gestational age	β 0.09 (-0.07–0.25)
PFHpA			
Li et al. 2017 General population (n=321 mother-infant pairs)	0.1 ng/mL (median cord serum PFHpA)	Gestational age	β 0.14 (-0.17–0.45)
PFBA			
Li et al. 2017 General population (n=321 mother-infant pairs)	0.1 ng/mL (median cord serum PFBA)	Gestational age	β 0.01 (-0.18–0.20)

Table 2-22. Summary of Pregnancy Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFDoDA			
Li et al. 2017 General population (n=321 mother-infant pairs)	0.1 ng/mL (median cord serum PFDoDA)	Gestational age	β 0.07 (-0.24 to 0.39)

[a]See the *Supporting Document for Epidemiological Studies for Perfluoroalkyls,* Table 13 for more detailed descriptions of studies.
[b]Participants in occupational exposure may have also lived near the site and received residential exposure; community exposure studies involved subjects living near PFOA facilities with known exposure to high levels of PFOA.
[c]Asterisk indicates association with perfluoroalkyl; unless otherwise specified, values in parenthesis are 95% confidence intervals.

OR = odds ratio; NS = not significant; PFDA = perfluorodecanoic acid; PFDoDA = perfluorododecanoic acid; PFHxS = perfluorohexane sulfonic acid; PFNA = perfluorononanoic acid; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid; PFUnA = perfluoroundecanoic acid; RR= risk ratio

Table 2-23. Summary of Birth Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFOA			
Darrow et al. 2013 Community (C8) (n=1,330 women)	≥37.2 ng/mL (5th PFOA quintile)	Birth weight	NS (p=0.70 for trend)
		Low birth weight	OR 0.92 (0.44–1.95)
Nolan et al. 2009 Community (n=1,555 singleton infants)	NR	Birth weight	NS (p>0.05)
		Low birth weight	**OR 0.37 (0.16–0.86)***
Nolan et al. 2010 Community (n=1,548 singleton infants)	NR	Congenital anomalies	OR 1.1 (0.34–3.3)
Savitz et al. 2012a Community (C8) (11,737 singleton infants)	63.1–934.3 ng/mL (4th maternal PFOA quartile)	Low birth weight	**OR 0.37 (0.16–0.86)***
		Birth defect	OR 1.0 (0.8–1.3)
Savitz et al. 2012b Community (13,243 cases stillbirth, preterm birth, low birth weight, or small for gestational age)	7.7 ng/mL (estimated maternal median PFOA)	Birth weight	β -14.80 (-42.28–13.68), per 100 ng/mL increase in PFOA
		Low birth weight	OR 1.0 (0.86–1.15), per 100 ng/mL increase in PFOA
		Small for gestational age	OR 0.86 (0.67–1.11), per 100 ng/mL increase in PFOA
Savitz et al. 2012b Community (4,547 infants)	13.4 ng/mL (estimated maternal median PFOA)	Low birth weight	OR 1.07 (0.96–1.18), per 100 ng/mL increase in PFOA
		Small for gestational age	**OR 1.08 (1.01–1.16)*, per 100 ng/mL increase in PFOA** OR 0.8 (0.6–1.2), for serum PFOA levels ≥80th percentile
		Birth weight	OR -12.76 (-26.08–0.57), per 100 ng/mL increase in PFOA
Stein et al. 2009 Community (C8) (n=1,845 pregnancies)	50.0–<120.6 and 120.6–894.4 ng/mL (3rd and 4th maternal PFOA quartile)	Low birth weight	OR 0.8 (0.3–1.9), 4th quartile
		Birth defects	OR 1.7 (0.8–3.6), 4th quartile

Table 2-23. Summary of Birth Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Stein et al. 2014c Community (C8) (n=10,262 infants)	61.3 ng/mL (estimated *in utero* mean PFOA)	Brain defects	OR 2.6 (1.3–5.1), interquartile range
		Gastrointestinal defects	OR 0.7 (0.3–1.4), interquartile range
		Kidney defects	OR 0.7 (0.3–1.8), interquartile range
		Craniofacial defects	OR 0.6 (0.3–1.3), interquartile range
		Eye defects	OR 1.1 (0.6–2.1), interquartile range
		Limb defects	OR 1.2 (0.7–2.0), interquartile range
		Genitourinary defects	OR 1.0 (0.6–1.7), interquartile range
		Heart defects	OR 1.2 (0.8–1.7), interquartile range
Alkhalawi et al. 2016 General population (n=156 mother-infant pairs)	2.43 ng/mL (geometric mean maternal PFOA)	Birth weight	NS (p>0.05)
		Birth length	NS (p>0.05)
		Ponderal index	**β -0.412 (-0.788 to -0.037)***
Apelberg et al. 2007b General population (n=341 singleton births)	1.6 ng/mL (cord serum median PFOA)	Birth weight	NS (p>0.05)
		Birth length	NS (p>0.05)
		Head circumference	**Inverse association (p>0.05)***
		Ponderal index	**Inverse association (p>0.05)***
Ashley-Martin et al. 2016 General population (n=1,723 pregnant women)	1.70 and 0.39 ng/mL (maternal and cord median PFOA)	Gestational weight gain	NS (p>0.1), serum PFOA **OR 1.04 (1.02–1.06)*, cord PFOA**
Ashley-Martin et al. 2017 General population (n=1,705 mother-infant pairs)	1.7 ng/mL (median maternal plasma PFOA)	Birth weight	β -0.10 (-0.34–0.13)
		Infant leptin levels	β 0.01 (-0.15–0.13)
		Infant adiponectin levels	β 0.04 (-0.05–0.12)
Bach et al. 2016 General population (n=1,507 nulliparous women)	2.0 ng/mL (median PFOA)	Birth weight	NS, investigators noted no consistent alterations across PFOA quartiles
		Birth length	NS, investigators noted no consistent alterations across PFOA quartiles
		Head circumference	NS, investigators noted no consistent alterations across PFOA quartiles

Table 2-23. Summary of Birth Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Bae et al. 2015 General population (n=233 couples)	5.01 and 4.05 ng/mL and 5.00 and 2.54 ng/mL (geometric mean PFOA in male and female nulliparous parents and male and female parous parents, respectively)	Male birth	OR 0.93 (0.68–1.26), maternal PFOA OR 0.94 (0.72–1.23), paternal PFOA
Callan et al. 2016 General population (n=98 pregnant women)	0.86 ng/mL (median maternal serum PFOA)	Birth weight	β -48 g (-203–108)
		Birth length	β 0.06 (-0.70–0.81)
		Head circumference	β -0.40 (-0.96–0.16)
		Ponderal index	β-0.06 (-0.16–0.05)
Cao et al. 2018 General population (n=337 newborns)	1.59 ng/mL (mean cord serum PFOA); >1.59 ng/mL (3rd tertile cord PFOA)	Birth weight	NS (p=0.58)
		Birth length	**β -0.45 (-0.79 to -0.10)*, 3rd tertile**
		Ponderal index	NS (p=0.21)
Chen et al. 2012a General population (n=429 infants)	1.84 ng/mL (cord blood geometric mean PFOA)	Birth weight	NS (p>0.05)
		Birth length	NS (p>0.05)
		Head circumference	NS (p>0.05)
		Ponderal index	NS (p>0.05)
		Small for gestational age	OR 1.24 (0.75–2.05)
		Low birth weight	OR 0.53 (0.18–1.55)
Fei et al. 2007, 2008a General population (n=1,400 pregnant women)	5.6 ng/mL (maternal median PFOA)	Birth weight	**β -10.63 (-20.79 to -0.47)***
		Birth length	**β -0.069 (-0.113 to -0.024)***
		Abdominal circumference	**β -0.059 (-0.106 to -0.012)***
		Head circumference	β-0.030 (-0.064–0.004)
		Low birth weight	OR 2.44 (0.27–22.25), 4th quartile
		Small for gestational age	OR 0.97 (0.55–1.70), 4th quartile
Govarts et al. 2016 General population (n=202 infants)	1.52 ng/mL (cord blood geometric mean PFOA)	Birth weight	NS (p=0.473)

Table 2-23. Summary of Birth Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Hamm et al. 2010 General population (n=252 pregnant women)	>2.1–18 ng/mL (maternal 3rd tertile PFOA)	Birth weight	Change in weight 14.80 (-107.29–136.89), 3rd tertile
		Small for gestational age	RR 0.99 (0.25–3.92), 3rd tertile
Kim et al. 2011 General population (n=44 pregnant women)	1.46 ng/mL (maternal median PFOA)	Birth weight	NS (p>0.05)
		Cord TSH	**Association (p<0.05)***
		Cord T3	NS (p>0.05)
		Cord T4	NS (p>0.05)
Kim et al. 2016a General population (n=27 infants with congenital hypothyroidism; n=13 controls)	5.398 and 2.12 ng/mL (mean PFOA in cases and controls)	Thyroid stimulating immunoglobulin levels	**Inverse association (p<0.05)***
		TSH	NS (p>0.05)
		T3	NS (p>0.05)
		T4	NS (p>0.05)
Kobayashi et al. 2017 General population (n=177 mother-infant pairs)	1.6 ng/mL (mean maternal serum PFOA)	Birth weight	β -49.4 (-130.4–31.6)
		Birth length	β 0.01 (-0.37–0.40)
		Ponderal index	β -0.44 (-0.99–0.12)
Lauritzen et al. 2017 General population (n=159 mother-infant pairs)	2.33 ng/mL (median maternal serum PFOA)	Birth weight	β -359 (-596 to -122; p=0.003)*
		Birth length	**β -1.3 (-2.3 to -0.3, p=0.010)***
		Head circumference	NS (p=0.115)
		Small for gestational age	**OR 5.25 (1.68–16.4)*** **OR 6.55 (1.14–37.45)*, boys only** OR 4.73 (0.79–28.3), girls only
Lauritzen et al. 2017 General population (n=265 mother-infant pairs)	1.62 ng/mL (median maternal serum PFOA)	Birth weight	NS (p=0.590)
		Birth length	NS (p=0.656)
		Head circumference	NS (p=0.354)
		Small for gestational age	OR 0.66 (0.33–1.33)
Lee et al. 2013 General population (n=59 pregnant women)	2.73 ng/mL (maternal mean PFOA)	Birth weight	OR 0.54 (0.17–3.03)
		Birth length	OR 0.44 (0.12–1.58)
		Ponderal index	OR 0.56 (0.16–2.01)
		Head circumference	OR 0.82 (0.24–13.65)

Table 2-23. Summary of Birth Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Lee et al. 2016 General population (n=85 infants)	1.11 ng/mL (cord blood mean PFOA)	Birth weight	NS (p>0.05)
Lenters et al. 2016a, 2016b General population (n=513 infants in Greenland subcohort, n=557 infants in Ukraine subcohort, and n=180 infants in Poland subcohort	1.84, 0.96, and 2.51 ng/mL (maternal median PFOA for Greenland, Ukraine, and Poland subcohorts)	Birth weight	**β -63.77 (-122.83 to -4.71, p=0.035)*, 2 SD increase in ln-transformed PFOA**
Li et al. 2017 General population (n=321 mother-infant pairs)	1.2 ng/mL (median cord serum PFOA)	Birth weight	**β -112.7 (-171.9 to -53.5)***
Lind et al. 2017a General population (n=649 pregnant women)	1.7 ng/mL (median maternal serum PFOA)	Birth weight	NS (p>0.05)
Maisonet et al. 2012 General population (n=447 girls)	3.7 ng/mL (maternal median PFOA)	Birth weight	**Inverse association (p=0.0120 for trend)***
		Birth length	NS (p=0.0978)
		Ponderal index	NS (p=0.5920)
		Body weight at 20 months	NS (p=0.4147)
Manzano-Salgado et al. 2017a General population (n=1,202 mother-infant pairs)	2.35 ng/mL (mean maternal serum PFOA)	Birth weight	β -9.33 (-38.81–20.16)
		Birth length	β -0.01 (-0.15–0.14)
		Head circumference	β 0.07 (-0.17–0.03)
		Small for gestational age	OR 0.92 (0.72–1.19)
		Low birth weight	OR 0.90 (0.63–1.29)
		Low birth weight at term	OR 0.85 (0.53–1.34)
Minatoya et al. 2017 General population (n=168 mother-infant pairs)	1.4 ng/mL (median maternal serum PFOA)	Birth weight	**β -197 (-391 to -3, p=0.047)***
		Ponderal index	β -1.32 (-2.66–0.02, p=0.054)
		Cord total adiponectin	NS (p=0.377)
		Cord leptin	NS (p=0.830)

Table 2-23. Summary of Birth Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Monroy et al. 2008 General population (n=101 pregnant women)	1.81 and 1.58 ng/mL (maternal and cord median PFOA)	Birth weight	NS (p>0.05), maternal serum and cord blood PFOA
Robledo et al. 2015a, 2015b General population (n=234 couples)	3.16 and 5.00 ng/mL (maternal and paternal geometric mean PFOA)	Birth weight	NS (p>0.05), maternal or paternal
		Birth length	NS (p>0.05), maternal or paternal
		Head circumference	NS (p>0.05), maternal or paternal
		Ponderal index	NS (p>0.05), maternal or paternal
Sagiv et al. 2018 General population (n=1,645 pregnant women)	5.8 ng/mL (median maternal plasma PFOA)	Birth weight for gestational age	β -0.02 (-0.08–0.03)
Shi et al. 2017 General population (n= 170 infants)	1.097 ng/mL (median cord serum PFOA)	Birth weight	β 163.28 (-127.66–454.23)
		Birth length	β 0.38 (-0.41–1.17)
		Ponderal index	β 0.06 (-0.10–0.22)
Starling et al. 2017 General population (n=604 mother-infant pairs)	1.1 ng/mL (median maternal serum PFOA); 1.4–17.0 ng/mL (3rd tertile maternal PFOA)	Birth weight	**β -92.4 g (-166.2 to -18.5)*, 3rd tertile**
		Adiposity at birth	β -0.97% fat mass (-0.33–0.49), 3rd tertile
Wang et al. 2016 General population (n=117 boys and 106 girls examined at 2, 5, 8, and 11 years of age)	2.37 and 2.34 ng/mL (median maternal PFOA for boys and girls)	Birth weight	NS (p>0.05)
		Birth length	NS (p>0.05)
		Head circumference	NS (p>0.05)
		Small for gestational age	NS (p>0.05)
Washino et al. 2009 General population (n=428 infants)	1.3 ng/mL (maternal median PFOA)	Birth weight	NS (p=0.207)
		Birth length	NS (p=0.631)
		Chest circumference	NS (p=0.460)
		Head circumference	NS (p=0.823)
Whitworth et al. 2012a General population (n=901 infants)	2.2 and ≥3.04 ng/mL (maternal median and 4th quartile PFOA)	Birth weight	NS (p=0.12)
		Small for gestational age	NS (p=0.92)
		Large for gestational age	NS (p=0.33)

Table 2-23. Summary of Birth Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Wu et al. 2012 General population (n=167 pregnant women at 2 hospitals)	18.32 and 9.76 ng/mL (mean maternal serum PFOA at each hospital)	Birth weight	**β -267.30 (-573.27 to -37.18, p<0.05)***
		Birth length	**β -1.91 (-3.31 to -0.52, p<0.01)***
		Ponderal index	β -0.095 (-0.200–0.389)
PFOS			
Grice et al. 2007 Occupational (n=263 females)	1,300–1,970 ng/mL (range of PFOS)	Birth weight	NS (p=0.15)
Darrow et al. 2013 Community (C8) (n=1,330 women)	15.6 ng/mL (mean PFOS)	Birth weight	NS (p=0.045 for trend), whole cohort **Association (p=0.006), women (n=783) who conceived after blood sample collection**
		Low birth weight	OR 1.33 (0.60–2.96)
Stein et al. 2009 Community (C8) (n=5,262 infants)	17.7–<23.2 and 23.2–83.4 ng/mL (75th–90th and >90th PFOS percentile)	Low birth weight	**OR 1.6 (1.1–2.3)*, 75th-90th percentile**
		Birth defects	OR 1.3 (0.8–2.1)
Alkhalawi et al. 2016 General population (n=156 mother-child pairs)	9.04 ng/mL (geometric mean maternal serum PFOS)	Birth weight	NS (p>0.05)
		Birth length	NS (p>0.05)
		Ponderal index	**β -0.355 (-0.702 to -0.008)***
Apelberg et al. 2007b General population (n=341 singleton births)	5 ng/mL (PFOS cord serum median)	Gestational age	NS (p>0.05)
		Birth weight	NS (p>0.05)
		Birth length	NS (p>0.05)
		Head circumference	**Inverse association (p>0.05)***
		Ponderal index	**Inverse association (p>0.05)***
Ashley-Martin et al. 2016 General population (1,723 pregnant women)	4.60 and 0.15 ng/mL (maternal and cord PFOS median)	Gestational weight gain	**Association (p<0.1), serum PFOS in underweight/normal weight subjects OR 1.03 (1.00–1.05)*, cord PFOS**
Ashley-Martin et al. 2017	4.6 ng/mL (median maternal plasma PFOS)	Birth weight	β 0.05 (-0.18–0.29)
		Infant leptin levels	β -0.09 (-0.23–0.04)

Table 2-23. Summary of Birth Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
General population (n=1,705 mother-infant pairs)		Infant adiponectin levels	β 0.02 (-0.11–0.07)
Bach et al. 2016 General population (n=1,507 nulliparous women)	8.3 ng/mL (PFOS median)	Birth weight	Inverse association reported by investigators
		Birth length	NS, investigators noted no consistent alterations across PFOS quartiles
		Head circumference	NS, investigators noted no consistent alterations across PFOS quartiles
Bae et al. 2015 General population (233 couples)	21.7 and 14.5 ng/mL and 21.5 and 10.8 ng/mL (geometric mean PFOS in male and female nulliparous parents and male and female parous parents, respectively)	Male birth	OR 1.16 (0.88–1.53), maternal PFOS OR 1.01 (0.78–1.33), paternal PFOS
Callan et al. 2016 General population (n=98 pregnant women)	1.99 ng/mL (median maternal serum PFOS)	Birth weight	β -69 g (-231–94)
		Birth length	β -0.22 (-1.0–0.57)
		Head circumference	β -0.39 (-0.98–0.20)
		Ponderal index	β -0.03 (-0.14–0.08)
Cao et al. 2018 General population (n=337 newborns)	1.43 ng/mL (mean cord serum PFOS)	Birth weight	NS (p=0.84)
		Birth length	NS (p=0.65)
		Ponderal index	NS (p=0.47)
Chen et al. 2012a General population (n=429 infants)	5.94 ng/mL (cord blood geometric mean PFOS)	Gestational age	**Inverse association (p<0.001)***
		Birth weight	**β -110.2 g (-176.0 to -44.5, p<0.001)*, per ln PFOS**
		Birth length	NS (p>0.05)
		Head circumference	**β -0.25 cm (-0.46–0.05 cm, p<0.05)*, per ln PFOS**
		Ponderal index	NS (p>0.05)
		Small for gestational age	**OR 2.27 (1.25–4.15)***
		Low birth weight	OR 2.61 (0.185–8.03)

Table 2-23. Summary of Birth Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
de Cock et al. 2014 General population (n=89 infants)	1.611 ng/mL (cord blood mean PFOS)	Weight	NS (p=0.802)
		Height	NS (p=0.975)
		BMI	NS (p=0.586)
		Head circumference	NS (p=0.649)
Fei et al. 2007, 2008a General population (n=1,400 pregnant women)	35.3 ng/mL (maternal median PFOS)	Birth weight	β -0.46 (-2.34–1.41)
		Birth length	β-0.002 (-0.011–0.006)
		Abdominal circumference	β -0.003 (-0.012–0.005)
		Head circumference	β 0.000 (-0.006–0.007)
		Gestation length	NS (p>0.01)
		Low birth weight	OR 4.82 (0.56–41.16), 4th quartile
		Small for gestation age	OR 0.98 (0.58–1.65), 4th quartile
Govarts et al. 2016 General population (n=202 infants)	2.63 ng/mL (cord blood geometric mean PFOS)	Birth weight	NS (p=0.798)
Hamm et al. 2010 General population (n=252 pregnant women)	>10–35 ng/mL (maternal 3rd tertile PFOS)	Birth weight	Change in weight 71.25 (54.97–197.48), 3rd tertile
		Small for gestational age	**RR 0.26 (0.10–0.70)*, 3rd tertile**
Kim et al. 2011 General population (n=44 pregnant women)	2.93 ng/mL (maternal median PFOS)	Birth weight	NS (p>0.05)
		Cord TSH	NS (p>0.05)
		Cord T3	**Inverse association (p<0.05)***
		Cord T4	NS (p>0.05)
Kim et al. 2016a General population (n=27 infants with congenital hypothyroidism; N=13 controls)	5.326 and 4.05 ng/mL (mean PFOS in cases and controls)	Thyroid stimulating immunoglobulin levels	NS (p>0.05)
		TSH	NS (p>0.05)
		T3	NS (p>0.05)
		T4	NS (p>0.05)
Kobayashi et al. 2017 General population (n=177 mother-infant pairs)	5.7 ng/mL (mean maternal serum PFOS)	Birth weight	β -56.0 (-162.8–50.8)
		Birth length	β 0.32 (-0.19–0.82)
		Ponderal index	**β -1.07 (-1.79 to -0.36)***

Table 2-23. Summary of Birth Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Lauritzen et al. 2017 General population (n=159 mother-infant pairs)	16.4 ng/mL (median maternal serum PFOS)	Birth weight	**β -292 (-500 to -84; p=0.006)***
		Birth length	**β -1.2 (-2.1 to -0.3, p=0.007)***
		Head circumference	NS (p=0.073)
		Small for gestational age	OR 2.51 (0.93–6.77)
Lauritzen et al. 2017 General population (n=265 mother-infant pairs)	9.74 ng/mL (median maternal serum PFOS)	Birth weight	NS (p=0.167)
		Birth length	NS (p=0.987)
		Head circumference	NS (p=0.189)
		Small for gestational age	OR 0.71 (0.42–1.20)
Lee et al. 2013 General population (n=59 pregnant women)	10.77 ng/mL (maternal mean PFOS)	Birth weight	OR 0.98 (0.32–3.03)
		Birth length	OR 0.97 (0.29–3.27)
		Ponderal index	**OR 0.22 (0.05–0.90)***
		Head circumference	OR 1.34 (0.20–8.90)
Lee et al. 2016 General population (n=85 infants)	0.87 ng/mL (cord blood mean PFOS)	Birth weight	NS (p>0.05)
Lenters et al. 2016a, 2016b General population (n=513 infants in Greenland subcohort, n=557 infants in Ukraine subcohort, and n=180 infants in Poland subcohort)	20.09, 5.04, and 7.81 ng/mL (maternal median PFOS for Greenland, Ukraine, and Poland subcohorts)	Birth weight	NS (p=0.109)
Li et al. 2017 General population (n=321 mother-infant pairs)	3.0 ng/mL (median cord serum PFOS)	Birth weight	**β -95.0 (-154.0 to -36.0)*** **β -150.6 (-225.4 to -75.7)*, boys only** β -26.6 (-125.1–71.8), girls only
Liew et al. 2014 General population (n=156 children diagnosed with congenital cerebral palsy (cases) and 550 controls)	28.90 and 27.50 ng/mL (maternal median PFOS in boy and girl cases) 27.60 and 26.20 ng/mL (maternal median PFOS in boy and girl controls)	Congenital cerebral palsy Boys Girls	 **RR 1.7 (1.0–2.8)*** RR 0.7 (0.4–1.4)

Table 2-23. Summary of Birth Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Lind et al. 2017a General population (n=649 pregnant women)	8.1 ng/mL (median maternal serum PFOS)	Birth weight	NS (p>0.05)
Maisonet et al. 2012 General population (n=447 girls)	19.6 ng/mL (maternal median PFOS)	Birth weight	**β -140.01 g (-238.14 to -41.89 g, p=0.0053 for trend)*, 3rd tertile**
		Birth length	**β -0.63 cm (-1.11 to -0.15 cm, p=0.103 for trend)* 3rd tertile**
		Ponderal index	NS (p=0.1120)
Manzano-Salgado et al. 2017a General population (n=1,202 mother-infant pairs)	6.05 ng/mL (mean maternal serum PFOS)	Birth weight	β 0.44 (-32.48–33.36)
		Birth length	β 0.03 (-0.12–0.17)
		Head circumference	β -0.00 (-0.10–0.10)
		Small for gestational age	OR 0.92 (0.70–1.22)
		Low birth weight	OR 1.06 (0.71–1.58)
		Low birth weight at term	OR 0.91 (0.55–1.50)
Minatoya et al. 2017 General population (n=168 mother-infant pairs)	5.1 ng/mL (median maternal serum PFOS)	Birth weight	β -29 (-289–232, p=0.828)
		Ponderal index	**β -2.25 (-4.01 to -0.50, p=0.012)***
		Cord total adiponectin	**β 0.12 (0.01–0.22, p=0.028)***
		Cord leptin	NS (p=0.691)
Monroy et al. 2008 General population (n=101 pregnant women)	14.54 and 6.08 ng/mL (maternal and cord median PFOS)	Birth weight	NS (p>0.05), maternal serum and cord blood PFOA
Robledo et al. 2015a, 2015b General population (n=234 couples)	12.44 and 21.6 ng/mL (maternal and paternal geometric mean PFOS)	Birth weight	NS (p>0.05), maternal or paternal
		Birth length	NS (p>0.05), maternal or paternal
		Head circumference	NS (p>0.05), maternal or paternal
		Ponderal index	NS (p>0.05), maternal or paternal
Sagiv et al. 2018 General population (n=1,645 pregnant women)	25.7 ng/mL (median maternal plasma PFOS)	Birth weight for gestational age	β -0.04 (-0.08–0.01)

Table 2-23. Summary of Birth Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Shi et al. 2017 General population (n= 170 infants)	0.974 ng/mL (median cord serum PFOS)	Birth weight	β 160.45 (-11.85–332.75)
		Birth length	β 0.33 (-0.14–0.79)
		Ponderal index	β 0.07 (-0.03–0.16)
Starling et al. 2017 General population (n=604 mother-infant pairs)	2.4 ng/mL (median maternal serum PFOS)	Birth weight	β -13.8 g (-53.8–26.3)
		Adiposity at birth	β 0.8 (-0.33–0.49)
Washino et al. 2009 General population (n=428 infants)	5.2 ng/mL (maternal median PFOS)	Birth weight Males Females	**β -148.8 g (-297.0 to -0.5 g, p=0.049)*, per log PFOS unit** NS (p=0.917) **β -269.4 g (-465.7 to -73.0 g, p=0.007)*, per log PFOS unit**
		Birth length	NS (p=0.167)
		Chest circumference	NS (p=0.718)
		Head circumference	NS (p=0.488)
Whitworth et al. 2012a General population (n=901 infants)	13.0 and ≥16.59 ng/mL (maternal median and 4th quartile PFOS)	Birth weight	NS (p=0.10)
		Small for gestational age	NS (p=0.51)
		Large for gestational age	NS (p=0.33)
PFHxS			
Alkhalawi et al. 2016 General population (n=156 mother-child pairs)	0.62 ng/mL (geometric mean maternal serum PFHxS)	Birth weight	NS (p>0.05)
		Birth length	NS (p>0.05)
		Ponderal index	NS (p>0.05)
Ashley-Martin et al. 2016 General population (n=1,723 pregnant women)	1.00 and 0.10 ng/mL (maternal and cord PFHxS median)	Gestational weight gain	NS (p>0.1), serum PFHxS OR 1.01 (10.99–1.03), cord PFHxS
Ashley-Martin et al. 2017 General population (n=1,705 mother-infant pairs)	1.0 ng/mL (median maternal plasma PFHxS)	Birth weight	β 0.04 (-0.12–0.20
		Infant leptin levels	β 0.01 (-0.08–0.10)
		Infant adiponectin levels	β 0.02 (-0.08–0.04)

Table 2-23. Summary of Birth Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Bach et al. 2016 General population (n=1,507 nulliparous women)	0.5 ng/mL (maternal PFHxS median)	Birth weight	**Inverse association reported by investigators**
		Birth length	NS, investigators noted no consistent alterations across PFHxS quartiles
		Head circumference	NS, investigators noted no consistent alterations across PFHxS quartiles
Callan et al. 2016 General population (n=98 pregnant women)	0.33 ng/mL (median maternal serum PFHxS)	Birth weight	β -103 g (-221–15)
		Birth length	β -0.20 (-0.78–0.38)
		Head circumference	β -0.31 (-0.74–0.12)
		Ponderal index	β -0.05 (-0.13–0.03)
Cao et al. 2018 General population (n=337 newborns)	0.16 ng/mL (mean cord serum PFHxS); 0.06–0.139 ng/mL (2nd tertile cord PFHxS)	Birth weight	NS (p=0.69)
		Birth length	NS (p=0.67)
		Head circumference	**β 1.33 (0.42–2.26)*, 2nd tertile**
		Ponderal index	NS (p=0.85)
Hamm et al. 2010 General population (n=252 pregnant women)	>1.4–43 ng/mL (maternal 3rd tertile PFHxS)	Birth weight	Change in weight (25.99, 95% CI -95.25–147.23), 3rd tertile
		Small for gestational age	RR 2.35 (0.63–8.72), 3rd tertile
Kim et al. 2011 General population (n=44 pregnant women)	0.55 ng/mL (maternal median PFHxS)	Birth weight	NS (p>0.05)
		Cord TSH	NS (p>0.05)
		Cord T3	NS (p>0.05)
		Cord T4	NS (p>0.05)
Kim et al. 2016a General population (n=27 infants with congenital hypothyroidism; n=13 controls)	1.228 and 1.17 ng/mL (mean PFHxS in cases and controls)	Thyroid stimulating immunoglobulin levels	**Association (p<0.05)***
		TSH	NS (p>0.05)
		T3	NS (p>0.05)
		T4	NS (p>0.05)

Table 2-23. Summary of Birth Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Lee et al. 2013 General population (n=59 pregnant women)	1.35 ng/mL (maternal mean PFHxS)	Birth weight	OR 0.57 (0.19–1.75)
		Birth length	OR 0.44 (0.12–1.58)
		Ponderal index	OR 0.64 (0.19–2.23)
		Head circumference	OR 0.90 (0.13–6.13)
Lee et al. 2016 General population (n=85 infants)	0.60 ng/mL (cord blood mean PFHxS)	Birth weight	NS (p>0.05)
Lenters et al. 2016a, 2016b General population (n=513 infants in Greenland subcohort, n=557 infants in Ukraine subcohort, and n=180 infants in Poland subcohort)	2.05, 1.56, and 2.28 ng/mL (maternal median PFHxS for Greenland, Ukraine, and Poland subcohorts)	Birth weight	NS (p=0.801)
Li et al. 2017 General population (n=321 mother-infant pairs)	3.9 ng/mL (median cord serum PFHxS)	Birth weight	β -30.0 (-83.4–23.5)
Liew et al. 2014 General population (n=156 children diagnosed with congenital cerebral palsy (cases) and 550 controls)	0.96 and 0.90 ng/mL (maternal median PFHxS in boy and girl cases) 0.92 and 0.92 ng/mL (maternal median PFHxS in boy and girl controls)	Congenital cerebral palsy Boys Girls	 RR 1.2 (0.9–1.7) RR 1.1 (0.6–1.9)
Lind et al. 2017a General population (n=649 pregnant women)	0.3 ng/mL (median maternal serum PFHxS)	Birth weight	NS (p>0.05)
Maisonet et al. 2012 General population (n=447 girls)	1.6 ng/mL (maternal median PFHxS)	Birth weight	**Inverse association (p=0.0314 for trend)***
		Birth length	**Inverse association (p=0.0008 for trend)***
		Ponderal index	NS (p=0.6802 for trend)

Table 2-23. Summary of Birth Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Manzano-Salgado et al. 2017a General population (n=1,202 mother-infant pairs)	0.58 ng/mL (mean maternal serum PFHxS)	Birth weight	β -8.60 (-32.00–14.80)
		Birth length	β -0.06 (-0.17–0.06)
		Head circumference	β -0.01 (-0.09–0.07)
		Small for gestational age	OR 0.98 (0.80–1.19)
		Low birth weight	OR 0.94 (0.71–1.23)
		Low birth weight at term	OR 0.97 (0.68–1.41)
Monroy et al. 2008 General population (n=101 pregnant women)	1.62 mg/mL (maternal median PFHxS)	Birth weight	NS (p>0.05)
Sagiv et al. 2018 General population (n=1,645 pregnant women)	2.4 ng/mL (median maternal plasma PFHxS)	Birth weight for gestational age	β 0.00 (-0.03–0.02)
Shi et al. 2017 General population (n= 170 infants)	0.157 ng/mL (median cord serum PFHxS)	Birth weight	β 108.80 (-53.84–271.45)
		Birth length	β 0.38 (-0.06–0.82)
		Ponderal index	β 0.03 (-0.06–0.12)
Starling et al. 2017 General population (n=604 mother-infant pairs)	0.8 ng/mL (median maternal serum PFHxS); 1.1–10.9 ng/mL (3rd tertile maternal PFHxS)	Birth weight	β -31.84 g (-105.8–42.2), 3rd tertile
		Adiposity at birth	**β -0.99% fat mass (-1.75 to -0.23)*, 3rd tertile**
PFNA			
Bach et al. 2016 General population (n=1,507 nulliparous women)	0.8 ng/mL (PFNA median)	Birth weight	NS, investigators noted no consistent alterations across PFNA quartiles
		Birth length	NS, investigators noted no consistent alterations across PFNA quartiles
		Head circumference	NS, investigators noted no consistent alterations across PFNA quartiles

Table 2-23. Summary of Birth Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Bae et al. 2015 General population (233 couples)	1.60 and 1.37 ng/mL and 1.55 and 1.09 ng/mL (geometric mean PFNA in male and female nulliparous parents and male and female parous parents, respectively)	Male birth	OR 0.94 (0.70–1.26), maternal PFNA OR 0.94 (0.71–1.24), paternal PFNA
Callan et al. 2016 General population (n=98 pregnant women)	0.30 ng/mL (median maternal serum PFNA)	Birth weight	β 14 g (-169–196)
		Birth length	β 0.20 (-0.68–1.09)
		Head circumference	β -0.14 (-0.80–0.52)
		Ponderal index	β -0.03 (-0.16–0.09)
Cao et al. 2018 General population (n=337 newborns)	0.13 ng/mL (mean cord serum PFNA)	Birth weight	NS (p=0.19)
		Birth length	NS (p=0.06)
		Ponderal index	NS (p=0.91)
Chen et al. 2012a General population (n=429 infants)	2.36 ng/mL (cord blood geometric mean PFNA)	Gestational age	NS (p>0.05)
		Birth weight	NS (p>0.05)
		Birth length	**Association (p<0.01)***
		Head circumference	NS (p>0.05)
		Ponderal index	**Inverse association (p<0.05)**
		Small for gestational age	OR 0.97 (0.74–1.26)
		Low birth weight	OR 0.76 (0.47–1.23)
Kim et al. 2016a General population (n=27 infants with congenital hypothyroidism; n=13 controls)	1.931 and 0.633 ng/mL (mean PFNA in cases and controls)	Thyroid stimulating immunoglobulin levels	NS (p>0.05)
		TSH	NS (p>0.05)
		T3	NS (p>0.05)
		T4	NS (p>0.05)
Lee et al. 2016 General population (n=85 infants)	0.36 ng/mL (cord blood mean PFNA)	Birth weight	NS (p>0.05)

Table 2-23. Summary of Birth Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Lenters et al. 2016a, 2016b General population (n=513 infants in Greenland subcohort, n=557 infants in Ukraine subcohort, and n=180 infants in Poland subcohort)	0.69, 0.61, and 0.56 ng/mL (maternal median PFNA for Greenland, Ukraine, and Poland subcohorts)	Birth weight	NS (p=0.065)
Li et al. 2017 General population (n=321 mother-infant pairs)	0.2 ng/mL (median cord serum PFNA)	Birth weight	β -45.6 (-106.9–15.8)
Liew et al. 2014 General population (n=156 children diagnosed with congenital cerebral palsy (cases) and 550 controls)	0.46 and 0.39 ng/mL (maternal median PFNA in boy and girl cases) 0.44 and 0.41 ng/mL (maternal median PFNA in boy and girl controls)	Congenital cerebral palsy Boys Girls	 RR 1.2 (0.6–2.5) RR 0.6 (0.3–1.2)
Lind et al. 2017a General population (n=649 pregnant women)	0.7 ng/mL (median maternal serum PFNA)	Birth weight	NS (p>0.05)
Manzano-Salgado et al. 2017a General population (n=1,202 mother-infant pairs)	0.66 ng/mL (mean maternal serum PFNA)	Birth weight	β -10.27 (-38.14–17.61)
		Birth length	β -0.00 (-0.13–0.13)
		Head circumference	β -0.04 (-0.13–0.05)
		Small for gestational age	OR 0.85 (0.68–1.07)
		Low birth weight	OR 0.86 (0.63–1.17)
		Low birth weight at term	OR 0.91 (0.60–1.38)
Monroy et al. 2008 General population (n=101 pregnant women)	0.69 mg/mL (maternal median PFNA)	Birth weight	NS (p>0.05)
Robledo et al. 2015a, 2015b General population (n=234 couples)	1.211 and 1.566 ng/mL (maternal and paternal geometric mean PFNA)	Birth weight	NS (p>0.05), maternal or paternal
		Birth length	NS (p>0.05), maternal or paternal
		Head circumference	NS (p>0.05), maternal or paternal
		Ponderal index	NS (p>0.05), maternal or paternal

Table 2-23. Summary of Birth Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Sagiv et al. 2018 General population (n=1,645 pregnant women)	0.7 ng/mL (median maternal plasma PFNA); 0.7–0.9 ng/mL (3rd quartile maternal PFNA)	Birth weight for gestational age	**β -0.20 (-0.33 to -0.06)*, 3rd quartile**
Shi et al. 2017 General population (n= 170 infants)	0.191 ng/mL (median cord serum PFNA)	Birth weight	β 52.68 (-206.01–311.36)
		Birth length	β 0.13 (-0.57–0.83)
		Ponderal index	β 0.01 (-0.13–0.15)
Starling et al. 2017 General population (n=604 mother-infant pairs)	0.4 ng/mL (median maternal serum PFNA); 0.5–6.0 ng/mL (2nd half maternal PFNA)	Birth weight	**β -92.1 g (-150.6 to -33.6)*, 2nd half**
		Adiposity at birth	**β -0.85% fat mass (-1.46 to -0.24)*, 2nd half**
Wang et al. 2016 General population (n=117 boys and 106 girls examined at 2, 5, 8, and 11 years of age)	1.55 and 1.58 ng/mL (median maternal PFNA for boys and girls)	Birth weight	**Inverse association (p>0.05)*, girls only**
		Birth length	NS (p>0.05)
		Head circumference	NS (p>0.05)
		Small for gestational age	NS (p>0.05)
		Growth during childhood	NS (p>0.05)
PFDA			
Bach et al. 2016 General population (n=1,507 nulliparous women)	0.3 ng/mL (PFDA median)	Birth weight	NS, investigators noted no consistent alterations across PFDA quartiles
		Birth length	NS, investigators noted no consistent alterations across PFDA quartiles
		Head circumference	NS, investigators noted no consistent alterations across PFDA quartiles
Bae et al. 2015 General population (233 couples)	0.46 and 0.38 ng/mL and 0.49 and 0.46 ng/mL (geometric mean PFDA in male and female nulliparous parents and male and female parous parents, respectively)	Male birth	OR 1.07 (0.81–1.42), maternal PFDA OR 1.02 (0.78–1.34), paternal PFDA

Table 2-23. Summary of Birth Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Callan et al. 2016 General population (n=98 pregnant women)	0.12 ng/mL (median maternal serum PFDA)	Birth weight	β 4 g (-161–170)
		Birth length	β 0.36 (-0.44–1.15)
		Head circumference	β -0.07 (-0.67–0.53)
		Ponderal index	β -0.06 (-0.18–0.05)
Cao et al. 2018 General population (n=337 newborns)	0.12 ng/mL (mean cord serum PFDA)	Birth weight	NS (p=0.26)
		Birth length	NS (p=0.24)
		Ponderal index	NS (p=0.55)
Kim et al. 2016a General population (n=27 infants with congenital hypothyroidism; n=13 controls)	0.523 and 0.298 ng/mL (mean PFDA in cases and controls)	Thyroid stimulating immunoglobulin levels	NS (p>0.05)
		TSH	NS (p>0.05)
		T3	NS (p>0.05)
		T4	NS (p>0.05)
Lee et al. 2016 General population (n=85 infants)	0.14 ng/mL (cord blood mean PFDA)	Birth weight	NS (p>0.05)
Lenters et al. 2016a, 2016b General population (n=513 infants in Greenland subcohort, n=557 infants in Ukraine subcohort, and n=180 infants in Poland subcohort)	0.40, 0.16, and 0.22 ng/mL (maternal median PFDA for Greenland, Ukraine, and Poland subcohorts)	Birth weight	NS (p=0.158)
Li et al. 2017 General population (n=321 mother-infant pairs)	0.1 ng/mL (median cord serum PFDA)	Birth weight	β -47.3 (-112.9–18.2)
Liew et al. 2014 General population (n=156 children diagnosed with congenital cerebral palsy (cases) and 550 controls)	0.18 and 0.16 ng/mL (maternal median PFDA in boy and girl cases) 0.17 and 0.16 ng/mL (maternal median PFDA in boy and girl controls)	Congenital cerebral palsy Boys Girls	 RR 1.1 (0.7–1.7) RR 0.6 (0.3–1.1)

Table 2-23. Summary of Birth Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Lind et al. 2017a General population (n=649 pregnant women)	0.3 ng/mL (median maternal serum PFDA)	Birth weight	NS (p>0.05)
Robledo et al. 2015a, 2015b General population (n=234 couples)	0.402 and 0.458 ng/mL (maternal and paternal geometric mean PFDA)	Birth weight	NS (p>0.05), maternal or paternal
		Birth length	NS (p>0.05), maternal or paternal
		Head circumference	NS (p>0.05), maternal or paternal
		Ponderal index	NS (p>0.05), maternal or paternal
Shi et al. 2017 General population (n= 170 infants)	0.075 ng/mL (median cord serum PFDA)	Birth weight	β -3.04 (-129.67–123.59)
		Birth length	β -0.002 (-0.354–0.34)
		Ponderal index	β -0.01 (-0.08–0.06)
Starling et al. 2017 General population (n=604 mother-infant pairs)	0.1 ng/mL (median maternal serum PFDA)	Birth weight	β 11.5 g (-37.3–60.4)
		Adiposity at birth	β 0.06 (-0.45–0.56)
Wang et al. 2016 General population (n=117 boys and 106 girls examined at 2, 5, 8, and 11 years of age)	0.46 and 0.43 ng/mL (median maternal PFDA for boys and girls)	Birth weight	**Inverse association (p>0.05)*, girls only**
		Birth length	NS (p>0.05)
		Head circumference	NS (p>0.05)
		Small for gestational age	**OR 3.14 (1.07–9.19)*, girls only**
PFUnA			
Bach et al. 2016 General population (n=1,507 nulliparous women)	0.3 ng/mL (PFUnA median)	Birth weight	NS, investigators noted no consistent alterations across PFUnA quartiles
		Birth length	NS, investigators noted no consistent alterations across PFUnA quartiles
		Head circumference	NS, investigators noted no consistent alterations across PFUnA quartiles
Callan et al. 2016 General population (n=98 pregnant women)	0.08 ng/mL (median maternal serum PFUnA)	Birth weight	β 102 g (-41–245)
		Birth length	β 0.32 (-0.37–1.02)
		Head circumference	β -0.29 (-0.81–0.24)
		Ponderal index	β 0.01 (-0.09–0.11)
		Optimal body weight	**β 5.3 (1.2–9.3)***

Table 2-23. Summary of Birth Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Cao et al. 2018 General population (n=337 newborns)	0.10 ng/mL (mean cord serum PFUnA); >0.11 ng/mL (3rd tertile cord PFUnA	Birth weight	NS (p=0.08)
		Birth length	**β 0.41 (0.06–0.77)*, 3rd tertile**
		Ponderal index	NS (p=0.56)
Chen et al. 2012a General population (n=429 infants)	10.26 ng/mL (cord blood geometric mean PFUnA)	Gestational age	NS (p>0.05)
		Birth weight	NS (p>0.05)
		Birth length	NS (p>0.05)
		Head circumference	NS (p>0.05)
		Ponderal index	NS (p>0.05)
		Small for gestational age	OR 0.93 (0.65–1.33)
		Low birth weight	OR 1.01 (0.53–1.91)
Kim et al. 2016a General population (n=27 infants with congenital hypothyroidism; n=13 controls)	0.982 and 0.438 ng/mL (mean PFUnA in cases and controls)	Thyroid stimulating immunoglobulin levels	NS (p>0.05)
		TSH	NS (p>0.05)
		T3	NS (p>0.05)
		T4	NS (p>0.05)
Lee et al. 2016 General population (n=85 infants)	0.22 ng/mL (cord blood mean PFUnA)	Birth weight	NS (p>0.05)
Lenters et al. 2016a, 2016b General population (n=513 infants in Greenland subcohort, n=557 infants in Ukraine subcohort, and n=180 infants in Poland subcohort)	0.70, 0.16, and 0.13 ng/mL (maternal median PFUnA for Greenland, Ukraine, and Poland subcohorts)	Birth weight	NS (p=0.275)
Li et al. 2017 General population (n=321 mother-infant pairs)	0.1 ng/mL (median cord serum PFUnA)	Birth weight	β -29.7 (-85.7–26.3)
Shi et al. 2017 General population (n= 170 infants)	0.063 ng/mL (median cord serum PFUnA)	Birth weight	β -28.87 (-128.16–70.42)
		Birth length	β -0.20 (-0.47–0.07)
		Ponderal index	β 0.01 (-0.04–0.06).

Table 2-23. Summary of Birth Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Wang et al. 2016 General population (n=117 boys and 106 girls examined at age 2, 5, 8, and 11 years of age)	3.52 and 3.31 ng/mL (median maternal PFUnA for boys and girls)	Birth weight	**Inverse association (p<0.05)*, girls only**
		Birth length	NS (p>0.05)
		Head circumference	NS (p>0.05)
		Small for gestational age	**OR 1.83 (1.01–3.32)*, girls only**
PFHpA			
Kim et al. 2016a General population (n=27 infants with congenital hypothyroidism; n=13 controls)	0.284 and 0.324 ng/mL (mean PFHpA in cases and controls)	Thyroid stimulating immunoglobulin levels	NS (p>0.05)
		TSH	NS (p>0.05)
		T3	NS (p>0.05)
		T4	NS (p>0.05)
Li et al. 2017 General population (n=321 mother-infant pairs)	0.1 ng/mL (median cord serum PFHpA)	Birth weight	β -103.7 (-211.3–3.8) **β -266.6 (-426.8 to -106.3)*, boys only** β 15.5 (-134.1–165.1), girls only
PFBA			
Kim et al. 2016a General population (n=27 infants with congenital hypothyroidism; n=13 controls)	0.464 and 0.220 ng/mL (mean PFBA in cases and controls)	Thyroid stimulating immunoglobulin levels	NS (p>0.05)
		TSH	NS (p>0.05)
		T3	NS (p>0.05)
		T4	NS (p>0.05)
Li et al. 2017 General population (n=321 mother-infant pairs)	0.1 ng/mL (median cord serum PFBA)	Birth weight	β -46.2 (-111.3–19.0)
PFDoDA			
Cao et al. 2018 General population (n=337 newborns)	0.04 ng/mL (mean cord serum PFDoDA)	Birth weight	NS (p=0.94)
		Birth length	NS (0.51)
		Ponderal index	NS (p=0.60)

Table 2-23. Summary of Birth Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Lee et al. 2016 General population (n=85 infants)	0.14 ng/mL (cord blood mean PFDoDA)	Birth weight	NS (p>0.05)
Lenters et al. 2016a, 2016b General population (n=513 infants in Greenland subcohort, n=557 infants in Ukraine subcohort, and n=180 infants in Poland subcohort)	0.13, 0.04, and 0.05 ng/mL (maternal median PFDoDA for Greenland, Ukraine, and Poland subcohorts)	Birth weight	NS (p=0.440)
Li et al. 2017 General population (n=321 mother-infant pairs)	0.1 ng/mL (median cord serum PFDoDA)	Birth weight	β -46.86 (-122.0–28.4) β 18.4 (-86.8–123.5), boys only **β -130.4 (-239.1 to -21.7)*, girls only**
Wang et al. 2016 General population (n=117 boys and 106 girls examined at 2, 5, 8, and 11 years of age)	0.37 and 0.37 ng/mL (median maternal PFDoDA for boys and girls)	Birth weight	**Inverse association (p<0.05)*, girls only**
		Birth length	NS (p>0.05)
		Head circumference	**Inverse association (p<0.05)*, girls only**
		Small for gestational age	NS (p>0.05)
FOSA			
Bae et al. 2015 General population (233 couples)	0.11 and 0.10 ng/mL and 0.10 and 0.12 ng/mL (geometric mean FOSA in male and female nulliparous parents and male and female parous parents, respectively)	Male birth	OR 1.07 (0.81–1.41), maternal FOSA OR 1.14 (0.86–1.51), paternal FOSA

Table 2-23. Summary of Birth Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Robledo et al. 2015a, 2015b General population (n=234 couples)	0.112 and 0.114 ng/mL (maternal and paternal geometric mean FOSA)	Birth weight	
		Boys	**Inverse association (p<0.05)*, maternal only**
		Girls	NS (p>0.05), maternal or paternal
		Birth length	NS (p>0.05), maternal or paternal
		Head circumference	NS (p>0.05), maternal or paternal
		Ponderal index	NS (p>0.05), maternal or paternal

[a]See the *Supporting Document for Epidemiological Studies for Perfluoroalkyls,* Table 13 for more detailed descriptions of studies.
[b]Participants in occupational exposure may have also lived near the site and received residential exposure; community exposure studies involved subjects living near PFOA facilities with known exposure to high levels of PFOA.
[c]Asterisk indicates association with perfluoroalkyl; unless otherwise specified, values in parenthesis are 95% confidence intervals.

BMI = body mass index; FSH = follicle stimulating hormone; FOSA = perfluorooctane sulfonamide; HR = hazard ratio; LH = luteinizing hormone; NS = not significant; NR = not reported; OR = odds ratio; PFDA = perfluorodecanoic acid; PFDoDA = perfluorododecanoic acid; PFHxS = perfluorohexane sulfonic acid; PFNA = perfluorononanoic acid; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid; PFUnA = perfluoroundecanoic acid; RR = relative risk; T3 = triiodothyronine; T4 = thyroxine; TSH = thyroid stimulating hormone

Table 2-24. Summary of Neurodevelopmental Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFOA			
Stein et al. 2013 Community (C8) (n=320 children 6–12 years old)	115.9 ng/mL (estimated *in utero* mean PFOA)	Full scale IQ	**β 4.61 (0.68–8.54)*, 4th quartile**
		Reading and math skills	NS
		Scores on tests of ADHD (improvement)	**β -8.49 (-16.14 to -0.84)*, 4th quartile**
Stein et al. 2014a, 2014b Community (C8) (n=321 children 6–12 years old)	94.1–838.6 ng/mL (4th PFOA quartile measured 3–4 years prior to behavioral assessment)	Executive function scores (mother completed survey)	**β -6.39 (-11.43 to -1.35)*, 4th quartile boys** β -6.39 (-0.03–8.87), 4th quartile girls
		Executive function scores (teacher completed survey)	β -6.42 (-13.29–0.45), 4th quartile boys β -1.92 (-10.39–6.55), 4th quartile girls
		ADHD-like behaviors (mother completed survey)	β -3.82 (-8.96–1.31), 4th quartile boys **β 6.99 (2.47–11.51)*, 4th quartile girls** β 2.30 (-1.18–5.77), 4th quartile boys and girls
		ADHD-like behaviors (teacher completed survey)	β -9.25 (-18.78–0.27), 4th quartile boys β -3.65 (-10.85–3.51) 4th quartile girls **β -6.03 (-11.40 to -0.66)*, 4th quartile boys and girls**
		Behavioral problems and emotional disturbances (mother completed survey)	β -1.55 (-5.91–2.82), 4th quartile boys **β 4.63 (0.72–8.53)*, 4th quartile girls**
		Behavioral problems and emotional disturbances (teacher completed survey)	β -2.47 (-8.24–3.30), 4th quartile boys β -0.91 (-6.19–4.37), 4th quartile girls
Stein and Savitz 2011 Community (C8) (n=10,546 children aged 5–18 year)	65.3–2,070.6 ng/mL (4th PFOA quartile)	ADHD	**OR 0.76 (0.64–0.90)*, 4th quartile**
		Learning problems 12–15 years old 5–18 years old	 OR 0.96 (0.73–1.26), 4th quartile OR 0.90 (0.76–1.06), 4th quartile

Table 2-24. Summary of Neurodevelopmental Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Braun et al. 2014 General population (n=175 children 4 and 5 years old)	5.5 ng/mL (maternal median PFOA)	Social responsiveness scale score (measure of autistic behaviors)	β -2.0 (-4.4–0.4)
Chen et al. 2013 General population (239 children 2 years of age)	2.5 ng/mL (mean cord PFOA)	Poor performance on tests	NS OR 0.6 (0.08–4.8), whole test OR1.3 (0.3–6.2), cognitive tests OR 0.5 (0.06–4.0), language tests OR 0.8 (0.1–4.7), gross motor tests OR 2.8 (0.6–13.5), fine motor tests OR 0.3 (0.02–2.7), social tests OR 3.2 (0.7–14.3), self-help tests
Donauer et al. 2015 General population (n=349 infants at 5 weeks of age)	5.49 ng/mL (maternal geometric mean PFOA)	Social/easy going	NS (p>0.05)
		Hypotonic	**OR 3.79 (1.1–12.8)* per 10-fold increase in PFOA**
		High arousal/difficult	NS (p=0.3533)
Fei et al. 2008b General population (n=1,400 infants)	5.6 ng/mL (maternal median PFOA)	Apgar scores <10	OR 1.14 (0.57–2.25)
		Motor and mental development at 6 months	NS (p>0.05)
		Neurobehavioral milestones at 18 months	NS (p>0.05)
Fei and Olsen 2011 General population (n=526–787 7-year-old children)	5.4 ng/mL (maternal median PFOA)	Behavioral problems	NS (p>0.15 for trend)
		Motor coordination	NS (p=0.89 for trend)
Forns et al. 2015 General population (n=843 infants)	40 ng/L (median PFOA breast milk level)	Risk of an abnormal score on neurobehavioral assessment questionnaire	OR 1.05 (0.77–1.44) at 6 months of age OR 1.0 (0.78–1.28) at 24 months of age
Goudarzi et al. 2016b General population (n=173 infants at 6 months and 133 at 18 months)	1.2 ng/mL (maternal median PFOA)	MDI/PDI at 6 months of age	NS (p>0.05), boys and girls **Inverse association (p<0.05)*, females**
		MDI/PDI at 18 months of age	NS (p>0.05)

Table 2-24. Summary of Neurodevelopmental Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Gump et al. 2011 General population (n=83 children aged 9–11 years)	3.23 ng/mL (mean PFOA)	Performance on task requiring behavioral inhibition	NS (p>0.05)
Hoffman et al. 2010 General population (NHANES) (n=571 children 12–15 years)	4.4 ng/mL (median PFOA)	ADHD (parent reported)	**OR 1.12 (1.01–1.23)*, per 1 ng/mL PFOA**
Høyer et al. 2015a General population (n=1,106 children aged 5–9 years)	1.4 and 1.9–9.8 ng/mL (maternal median and 3rd tertile PFOA)	Motor skills	β -0.2 (-1.2–0.9)
		Abnormal behavior	**OR 2.7 (1.2–6.3)*, 3rd tertile**
		Hyperactivity	**OR 3.1 (1.3–7.2)*, 3rd tertile**
Lien et al. 2016 General population (n=282 children aged 7 years)	1.55 ng/mL (cord blood weighted average PFOA)	Inattention	NS (p=0.7758)
		Hyperactivity/impulsivity	NS (p=0.2997)
		Emotional symptoms	NS (p=0.691)
		Conduct problems	NS (p=0.2664)
		Hyperactivity/inattention	NS (p=0.774)
Jeddy et al. 2017 General population (n=432 mother-daughter pairs)	3.7 ng/mL (maternal median serum PFOA)	Verbal comprehension (15-month-olds)	NS (p>0.05)
		Vocabulary comprehension and production (15-month-olds)	NS (p>0.05)
		Nonverbal communication (15-month-olds)	NS (p>0.05)
		Social development (15-month-olds)	NS (p>0.05)
		Intelligibility scores (38-month-olds)	**β -0.04 (-0.08 to -0.01)***
		Language scores (38-month-olds)	NS (p>0.05)
		Communicative scores (38-month-olds)	NS (p>0.05)

Table 2-24. Summary of Neurodevelopmental Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Liew et al. 2015 General population (n=215, ADHD cases, 213 autism cases, 545 controls)	4.06, 3.88, and 4.00 ng/mL (maternal median PFOA for ADHD, autism, and controls)	ADHD	RR 0.98 (0.82–1.16)
		Autism	RR 0.98 (0.73–1.31)
Ode et al. 2014 General population (n=206 children with ADHD and 206 controls; children were 5–17 years old at time of diagnosis)	1.80 and 1.83 ng/mL (cord blood median PFOA in cases and controls)	ADHD	OR 0.98 (0.91–1.02), per 1 ng/mL increase in PFOA
Oulhote et al. 2016 General population (n=567 7-year-old children)	3.19 ng/mL (maternal geometric mean PFOA)	Behavioral development scores	No associations
Oulhote et al. 2016 General population (n=567 7-year-old children)	4.09 ng/mL (geometric mean PFOA in 5-year-old children)	Total behavioral scores and higher internalizing problems, peer relationship, and autism screening scores	Associations
Oulhote et al. 2016 General population (n=567 7-year-old children)	4.51 ng/mL (geometric mean PFOA in 7-year-old children)	Behavioral development scores	No associations
Quaak et al. 2016 General population (n=76 infants 18 months of age)	0.9056 ng/mL (cord mean PFOA)	Score on test evaluating ADHD	NS (p=0.72), 3rd tertile
		Males	NS (p=0.22), 3rd tertile
		Females	NS (p=0.31), 3rd tertile
		Scores on test evaluating externalizing problem	NS (p=0.31), 3rd tertile
		Males	**Association (p=0.05 and 0.09)*, 2nd and 3rd tertiles**
		Females	NS (p=0.74), 3rd tertile
Strøm et al. 2014 General population (n=876 adults age 20 years)	3.7 ng/mL (median maternal PFOA)	ADHD	NS (p=0.45 for trend of 3rd tertile)
		Depression	NS (p=0.28 for trend of 3rd tertile)
		Scholastic achievement	NS (p=0.21 for trend of 3rd tertile)

Table 2-24. Summary of Neurodevelopmental Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Vuong et al. 2016 General population (n=256 children aged 5 or 8 years)	5.4 ng/mL (maternal median PFOA)	Behavioral regulation	β 1.11(-1.22–3.44)
		Metacognition	β 0.58 (-1.77–2.93)
		Global executive functioning	β 1.06 (-1.33–3.45)
Vuong et al. 2018 General population (n=208 8-year-old children)	2.4 ng/mL (mean serum PFOA)	Metacognition index score	NS (p>0.05)
		Behavior regulation index score	NS (p>0.05)
		Global executive functioning score	NS (p>0.05)
		At risk metacognition score	**OR 3.18 (1.17–8.60)***
		At risk behavior regulation score	OR 1.56 (0.49–4.92)
		At risk global executive score	OR 2.69 (0.92–7.90)
Wang et al. 2015b General population (n=120 children age 5 years and 120 children aged 8 years)	2.50 and 2.50 ng/mL (maternal median PFOA for 5- and 8-year-old children)	IQ score Age 5 years Age 8 years	 NS NS
Wu et al. 2012 General population (n=167 pregnant women at 2 hospitals)	18.32 and 9.76 ng/mL (mean maternal serum PFOA at each hospital)	5-minute Apgar score	**β -1.37 (-2.42 to -0.32, p<0.05)***
Zhang et al. 2018 General population (n=167 mother-child pairs)	5.4 ng/mL (median maternal PFOA)	Reading scores At 5 years of age At 8 years of age	 NS (p>0.05) NS (p>0.05)
Zhang et al. 2018 General population (n=167 mother-child pairs)	5.5 ng/mL (median PFOA in 3-year-old children)	Reading scores At 5 years of age At 8 years of age	 **Association (p<0.05)*** NS (p>0.05)
Zhang et al. 2018 General population (n=167 mother-child pairs)	2.4 ng/mL (median PFOA in 5-year-old children)	Reading scores At 8 years of age	 **Association (p<0.05)***

Table 2-24. Summary of Neurodevelopmental Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFOS			
Stein and Savitz 2011 Community (C8) (n=10,546 children aged 5–18 years)	14.8–<20.2, 20.22–<27.9, and 27.9–202.1 ng/mL (2nd, 3rd, and 4th PFOS quartiles)	ADHD	OR 0.99 (0.76–1.30), 4th quartile
		Learning problems 5–18 years old 12–15 years old	 **OR 0.83 (0.70–0.98)*, 2nd quartile** **OR 0.68 (0.52–0.89)*, 3rd quartile**
Braun et al. 2014 General population (n=175 children 4 and 5 years old)	13 ng/mL (maternal PFOS median)	Social responsiveness scale score (test of autism)	No association
Donauer et al. 2015 General population (n=349 infants)	13.25 ng/mL (maternal geometric mean PFOS)	Neurobehavioral outcomes	NS (p>0.05)
		Hypotonic	NS (p=0.3996)
		High arousal/difficult	NS (p=0.4678)
Fei et al. 2008b General population (n=1,400 infants)	35.3 ng/mL (maternal median PFOS)	Apgar scores <10	OR 1.20 (0.67–2.14)
		Neurobehavioral milestones Delay in age of sitting Earlier use of word-like sounds Delays in using 2-word sentences Other milestones	 **Association (p=0.041 for trend)*** **Association (p=0.039 for trend)*** **Association (p=0.050 for trend)*** NS (p>0.05)
		Motor and mental development at 6 months	NS (p>0.05)
Fei and Olsen 2011 General population (n=526–787 children)	34.4 ng/mL (maternal median PFOS)	Behavioral health	NS (p>0.39 for trend)
		Motor coordination	NS (p=0.41 for trend)
Forns et al. 2015 General population (n=843 infants)	110 ng/L (median PFOS breast milk level)	Risk of an abnormal score on neurobehavioral assessment questionnaire	OR 0.96 (0.76–1.20) at 6 months of age OR 0.93 (0.74–1.17) at 24 months of age
Goudarzi et al. 2016b General population (n=173 at 6 months and 133 at 18 months)	5.7 ng/mL (maternal median PFOS)	MDI/PDI at 6 months of age	NS (p>0.05)
		MDI/PDI at 18 months of age	NS (p>0.05)

Table 2-24. Summary of Neurodevelopmental Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Gump et al. 2011 General population (n=83 children aged 9–11 years)	9.90 ng/mL (mean PFOS)	Performance on task requiring behavioral inhibition	**Inverse association (p<0.05)***
Hoffman et al. 2010 General population (NHANES) (n=571 children 12–15 years)	22.6 ng/mL (median PFOS)	ADHD (parent reported)	**OR 1.03 (1.01–1.05)*, per 1 ng/mL PFOS**
Høyer et al. 2015a General population (n=1,106 children)	10.0 and 16.6–87.3 ng/mL (maternal median and 3rd tertile PFOS)	Motor skills	β -0.1 (-1.2–1.1)
		Abnormal behavior	OR 1.5 (0.5–4.8), 3rd tertile
		Hyperactivity	OR 1.4 (0.4–4.9), 3rd tertile
Jeddy et al. 2017 General population (n=432 mother-daughter pairs)	19.8 ng/mL (maternal median serum PFOS)	Verbal comprehension (15-month-olds)	**β 0.03 (0.01–0.05)***
		Vocabulary comprehension and production (15-month-olds)	NS (p>0.05)
		Nonverbal communication (15-month-olds)	NS (p>0.05)
		Social development (15-month-olds)	NS (p>0.05)
		Intelligibility scores (38-month-olds)	**β -0.01 (-0.01–0.00)***
		Language scores (38-month-olds)	NS (p>0.05)
		Communicative scores (38-month-olds)	NS (p>0.05)
Lien et al. 2016 General population (n=282 children aged 7 years)	4.79 ng/mL (cord blood weighted average PFOS)	Inattention	NS (p=0.8508)
		Hyperactivity/impulsivity	NS (p=0.6857)
		Emotional symptoms	NS (p=0.9431)
		Conduct problems	NS (p=0.4938)
		Hyperactivity/inattention	NS (p=0.5226)

Table 2-24. Summary of Neurodevelopmental Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Liew et al. 2015 General population (n=215, ADHD cases, 213 autism cases, 545 controls)	26.80, 25.40, and 27.40 ng/mL (maternal median PFOS for ADHD, autism, and controls)	ADHD	RR 0.87 (0.74–1.02) **RR 0.79 (0.64–0.98)*, 4th quartile**
		Autism	RR 0.92 (0.69–1.22)
Ode et al. 2014 General population (n=206 children with ADHD and 206 controls)	6.92 and 6.77 ng/mL (cord blood median PFOS in cases and controls)	ADHD	OR 0.98 (0.92–1.04), per 1 ng/mL increase in PFOS
Oulhote et al. 2016 General population (n=567 7-year-old children)	27.42 ng/mL (maternal geometric mean PFOS)	Behavioral development scores	No associations
Oulhote et al. 2016 General population (n=567 7-year-old children)	16.75 ng/mL (geometric mean PFOS in 5-year-old children)	Behavioral development scores	No associations
Oulhote et al. 2016 General population (n=567 7-year-old children)	15.27 ng/mL (geometric mean PFOS in 7-year-old children)	Behavioral development scores	No associations
Quaak et al. 2016 General population (n=76 infants 18 months of age)	1.5836 ng/mL (cord mean PFOS)	Score on test evaluating ADHD	NS (p=0.19), 3rd tertile
		Males	NS (p=0.35), 3rd tertile
		Females	NS (p=0.43), 3rd tertile
		Scores on test evaluating externalizing problem	NS (p=0.31), 3rd tertile
		Males	NS (p=0.74), 3rd tertile
		Females	NS (p=0.31), 3rd tertile
Strøm et al. 2014 General population (n=876 adults age 20 years)	21.4 ng/mL (median maternal PFOS)	ADHD	NS (p=0.38 for trend of 3rd tertile)
		Depression	NS (p=0.14 for trend of 3rd tertile)
		Scholastic achievement	NS (p=0.59 for trend of 3rd tertile)

Table 2-24. Summary of Neurodevelopmental Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Vuong et al. 2016 General population (n=256 children 5 or 8 years of age)	13.2 ng/mL (maternal median PFOS)	Behavioral regulation	**β 3.14 (0.68–5.61)***
		Metacognition	**β 3.10 (0.62–5.58)***
		Global executive functioning	**β 3.38 (0.86–5.90)***
		Global executive functioning composite score >60	**OR 2.19 (1.03–4.66)***
Vuong et al. 2018 General population (n=208 8-year-old children)	3.9 ng/mL (mean serum PFOS)	Metacognition index score	NS (p>0.05)
		Behavior regulation index score	NS (p>0.05)
		Global executive functioning score	NS (p>0.05)
		At risk metacognition score	OR 1.53 (0.67–3.52)
		At risk behavior regulation score	OR 0.40 (0.14–1.14)
		At risk global executive score	OR 1.04 (0.41–2.68)
Wang et al. 2015b General population (n=120 children age 5 years and 120 children aged 8 years)	13.25 and 12.28 ng/mL (maternal median PFOS for 5- and 8-year-old children)	IQ score Age 5 years Age 8 years	 NS (p>0.05) NS (p>0.05)
Zhang et al. 2018 General population (n=167 mother-child pairs)	13.0 ng/mL (median maternal PFOS)	Reading scores At 5 years of age At 8 years of age	 NS (p>0.05) NS (p>0.05)
Zhang et al. 2018 General population (n=167 mother-child pairs)	6.6 ng/mL (median PFOS in 3-year-old children)	Reading scores At 5 years of age At 8 years of age	 **Association (p<0.05)*** NS (p>0.05)
Zhang et al. 2018 General population (n=167 mother-child pairs)	3.6 ng/mL (median PFOS in 5-year-old children)	Reading scores At 8 years of age	 **Association (p<0.05)***

Table 2-24. Summary of Neurodevelopmental Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFHxS			
Stein and Savitz 2011 Community (C8) (n=10,546 children aged 5–18 years)	2.9–<5.2 and 10.1–276.4 ng/mL (2nd and 4th PFHxS quartiles)	ADHD 5–18 years 12–15 years	 **OR 1.27 (1.06–1.52)*, 2nd quartile** **OR 1.46 (1.10–1.93)*, 2nd quartile**
		Learning problems 5–18 years old 12–15 years old	 OR 1.19 (1.00–1.41), 4th quartile OR 1.05 (0.79–1.40), 4th quartile
Braun et al. 2014 General population (n=175 children 4 and 5 years old)	1.6 ng/mL (maternal PFHxS median)	Social responsiveness scale score (test for autism)	No association
Gump et al. 2011 General population (n=83 children aged 9–11 years)	6.06 ng/mL (mean PFHxS)	Performance on task requiring behavioral inhibition	**Inverse association (p<0.01)***
Hoffman et al. 2010 General population (NHANES) (n=571 children 12–15 years)	2.2 ng/mL (median PFHxS)	ADHD (parent reported)	**OR 1.06 (1.02–1.11)*, per 1 ng/mL PFOS**
Jeddy et al. 2017 General population (n=432 mother-daughter pairs)	1.6 ng/mL (maternal median serum PFHxS)	Verbal comprehension (15-month-olds)	NS (p>0.05)
		Vocabulary comprehension and production (15-month-olds)	NS (p>0.05)
		Nonverbal communication (15-month-olds)	NS (p>0.05)
		Social development (15-month-olds)	NS (p>0.05)
		Intelligibility scores (38-month-olds)	NS (p>0.05)
		Language scores (38-month-olds)	NS (p>0.05)

Table 2-24. Summary of Neurodevelopmental Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
		Communicative scores (38-month-olds)	NS (p>0.05)
Liew et al. 2015 General population (n=215, ADHD cases, 213 autism cases, 545 controls)	0.84. 0.92, and 0.92 ng/mL (maternal median PFHxS for ADHD, autism, and controls)	ADHD	**RR 0.67 (0.54–0.83)*, 4th quartile**
		Autism	RR 1.10 (0.92–1.33)
Oulhote et al. 2016 General population (n=567 7-year-old children)	4.43 ng/mL (maternal geometric mean PFHxS)	Behavioral development scores	No associations
Oulhote et al. 2016 General population (n=567 7-year-old children)	0.54 ng/mL (geometric mean PFHxS in 5-year-old children)	Behavioral development scores	No associations
Oulhote et al. 2016 General population (n=567 7-year-old children)	0.53 ng/mL (geometric mean PFHxS in 7-year-old children)	Behavioral development scores	No associations
Vuong et al. 2016 General population (n=256 children 5 or 8 years of age)	1.5 ng/mL (maternal median PFHxS)	Behavioral regulation	β 1.19 (-0.54–5.40)
		Metacognition	β 1.31 (-0.43–3.04)
		Global executive functioning	β 1.36 (-0.41–3.12)
		Global executive functioning composite score >60	**OR 1.71 (1.05–2.77)***
Vuong et al. 2018 General population (n=208 8-year-old children)	1.4 ng/mL (mean serum PFHxS)	Metacognition index score	NS (p>0.05)
		Behavior regulation index score	NS (p>0.05)
		Global executive functioning score	NS (p>0.05)
		At risk metacognition score	OR 1.10 (0.58–2.09)
		At risk behavior regulation score	OR 0.54 (0.22–1.32)
		At risk global executive score	OR 0.65 (0.32–1.32)

Table 2-24. Summary of Neurodevelopmental Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Wang et al. 2015b General population (n=120 children age 5 years and 120 children aged 8 years)	0.69 and 0.69 ng/mL (maternal median PFHxS for 5- and 8-year-old children)	IQ score Age 5 years Age 8 years	 NS (p>0.05) NS (p>0.05)
Zhang et al. 2018 General population (n=167 mother-child pairs)	1.5 ng/mL (median maternal PFHxS)	Reading scores At 5 years of age At 8 years of age	 NS (p>0.05) NS (p>0.05)
Zhang et al. 2018 General population (n=167 mother-child pairs)	1.9 ng/mL (median PFHxS in 3-year-old children)	Reading scores At 5 years of age At 8 years of age	 NS (p>0.05) NS (p>0.05)
Zhang et al. 2018 General population (n=167 mother-child pairs)	1.2 ng/mL (median PFHxS in 5-year-old children)	Reading scores At 8 years of age	 NS (p>0.05)
PFNA			
Stein and Savitz 2011 Community (C8) (n=10,546 children aged 5–18 years)	1.2–<1.5, 1.5–<2.0, and 2.0–24.1 ng/mL (2nd, 3rd, and 4th PFNA quartiles)	ADHD 5–18 years 12–15 years	 OR 0.99 (0.84–1.18), 4th quartile OR 1.00 (0.75–1.32), 4th quartile
		Learning problems 5–18 years old 12–15 years old	 **OR 0.81 (0.69–0.95)*, 3rd quartile** **OR 0.73 (0.55–0.98)*, 4th quartile**
Braun et al. 2014 General population (n=175 children 4 and 5 years old)	0.9 ng/mL (maternal PFNA median)	Social responsiveness scale score (tests for autism)	No association
Gump et al. 2011 General population (n=83 children aged 9–11 years)	0.82 ng/mL (mean PFNA)	Performance on task requiring behavioral inhibition	**Inverse association (p<0.05)***
Hoffman et al. 2010 General population (NHANES) (n=571 children 12–15 years)	0.6 ng/mL (median PFNA)	ADHD (parent reported)	OR 1.32 (0.86–2.02), per 1 ng/mL PFNA

Table 2-24. Summary of Neurodevelopmental Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Jeddy et al. 2017 General population (n=432 mother-daughter pairs)	0.5 ng/mL (maternal median serum PFNA)	Verbal comprehension (15-month-olds)	NS (p>0.05)
		Vocabulary comprehension and production (15-month-olds)	NS (p>0.05)
		Nonverbal communication (15-month-olds)	NS (p>0.05)
		Social development (15-month-olds)	NS (p>0.05)
		Intelligibility scores (38-month-olds)	NS (p>0.05)
		Language scores (38-month-olds)	NS (p>0.05)
		Communicative scores (38-month-olds)	NS (p>0.05)
Lien et al. 2016 General population (n=282 children aged 7 years)	4.49 ng/mL (cord blood weighted average PFNA)	Inattention	**Inverse association (p=0.0129)***
		Hyperactivity/impulsivity	NS (p=0.0588)
		Emotional symptoms	NS (p=0.1902)
		Conduct problems	NS (p=0.6931)
		Hyperactivity/inattention	**Inverse association (p=0.0484)***
Liew et al. 2015 General population (n=215, ADHD cases, 213 autism cases, 545 controls)	0.42, 0.41, and 0.43 ng/mL (maternal median PFNA for ADHD, autism, and controls)	ADHD	RR 0.80 (0.62–1.03)
		Autism	RR 0.80 (0.58–1.11)
Oulhote et al. 2016 General population (n=567 7-year-old children)	0.61 ng/mL (maternal geometric mean PFNA)	Behavioral development scores	No association
Oulhote et al. 2016 General population (n=567 7-year-old children)	1.01 ng/mL (geometric mean PFNA in 5-year-old children)	Total behavioral development score and higher externalizing problems score	**Association***

Table 2-24. Summary of Neurodevelopmental Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Oulhote et al. 2016 General population (n=567 7-year-old children)	1.2 ng/mL (geometric mean PFNA in 7-year-old children)	Behavioral development scores	No association
Vuong et al. 2016 General population (n=256 children 5 or 8 years of age)	0.9 ng/mL (maternal median PFNA)	Behavioral regulation	β 2.57 (-0.26–5.40)
		Metacognition	β 1.37 (-1.49–4.23)
		Global executive functioning	β 2.01 (-0.89–4.92)
Vuong et al. 2018 General population (n=208 8-year-old children)	0.8 ng/mL (mean serum PFNA)	Metacognition index score	**β 3.4 (0.4–6.3, p<0.05)***
		Behavior regulation index score	NS (p>0.05)
		Global executive functioning score	**Association (p<0.05)***
		At risk metacognition score	**OR 2.94 (1.52–5.69)***
		At risk behavior regulation score	**OR 2.75 (1.30–5.79)***
		At risk global executive score	**OR 3.07 (1.60–5.90)***
Wang et al. 2015b General population (n=120 children age 5 years and 120 children aged 8 years)	1.59 and 1.44 ng/mL (maternal median PFNA for 5- and 8-year-old children)	IQ score	NS (p>0.05)
		IQ scores, age 8 years	
		Full scale IQ	NS (p>0.05)
		Visual IQ	**Association (p<0.05)***
		Performance IQ	NS (p>0.05)
Zhang et al. 2018 General population (n=167 mother-child pairs)	0.9 ng/mL (median maternal PFNA)	Reading scores	
		At 5 years of age	NS (p>0.05)
		At 8 years of age	NS (p>0.05)
Zhang et al. 2018 General population (n=167 mother-child pairs)	1.2 ng/mL (median PFNA in 3-year-old children)	Reading scores	
		At 5 years of age	**Association (p<0.05)***
		At 8 years of age	NS (p>0.05)
Zhang et al. 2018 General population (n=167 mother-child pairs)	0.7 ng/mL (median PFNA in 5-year-old children)	Reading scores	
		At 8 years of age	NS (p>0.05)

Table 2-24. Summary of Neurodevelopmental Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFDA			
Gump et al. 2011 General population (n=83 children aged 9–11 years)	0.26 ng/mL (mean PFDA)	Performance on task requiring behavioral inhibition	**Inverse association (p<0.05)***
Liew et al. 2015 General population (n=215, ADHD cases, 213 autism cases, 545 controls)	0.15, 0.15, and 0.17 ng/mL (maternal median PFDA for ADHD, autism, and controls)	ADHD	**RR 0.76 (0.64–0.91)*** **RR 0.53 (0.43–0.66)*, 4th quartile**
		Autism	RR 0.79 (0.63–1.01) **RR 0.52 (0.35–0.77)*, 4th quartile**
Oulhote et al. 2016 General population (n=567 7-year-old children)	0.28 ng/mL (maternal geometric mean PFDA)	Behavioral development scores	No associations
Oulhote et al. 2016 General population (n=567 7-year-old children)	0.28 ng/mL (geometric mean PFDA in 5-year-old children)	Total behavioral development score and higher externalizing problems and hyperactivity/ inattention scores	**Associations***
Oulhote et al. 2016 General population (n=567 7-year-old children)	0.36 ng/mL (geometric mean PFDA in 7-year-old children)	Behavioral development scores	No associations
Vuong et al. 2016 General population (n=256 children 5 or 8 years of age)	0.2 ng/mL (maternal median PFDA)	Behavioral regulation	β 0.70 (-3.31–1.92)
		Metacognition	β 1.24 (-3.87–1.39)
		Global executive functioning	β -1.13 (-3.79–1.54)
Wang et al. 2015b General population (n=120 children age 5 years and 120 children aged 8 years)	0.44 and 0.44 ng/mL (maternal median PFDA for 5- and 8-year-old children)	IQ score Age 5 years Age 8 years	 NS (p>0.05) NS (p>0.05)

Table 2-24. Summary of Neurodevelopmental Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFUnA			
Lien et al. 2016 General population (n=282 children aged 7 years)	7.96 ng/mL (cord blood weighted average PFUnA)	Inattention	NS (p=0.6177)
		Hyperactivity/impulsivity	NS (p=0.3642)
		Emotional symptoms	NS (p=0.0517)
		Conduct problems	NS (p=0.1207)
		Hyperactivity/inattention	NS (p=0.9991)
Wang et al. 2015b General population (n=120 children age 5 years and 120 children aged 8 years)	3.42 and 3.13 ng/mL (maternal median PFUnA for 5- and 8-year-old children)	IQ score	NS (p>0.05)
		IQ scores, age 8 years	
		Full scale IQ	NS (p>0.05)
		Visual IQ	NS (p>0.05)
		Performance IQ	**Inverse association (p<0.05)***
PFDoDA			
Wang et al. 2015b General population (n=120 children age 5 years and 120 children aged 8 years)	0.38 and 0.37 ng/mL (maternal median PFDoDA for 5- and 8-year-old children)	IQ score	
		Age 5 years	NS (p>0.05)
		Age 8 years	NS (p>0.05)
FOSA			
Gump et al. 2011 General population (n=83 children aged 9–11 years)	0.75 ng/mL (mean FOSA)	Performance on task requiring behavioral inhibition	**Inverse association (p<0.05)***

[a]See the *Supporting Document for Epidemiological Studies for Perfluoroalkyls,* Table 13 for more detailed descriptions of studies.
[b]Participants in occupational exposure may have also lived near the site and received residential exposure; community exposure studies involved subjects living near PFOA facilities with known exposure to high levels of PFOA.
[c]Asterisk indicates association with perfluoroalkyl; unless otherwise specified, values in parenthesis are 95% confidence intervals.

ADHD = attention deficit hyperactivity disorder; FOSA = perfluorooctane sulfonamide; MDI/PDI = mental and psychomotor development indices; NHANES = National Health and Nutrition Examination Survey; NS = not significant; OR = odds ratio; PFDA = perfluorodecanoic acid; PFDoDA = perfluorododecanoic acid; PFHxS = perfluorohexane sulfonic acid; PFNA = perfluorononanoic acid; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid; PFUnA = perfluoroundecanoic acid

Table 2-25. Summary of Effects on the Development of the Reproductive System in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFOA			
Lopez-Espinosa et al. 2011 Community (C8) (n=3,076 boys and 2,931 girls aged 8–18 years)	26 and 20 ng/mL (median PFOA in boys and girls)	Age of puberty Boys Girls	 OR 0.95 (0.84–0.07) **OR 0.54 (0.35–0.84)*, 2nd quartile**
Lopez-Espinosa et al. 2016 Community (C8) (n=1,169 boys and 1,123 girls aged 6–9 years)	34.8 and 30.1 ng/mL (median PFOA in boys and girls)	Estradiol	NS (interquartile difference of 4.3, 95% CI -0.4–9.1), boys NS (4.2, 95% CI -0.7–9.4), girls
		Total testosterone	**Inverse association (-4.9, 95% CI -8.7 to -0.8)*, boys** NS (-2.5, 95% CI -6.7–1.8), girls
		Insulin-like growth factor-1	NS (-0.4, 95% CI -3.4–2.7), boys **Inverse association (-3.6, 95% CI -6.6 to -0.5)*, girls**
Christensen et al. 2011 General population (n=448 girls)	3.7 ng/mL (maternal median PFOA)	Earlier age of menarche	OR 1.01 (0.61–1.68)
Itoh et al. 2016 General population (n=189 infants)	1.4 ng/mL (maternal median PFOA)	Cord estradiol	NS (p>0.05)
		Cord testosterone	NS (p>0.05)
		Cord testosterone: estradiol ratio	NS (p>0.05)
		Cord progesterone	NS (p>0.05)
		Cord prolactin	NS (p>0.05)
		Cord LH	NS (p>0.05)
		Cord FSH	NS (p>0.05)
		Cord SHBG	NS (p>0.05)
		Cord insulin-like factor 3	NS (p>0.05)
		Cord inhibin Males Females	 **Association (p=0.040)*** NS (p>0.05)

Table 2-25. Summary of Effects on the Development of the Reproductive System in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Kristensen et al. 2013 General population (n=343 females approximately 20 years of age)	3.6 and 4.4–19.8 ng/mL (maternal median PFOA and 3rd PFOA tertile)	Age of menarche	**Association (p=0.01)***
		Menstrual cycle length	NS (p>0.05)
		Total testosterone	NS (p>0.05)
		SHBG	NS (p>0.05)
		Free androgen index	NS (p>0.05)
		Dehydroepiandrosterone sulphate	NS (p>0.05)
		Anti-Müllerian hormone	NS (p>0.05)
		Number of follicles/ovary	NS (p>0.05)
Lind et al. 2017a General population (n=649 pregnant women)	1.7 ng/mL (median maternal serum PFOA)	Anogenital distance	NS (p=0.71), boys NS (p=0.71), girls
Maisonet et al. 2015 General population (n=72 girls aged 15 years)	>4.1 ng/mL (maternal 3rd tertile PFOA)	Testosterone	**β 0.24 (0.05–0.43)*, 3rd tertile**
		SHBG	β 5.02 (-13.07–11.00), 3rd tertile
Vesterholm Jensen et al. 2014 General population (n=107 cases cryptorchidism [29 from Denmark and 78 from Finland] and 108 matched controls from Denmark and Finland)	2.6 and 2.1 ng/mL (median cord blood PFOA Denmark and Finland cohorts)	Cryptorchidism	OR 0.51 (0.21–1.20), whole cohort **OR 0.35 (0.12–0.99, p=0.04 for trend)*, Finland cohort 3rd tertile**
PFOS			
Lopez-Espinosa et al. 2011 Community (C8) (n=3,076 boys and 2,931 girls aged 8–18)	20 and 18 ng/mL (PFOS median in boys and girls)	Age of puberty Boys Girls	 **OR 0.58 (0.37–0.90)*, 3rd quartile** **OR 0.55 (0.35–0.86)*, 3rd quartile**

Table 2-25. Summary of Effects on the Development of the Reproductive System in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Lopez-Espinosa et al. 2016 Community (C8) (n=1,169 boys and 1,123 girls aged 6–9 years)	22.4 and 20.9 ng/mL (PFOS median in boys and girls)	Estradiol	**Inverse association (interquartile difference of -4.0, 95% CI -7.7 to -0.1)*, boys** NS (-0.3, 95% CI -4.6–4.2), girls
		Total testosterone	**Inverse association (-5.8, 95% CI -9.4 to -2.0)*, boys** **Inverse association (-6.6, 95% CI -10.1 to -2.8)*, girls**
		Insulin-like growth factor-1	**Inverse association (-5.9, 95% CI -8.3 to -3.3)*, boys** **Inverse association (-5.6, 95% CI -8.2 to -2.9)*, girls**
Christensen et al. 2011 General population (n=448 girls)	19.8 ng/mL (maternal median PFOS)	Earlier age of menarche	OR 0.68 (0.40–1.13)
Itoh et al. 2016 General population (n=189 infants)	5.2 ng/mL (maternal median PFOS)	Cord estradiol Males Females	 **Association (p=0.021)*** NS (p>0.05)
		Cord testosterone	NS (p>0.05)
		Cord testosterone: estradiol ratio Males Females	 **Inverse association (p=0.008)*** NS (p>0.05)
		Cord progesterone Males Females	 **Association (p=0.043)*** **Association (p=0.002)***
		Cord prolactin Males Females	 NS (p>0.05) **Association (p=0.001)***
		Cord LH	NS (p>0.05)
		Cord FSH	NS (p>0.05)
		Cord SHBG	NS (p>0.05)

Table 2-25. Summary of Effects on the Development of the Reproductive System in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
		Cord insulin-like factor 3	NS (p>0.05)
		Cord inhibin Males Females	 **Association (p<0.001)*** NS (p>0.05)
Kristensen et al. 2013 General population (n=343 young women approximately 20 years of age)	21.1 ng/mL (maternal median PFOS)	Age of menarche	NS (p=0.28)
		Menstrual cycle length	NS (p>0.05)
		Total testosterone	NS (p>0.05)
		SHBG	NS (p>0.05)
		Free androgen index	NS (p>0.05)
		Dehydroepiandrosterone sulphate	NS (p>0.05)
		Anti-Müllerian hormone	NS (p>0.05)
		Number of follicles/ovary	NS (p>0.05)
Lind et al. 2017a General population (n=649 pregnant women)	8.1 ng/mL (median maternal serum PFOS)	Anogenital distance	β 0.5 (-1.2–2.2, p=0.55), boys **β -0.4(-3.8 to -0.7, p<0.01)*, girls**
Toft et al. 2016 General population (270 cases cryptorchidism, 75 cases hypospadias, and 300 controls)	>1.4 ng/mL (amniotic fluid 3rd tertile PFOS)	Cryptorchidism	OR 1.01 (0.66–1.53), 3rd tertile
		Hypospadias	OR 0.69 (0.35–1.38), 3rd tertile
		Testosterone	**Association (p=0.002)***
		Androstenedione	**Association (p=0.001)***
		Progesterone	**Association (p=0.001)***
		Cortisol	**Association (p<0.001)***
		DHEAS	NS (p=0.93)
		Insulin-like factor 3	**Inverse association (p<0.001)***
Vesterholm Jensen et al. 2014 General population (n=107 cases cryptorchidism [29 from Denmark and 78 from Finland] and 108 matched controls from Denmark and Finland)	9.1 and 5.2 ng/mL (median cord blood PFOS Denmark and Finland cohorts)	Cryptorchidism	OR 0.83 (0.44–1.58), whole cohort

Table 2-25. Summary of Effects on the Development of the Reproductive System in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFHxS			
Lopez-Espinosa et al. 2016 Community (C8) (n=1,169 boys and 1,123 girls aged 6–9 years)	8.1 and 7.0 ng/mL (PFHxS median in boys and girls)	Estradiol	NS (interquartile difference of -1.3, 95% CI -5.5–3.1), boys NS (2.1, 95% CI -2.2–6.5), girls
		Total testosterone	NS (-2.7, 95% CI -6.4–1.2), boys NS (0.2, 95% CI -3.5–4.0), girls
		Insulin-like growth factor-1	NS (-2.5, 95% CI -5.2–0.3), boys NS (-2.1, 95% CI -4.8–0.7), girls
Christensen et al. 2011 General population (n=448 girls)	1.6 ng/mL (maternal median PFHxS)	Earlier age of menarche	OR 0.89 (0.65–1.22)
Lind et al. 2017a General population (n=649 pregnant women)	0.3 ng/mL (median maternal serum PFHxS)	Anogenital distance	NS (p=0.56), boys NS (p=0.10), girls
Maisonet et al. 2015 General population (n=72 girls aged 15 years)	>1.9 ng/mL (maternal 3rd tertile PFHxS)	Testosterone	β 0.18 (0.00–0.35), 3rd tertile
		SHBG	β 5.31 (-21.61–11.00), 3rd tertile
PFNA			
Lopez-Espinosa et al. 2016 Community (C8) (n=1,169 boys and 1,123 girls aged 6–9 years)	1.7 and 1.7 ng/mL (PFNA median in boys and girls)	Estradiol	NS (interquartile difference of -2.5, 95% CI -6.2–1.4), boys NS (-2.4, 95% CI -6.3–1.7), girls
		Total testosterone	NS (-2.1, 95% CI -5.5–1.3), boys NS (-1.9, 95% CI -5.5–1.9), girls
		Insulin-like growth factor-1	**Inverse association (-3.5, 95% CI -6.0 to -1.0)*, boys** **Inverse association (-3.8, 95% CI -6.4 to -1.2)*, girls**
Lind et al. 2017a General population (n=649 pregnant women)	0.7 ng/mL (median maternal serum PFNA)	Anogenital distance	β -0.5 (-2.1–1.1, p=0.63), boys **β -1.8 (-3.5 to -0.1, p=0.05)*, girls**

Table 2-25. Summary of Effects on the Development of the Reproductive System in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Maisonet et al. 2015 General population (n=72 girls aged 15 years)	>0.6 ng/mL (maternal 3rd tertile PFNA)	Testosterone	β 0.05 (-0.14–0.24), 3rd tertile
		SHBG	β 7.91 (-8.69–24.52), 3rd tertile
PFDA			
Lind et al. 2017a General population (n=649 pregnant women)	0.3 ng/mL (median maternal serum PFDA)	Anogenital distance	β -0.6 (-2.0–0.9, p=0.97), boys **β -1.3 (-2.8–0.2, p=0.04)*, girls**
FOSA			
Christensen et al. 2011 General population (n=448 girls)	0.2 ng/mL (maternal median FOSA)	Earlier age of menarche	OR 0.91 (0.67–1.24)

[a]See the *Supporting Document for Epidemiological Studies for Perfluoroalkyls,* Table 13 for more detailed descriptions of studies.
[b]Participants in occupational exposure may have also lived near the site and received residential exposure; community exposure studies involved subjects living near PFOA facilities with known exposure to high levels of PFOA.
[c]Asterisk indicates association with perfluoroalkyl; unless otherwise specified, values in parenthesis are 95% confidence intervals.

CI = confidence interval; DHEAS = dihydroepiandrosterone sulfate; FOSA = perfluorooctane sulfonamide; FSH = follicle stimulating hormone; LH = luteinizing hormone; NS = not significant; OR = odds ratio; PFDA = perfluorodecanoic acid; PFHxS = perfluorohexane sulfonic acid; PFNA = perfluorononanoic acid; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid; SHBG = sex hormone binding globulin

alterations in birth weight were found for other perfluoroalkyls (PFHxS, PFNA, PFDA, PFUnA, PFDoDA). Overall, no associations were found between serum PFOA, PFOS, PFHxS, PFNA, or PFUnA and increases in the risk of low birth weight or small for gestational age infants. The small number of studies (2 or less) examining potential developmental effects of PFHpA, PFBA, and FOSA do not allow for assessing possible associations with pregnancy outcomes or birth outcomes.

No consistent results for risks of birth defects have been found; these potential endpoints were only examined for a few perfluoroalkyls. The available epidemiological data do not suggest associations between perfluoroalkyls and IQ or scholastic achievement for PFOA, PFOS, PFHxS, PFNA, PFDA, PFUnA, or PFDoDA. Similarly, no associations were found between PFOA, PFOS, PFHxS, PFNA, or PFDA and increased risk of ADHD; several studies found decreased risk of ADHD. Inconsistent results have been found between PFOA and PFOS and delays in puberty or age of puberty, especially in girls.

Summaries of laboratory animal studies are presented in Tables 2-1, 2-3, 2-4, and 2-5 and the NOAEL and LOAEL values are presented in Figures 2-6, 2-8, 2-9, and 2-10; no data were available for PFHpA or FOSA. Laboratory animal studies provide strong evidence of the developmental toxicity of a number of perfluoroalkyls. Prenatal losses and decreases in pup survival were observed following exposure to PFOA, PFOS, PFNA, PFDA, PFDoDA and PFHxA; no deaths were observed in a single study of PFBS. Decreases in fetal weights, birth weight, and pup weight were observed in studies of PFOA, PFOS, PFNA, PFDA, PFUnA, PFBS, and PFHxA; no effects on weight were observed in studies on PFHxS or PFDoDA. In PFOA studies, delays in mammary gland development were observed at fairly low doses. Several studies have demonstrated biphasic alterations in motor activity in rodents exposed to PFOA, PFOS, and PFHxS; no effects on locomotor activity were observed in a study of PFDA. Studies in laboratory animals have examined a number of developmental endpoints, including pup survival, malformations, birth weight, mammary gland development, and neurodevelopment.

PFOA

Epidemiological Studies—Pregnancy Outcomes. The results of available epidemiological studies of women living near a PFOA facility and the general population do not suggest an association between serum PFOA levels and adverse pregnancy outcomes. No increases in risk of miscarriage (Darrow et al. 2014; Jensen et al. 2015; Savitz et al. 2012b; Stein et al. 2009), stillbirths (Savitz et al. 2012b), pregnancy loss (Buck Louis et al. 2016), or pre-term birth (Chen et al. 2012a; Darrow et al. 2013; Hamm et al. 2010; Manzano-Salgado et al. 2017a; Sagiv et al. 2018; Stein et al. 2009; Whitworth et al. 2012a) were found.

The Whitworth et al. (2012a) general population study reported a decrease in the risk of preterm births among women with serum PFOA levels in the 4th quartile. Most studies did not find an association between maternal PFOA levels and gestational age (Apelberg et al. 2007b; Chen et al. 2012a; Lauritzen et al. 2017; Li et al. 2017; Manzano-Salgado et al. 2017a) or gestational length (Lind et al. 2017; Sagiv et al. 2018). The exception is a study by Wu et al. (2012) of pregnant women with higher serum PFOA levels which found an inverse association between maternal serum PFOA levels and gestational age.

Epidemiological Studies—Birth Outcomes. Community and general population exposure studies have evaluated a number of birth outcomes including birth weight; risk of low birth weight; risk of small for gestational age; birth length; head, chest, and abdominal circumferences; ponderal index; sex ratio; and birth defects. In highly exposed populations, no association between maternal serum PFOA levels and birth weight were found (Darrow et al. 2013; Nolan et al. 2009; Savitz et al. 2012b). Several general population studies have found associations between maternal serum PFOA and birth weight. Fei et al. (2007, 2008a), Lauritzen et al. (2017), Lenters et al. (2016a), Maisonet et al. (2012), Minatoya et al. (2017), Starling et al. (2017), and Wu et al. (2012) found inverse associations between maternal serum PFOA and birth weight. However, 23 other general population studies did not find associations (Alkhalawi et al. 2016; Ashley-Martin et al. 2017; Bach et al. 2016; Callan et al. 2016; Cao et al. 2018; Chen et al. 2012a; Govarts et al. 2016; Hamm et al. 2010; Kim et al. 2011; Kobayashi et al. 2017; Lauritzen et al. 2017; Lee et al. 2013, 2016; Li et al. 2017; Lind et al. 2017; Manzano-Salgado et al. 2017a; Monroy et al. 2008; Robledo et al. 2015a; Sagiv et al. 2018; Shi et al. 2017; Wang et al. 2016; Washino et al. 2009; Whitworth et al. 2012a). As illustrated in Figure 2-35, most studies found no association between maternal serum PFOA levels and the risk of low birth weight infants (typically defined as <2,500 g) (Chen et al. 2012a; Darrow et al. 2013; Fei et al. 2007, 2008a; Manzano-Salgado et al. 2017a; Savitz et al. 2012b; Stein et al. 2009) or found a decreased risk of low birth weight infants (Nolan et al. 2009; Savitz et al. 2012a). Similarly, most studies found no increases in the risk for small for gestational age (Chen et al. 2012a; Fei et al. 2007, 2008a; Hamm et al. 2010; Lauritzen et al. 2017; Manzano-Salgado et al. 2017a; Savitz et al. 2012b; Wang et al. 2016; Whitworth et al. 2012a); these data are presented in Figure 2-36. One study (Savitz et al. 2012b) of C8 participants did find an increase in the risk of small for gestational age; however, when the maternal serum PFOA levels were categorized into percentiles, the risk was not increased in infants whose maternal serum PFOA levels were ≥80th percentile (21.0–717.6 ng/mL). A general population study (Lauritzen et al. 2017) also found an increased risk of small for gestational age (Lauritzen et al. 2017). Using data compiled from four European birth cohort studies in which cord serum PFOA was measured or estimated from breast milk levels, Govarts et al. (2018) did not find an association between cord PFOA and the risk of small for gestational age.

Figure 2-35. Risk of Low Birth Weight Infant Relative to PFOA Levels (Presented as Adjusted Odds Ratios)

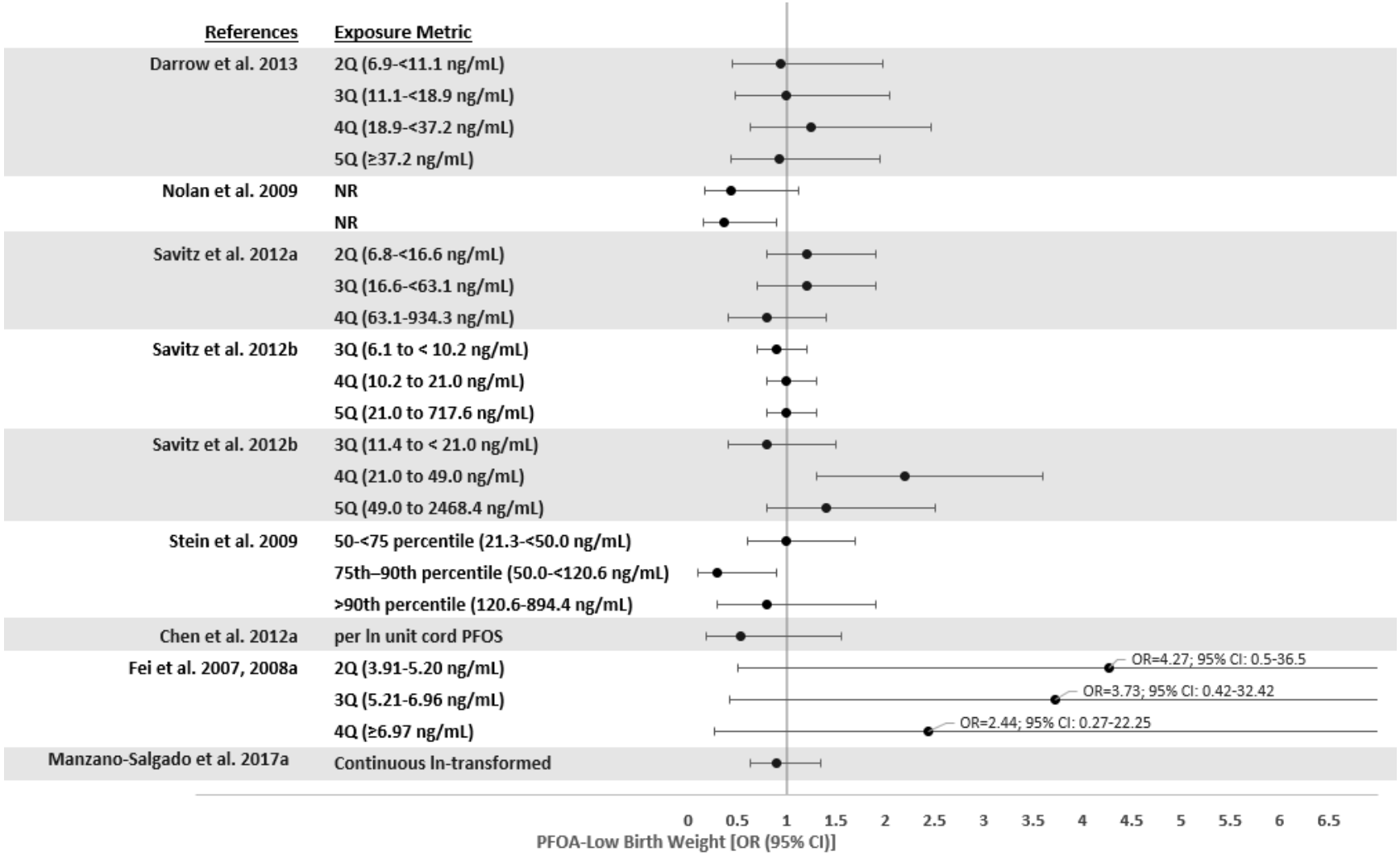
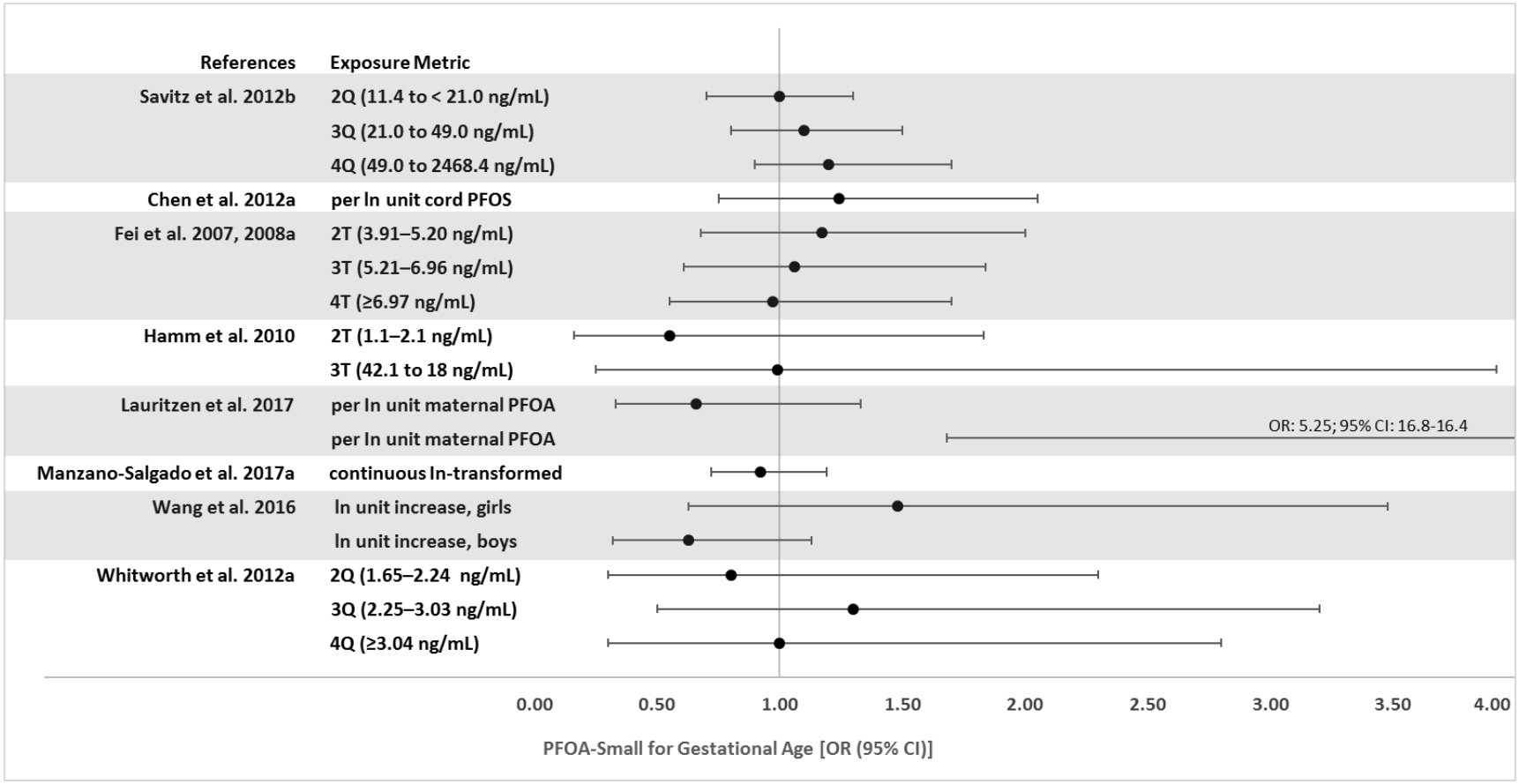


Figure 2-36. Risk of Small for Gestational Age Infant Relative to PFOA Levels (Presented as Adjusted Odds Ratios)



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However, among women who smoked during pregnancy, cord serum PFOA was associated with an increased risk of small for gestational age infants (OR 2.177, 95% CI 1.022–4.643); no association was found among nonsmoking women (OR 0.511, 95% CI 0.869–2.632). Six general population studies found inverse associations between maternal serum PFOA levels and birth length, abdominal circumference, and/or ponderal index (ratio of birth weight to birth length) (Alkhalawi et al. 2016; Apelberg et al. 2007b; Cao et al. 2018; Fei et al. 2007, 2008a; Lauritzen et al. 2017; Wu et al. 2012). However, most studies did not find associations between maternal serum PFOA levels and birth length; head, chest, or abdominal circumference; or ponderal index (Alkhalawi et al. 2016; Bach et al. 2016; Callan et al. 2016; Cao et al. 2018; Chen et al. 2012a; Kobayashi et al. 2017; Lauritzen et al. 2017; Lee et al. 2013; Maisonet et al. 2012; Manzano-Salgado et al. 2017a; Minatoya et al. 2017; Robledo et al. 2015a; Shi et al. 2017; Wang et al. 2016). Studies examining newborn leptin and adiponectin levels (Ashley-Martin et al. 2017; Minatoya et al. 2017) and adiposity (Starling et al. 2017) have not found associations with maternal PFOA levels.

In a systematic review of 19 epidemiological studies discussed above, Johnson et al. (2014) evaluated the possible association between PFOA exposure and fetal growth and concluded that there was sufficient evidence that PFOA reduces fetal growth based on a moderate rating of the human evidence. A meta-analysis of the Apelberg et al. (2007b), Chen et al. (2012a), Fei et al. (2007, 2008a), Fromme et al. (2010), Hamm et al. (2009), Kim et al. (2011), Maisonet et al. (2012), Washino et al. (2009), and Whitworth et al. (2012) studies showed an association between PFOA and birth weight; a 1 ng/mL increase in serum or plasma PFOA was associated with a -18.9 g (95% CI -29.8 to -7.9) change in birth weight. The results of this meta-analysis are also reported in Lam et al. (2014). Johnson et al. (2014) and Lam et al. (2014) discuss whether glomerular filtration rate was a possible confounder in evaluating the association between serum PFOA and birth weight. They concluded that there was insufficient evidence of an association between glomerular filtration rate and birth weight.

A second meta-analysis (Verner et al. 2015) of the Apelberg et al. (2007b), Chen et al. (2012a), Fei et al. (2007), Hamm et al. (2010), Maisonet et al. (2012), Washino et al. (2009), and Whitworth et al. (2012a) studies found a similar result, a 1 ng/mL increase in PFOA levels was associated with a 14.72 g (95% CI -21.66 to -7.78) decrease in birth weight. Verner et al. (2015) also utilized a PBPK model to simulate maternal PFOA levels at delivery and evaluate the influence of glomerular filtration rate on the association between maternal PFOA and birth weight. In contrast to the conclusions of Johnson et al. (2014) and Lam et al. (2014), Verner et al. (2015) found that a 1 ng/mL increase in PFOA was associated

with a 7.13 g (95% CI -8.46 to -5.80) decrease in birth weight; suggesting that glomerular filtration rate may be a confounding factor.

A third meta-analysis conducted by Negri et al. (2017) of the Apelberg et al. (2007b), Bach et al. (2016), Chen et al. (2012a), Darrow et al. (2013), Fei et al. (2007), Fromme et al. (2010), Hamm et al. 2009), Kim et al. (2011), Maisonet et al. (2012), Monroy et al. (2008), Washino et al. (2009), and Whitworth et al. (2012a) studies reported a -12.80 g (95% CI -23.21 to -2.38) change in birth weight associated with a 1 ng/mL increase in serum PFOA.

A fourth meta-analysis conducted by Steenland et al. (2018) included 24 studies; 11 of the 12 studies included by Negri et al. (2017) (the Monroy et al. 2008 study was excluded) plus studies by Wu et al. (2012), Robledo et al. (2015a), Callan et al. (2016), Lee et al. (2016), Wang et al. (2016), Lenters et al. (2016a); Minatoya et al. (2017), Shi et al. (2017), Li et al. (2017), Manzano-Selgado et al. (2017); Starling et al. (2017), and Sagiv et al. (2018). The study found a that a 1 ng/mL increase in serum PFOA was associated with a -10.5 g (95% CI -16.7 to -4.4) change in birth weight. In sensitivity analysis, inclusion of the Savitz et al. (2012b) study, which used predicted maternal serum concentrations based on estimated environmental exposure, resulted in a birth weight change of -1.0 g (95% CI -2.4–0.4) per 1 ng/mL increase in serum PFOA. Categorizing studies based on when maternal serum PFOA levels were sampled resulted in differences in birth weight change; -3.3 g (95% CI -9.6–3.0) when sampled early in pregnancy or shortly after conception and -17.8 g (-25.0 to -10.6) when sampled late in pregnancy. The investigators suggested that this may be indicative of reverse causality or confounding.

A small number of studies have examined the potential associations between PFOA exposure and risks of birth defects. In a study of C8 Health Study participants, no increases in the risk of brain, gastrointestinal, kidney, craniofacial, eye, limb, genitourinary, or heart defects were found (Stein et al. 2014c).

Epidemiological Studies—Neurodevelopmental Outcomes. A number of epidemiological studies have evaluated neurodevelopment at various ages using maternal serum PFOA or cord blood PFOA as a biometric of exposure. Fei et al. (2008b) did not find an increased risk of Apgar scores of <10 in newborns. Another study found an inverse association between maternal serum PFOA and the 5-minute Apgar score (Wu et al. 2012). Utilizing the Neonatal Intensive Care Unit Network Neurobehavioral Scale (NNNS) in 5-week-old infants, Donauer et al. (2015) found an increased risk of reduced muscle tone (hypotonia), which was associated with maternal serum PFOA levels, but found no associations on tests of social/easy going or high arousal/difficult. Goudarzi et al. (2016b) reported lower scores on tests of

mental and psychomotor development in female 6-month-old infants; no association was found when male and female infants were grouped together. When the infants were tested at 18 months of age, no association between maternal PFOA levels and mental and psychomotor indices were found. Fei et al. (2008b) did not find associations between maternal PFOA levels and the risk of delays in motor, cognitive, or language development in 6- and 18-month-old infants. It is noted that in the Fei et al. (2008b) study, the mothers were asked to recall at what age the infants reached a developmental milestone, whereas standardized tests of development were used in the other two studies. Although the Donauer et al. (2015) and Goudarzi et al. (2016b) studies suggest some delays in neurodevelopment in young infants, more research is needed before establishing a possible relationship with PFOA.

Studies in children have examined possible associations between PFOA and IQ, motor skills, behavior, and ADHD. An association between estimated *in utero* PFOA levels and IQ was found in 6–12-year-old children participating in the C8 Health Studies (Stein et al. 2013); higher IQ scores were found in children with the highest estimated PFOA exposure levels. The study did not find an association with reading or math skills. A general population study (Wang et al. 2015b) did not find an association between maternal serum PFOA levels and IQ scores in children 5 or 8 years of age. Jeddy et al. (2017) did not find an association between maternal PFOA levels and early communication development in 15-month-olds; among 38-month-olds, an inverse association was found for intelligibility scores, but there were no associations with other scores of communication development. In a prospective study, maternal PFOA levels were not associated with reading scores in 5- or 8-year-old children (Zhang et al. 2018). Reading scores at age 5 years were associated with serum PFOA levels when the children were 3 years of age and serum PFOA levels in 5-year-olds were not associated with reading scores at 8 years of age (Zhang et al. 2018). In a study of adults (20 years of age), Strøm et al. (2014) did not find an association between maternal PFOA levels and scholastic achievement. A community study of children and adolescents did not find an association between serum PFOA levels and learning problems in 12–15- or 5–18-year-olds (Stein and Savitz 2011). Two studies (Fei and Olsen 2011; Høyer et al. 2015a) did not find associations between maternal PFOA levels and motor coordination in 7-year-old children or motor skills in 5–9-year-old children.

Several studies have examined possible associations between maternal or child PFOA levels and scores on tests/surveys that assess behavioral problems. No associations were found between maternal PFOA levels and behavioral problems in 7-year-old children (Fei and Olsen 2011) or behavioral regulation problems in 5- or 8-year-old children (Vuong et al. 2016) or 7-year-old children (Oulhote et al. 2016). Similarly, no associations between serum PFOA levels and scores on behavioral tests were observed in 7-

year-old children (Oulhote et al. 2016) or 9–11-year-old children (Gump et al. 2011). No associations between breast milk PFOA levels and behavioral development in 6- and 24-month-old infants were observed (Forns et al. 2015). In contrast, Høyer et al. (2015a) found an association between maternal PFOA levels and behavioral problems in 5–9-year-old children; the risk was increased in children with maternal PFOA levels in the 3rd tertile. Stein et al. (2014a) found an association between the children's serum PFOA levels and survey results on behavioral problems and emotional disturbances in girls aged 6–12 years of age; this association was not found in boys or in boys and girls combined. Additionally, the association was only found when the survey was completed by mothers, but not when completed by the child's teacher. Oulhote et al. (2016) found associations between serum PFOA in 5-year-old children and behavioral survey scores, particularly for internalizing problems, peer relationships, and autism screening scores. In a study of 8-year-old children, Vuong et al. (2018) found an association between PFOA and at risk metacognition scores, but no associations with at risk behavior regulation or global executive scores.

Ten studies have looked for a possible association between PFOA and ADHD in children. Two studies of participants of the C8 Health Study found lower scores on tests for ADHD (Stein et al. 2013) or lower risks of ADHD (Stein and Savitz 2011) associated with estimated *in utero* PFOA or child PFOA levels, respectively. In a third community study in which parents and teachers completed surveys regarding ADHD-like behaviors (Stein et al. 2014a), no association between the child's serum PFOA (measured 3–4 years before the surveys were completed) and ADHD-like behaviors were found when the mothers completed the survey and an inverse association was found when the teachers completed the survey. Segregating the children by sex resulted in an association in girls (mother-completed survey only) and no associations in boys. Two general population studies have found associations between the risk of ADHD or increases in ADHD behavior in children. An increase in the risk of parent-reported ADHD diagnosis was observed in a study of 12–15-year-old NHANES participants (Hoffman et al. 2010). The second study (Høyer et al. 2015a) found increases in hyperactivity among 5–9-year-old children with maternal serum PFOA levels in the 3rd tertile. When this multinational cohort was segregated by country, the association was only found in the group of children from Greenland, but not in the Ukrainian cohort. Median serum PFOA levels were slightly higher in the Greenland cohort; it is also noted that the median maternal PFOS levels were 4 times higher in the Greenland cohort than in the Ukraine cohort. Other general population studies have not found associations. Two case-control studies of children did not find increased risks of being diagnosed with ADHD associated with maternal PFOA levels (Liew et al. 2015) or cord blood PFOA levels (Ode et al. 2014). Two studies did not find associations between cord blood PFOA levels and performance on tests evaluating for ADHD symptoms in 7-year-old children (Lien et al. 2016) or 18-month-old infants (Quaak et al. 2016). A third study found no association between maternal

PFOA levels and ADHD in 20-year-olds (Strøm et al. 2014). In addition to looking at possible relationships between PFOA and ADHD, two studies did not find associations between maternal PFOA levels and autism behaviors (Braun et al. 2014) or the risk of autism diagnosis (Liew et al. 2015).

Epidemiological Studies—Development of the Reproductive System. Studies exploring possible associations between PFOA and alterations in the development of the reproductive system have examined several outcomes including hormone levels in cord blood, hormone levels in children and adolescents, anogenital distance, congenital malformations of reproductive organs, and age of puberty in boys and girls.

A multinational case-control general population study (Vesterholm Jensen et al. 2014) found a decrease in the risk of cryptorchidism in the Finnish cohort, but not in the Danish cohort or in the combined cohort. With the exception of inhibin levels, no associations between maternal serum PFOA levels and cord blood levels of reproductive hormones were found (Itoh et al. 2016). Cord inhibin was associated with maternal serum PFOA levels in male infants, but not in female infants (Itoh et al. 2016). Some alterations in reproductive hormone levels were found in 6–9-year-old boys and girls participating in the C8 Health Study (Lopez-Espinosa et al. 2016). In boys, an inverse association between serum PFOA levels and total testosterone levels were observed; no associations were found for estradiol levels or insulin-like growth factor 1. In girls, an inverse association was found for insulin-like growth factor 1 levels and no associations were found for estradiol or testosterone levels. In adolescent girls, an association between maternal PFOA levels and testosterone levels was found (Maisonet et al. 2015a). This association was not found in young adult females (Kristensen et al. 2013). Other reproductive hormones were not shown to be associated with maternal PFOA levels (Kristensen et al. 2013; Maisonet et al. 2015a). Lind et al. (2017a) found no association between maternal PFOA levels and anogenital distance in boys or girls.

In a community exposure study (Lopez-Espinosa et al. 2011), increasing levels of serum PFOA were associated with delays in menarche in girls aged 8–18 years. Serum PFOA levels in the 2nd, 3rd, and 4th quartiles were associated with 142-, 163-, and 130-day delays in the onset of menarche, respectively. Using PBPK modeling, Wu et al. (2015) examined whether the association between serum PFOA and delays in the onset of menarche observed in the Lopez-Espinosa et al. (2011) study were due to reverse causality using a Monte Carlo PBPK model. They found that rapid growth around the time of menstruation onset may contribute to the apparent association between PFOA and delay of menarche. In the PBPK simulated study, the delay in the onset of menarche was 48 days for the 4th quartile (OR 0.82, 95% CI 0.76–0.88). A delay in menarche was also observed in a general population study; a 162-day

delay was estimated in the daughters of women with maternal serum PFOA levels in the 3rd tertile (Kristensen et al. 2013). A second general population study did not find an association between maternal serum PFOA levels and an earlier age of menarche (Christensen et al. 2011).

The only study available on age of puberty in males (Lopez-Espinosa et al. 2011) did not find an association with serum PFOA levels.

Laboratory Animal Exposure Studies. Exposure of pregnant Sprague-Dawley rats to 25 mg/m^3 APFO on GDs 6–15 resulted in a statistically significant reduction (10.3%) in neonatal body weight on PND 1, but the difference over controls was no longer significant on PND 4 (Staples et al. 1984). Exposure concentrations ≤10 mg/m^3 did not affect neonatal body weight. The incidence of malformations and variations among the exposed groups and controls was comparable.

In utero exposure to PFOA resulted in prenatal losses and decreases in pup survival. An increase in resorbed embryos were observed in mice administered 10 mg/kg on GD 13 (Chen et al. 2017b). An increase in resorptions was observed in mice administered ≥5 mg/kg/day throughout gestation (Lau et al. 2006) or 2 mg/kg/day on GDs 11–16 (Suh et al. 2011). Prenatal losses were also observed in PFOA mouse studies administering ≥6 mg/kg/day (Abbott et al. 2007), 5 mg/kg/day (White et al. 2011), or 20 mg/kg/day (Lau et al. 2006) throughout gestation; an increase in the percentage of dams with total litter loss was also observed at 5 mg/kg/day (Wolf et al. 2007). Administration of 20 mg/kg/day PFOA on GDs 7–17 or 10–17 did not result in litter loss (Wolf et al. 2007); no effect on litter size was observed as a result of administration of 5 mg/kg/day on GDs 8–17 (White et al. 2009). Gestational exposure (GDs 1–17) to PFOA also resulted in perinatal losses in mice administered 3 mg/kg/day PFOA (Ngo et al. 2014) and decreases in pup survival in mice exposed to ≥0.6 mg/kg/day (Abbott et al. 2007), 3 mg/kg/day (Albrecht et al. 2013), or 5 mg/kg/day (Lau et al. 2006; Yahia et al. 2010; White et al. 2011, Wolf et al. 2007); 100% pup mortality was observed in the offspring of mice exposed to 10 mg/kg/day throughout gestation (Yahia et al. 2010). Decreased pup survival was also observed in mice exposed to 5 mg/kg/day PFOA on GDs 15–17 (Wolf et al. 2007). No alterations in fetuses/litter or survival were observed at 1 mg/kg/day PFOA (Lau et al. 2006; White et al. 2011). Butenhoff et al. (2004b) also reported increases in pup mortality on PNDs 6–8 in the offspring of rats administered 30 mg/kg/day PFOA throughout gestation and during lactation.

Decreases in birth weight have not been consistently found in mouse studies with PFOA. No significant alterations in birth weight were observed in mice exposed to 3 mg/kg/day (Albrecht et al. 2013), 5 or

10 mg/kg/day (Lau et al. 2006), or 20 mg/kg/day (Abbott et al. 2007); decreases in birth or fetal weight were observed at 5 mg/kg/day (Hines et al. 2009; Yahia et al. 2010), 10 mg/kg/day (Suh et al. 2011), and 20 mg/kg/day (Lau et al. 2006). A decrease in mean litter weight on PNDs 2–14 was observed in mice administered ≥0.5 mg/kg/day PFOA on GDs 6–17 (Hu et al. 2010) and a decrease in pup body weight on PND 20 was observed in mice exposed to 5 mg/kg/day on GDs 8–17 or 12–17 (White et al. 2007). *In utero* exposure of mice to PFOA throughout gestation resulted in decreases in pup body weight in mice exposed to 1 mg/kg/day (Abbott et al. 2007; Hines et al. 2009), ≥3 mg/kg/day (Lau et al. 2006; Wolf et al. 2007), and 5 mg/kg/day (Yahia et al. 2010; White et al. 2007, 2011). In a cross-fostering study, lactation-only exposure (maternal dose of 5 mg/kg/day PFOA) resulted in decreased body weight in female pups on some PNDs (2, 3, 4, and 22, but not on PNDs 7, 10, 15, or 17) (Wolf et al. 2007). Hines et al. (2009) monitored body weights from birth to 18 months of age in female mice exposed *in utero* to PFOA on GDs 1–17. At weaning, decreases in body weight were observed at 1 and 5 mg/kg/day; by 10 weeks of age, there were no differences in body weight between the controls and mice exposed to ≥1 mg/kg/day. Significant increases in body weight were observed in mice exposed to 0.1 and 0.3 mg/kg/day, and by 20–29 weeks of age, the increases in body weight were observed in mice exposed to 0.01, 0.1, or 0.3 mg/kg/day. The largest increase in body weight gain (9.6%) was observed at 0.1 mg/kg/day; because the weight increase was less than 10%, the 0.1 mg/kg/day was considered a NOAEL. At 40 weeks of age, the increased body weight was observed in the 0.1 and 0.3 mg/kg/day groups. At termination (18 months of age), there were no differences in body weight between the controls and mice exposed to 0.01–3 mg/kg/day; a decrease in body weight was observed at 5 mg/kg/day. During the period of increased body weight in the lower-dose animals, there were no changes in serum glucose levels or the response to a glucose challenge, but there were significant increases in insulin and leptin levels at 0.01 and 0.1 mg/kg/day. Although there were no changes in the percentage of body fat to body weight measurements in mice at 42 weeks of age, at 18 months of age, significant decreases in abdominal body fat and increases in intrascapular brown fat was observed at ≥1 mg/kg/day PFOA (Hines et al. 2009). Based on systematic review of pup body weight data from the Abbott et al. (2007), Hines et al. (2009), Lau et al. (2006), White et al. (2007, 2009, 2011), and Wolf et al. (2007) mouse studies, Koustas et al. (2014) concluded that there was sufficient evidence that exposure to PFOA adversely affected fetal growth in animals. A meta-analysis estimate was a decrease of 0.023 g pup body weight per 1 mg/kg/day increase in PFOA dose.

A few studies have examined the potential of PFOA to induce malformations/variations. Lau et al. (2006) reported reductions in ossification of the proximal phalanges at ≥1 mg/kg/day and supraoccipital at 10 or 20 mg/kg/day. This study also reported enlarged fontanels in pups exposed to ≥1 mg/kg/day and tail and

limb defects at ≥5 mg/kg/day; however, there was no clear dose-response for these effects. Koskela et al. (2016) found altered femur and tibial bone morphology and decreased tibial mineral density in the offspring of mice exposed to 0.3 mg/kg/day in the diet on GDs 1–21. An increased percentage of litters with microcardia was also observed in the offspring of mice exposed to 10 or 20 mg/kg/day (Lau et al. 2006). No increases in the occurrence of malformations/variations were observed in the offspring of rats administered 100 mg/kg/day on GDs 6–15 (Staples et al. 1984) or in a 2-generation study at doses as high as 30 mg/kg/day (Butenhoff et al. 2004b).

Delayed eye opening was observed in the offspring of mice administered ≥1 mg/kg/day PFOA on GDs 1–17 (Abbott et al. 2007) and in mice administered 5 mg/kg/day throughout gestation (Lau et al. 2006; Wolf et al. 2007). Neither Albrecht et al. (2013) nor Lau et al. (2006) found alterations in eye opening in mice exposed to 3 mg/kg/day PFOA on GDs 1–17. Lau et al. (2006) also reported advanced (earlier than controls) preputial separation at ≥1 mg/kg/day and delayed vaginal opening at 20 mg/kg/day. The effect in the male offspring is in contrast to the Butenhoff et al. (2004b) study, which found delays in preputial separation in rats exposed to 30 mg/kg/day PFOA; a delay in vaginal patency was also observed at this dose.

A series of studies conducted by White and associates found significant delays in mammary gland development in the offspring of mice administered 1 mg/kg/day PFOA via gavage on GDs 8–17 (White et al. 2011) or 5 mg/kg/day PFOA on GDs 1–17, 8–17, 12–17, 10–17, 13–17, or 15–17 (White et al. 2007, 2009, 2011). The delay was characterized as reduced ductal elongation and branching and delays in timing and density of terminal end buds and was observed at all observational periods (PNDs 10, 20, 22, and 42, and 63 and 18 months of age). Decreases in mammary epithelial growth, as assessed by developmental scoring, were observed in the offspring of mice exposed to 0.01 mg/kg/day on GDs 1–17 (Tucker et al. 2015), 0.3 mg/kg/day on GDs 1–17 (Macon et al. 2011), or 0.01 mg/kg/day on GDs 10–17 (Macon et al. 2011). Tucker et al. (2015) noted that the delays in mammary gland development began at puberty and continued during young adulthood. Albrecht et al. (2013) did not find any alterations in mammary gland development on PND 20 in mouse offspring following *in utero* exposure to PFOA on GDs 1–17. Delayed mammary gland development was also observed in offspring only exposed via lactation (maternal dose of 3 mg/kg/day PFOA on GDs 1–17); the effects were observed on PNDs 42 and 63, but not on PND 22 (White et al. 2009). In a multigeneration study conducted by White et al. (2011), delays in mammary gland development were not consistently observed in the F2 offspring of F1 females that were exposed *in utero* to 1 or 5 mg/kg/day PFOA. However, delays in mammary gland development were observed in the F1 and F2 offspring exposed to 0.001 mg/kg/day *in utero* (GDs 7–17) and

postnatally. The investigators (White et al. 2011) noted that the delay in mammary gland development did not appear to affect lactational support based on normal survival and growth of the F2 pups. Tucker et al. (2015) noted dose-related strain differences on the effect of PFOA on mammary gland differences; effects were observed in CD-1 mice at ≥0.01 mg/kg/day and in C57BL/6 mice at ≥0.3 mg/kg/day (the highest NOAEL for this strain was 0.1 mg/kg/day); it is noted that the serum PFOA concentrations at a given dose were lower in the C57Bl/6 mice than in the CD-1 mice. Yang et al. (2009) reported strain differences in mammary gland effects in peripubertal mice administered PFOA for 4 weeks beginning on PND 21. In BALB/c mice, reductions in ductal length and decreased numbers of terminal end buds and stimulated terminal ducts were observed at 5 and 10 mg/kg. In contrast, 5 mg/kg resulted in mammary gland growth stimulation in C57BL/6 mice, as evidenced by increased number of terminal end buds with no alterations in ductal length. Mammary gland inhibition was observed in the C57BL/6 mice administered 10 mg/kg. Stimulation of mammary gland growth was also observed in PPARα knockout mice similarly administered 5 mg/kg (Zhao et al. 2010). In a series of experiments to evaluate the mechanism of PFOA-induced alterations in mammary gland development, Zhao et al. (2010) found that PFOA did not result in alterations in ovariectomized C57BL/6 mice administered 5 mg/kg 5 days/week for 4 weeks. In ovary-intact mice, PFOA enhanced mammary gland responses to exogenous estradiol and progesterone. Increased levels of epidermal growth factor receptor, hepatocyte growth factor, cyclin D1, and proliferating cell nuclear antigen levels were also found in PFOA-exposed C57BL/6 and PPARα-knockout mice (Zhao et al. 2010).

A consistent finding in the five mouse studies evaluating the neurodevelopmental toxicity of PFOA is an increase in motor activity. Increases in horizontal and ambulatory locomotor activity (tested on PND 60) were observed in the offspring of mice exposed to 0.1 mg/kg/day in the diet on GD 7 through PND 21 (Sobolewski et al. 2014); a decrease in resting time was also observed in the males. Increased ambulatory activity was observed on PND 18 in the offspring of mice administered 1 mg/kg/day on GDs 1–17 (Goulding et al. 2017). Significant increases in open field activity were observed at PND 36 in the offspring of mice exposed to 1.6 mg/kg/day throughout gestation and lactation (Cheng et al. 2013). Johansson et al. (2008) and Onishchenko et al. (2011) demonstrated a biphasic alteration in motor activity: an initial period of decreased activity followed by increased activity. Johansson et al. (2008) administered a single dose of 8.7 mg/kg/day PFOA to mice on PND 10 and monitored spontaneous activity for a 1-hour period when the mice were 2 or 4 months of age. In the first 20-minute period, there was a decrease in spontaneous activity, followed by a 20-minute period with an activity level similar to controls, and a 20-minute period with significantly increased spontaneous activity. Similarly, Onishchenko et al. (2011) reported an increase in activity in a 48-hour period in the adult offspring of

mice exposed to 0.3 mg/kg/day PFOA throughout gestation; however, there was a decrease in activity during the initial 3 hours of testing. Johansson et al. (2008) also found an increased susceptibility of the cholinergic system in mice exposed to 0.58 or 8.7 mg/kg/day PFOA on PND 10. In control mice, an injection of nicotine resulted in increases in activity; mice exposed to 0.58 mg/kg/day also responded with an increase in activity, although the increase was less than that observed in the controls. In contrast, nicotine resulted in a decrease in activity in mice exposed to 8.7 mg/kg/day. Exposure to PFOA did not alter learning or memory, as evidenced by the lack of effect on maze tests (Cheng et al. 2013; Johansson et al. 2008). Tests of neurobehavioral development found altered motor coordination and impaired negative geotaxis reflex, but no effect on righting reflex or cliff avoidance, in the offspring of mice exposed to 1.6 mg/kg/day throughout gestation and lactation (Cheng et al. 2013). Decreases in initial novel object exploratory behavior were also observed at 0.1 mg/kg/day, but there were no alterations in recognition time for novel objects (Sobolewski et al. 2014).

Support for the heart effects observed in the mouse study conducted by Lau et al. (2006) comes from a series of studies in chicken embryos and hatchlings that demonstrate the developmental cardiotoxicity of PFOA (Jiang et al. 2012, 2013, 2016). The avian model was selected due to the similarity between avian and mammalian cardiovascular development and the lack of direct maternal influence (Jiang et al. 2012). The effects following *in ovo* exposure include thinning of the right ventricular wall in chick embryos and alterations in left ventricular posterior wall dimension, volume, heart rate, stroke volume, and ejection fraction in the hatchlings (Jiang et al. 2012). Tests with WY 14,643, a PPARα agonist, and PFOA provide evidence that the cardiotoxicity involves both PPARα and bone morphorgenic protein 2 (BMP2) pathways (Jiang et al. 2013). Comparisons of results following *in ovo* exposure and *in vitro* exposure suggest that the cardiotoxicity was not likely due to cytotoxicity, but rather an alteration in early cardio morphology and function processes (Jiang et al. 2016).

Summary. Epidemiological studies have examined a number of potential developmental outcomes in communities living near a PFOA facility and in general populations. Although not consistently reported, the available general population studies suggest an inverse association between maternal serum PFOA levels and birth weight; a number of studies have not found this association. Several systematic reviews of these data have concluded that there was sufficient evidence that maternal PFOA levels are associated with reductions in fetal growth. After correcting for glomerular filtration rate, a small decrease in birth weight was associated with increases in maternal serum PFOA. Two of the three studies evaluating possible effects of sexual maturation found small delays in the start of menarche associated with maternal serum PFOA levels. Overall, the data do not suggest associations between serum PFOA levels and

adverse pregnancy outcomes such as miscarriages or stillbirths, most birth outcomes (e.g., risk of low birth weight, risk of small for gestational age, birth length, ponderal index, sex ratio, or birth defects), or neurodevelopmental outcomes (IQ or scholastic achievement, motor skills, and risk of ADHD). Animal studies provide strong evidence that developmental toxicity is a sensitive target of PFOA toxicity. Observed effects include prenatal losses and decreases in pup survival, decreases in birth weight, developmental delays such as delayed eye opening, delays in mammary gland development, and increased motor activity.

PFOS

Epidemiological Studies—Pregnancy Outcomes. No associations between maternal PFOS levels and the risk of miscarriages were observed in several studies (Darrow et al. 2014; Jensen et al. 2015; Stein et al. 2009). Three studies reported increases in the risk of preterm birth associated with maternal serum PFOS levels in the >90th percentile (>23.2 ng/mL) (Stein et al. 2009), maternal serum levels in the 2nd, 3rd, or 4th quartiles (≥18.9 ng/mL) (Sagiv et al. 2018), or cord blood PFOS levels in the 3rd and 4th quartiles (≥5.68 ng/mL) (Chen et al. 2012a), and one study reported a decrease risk in preterm birth (Whitworth et al. 2012a). Three other studies did not find associations for preterm birth (Fei et al. 2007, 2008a; Hamm et al. 2010; Manzano-Salgado et al. 2017a), one study found no association between serum PFOS and pregnancy loss (Buck Louis et al. 2016), and five studies found no associations between maternal PFOS levels and gestational age or length (Lauritzen et al. 2017; Li et al. 2017; Lind et al. 2017a; Manzano-Salgado et al. 2017a).

Epidemiological Studies—Birth Outcomes. Occupational, community, and general population exposure studies have examined the possible associations between maternal PFOS levels and a number of birth outcomes including birth weight; risk of low birth weight; risk of small for gestational age; birth length; head, chest, and abdominal circumferences; ponderal index; sex ratio; and birth defects. Most studies did not find associations between maternal serum PFOS levels and birth weight (Alkhalawi et al. 2016; Apelberg et al. 2007b; Ashley-Martin et al. 2016, 2017; Callan et al. 2016; Cao et al. 2018; Darrow et al. 2013; Bach et al. 2016; Fei et al. 2007, 2008a; Govarts et al. 2016; Hamm et al. 2010; Kim et al. 2011; Kobayashi et al. 2017; Lauritzen et al. 2017; Lee et al. 2013, 2016; Lenters et al. 2016a; Lind et al. 2017a; Maisonet et al. 2012; Manzano-Salgado et al. 2017a; Minatoya et al. 2017; Monroy et al. 2008; Robledo et al. 2015a; Sagiv et al. 2018; Shi et al. 2017; Starling et al. 2017; Whitworth et al. 2012a), including an occupational exposure study (Grice et al. 2007) in which female workers were exposed to very high levels of PFOS (serum levels ranged from 1,300 to 1,970 ng/mL). Five studies did find inverse associations

between birth weight and maternal serum PFOS levels. In the Washino et al. (2009) study, an inverse association was found between maternal serum PFOS levels and birth weight; segregating by sex resulted in an inverse association in girls, but not in boys. The magnitude of the change was small, 148.8 g decrease in birth weight per log unit increase in maternal PFOS for combined. Maisonet et al. (2012) also reported small decreases in birth weight (140.1 g) in infants whose mother's serum PFOS levels were in the 3rd tertile. Lauritzen et al. (2017) also reported an inverse association between birth weight and maternal serum PFOS levels (292 g per ln unit increase in PFOS). Similarly, Chen et al. (2012a) reported an inverse association between cord blood PFOS and birth weight, but the magnitude was small (110.2 g decrease per ln unit increase in cord PFOS levels). Li et al. (2017) also reported a small decrease in birth weight associated with cord PFOS levels (95 g decrease per ln increase in cord PFOS levels); when infants were categorized by sex, the association was only found among boys. Although these studies found decreases in birth weight associated with PFOS levels, no studies found increases in the risk of low birth weight infants (Chen et al. 2012a; Darrow et al. 2013; Fei et al. 2007, 2008a; Manzano-Salgado et al. 2017a; Stein et al. 2009) or small for gestational age infants (Chen et al. 2012a; Fei et al. 2007, 2008a; Hamm et al. 2010; Lauritzen et al. 2017; Manzano-Salgado et al. 2017a; Whitworth et al. 2012a). The ORs for low birth weight and small for gestational age risks are presented in Figures 2-37 and 2-38. Analysis of data compiled from four European birth cohort studies found an inverse association between cord PFOS levels (measured levels and levels estimated from breast milk PFOS levels) and small for gestational age (OR 0.823, 95% CI 0.741–0.913) (Govarts et al. 2018). When subjects were segregated based on whether they smoked during pregnancy, a positive association was found among smokers (OR 1.627, 95% CI 1.024–2.588) and an inverse association was found among nonsmokers (OR 0.661, 95% CI 0.644–0.717). Three studies have evaluated leptin and adiponectin hormone levels or adiposity in newborns. Maternal PFOS levels were not associated with alterations in leptin levels (Ashley-Martin et al. 2017; Minatoya et al. 2017). Mixed results were found for adiponectin levels with one study finding no alterations (Ashley-Martin et al. 2017) and another finding an association (Minatoya et al. 2017). No association was found between maternal PFOS levels and adiposity at birth (Starling et al. 2017).

Verner et al. (2015) conducted a meta-analysis of the Apelberg et al. (2007b), Chen et al. (2012a), Fei et al. (2007), Hamm et al. (2010), Maisonet et al. (2012), Washino et al. (2009), and Whitworth et al. (2012a) studies and found that a 1 ng/mL increase in maternal PFOS levels was associated with a 5.00 g (95% CI -8.92 to -1.09) decrease in birth weight. When the data were re-analyzed utilizing a PBPK model to account for glomerular filtration rate, the magnitude of the effect of PFOS on birth weight decreased (Verner et al. 2015). A 1 ng/mL increase in PFOS was associated with a 2.72 g (95% CI -3.40 to -2.04) decrease in birth weight. A second meta-analysis conducted by Negir et al. (2017)

Figure 2-37. Risk of Low Birth Weight Infant Relative to PFOS Levels (Presented as Adjusted Odds Ratios)

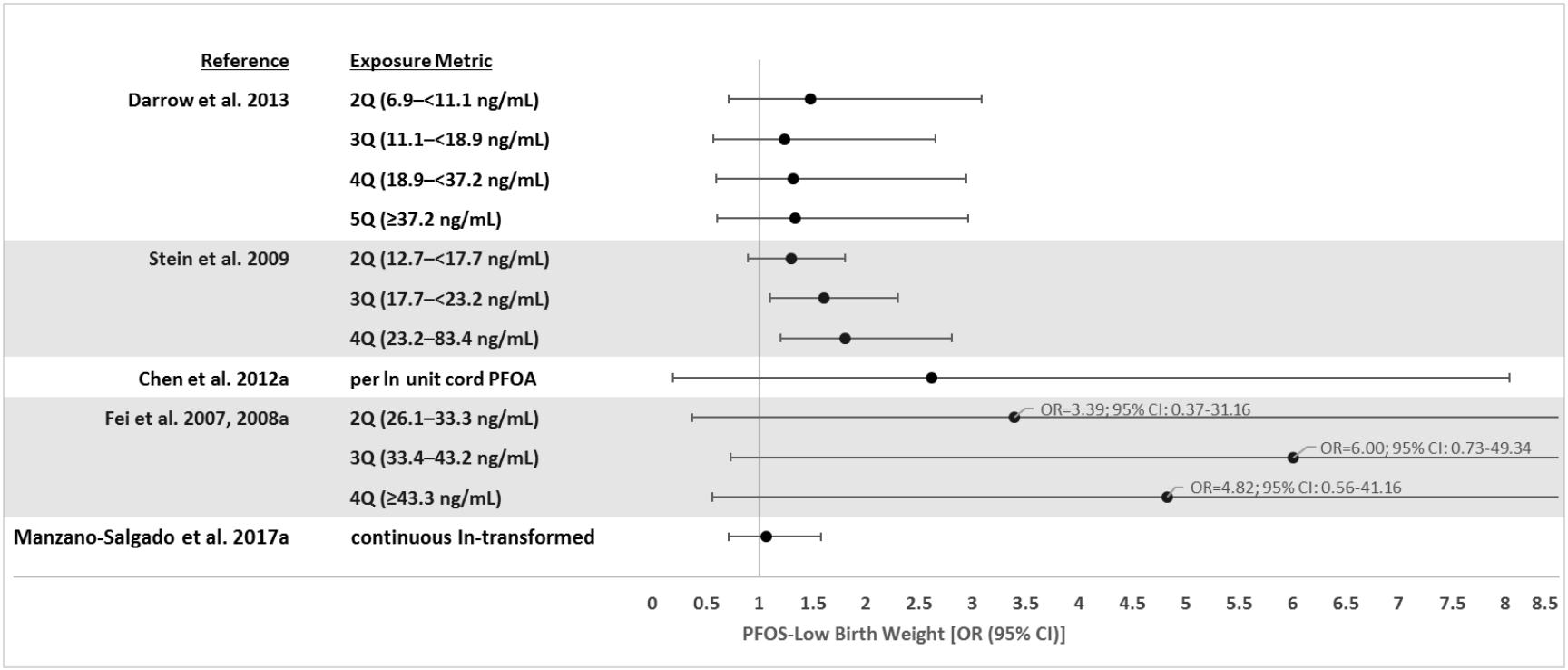
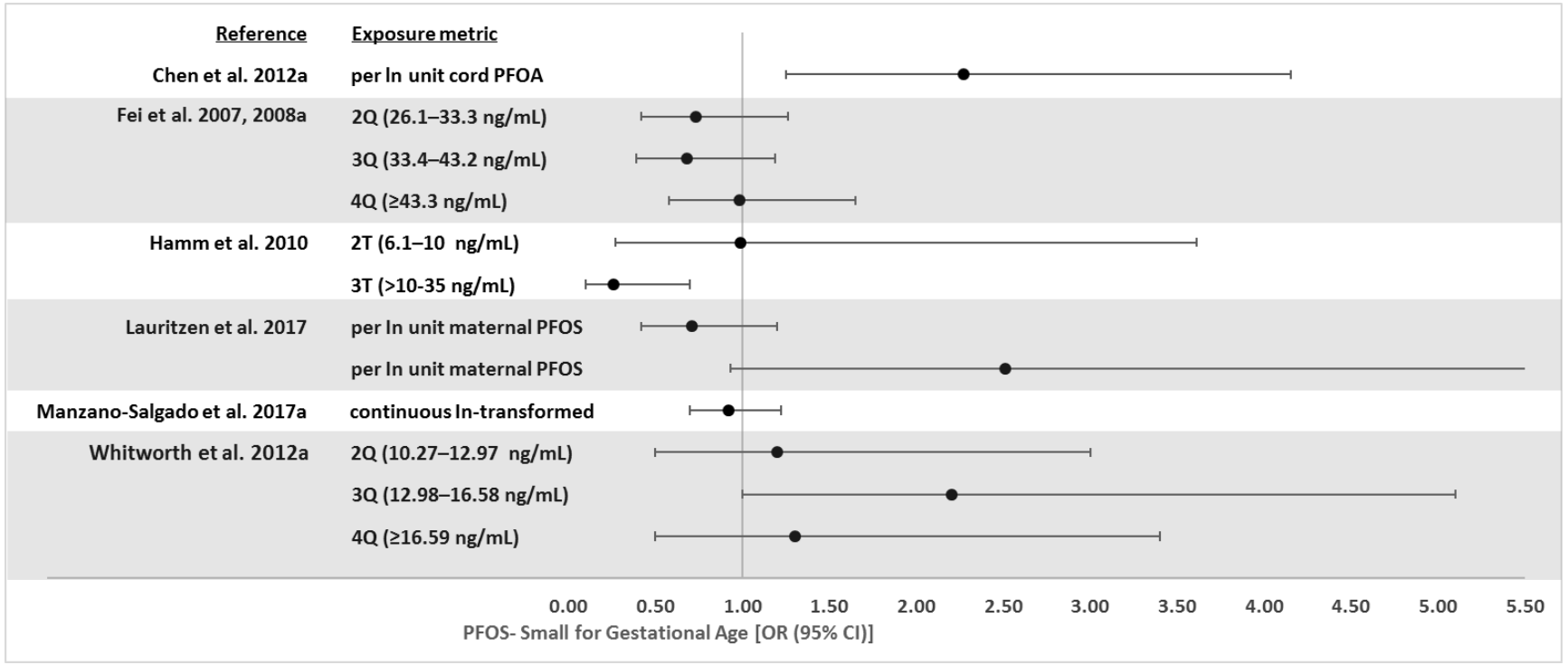


Figure 2-38. Risk of Small for Gestational Age Infant Relative to PFOS Levels (Presented as Adjusted Odds Ratios)



utilized data from the Fei et al. (2007), Monroy et al. (2008), Washino et al. (2009), Hamm et al. (2010), Chen et al. (2012a), Maisonet et al. (2012), Whitworth et al. (2012a), and Bach et al. (2016) studies. The investigators found a -0.92 g (95% CI -3.43–1.60) change in birth weight per 1 ng/mL increase in serum PFOS.

Maternal PFOS was not associated with birth length (Alkhalawi et al. 2016; Apelberg et al. 2007b; Bach et al. 2016; Callan et al. 2016; Cao et al. 2018; Chen et al. 2012a; Kobayashi et al. 2017; Laurizten et al. 2017; Lee et al. 2013; Manzano-Salgado et al. 2017a; Robledo et al. 2015a; Shi et al. 2017; Washino et al. 2009) with the exception of the finding of small decreases in birth length (≤1.2 cm) that was associated with serum PFOS levels (Fei et al. 2007, 2008a; Lauritzen et al. 2017). Four studies reported inverse associations between ponderal index and cord blood PFOS levels (Apelberg et al. 2007b) or maternal serum PFOS levels (Alkhalawi et al. 2016; Lee et al. 2013; Minatoya et al. 2017); other studies did not find this effect (Callan et al. 2016; Cao et al. 2018; Chen et al. 2012a; Maisonet et al. 2012; Robledo et al. 2015a; Shi et al. 2017). Two studies reported small decreases in head circumference, which were associated with maternal serum PFOS levels (Apelberg et al. 2007b) and cord blood PFOS (Chen et al. 2012a); other studies have not found associations (Bach et al. 2016; Callan et al. 2016; de Cock et al. 2014; Fei et al. 2007, 2008a; Lauritzen et al. 2017; Lee et al. 2013; Manzano-Salgado et al. 2017a; Robledo et al. 2015a; Washino et al. 2009).

One study reported no increases in the risk of birth defects associated with maternal serum PFOS levels (Stein et al. 2009); a second study found an increased risk of congenital cerebral palsy in girls, but not in boys (Liew et al. 2014). Bae et al. (2015) did not find associations between the odds of having a boy and paternal or maternal serum PFOS levels.

Epidemiological Studies—Neurodevelopmental Outcomes. Epidemiological studies examined several aspects of neurodevelopment, including age of reaching neurobehavioral milestones, IQ, motor development, behavior, ADHD, and autism. Fei et al. (2008b) did not find associations between maternal PFOS levels and the risk of having an Apgar score of <10 or in motor and mental development at 6 months. However, the study did find that some neurobehavioral milestones (delay in sitting, early use of word-like sounds, and delays in using two-word sentences) were associated with maternal PFOS levels. Goudarzi et al. (2016b) did not find alterations on mental and psychomotor development in 6- and 18-month-old infants that were associated with maternal serum PFOS levels. A third study of infants did not find alterations in neurobehavioral or muscle coordination tests (Donauer et al. 2015).

In the only study evaluating IQ, Wang et al. (2015b) did not find associations between maternal PFOS levels and IQ score in children 5 or 8 years of age. Zhang et al. (2018) did not find associations between maternal PFOS levels and reading scores in 5- or 8-year-old children. However, associations were found between the child's serum PFOS levels at age 3 years and reading scores at 5 years of age and serum PFOS levels at 5 years of age and reading scores at 8 years of age. Strøm et al. (2014) found no associations between scholastic achievement in 20-year-olds and maternal PFOS levels. In a study of children living in a community with high PFOA contamination, Stein and Savitz (2011) found decreases in the risk of learning problems in children 5–18 or 12–15 years of age. In contrast, Vuong et al. (2016) found increased risks of global executive functioning and metacognition problems that were associated with maternal PFOS levels. Another study found an association between maternal PFOS levels and verbal comprehension in 15-month-olds, but an inverse association with intelligibility scores in 38-month-olds (Jeddy et al. 2017). A subsequent study by Vuong et al. (2018) did not find associations between serum PFOS levels in 8-year-old children and metacognition or global executive functioning scores. Four studies have not found associations between maternal PFOS levels and behavioral health and motor coordination/skills in children (Fei and Olsen 2011; Høyer et al. 2015a; Oulhote et al. 2016), between breast milk PFOS levels and behavioral development in 6- and 24-month-old infants (Forns et al. 2015), or between serum PFOS levels age 5 or 7 years and behavioral development in 7-year-old children (Oulhote et al. 2016). A fifth study (Vuong et al. 2016) found an increased risk for problems with behavioral regulation. The available data do not suggest an association between maternal PFOS levels or cord blood PFOS levels and the risk of ADHD or ADHD behaviors (Hoffman et al. 2010; Liew et al. 2015; Ode et al. 2014; Quaak et al. 2016; Stein and Savitz 2011; Strøm et al. 2014), although Liew et al. (2015) found a decreased risk of ADHD diagnosis in children whose mothers had serum PFOS levels in the 4th quartile. Similarly, Høyer et al. (2015a) did not find increases in the risk of hyperactivity in children and Gump et al. (2011) found a decrease in impulsivity. Braun et al. (2014) and Liew et al. (2015) did not find associations between maternal PFOS and autism risk.

Epidemiological Studies—Development of Reproductive System. Several epidemiological studies have examined the possible associations between PFOS and the development of the reproductive system, including the risk of congenital defects to reproductive organs, alterations in reproductive hormone levels, and age of puberty; the results of these studies are summarized in Table 2-25. No alterations in the risk of cryptorchidism (Toft et al. 2016; Vesterholm Jensen et al. 2014) or hypospadias (Toft et al. 2016) were found in two studies. No association between maternal PFOS levels and anogenital distance was found in boys and an inverse association was found in girls (Lind et al. 2017a). Itoh et al. (2016) reported associations between maternal PFOS levels and alterations in cord blood hormone levels, in particular

estradiol in males, testosterone:estradiol ratio in males (inverse association), progesterone levels in males and females, prolactin levels in females, and inhibin levels in males. Similarly, Toft et al. (2016) found associations between amniotic fluid PFOS levels and levels of testosterone, androstenedione, progesterone, and insulin-like factor 3 (inverse association) in amniotic fluid. Lopez-Espinosa et al. (2016) also found a number of alterations in reproductive hormone levels in 6–9-year-old boys and girls. In the boys, inverse associations between serum PFOS levels and estradiol, total testosterone, and insulin-like growth factor 1 were observed. Inverse associations between total testosterone and insulin-like growth factor 1 and serum PFOS levels were also observed in the girls. A study of young adult women found no associations between reproductive hormone levels and maternal PFOS levels (Kristensen et al. 2013).

A study of 8–18-year-old children found delays in the age of puberty in boys and girls (Lopez-Espinosa et al. 2011) that were associated with serum PFOS levels. In the children with serum PFOS levels in the 3rd and 4th quartiles, the respectively delays were 131 and 190 days in boys and 141 and 138 days in girls. In contrast, two other studies have not found alterations in either the age of menarche or an earlier age of menarche that were associated with maternal PFOS levels (Christensen et al. 2011; Kristensen et al. 2013). The differences in the biomarker of exposure and the potential exposure to high levels of PFOA in the Lopez-Espinosa et al. (2011) community study make it difficult to compare the results of these three studies. As discussed in the PFOA section, Wu et al. (2009) reanalyzed the Lopez-Espinosa et al. (2011) data using a Monte Carlo PBPK model, which accounted for rapid growth occurring around puberty, and found much shorter delays in the age of menarche than found in the Lopez-Espinosa et al. (2011) study. In the girls with simulated serum PPFOS levels in the 4th quartile, the delay was 72 days (OR 0.75, 95% CI 0.70–0.81).

Laboratory Animal Exposure Studies. Increases in fetal mortality and decreases in pup survival have also been observed in rats and mice exposed to PFOS *in utero* (Abbott et al. 2009; Chen et al. 2012b; Fuentes et al. 2006; Grasty et al. 2003, 2005; Lau et al. 2003; Lee et al. 2015a; Luebker et al. 2005a, 2005b; Ngo et al. 2014; Thibodeaux et al. 2003; Xia et al. 2011; Yahia et al. 2008). Increases in the number of resorptions and dead fetuses were observed in mice administered ≥0.5 mg/kg/day (Lee et al. 2015a); increases in abortions between GD 22 and 28 were observed in rabbits treated with 3.75 mg/kg/day PFOS by gavage on GDs 6–20 (Case et al. 2001). Decreases in the number of live fetuses were observed in mice exposed to ≥2.0 mg/kg/day on GDs 11–16 and 20 mg/kg/day on GDs 1–17 (Thibodeaux et al. 2003) or GDs 0–17 (Yahia et al. 2008). Increases in perinatal losses were observed in the litters of mice administered ≥0.1 mg/kg/day PFOS on GDs 1–17 (Ngo et al. 2014). Pup survival is

affected at lower maternal doses. Significant decreases in pup survival were observed in rats at 1.6 mg/kg/day (dams were exposed for 6 weeks prior to mating and during gestation through lactation days 4 or 21) (Luebker et al. 2005a, 2005b) and in mice exposed to 4.5 mg/kg/day on GDs 15–18 (Abbott et al. 2009); no alterations in pup survival were observed in rats or mice exposed to 1 mg/kg/day (Luebker et al. 2005b; Yahia et al. 2008). A series of studies by Grasty et al. (2003) in rats that were exposed for 4 days during different gestational periods showed that the pup was more susceptible if exposure occurred later in gestation. On PND 4, pup survival was 70, 50, 60, 20, or 5% for exposures on GDs 2–5, 6–9, 10–13, 14–17, or 17–20, respectively. Grasty et al. (2003) and others (Abbott et al. 2009; Chen et al. 2012b; Lau et al. 2003) also noted that most deaths occurred within the first 4 PNDs, with the highest rates occurring on PND 1. Lau et al. (2003) and Luebker et al. (2005a) found that cross fostering did not significantly improve pup survival; deaths were observed in the *in utero* only exposure group. However, Luebker et al. (2005a) showed that rats exposed *in utero* and during lactation had the highest pup mortality, as compared to other cross-fostered groups. The mechanism involved in the early pup mortality has not been identified, but there is some indication that pulmonary deficits may be a contributing factor. At high doses (50 mg/kg/day administered on GDs 19–20), pups demonstrated difficulty breathing within minutes of birth (Grasty et al. 2003). Histological examination of the lungs of pups exposed to 25 or 50 mg/kg/day on GDs 19–20 showed evidence of delayed lung maturation (Grasty et al. 2003, 2005), specifically, an increase in the proportion of solid lung tissue and a decrease in the proportion of small airway tissue. A comparison of the lungs of PFOS-exposed neonates to control fetuses (GD 21) showed that 17 and 50% of the lung tissue in the neonates exposed to 25 or 50 mg/kg/day, respectively, on GDs 19–20 was not histologically different from the control fetuses (Grasty et al. 2005). Administration of therapeutic agents known to enhance terminal lung maturation and accelerate surfactant production did not improve pup survival (Grasty et al. 2005). Histological damage has also been reported in pups exposed to lower PFOS levels. Lung atelectasis was observed in pups exposed to 10 mg/kg/day on GDs 0–18 (Yahia et al. 2008). No lung effects were observed in pups exposed to 1 mg/kg/day or in fetuses exposed to 20 mg/kg/day on GDs 0–17 (Yahia et al. 2008). Alveolar hemorrhage, thickened epithelial walls of the pulmonary alveolus, focal lung consolidation, and focal infiltration of inflammation cells were observed in pups exposed to 2 mg/kg/day on GDs 0–21; no lung effects were observed at 0.1 mg/kg/day (Chen et al. 2012b).

Decreases in fetal body weight, birth weight, and pup body weight have been observed in rats, mice, and rabbits exposed to PFOS (Case et al. 2001; Chen et al. 2012b; Era et al. 2009; Fuentes et al. 2006, 2007b; Grasty et al. 2003; Lau et al. 2003; Lee et al. 2015a; Li et al. 2016; Luebker et al. 2005a, 2005b; Rogers et al. 2014; Xia et al. 2011; Yahia et al. 2008). In rats, the lowest-adverse-effect level for decrease in fetal

body weight was 10 mg/kg/day following administration on GDs 2–20 (Thibodeaux et al. 2003) and the highest no-effect level was 5 mg/kg/day, also identified in the Thibodeaux et al. (2003) study. Decreases in rat pup birth weight and body weight on PND 4 were observed in the offspring of rats exposed to 0.4 mg/kg/day for 42 days prior to mating and gestation through lactation day 4 (Luebker et al. 2005b). Mice appear to be less sensitive to the effect of PFOS on pup body weight than rats (Lau et al. 2003). Exposure of rats to 2 mg/kg/day PFOS on GDs 2–21 resulted in significant decreases in birth weight and pup body weight on PNDs 1–3; exposure to 5 mg/kg/day resulted in decreases in pup body weight through PND 19. In contrast, no alterations in birth weight or pup body weight were observed in mice exposed to doses as high as 5 mg/kg/day on GDs 1–18. Fuentes et al. (2007b) reported the lowest LOAEL of 6 mg/kg/day for decreases in pup weight in mice exposed on GDs 12–18. Decreases in fetal body weight were observed in mice exposed to 10 mg/kg/day on GDs 0–17 (Yahia et al. 2008). Fuentes et al. (2006) did not find decreases in fetal body weight following exposure to 6 mg/kg/day on GDs 6–18. In rabbits, a decrease in fetal body weight was observed following exposure to 2.5 mg/kg/day on GDs 6–20, but not at 1 mg/kg/day (Case et al. 2001).

Several studies also reported delays in developmental milestones. Delays in eye opening were observed in rats exposed to 2 mg/kg/day on GDs 2–21 (Lau et al. 2003) or 0.4 mg/kg/day for 42 days prior to mating and throughout the gestation and lactation periods (Luebker et al. 2005a) and in mice exposed to 8.5 mg/kg/day on GDs 15–18 (Abbott et al. 2009). Fuentes et al. (2007b) did not find a delay in eye opening in mouse pups exposed to 6 mg/kg/day on GDs 12–18, but did find a delay in pinna detachment at this dose level. A decrease in neuromuscular development, as evidenced by a delay in tail pull reflex, climbing ability, and forelimb grip strength, was observed in mice exposed to 6 mg/kg/day on GDs 12–18 (Fuentes et al. 2007b).

Prenatal exposure to PFOS has resulted in malformations/anomalies/variations in rats, mice, and rabbits (Case et al. 2001; Era et al. 2009; Thibodeaux et al. 2003; Yahia et al. 2008). An increased incidence of cleft palate was observed in rats exposed to 10 mg/kg/day on GDs 2–20 (Thibodeaux et al. 2003) and in mice exposed to 10 mg/kg/day on GDs 0–17 (Yahia et al. 2008), 15 mg/kg/day on GDs 1–17 (Thibodeaux et al. 2003), 20 mg/kg/day on GDs 1–17 (Era et al. 2009), and 50 mg/kg/day on GDs 11–15 (Era et al. 2009). Other skeletal and external alterations included sternal defects in rats exposed to 10 mg/kg/day on GDs 2–20 (Thibodeaux et al. 2003) and mice exposed to 1 mg/kg/day on GDs 0–17 (Yahia et al. 2008), delayed skeletal ossification in rabbits exposed to 2.5 mg/kg/day on GDs 6–20 (Case et al. 2001), wavy ribs and spina bifida occulta in mice exposed to 10 mg/kg/day on GDs 1–17 (Yahia et al. 2008), and tail abnormalities and delayed ossification of phalanges at 20 mg/kg/day (Yahia et al.

2008). Visceral abnormalities, consisting of enlarged right atrium at 10 mg/kg/day, and ventricular septal defects at 20 mg/kg/day were observed in mice exposed on GDs 1–17 (Thibodeaux et al. 2003). No malformations/anomalies/variations were found by Thibodeaux et al. (2003) in mice exposed to 1 mg/kg/day on GDs 1–17 or by Fuentes et al. (2006) in mice exposed to 6 mg/kg/day on GDs 6–18. In addition to the previously discussed histological alterations observed in the pups exposed to lethal doses, mild to severe intracranial dilatation of blood vessels was observed in fetuses exposed to 20 mg/kg/day on GDs 0–17 and in pups exposed to 10 mg/kg/day on GDs 0–18 (Yahia et al. 2008). No histological alterations were observed in the heart of rat pups exposed to 2 mg/kg/day on GDs 2–21 (Xia et al. 2011); the study also found no alterations in heart rate or blood pressure. Lee et al. (2015b) found increases in cholesterol levels in fetal livers of mice exposed to PFOA on GDs 1–17 and Wan et al. (2014b) found increases in relative liver weights in pups on PND 21.

A study with wild-type mice (129S1/Svlm) and PPARα-null mice evaluated the influence of PPARα on developmental toxicity of PFOS (Abbott et al. 2009). Decreases in pup survival and delays in eye opening were observed in both strains, although lower LOAELs were identified in the wild-type mice. The investigators concluded that neonatal lethality and delayed eye opening was not dependent on PPARα activation.

Neurodevelopmental studies have shown that prenatal and/or postnatal exposure to PFOS can affect motor activity, but does not appear to affect learning or memory. A significant decrease in locomotion was observed in male mice aged 5–8 weeks exposed to 0.3 mg/kg/day on GDs 1–17 when they were placed in a novel environment (Onishchenko et al. 2011). Hallgren et al. (2015) reported biphasic alterations in spontaneous activity in 2-month-old mice administered a single dose of 11.3 mg/kg on PND 10; locomotor activity was reduced during the first 20-minute period, was unchanged in the second period, and increased during the third period. Decreases in circadian activity were noted in males and increases in the number of inactive periods were noted in males and females when they were observed over a 48-hour period. The study also found increased inactivity in an elevated plus maze test. In an open field test of 70-day-old mice exposed to 6 mg/kg/day on GDs 12–18, an increase in the amount of time spent in the center of the field was found; no changes in vertical movement were found (Ribes et al. 2010). In 3-month-old mice exposed to 6 mg/kg/day on GDs 12–18, a decrease in the distance traveled was observed after 20–25 minutes in an open field apparatus; activity was not affected during the first 5 minutes of the test (Fuentes et al. 2007a). In a 15-minute open field test, prenatal exposure to 6 mg/kg/day PFOS on GDs 12–18 did not alter motor activity in 3-month-old mice (Fuentes et al. 2007b). In contrast, Butenhoff et al. (2009b) found a significant increase in locomotion in male rats exposed to

0.3 or 1.0 mg/kg/day PFOS throughout gestation and lactation. However, this effect was only observed in male rats on PND 17; no significant alterations were observed on PNDs 13, 21, or 61. An increase in locomotion was observed in female rats on PND 21 exposed to 1.0 mg/kg/day, but not at other time points. To evaluate the biological relevance of the increased activity, activity was analyzed by 1-minute sequential time periods. The investigators concluded that the increased activity observed in the 0.3 mg/kg/day males at PND 17 and 1.0 mg/kg/day females at PND 21 was not treatment-related due to the lack of significant changes in total or ambulatory activity and the similarity in habituation pattern between the treated groups and controls. In the 1.0 mg/kg/day PND 17 males, the pattern of habituation differed from controls and there was an increase in ambulatory activity; this increase in locomotor activity was considered to be related to PFOS exposure. The increased activity was observed in the last three time periods. Postnatal exposure (PND 10) to 11.3 mg/kg/day resulted in an initial decrease in motor activity followed by an increase in activity in 2- and 4-month-old mice (Johansson et al. 2008). In 2-month-old mice exposed to 0.75 mg/kg/day, there was a decrease in total activity during the first 20 minutes of testing, but not during the remaining 40 minutes of the test; no changes in activity were observed in the 4-month-old mice exposed to 0.75 mg/kg/day. Johansson et al. (2009) also found an altered response to nicotine exposure. Exposure to 11.3 mg/kg/day PFOS resulted in a decrease in motor activity in response to nicotine exposure, as compared to the increased activity observed in controls; no significant alteration was observed at 0.75 mg/kg/day. Two studies testing muscle coordination did not find alterations in the offspring of rats exposed to 3.2 mg/kg/day for 6 weeks prior to mating and throughout gestation and lactation (Luebker et al. 2005a) or mice exposed to 6 mg/kg/day on GDs 12–18 (Fuentes et al. 2007b). A decrease in muscle coordination was observed in mice exposed to 0.3 mg/kg/day on GDs 1–17 (Onishchenko et al. 2011). Perinatal exposure to PFOS did not significantly alter learning or memory in rats exposed to 2 mg/kg/day on GDs 2–21 and tested on PND 21 (Lau et al. 2003), the offspring of rats exposed to 3.2 mg/kg/day for 6 weeks prior to mating and throughout gestation and lactation and tested on PNDs 21 and 70 (Luebker et al. 2005a), or mice exposed to 6 mg/kg/day on GDs 12–18 and tested at 3 months of age (Fuentes et al. 2007a). In contrast, decreases in spatial learning ability were observed in the offspring of mice exposed to 0.8 mg/kg/day on GD 1 through PND 1 or on PNDs 1–7 (Wang et al. 2015b).

The effect of pre- and/or postnatal exposure to PFOS on serum lipid levels, thyroid function, and immune function has also been evaluated by a small number of studies. In the offspring of rats exposed to 1.6 mg/kg/day for 6 weeks prior to mating through GD 20, a significant decrease in fetal serum cholesterol levels and an increase in LDL cholesterol levels were observed (Luebker et al. 2005b). In rats exposed through PND 4, there was a decrease in serum triglyceride levels in the pups exposed to

1 mg/kg/day (Luebker et al. 2005b). No alterations in thyroid histology or follicular morphology were observed in rats exposed to 1 mg/kg/day on GD 0–PND 20 (Chang et al. 2009), and no alterations in TSH levels were observed in the Chang et al. (2009) study or in rats exposed to 2 mg/kg/day on GDs 2–21 (Lau et al. 2003). Decreases in total and free T4 levels were observed in rats exposed to 1 mg/kg/day on GDs 2–21 (Lau et al. 2003); free T4 levels remained low through PND 35. Similarly, a cross-fostering study found decreases in T4 levels in rats exposed to 3.2 mg/kg/day *in utero*, during lactation only, and throughout gestation and lactation (Yu et al. 2009b). Altered immune function was observed in mice exposed to PFOS on GDs 1–17 (Keil et al. 2008). At 5 mg/kg/day, an altered IgM antibody response to sRBCs was observed in 8-week-old males; decreases in CD3+ and CD4+ lymphocytes were also observed. At 1 mg/kg/kg/day, there was decreased in NK cell activity in males; no effects were observed at 0.1 mg/kg/day.

Summary. A number of epidemiological studies have evaluated developmental outcomes in occupational, community (living near a PFOA facility), and general exposure populations. Overall, these studies have not found associations between serum PFOS and adverse pregnancy outcomes (miscarriage, preterm birth), most birth outcomes (risks of low birth weight or small for gestational age, birth length, head, chest or abdominal circumferences, ponderal index, sex ratio, or birth defects), or neurodevelopmental outcomes (IQ, motor development, behavior, ADHD, or autism). It is noted that some studies have found associations for these effects and for some effects, only a couple of studies examined the endpoint. Although most studies did not find associations between maternal PFOS and birth weight, a meta-analysis did find a small decrease in birth weight was associated with increasing maternal PFOS levels, after adjustment for glomerular filtration rate. There is also some suggestive evidence that PFOS levels may be associated with small delays in the age of puberty in boys and girls. Studies in laboratory animals clearly indicate that developmental toxicity is a sensitive outcome of PFOS exposure. Oral exposure studies have reported increases in fetal mortality and decreases in pup survival; decreases in fetal body weight, birth weight, and pup body weight; delays in developmental milestones such as eye opening; increases in skeletal malformations/anomalies/variations such as cleft palate and delayed skeletal ossification; and decreases in offspring motor activity.

PFHxS

Epidemiological Studies—Pregnancy Outcomes. Six studies, summarized in Table 2-22 have evaluated possible associations between pregnancy outcomes and maternal PFHxS levels. Jensen et al. (2015) did not find an association between maternal PFHxS levels and the risk of miscarriage. Hamm et al. (2010)

found a decreased risk of preterm births among women with serum PFHxS levels in the 3rd tertile and Manzano-Salgado et al. (2017a) and Sagiv et al. (2018) found no associations with risk of preterm birth. Other studies have found no associations with gestational age (Li et al. 2017; Manzano-Salgado et al. 2017a) or length (Lind et al. 2017a; Sagiv et al. 2018).

Epidemiological Studies—Birth Outcomes. General population studies have evaluated possible associations between maternal PFHxS levels and birth outcomes including birth weight, length, small for gestation age, and birth defects; studies are summarized in Table 2-23. Bach et al. (2016) and Maisonet et al. (2012) reported inverse associations between maternal PFHxS levels and birth weight; however, other studies have not found associations (Alkhalawi et al. 2016; Ashley-Martin et al. 2017; Callan et al. 2016; Cao et al. 2018; Hamm et al. 2010; Kim et al. 2011; Li et al. 2017; Lee et al. 2013, 2016; Lenters et al. 2016a; Lind et al. 2017a; Manzano-Salgado et al. 2017a; Monroy et al. 2008; Sagiv et al. 2018; Shi et al. 2017; Starling et al. 2017). Manzano-Salgado et al. (2017a) did not find an association between maternal PFHxS levels and the risk of low birth weight infants. Hamm et al. (2010) and Manzano-Salgado et al. (2017a) did not find an association between maternal PFHxS level and the relative risk of small for gestational age. Ashley-Martin et al. (2017) did not find an association between maternal PFHxS levels and infant leptin or adiponectin levels, but Starling et al. (2017) found an inverse association between maternal PFHxS levels and adiposity at birth. Several studies did not find associations between maternal PFHxS levels and birth length, head circumference, or ponderal index (Alkhalawi et al. 2016; Bach et al. 2016; Callan et al. 2016; Cao et al. 2018; Lee et al. 2013; Manzano-Salgado et al. 2017a; Shi et al. 2017). Maisonet et al. (2012) found an inverse association for birth length, but no association for ponderal index. Cao et al. (2018) found an association between head circumference and cord PFHxS levels. Only one study examined possible birth defects; Liew et al. (2014) did not find an association between maternal PFHxS levels and the risk of congenital cerebral palsy in a case-control study.

Epidemiological Studies—Neurodevelopmental Outcomes. Epidemiological studies, summarized in Table 2-24, have examined PFHxS-related alterations in risks of ADHD, autism, intelligence, and behavior. Wang et al. (2015b) did not find associations between maternal PFHxS levels and IQ in 5- or 8-year-old children and Jeddy et al. (2017) did not find associations between maternal PFHxS levels and verbal comprehension or vocabulary comprehension production in 15-month-old infants or intelligibility, language, or communication scores in 38-month-old children. Zhang et al. (2018) did not find associations between reading scores at 5 or 8 years of age and maternal PFHxS levels or PFHxS levels at age 3 or 5 years. Vuong et al. (2016) found a higher risk of performing poorly on tests of global executive function with increasing maternal PFHxS levels. However, no association was found between

serum PFHxS levels and metacognition or global executive function in 8-year-old children (Vuong et al. 2018). No association between serum PFHxS levels and the risk of learning problems was found in children living in a community with high PFOA levels (Stein and Savitz 2011). Gump et al. (2011) found an inverse association between serum PFHxS levels and performance on tasks requiring behavioral inhibition; Vuong et al. (2016, 2018) did not find alterations in behavioral regulation associated with maternal PFHxS levels or 8-year-old's PFHxS levels and Oulhote et al. (2016) did not find associations between behavioral development scores in 7-year-old children and maternal PFHxS levels or PFHxS levels at 5 or 7 years of age. Two studies evaluated the risk of ADHD and reported conflicting findings. Stein and Savitz (2011) reported increases in risk of ADHD in 5–18- and 12–15-year-olds with serum PFHxS levels in the 2nd, 3rd, or 4th quartile, whereas Liew et al. (2015) reported an inverse association between maternal PFHxS levels and risk of ADHD. This study also did not find an increase in the risk of autism; Braun et al. (2014) also found no association between maternal PFHxS levels and performance on tests assessing autism.

Epidemiological Studies—Development of the Reproductive System. No associations between reproductive hormone levels and serum PFHxS levels (Lopez-Espinosa et al. 2016) or maternal serum PFHxS levels (Maisonet et al. 2015a) were found in boys and girls 6–9 years of age or in girls 15 years of age. Lind et al. (2017a) did not find an association between maternal PFHxS levels and anogenital distance in boys or girls. Christensen et al. (2011) did not find an association between maternal PFHxS levels and risk of an earlier menarche. Summaries of these epidemiological studies are presented in Table 2-25.

Laboratory Animal Studies. Administration of 9.2 mg/kg/day PFHxS on PND 10 resulted in a decrease in spontaneous motor activity during the first 20 minutes of the test and an increase in activity in the last 20 minutes of the test (Viberg et al. 2013). The study also assessed the influence of PFHxS on nicotine-induced behavior. In the 9.2 mg/kg/day PFHxS group, exposure to nicotine did not significantly affect spontaneous motor activity, which was in contrast to the nicotine-induced increases in spontaneous motor activity observed in the controls and lower PFHxS groups. Studies evaluating the developmental toxicity of PFHxS did not find alterations in litter size, pup survival, or pup body weight in rats exposed to 10 mg/kg/day PFHxS or mice exposed to 3 mg/kg/day for 14 days prior to mating and throughout gestation and lactation (Butenhoff et al. 2009a; Chang et al. 2018). Although the rat study did not find alterations in litter size (Butenhoff et al. 2009a), the mouse study found a decrease in the number of pups per litter, without a change in the pup to implantation site ratio at ≥1 mg/kg/day (Chang et al. 2018). Similarly, no alterations in litter size, perinatal loss, or sex ratio were observed in the offspring of rats

administered up to 25 mg/kg/day PFHxS on GDs 7–22 (Ramhøj et al. 2018). The study did find decreases in male birth weights (3.5%) at ≥5 mg/kg/day and 30 and 45% decreases in pup serum thyroxine levels at 5 and 25 mg/kg/day (Ramhøj et al. 2018).

PFNA

Epidemiological Studies—Pregnancy Outcomes. Seven studies (summarized in Table 2-22) have examined pregnancy outcomes. Jensen et al. (2015) found an increase in the risk of having a miscarriage before gestation week 12, which was associated with maternal serum PFNA levels. Another study found no alteration in the risk of pregnancy loss (Buck Louis et al. 2016). No alterations in the risk of preterm birth was found in studies conducted by Chen et al. (2012a), Manzano-Salgado et al. (2017a), and Sagiv et al. (2018). Other studies found no association between PFNA and gestational age (Li et al. 2017; Manzano-Salgado et al. 2017a) or length (Lind et al. 2017a; Sagiv et al. 2018)

Epidemiological Studies—Birth Outcomes. Several studies have examined the possible associations between birth outcomes and maternal PFNA levels, these studies are summarized in Table 2-23. Most studies did not find an association between birth weight and maternal PFNA levels (Bach et al. 2016; Callan et al. 2016; Cao et al. 2018; Chen et al. 2012a; Lee et al. 2016; Lenters et al. 2016a; Li et al. 2017; Lind et al. 2017a; Manzano-Saldago et al. 2017a; Monroy et al. 2008; Robledo et al. 2015a; Shi et al. 2017). No alterations in the risk of low birth weight or small for gestational age were found in studies conducted by Chen et al. (2012a) and Manzano-Salgado et al. (2017a). Wang et al. (2016) did find an inverse association between maternal PFNA levels and birth weight in girls only and Starling et al. (2017) and Sagiv et al. (2018) found inverse associations in boys and girls combined; Starling et al. (2017) also found an inverse association between maternal PFNA levels and adiposity. Chen et al. (2012a) found an association between maternal PFNA levels and birth length, but other studies have not found alterations (Bach et al. 2016; Callan et al. 2016; Cao et al. 2018; Manzano-Salgado et al. 2017a; Robledo et al. 2015a; Shi et al. 2017; Wang et al. 2016). Most studies did not find alterations in ponderal index or head circumference (Bach et al. 2016; Callan et al. 2016; Cao et al. 2018; Chen et al. 2012a; Manzano-Salgado et al. 2017a; Robledo et al. 2015a; Shi et al. 2017; Wang et al. 2016); Chen et al. (2012a) reported an inverse association between cord PFNA levels on ponderal index. No associations between maternal PFNA or paternal PFNA levels and the odds of a male birth were observed in a general population study (Bae et al. 2015). Liew et al. (2014) did not find alterations in the risk of congenital cerebral palsy that were associated with maternal PFNA levels.

Epidemiological Studies—Neurodevelopmental Outcomes. Several potential neurodevelopmental outcomes have been examined in epidemiological studies; these studies are summarized in Table 2-24. No association between maternal PFNA levels and full-scale IQ scores were observed in 8-year-old children (Wang et al. 2015b); however, an association was found for visual IQ. Maternal PFNA levels were not associated with IQ scores in 5-year-old children (Wang et al. 2015b). Stein and Savitz (2011) found a decrease in the risk of learning problems in 5–18- or 12–15-year-olds with serum PFNA levels in the two highest quartiles or in the 4th quartile, respectively. No associations were found between maternal PFNA levels and verbal and vocabulary comprehension in 15-month-olds or language skills and intelligence scores in 38-month-olds (Jeddy et al. 2017). Vuong et al. (2016) did not find an association between maternal PFNA levels and metacognition or global executive functioning in 5- or 8-year-old children. In a subsequent study (Vuong et al. 2018), associations were found between serum PFNA levels at age 8 years and metacognition and global executive function scores, which were indicative of poorer performance; When the children were categorized by sex, the associations were only found in boys. The study also found associations between PFNA levels and at risk metacognition and global executive functioning scores. Reading scores in 5-year-old children were associated with serum PFNA levels when the children were 3 years of age but were not associated with maternal PFNA levels (Zhang et al. 2018), and reading levels at 8 years of age were not associated with maternal, 3-year-old, or 5-year-old serum PFNA levels (Zhang et al. 2018).

Mixed results have been found in studies on behavior. Gump et al. (2011) found a decrease in behavioral response inhibition that was associated with serum PFNA levels in children aged 9–11 years, and Lien et al. (2016) reported inverse associations between cord blood PFNA levels in inattention and hyperactivity/ inattention in 7-year-old children, but no effect on hyperactivity/impulsivity. Vuong et al. (2016) did not find an association between maternal PFNA levels and behavior regulation, but serum PFNA levels in 8-year-old children were associated with higher at risk behavioral regulation scores (Vuong et al. 2018). Three studies have not found associations between PFNA levels and ADHD risk (Hoffman et al. 2010; Liew et al. 2015; Stein and Savitz 2011). Similarly, maternal PFNA levels do not appear to be associated with autism (Braun et al. 2014; Liew et al. 2015).

Epidemiological Studies—Development of the Reproductive System. An inverse association between PFNA levels and insulin-like growth factor 1 was found in boys and girls aged 6–9 years (Lopez-Espinosa et al. 2016). No associations were found between PFNA and estradiol or total testosterone in 6–9 years olds (Lopez-Espinosa et al. 2016) or between maternal PFNA and testosterone or sex hormone binding globulin levels in 15-year-old girls (Maisonet et al. 2015a). Additionally, no association between

maternal serum PFHxS levels and risk of earlier age of menarche were observed in girls (Christensen et al. 2011). Summaries of these three studies are presented in Table 2-25.

Laboratory Animal Studies. Three studies were identified that examined the developmental toxicity of PFNA in laboratory animals. Full litter resorptions were observed in mice administered 10 mg/kg/day on GDs 1–17; maternal weight loss was also observed at this dose level (Das et al. 2015). At ≤1.5 mg/kg/day, decreases in postnatal survival were observed (Das et al. 2015; Wolf et al. 2010). Decreases in birth weight were observed in female offspring of rats administered 5 mg/kg/day PFNA on GDs 1–20 (Rogers et al. 2014). Postnatal growth was decreased on PNDs 1–24 in the offspring of mice administered ≥3 mg/kg/day PFNA on GDs 1–17 (Das et al. 2015); the decreases in body weight persisted in the males through PND 287 and in the females through PND 50. No skeletal or visceral abnormalities were observed in mouse pups (Das et al. 2015). Reductions in nephron endowment (number of functioning nephrons at birth) were observed in male rat pups on PND 22 (Rogers et al. 2014). This study also found increases in systolic blood pressure in pups at 10 weeks of age; no alterations were observed at 26 or 52 weeks of age. Delays in eye opening and decreased in pup body weight gain were observed in offspring of mice administered 2.0 mg/kg/day on GDs 1–18 (Wolf et al. 2010). Studies in PPARα knockout mice did not find alterations in pup survival, birth weight, pup body weight gain, or day of eye opening at maternal doses as high as 2.0 mg/kg/day (Wolf et al. 2010). Comparison between the results in tests using wild-type mice and knockout mice suggests that PPARα plays a role in PFNA developmental toxicity (Wolf et al. 2010).

PFDA

Epidemiological Studies—Pregnancy Outcomes. Four epidemiological studies examined pregnancy outcomes. Jensen et al. (2015) found an increased risk of miscarriage that was associated with maternal PFDA levels. The remaining studies found no associations between maternal PFDA levels and pregnancy loss (Buck Louis et al. 2016), gestational age (Li et al. 2017), or gestational length (Lind et al. 2017a).

Epidemiological Studies—Birth Outcomes. A small number of epidemiological studies examined risks of adverse birth outcomes associated with maternal PFDA exposure; these studies are summarized in Table 2-23. Wang et al. (2016) found an inverse association between maternal PFDA levels and birth weight in female infants only. This study also found an increased risk for small for gestational age among female infants. Other studies have not found associations (Bach et al. 2016; Callan et al. 2016; Cao et al. 2018; Lee et al. 2016; Lenters et al. 2016a; Li et al. 2017; Lind et al. 2017a; Robledo et al. 2015a; Shi et

al. 2017; Starling et al. 2017). Starling et al. (2017) also found no association with adiposity at birth. Epidemiological studies have not found associations between birth length, ponderal index, and/or head circumference and maternal PFDA levels (Bach et al. 2016; Callan et al. 2016; Cao et al. 2018; Robledo et al. 2015a; Shi et al. 2017; Wang et al. 2016). Liew et al. (2014) did not find alterations in the risk of congenital cerebral palsy in boys or girls and Bae et al. (2015) did not find alterations in odds of a male birth associated with maternal or paternal PFDA levels. Additionally, Kim et al. (2016b) did not find associations between serum PFDA levels and thyroid parameters.

Epidemiological Studies—Neurodevelopmental Outcomes. Several studies have evaluated the potential of PFDA to adversely affect neurodevelopment; see Table 2-24 for a summary of the studies. Wang et al. (2015b) did not find associations between maternal PFDA levels and IQ in 5- and 8-year-old children. Similarly, Vuong et al. (2016) did not find alterations in scores on tests of global executive functioning and metacognition in 5- or 8-year-old children. This study also found no alteration in behavioral regulation. In contrast, Gump et al. (2011) found increases in impulsivity. Oulhote et al. (2016) found an association between serum PFDA levels in 5-year-old children and total behavioral development score and higher externalizing and hyperactivity/inattention scores in 7-year-old children; the study did not find associations between behavioral development at age 7 years and maternal PFDA levels or 7-year-old PFDA levels. Liew et al. (2015) found decreases in the risk of ADHD and autism in children.

Epidemiological Studies—Developmental of the Reproductive System. In the only study examining reproductive outcomes, Lind et al. (2017a) found an inverse association between maternal PFDA levels and anogenital distance in girls, but not in boys.

Laboratory Animal Studies. An increase in fetal mortality was observed in mice exposed to 12.8 mg/kg/day PFDA on GDs 6–15 (Harris and Birnbaum 1989); this dose level was also associated with a marked decrease in fetal weight/litter (50% lower than controls), 100% incidence of variations in ossification of the braincase, decreases in maternal body weight, and maternal mortality. Decreases in fetal body weight/litter were observed at ≥1 mg/kg/day. The study did not find alterations in the occurrence of cleft palate, soft tissue malformations, or skeletal malformations. In mice exposed to 10.8 mg/kg/day PFDA on PND 10, there was no effect on spontaneous activity, habituation, performance on an elevated maze test, or response to a nicotine injection (Johansson et al. 2008). These results differ from the Johansson et al. (2008) findings when mice were exposed to PFOA or PFOS and the findings of Viberg et al. (2013) in mice exposed to PFHxS.

PFUnA

Epidemiological Studies—Pregnancy Outcomes. A limited number of epidemiological studies evaluated pregnancy outcomes. Jensen et al. (2015) did not find an alteration in the risk of miscarriage before gestation week 12. No association between gestational age and maternal PFUnA levels were found in a study conducted by Li et al. (2017).

Epidemiological Studies—Birth Outcomes. The results from a study conducted by Wang et al. (2016) found an inverse association between maternal PFUnA levels and birth weight and an increased risk of small for gestation age among female infants. Callan et al. (2016) reported an association between maternal PFUnA levels and optimal body weight but did not find an association with birth weight. The remaining epidemiological studies have not found alterations in infant size (birth weight, birth length, ponderal index, head circumference) (Bach et al. 2016; Callan et al. 2016; Cao et al. 2018; Chen et al. 2012a; Lee et al. 2016; Lenters et al. 2016a; Li et al. 2017; Shi et al. 2017) or the risks of low birth weight (Chen et al. 2012a) or small for gestational age (Chen et al. 2012a). No association between serum PFUnA levels and thyroid parameters were observed in infants (Kim et al. 2016a). The results of the epidemiological studies examining associations between birth outcome and PFUnA are presented in Table 2-23.

Epidemiological Studies—Neurodevelopmental Outcomes. The results of two studies examining possible associations between neurodevelopmental outcome and PFUnA are summarized in Table 2-24. Wang et al. (2015b) found no association between maternal PFUnA levels and IQ score in 5- and 8-year-old children; the study did find an inverse association with scores on tests assessing performance IQ. Lien et al. (2016) found no associations between cord blood PFUnA levels and performance on behavioral tests.

Laboratory Animal Studies. One study was identified that examined the potential developmental toxicity of PFUnA (Takahashi et al. 2014); the study found decreases in pup body weight at birth and on PND 4 in the offspring of rats administered via gavage 1.0 mg/kg/day PFUnA.

PFHpA

Epidemiological Studies. In the only epidemiological study evaluating developmental outcomes, Li et al. (2017) found no association between cord PFHpA levels and gestational age. The study did find an inverse association for birth weight in boys only, but not in girls and in boys and girls combined.

PFBS

Laboratory Animal Studies. No alterations in pup survival, body weight, or development were observed at doses as high as 1,000 mg/kg/day in a 2-generation rat study of potassium PFBS (Lieder et al. 2009b). In contrast to these findings, Feng et al. (2017) reported decreases in pup body weight, delays in eye opening, vaginal opening, and first estrous in the offspring of mice administered PFBS on GDs 1–20. York (2002) reported decreases in fetal body weight at 1,000 mg/kg/day in a rat study; however, a subsequent study (York 2003a) found decreases in body weights in the fetuses of rats administered 2,000 mg/kg/day, but not 1,000 mg/kg/day.

Reproductive and endocrine effects were also observed in the offspring at 200 and 500 mg/kg/day; these effects consisted of decreases in number of ovarian follicles and corpora lutea at diestrus, decreases in uterine weight and endometrial and myometrial thickness; increases in the average number of days in estrous stage; decreases in estrogen and progesterone levels; increases in luteinizing hormone levels; decreases in total T4, free T4, and total T3; and increases in TSH levels (Feng et al. 2017).

PFBA

Epidemiological Studies. Li et al. (2017) did not find an association between cord PFBA levels and gestational age or birth weight. In a study conducted by Kim et al. (2016a), no associations were found between serum PFBA levels and thyroid parameters in infants.

Laboratory Animal Studies. A delay (approximately 1 day) in eye opening was observed in the offspring of mice administered via gavage 35 mg/kg/day PFBA on GDs 1–17 (Das et al. 2008).

PFDoDA

Epidemiological Studies—Pregnancy Outcomes. In the only study examining pregnancy outcome (Table 2-22), Li et al. (2017) found no association between cord serum PFDoDA levels and gestational age.

Epidemiological Studies—Birth Outcomes. General population studies conducted by Cao et al. (2018), Lee et al. (2016), and Lenters et al. (2016a) did not find associations between cord blood PFDoDA or maternal PFDoDA levels and birth weight, birth length, and/or ponderal index. Wang et al. (2016) found an inverse association between maternal PFDoDA levels and birth weight and head circumference in female infants; no alteration in the risk of small for gestation age was found. Li et al. (2017) also found an association between cord PFDoDA levels and birth weight in girls only; no association was found in boys or in boys and girls combined. The results of these three studies are summarized in Table 2-23.

Epidemiological Studies—Neurodevelopmental Outcomes. As summarized in Table 2-24, only one study examined neurodevelopmental outcomes. In this study, maternal PFDoDA levels were not associated with IQ scores in 5- or 8-year-old children (Wang et al. 2015b).

Laboratory Animal Studies. One study evaluated the developmental toxicity of PFDoDA; no alterations in the number of live pups born, birth weight, growth, or the prevalence of external, visceral, or skeletal anomalies were observed at 0.1 or 0.5 mg/kg/day (Kato et al. 2015). At the next highest dose (2.5 mg/kg/day), only 1 of the 12 dams delivered live pups; 2 of these pups died on PND 0 and decreases in body weight gain were observed in the remaining pups.

PFHxA

Laboratory Animal Studies. Administration of 500 mg/kg/day NaPFHx on GDs 1–20 resulted in 10% decreases in fetal weight in rats (Loveless et al. 2009). Similarly, decreases in pup body weight (17–18% during the lactation period) were observed in the offspring of rats administered 500 mg/kg/day NaPFHx for 70 days prior to mating, during mating, and throughout gestation and lactation (Loveless et al. 2009). This study also found no alterations in pup clinical signs, survival, or developmental landmarks. No alterations in litter size, pup survival, or pup body weight, or occurrence of internal malformations were observed in the offspring of rats administered 315 mg/kg/day PFHxA (TWA dose) prior to mating through lactation day 4 (Kirkpatrick 2005).

FOSA

Epidemiological Studies. Robledo et al. (2015a) found an inverse association between maternal FOSA levels and birth weight in boys, but not in girls; paternal FOSA levels were not associated with birth weight. The study did not find alterations in birth length, head circumference, or ponderal index (see Table 2-23). Bae et al. (2015) did not find alterations in the odds of a male birth that was associated with maternal or paternal FOSA levels. As summarized in Table 2-24, only one study evaluated possible associations between FOSA and neurodevelopmental outcomes. Gump et al. (2011) reported an inverse association between serum FOSA levels and performance on tasks requiring behavioral inhibition. In the only study examining development of the reproductive system, Christensen et al. (2011) did not find an association between maternal serum FOSA levels and the risk of an earlier age of menarche in girls (see Table 2-25).

2.18 OTHER NONCANCER

Overview. A number of epidemiological studies have examined the possible associations between perfluoroalkyls and outcomes related to diabetes; the results of these studies are summarized in Table 2-26, with additional study details presented in the *Supporting Document for Epidemiological Studies for Perfluoroalkyls*, Table 14. Overall, the epidemiological studies do not provide support for an association between serum perfluoroalkyl levels and increases in the risk of diabetes or related outcomes (e.g., increases in blood glucose, glucose tolerance) for PFOA, PFOS, PFHxS, PFNA, PFDA, PFUnA, PFHpA, or FOSA. Additionally, results of studies on PFOA, PFOS, and PFHxS do not suggest an association between perfluoroalkyls and gestational diabetes. No epidemiological studies examining other noncancer endpoints were identified for PFBS, PFBA, PFDoDA, or PFHxA. Only four laboratory animal studies examined other noncancer endpoints reporting inflammation of the salivary glands in rats exposed to PFOA, pancreatic acinar cell hyperplasia in rats exposed to PFOA, and an increase in serum glucose levels in rats administered PFNA (Table 2-5). The fourth study did not find increases in serum glucose in rats exposed to PFOS (Table 2-4).

PFOA

Epidemiological Studies. A cohort mortality study conducted by Leonard et al. (2008; Leonard 2006) of workers at the Washington Works facility found a significant increase in deaths from diabetes, as

Table 2-26. Summary of Outcomes Related to Diabetes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFOA			
Leonard 2006 Occupational (n=6,027)	>250–≤750 ng/mL PFOA	Diabetes deaths	**SMR 183 (112–283)* (males only)**
Leonard et al. 2008 Occupational (n=6,027)	NR	Diabetes deaths	**SMR 197 (123–298)***
Lundin et al. 2009 Occupational (n=3,992)	Probable exposure	Diabetes deaths	**SMR 2.0 (1.2–3.2)***
Raleigh et al. 2014 Occupational (n=9,027)	NR	Diabetes deaths	SMR 0.76 (0.50–1.11)
Steenland et al. 2015 Occupational (n=3,713)	Estimated cumulative	Risk of diabetes	RR 1.10 (0.77–1.57) no lag RR1.12 (0.76–1.66) 10-year lag
Steenland and Woskie 2012 Occupational (n=1,088)	580 ng/mL (median PFOA)	Diabetes deaths	**SMR 1.90 (1.35–2.61)* no lag** SMR 1.90 (0.98–3.33) 10-year lag SMR 1.73 (0.83–3.18) 20-year lag
Anderson-Mahoney et al. 2008 Community (n=566)	NR	Self-reported diabetes	**SPR 1.54 (1.16–2.05)***
Conway et al. 2016 Community (n=820 with type 1 diabetes, 4,291 with type 2 diabetes, 1,349 with uncategorized, and 60,439 without diabetes)	68.4 ng/mL, 92.8 ng/mL, 86.5 ng/mL, 82.3 ng/mL (mean and serum PFOA in type 1 diabetics, type 2 diabetics, uncategorized diabetics, and no diabetes groups)	Type 1 diabetes (all) Adults (>20 years) Youth (≤20 years)	**OR 0.69 (0.65–0.74)*** **OR 0.74 (0.70–0.79)*** **OR 0.72 (0.54–0.97)***
		Type 2 diabetes Adults (>20 years) Youth (≤20 years)	**OR 0.87 (0.89–0.91)*** **OR 0.91 (0.89–0.94)*** **OR 0.92 (0.88–0.96)***
		Uncategorized diabetes Adults (>20 years) Youth (≤20 years)	**OR 0.92 (0.88–0.97)*** OR 1.13 (0.82–1.56) OR 1.18 (0.90–1.55)

Table 2-26. Summary of Outcomes Related to Diabetes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Karnes et al. 2014 Community (n=32,254 C8 participants)	Estimated cumulative	Self-reported diabetes	HR 1.00 (0.99–1.00, p=0.60), retrospective analysis HR 1.00 (1.00–1.01, p=0.31), prospective analysis
		Fasting blood glucose	NS (p>0.05)
MacNeil et al. 2009 Community (n=13,922 C8 participants)	122.7 ng/mL (mean PFOA)	Validated diabetes	OR 0.72 (0.52–1.00) (10th decile)
Cardenas et al. 2017 General population (n=957 adults at high risk of developing type 2 diabetes)	4.82 ng/mL (geometric mean serum PFOA)	Type 2 diabetes	HR 1.06 (0.89–1.28, p=0.50)
		Fasting blood glucose	**Association (p<0.05)***
		Fasting insulin	**Association (p<0.05)***
		HOMA-IR	**Association (p<0.05)***
		HOMA-β	**Association (p<0.05)***
		HbA1c	**Association (p<0.05)***
Domazet et al. 2016 General population (n=501children assessed at ages 9, 15, and 21 years)	9.7 and 9.0 ng/mL (median serum PFOA in males and females at age 9 years)	Glucose At age 15 At age 21	 β 1.87 (-1.19–4.93) β -1.01 (-14.62–30.07)
		Insulin At age 15 At age 21	 β -12.99 (-25.95–2.23) β -13.98 (-36.23–16.00)
		HOMA-IR At age 15 At age 21	 β -12.54 (-25.59–2.77) β -14.16 (-36.60–16.28)
		HOMA-β At age 15 At age 21	 **β -11.10 (-20.28 to -1.01)*** β -7.82 (-22.44–9.66)

Table 2-26. Summary of Outcomes Related to Diabetes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Domazet et al. 2016 General population (n=501children assessed at ages 9, 15, and 21 years)	3.7 and 3.4 ng/mL (median serum PFOA in males and females at age 15 years)	Glucose at age 21	β 5.83 (-3.70–16.92)
		Insulin at age 21	β -0.59 (-44.76–79.43)
		HOMA-IR at age 21	β 0.93 (-44.45–83.55)
		HOMA-β at age 21	β -11.70 (-37.16–24.67)
Fisher et al. 2013 General population (n=2,700)	2.46 ng/mL (geometric mean PFOA)	Insulin	NS (p=0.12)
		Blood glucose	NS (p=0.17)
		HOMA-IR	NS (p=0.10)
Fleisch et al. 2017 General population (n=665 children 7.7 (median) years of age)	5.3 ng/mL (geometric mean maternal PFOA)	HOMA-IR	β -0.7 (-9.8–9.4)
Fleisch et al. 2017 General population (n=665 children 7.7 (median) years of age)	4.2 ng/mL (geometric mean PFOA in child)	HOMA-IR	**β -10.1 (-17.3 to -2.3)***
He et al. 2018 General population (NHANES) (n=7,904 adults)	2.1–3.34, 3.34–5.1, and >5.1 ng/mL (serum PFOA for 2nd, 3rd, and 4th quartiles)	Diabetes	**OR 2.13 (1.30–3.46)*, 2nd quartile males** OR 1.47 (0.87–2.48), 4th quartile, females
Jensen et al. 2018 General population (n=158 pregnant women with high risk of gestational diabetes mellitus)	1.67 ng/mL (maternal median serum PFOA)	Fasting glucose	β-1.3 (3.0–0.5),
		Fasting insulin	β -4.0 (-12.2–5.0)
		2-hour glucose in oral glucose tolerance test	β-2.6 (-6.9–1.8)
		HOMA-IR	β -5.2 (-14.2–4.7)
		HOMA-β	β -0.4 (-8.0–8.0)
Kang et al. 2018 General population (n=150 children, ages 3–18 years)	1.88 ng/mL (median serum PFOA)	Fasting blood glucose	β 1.262 (-1.108–3.633, p=0.294)

Table 2-26. Summary of Outcomes Related to Diabetes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Koshy et al. 2017 General population (n=180 children enrolled in the WTCHR)	1.81 and 1.39 ng/mL (median serum PFOA in WTCHR group and comparison group)	HOMA-IR	β -0.05 (-0.21–0.12, p=0.58)
Lin et al. 2009 General population (NHANES) (n=474 adolescents)	1.51 ng/mL (mean log PFOA)	Insulin	NS (p>0.05)
		β-cell function	NS (p>0.05)
		Fasting blood glucose	NS (p>0.05)
		HOMA-IR	NS (p>0.05)
General population (NHANES) (n=969 adults)	1.48 ng/mL (mean log PFOA)	Insulin	Association (p<0.05)
		β-cell function	Association (p<0.05)
		Fasting blood glucose	NS (p>0.05)
		HOMA-IR	NS (p>0.05)
Lind et al. 2014 General population (n=1,016)	3.3 ng/mL (median PFOA)	Diabetes	OR 0.97 (0.61–1.53, p=0.88)
		HOMA-IR	NS (p=0.20)
Liu et al. 2018b General population (NHANES) (n=1,871 adults)	1.86 ng/mL (geometric mean serum PFOA)	Fasting glucose	NS (p>0.05)
		Insulin	NS (p>0.05)
		2-hour glucose in glucose tolerance test	NS (p>0.05)
		HOMA-IR	NS (p>0.05)
		HbA1C	**Association (p<0.05)***
		β cell function	**Association (p<0.05)***
Melzer et al. 2010 General population (NHANES) (n=3,966)	10.39 ng/mL (M) 9.47 ng/mL (F) (4th PFOA quartile)	Self-reported diabetes	OR 0.69 (0.41–1.16, p=0.158)
Nelson et al. 2010 General population (NHANES) (n=306 adolescent and 524 adults)	4.6 ng/mL (mean PFOA)	HOMA (adolescent)	NS (p=0.16) (M), NS (p=0.11) (F)
		HOMA (adult)	NS (p>0.05)

Table 2-26. Summary of Outcomes Related to Diabetes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Shapiro et al. 2016 General population (1,274 pregnant women)	1.68 ng/mL (geometric mean PFOA)	Gestational diabetes	NS (p=0.86 for trend)
		Impaired glucose tolerance	NS (p=0.36 for trend)
Starling et al. 2017 General population (n=604 mother-infant pairs)	1.4–17.0 ng/mL (3rd quartile maternal serum PFOA)	Maternal glucose levels	**β -0.025 (-0.046 to -0.004)*, 3rd quartile**
Su et al. 2016 General population (n=571)	5.8–8.0 ng/mL (2nd PFOA quartile)	Diabetes	**OR 0.39 (0.16–0.96)* (inverse association)**
		Fasting blood glucose	**Inverse association (p<0.01 for trend)***
		Glucose tolerance	**Inverse association (p<0.01 for trend)***
		Glycated hemoglobin	**Inverse association (p=0.04 for trend)***
Sun et al. 2018 General population (n=793 female cases and 793 female controls)	5.48–112 ng/mL (3rd tertile serum PFOA)	Type 2 diabetes	**OR 1.54 (1.04–2.28)*, 3rd tertile**
Wang et al. 2018 General population (n=385 pregnant women)	7.3 ng/mL (median maternal serum PFOA); ≥10.1 ng/mL (3rd tertile serum PFOA)	Fasting blood glucose	β -0.005 (-0.018–0.008, p=0.465)
		Fasting insulin	β 0.069 (-0.005–0.143, p=0.068)
		HOMA-IR	β 0.074 (-0.011–0.158, p=0.087)
		Blood glucose in oral glucose tolerance test	β 0.014 (-0.013–0.041, p=0.305)
		Gestational diabetes mellitus	HR 2.11 (0.76–5.86, p=0.151), 3rd tertile
Yang et al. 2018 General population (n=148 men, 81 diagnosed with metabolic syndrome)	1.90 ng/mL (median serum PFOA)	Fasting blood glucose	β 0.545 (-1.8–2.887)

Table 2-26. Summary of Outcomes Related to Diabetes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Zhang et al. 2015a General population (n=258)	3.07 and 3.94 ng/mL (geometric mean PFOA in women with or without gestational diabetes)	Gestational diabetes	**OR 1.86 (1.14–3.02)***
PFOS			
Conway et al. 2016 Community (n=820 with type 1 diabetes, 4,291 with type 2 diabetes, 1,349 with uncategorized, and 60,439 with no diabetes)	21.8, 25.2, 25.1, 23.1 ng/mL (mean and serum PFOS in type 1 diabetics, type 2 diabetics, uncategorized diabetics, and no diabetes groups)	Type 1 diabetes (all)	**OR 0.65 (0.61–0.70)***
		Type 2 diabetes	**OR 0.86 (0.82–0.90)***
		Uncategorized diabetes	OR 0.93 (0.86–1.03)
Cardenas et al. 2017 General population (n=957 adults at high risk of developing type 2 diabetes)	4.82 ng/mL (geometric mean serum PFOS)	Type 2 diabetes	HR 0.87 (0.74–1.02, p=0.08)
		Fasting blood glucose	**Association (p<0.05)***
		Fasting insulin	**Association (p<0.05)***
		HOMA-IR	**Association (p<0.05)***
		HOMA-β	**Association (p<0.05)***
		HbA1c	**Association (p<0.05)***
Domazet et al. 2016 General population (n=501 children assessed at ages 9, 15, and 21 years)	44.5 and 39.9 ng/mL (median serum PFOS in males and females at age 9 years)	Glucose	
		At age 15	**β 0.88 (0.07–1.60)***
		At age 21	β 0.64 (-0.55–1.84)
		Insulin	
		At age 15	β -0.29 (-4.26–3.67)
		At age 21	β -1.01 (-8.00–6.62)
		HOMA-IR	
		At age 15	β -12.54 (-25.59–2.77)
		At age 21	β -0.83 (-7.91–6.71)
		HOMA-β	
		At age 15	β -0.29 (-4.17–3.76)
		At age 21	β -1.66 (-5.70–2.67)

Table 2-26. Summary of Outcomes Related to Diabetes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Domazet et al. 2016 General population (n=501children assessed at ages 9, 15, and 21 years)	22.3 and 20.8 ng/mL (median serum PFOS in males and females at age 15 years)	Glucose at age 21	β 0.81 (-1.27–2.72)
		Insulin at age 21	β 4.78 (-6.82–17.77)
		HOMA-IR at age 21	β 4.74 (-6.69–17.91)
		HOMA-β at age 21	β 1.81 (-4.77–9.09)
Fisher et al. 2013 General population (n=2,700)	8.04 ng/mL (geometric mean PFOS)	Insulin	NS (p=0.88)
		Blood glucose	NS (p=0.96)
		HOMA-IR	NS (p=0.25
Fleisch et al. 2017 General population (n=665 children 7.7 (median) years of age)	24.4 ng/mL (geometric mean maternal PFOS)	HOMA-IR	β -0.6 (-8.2–7.6)
Fleisch et al. 2017 General population (n=665 children 7.7 (median) years of age)	6.2 ng/mL (geometric mean PFOS in child)	HOMA-IR	**β -10.1 (-16.4 to -3.3)***
He et al. 2018 General population (NHANES) (n=7,904 adults)	>25.5 ng/mL (4th quartile serum PFOS)	Diabetes	OR 1.75 (1.00–3.04), 4th quartile males OR1.41 (0.82–2.41), 4th quartile, females
Jensen et al. 2018 General population (n=158 pregnant women with high risk of gestational diabetes mellitus)	8.37 ng/mL (maternal median serum PFOS)	Fasting glucose	β -0.1 (-2.3–2.2)
		Fasting insulin	β 2.7 (-8.5–15.2)
		2-hour glucose in oral glucose tolerance test	β 2.9 (-2.8–8.9)
		HOMA-IR	β -2.9 (-7.1–14.1)
		HOMA-β	β -2.6 (-9.7–16.6)
Kang et al. 2018 General population (n=150 children, ages 3–18 years)	5.68 ng/mL (median serum PFOS)	Fasting blood glucose	β 0.707 (-1.921–3.336, p=0.595)

Table 2-26. Summary of Outcomes Related to Diabetes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Koshy et al. 2017 General population (n=180 children enrolled in the WTCHR)	3.72 and 2.78 ng/mL (median serum PFOS in WTCHR group and comparison group)	HOMA-IR	β -0.06 (-0.18–0.06, p=0.31)
Lin et al. 2009 General population (NHANES) (n=474 adolescents)	3.11 ng/mL (log mean PFOS)	Insulin	NS (p>0.05)
		HOMA-IR	NS (p>0.05)
		β-cell function	NS (p>0.05)
		Blood glucose	NS (p>0.05)
General population (NHANES) (969 adults)	3.19 ng/mL (log mean PFOS)	Insulin	**Association (p<0.05)***
		HOMA-IR	**Association (p<0.05)***
		β-cell function	**Association (p<0.05)***
		Blood glucose	NS (p>0.05)
Lind et al. 2014 General population (n=1,016)	13.2 ng/mL (median PFOS)	Diabetes	OR 1.43 (0.94–22.16, p=0.09)
		HOMA	NS (p=0.51)
Liu et al. 2018b General population (NHANES) (n=1,871 adults)	5.28 ng/mL (geometric mean serum PFOS)	Fasting glucose	**Inverse association (p<0.05)***
		Insulin	NS (p>0.05)
		2-hour glucose in glucose tolerance test	NS (p>0.05)
		HOMA-IR	NS (p>0.05)
		HbA1C	NS (p>0.05)
		β cell function	NS (p>0.05)
Melzer et al. 2010 General population (NHANES) (n=3,966)	57.73 ng/mL (M) 50.96 ng/mL (F) (4th quartile mean PFOS)	Self-reported diabetes	OR 0.87 (0.57–1.31, p=0.491)
Nelson et al. 2010 General population (NHANES) (n=306 adolescent and 524 adults)	25.3 ng/mL (mean PFOS)	HOMA (adolescent)	NS (p=0.18) (M), NS (p=0.22) (F)
		HOMA (adult)	NS (p>0.05)

Table 2-26. Summary of Outcomes Related to Diabetes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Shapiro et al. 2016 General population (1,274 pregnant women)	4.58 ng/mL (geometric mean PFOS)	Gestational diabetes	NS (p=0.70 for trend)
		Impaired glucose tolerance	NS (p=0.74 for trend)
Starling et al. 2017 General population (n=604 mother-infant pairs)	2.4 ng/mL (maternal median serum PFOS)	Maternal glucose levels	β -0.009 (-0.020–0.003)
Su et al. 2016 General population (n=571)	>4.8 ng/mL (4^{th} PFOS quartile)	Diabetes	**OR 3.37 (1.18–9.65)***
		Fasting blood glucose	**Association (p<0.01 for trend)***
		Glucose tolerance test	**Association (p≤0.01 for trend)***
		Glycated hemoglobin	**Association (p=0.04 for trend)***
Sun et al. 2018 General population (n=793 female cases and 793 female controls)	26.3–41.4 ng/mL (2^{nd} tertile serum PFOS)	Type 2 diabetes	**OR 1.63 (1.25–2.12)*, 2^{nd} tertile**
Wang et al. 2018 General population (n=385 pregnant women)	5.4 ng/mL (median maternal serum PFOS); ≥7.3 ng/mL (3^{rd} tertile serum PFOS)	Fasting blood glucose	β -0.009 (-0.019–0.002, p=0.108)
		Fasting insulin	β 0.013 (-0.048–0.074, p=0.672)
		HOMA-IR	β 0.074 (-0.011–0.158, p=0.087)
		Blood glucose in oral glucose tolerance test	β 0.006 (-0.015–0.028, p=0.562)
		Gestational diabetes mellitus	HR 0.71 (0.29–0.75, p=0.453), 3^{rd} tertile
Yang et al. 2018 General population (n=148 men, 81 diagnosed with metabolic syndrome)	3.00 ng/mL (median serum PFOS)	Fasting blood glucose	β -1.237 (-2.63–1.59)
Zhang et al. 2015a General population (n=258)	13.10 and 12.04 ng/mL (geometric mean PFOS in women with or without gestational diabetes)	Gestational diabetes	OR 1.13 (0.75–1.72)

Table 2-26. Summary of Outcomes Related to Diabetes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFHxS			
Conway et al. 2016 Community (n=820 with type 1 diabetes, 4,291 with type 2 diabetes, 1,349 with uncategorized, and 60,439 with no diabetes)	3.4, 3.8, 4.2, and 5.2 ng/mL (mean and serum PFHxS in type 1 diabetics, type 2 diabetics, uncategorized diabetics, and no diabetes groups)	Type 1 diabetes (all)	**OR 0.59 (0.54–0.64)***
		Type 2 diabetes	**OR 0.74 (0.71–0.77)***
		Uncategorized diabetes)	**OR 0.84 (0.78–0.90)***
Cardenas et al. 2017 General population (n=957 adults at high risk of developing type 2 diabetes)	4.82 ng/mL (geometric mean serum PFOA)	Type 2 diabetes	HR 0.99 (0.87–1.12, p=0.82)
		Fasting blood glucose	**Association (p<0.05)***
		Fasting insulin	NS (p>0.05)
		HOMA-IR	NS (p>0.05)
		HOMA-β	NS (p>0.05)
		HbA1c	NS (p>0.05)
Fisher et al. 2013 General population (n=2,700)	2.18 ng/mL (geometric mean PFHxS)	Insulin	NS (p=0.89)
		Blood glucose	NS (p=0.98)
		HOMA-IR	NS (p=0.20)
Fleisch et al. 2017 General population (n=665 children 7.7 (median) years of age)	2.5 ng/mL (geometric mean maternal PFHxS)	HOMA-IR	β -2.07 (-5.9–2.0)
Fleisch et al. 2017 General population (n=665 children 7.7 (median) years of age)	2.2 ng/mL (geometric mean PFHxS in child)	HOMA-IR	β -1.7 (-3.8–0.5)
He et al. 2018 General population (NHANES) (n=7,904 adults)	0.9–1.64, 1.64–2.9, and >2.9 (2^{nd}, 3^{rd}, and 4^{th} quartile serum PFHxS)	Diabetes	**OR 1.99 (1.19–3.33)*, 2^{nd} quartile males** OR 1.22 (0.71–2.11), 4^{th} quartile, females

Table 2-26. Summary of Outcomes Related to Diabetes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Jensen et al. 2018 General population (n=158 pregnant women with high risk of gestational diabetes mellitus)	0.31 ng/mL (maternal median serum PFHxS)	Fasting glucose	**β 1.7 (0.2–3.2)***
		Fasting insulin	**β 7.7 (0.1–15.9)***
		2-hour glucose in oral glucose tolerance test	β 2.9 (-0.8–6.8)
		HOMA-IR	**β 9.5 (1.0–18.8)***
		HOMA-β	β 2.3 (-4.3–9.4)
Kang et al. 2018 General population (n=150 children, ages 3–18 years)	0.793 ng/mL (median serum PFHxS)	Fasting blood glucose	β 0.925 (-1.779–2.164, p=0.500)
Koshy et al. 2017 General population (n=180 children enrolled in the WTCHR)	0.67 and 0.53 ng/mL (median serum PFHxS in WTCHR group and comparison group)	HOMA-IR	β -0.09 (-0.18–0.003, p=0.04)
Lin et al. 2009 General population (NHANES) (n=474 adolescents);	0.95 ng/mL (log mean)	Insulin	NS (p>0.05)
		HOMA-IR	NS (p>0.05)
		β-cell function	NS (p>0.05)
		Blood glucose	NS (p>0.05)
General population (NHANES) (n=969 adults)	0.60 ng/mL (log mean PFHxS)	Insulin	NS (p>0.05)
		HOMA-IR	NS (p>0.05)
		B-cell function	NS (p>0.05)
		Blood glucose	NS (p>0.05)
Lind et al. 2014 General population (n=1,016)	2.1 ng/mL (median PFHxS)	Diabetes	OR 1.00 (0.74–1.35, p=0.98)
		HOMA	NS (p=0.29)
Nelson et al. 2010 General population (NHANES) (n=306 adolescent and 524 adults)	2.6 ng/mL (mean PFHxS)	HOMA (adolescent)	NS (p=0.20) (M), **Inverse association (p=0.001)* (F)**
		HOMA (adult)	NS (p>0.05)

Table 2-26. Summary of Outcomes Related to Diabetes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Shapiro et al. 2016 General population (n=1,274 pregnant women)	1.02 ng/mL (geometric mean PFHxS)	Gestational diabetes	NS (p=0.73 for trend)
		Impaired glucose tolerance	NS (p=0.44 for trend)
Starling et al. 2017 General population (n=604 mother-infant pairs)	1.1–10.9 ng/mL (3rd quartile maternal serum PFHxS)	Maternal glucose levels	**β -0.023 (-0.044 to -0.002)*, 3rd quartile**
Sun et al. 2018 General population (n=793 female cases and 793 female controls)	2.15 and 2.01 ng/mL (serum PFHxS in cases and controls, respectively)	Type 2 diabetes	OR 1.26 (0.86–1.86), 3rd tertile
Yang et al. 2018 General population (n=148 men, 81 diagnosed with metabolic syndrome)	3.80 ng/mL (median serum PFHxS)	Fasting blood glucose	β -0.29 (-1.9–1.32)
PFNA			
Conway et al. 2016 Community (n=820 with type 1 diabetes, 4,291 with type 2 diabetes, 1,349 with uncategorized, and 60,439 with no diabetes)	1.4, 1.5, 1.5, and 1.6 ng/mL (mean and serum PFNA in type 1 diabetics, type 2 diabetics, uncategorized diabetics, and no diabetes groups)	Type 1 diabetes (all)	**OR 0.65 (0.57–0.74)***
		Type 2 diabetes	**OR 0.94 (0.88–1.00)***
		Uncategorized diabetes)	OR 0.95 (0.85–1.06)
Cardenas et al. 2017 General population (n=957 adults at high risk of developing type 2 diabetes)	0.53 ng/mL (geometric mean serum PFNA)	Type 2 diabetes	HR 0.99 (0.87–1.12, p=0.82)
		Fasting blood glucose	**Association (p<0.05)***
		Fasting insulin	NS (p>0.05)
		HOMA-IR	NS (p>0.05)
		HOMA-β	NS (p>0.05)
		HbA1c	NS (p>0.05)
Fleisch et al. 2017 General population (n=665 children 7.7 (median) years of age)	0.6 ng/mL (geometric mean maternal PFNA)	HOMA-IR	β 1.4 (-8–11.7)

Table 2-26. Summary of Outcomes Related to Diabetes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Fleisch et al. 2017 General population (n=665 children 7.7 (median) years of age)	1.7 ng/mL (geometric mean PFNA in child)	HOMA-IR	β -0.6 (-3.2–2.6)
He et al. 2018 General population (NHANES) (n=7,904 adults)	>1.64 (4th quartile serum PFNA)	Diabetes	OR 1.19 (0.73–1.95), 4th quartile males OR 1.01 (0.62–1.65), 4th quartile, females
Jensen et al. 2018 General population (n=158 pregnant women with high risk of gestational diabetes mellitus)	0.65 ng/mL (maternal median serum PFNA)	Fasting glucose	β 0.03 (-2.1–2.2)
		Fasting insulin	**β 12.1 (0.7–24.8)***
		2-hour glucose in oral glucose tolerance test	β 1.3 (-6.5–4.2)
		HOMA-IR	β 12.2 (-0.5–26.4)
		HOMA-β	**β 12.4 (0.2–23.7)***
Kang et al. 2018 General population (n=150 children, ages 3–18 years)	0.938 ng/mL (median serum PFNA)	Fasting blood glucose	β 0.428 (-1.785–2.641, p=0.703)
Koshy et al. 2017 General population (n=180 children enrolled in the WTCHR)	0.61 and 0.49 ng/mL (median serum PFNA in WTCHR group and comparison group)	HOMA-IR	β 0.01 (-0.13–0.14, p=0.89)
Lin et al. 2009 General population (NHANES) (n=474 adolescents)	0.35 ng/mL (log mean PFNA)	Insulin	**Inverse association (p<0.05)***
		HOMA-IR	NS (p>0.05)
		β-cell function	**Inverse association (p<0.05)***
		Blood glucose	NS (p>0.05)
General population (NHANES) (n=969 adults)	0.21 ng/mL (log mean PFNA)	Insulin	NS (p>0.05)
		HOMA-IR	NS (p>0.05)
		β-cell function	NS (p>0.05)
		Blood glucose	NS (p>0.05)

Table 2-26. Summary of Outcomes Related to Diabetes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Lind et al. 2014 General population (n=1,016)	0.7 ng/mL (median PFNA)	Diabetes	OR 1.30 (0.85–1.97, p=0.22)
		HOMA	NS (p=0.90)
Nelson et al. 2010 General population (NHANES) (n=306 adolescents and 524 adults)	1.3 ng/mL (mean PFNA)	HOMA (adolescent)	NS (p=0.83) (M), (p=0.20) (F)
		HOMA (adult)	NS (p>0.05)
Starling et al. 2017 General population (n=604 mother-infant pairs)	0.5–6.0 ng/mL (2nd half maternal serum PFNA)	Maternal glucose levels	**β -0.025 (-0.042 to -0.009)*, 2nd half**
Su et al. 2016 General population (n=571)	>5.1 ng/mL (4th PFNA quartile)	Diabetes	**OR 0.31 (0.11–0.88)* 4th quartile (inverse association)**
		Fasting blood glucose	NS (p=0.10 for trend)
		Glucose tolerance test	**Inverse association (p<0.01 for trend)***
		Glycated hemoglobin	NS (p=0.11 for trend)
Sun et al. 2018 General population (n=793 female cases and 793 female controls)	0.60 and 0.61 ng/mL (serum PFNA in cases and controls, respectively)	Type 2 diabetes	OR 0.99 (0.67–1.48), 3rd tertile
Yang et al. 2018 General population (n=148 men, 81 diagnosed with metabolic syndrome)	0.50 ng/mL (median serum PFNA)	Fasting blood glucose	β -0.627 (-2.54–1.29)
Zhang et al. 2015a General population (n=258)	1.23 and 1.20 ng/mL (geometric mean PFNA in women with or without gestational diabetes)	Gestational diabetes	OR 1.06 (0.70–1.60)
PFDA			
Fleisch et al. 2017 General population (n=665 children 7.7 (median) years of age)	0.3 ng/mL (geometric mean PFDA in child)	HOMA-IR	**β -14.7 (-22.1 to -6.5)***

Table 2-26. Summary of Outcomes Related to Diabetes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Jensen et al. 2018 General population (n=158 pregnant women with high risk of gestational diabetes mellitus)	0.26 ng/mL (maternal median serum PFDA)	Fasting glucose	β 1.3 (-3.6–1.0)
		Fasting insulin	β -0.2 (-11.2–12.1)
		2-hour glucose in oral glucose tolerance test	β -3.3 (-8.7–2.5)
		HOMA-IR	β -1.5 (-13.5–12.1)
		HOMA-β	β 3.9 (-6.4–15.2)
Kang et al. 2018 General population (n=150 children, ages 3–18 years)	0.0592 ng/mL (median serum PFDA)	Fasting blood glucose	β -0.201 (-1.280–0.878, p=0.713)
Koshy et al. 2017 General population (n=180 children enrolled in the WTCHR)	0.14 and 0.11 ng/mL (median serum PFDA in WTCHR group and comparison group)	HOMA-IR	β -0.04 (-0.11–0.03, p=0.26)
Starling et al. 2017 General population (n=604 mother-infant pairs)	0.2–3.5 ng/mL (2nd half maternal serum PFDA)	Maternal glucose levels	**β -0.024 g (-0.041 to -0.007)*, 2nd half**
Sun et al. 2018 General population (n=793 female cases and 793 female controls)	0.13 and 0.16 ng/mL (serum PFDA in cases and controls, respectively)	Type 2 diabetes	OR 0.71 (0.48–1.05), 3rd tertile
Yang et al. 2018 General population (n=148 men, 81 diagnosed with metabolic syndrome)	0.40 ng/mL (median serum PFDA)	Fasting blood glucose	β -2.543 (-4.65 to -0.44)

Table 2-26. Summary of Outcomes Related to Diabetes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Zhang et al. 2015a General population (n=258)	0.41 and 0.40 ng/mL ng/mL (geometric mean PFDA in women with or without gestational diabetes)	Gestational diabetes	OR 1.04 (0.70–1.53)
PFUnA			
Kang et al. 2018 General population (n=150 children, ages 3–18 years)	0.652 ng/mL (median serum PFUnA)	Fasting blood glucose	β 1.350 (-0.020–2.721, p=0.053)
Koshy et al. 2017 General population (n=180 children enrolled in the WTCHR)	0.12 and 0.04 ng/mL (median serum PFUnA in WTCHR group and comparison group)	HOMA-IR	β -0.04 (-0.10–0.02, p=0.21)
Lind et al. 2014 General population (n=1,016)	0.3 ng/mL (median PFUnA)	Diabetes	OR 0.95 (0.59–1.52; p=0.81)
		HOMA	NS (p=0.32)
Su et al. 2016 General population (n=571)	6.4–9.2 ng/mL (3rd PFUnA quartile)	Diabetes	**OR 0.24 (0.08–0.78)* 3rd quartile (inverse association)**
		Fasting blood glucose	**Inverse association (p<0.01 for trend)***
		Glucose tolerance test	**Inverse association (p<0.01 for trend)***
		Glycated hemoglobin	NS (p=0.17 for trend)
Yang et al. 2018 General population (n=148 men, 81 diagnosed with metabolic syndrome)	0.30 ng/mL (median serum PFUnA)	Fasting blood glucose	**β -1.821 (-3.45 to -0.189)***

Table 2-26. Summary of Outcomes Related to Diabetes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFHpA			
Lind et al. 2014 General population (n=571)	0.05 ng/mL (median PFHpA)	Diabetes	OR 1.02 (0.77–1.34, p=0.90)
		HOMA	NS (p=0.56)
Yang et al. 2018 General population (n=148 men, 81 diagnosed with metabolic syndrome)	0.20 ng/mL (median serum PFHpA)	Fasting blood glucose	β -1.101 (-5.54–3.34)
FOSA			
Lind et al. 2014 General population (n=571)	0.11 ng/mL (median FOSA)	Diabetes	OR 1.07 (0.75–1.53, p=0.71)
		HOMA	NS (p=0.070)

[a]See the *Supporting Document for Epidemiological Studies for Perfluoroalkyls,* Table 14 for more detailed descriptions of studies.
[b]Participants in occupational exposure may have also lived near the site and received residential exposure; community exposure studies involved subjects living near PFOA facilities with known exposure to high levels of PFOA.
[c]Asterisk indicates association with perfluoroalkyl; unless otherwise specified, values in parenthesis are 95% confidence intervals.

(F) = females; FOSA = perfluorooctane sulfonamide; HOMA = homeostatic model assessment; HR = hazard ratio; IR = insulin resistance; (M) = males; NHANES = National Health and Nutrition Examination Survey; NR = not reported; NS = not significant; OR = odds ratio; PFDA = perfluorodecanoic acid; PFHpA = perfluoroheptanoic acid; PFHxS = perfluorohexane sulfonic acid; PFNA = perfluorononanoic acid; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid; PFUnA = perfluoroundecanoic acid; SMR = standardized mortality ratio; SPR = standardized prevalence ratio; WTCHR = World Trade Center Health Registry

compared to workers at other DuPont facilities in the region. In an update of the Leonard et al. (2008) study, Steenland and Woskie (2012) found an increased risk of diabetes deaths when compared to other regional DuPont employees, but not when compared to the U.S. population. However, when the workers were categorized by estimated cumulative exposure levels, the exposure-response trend was not statistically significant. Lundin et al. (2009) also found an increase in deaths from diabetes in workers exposed to APFO at the 3M Cottage Grove facility in Minnesota, as compared to Minnesota death rates. The increase was only found in workers with probable exposure to APFO, but not with definite exposure; no deaths from diabetes were observed in the workers with definite exposure to APFO. As noted by Steenland and Woskie (2012), diabetes mortality may not be a good surrogate for the underlying diabetes incidence data. Raleigh et al. (2014) did not find an increase in diabetes deaths at the Cottage Grove facility and Steenland et al. (2015) did not find an increased risk of diabetes associated with estimated cumulative PFOA exposure at the Washington Works facility.

In community exposure studies, Anderson-Mahoney et al. (2008) found an increased prevalence of self-reported diabetes in residents living near the Washington Works facility, as compared to expected rates taken from NHANES. Conway et al. (2016) found increases in the prevalence of type 1 diabetes, type 2 diabetes, and uncategorized diabetes in C8 Health Study participants. When the participants were categorized by age, the increases in type 1 diabetes and type 2 diabetes prevalences were found in adults and children; uncategorized diabetes was not increased in either group. In contrast, Karnes et al. (2014) did not find an increased risk of self-reported diabetes associated with estimated cumulative PFOA levels and MacNeil et al. (2009) did not find an increased risk of validated diabetes in C8 Health Study participants.

General population studies found either an inverse association between serum PFOA and risk of diabetes (Su et al. 2016), an association (He et al. 2018; Sun et al. 2018), or no association (Cardenas et al. 2017; Lind et al. 2014; Melzer et al. 2010). Additionally, most general population studies have not found associations between serum PFOA levels and insulin (Fisher et al. 2013; Lin et al. 2009; Liu et al. 2018b), blood glucose levels (Fisher et al. 2013; Lin et al. 2009; Liu et al. 2018b; Su et al. 2016; Yang et al. 2018), homeostatic model assessment for insulin resistance (HOMA-IR) (Fisher et al. 2013; Lin et al. 2009; Lind et al. 2014; Liu et al. 2018b; Nelson et al. 2010), or glucose tolerance (Liu et al. 2018b; Su et al. 2016). Cardenas et al. (2017) did find associations between serum PFOA and glycemic parameters in cross-sectional analyses; however, in longitudinal analyses, no associations were found between serum PFOA and fasting blood glucose, fasting insulin, HOMA-IR, HOMA-β, or HbA1c. Studies in children

have not found associations between serum PFOA and blood glucose, blood insulin, HOMA-IR, and/or HOMA-β (Domazet et al. 2016; Fleisch et al. 2017; Kang et al. 2017; Koshy et al. 2017).

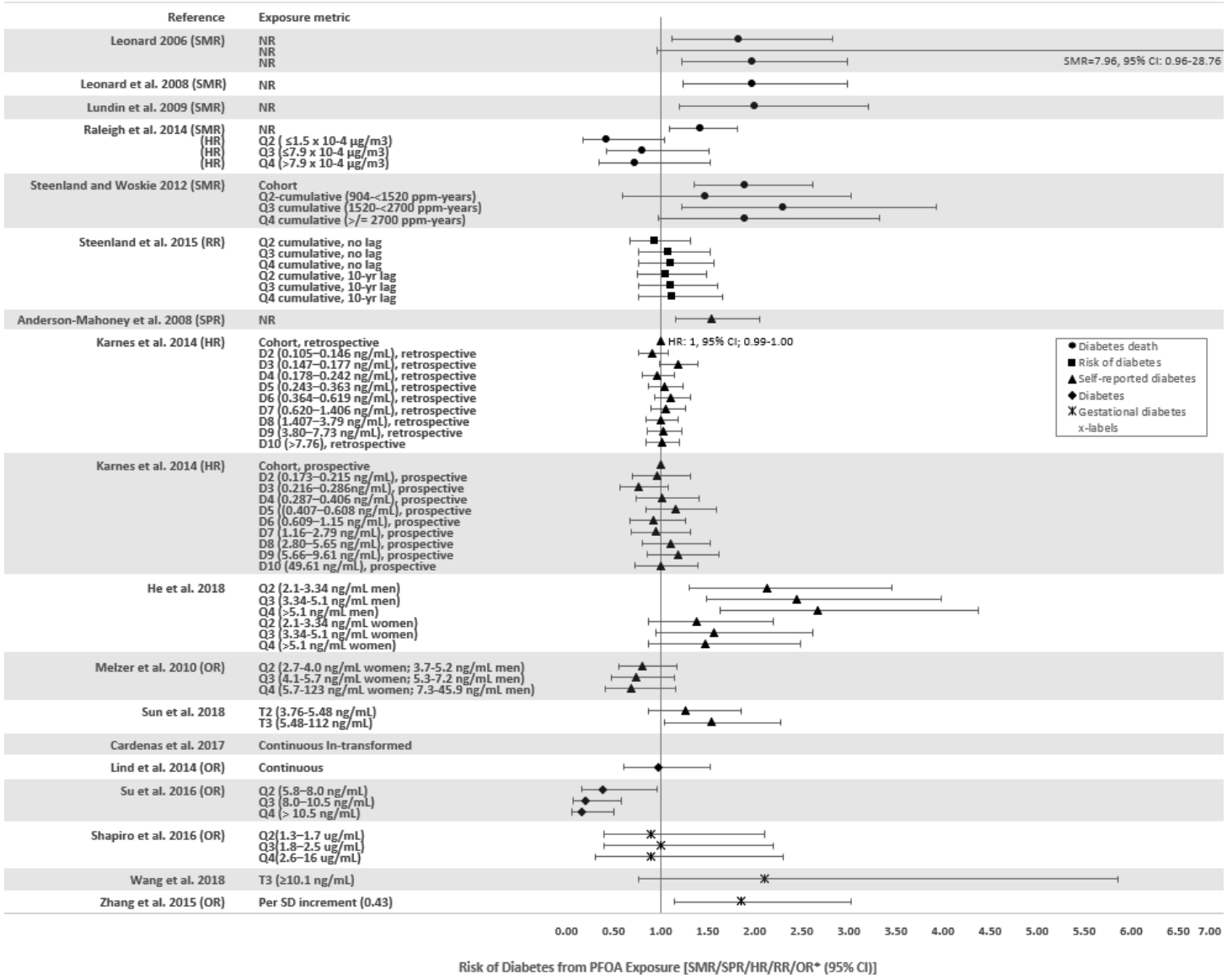
Three studies evaluated the risk of gestational diabetes and found mixed results. In a case-control study, Zhang et al. (2015a) found an increased risk of gestational diabetes associated with serum PFOA, whereas Shapiro et al. (2016) and Wang et al. (2018) did not find associations between serum PFOA and gestational diabetes or impaired glucose tolerance. Additionally, Starling et al. (2017) found an inverse association between blood glucose levels and serum PFOA in pregnant women. In contrast, Jensen et al. (2018) did not find associations between serum PFOA and fasting glucose, fasting insulin, HOMA-IR, HOMA-β, or blood glucose levels in a glucose tolerance test in pregnant women. The ORs for the risk of diabetes and gestational diabetes are graphically presented in Figure 2-39.

Laboratory Animal Studies. Two chronic-duration oral studies examined other noncancer endpoints. Inflammation of the salivary gland was observed in rats exposed to 1.5 mg/kg/day (3M 1983; Butenhoff et al. 2012c) and an increased incidence of acinar cell hyperplasia was observed in rats exposed to 13.6 mg/kg/day (Biegel et al. 2001).

PFOS

Epidemiological Studies. Inverse associations between serum PFOS and the prevalence of type 1 diabetes and type 2 diabetes were observed among participants of the C8 Health Study (Conway et al. 2016). In a general population study conducted by Su et al. (2016), an increased risk of diabetes was noted, as well as associations between serum PFOS levels and fasting blood glucose, response to glucose tolerance test, and glycated hemoglobin levels. Cardenas et al. (2017) also found associations between serum PFOS and fasting blood glucose, fasting insulin, HOMA-IR, and HOMA-β; however, these associations were not found in longitudinal analyses over a 3-year period. In a prospective case-control study, Sun et al. (2018) reported an association between serum PFOS and type 2 diabetes. Four other general population studies did not find increased risks of diabetes (Cardenas et al. 2017; He et al. 2019; Lind et al. 2014; Melzer et al. 2010). Several studies have not found associations between serum PFOS levels and insulin, blood glucose, or HOMA-IR levels (Fisher et al. 2013; Lin et al. 2009; Lind et al. 2014; Liu et al. 2018b; Nelson et al. 2010; Yang et al. 2018). In NHANES adult participants, Lin et al. (2009) found associations between serum PFOS and insulin and HOMA-IR and Liu et al. (2018b) found an inverse association with fasting glucose levels. No associations were found in adolescent participants

Figure 2-39. Diabetes Risk Relative to Serum PFOA Levels (Presented as Adjusted Ratios)



(Lin et al. 2009). Studies in children have not found associations between serum PFOS and fasting blood glucose, fasting blood insulin, HOMA-IR, and/or HOMA-β (Domazet et al. 2016; Fleisch et al. 2017; Kang et al. 2018; Koshy et al. 2017).

No alterations in the risk of gestational diabetes were observed in three general population studies (Shapiro et al. 2016; Wang et al. 2018' Zhang et al. 2015a). Shapiro et al. (2016), Starling et al. (2017), and Wang et al. (2018) studies also found no association between serum PFOS and blood glucose levels, glucose tolerance or other glycemic measurements in pregnant women. The ORs for the risk of diabetes and gestational diabetes are graphically presented in Figure 2-40.

Laboratory Animal Studies. Perinatal exposure to 3 mg/kg/day PFOS did not result in alterations in serum insulin or glucose levels in the offspring on PND 63 (Wan et al. 2014b). However, when the offspring were fed a high fat diet, increases in fasting glucose levels were observed at 0.3 and 3 mg/kg/day and fasting serum insulin levels were increased at 3 mg/kg/day.

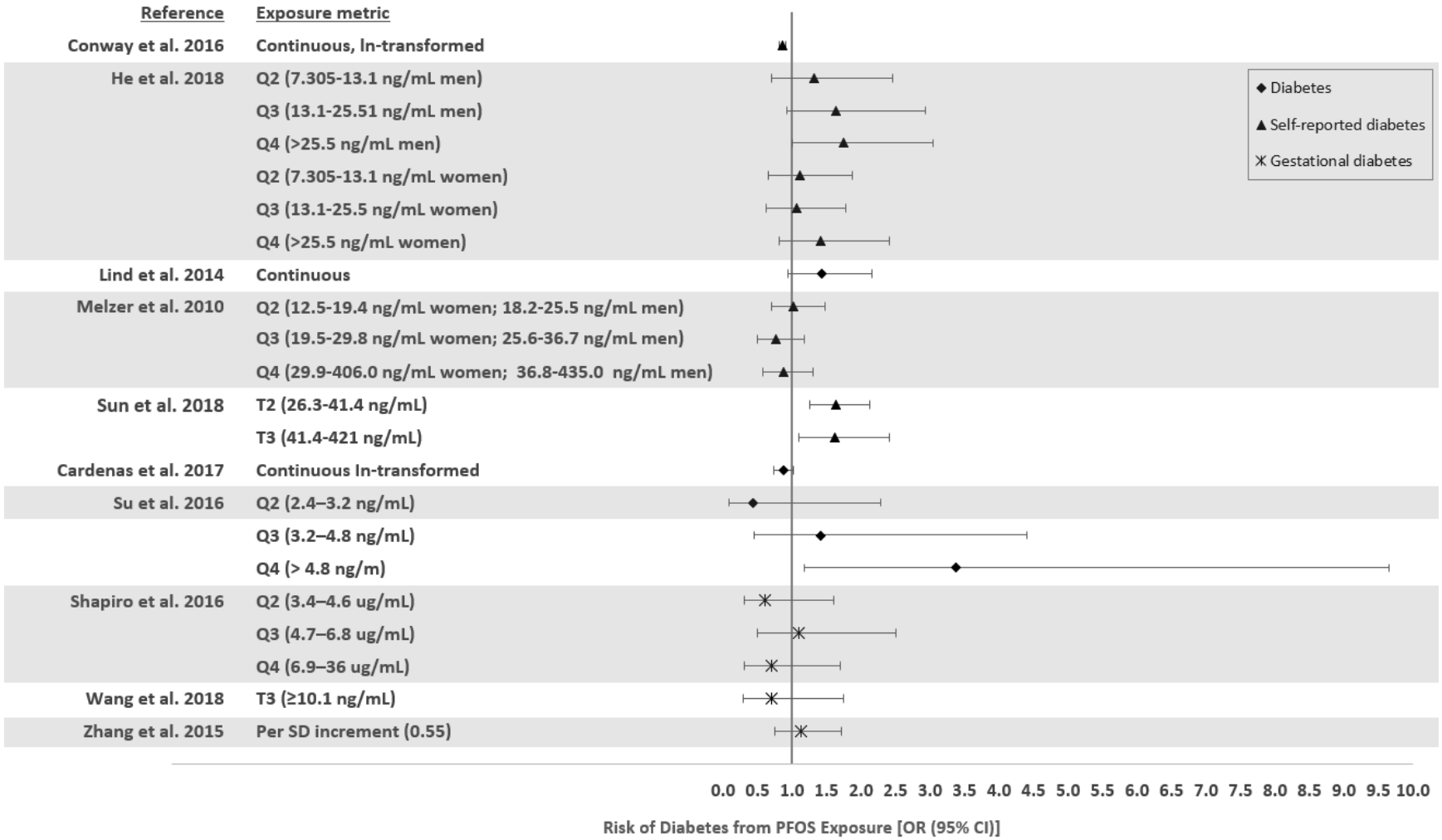
PFHxS

Epidemiological Studies. A study of C8 Health Project participants found inverse associations between serum PFHxS levels and the prevalence of type 1 diabetes, type 2 diabetes, and uncategorized diabetes (Conway et al. 2016). General population studies have examined diabetes-related outcomes and have not found associations between serum PFHxS levels and diabetes risk (Cardenas et al. 2017; He et al. 2018; Lind et al. 2014; Sun et al. 2018), gestational diabetes (Shapiro et al. 2016) or insulin, blood glucose, or HOMA-IR levels (Cardenas et al. 2017; Fisher et al. 2013; Jensen et al. 2018; Lin et al. 2009; Lind et al. 2014; Nelson et al. 2010; Yang et al. 2018). An inverse association between serum PFHxS levels and blood glucose levels was found in pregnant women (Starling et al. 2017). No associations between serum PFHxS and glycemic parameters were found in children (Fleisch et al. 2017; Kang et al. 2018; Koshy et al. 2017).

PFNA

Epidemiological Studies. An inverse association between serum PFNA levels and the risk of diabetes was observed in a general population study (Su et al. 2016) and for type 1 diabetes and type 2 diabetes in C8 Health Study participants (Conway et al. 2016). Four other studies did not find associations for diabetes (He et al. 2018; Lind et al. 2014; Sun et al. 2018) or gestational diabetes (Zhang et al. 2015a).

Figure 2-40. Diabetes Risk Relative to Serum PFOS Levels (Presented as Adjusted Odds Ratios)



The Su et al. (2016) study also reported an inverse association between PFNA levels and response on a glucose tolerance test. A study by Starling et al. (2017) also found an inverse association between PFNA levels and blood glucose levels in pregnant women. A study of adolescent NHANES participants found decreasing levels of insulin with increasing serum PFNA levels (Lin et al. 2009); this association was not found in adult NHANES participants (Lin et al. 2009). Several studies did not find associations between serum PFNA levels and fasting blood glucose, glucose tolerance, HOMA-IR, and/or HOMA-β (Cardenas et al. 2017; Fleisch et al. 2017; Jensen et al. 2018; Kang et al. 2018; Koshy et al. 2017; Lin et al. 2009; Lind et al. 2014; Nelson et al. 2010; Yang et al. 2018).

Laboratory Animal Studies. An increase in serum glucose levels was observed in rats administered via gavage 1 mg/kg/day PFNA for 14 days (Fang et al. 2012a).

PFDA

Epidemiological Studies. Two studies evaluated the potential association between PFDA and diabetes risk. Sun et al. (2018) did not find an association for type 2 diabetes risk and Zhang et al. (2015a) did not find an association between serum PFDA levels and the risk of gestational diabetes. Other studies have examined possible associations between serum PFDA and glycemic measurements. Fleisch et al. (2017) found an inverse association with HOMA-IR in children. Other studies in children (Kang et al. 2018; Koshy et al. 2017), adults (Yang et al. 2018), and pregnant women (Jensen et al. 2018) did not find associations for fasting blood glucose, fasting insulin, glucose tolerance, HOMA-IR, and/or HOMA-β.

PFUnA

Epidemiological Studies. Six epidemiological studies evaluating associations between PFUnA and diabetes-related outcomes have found conflicting results. Su et al. (2016) found inverse associations between serum PFUnA levels and diabetes risk, fasting blood glucose levels, and glucose tolerance test results, Yang et al. (2018) found an inverse association with fasting blood glucose levels, and Starling et al. (2017) found an inverse association with blood glucose levels in pregnant women. Whereas Lind et al. (2014) found no alterations in the risk of diabetes or HOMA, and Kang et al. (2018) and Koshy et al. (2017) found no associations between serum PFUnA levels and fasting blood glucose and HOMA-IR, respectively, in studies in children.

PFHpA

Epidemiological Studies. Lind et al. (2014) did not find associations between serum PFHpA levels and the risk of diabetes or HOMA alterations and Yang et al. (2018) did not find an association with fasting blood glucose levels.

FOSA

Epidemiological Studies. In the one epidemiological study identified, no associations between serum FOSA levels and the risk of diabetes or HOMA were found (Lind et al. 2014).

2.19 CANCER

Overview. A number of occupational exposure, community, and general population studies have examined possible associations between perfluoroalkyls and cancer risk; these studies are summarized in Table 2-27 and the *Supporting Document for Epidemiological Studies for Perfluoroalkyls*, Table 15. Occupational and community exposure studies have found increases in the risk of testicular and kidney cancer associated with PFOA. No consistent epidemiologic evidence for other cancer types were found for PFOA. For PFOS, one occupational exposure study reported an increase in bladder cancer, but this was not supported by subsequent occupational studies. General population studies have not consistently reported increases in malignant tumors for PFOS. A small number of epidemiology studies examined possible associations between other perfluoroalkyls and cancer risk. No consistent associations were observed for breast cancer risk for PFHxS, PFNA, PFHpA, or PFDoDA; increased breast cancer risks were observed for PFDA and FOSA, but this was based on a single study. No associations between PFOA, PFOS, PFHxS, PFNA, PFDA, or PFUnA and prostate cancer risk were found. However, among men with a first-degree relative with prostate cancer, associations were found for PFOA, PFOS, PFHxS, PFDA, and PFUnA, but not for PFNA. Epidemiological studies examining potential cancer effects were not identified for PFBS, PFBA, or PFHxA.

Laboratory animal studies have evaluated the carcinogenicity of PFOA and PFOS; the results of these studies are summarized in Tables 2-3 and 2-4. In laboratory animals, there is some evidence for increases in Leydig cell adenomas, pancreatic acinar cell adenomas, and hepatocellular adenomas in male rats exposed to PFOA in the diet. An increase in hepatocellular adenomas was observed in male rats exposed to dietary PFOS for 2 years; thyroid follicular cell adenomas were observed in rats exposed to PFOS for

Table 2-27. Summary of Cancer Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFOA			
Gilliland 1992; Gilliland and Mandel 1993 Occupational (n=389 deaths) Reference population: Minnesota general population	NR	All cancer deaths	SMR 0.86 (0.72–1.01), males SMR 0.75 (0.56–0.99), females
		Prostate cancer	SMR 2.03 (0.55–4.59) RR 1.13 (1.01–1.27) for a 1-year increase in employment length **RR 3.3 (1.02–10.6)* for a 10-year employment length**
Leonard 2006; Leonard et al. 2008 Occupational (n=6,027) Reference population: DuPont workers at other regional facilities	5–9,550 ng/mL (estimated range of PFOA)	All cancer deaths	SMR 100 (88–114), males SMR 149 (77–260), females
		Kidney cancer deaths	SMR 185 (95–323), males
		Biliary passages and liver cancer deaths	SMR 133 (53–274)
		Pancreatic cancer deaths	SMR 100 (50–180)
		Bladder or other urinary organ cancer deaths	SMR 131 (53–269)
		Prostate cancer deaths	SMR 65 (34–114)
		Bronchus, trachea, lung cancer deaths	SMR 81 (63–104)
Lundin et al. 2009 Occupational (n=3,993) Reference population: Minnesota general population; for HR analysis comparisons with workers with low exposure or <1 year of exposure	2,600–5,200 and 300–1,500 ng/mL (range of PFOA in subset of current workers with definite exposure jobs and probable exposure jobs)	All cancer deaths	SMR 0.9 (0.5–1.4), definite exposure SMR 0.9 (0.8–1.1), probable exposure
		Pancreas cancer deaths	SMR 0.9 (0.0–4.7), definite exposure SMR 1.0 (0.4–2.1), probable exposure
		Trachea, bronchus, and lung cancer deaths	SMR 1.2 (0.5–2.3), definite exposure SMR 1.0 (0.7–1.4), probable exposure
		Prostate cancer deaths	SMR 2.1 (0.4–6.1), definite exposure SMR 0.9 (0.4–1.8), probable exposure **HR 6.6 (1.1–37.7), high exposure ≥6 months** **HR 3.7 (1.3–10.4), definite exposure for ≥5 years**

Table 2-27. Summary of Cancer Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Raleigh et al. 2014 Occupational (n=9,207) Reference population: Minnesota general population	>7.9x10^{-4} µg/m^3 (cumulative exposure, 4th PFOA quartile)	All cancer deaths	SMR 0.87 (0.78–0.97)
		Pancreatic cancer deaths	SMR 0.85 (0.50–1.34)
		Prostate cancer deaths	SMR 0.83 (0.53–1.23)
		Kidney cancer deaths	SMR 0.53 (0.20–1.16)
		Liver cancer deaths	SMR 0.81 (0.35–1.59)
		Breast cancer deaths	SMR 0.82 (0.41–1.47)
		Bladder cancer deaths	SMR 0.89 (0.38–1.76)
Raleigh et al. 2014 Occupational (n=9,207) Reference population: non-APFO exposed workers at a St. Paul facility	≤7.9x10^{-4} and >7.9x10^{-4} µg/m^3 (cumulative exposure,3rd and 4th PFOA quartile)	Pancreatic cancer deaths	HR 1.23 (0.50–3.00), 3rd and 4th quartiles combined
		Pancreatic cancer	HR 1.36 (0.59–3.11), 3rd and 4th quartiles combined
		Prostate cancer deaths	HR 1.32 (0.61–2.84), 4th quartile
		Prostate cancer	HR 1.11 (0.82–1.49), 4th quartile
		Kidney cancer deaths	HR 0.39 (0.11–1.32), 3rd and 4th quartiles combined
		Kidney cancer	HR 0.73 (0.21–2.48), 4th quartile
		Liver cancer deaths	HR 0.67 (0.14–3.27), 3rd and 4th quartiles combined
		Breast cancer deaths	HR 0.54 (0.15–1.94), 3rd and 4th quartiles combined
		Breast cancer	HR 1.27 (0.70–2.31), 4th quartile
		Bladder cancer deaths	HR 1.96 (0.63–6.15), 3rd and 4th quartiles combined
		Bladder cancer	HR 1.66 (0.86–3.18), 4th quartile
Steenland et al. 2015 Occupational (n=3,713)	Estimated cumulative exposure	Bladder cancer	**Inverse association (p=0.04 or p=0.06 for trend) with no lag or 10-year lag** **RR 0.23 (0.05–0.93), 4th quartile with no lag**

Table 2-27. Summary of Cancer Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
		Colorectal cancer	NS (p=0.91 and 0.86 for trend), with no lag or 10-year lag
		Prostate cancer	NS (p=0.83 and 0.91 for trend), no lag or 10-year lag
		Melanoma	NS (p=0.16 and 0.55 for trend), no lag or 10-year lag
Steenland and Woskie 2012 Occupational (n=1,084 deceased workers) Reference population: DuPont workers at other regional facilities	≥2,700,000 ng/mL-years (estimated cumulative 4th PFOA quartile)	All cancer deaths	SMR 0.94 (0.76–1.16), 4th quartile
		Pancreatic cancer deaths	SMR 0.92 (0.30–2.16), 4th quartile
		Lung cancer deaths	SMR 0.75 (0.48–1.11), 4th quartile
		Prostate cancer deaths	SMR 0.57 (0.16–1.46), 4th quartile
		Bladder cancer deaths	SMR 0.36 (0.10–2.01), 4th quartile
		Kidney cancer deaths	**SMR 2.66 (1.15–5.24)*, 4th quartile** **SMR 2.82 (1.13–5.81)*, 10-year lag** **SMR 3.67 (1.48–7.57)*, 20-year lag**
Barry et al. 2013 Community and occupational (n=32,254)	Estimated cumulative exposure 24.2 and 112.7 ng/mL (median PFOA)	Testicular cancer	**HR 1.34 (1.00–1.79, p=0.05)* no lag** HR 1.28 (0.95–1.73, p=0.10) 10-year lag **HR 3.17 (0.75–13.45, p=0.04 for trend)*, 4th quartile**
		Kidney cancer	HR 1.10 (0.98–1.24, p=0.10), no lag (continuous) HR 1.58 (0.88–2.84, p=0.18 for trend), 4th quartile
		Breast cancer	**HR 0.94 (0.89–1.00, p=0.05)*, no lag** **HR 0.93 (0.88–0.99, p=0.03)*, 10-year lag**
		Colorectal cancer	HR 0.99 (0.92–1.07, p=0.84), no lag

Table 2-27. Summary of Cancer Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Ducatman et al. 2015a, 2015b Community (C8) (n=25,412 men)	86.6 ng/mL (mean PFOA)	Prostate specific antigen	NS (p<0.05)
Innes et al. 2014 Community (n=208 cases of colorectal cancer and 47,359 cancer-free adults)	13.5–27.8 ng/mL (2nd PFOA quartile)	Colorectal cancer	**OR 0.47 (0.31–0.74)*, 2nd quartile**
Vieira et al. 2013 Community (n=25,107)	30.8–109 and 110–655 ng/mL (estimated PFOA in high and very high exposure groups)	Kidney cancer	**AOR 2.0 (1.3–3.2)*, high exposure group** AOR 2.0 (1.0–3.9), very high exposure group
		Testes cancer	AOR 2.8 (0.8–9.2), very high exposure group
		Prostate cancer	AOR 1.5 (0.9–2.5), very high exposure group
		Breast cancer	AOR 1.4 0.9–2.3) very high exposure group, females only
Bonefeld-Jorgensen et al. 2011 General population (n=31 breast cancer cases and115 matched controls)	2.5 and 1.6 ng/mL (median PFOA in cases and controls)	Breast cancer	AOR 1.20 (0.77–1.88, p=0.43)
Bonefeld-Jorgensen et al. 2014 General population (n=250 breast cancer cases and 233 matched controls)	5.2 ng/mL (mean PFOA)	Breast cancer	RR 1.00 (0.90–1.11).
Eriksen et al. 2009 General population (n=713 for prostate cancer, n=332 for bladder cancer, n=128 for pancreatic cancer, n=67 for liver cancer, and n=772 controls)	6.8 and 6.0 ng/mL (median PFOA in male and female cancer patients) 6.9 and 5.4 ng/mL (median PFOA in male and female controls)	Prostate cancer	IRR 1.18 (0.84–1.65)
		Bladder cancer	IRR 0.81 (0.53–1.24)
		Pancreas cancer	IRR 1.55 (0.85–2.80)
		Liver cancer	IRR 0.60 (0.26–1.37)

Table 2-27. Summary of Cancer Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Hardell et al. 2014 General population (n=201 cases and 186 controls)	2.3 and 1.9 ng/mL (mean PFOA in cases and controls)	Prostate cancer	OR 1.1 (0.7–1.7) **OR 2.6 (1.2–6.0)*, among subjects with a heredity risk and serum PFOA above the median**
Wielsøe et al. 2017 General population (n=77 cases and 84 controls)	2.08 and 1.48 ng/mL (median serum PFOA in cases and controls, respectively)	Breast cancer	**OR 1.26 (1.01–1.58, p=0.039)*, continuous** **OR 2.64 (1.17–5.97, p=0.019)*, 3rd tertile**
PFOS			
Alexander et al. 2003 Occupational (n=2,083; 145 deaths) Reference population: Alabama general population	NR	All cancer deaths	SMR 0.84 (0.50–1.32), high potential exposure group
		Bladder and other urinary organs cancer	**SMR 12.77 (2.63–37.35)*, high potential exposure group** **SMR 16.12 (3.32–47.14)*, high exposure group ≥1 year exposure**
Alexander and Olsen 2007 Occupational (n=1,895; 1,488 deaths) Reference population: NIOSH SEER referent data	NR	Bladder cancer	SIR 1.74 (0.64–3.79), high potential exposure group SIR 1.43 (0.16–5.15) ≥10-year exposure group
Grice et al. 2007 Occupational (n=1,400 current, retired, or former workers)	1,300–1,970 ng/mL (PFOS levels in high potential exposure group)	Colon cancer	OR 1.69 (0.68–4.17)
		Melanoma	OR 1.01 (0.25–4.11)
		Prostate cancer	OR 1.08 (0.44–2.69)
Olsen et al. 2004a Occupational (current and retired workers)	NR	Malignant melanoma of the skin	RRE_pC 12 (1.0–>100) RRE_pC 10 (0.7–>100), >10 years employment
		Malignant neoplasm of the colon	RRE_pC 5.4 (0.5–>100) RRE_pC 12 (0.8–>100), >10 years employment
Ducatman et al. 2015a, 2015b Community (C8) (n=25,412 men)	22.18 ng/mL (mean PFOS)	Prostate specific antigen	NS ($p<0.05$)

Table 2-27. Summary of Cancer Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Innes et al. 2014 Community (n=208 cases of colorectal cancer and 47,359 cancer-free adults)	13.6–20.1 ng/mL (2nd PFOS quartile)	Colorectal cancer	**OR 0.35 (0.24–0.53)***
Bonefeld-Jorgensen et al. 2011 General population (n=31 breast cancer cases and 115 matched controls)	45.6 and 21.9 ng/mL (median PFOS in cases and controls)	Breast cancer	**OR 1.03 (1.001–1.07, p=0.05)***
Bonefeld-Jorgensen et al. 2014 General population (n=250 breast cancer cases and 115 matched controls)	30.6 ng/mL (mean PFOS)	Breast cancer	RR 0.99 (0.98–1.01)
Eriksen et al. 2009 General population (n=713 for prostate cancer, n=332 for bladder cancer, n=128 for pancreatic cancer, n=67 for liver cancer, and n=772 controls)	35.1 and 32.1 ng/mL and 35.0 and 29.3 ng/mL (median PFOS in male and female cancer patients and males and females in the comparison group)	Prostate cancer	IRR 1.05 (0.97–1.14)
		Bladder cancer	IRR 0.93 (0.83–1.03)
		Pancreas cancer	IRR 0.99 (0.86–1.14)
		Liver cancer	IRR 0.59 (0.27–1.27)
Hardell et al. 2014 General population (n=201 cases of prostate cancer and 186 controls)	11 and 10 ng/mL (mean PFOS in cases and controls)	Prostate cancer	OR 1.0 (0.6–1.5) **OR 2.7 (1.04–6.8)*, among subjects with a heredity risk and serum PFOS above the median**
Wielsøe et al. 2017 General population (n=77 cases and 84 controls)	35.50 and 18.2 ng/mL (median serum PFOS in cases and controls, respectively)	Breast cancer	**OR 1.02 (1.01–1.03, p=0.005)*, continuous** **OR 3.13 (1.20–8.15, p=0.020)*, 2nd tertile**
PFHxS			
Ducatman et al. 2015a, 2015b Community (C8) (n=25,412 men)	3.58 ng/mL (mean PFHxS)	Prostate specific antigen	NS (p<0.05)
Bonefeld-Jorgensen et al. 2014 General population (n=250 breast cancer cases and 115 matched controls)	1.2 ng/mL (mean PFHxS)	Breast cancer	**RR 0.66 (0.47–0.94)***

Table 2-27. Summary of Cancer Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Hardell et al. 2014 General population (n=201 cases of prostate cancer and 186 controls)	1.1 and 0.940 ng/mL (mean PFHxS in cases and controls)	Prostate cancer	OR 1.3 (0.8–1.9) **OR 4.4 (1.7–12)*, among subjects with a heredity risk and serum PFHxS above the median**
Wielsøe et al. 2017 General population (n=77 cases and 84 controls)	2.52 and 1.14 ng/mL (median serum PFHxS in cases and controls, respectively)	Breast cancer	**OR 1.16 (1.02–1.32, p=0.029)*, continuous** **OR 2.69 (1.23–5.88, p=0.013)*, 3rd tertile**
PFNA			
Ducatman et al. 2015a, 2015b Community (C8) (n=25,412 men)	1.47 ng/mL (mean PFNA)	Prostate specific antigen	NS (p<0.05)
Bonefeld-Jorgensen et al. 2014 General population (n=250 breast cancer cases and 115 matched controls)	0.5 ng/mL (mean PFNA)	Breast cancer	RR 0.76 (0.30–1.94)
Hardell et al. 2014 General population (n=201 cases of prostate cancer and 186 controls)	0.679 and 0.631 ng/mL (mean PFNA in cases and controls)	Prostate cancer	OR 1.2 (0.8–1.8) OR 2.1 (0.9–4.8), among subjects with a heredity risk and serum PFNA above the median
Wielsøe et al. 2017 General population (n=77 cases and 84 controls)	3.28 and 1.83 ng/mL (median serum PFNA in cases and controls, respectively)	Breast cancer	OR 1.07 (0.98–1.17, p=0.116), continuous **OR 2.43 (1.07–5.51, p=0.034)*, 2nd tertile** OR 2.07 (0.90–4.76, p=0.056), 3rd tertile
PFDA			
Hardell et al. 2014 General population (n=201 cases of prostate cancer and 186 controls)	0.338 and 0.291 ng/mL (mean PFDA in cases and controls)	Prostate cancer	OR 1.4 (0.9–2.1) **OR 2.6 (1.1–6.1)*, among subjects with a heredity risk and serum PFDA above the median**

Table 2-27. Summary of Cancer Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Wielsøe et al. 2017 General population (n=77 cases and 84 controls)	1.30 n and 1.01 ng/mL (median serum PFDA in cases and controls, respectively)	Breast cancer	OR 1.17 (0.97–1.40, p=0.094), continuous **OR 2.36 (1.04–5.36, p=0.041)*, 3rd tertile**
PFUnA			
Hardell et al. 2014 General population (n=201 cases of prostate cancer and 186 controls)	0.308 and 0.285 ng/mL (mean PFUnA in cases and controls)	Prostate cancer	OR 1.2 (0.8–1.9) **OR 2.6 (1.1–5.9)*, among subjects with a heredity risk and serum PFUnA above the median**
Wielsøe et al. 2017 General population (n=77 cases and 84 controls)	2.23 and 2.02 ng/mL (median serum PFUnA in cases and controls, respectively)	Breast cancer	OR 1.06 (0.97–1.15, p=0.207), continuous **OR 2.00 (0.88–4.53, p=0.019)*, 3rd tertile**
PFHpA			
Wielsøe et al. 2017 General population (n=77 cases and 84 controls)	0.11 and 0.08 ng/mL (median serum PFHpA in cases and controls, respectively)	Breast cancer	OR 6.98 (0.61–80.0, p=0.119), continuous OR 1.52 (0.54–4.24, p=0.425), 3rd tertile
PFDoDA			
Wielsøe et al. 2017 General population (n=77 cases and 84 controls)	0.40 and 0.21 ng/mL (median serum PFDoDA in cases and controls, respectively)	Breast cancer	OR 1.03 (1.01–1.06, p=0.447), continuous OR 0.93 (0.45–1.91, p=0.839), 3rd tertile

Table 2-27. Summary of Cancer Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
FOSA			
Bonefeld-Jorgensen et al. 2014 General population (n=250 breast cancer cases and 115 matched controls)	3.5 ng/mL (mean FOSA)	Breast cancer	**RR 1.89 (1.01–3.54)*, among women with serum FOSA >5.75 ng/mL**

[a]See the *Supporting Document for Epidemiological Studies for Perfluoroalkyls,* Table 15 for more detailed descriptions of studies.
[b]Participants in occupational exposure may have also lived near the site and received residential exposure; community exposure studies involved subjects living near PFOA facilities with known exposure to high levels of PFOA.
[c]Asterisk indicates association with perfluoroalkyl; unless otherwise specified, values in parenthesis are 95% confidence intervals.

AOR = adjusted odds ratio; CI = confidence interval; FOSA = perfluorooctane sulfonamide; HR = hazard ratio; IRR = incidence rate ratio; NIOSH = National Institute for Occupational Safety and Health; NR = not reported; NS = not significant; OR = odds ratio; PFDA = perfluorodecanoic acid; PFHxS = perfluorohexane sulfonic acid; PFNA = perfluorononanoic acid; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid; PFUnA = perfluoroundecanoic acid; RR = relative risk; RRE_pC = risk ratio episodes of care; SEER = Surveillance Epidemiology and End Results; SIR = standardized incidence ratio; SMR = standardized mortality ratio

1 year and allowed to recover for an additional year. A discussion of the relevance of the rodent carcinogenicity data to humans is included in Section 2.20.6.

EPA (2016e, 2016f) has concluded that there is suggestive evidence of the carcinogenic potential of PFOA and PFOS in humans. IARC (2017) concluded that PFOA is possibly carcinogenic to humans (Group 2B).

PFOA

Epidemiological Studies. Several studies have examined the possible association between occupational exposure to PFOA and increased cancer risk in workers at two U.S. facilities—3M facility in Cottage Grove, Minnesota (Gilliland and Mandel 1993; Lundin et al. 2009; Raleigh et al. 2014) and DuPont Washington Works facility in West Virginia (Leonard 2006; Leonard et al. 2008; Steenland and Woskie 2012; Steenland et al. 2015). In addition, the potential carcinogenicity of PFOA has been assessed in the community near the Washington Works facility (Barry et al. 2013; Innes et al. 2014; Vieira et al. 2013) and in the general population (Bonefeld-Jorgensen 2011, 2014; Eriksen et al. 2009; Hardell et al. 2014).

Occupational exposure studies have not found increases in the risk of all cancer deaths (Gilliland and Mandel 1993; Leonard 2006; Leonard et al. 2008; Lundin et al. 2009; Raleigh et al. 2014; Steenland and Woskie 2012). The occupational exposure studies have consistently found no increases in the risk of pancreatic, liver, or respiratory tract cancers or deaths from these cancers (Leonard 2006; Leonard et al. 2008; Lundin et al. 2009; Raleigh et al. 2014; Steenland and Woskie 2012); a general population case-control study also found no associations between serum PFOA and pancreas or liver cancer (Eriksen et al. 2009). Additionally, two case-control studies did not find associations between serum PFOA levels and risk of breast cancer (Bonefeld-Jorgensen et al. 2011, 2014); a third case-control study found an association between serum PFOA and breast cancer (Wielsøe et al. 2017). Steenland et al. (2015) found an inverse association between estimated cumulative PFOA exposure and bladder cancer in workers; other studies have not found associations (Eriksen et al. 2009; Gilliland and Mandel 1993; Leonard 2006; Leonard et al. 2008; Raleigh et al. 2014; Steenland and Woskie 2012).

Some associations between PFOA and cancer effects have been observed, including prostate, kidney, and testicular cancers. Ten years of employment in the Chemical Division of the 3M Cottage Grove facility was associated with a 3.3-fold increase in the relative risk of prostate cancer mortality, as compared to no employment in PFOA production areas (Gilliland and Mandel 1993; data also reported in Gilliland 1992);

no increase in prostate cancer risk was observed when all workers in the Chemical Division were analyzed. The investigators noted that the prostate cancer findings are based on a small number of cases and could have resulted from chance or unrecognized confounding from exposure to other factors. An update of this study conducted by Lundin et al. (2009) did not find an increase in prostate cancer deaths in workers with definite PFOA exposure. When the cohort was divided into the three exposure categories and duration of definite exposure, increased risks for prostate cancer were found in the high-exposure category and in workers with definite exposure for at least 5 years, as compared with workers in the low-exposure category and with the shortest cumulative exposure duration, respectively. Interpretation of the Gilliland and Mandel (1993) and Lundin et al. (2009) studies is limited by the qualitative assessment of potential exposure and the fact that workers in the low exposure categories were likely research-and-development professionals rather than production workers (Raleigh et al. 2014). In the most recent evaluation of the Cottage Grove facility, which involved extensive exposure assessment, Raleigh et al. (2014) did not find increases in prostate cancer deaths when compared to the general population or to workers at another facility and did not find an increase in the incidence of prostate cancer when the workers were categorized by cumulative exposure levels. Studies of the Washington Works facility workers did not find increases in prostate cancer deaths (Leonard et al. 2008; Steenland and Woskie 2012) or incidence (Steenland et al. 2015). A case-control general population study by Hardell et al. (2014) did find an increase in prostate risk only among subjects with a heredity risk (first-degree relative with prostate cancer) and serum PFOA levels above the median. In a study of community members, Ducatman et al. (2015b) did not find an association between prostate-specific antigen (PSA) levels and serum PFOA levels in men 20–49 or 50–69 years of age.

In the earliest cancer assessment of workers at the Washington Works facility (Leonard 2006; Leonard et al. 2008), an increase in the number of deaths from kidney cancer relative to workers at other regional DuPont facilities was observed; however, the CI included unity. In a follow-up study that used serum PFOA levels collected in current workers to assess job title exposure (Steenland and Woskie 2012), an increase in kidney cancer deaths was observed in workers with the highest exposures when analyzed with no lag, a 10-year lag, or a 20-year lag. Steenland and Woskie (2012) also found an increase in deaths from mesothelioma; the investigators noted that this was likely due to asbestos exposure. Steenland and Woskie (2012) noted that tetrafluoroethylene, a rodent kidney carcinogen, is used in the manufacture of a variety of fluoropolymers and noted that the tetrafluoroethylene is well controlled due to its volatile and explosive properties. It is noted that in a multisite study of tetrafluoroethylene workers, which included workers at the Washington Works facility (Consonni et al. 2013), an increased risk of renal cancer (SMR 1.44, 95% CI 0.69–2.65) was found, although the CI included unity. Consonni et al. (2013) noted that

88% of the workers were also exposed to PFOA. When PFOA exposure was used as an exposure variable, the findings were the similar as when tetrafluoroethylene was used as the exposure variable, and thus, it was difficult for the investigators to evaluate separate associations for each compound. It is noted that increases in kidney deaths were not observed in the Cottage Grove facility (Raleigh et al. 2014), which did not use tetrafluoroethylene (Chang et al. 2014).

Three studies have examined the community living near the Washington Works facilities; some of these studies also included workers at the facility. Barry et al. (2013) reported an increased risk of testicular cancer that was associated with estimated cumulative PFOA exposure. Vieira et al. (2013) also reported an increase in testicular cancer, but the CIs of the adjusted odds ratio (AOR) included unity. When the participants were grouped by water district, an increased risk of testicular cancer (AOR 5.1, 95% CI 1.6–15.6) was observed in the Little Hocking water district, which had the highest PFOA levels in the water. The Vieira et al. (2013) study also found increased risks of kidney cancer among participants with high or very high exposure to PFOA; Barry et al. (2013) also concluded that there was an association between estimated cumulative PFOA exposure and kidney cancer, although the CIs for the hazard ratio included unity. The third study of the Washington Works community found an inverse association between serum PFOA and risk of colorectal cancer (Innes et al. 2014).

In their review of the available epidemiological data, IARC (2017) concluded that the evidence for testicular cancer was "considered credible and unlikely to be explained by bias and confounding, however, the estimate was based on small numbers." Similarly, IARC (2017) concluded that the evidence for kidney cancer was also credible but noted that chance, bias, and confounding could not be ruled out with reasonable confidence. They considered that there was limited evidence in humans for the carcinogenicity of PFOA.

Laboratory Animal Exposure Studies. Two studies have examined the carcinogenic potential of PFOA in rats. In the first study of male and female Sprague-Dawley rats exposed to PFOA in the diet for 2 years (3M 1983; Butenhoff et al. 2012c), significant increases in the incidence of fibroadenoma of the mammary gland in females and Leydig cell adenoma were found in males exposed to 15 mg/kg/day. A high incidence of pituitary adenoma occurred among all groups, including controls. The incidence of hepatocellular carcinoma was not significantly increased. The investigators noted that the incidence of fibroadenoma in the mammary gland in the 15 mg/kg/day group was similar to the incidence found in untreated aging rats and that the incidence of Leydig cell adenoma was similar to the spontaneous incidence of this tumor in aged rats. The mammary gland pathology slides from this study (3M 1983;

Butenhoff et al. 2012c) study were re-examined in 2005 by a Pathology Working Group (PWG) using current diagnostic criteria (Hardisty et al. 2010). The incidences of fibroadenoma found by the PWG were 36, 44, and 46% in the 0, 1, and 15 mg/kg/day groups, respectively; there were no statistically significant differences between the groups (Hardisty et al. 2010). Additionally, there were no significant differences in the incidence of adenocarcinoma, total benign neoplasms, or total malignant neoplasms between the groups. In the second study of male Sprague-Dawley rats exposed to PFOA in the diet for 2 years (Biegel et al. 2001), an increase in the incidence of hepatocellular adenomas was found, but there were no hepatocellular carcinomas in the treated group. PFOA also increased the incidence of Leydig cell adenomas. In addition, PFOA increased the incidence of pancreatic acinar cell adenomas; a pancreatic carcinoma was observed in one treated rat. Hepatic peroxisome proliferation was increased significantly at all interim evaluation time points (1, 3, 6, 9, 12, 15, 18, and 21 months), but there was no increase in cell proliferation. In Leydig cells, neither peroxisome proliferation nor cell proliferation were increased.

PFOA was a positive modulator of hepatocarcinogenesis in male Wistar rats in a biphasic (initiation with diethylnitrosamine followed by oral treatment with PFOA) or triphasic (initiation with diethylnitrosamine [DEN] followed by dosing with 2-acetylaminofluorene and then PFOA) promotion protocol (Abdellatif et al. 1991, 2004). PFOA induced a marked increase in acylCoA oxidase activity and only a slight increase in catalase activity (Abdellatif et al. 2004). Since PFOA did not significantly increase 8-hydroxy-deoxyguanosine (a marker of oxidative DNA damage *in vivo*) in isolated liver DNA, it appeared that PFOA did not require extensive DNA damage for its promoting activity (Abdellatif et al. 2004). PFOA was also found to act as a promoter in male Wistar rats in an initiation-selection-promotion protocol (Nilsson et al. 1991).

IARC (2017) concluded that there was limited evidence in experimental animals for the carcinogenicity of PFOA.

PFOS

Epidemiological Studies. Four studies have evaluated the carcinogenic potential in workers at a Decatur, Alabama perfluorooctanesulphonyl fluoride (PFOSF) based fluorochemical production facility. In the earliest study, no increase in all cancer deaths was found, as compared to the Alabama general population (Alexander et al. 2003). An increased risk of bladder cancer was observed in workers with high potential exposure and in workers with a high potential exposure for ≥1 year; the mortality ratio was based on three

cases in the high exposure group. In a reanalysis of workers at this facility conducted by Alexander and Olsen (2007), 11 cases of bladder cancer were identified from worker surveys (n=6) and death certificates (n=5). Only two of the six self-reported bladder cancer diagnosis were confirmed via medical records; the other four subjects declined to give consent for medical verification. When compared to incidence data from the National Institute for Occupational Safety and Health (NIOSH) Surveillance Epidemiology and End Results (SEER) referent data, the standardized incidence ratios for the high potential exposure group were elevated, but the CIs included unity. When compared with workers with <1 year of high exposure, workers with 5–<10 and ≥10 years of high exposure had relative risks of 1.92 (95% CI 0.30–12.06) or 1.52 (95% CI 0.21–10.99). Although the study did not adjust for smoking, the investigators noted that 83% of the living bladder cancer cases (five of the six subjects) reported cigarette use, as compared to 56% reported in the noncases. An additional limitation of the study is inclusion of four cases of bladder cancer that were not verified by medical records. The results of this study do not appear to confirm the findings of increased bladder cancer in the mortality study (Alexander et al. 2003). In a subsequent study of this facility, treatment for bladder cancer was not reported among current workers (Olsen et al. 2004a). The study did find increases in the number of episodes of care for malignant neoplasm of the prostate or malignant neoplasms of the colon, as compared to long-term workers in another division, but the CIs included unity. No increases in the risk ratio episodes of care were found for liver, rectum, or respiratory tract (Olsen et al. 2004a). A fourth study of this facility (Grice et al. 2007) examined possible associations between colon cancer, melanoma, and prostate cancer and PFOS exposure. The risks of these cancers were not associated with any of the PFOS-exposure categories for analyses that included all self-reported or only validated cancers.

General population case-control studies have evaluated several cancer types. Innes et al. (2014) reported an inverse association between PFOS and colorectal cancer. A small-scale study of 31 cases by Bonefeld-Jorgensen et al. (2011) found a slight increase in breast cancer risk, a finding not replicated in another larger study of a different population (Bonefeld-Jorgensen et al. 2014). A third case-control study found associations between serum PFOS and breast cancer in subjects with serum PFOS levels in the second tertile and higher (Wielsøe et al. 2017). Eriksen et al. (2009) and Hardell et al. (2014) did not find increases in the risk of prostate cancer associated with serum PFOS. However, an increased risk of prostate cancer was found among subjects with a first-degree relative with prostate cancer and PFOS levels above the median level (Hardell et al. 2014). Eriksen et al. (2009) also found no associations between serum PFOS and the risk of bladder cancer, pancreatic cancer, or liver cancer. Ducatman et al. (2015b) did not find an association between serum PFOS levels and PSA levels in men participating in the C8 studies.

Laboratory Animal Studies. In a 2-year PFOS dietary exposure study bioassay in male and female Sprague-Dawley rats (Butenhoff et al. 2012b; unpublished study by Thomford 2002b), a significant positive trend of hepatocellular adenoma was observed in males; the incidence was significantly higher than controls at 1.04 mg/kg/day. No hepatocellular adenomas were seen in a group of rats exposed to 1.17 mg/kg/day for 1 year and allowed to recover for the second year. High-dose males from the recovery group showed a significant increase in thyroid follicular cell adenoma relative to controls. No significant increase in this type of tumor was observed in rats exposed for 2 years. In females, there was a significant positive trend for incidences of hepatocellular adenoma, which was associated with a significant increase in the 1.04 mg/kg/day group. In females, there were also significant negative trends for mammary adenoma and fibroadenoma carcinoma combined.

PFHxS

Epidemiological Studies. Three case-control studies have examined the possible association between serum PFHxS and cancer. Bonefeld-Jorgensen et al. (2014) found in inverse association between PFHxS levels and breast cancer risk. In contrast, Wielsøe et al. (2017) found a positive association between serum PFHxS levels and breast cancer risk. No association between PFHxS and prostate cancer was observed (Hardell et al. 2014), with the exception of increased risk in men with a first-degree relative with prostate cancer and above-median serum PFHxS levels. No associations between serum PFHxS and PSA levels were observed in a cross-sectional study of men 20–49 or 50–69 years of age participating in the C8 Health Studies (Ducatman et al. 2015b).

PFNA

Epidemiological Studies. The carcinogenic potential of PFNA has been examined in three case-control studies. No consistent associations between serum PFNA levels and breast cancer (Bonefeld-Jorgensen et al. 2014; Wielsøe et al. 2017) or prostate cancer (Hardell et al. 2014) were found. Serum PSA levels were not associated with serum PFNA levels in men participating in the C8 Health Study (Ducatman et al. 2015b).

PFDA

Epidemiological Studies. Hardell et al. (2014) examined the possible association between the serum PFDA level and risk of prostate cancer and only found an association in men with a heredity risk factor and PFDA levels above the median. In a case-control study of breast cancer, Wielsøe et al. (2017) found an association among women with serum PFDA levels in the third quartile.

PFUnA

Epidemiological Studies. An increased risk of prostate cancer was found in men with first-degree relatives with prostate cancer and serum PFUnA levels above the median (Hardell et al. 2014). An increased breast cancer risk was found in women with serum PFUnA levels in the third quartile (Wielsøe et al. 2017).

PFHpA

Epidemiological Studies. One study evaluated possible associations between serum PFHpA and cancer risk and found no association for breast cancer (Wielsøe et al. 2017).

PFDoDA

Epidemiological Studies. In the only cancer study for PFDoDA, Wielsøe et al. (2017) did not find an increased risk of breast cancer in women associated with serum PFDoDA levels.

FOSA

Epidemiological Studies. Bonefeld-Jorgensen et al. (2014) reported an increased risk of breast cancer among women with serum FOSA levels >5.75 ng/mL.

2.20 MECHANISM OF TOXICITY

The primary effects observed in rodents exposed to perfluoroalkyls are liver toxicity, developmental toxicity, and immune toxicity. The cellular mechanisms by which hepatic effects are induced have been extensively studied, while more limited data are available on mechanisms for other effects. The available

data indicate that perfluoroalkyls produce a number of adverse effects through activation of the PPARα, a member of the nuclear receptor superfamily that mediates a broad range of biological responses (Issemann and Green 1990). However, some adverse effects of perfluoroalkyls occur through PPARα-independent mechanisms, which may include activation of other nuclear receptors, increased oxidative stress, dysregulation of mitochondrial function, and inhibition of gap junction intercellular communication (GJIC). In the sections below, cellular mechanisms of action that are mediated by PPARα and independent of PPARα are discussed, followed by discussions of mechanisms specific to the hepatic, developmental, immunotoxic, and hormone effects of perfluoroalkyls.

2.20.1 Cellular Mechanisms of Toxicity

PPARα-Dependent Mechanisms

Activation of the PPARα receptor in rodents initiates a characteristic sequence of morphological and biochemical events, principally, but not exclusively, in the liver. These events include marked hepatocellular hypertrophy due to an increase in number and size of peroxisomes, a large increase in peroxisomal fatty acid β oxidation, increased cytochrome 450-mediated ω hydroxylation of lauric acid, and alterations in lipid metabolism. Although there is uncertainty regarding the exact and possibly, multiple mechanisms for liver effects of perfluoroalkyls, peroxisome proliferation mediated by PPARα is a contributing mechanism. Proliferation of peroxisomes in laboratory animals exposed to perfluoroalkyls is discussed in Section 2.9 (Hepatic); as discussed in that section, hepatic peroxisome proliferation has been shown in rats exposed to PFOA and in mice exposed to PFDA.

Many, but not all, of the adverse effects induced by perfluoroalkyls in rodents are mediated through activation of the PPARα. Ligands, including perfluoroalkyls, bind to and activate PPARα, causing a conformational change in the receptor that leads to dissociation of co-repressors and enables heterodimerization with the retinoid X receptor (Corton et al. 2014). The activated receptor complex binds to a DNA direct repeat motif (the peroxisome proliferator response element or PPRE) located in the promoters of peroxisome proliferator responsive genes. The binding of the receptor complex leads to recruitment of co-activators, which acetylate histones and remodel chromatin, enabling RNA polymerase to transcribe the target gene(s). PPARα regulates lipid homeostasis by modulating the expression of genes involved in fatty acid uptake, activation, and oxidation. Activation of nuclear receptors including PPARα is a complex, dynamic process that depends on levels of expression of the receptors in different

tissues, competition among receptors for endogenous and exogenous ligands and for binding sites on chromatin, and availability and abundance of co-activators and/or co-repressors (Corton et al. 2014).

PPARα Receptor Activation. Many perfluoroalkyls, including PFOA, PFOS, PFUnA, PFHpA, and PFDoDA have been shown to activate PPARα in mammalian cells *in vitro* (Bjork and Wallace 2009; Bjork et al. 2011; Shipley et al. 2004; Takacs and Abbott 2007; Vanden Heuvel et al. 2006; Wolf et al. 2008b, 2012). Cell systems used in these studies include COS-1 cells expressing mouse, rat, or human PPARα, and cultured rat, mouse, and human hepatocytes. In these studies, perfluoroalkyl sulfonate compounds were less potent than perfluoroalkyl carboxylate compounds in activating PPARα-induced gene expression, and the potency of stimulation within each class increased with carbon chain length (Bjork and Wallace 2009; Wolf et al. 2008b, 2012). In comparison with naturally occurring long-chain fatty acids such as linoleic and α linoleic acids, PFOA and PFOS are relatively weak ligands for PPARα (Vanden Heuvel et al. 2006)

PPARα-Dependent Gene Expression Changes. Perfluoroalkyls have been shown to induce changes in the expression of genes under the regulation of PPARα. The expression of PPARα target genes in the liver involved in fatty acid metabolism, cell cycle control, peroxisome biogenesis, and proteasome structure and organization were upregulated, while inflammatory response genes in the liver were downregulated in wild-type mice exposed orally to PFOA or the PPARα agonist WY-14,643 (Rosen et al. 2008a). Furthermore, PFOA and PFDA have been shown to downregulate, via PPARα activation, genes involved in bile transport in the livers of wild-type mice exposed by intraperitoneal administration (Cheng and Klaassen 2008a). Both compounds decreased expression of organic anion transporting polypeptides [*OATP1a1*, *1a4,* and *1b2*], and PFDA also downregulated sodium-taurocholate cotransporting polypeptide [*Nctp*], via activation of PPARα. Many of these expression changes may play roles in the hepatic effects of perfluoroalkyls.

Gene expression changes induced by perfluoroalkyls have been extensively studied in experiments aimed at determining the extent to which the adverse effects of these compounds are dependent on activation of PPARα or interaction with other nuclear receptors (Foreman et al. 2009; Rosen et al. 2008a, 2008b, 2010, 2017). These studies, comparing gene expression changes in wild-type and PPARα-null mice exposed to perfluoroalkyls, demonstrate the following:

- A majority of the gene expression changes induced in rodents by perfluoroalkyls tested to date, especially PFOA and PFNA, are dependent on activation of PPARα.

- Perfluoroalkyls also induce gene expression changes that are independent of PPARα.
- The extent to which gene expression changes induced by perfluoroalkyls are dependent on activation of PPARα varies by compound.

Species Differences in PPARα Activation. Species differences in response to PPARα activators have been reviewed by Corton et al. (2014). Studies of PPARα activation by structurally diverse ligands in various species have shown that rats and mice are the most sensitive species to PPARα agonists, whereas guinea pigs, hamsters, nonhuman primates, and humans are less responsive (Corton et al. 2014). However, the differences do not appear to result from differences in the PPARα gene itself: PPARα cDNA from humans is indistinguishable from the rodent PPARα. Species differences in response to exogenous PPARα activators may stem from any or all of the following: (1) differences in the expression of PPARα in a given tissue; (2) differences in the gene product structure; and (3) differences in the ligand-mediated transactivation of PPARα. Experiments quantifying mRNA and/or protein levels of PPARα show ~10-fold higher expression of PPARα in the livers of mice and rats compared with humans and guinea pigs, but available data are limited and require further study to validate these differences (Corton et al. 2014). In humans, variants of PPARα that may affect its transactivation potential have been identified. For example, humans produce higher levels of a truncated PPARα (that lacks a ligand binding domain) compared with mice and rats (Corton et al. 2014). The truncated form appears to inhibit the activity of the full-length receptor, possibly via sequestering critical co-activators. Other, non-truncated variants of PPARα have been identified in humans, but the sensitivity of these variants to PPARα activators does not differ markedly from that of the wild-type receptor.

Species and compound-related differences in PPARα transactivation by perfluoroalkyls have been demonstrated *in vitro* (Shipley et al. 2004; Takacs and Abbott 2007; Vanden Heuvel et al. 2006; Wolf et al. 2008b, 2012). In a comparison of human and mouse PPARα transactivation by different perfluoroalkyls in transfected COS-1 cells, Wolf et al. (2008b, 2012; see Table 2-28) found that some perfluoroalkyls exhibited marked species differences in transactivation potency (for example, PFUnA, PFDA, PFDoDA), while other compounds showed similar transactivation potency for both human and mouse PPARα (for example, PFNA, PFOA, perfluoropentanoic acid [PFPeA]).

Table 2-28. Transactivation of Human and Mouse PPARα in Transfected Cos-1 Cells Exposed to Perfluoroalkyls (In Order of Decreasing C_{20max} in the Mouse)

		Human		Mouse		C_{20max} (μM)[a]	
Perfluoroalkyl	Carbon number	NOEC (μM)	LOEC (μM)	NOEC (μM)	LOEC (μM)	Human	Mouse
PFNA	9	1	5	1	5	11	5
PFOA[b]	8	5, 0.5	10,1	0.5, 1	1, 3	7	6
PFUnA	11	50	75	5	10	86	8
PFHpA	7	<0.5	0.5	3	5	15	11
PFDA	10	100	>100[c]	<5	5	–	20
PFDoDA	12	75	90	3	5	NA	33
PFPeA	5	0.5	1	1	5	52	45
PFHxA	6	5	10	10	20	86	45
PFBA	4	30	40	30	40	75	51
PFHxS	6	5	10	10	20	81	76
PFOS	9	20	30	60	90	262	94
PFBS	4	20	30	120	150	206	317

[a]Perfluoroalkyl concentration yielding 20% of maximum response given by the most active compound (PFNA).
[b]Results from two separate experiments.
[c]Slope for human PPARα dose-response line was not significant.

– = not active; LOEC = lowest-observed-effect concentration; NA = not available; NOEC = no-observed-effect concentration; PFBA = perfluorobutanoic acid; PFBS = perfluorobutane sulfonic acid; PFDA = perfluorodecanoic acid; PFDA = perfluorodecanoic acid; PFDoDA = perfluorododecanoic acid; PFHpA = perfluoroheptanoic acid; PFHxA = perfluorohexanoic acid; PFHxS = perfluorohexane sulfonic acid; PFNA = perfluorononanoic acid; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid; PFPeA = perfluoropentanoic acid; PFUnA = perfluoroundecanoic acid; PPAR = peroxisome proliferator activated receptor

Source: Wolf et al. 2008b, 2012

In addition to differences in transactivation of PPARα, Corton et al. (2014) noted that there are species differences in the transcripts controlled by PPARα. While PPARα activation leads to hypolipidemic changes in both humans and laboratory rodents, the gene sets responsible for these changes may differ. In a comparison between human and mouse hepatocytes exposed to the prototypical PPARα ligand WY-14,643, some genes (ACOX1, ECH1, PEX11A, and ACAA1) were induced in both species, while some (*Ehhadh*, *Pxmp4*, *Acot4*, and *Peci*) were induced only in mouse hepatocytes (Corton et al. 2014). Importantly, PPARα activators induce large increases in the expression of fatty acyl-CoA oxidase (ACO, which is believed to play a role in oxidative stress-induced liver cancer) in rodent hepatocytes, but relatively weak increases in human hepatocytes (Corton et al. 2014). Other hypothesized explanations for the species difference in response to exogenous PPARα ligands include variations in the structure of the

PPRE that alter the response of the human genes compared with rodents; differences between humans and rodents in the functions of genes under the regulation of PPARα; and differences in the ability of ligand-bound human and mouse PPARα receptor complex to recruit or interact with co-activators (Corton et al. 2014).

PPARα-Independent or Associative Mechanisms

Experiments using PPARα-null mice have demonstrated that perfluoroalkyls exert some adverse effects, including developmental and hepatic effects, through mechanisms other than activation of PPARα. These may include activation of other nuclear receptors, increased oxidative stress, dysregulation of mitochondrial function, and inhibition of GJIC. While some of these effects have been seen after exposure to PPARα activators (Corton et al. 2014), these mechanisms may also occur independent of PPARα activation.

Activation of Other Nuclear Receptors. Examination of gene expression changes, as well as studies using other knock-out mice, have shown that some of the PPARα-independent effects induced by perfluoroalkyls may be mediated by activation of other nuclear receptors, especially PPARγ, CAR, and ERα. In a series of experiments, Rosen et al. (2008b, 2010, 2017) compared the gene expression changes induced by perfluoroalkyls in wild-type and PPARα -null mice with gene expression changes induced by known agonists of PPARγ, CAR, and ERα. Using these data, the study authors estimated the percentage of gene expression changes that were independent of activation of PPARα, and identified other nuclear receptors potentially involved in the changes induced by the perfluoroalkyls. The results, summarized in Table 2-29, show that between 10 and 24% of gene expression changes induced by perfluoroalkyls are independent of PPARα. All four compounds tested (PFOA, PFOS, PFNA, and PFHxS) were shown to alter the expression of PPARγ- and CAR-regulated genes in PPARα-null mice, and PFNA and PFHxS also altered the expression of ERα-regulated genes in the knock-out mice. In contrast, none of the compounds altered the expression of genes commonly affected by an agonist of LXR in either wild-type or null mice.

Table 2-29. Gene Expression Changes Induced by Perfluoroalkyls

	Dose (mg/kg/day for 7 days)	% PPARα-independent gene changes	Gene expression changes similar to those induced by prototypical agonist							
			PPARγ		CAR		ERα		LXR	
			WT	PPARα-null	WT	PPARα-null	WT	PPARα-null	WT	PPARα-null
PFOA	3	~14	+	+	+	+	+	–	–	–
PFOS	10	~16	+	+	+	+	+	–	–	–
PFNA	1	~10	+	+/–	+	–	+	–	–	–
	3	~17	+	+	+	+	+	+	–	–
PFHxS	3	24	+	–	+	–	+	+	–	–
	10	22	+	+	+	+	+	+	–	–
WY-14,643[a]	0.1% in diet	2	+	–	–	–	+	–	–	–

[a]WY-14,643 is a PPARα agonist.

+ = significant ($p<0.0001$) similarity to gene expression changes induced by prototypical receptor agonist as assessed by running Fisher test; +/– = equivocal evidence; CAR = constitutive androstane receptor; ER = estrogen receptor; LXR = liver X receptor; PFHxS = perfluorohexane sulfonic acid; PFNA = perfluorononanoic acid; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid; PPAR = peroxisome proliferator activated receptor; WT = wild-type

Sources: Rosen et al. 2017, 2008b, 2013; Wolf et al. 2008b

Gene expression changes typical of CAR and PXR activators (phenobarbital and pregnenolone 16 α-carbonitrile [PCN]) were also observed in rat liver after oral exposure to PFOA and PFOS (Ren et al. 2009). In addition, PFDA was shown to activate CAR-dependent genes in a study comparing wild-type and CAR-null mice exposed by intraperitoneal injection (Cheng and Klaassen 2008b).

These data suggest that perfluoroalkyls may induce gene expression changes through activation of other nuclear receptors including PPARγ, CAR, and ERα. Support for these findings are available from *in vitro* studies demonstrating binding and/or transactivation of PPARγ, CAR, and ERα by perfluoroalkyls. Both PFOA and PFOS activated PPARγ in cultured human, mouse, and rat hepatocytes, albeit with much lower potency than the known agonist rosiglitazone; neither LXRβ nor RXRα was activated in this system (Vanden Heuvel et al. 2006). Zhang et al. (2014) observed binding of PFOA and PFOS to human PPARγ in transfected *Escherichia coli*. However, in experiments conducted by Takacs and Abbott (2007), neither PFOA nor PFOS activated the mouse or human PPARγ.

Oxidative Stress. Perfluoroalkyls increase oxidative stress in the liver, kidney, and brain. Increases in oxidative stress may be mediated in part via PPARα activation, but may also result from activation of the Nrf2 receptor (Xu et al. 2016).

Oxidative stress may contribute to oxidative DNA damage, tumor promotion, perturbation of lipid homeostasis, and stimulation of inflammation, among other changes; thus, increases in oxidative stress can have diverse physiological effects. Evidence that perfluoroalkyls increase oxidative stress is available from *in vivo* and *in vitro* studies. For example, oxidative DNA damage (measured as 8-OH-dG levels) was significantly increased in the liver, but not the kidneys, of male rats exposed to PFOA via feed for 2 weeks (Takagi et al. 1991). In HepG2 cells cultured with PFOA or PFOS, significant increases in reactive oxygen species (ROS) (measured as 2'7'-dichlorofluorescein diacetate fluorescence) were observed, but there was no evidence of DNA damage measured with the comet assay (Eriksen et al. 2010). In this system, PFNA, PFBS, and PFHxA did not induce ROS production, but a significant increase in DNA damage was seen in cells exposed to PFNA (Eriksen et al. 2010).

In male, but not female, KM mouse pups administered a single subcutaneous injection of PFOS at 1, 2, 3, 4, or 5 weeks of age, brain total antioxidant capacity (T-AOC) was lower than controls at most time points, and significantly decreased after treatment on PND 21 (Liu et al. 2009). In the liver, T-AOC was decreased in male pups treated on PNDs 7 and 14, and in females treated on PND 21. Significant decreases in superoxide dismutase (SOD) activity were noted in the brain of males treated on PNDs 7 and 21, and in the liver of females treated on PND 14.

Increases in oxidative stress can lead to NFκB activation (Corton et al. 2014). NFκB activation plays a role in tumorigenesis, and NFκB transcription factors coordinate immune responses. Few studies have examined NFκB activation after exposure to perfluoroalkyls. An increase in NFκB mRNA level was seen in the hippocampus of neonatal rats exposed to PFOS *in utero* (Zeng et al. 2011). In addition, NFκB nuclear translocation was accelerated, and NFκB was activated, in breast cancer cells exposed to PFOA (Zhang et al. 2014). The activation of NFκB was associated with increased invasiveness of the breast cancer cells, as coexposure to an inhibitor of NFκB reduced the invasiveness induced by PFOA.

Gap Junction Intercellular Communication (GJIC) Inhibition. Perfluoroalkyls also have been shown to inhibit GJIC both *in vivo* and *in vitro* in rats (Corton et al. 2014). GJIC plays an important role in maintenance of tissue homeostasis, intercellular transmission of regulatory signals, and metabolic cooperation. Disruption of GJIC is thought to be involved in neurological, reproductive, and endocrine

abnormalities, as well as in carcinogenesis (Corton et al. 2014; EPA 2016h). There are limited data examining the effects of perfluoroalkyls on GJIC. The available studies showed that both PFOA and PFOS inhibited GJIC in the livers of rats exposed via diet for 1 week or 3 or 21 days, respectively (Hu et al. 2002; Upham et al. 1998, 2009). *In vitro* studies in WB-344 rat liver epithelial cells also showed inhibition of GJIC after exposure to PFOS (Hu et al. 2002) and to perfluorinated fatty acids with 7–10 carbons (Upham et al. 1998, 2009). In this system, PFOA activated extracellular receptor kinase, which may play a role in the inhibition of GJIC. In addition, inhibition of phosphatidylcholine-specific phospholipase C partially mitigated the GJIC inhibition, suggesting that PFOA-induced activation of this enzyme may also be involved in GJIC inhibition (Upham et al. 1998, 2009).

PFOS was also shown to inhibit GJIC in dolphin kidney epithelial cells and rat Sertoli cells *in vitro* (Hu et al. 2002; Wan et al. 2014a). In Sertoli cells, GJIC plays an important role in maintenance of the blood:testes barrier and in intercellular communication during spermatogenesis (EPA 2016i).

Impaired Mitochondrial Function. Mitochondrial function, including cellular respiration as well as mitochondrial membrane potential, has been shown to be perturbed by perfluoroalkyls. Available data suggest that PFOA and PFOS are relatively weak mitochondrial toxicants (EPA 2016h, 2016i). Mitochondrial proliferation was observed in rats exposed orally to PFOA for 28 days and in mice exposed to PFOA during gestation and lactation (Quist et al. 2015a, 2015b; Waters et al. 2009). In isolated rat liver mitochondria, higher concentrations of either PFOA or PFOS were noted to slightly increase resting respiration rate and decrease membrane potential, possibly due to these compounds' effects on membrane fluidity (Starkov and Wallace 2002). Testing of other perfluoroalkyls for effects on mitochondrial respiration rate and oxidative phosphorylation showed a wide range of inhibitory activities, with PFOS demonstrating the highest potency (3-fold higher than PFOA and 20–30-fold higher than PFBS and PFHxA) (Wallace et al. 2013).

2.20.2 Hepatic Toxicity Mechanisms

Hepatic effects of perfluoroalkyls in rodents likely result from a combination of PPARα-dependent and independent changes; see Table 2-30. For example, increased liver weight has been observed in both wild-type and PPARα-null mice orally exposed to PFOA or APFO (Nakagawa et al. 2012; Rosen et al. 2008a), PFOS (Qazi et al. 2009b; Rosen et al. 2010), PFNA (Das et al. 2017; Rosen et al. 2017), or PFHxS (Das et al. 2017; Rosen et al. 2017), but not in null mice exposed to PFBA by intraperitoneal injection (Foreman et al. 2009). Similarly, both wild-type and PPARα-null mice exposed to APFO

exhibited increased hepatocyte vacuolation and proliferation, while exposure to WY-14,643 did not induce such changes in the null mice (Wolf et al. 2008b). Das et al. (2017) showed that PFOA, PFNA, and PFHxS also increased hepatocyte cell size, percent lipid, and hepatic triglyceride levels, and decreased hepatic DNA content, in both wild-type and PPARα-null mice, while WY-14,643 did not, indicating that these effects were not dependent on PPARα activation. Similarly, Nakagawa et al. (2012) showed that at a lower APFO dose (1.0 mg/kg/day for 6 weeks), increases in hepatic triglyceride levels were observed in wild-type, PPARα-null, and humanized PPAR (hPPAR) mouse strains; however, at a higher dose (5 mg/kg/day), hepatic triglyceride levels were still increased in PPARα-null and hPPAR mice, but decreased in wild-type mice.

Table 2-30. Hepatic Effects of Perfluoroalkyls in Wild-Type and PPARα-Null Mice Exposed Orally

		↑ Relative liver weight		↑ % Lipid by cell area		↑ Hepatic triglycerides		↑ Hepatocyte cell size		↓ Hepatic DNA content	
	Dose (mg/kg/day)	WT	PPARα-null	WT	PPARα-null	WT	PPARα-null	WT	PPARα-null	WT	PPARα-null
PFOA	3	+++	+++	+++	-	+++	-	+++	+++	+	+
PFOS	10	++	++	ND							
PFNA	1	++	+	ND							
	3	+++	++								
	10	+++	+++	+++	+++	+++	+++	+++	++	+	+
PFHxS	3	+	+	ND							
	10	+++	+++	+++	+++	+++	–	+++	+++	+	+
WY-14,643[a]	50	+++	–	+++	–	–	–	+++	–	+	–

[a]WY-14,643 is a PPARα agonist.

+ = statistically significant change from control (the number of plus signs indicates degree of change from controls); – = not statistically significantly different from control; DNA = deoxyribonucleic acid; ND = no data; PFHxS = perfluorohexane sulfonic acid; PFNA = perfluorononanoic acid; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid; PPAR = peroxisome proliferator activated receptor; WT = wild type

Sources: Das et al. 2017; Rosen et al. 2008a, 2010, 2017

Lipid homeostasis is maintained through a balance between fatty acid synthesis or accumulation and fatty acid oxidation. Available data indicate that perfluoroalkyls affect both sides of this balance, but a growing body of evidence indicates that fatty acid accumulation induced by perfluoroalkyls tips the balance in favor of hepatic steatosis (Das et al. 2017). As discussed above, perfluoroalkyls alter lipid homeostasis via PPARα activation, which upregulates genes involved in fatty acid oxidation and reduces lipid levels. However, as noted above, Das et al. (2017) indicate that perfluoroalkyls also perturb lipid homeostasis via PPARα-independent mechanisms. In addition to the effects noted in Table 2-30, increased incidences of hepatic steatosis were seen in PPARα-null mice exposed to perfluoroalkyls (Das

et al. 2017; Minata et al. 2010; Nakagawa et al. 2012), but not in those exposed to the PPARα agonist WY-14,643 (Das et al. 2017). Additionally, microvesicular steatosis was observed in hPPAR mice (Nakagawa et al. 2012). The findings are consistent with earlier studies showing triglyceride accumulation in rodent livers after exposure to perfluoroalkyls (Kudo and Kawashima 1997, 2003; Kudo et al. 1999); hepatic steatosis and glucose intolerance in adult rats exposed to PFOS during the prenatal and postnatal periods (Lv et al. 2013); and inhibited hepatic secretion of VLDL, resulting in steatosis, in APOE3-Leiden mice (a rodent model with lipoprotein metabolism similar to humans) exposed to PFOS or PFHxS (Bijland et al. 2011).

Das et al. (2017) investigated whether the steatosis induced by PFOA, PFNA, and PFHxS was mediated by increased fatty acid or triglyceride synthesis or by inhibition of mitochondrial fatty acid transport or β-oxidation. Microarray analysis of mouse liver after exposure to these compounds showed upregulation of genes involved in fatty acid and triglyceride synthesis in both wild-type and PPARα-null mice. In contrast, *in vitro* experiments demonstrated that these perfluoroalkyls did not affect mitochondrial fatty acid oxidation in isolated rat liver mitochondria, and neither PFOA nor PFOS altered fatty acid oxidation in HepG2/C3A human liver cells. The authors suggested that perfluoroalkyls induce hepatic steatosis by perturbing lipid homeostasis in favor of the accumulation of fatty acids and triglycerides in the liver.

Data are also available to suggest that proinflammatory cytokines may also contribute to the hepatotoxicity of perfluoroalkyls. Studies in rodents have shown that *in vivo* exposure to PFOA (Qazi e al. 2013; Yang et al. 2014) or PFNA (Fang et al. 2012b, 2012c) have resulted in increases in IL-6, IL-1β, tumor necrosis factor-α (TNFα), C-reactive protein, and COX-2 at higher perfluoroalkyl doses (Fang et al. 2012b, 2012c; Yang et al. 2014) and decreases in TNFα, interferon-γ (IFN-γ), IL-4, and IL-6 levels at lower doses (Fang et al. 2012b; Qazi et al. 2013). Exposure to PFNA also resulted in increased expression of TNFα, IL-1β, and IL-6 mRNA (Fang et al. 2012b). Nakagawa et al. (2012) found increases in TNFα-mRNA in wild-type (2.9-fold), PPARα-null (1.9-fold), and humanized PPARα (1.9-fold) mouse strains exposed to 5 mg/kg/day doses of PFOA. Fang et al. (2012c) suggested that PFNA exposure stimulated liver Kupffer cells to release large amounts of TNFα and IL-1β and that the release of these cytokines activated the NFκB p65 pathway causing suppression of PPARα promoter activity and resulting in increases in liver triglyceride levels and steatosis.

2.20.3 Developmental Toxicity Mechanisms

Developmental effects observed in laboratory rodents exposed to perfluoroalkyls include prenatal loss, reduced neonate weight and viability, neurodevelopment toxicity, and delays in mammary gland differentiation, eye opening, vaginal opening, and first estrus (see Section 2.17 Developmental). During development, PPARα, PPARβ, and PPARγ mRNA and protein are expressed in the embryo of rodents and humans (Abbott 2009; Abbott et al. 2010). In humans, the fetal expression levels are equivalent to levels in adult tissues (Abbott et al. 2010). PPARα activation also appears to be involved in some, but not all, of the developmental effects of perfluoroalkyls in mice, and the role of PPARα in mediating developmental toxicity differs among the various compounds. For example, a gestational exposure study of PFOA resulted in decreases in postnatal survival in wild-type mice, but not in PPARα-null mice, while the occurrence of full-litter resorptions was similar between the two genotypes (Abbott 2009; Abbott et al. 2007). In contrast, gestational exposure to PFOS resulted in decreased pup survival in both wild-type and PPARα-null mice (Abbot et al. 2009). The developmental effects of PFNA, including reduced pup survival and body weight and delayed eye opening, were seen only in wild-type, and not in PPARα-null mice; however, maternal pregnancy rate was affected only in the null mice (Wolf et al. 2010). No alterations in postnatal survival or growth were observed in wild-type mice exposed to PFBA *in utero* (Das et al. (2008). The investigators suggested that the contrast of these findings to that of PFOA may be due to the shorter half-life of PFBA (daily administration did not result in reaching steady-state) and that PFBA is a less potent agonist of PPARα than PFOA.

Abbott et al. (2012) showed that PFOA altered expression of genes that are involved in homeostatic control of lipids and glucose, and postulated that decreased neonatal survival and body weights may be, in part, due to metabolic disruption. It has been suggested that PFOS interacts with pulmonary surfactants, and that this effect is responsible for neonatal mortality seen in rats. Grasty et al. (2003, 2005) showed that neonatal mortality in PFOS-exposed rats was highest when exposure occurred during the gestational period of lung maturation (GDs 17–20), and that the morphometry of the lungs in exposed neonates was consistent with immaturity. However, treatment of neonates with rescue agents that hasten lung maturation did not prevent neonatal mortality induced by PFOS, and examination of the pulmonary surfactant profile in exposed animals showed no difference from controls, leading Grasty et al. (2005) to conclude that neonatal mortality in neonatal rats exposed to PFOS was not due to immaturity. Other hypotheses pertaining to the mechanisms of developmental toxicity of perfluoroalkyls were not located. However, other molecular- and cellular-level effects of perfluoroalkyls, including increased oxidative

stress, dysregulation of mitochondrial function, and receptor-mediated events, may be involved in the observed developmental effects of these compounds.

2.20.4 Immunotoxicity Mechanisms

NTP (2016b) conducted a systematic review of the human, animal, and *in vitro* data examining immunotoxic effects of PFOA and PFOS. The conclusion of the systematic review was that both PFOA and PFOS are "presumed to be immune hazards to humans." Evidence was considered strong that both compounds were associated with suppression of the antibody response, while there was weaker evidence for PFOA-induced impairment of infectious disease resistance, increased hypersensitivity-related outcomes, and increased autoimmune disease incidence, and for PFOS-induced suppression of natural killer cell activity. A recent study comparing the T-cell dependent antibody response (TDAR) in female wild-type and PPARα knock-out mice after exposure to PFOA with or without antigen exposure showed that PFOA suppressed TDAR in both wild-type and knock-out mice, indicating that the mechanism for antibody response suppression is independent of PPARα activation (DeWitt et al. 2016). These investigators observed no treatment-related changes in splenic lymphocyte subpopulations in exposed mice of either genotype, suggesting that PFOA suppressed TDAR via impairment of B-cell/plasma cell function rather than by altering lymphocyte numbers. DeWitt et al. (2012) and Corsini et al. (2014) reviewed mechanistic data for perfluoroalkyl-induced suppression of antibody response, and postulated that perfluoroalkyls may modulate cell-signaling responses critical to antibody production, including c-Jun, NF-κB, and IL-6.

2.20.5 Endocrine Mechanisms

Perfluoroalkyls have been shown to induce alterations in thyroid hormone levels in rats, and associations between serum perfluoroalkyl concentrations and thyroid hormone levels have been reported in human epidemiological studies (see Section 2.13). Few data examining mechanisms of thyroid hormone disruption are available, but suggest that effects of perfluoroalkyls on thyroid function may be mediated by binding to the thyroid hormone receptor, and/or by altering expression of genes involved in thyroid function or thyroid hormone regulation. Several perfluoroalkyls were shown to bind to the human thyroid hormone receptor in cultured GH2 cancer cells and in molecular docking experiments (Ren et al. 2015). In the *in vitro* tests, all 16 of the tested compounds exhibited lower affinity for the receptor than T3 (Ren et al. 2015). Among the tested compounds, PFOS exhibited the strongest agonist activity (Ren et al. 2015). Alterations in the mRNA or protein levels of thyroid-regulating genes have been observed after oral exposure of male Sprague-Dawley rats to PFOS. PFOS exposure for 5 or 90 days resulted in

decreased hepatic levels of mRNA type 1 deiodinase (DIO1, which bioactivates T3 by deiodination of T4) (Martin et al. 2007; Yu et al. 2009a); after 5 days of exposure, hepatic mRNA for type 3 deiodinase (DIO3, which inactivates T3) was increased relative to controls (Martin et al. 2007). After 90 days, hepatic levels of uridine diphosphoglucuronosyl transferase 1A1 (UGT1A1, which plays a role in T4 turnover) mRNA and thyroid levels of DOI1 protein were increased, while there were no changes in thyroid levels of the sodium iodide symporter, thyrotropin (THS) receptor, or activity of thyroid peroxidase (Yu et al. 2009a).

Limited data from *in vitro* studies suggest the possibility that perfluoroalkyls may interact with the estrogen and androgen receptors. PFOA, PFOS, PFHxS, PFNA, and PFDA were all shown to be antagonists of the androgen receptor, while PFOA, PFOS, and PFHxS induced transactivation of the estrogen receptor (Kjeldsen and Bonefeld-Jorgensen 2013). Recently, analysis of gene expression data from the livers of wild-type and PPARα-null mice exposed to PFOA, PFOS, PFHxS, and PFNA by gavage for 7 days indicated similarities to gene expression changes induced by known ERα agonists (Rosen et al. 2017), providing indirect evidence for perfluoroalkyl changes in the liver mediated via ER activation. However, at oral doses up to 1 mg/kg, PFOA failed to induce treatment-related alterations in uterine weight, ER-dependent gene expression, or morphology of reproductive organs in uterotrophic assays using immature CD-1 mice (Dixon et al. 2012; Yao et al. 2014), suggesting that PFOA is either inactive *in vivo* or of very low estrogenic potency.

2.20.6 Cancer Mechanisms

PFOA induced hepatocellular adenomas, Leydig cell adenomas, and pancreatic acinar cell adenomas in rats (Biegel et al. 2001). Liver tumors induced by PFOA are believed to be mediated largely through PPARα activation, and considered to be of limited or no relevance to humans (EPA 2016h), based on species differences in response to PPARα (see details above under PPARα activation). An expert panel convened by EPA's Science Advisory Board in 2006 to review issues related to the toxicity of PFOA agreed that the weight of evidence supports the hypothesis that induction of liver tumors in rats by PFOA is mediated by a PPARα agonism mode of action (EPA 2006); this conclusion is also reflected in the EPA Health Effects Support Document for PFOA (EPA 2016h). A recent review by a panel of experts from academia, government, industry, and consulting groups updated the Klaunig et al. (2003) assessment of PPARα agonism as a liver cancer mode of action, and drew the same conclusion: while the PPARα mode of action for liver tumors is biologically plausible, species differences in response to PPARα activation indicate that liver tumors are unlikely to be induced by PPARα induction in humans (Corton et al. 2014).

Studies conducted in rainbow trout, an animal model that is similar to humans in terms of insensitivity to peroxisome proliferators, suggest that some perfluoroalkyls may induce liver cancer by alternate mechanisms (Benninghoff et al. 2011, 2012). The investigators (Benninghoff et al. 2011) found that PFOA, PFNA, PFDA, and PFUnA were potent inducers of vitellogenin, an estrogen-responsive biomarker protein at fairly high doses. Neither PFOA nor PFDA exposure resulted in vitellogenin expression at serum levels corresponding to general population serum levels of 2–7 ng/L. *In vitro*, PFOA, PFOS, PFHpA, PFNA, PFUnA, and PFDA also had weak to very weak affinities for estrogen receptors (ERα) for several species including humans, mice, and rats (Benninghoff et al. 2011). *In vivo* studies demonstrated that PFOA, PFOS, PFNA, and PFDA enhanced liver carcinogenesis in AFB_1 initiated fish via a mechanism that likely involves interactions with hepatic estrogen receptors (Benninghoff et al. 2012).

Although Leydig cell tumors are commonly induced by peroxisome proliferating agents such as perfluoroalkyls, the mode of action by which these tumors are induced, and thus their relevance to humans, is much less clear (Corton et al. 2014; EPA 2016h; Klaunig et al. 2003). One mode of action proposed for the induction of Leydig cell tumors involves PFOA-induced inhibition of testosterone biosynthesis, leading to increased production of gonadotropin releasing hormone and circulating LH, which promotes Leydig cell proliferation. Activation of PPARα may be involved in the decreased serum testosterone levels; PPARα-null mice did not exhibit the reduction in testosterone concentration seen in wild-type mice exposed to PFOA (Li et al. 2011). Evidence of decreased serum testosterone and increased serum estradiol was seen in studies of male rats exposed orally to PFOA for 14 days (Biegel et al. 1995; Cook et al. 1992; Liu et al. 1996). Reduced testosterone levels may occur through the conversion of testosterone to estradiol via the enzyme aromatase. Hepatic aromatase activity was shown to be markedly increased in male rats exposed to APFO by gavage for 14 days, and aromatase activity was positively correlated with serum estradiol levels in these animals (Liu et al. 1996). The relevance of Leydig cell tumors induced by PFOA to human risk assessment is uncertain. For example, an intermediate-duration study in Cynomolgus monkeys exposed to PFOA did not find treatment-related alterations in serum estradiol, estrone, estriol, or testosterone (Butenhoff et al. 2002). Studies of humans occupationally exposed to PFOA have not consistently reported alterations in estradiol or testosterone levels (Klaunig et al. 2012). In addition, humans are less sensitive than rats to LH stimulation, and the average number of LH receptors per Leydig cell is 13-fold higher in rats than humans (Klaunig et al. 2012). In summary, the induction of Leydig cell tumors by PFOA may be mediated by effects on aromatase activity or testosterone biosynthesis, both of which may be related to PPARα activation (EPA

2016h). While the relevance of the PPARα mode of action to humans is uncertain, the data supporting this mode of action for Leydig cell tumors is not sufficient to rule out human relevance (EPA 2016h).

The mechanism of PFOA-induced pancreatic acinar cell tumors in rodents has not been elucidated, and relevant data are limited. A proposed mode of action involves stimulation of PPARα leading to reduced bile flow and/or changes in bile acid composition with subsequent increase in cholecystokinin (CCK), which stimulates pancreatic cell proliferation and tumor formation (EPA 2016h). Effects on bile acid composition induced by PFOA may be mediated by effects on bile acid transporters. PFOA exposure has been shown to decrease expression of OATPs and increase expression of MRP3 and MRP4 (Cheng and Klassen 2008a; Maher et al. 2008). In a study using wild-type and PPARα-null mice, increased biliary excretion of PFOA was seen in wild-type mice compared with null mice, and biliary excretion of bile acids was highest in the null mice (Minata et al. 2010). These observations suggest the possibility that increased excretion of PFOA could diminish the excretion of bile acids that require the same transporters. However, given the limitations in available data, information is insufficient to fully characterize the mode of action for PFOA-induced pancreatic tumors (EPA 2016e).

Mechanisms of carcinogenicity of PFOA are unknown. Liver and Leydig cell tumors produced by PFOS may be associated with PPARα activation or may involve other mechanisms. PFOS activates PPARβ/δ, γ, and CAR and PXR (Ren et al. 2009).

2.21 GENOTOXICITY

The genotoxicity of perfluoroalkyls has not been extensively studied, with the most information available for PFOA and PFOS. To supplement the information reported in the published literature, results of unpublished studies taken from publicly available reviews have been included in the following discussions. No studies of genotoxicity in humans exposed to perfluoroalkyls were located.

PFOA

The genotoxicity of PFOA has been examined in bacterial and mammalian *in vitro* systems and in mammalian *in vivo* assays. In general, results show that PFOA can produce DNA damage, but is not mutagenic at noncytotoxic concentrations.

Results of *in vitro* studies in bacteria show that PFOA induces DNA damage but is not mutagenic. DNA damage was observed in *Paramecium caudatum* following exposure to 100 µM for 12 and 24 hours (Kawamoto et al. 2010). Intracellular ROS was significantly increased but DNA damage was not reversed by the application of glutathione, a ROS inhibitor, indicating that intracellular ROS may not be the cause of PFOA-induced DNA damage. PFOA was not mutagenic in *Salmonella typhimurium* TA1535/pSK1002 (*hisG46, rfa, uvrB*) with or without metabolic activation using the *umu* test (Oda et al. 2007) or in *S. typhimurium* TA98, TA100, TA102, and TA104 strains with or without metabolic activation using an Ames assay (Fernández Freire et al. 2008). Butenhoff et al. (2014) and Kennedy et al. (2004) summarized the results of various unpublished mutagenicity studies with PFOA showing negative results in reverse mutation assays using *S. typhimurium* (strains TA98, TA100, TA1535, TA1537, and TA1538), *Saccharomyces cerevisiae*, and *Escherichia coli* (WP2uvrA strain) with or without metabolic activation.

In vitro genotoxicity assays in mammalian cells show that PFOA induced DNA damage, although conflicting results have been reported for mutagenicity and increased micronuclei formation. Incubation of human hepatoma HepG2 cells with 50–400 µM PFOA caused DNA strand breaks and 100–400 µM increased the incidence of micronuclei, in a dose-related manner in both cases (Yao and Zhong 2005). These effects were accompanied by a significant increase in ROS, which the investigators suggested caused the DNA damage. Bjork and Wallace (2009) measured mRNA expression for DNA damage inducible *Ddit3* to assess DNA damage in primary rat and human hepatocyte cultures and in HepG2/C3a hepatoma cells. Significant increases in mRNA transcription for *Ddit3* were found in primary rat hepatocytes at 100 µM PFOA and in primary human hepatocytes and HepG2/C3a hepatoma cells at 200 µM PFOA. Although both studies provide evidence of DNA damage, the tested concentrations were very high as compared to what could be expected to occur in the environment. A significant increase in mutation frequencies was observed in hamster-human hybrid cells exposed to 200 µM PFOA for 1–16 days; a 79% decrease in cell viability was also observed at this concentration (Zhao et al. 2011). Concurrent treatment with a ROS inhibitor significantly decreased mutations, indicating that ROS may play an important role in mediating the genotoxic effects of PFOA. In contrast, Butenhoff et al. (2014) and Kennedy et al. (2004) summarized the results of various unpublished mutagenicity studies with PFOA. In mammalian cells, PFOA was negative for forward mutations using Chinese hamster ovary cells, for chromosomal aberrations in Chinese hamster ovary cells and human lymphocytes, and for cell transformation in C3H 10T1/2 cells.

Results of *in vivo* exposure of laboratory animals show that PFOA induced DNA damage, but not micronuclei formation. Administration of a single intraperitoneal injection of 100 mg/kg PFOA to male Fischer-344 rats resulted in a significant increase in the levels of 8-hydroxydeoxyguanosine (a marker of oxidative DNA damage) in liver DNA, but not in kidney DNA (Takagi et al. 1991). Oral administration of approximately 20 mg/kg/day PFOA in the diet for 2 weeks to male Fischer-344 rats induced hepatomegaly and increased the levels of 8-hydroxydeoxyguanosine in liver DNA but not in kidney DNA (Takagi et al. 1991). Unpublished studies summarized by Butenhoff et al. (2014) and Kennedy et al. (2004) did not find increased micronuclei formation in mice orally exposed to PFOA.

PFOS

The genotoxicity of PFOS has been examined in bacterial and mammalian *in vitro* systems and in mammalian *in vivo* assays. However, compared to PFOA, less information is available. Results do not provide evidence for genotoxicity of PFOS, except for one *in vitro* study showing cell transformation and one report of increased micronuclei formation following *in vivo* exposure.

Results of *in vitro* studies in bacteria and mammalian cells show that PFOS did not induce DNA damage, mutagenicity or chromosome damage. In bacterial cell assays, as reviewed by OECD (2002), PFOS did not induce reverse mutations in *S. typhimurium* or *E. coli* with or without metabolic activation. A study published after this review also found that PFOS was not mutagenic in *S. typhimurium* TA1535/pSK1002 (*hisG46, rfa, uvrB*) with or without metabolic activation using the *umu* test (Oda et al. 2007).

In vitro genotoxicity assays of PFOS in mammalian cells were negative for DNA damage, mutagenicity, micronuclei formation, and chromosome damage, although one *in vitro* study reported cell transformation. PFOS did not result in DNA damage in Syrian hamster embryo cells at concentrations up to 50 µg/mL but did induce cell transformation at noncytotoxic concentrations (0.2 and 2 µg/mL) following 5 and 24 hours of exposure (Jacquet et al. 2012). Similarly, PFOS did not induce DNA damage or increased micronuclei formation in human hepatoma HepG2 cells following a 24-hour exposure to PFOS concentrations as high as 600 µM; cytotoxicity was observed at ≥300 µM (Florentin et al. 2011). Another study of with HepG2 cells did not find evidence of DNA damage at concentrations of 100 and 400 µM PFOS (Eriksen et al. 2010). As summarized by OECD (2002), PFOS did not induce chromosomal aberrations in human lymphocytes with or without metabolic activation and did not induce unscheduled DNA synthesis in primary cultures of rat hepatocytes.

Conflicting results have been reported on micronuclei formation following *in vivo* exposure to PFOS. Micronuclei frequency was increased and the ratio of polychromatic erythrocytes to normochromatic erythrocytes was decreased in bone marrow of rats following oral exposure to 0.6–2.5 mg/kg PFOS for 30 days (Celik et al. 2013; Eke and Celik 2016). As summarized by OECD (2002), PFOS did not induce micronuclei in the bone marrow of CD-1 mice in an *in vivo* assay.

Other Perfluoroalkyls

Little information is available on the genotoxicity of other perfluoroalkyl compounds, with available studies focused on DNA damage. No DNA damage was found in HepG2 cells incubated with 100 or 400 µM PFHxS or PFBS for 24 hours, although a "modest" increase in DNA damage was observed at 400 µM PFNA, a cytotoxic concentration (Eriksen et al. 2010). Oral administration of approximately 10 mg/kg/day PFDA in the diet for 2 weeks to male Fischer-344 rats induced hepatomegaly and also increased the levels of 8-hydroxydeoxyguanosine in liver DNA but not in kidney DNA (Takagi et al. 1991). In contrast, no DNA damage in liver or kidney was observed following administration of a single intraperitoneal injection of 100 mg/kg PFBA to male Fischer-344 rats (Takagi et al. 1991).

CHAPTER 3. TOXICOKINETICS, SUSCEPTIBLE POPULATIONS, BIOMARKERS, CHEMICAL INTERACTIONS

3.1 TOXICOKINETICS

Toxicokinetic data on perfluoroalkyls examined in this profile are available from studies in humans and animals. Most studies in animals administered perfluoroalkyls by the oral route. These data are briefly summarized below.

- Absorption
  - Perfluoroalkyls are absorbed following oral, inhalation, and dermal exposure.
  - Quantitative estimates of the fractional absorption of orally administered perfluoroalkyls in animals range from >50% for PFHxS to >95% for PFOA, PFBA, PFNA, PFDA, PFUnA, and PFDoDA.
  - No quantitative estimates of the fractional absorption of perfluoroalkyls following inhalation or dermal exposure were identified.
- Distribution
  - Perfluoroalkyls are widely distributed in the body, with the highest concentrations in the liver, kidneys, and blood.
  - In the blood, perfluoroalkyls bind to albumin and other proteins.
  - Perfluoroalkyls can be transferred to the fetus during pregnancy and to nursing infants.
- Metabolism
  - Results of available oral and *in vitro* studies suggest that perfluoroalkyls are not metabolized and do not undergo chemical reactions in the body.
  - Although no studies examining metabolism of perfluoroalkyls following inhalation or dermal exposure were identified, metabolism by these exposure routes is not expected.
- Excretion
  - Studies of elimination rates (i.e., half-lives) of perfluoroalkyls show that elimination $t_{1/2}$ values are similar following intravenous, intraperitoneal, and oral exposures. Findings suggest that the route of absorption has no substantial effect on rates of elimination of absorbed perfluoroalkyls.
  - Perfluoroalkyls are primarily eliminated in the urine, with smaller amounts eliminated in feces and breast milk.

- Perfluoroalkyls undergo biliary excretion, but substantial reabsorption occurs; therefore, biliary excretion is not a major elimination pathway.
- Rates of elimination of perfluoroalkyls vary substantially across chemical species and animal species, and show sex differences and age-dependencies within certain species.
- In general, perfluoroalkyl sulfonates are eliminated more slowly than perfluoroalkyl carboxylates; elimination rate decreases with increasing chain length, and increases with increased branching.
- In humans, estimates for elimination $t_{1/2}$ range from hours (PFBA: 72–81 hours) to several years (PFOA: 2.1–8.5 years; PFOS: 3.1–7.4 years; PFHxS: 4.7–15.5 years).
- Evidence for sex differences in elimination of perfluoroalkyls in humans is not as strong as in rats. Menstruation may contribute to faster elimination of PFOS in younger women (≤50 years) when compared to men and older women.

3.1.1 Absorption

Inhalation Exposure. Studies of the absorption of perfluoroalkyls in humans following inhalation exposure were not located; elevated serum concentrations of perfluoroalkyls in workers in fluorochemical production industry have been reported (see Table 5-22), indicating that perfluoroalkyls are absorbed following inhalation exposure. Occupational exposures in these workers are likely to have included inhalation of aerosols of perfluoroalkyls complexed with airborne dusts. Higher serum levels in workers compared to the general population (see Table 5-20) probably reflect a predominant contribution from inhaled perfluoroalkyls.

Studies conducted in rodents provide direct evidence for absorption of inhaled perfluoroalkyls. PFOA was detected in plasma of rats within 30 minutes of initiating nose-only exposures to aerosols (mass median aerodynamic diameter [MMAD]=1.9–2.1 µm) of 1–25 mg ammonium PFOA/m^3. Plasma concentrations increased during the 6-hour exposure, with the highest concentrations observed at 9 hours (3 hours after cessation of exposure) in male rats and at 7 hours (1 hour after cessation of exposure) in females (Hinderliter et al. 2006a). Assuming an elimination $t_{1/2}$ of absorbed PFOA of approximately 160 hours in male rats, a peak plasma concentration at 9 hours would correspond to an absorption $t_{1/2}$ of approximately 1.3 hours (see discussion below, Equations 3-1 and 3-2). The earlier time of highest plasma concentration observed in female rats appears to be associated with faster elimination of absorbed PFOA in female rats, compared to male rats (see Section 3.1.4).

Nose-only exposure of male rats to dusts of ammonium perfluorononanoate induced significant increases in absolute and relative liver weight, assessed 5 and 12 days after exposure, providing indirect evidence of absorption of this compound through the respiratory airways (Kinney et al. 1989).

Oral Exposure. Studies of absorption of perfluoroalkyls through the gastrointestinal tract in humans are not available. A study of the general population of Europe and North America estimated that the greatest portion of the chronic exposure to PFOS and PFOA results from the intake of contaminated food, including drinking water (Trudel et al. 2008). Direct evidence of oral absorption of perfluoroalkyls was provided in studies that found associations between environmental levels (e.g., drinking water) and perfluoroalkyl concentrations in human serum (Emmett et al. 2006a; Hoffman et al. 2011; Hölzer et al. 2008; Seals et al. 2011; Wilhelm et al. 2008) and by reductions in serum levels after exposures from water were eliminated or reduced (Bartell et al. 2010; Emmett et al. 2009).

Animal data provide quantitative estimates of the fractional absorption of orally administered PFOA, PFOS, PFBA, PFHxA, PFHxS, PFHpA, PFNA, PFDA, PFUnA, and PFDoDA, with estimates ranging from >50% for PFHxS to >95% for PFOA, PFBA, PFNA, PFDA, PFUnA, and PFDoDA. Greater than 95% of an oral dose of ammonium [^{14}C]PFOA was absorbed in rats that received single gavage doses ranging from 0.1 to 25 mg/kg (Kemper 2003). In male and female mice, comparison of the 24-hour area under the curve (AUC) for oral and intravenous administration showed that 90–100% of the oral dose was absorbed for PFOA (females), PFNA (males and female), PFDA (males and females), PFUnA (males and females), and PFDoDA (males and female); however, absorption of PFOA in males was 80%, compared to 100% in females (Fujii et al. 2015a, 2015b). Gannon et al. (2011) estimated an absorption fraction of 99% based on 168-hour urinary excretion of ^{14}C in male and female rats and mice following single oral doses of 2 or 100 mg/kg ^{14}C-PFHxA. Based on comparison of the AUC for oral and intravenous administration, the estimated oral absorption fractions were 50% in female rats administered a single 10 mg/kg dose of potassium [$^{18}O_{3-}$]PFHxS (Sundström et al. 2012) and 79 and 55% in male and female rats administered a single dose of 4 mg/kg sodium [$^{18}O_{3-}$]PFHxS (Kim et al. 2016b). Sundström et al. (2012) stated that this estimate may not be reliable due to the short (24 hours) observation period. Based on 72-hour urinary excretion of ^{14}C, the estimated fractional absorption of a single dose (50 mg/kg) of ^{14}C-PFHxA was approximately 74% in male rats, 90% in female rats, and 80% in male and female mice (Iwai et al. 2011). A comparison of ^{14}C disposition in rats, mice, hamsters, and rabbits following an oral dose of 10 mg ammonium [^{14}C]PFOA/kg showed that similar fractions of the dose were absorbed (Hundley et al. 2006). The estimated absorbed fractions (i.e., ^{14}C in tissues, urine, and exhaled air measured 120–168 hours after the dose) in males were 89% in rats, 82% in mice, 92% in hamsters, and

88% in rabbits. Corresponding values for females were 76% in rats, 61%, in mice, 75% in hamsters, and 88% in rabbits. These estimates exclude ^{14}C excreted in feces, which may have been absorbed and secreted in bile before excretion (see Section 3.1.4). Fasting appears to increase absorption of PFOA. Plasma PFOA concentrations in rats, 24 hours following a gavage dose of 10 mg ammonium PFOA/kg, were 2–3 times higher when administered to fasted rats, compared to fed rats (Hinderliter et al. 2006b). The estimated absorption fractions of ingested ammonium [^{14}C]PFOA or potassium [^{14}C]PFOS (administered as a 4.2 mg/kg oral dose) were >93 and >95% in rats, respectively (Johnson and Ober 1979, 1999a). Based on combined urinary excretion and retention in the carcass (excluding the gastrointestinal tract and its contents), the estimated oral absorption fraction of [^{14}C]PFOS (administered as a single 4.2 mg/kg dose of potassium [^{14}C]PFOS) in male rats was >95% (Chang et al. 2012). The estimated absorption fraction of PFBA (administered as 30 mg/kg oral dose of PFBA) was >95% in rats (Chang et al. 2008a). Cumulative excretion of PFBA 24 hours after an oral dose (administered as 10, 30, or 100 mg/kg ammonium PFBA) was approximately 35% in urine and 4–11% in feces in male mice; and 65–69% in urine, and 5–7% in feces in female mice (Chang et al. 2008a).

Studies examining the rate of absorption of PFOA, PFHxA, PFBA, and PFBS show rapid absorption from the gastrointestinal tract, with values for absorption $t_{1/2}$ of <2 hours. For PFOA, the highest observed concentrations of ^{14}C in plasma occurred in male rats at approximately 10 hours (range 7.5–15 hours) following single oral doses ranging from 0.1 to 25 mg ammonium PFOA/kg (Kemper 2003). The elimination $t_{1/2}$ of ^{14}C in plasma estimated in these same animals was approximately 170 hours (range 138–202 hours), corresponding to an elimination rate constant (k_e) of 0.0044 hour^{-1} (range 0.004–0.005 hour^{-1}). The corresponding absorption $t_{1/2}$ of approximately 1.5 hours (k_a=0.45 hour^{-1}) can be calculated from these observations (Equations 3-1 and 3-2):

$$t_{\max} = \ln\frac{k_a}{k_e} \cdot \frac{1}{(k_a - k_e)} \qquad \text{Eq. (3-1)}$$

$$t_{1/2} = \frac{\ln(2)}{k_e} \qquad \text{Eq. (3-2)}$$

Where t_{max} = time of maximum concentration of ^{14}C; k_e = elimination rate constant; and k_a = absorption constant. The absorption rate of PFOA appears to be greater in female rats compared to male rats. The time to peak concentrations of ^{14}C in plasma occurred at approximately 1.1 hour (range 0.6–1.5 hours) in female rats and 10 hours (range 7–15 hours) in male rats following single oral doses ranging from 0.1 to 25 mg ammonium PFOA/kg (Kemper 2003). The elimination $t_{1/2}$ of ^{14}C in plasma estimated in these same animals varied with dose and ranged from 3.2 hours at the lowest dose (k_e=0.23 hour^{-1}) to 16.2 hours at the highest dose (k_e=0.059 hour^{-1}). The estimated absorption $t_{1/2}$ from the observations

made at all doses (0.1, 1, 5, and 25 mg/kg), based on Equations 3-1 and 3-2, was approximately 0.25 hours (range 0.12–0.38 hours). The absorption $t_{1/2}$ of PFBA in male and female rats following administration of a single oral dose (30 mg/kg ammonium PFBA) was 0.23 hours (3.04 $hour^{-1}$) in males and 0.17 hours (4.15 $hour^{-1}$) in females (Chang et al. 2008a). In male and female mice administered 10–30 mg/kg ammonium PFBA, the absorption $t_{1/2}$ was <1 hour, although the absorption rate may be dose-dependent in males, with higher absorption $t_{1/2}$ at doses >30 mg/kg (Chang et al. 2008a). Estimated t_{max} values following administration of single doses (2 or 100 mg/kg) of ^{14}C-PFHxA to rats and mice ranged from 0.3 to 0.8 hours (Gannon et al. 2011). Similar results for were reported by Olsen et al. (2009) based on estimated compartmental pharmacokinetic parameters for PFBS in serum of male and female rats following a single intravenous or gavage dose of 30 mg potassium PFBS. Plasma concentration-time profiles were fit to a two-compartment elimination model. The absorption $t_{1/2}$ can be approximated from these data using Equation 3-1, with the elimination rate constant represented by the fast-phase elimination rate constant estimated for either the oral or intravenous dose. Using the oral or intravenous parameters yielded similar values for the absorption $t_{1/2}$ (0.12–0.16 hours). The estimated t_{max} values following the gavage dose were 0.42 hours in males and 0.33 hours in females. The fast-phase elimination rate constants following the gavage dose were 0.892 $hours^{-1}$ ($t_{1/2}$=0.79 hours) in males and 1.308 $hours^{-1}$ ($t_{1/2}$=0.53 hours) in females. The corresponding values for absorption $t_{1/2}$ were 0.14 hours (k_a=5.0 $hours^{-1}$) in males and 0.12 hours (k_a=5.8 $hours^{-1}$) in females. Use of the fast-phase elimination rate constants estimated following intravenous administration (male: 1.143 $hours^{-1}$; female: 1.956 $hours^{-1}$) yielded values for the absorption $t_{1/2}$ of 0.16 hours in males (k_a=4.30 $hours^{-1}$) and females (k_a=4.45 $hours^{-1}$).

Mechanisms of oral absorption of perfluoroalkyls have not been elucidated.

Dermal Exposure. Dermal exposures of rats to ammonium PFOA have been shown to produce systemic (e.g., liver, immunotoxicity) toxicity in animals (see Chapter 2). Estimates of the amount or rates of dermal absorption in humans or animals have not been reported. PFOA was detected in serum of mice following dermal application of PFOA dissolved in acetone (Franko et al. 2012). The investigators noted PFOA ingestion may have occurred during grooming and may have contributed to the body burden. Dermal absorption of PFOS was assessed following application of single doses of potassium PFOS (doses up to 0.30 mg/kg) and the diethanolamine salt of PFOS (doses up to 20 µg/kg) to clipped, intact skin of rabbits (Johnson 1995a, 1995b). Analysis of the liver 28 days after application showed no increase in content of total organic fluoride compared to controls, indicating that dermal absorption was not detectable at low dose levels using this methodology. Dermal penetration of PFOA has been studied in preparations of isolated rat, mouse, and human epidermis (Fasano et al. 2005; Franko et al. 2012). These

studies indicate that the rat and mouse skin may be more permeable to PFOA than human skin. Approximately 24% of a dermal dose of PFOA (0.5 mg in 1% acetone) was absorbed across isolated full thickness human skin in 24 hours and 45% of the dose was retained in skin (Franko et al. 2012); it is noted that the acetone, as well as the glycerol used to pretreat the skin may have enhanced PFOA absorption. Permeability was sensitive to pH and was higher when the skin was buffered at pH 2.5 ($5.5x10^{-2}$ cm/hour) compared to pH 5.5 ($4.4x10^{-5}$ cm/hour), well above the pKa for the terminal carboxylic acid of PFOA (Franko et al. 2012). This suggests that permeability of the unionized acid is greater than that of the dissociated anion. Lower permeability of ionized PFOA is also suggested by relatively low permeability of the ammonium salt of PFOA in isolated preparations of rat and human skin. Following application of the ammonium salt of PFOA to isolated human or rat epidermis (150 µL/cm^2 of a 20% aqueous solution of ammonium PFOA; approximately 30 mg ammonium PFOA/cm^2), approximately 0.048% of the dose was absorbed across human epidermis and 1.44% was absorbed across rat epidermis in 40 hours. The estimated dermal penetration coefficients were $9.49x10^{-7}$ cm/hour in the isolated human epidermis and $3.25x10^{-5}$ cm/hour in the isolated rat epidermis.

The available data suggest that absorption of PFOA and PFOS through the skin is limited and is of minimal concern as an exposure route. No dermal absorption data were located for other perfluoroalkyls.

3.1.2 Distribution

Available information on the distribution of perfluoroalkyls is obtained from oral exposure studies in laboratory animals and occupational exposure studies in which exposure is predominantly by inhalation. Studies specifically examining the distribution of perfluoroalkyls by inhalation or dermal exposure were not identified. As discussed in Section 3.1.3 (Metabolism), perfluoroalkyls do not undergo metabolism. Therefore, distribution is expected to be the same regardless of the route of administration.

Distribution in Blood. In a study of 60 healthy Chinese participants from the general population, whole blood:plasma ratios for PFOS, PFOA, PFHxA, and PFHxS were 0.65, 0.83, 3.0, and 0.57 (Jin et al. 2016). These results indicate that PFHxA, but not PFOA, PFOS, or PFHxS, enters cellular components of blood. In a study of perfluoroalkyl workers, serum:plasma ratios for PFHxS, PFOS, and PFOA were 1:1, and this ratio was independent of the concentrations measured (Ehresman et al. 2007). The ratio of whole blood:plasma (or serum) was approximately one-half, which corresponded to volume displacement by red blood cells, suggesting that these perfluoroalkyls do not enter cellular components of blood. In studies conducted in animals, most of the PFOA in blood is in the plasma fraction. In rats, 24 or 48 hours

following an oral dose of 11.4 mg ammonium [^{14}C]PFOA/kg, the red blood cell:plasma PFOA concentration ratio ranged from 0.2 to 0.3, suggesting that there was no selective retention of PFOA by red blood cells (Johnson and Ober 1999a). Blood:plasma (or serum) ratios of approximately 0.5 have also been observed in rats following intravenous injection of PFOA (Kudo et al. 2007).

Perfluoroalkyls in plasma bind to serum albumin. The dissociation constant for binding of PFOA to serum albumin is approximately 0.4 mM (0.38 mM, ±0.04 standard deviation [SD] for human serum albumin; 0.36 mM, ±0.08 SD for rat serum albumin) and involves 6–9 binding sites (Han et al. 2003). Given a dissociation constant (K_D) of 0.4 mM and an albumin concentration of approximately 0.6 mM, >90% of PFOA in serum would be expected to be bound to albumin when the serum concentration of PFOA is <1 mM (<440 mg/L). This is consistent with observations of the bound fraction of perfluoroalkyls in plasma of rats that received a gavage dose of 25 mg PFOA/kg (Han et al. 2003, 2005; Ylinen and Auriola 1990), and in human, rat, and monkey plasma incubated *in vitro* with perfluoroalkyls (e.g., PFHxA, PFOA, PFOS, PFNA, PFDA) (Kerstner-Wood et al. 2003; Ohmori et al. 2003). Comparison of dissociation constants for binding of PFOA and PFOS to human serum albumin indicates that PFOS (K_D: $8x10^{-8}$) has a higher binding affinity than PFOA (K_D: $1x10^{-4}$) for albumin, consistent with the longer $t_{1/2}$ of PFOS versus PFOA in humans (Beesoon and Martin 2015; see Section 3.1.4 for additional information). PFOS has also been shown to bind to human hemoglobin *in vitro* (Wang et al. 2016). PFBS was found to bind only to albumin, whereas PFOS, PFOA, and PFHxS were found to have the potential to bind to other human serum binding proteins, including plasma gamma-globulin, alpha-globulin, alpha-2-macroglobulin, transferrin, and beta-lipoproteins (Kerstner-Wood et al. 2003).

Distribution to Extravascular Tissues. Absorbed perfluoroalkyls distribute from plasma to soft tissues, with the highest extravascular concentrations achieved in liver. An analysis of samples from human cadavers attempted to quantify PFOA, PFOS, FOSA, and PFHxA concentrations in serum and liver (Olsen et al. 2003c). The route of exposure was unknown. Mean serum PFOS concentration was 17.7 ng/mL (95% CI 13.0–22.5, range of <6.9 [limit of quantification] to 57 ng/mL, n=24) and was not different in males (18.2 ng/mL, n=13) and females (17.2 ng/mL, n=11). The mean liver concentration was 18.8 ng/g (95% CI 14.1–23.5; range <7.3–53.8 ng/g, n=30). The mean liver:serum concentration ratio was 1.3 (95% CI 0.9–1.7, n=23) and was not different in males (1.3, n=13) and females (1.3, n=10). Most liver and serum concentrations for PFOA, FOSA, and PFHxA were below the limit of quantification; these limits were <17.9–<35.9 ng/mL for PFOA, <7.5–<19.6 ng/g for FOSA, and <3.4–<18.5 ng/mL for PFHxA.

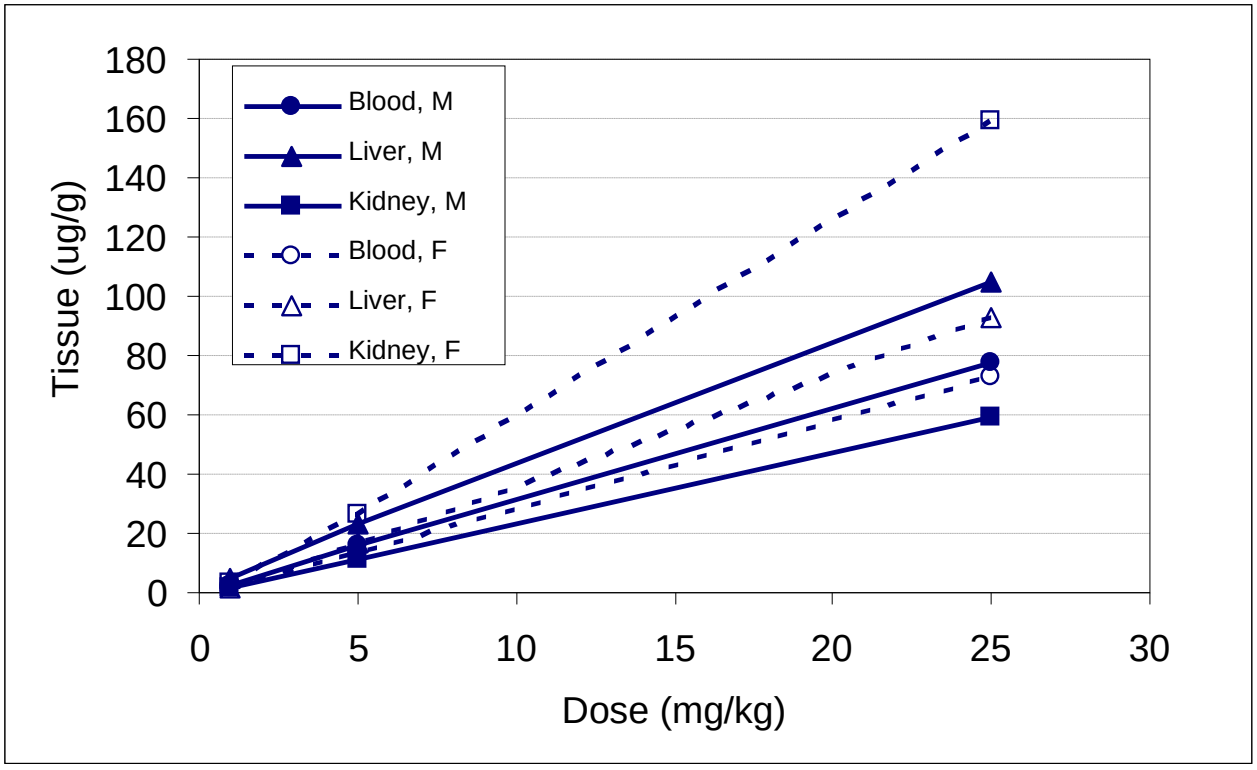
Studies conducted in nonhuman primates and rodents have provided additional information on the distribution of absorbed perfluoroalkyls to extravascular tissues. Distribution, as assessed from tissue perfluoroalkyl concentrations and tissue:serum ratios, exhibits profound species and sex differences as well as dose-dependencies (e.g., tissue levels that change disproportionately with dose). These differences have been attributed, in part, to species and sex differences in elimination kinetics of absorbed perfluoroalkyls and dose-dependence of elimination kinetics (see Section 3.1.4). In general, a consistent finding across species is that the liver receives a relatively high fraction of the absorbed dose and may also experience relatively high tissue concentrations compared with other tissues, with blood (i.e., plasma) and kidney also showing relatively high concentrations. The most extensive investigations of tissue distribution have been conducted in rodents.

Bogdanska et al. (2011) examined distribution of ^{35}S following dietary exposure to adult male C57/BL6 mice to low (environmentally relevant; 0.031 mg/kg/day) and high (experimentally relevant; 23 mg/kg/day) doses of [^{35}S]PFOS for 1–5 days. For both low and high doses after 1, 3, and 5 days of exposure, ^{35}S was distributed to the following tissues: blood, liver, lung, kidney, skin, whole bone, pancreas, spleen, thymus, heart, testes, epididymal fat, fat pads, brain, and muscle; ^{35}S was also detected in tissues throughout the gastrointestinal tract. Similar tissue:blood ratios were observed in both dose groups. In low-dose animals after 5 days of treatment, the highest tissue concentrations (excluding the gastrointestinal tract) were liver (tissue:blood=5.8), followed by lung (tissue:blood=1.4), whole bone, including marrow (tissue:blood=1.1), blood, and kidney (tissue:blood=0.94). In high-dose animals, the highest tissue concentrations were liver (tissue:blood=3.6), followed by lung (tissue:blood=1.6), blood, kidney (tissue:blood=0.81), and whole bone, including marrow (tissue:blood=0.72). A similar pattern of distribution was observed following intravenous administration of [^{14}C]potassium PFOS (4.2 mg/kg) to male rats (Johnson and Ober 1980). For both dose groups, the tissue:blood ratios for all other tissues were <1. In male and female CD-1 mice administered a single oral dose (4.2 mg/kg) of [^{14}C]PFOS, the highest concentrations of ^{14}C was observed in the liver, followed by serum, and then kidney, with similar tissue levels observed in males and females (Chang et al. 2012). In male and female rats fed diets containing 0, 2, 20, 50, or 100 mg/kg [^{13}C]sodium PFOS (equivalent to 0, 0.14, 1.33, 3.21, and 6.34 and 0, 0.15, 1.43, 3.73, and 7.58 mg/kg/day in males and females, respectively) for 28 days, PFOS levels were highest in liver, followed by spleen, heart, and serum. Liver:serum ratios for the 2, 20, 50, and 100 mg/kg/day diets were approximately 52, 42, 41, and 35, respectively, in males and 30, 47, 20, and 23, respectively, in females (Curran et al. 2008). Except for rats fed diets containing 20 mg/kg, the liver:serum ratio in males was higher than in females. No additional data were reported to determine if PFOS distribution differed between male and female rats.

Kemper (2003) determined the distribution of ^{14}C in male and female rats at the approximate time of maximum plasma concentration in both sexes, following single gavage doses of [^{14}C]PFOA (as ammonium PFOA, 0.1–25 mg/kg). This design allows a more direct comparison of patterns of tissue distribution in male and female rats at similar plasma concentrations, even though the elimination kinetics in the female rat are substantially faster than in male rats (see Section 3.1.4). The highest concentrations of ^{14}C were observed in blood, liver, and kidney (Figure 3-1). Liver, blood, and kidney accounted for approximately 22, 22, and 2% of the administered dose of 1 mg/kg in male rats; and 6, 7, and, 3% in female rats (the sex difference reflected more rapid excretory elimination in females). Although blood, liver, and kidney concentrations appeared to increase proportionately with increasing dose in male rats, in female rats, a disproportionately higher concentration in kidney was observed following the 25 mg/kg dose (Figure 3-1). Concentrations in other tissues ranged from 0.1 to 0.25 of that in liver or kidney; concentrations in bone and fat were <0.1 of that in liver or kidney. Profound sex differences and dose-dependencies in tissue concentrations of PFOA were also observed in rats that received oral doses of PFOA for 28 days at doses of 3, 10, or 30 mg PFOA/kg/day (Ylinen et al. 1990; Figure 3-2). Mean serum, kidney, or liver concentrations did not increase proportionally with dose in either sex. Kidney concentrations exhibited a disproportionate increase as the dose increased from 3 to 10 mg/kg/day, with little further increase at the 30 mg/kg/day dose. Sex differences in tissue distribution of PFOA in rats are not explained by sex differences in bioavailability since the differences persist in animals that received parenteral doses of PFOA (Johnson and Ober 1999b; Vanden Heuvel et al. 1991b, 1991c). The differences have been attributed to more rapid elimination of PFOA in female rats, compared to male rats (see Section 3.1.4).

A comparison of PFOA disposition in rats, mice, hamsters, and rabbits showed pronounced species and sex differences (Hundley et al. 2006; Table 3-1). In this study, rats, mice, hamsters, or rabbits received an oral dose of 10 mg ammonium [^{14}C]PFOA/kg and ^{14}C in tissues was measured at 120 or 168 hours (rabbits) hours post-dosing. In male rats, the highest concentrations of ^{14}C occurred in blood, liver and kidney, and all tissues combined accounted for approximately 60% of the dose. However, in female rats, concentrations of ^{14}C in all tissues were below limits of quantification. In mice, liver concentrations were similar in males and females, and liver showed the highest concentrations; ^{14}C levels in all tissues combined were lower in females compared to males. The opposite pattern was evident in hamsters and rabbits, with males having lower tissue levels than females, although, in common with rats and mice,

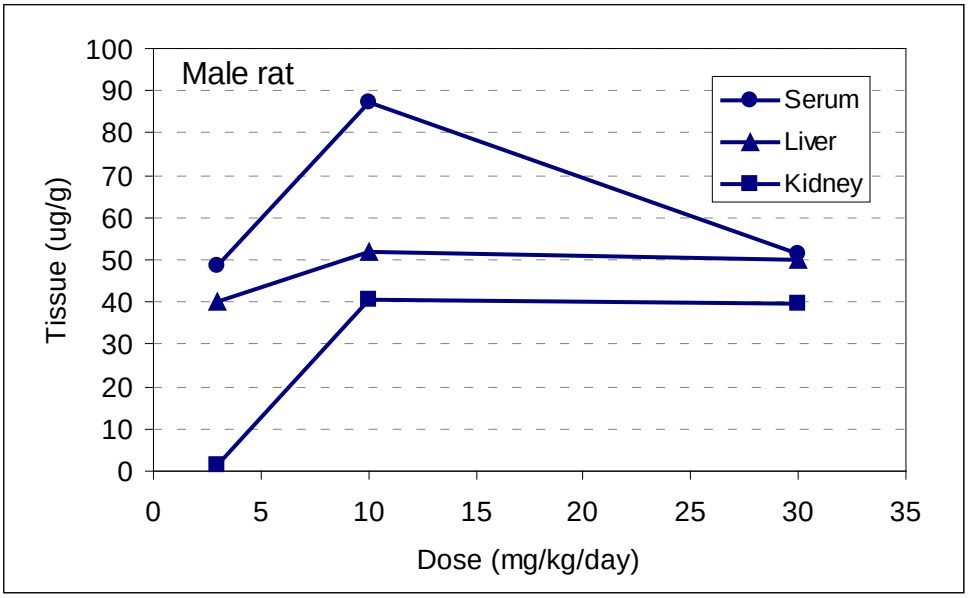
Figure 3-1. Tissue Concentrations of ^{14}C in Male and Female Rats Following a Single Gavage Dose of [^{14}C]PFOA at 1, 5, or 25 mg/kg*

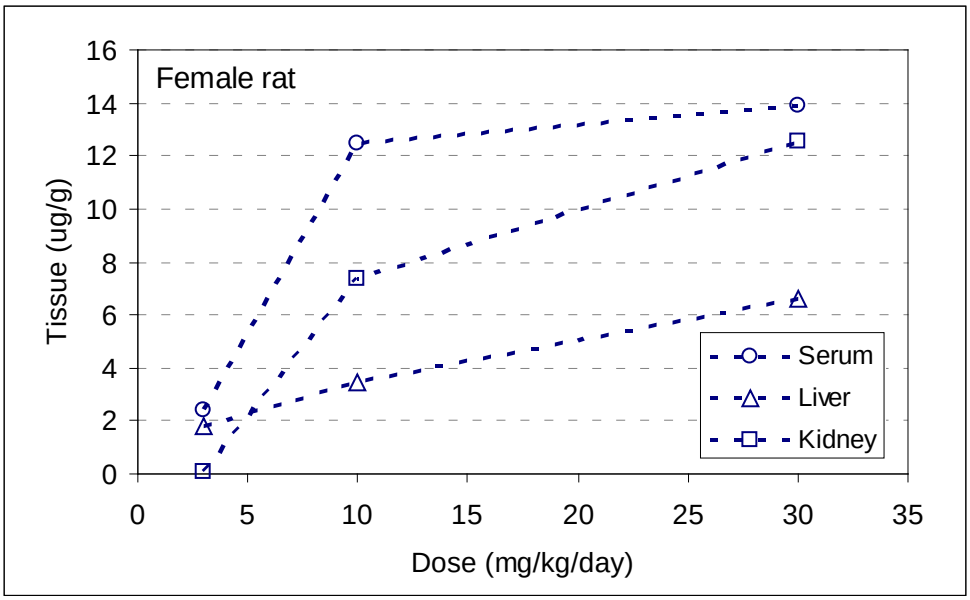


*Tissue levels are measured at time of maximum concentration in each tissue.

Source: Kemper 2003

Figure 3-2. Tissue Concentrations of ^{14}C in Male (Upper Panel) and Female (Lower Panel) Rats Following Oral Doses of PFOA for 28 Days at Doses of 3, 10, or 30 mg/kg/day





Source: Ylinen et al. 1990

Table 3-1. Tissue Distribution and Excretion of ^{14}C-Radioactivity from Both Sexes of Rats, Mice, Hamsters, and Rabbits Dosed with ^{14}C-Labeled APFO[a]

	µg Equivalent per g (mL) wet weight[b]							
	Rat		Mouse		Hamster		Rabbit	
Sample	Male	Female	Male	Female	Male	Female	Male	Female
Blood	23.5	<0.1	13.8	10.1	0.1	8.8	<0.1	0.1
Liver	40.0	<0.1	43.2	45.3	0.3	7.3	0.1	1.5
Kidneys	24.0	<0.1	2.9[c]	2.2[c]	0.2	7.1	0.1	0.4
Lungs	8.7	<0.1	1.4[c]	1.3[c]	<0.1	3.8	<0.1	0.1
Heart	6.4	<0.1	1.2[c]	0.6[c]	<0.1	2.9	<0.1	<0.1
Skin	4.8	<0.01	3.5	0.2	<0.1	3.4	<0.1	<0.1
Testes	3.2	–	0.9[c]	–	<0.1	–	<0.1	–
Muscle	1.9	<0.1	1.1	0.5	<0.1	0.9	<0.1	<0.1
Fat	1.7	<0.1	1.6	1.3	<0.1	1.5	<0.1	<0.1
Brain	0.6	<0.1	0.2[c]	0.8[c]	<0.1	0.3	<0.1	<0.1
	Percent of dose							
Tissues	59.6	0.6	73.6	50.0	0.7	26.5	<0.1	0.3
Urine	25.6	73.9	3.4	6.7	90.3	45.3	76.8	87.9
Feces	9.2	27.8	8.3	5.4	8.2	9.3	4.2	4.6
Expiration	3.6	1.5	5.2	4.4	1.3	2.9	No data	No data
Cage wash	0.6	0.8	4.9	4.9	0.6	2.1	0.5	4.8
Percent recovered	98.5	104.6	95.4	71.4	101.1	86.1	81.6	97.6

[a]The rabbits were sacrificed 168 hours after dosing; all other animals were sacrificed 120 hours after dosing.
[b]The µg equivalent calculations were based on the specific activity of ^{14}C-labeled APFO, which was 1.1×10^6 DPM/mg. The µg equivalent per g wet weight could not accurately be determined below 0.1 µg/g.
[c]Represents the µg equivalents for the entire organ.

APFO = ammonium perfluorooctanoate

Source: Hundley et al. 2006

blood, liver and kidney had the highest concentrations. Male rats that received a single oral dose of 5 mg FOSA/kg had liver FOSA concentrations that were 3–5 times higher than serum concentrations 1 day post-dosing (Seacat and Luebker 2000).

Sex differences in elimination that give rise to sex differences in tissue levels following oral exposure to perfluoroalkyls in rats are not evident in studies conducted with nonhuman primates. Rhesus monkeys that received 3 or 10 mg ammonium PFOA/kg/day for 90 days had liver concentrations of 48 µg/g (one male) or 50 µg/g (one female) at the low dose and 45 µg/g (one male) and 72 µg/g (one female) at the higher dose, with corresponding serum concentrations of 3 and 7 µg/mL, and 9 and 10 µg/mL, respectively (Griffith and Long 1980). Although limited to only one animal per sex, these results suggest

that liver levels did not increase proportionately with increasing dose. A similar observation was made in a study of male Cynomolgus monkeys (Butenhoff et al. 2004c). In male monkeys that received daily oral doses of 3 or 10 mg ammonium PFOA kg/day for 27 weeks, liver PFOA concentrations ranged from 11 to 18 µg/g at the low dose and from 6 to 22 µg/g at the higher dose. Mean serum concentrations measured after 6 weeks of exposure (which may have represented steady-state concentrations) were 77,000 ng/mL in the low-dose group and 86,000 ng/mL in the higher dose group. In this same study, an analysis of serum PFOA kinetics following an intravenous dose of PFOA revealed similar elimination kinetics in males and females (Butenhoff et al. 2004c; see Section 3.1.4). In Cynomolgus monkeys that received daily oral doses of PFOS (0, 0.03, 0.15, or 0.75 mg PFOS/kg/day) for 26 weeks, liver concentrations of PFOS and serum concentration were similar in males and females (liver:serum ratios ranged from 1 to 2) and increased in approximate proportion to the administered dose (Seacat et al. 2002).

Bogdanska et al. (2014) examined distribution of ^{35}S in 20 tissues following dietary exposure of adult male C57/BL6 mice to PFBS (16 mg/kg/day) for 1–5 days. ^{35}S was detected in all tissues and concentrations reached plateau levels after 3 days of exposure. After 5 days, tissue:blood ratios (excluding stomach and small intestine) were >1 for liver (tissue:blood=1.6), kidney (tissue:blood=1.3), whole bone (tissue:blood=1.1), and cartilage (tissue:blood=1.1). At all-time points, approximately 90% of the ingested ^{35}S was recovered in combined blood, liver, bone, skin, and muscle.

Iwabuchi et al. (2017) compared tissue distribution following single doses or 3-month dosing of PFOS (100 µg/kg), PFOA (100 µg/kg), PFHxA (100 µg/kg), and PFNA (50 µg/kg). Following administration of single doses, the tissue:serum (and/or whole blood) ratio was >1 for the liver for PFOS, PFOA, and PFNA, with tissue:serum ratios <1 for kidney, spleen, heart, and brain. For PFNA, the only tissue with a tissue:serum ratio >1 was kidney. After 3 months of exposure, tissue:serum ratios >1 were observed for the liver for PFOA and PFNA, and the liver and kidney for PFOS. For PFHxA, all tissue:serum ratios were <1. Similar to the single dose study, the lowest serum:tissue ratio for all compounds was observed for brain.

Subcellular Distribution. The subcellular distribution of perfluoroalkyls has been examined in rats (Han et al. 2004, 2005; Kudo et al. 2007; Vanden Heuvel et al. 1992b). Two hours following an oral dose of 25 mg ammonium [^{14}C]PFOA/kg, sex differences were noted in the subcellular distribution of ^{14}C in liver; females had approximately 50% of total ^{14}C in the cytosolic fraction compared to 26% in males (Han et al. 2005). The distributions to other cell fractions were: nuclear/cell debris fraction, 30% females, 40% males; lysosomes, 12% females, 14% males; mitochondria, 8% females, 16% males; and

ribosomes, <3% males and females. In kidney, 80 and 70% of the ^{14}C was associated with the cytosolic fraction in males and females, respectively, 16–22% in the nuclear/cell debris fraction, and the remainder in lysosome/mitochondria/ribosome fractions. In liver, approximately 55% of cytosolic ^{14}C was bound to proteins (>6,000 Da) in both males and females, whereas in kidney, 42% of the cytosolic fraction was bound to protein in males and 17% in females. The subcellular distribution of PFOA is dose-dependent. In rats, 2 hours following an intravenous dose of 0.041 mg [^{14}C]PFOA/kg, approximately 5% ^{14}C in the liver was associated with the cytosolic fraction, whereas approximately 45% was in the cytosolic fraction following a dose of 16.6 mg/kg (Kudo et al. 2007). A small component of tissue-associated PFOA and PFDA appeared to be bound covalently to protein. Following an intraperitoneal dose of 9.4 µmol/kg [^{14}C]PFDA or [^{14}C]PFOA (4.2 mg/kg), approximately 0.1–0.5% of liver ^{14}C was bound covalently (i.e., was not removed by repeated extraction with a methanol/ether and ethyl acetate; Vanden Heuvel et al. 1992b). Covalent binding was detected when cytosolic or microsomal fractions of rat liver were incubated *in vitro* with [^{14}C]PFDA (Vanden Heuvel et al. 1992b).

PFOA binds to rat kidney and urine α2u-globulin; dissociation constants were estimated to be approximately 1.5 and >2 mM (for a single binding site) for the proteins isolated from rat kidney and urine, respectively. These values suggest relatively low affinity for the protein, compared to other ligands that are known to induce hyaline droplet nephropathy (10^{-4}–10^{-7} M; Han et al. 2004).

Maternal-fetal Transfer. Perfluoroalkyls can be transferred to the fetus during pregnancy (Cariou et al. 2015; Chen et al. 2017a; Fei et al. 2007; Fisher et al. 2016; Fromme et al. 2010; Glynn et al. 2012; Gützkow et al. 2012; Hanssen et al. 2010, 2013; Inoue et al. 2004; Kato et al. 2014; Kim et al. 2011, Lee et al. 2013; Lien et al. 2013; Liu et al. 2011; Manzano-Salgado et al. 2015; Midasch et al. 2007; Monroy et al. 2008; Needham et al. 2011; Ode et al. 2013; Porpora et al. 2013; Yang et al. 2016a, 2016b). Studies that measured perfluoroalkyls in maternal and fetal cord blood of matched mother-infant pairs found relatively strong correlations (r>0.8) between maternal and fetal serum (or plasma); however, fetal/maternal serum ratios vary depending on the structure of the perfluoroalkyl (Table 3-2). With some exceptions, longer fluoroalkyl chain length and a terminal sulfonate group are associated with lower fetal/maternal ratios (Glynn et al. 2012; Gützkow et al. 2012; Hanssen et al. 2013; Kim et al. 2011; Liu et al. 2011; Needham et al. 2011). PFOS was detected in amniotic fluid obtained from amniocentesis (Jensen et al. 2012). The median concentration in amniotic fluid samples from 300 pregnancies (from the Danish amniotic fluid pregnancy-screening biobank) was 1.1 ng/mL.

Table 3-2. Serum (or Plasma) Concentrations in Matched Human Maternal-Infant Pairs

Study	Perfluoro-alkyl	Perfluoroalkyl chain length	N	Maternal (ng/mL)	Cord (ng/mL)	Ratio[a]	r
Glynn et al. 2012	PFOA	7	413	4	1	NR	0.89
	PFOS	8	413	29	5	NR	0.86
	PFNA	8	413	0.6	0.1	NR	0.53
Cariou et al. 2015	PFHxS	6	59	0.62	0.34	0.56	0.99
	PFOA	7	89	1.05	0.86	0.78	0.83
	PFOS	8	94	3.07	1.11	0.38	0.88
	PFNA	8	22	0.43	0.27	0.51	0.92
Chen et al. 2017a	PFHxS	6	32	0.53	0.33	0.62	ND
	PFOA	7	32	8.67	3.67	0.42	ND
	PFOS	8	32	1.56	1.24	0.79	ND
Fisher et al. 2016	PFHxS	6	315	NR	NR	0.23	NR
	PFOA	7	865	NR	NR	0.28	NR
	PFOS	8	648	NR	NR	0.14	NR
Fromme et al. 2010	PFHxS	6	53	0.60	0.30	0.50	0.89
	PFOA	7	53	2.60	1.70	0.65	0.94
	PFOS	8	53	3.50	1.10	0.31	0.89
	PFNA	8	53	0.60	<0.4	ND	ND
Gützkow et al. 2012	PFHxS	6	123	0.34	0.23	0.68	0.70
	PFOA	7	123	1.25	1.03	0.82	0.82
	PFOS	8	123	5.37	1.78	0.33	0.74
	PFNA	8	123	0.40	0.16	0.40	0.64
	PFDA	9	123	0.10	0.04	ND	ND
	PFUnA	10	123	0.19	0.06	0.32	0.67
Hanssen et al. 2013	PFHxS	6	7	0.26	0.17	0.65	ND
	PFOA	7	7	1.50	1.26	0.84	ND
	PFOS	8	7	10.70	3.93	0.37	ND
	PFNA	8	7	0.89	0.50	0.56	ND
	FOSA	8	7	0.41	0.45	1.10	ND
	PFUnA	10	7	0.33	0.16	0.48	ND
Han et al. 2018	PFBS	4	369	0.19	0.19	1.00	ND
	PFHxS	6	369	0.32	0.31	1.03	ND
	PFHpA	6	369	0.06	0.09	1.50	ND
	PFOA	7	369	42.83	34.67	0.81	ND
	PFOS	8	369	4.55	1.39	0.31	ND
	FOSA	8	369	0.13	0.13	1.00	ND
	PFNA	8	369	0.81	0.44	0.54	ND
	PFDA	9	369	0.55	0.21	0.38	ND
	PFUnA	10	369	0.47	0.17	0.36	ND
	PFDoDA	11	369	0.17	0.14	0.82	ND

Table 3-2. Serum (or Plasma) Concentrations in Matched Human Maternal-Infant Pairs

Study	Perfluoro-alkyl	Perfluoroalkyl chain length	N	Maternal (ng/mL)	Cord (ng/mL)	Ratio[a]	r
Inoue et al. 2004	PFOA	7	15	8.90	2.90	0.32	0.94
Kim et al. 2011	PFHxS	6	20	0.89	0.58	0.65	ND
	PFOA	7	20	1.60	1.10	0.69	ND
	PFOS	8	20	5.60	2.00	0.36	ND
	PFNA	8	20	0.79	0.37	0.47	ND
	PFDA	9	20	0.36	0.01	0.03	ND
	PFUnA	10	20	1.60	0.46	0.29	ND
Kato et al. 2014	PFHxS	6	78	1.20	0.60	0.50	0.89
	PFOA	7	78	3.30	3.10	0.89	0.88
	PFOS	8	78	8.50	3.50	0.31	0.82
	PFNA	8	78	0.66	0.41	0.62	0.79
	PFDA	9	78	0.20	ND	ND	ND
Lee et al. 2013	PFHS	6	70	1.35	0.67	0.57	ND
	PFOA	7	70	2.73	2.09	0.84	ND
	PFOS	8	70	10.77	3.44	0.35	ND
Liu et al. 2011	PFHxS	6	50	0.08	0.06	0.79	0.59
	PFOA	7	50	1.66	1.50	0.91	0.91
	PFOS	8	50	3.18	1.69	0.53	0.75
	PFNA	8	50	0.55	0.33	0.61	0.82
	PFDA	9	50	0.58	0.24	0.41	0.82
	PFUnA	10	50	0.56	0.30	0.53	0.70
	PFDoDA	11	50	0.08	ND	ND	ND
Manzano-Salgado et al. 2015	PFHxS	6	66	0.84	0.40	0.446	NR
	PFOA	7	66	2.97	1.90	0.746	NR
	PFOS	8	66	6.99	1.86	0.299	NR
	PFNA	8	66	0.85	0.32	0.4	NR
Midasch et al. 2007	PFOA	7	11	2.70	3.40	1.30	0.42
	PFOS	8	11	12.10	7.20	0.60	0.72
Monroy et al. 2008	PFHxS	6	101	4.05	5.05	1.25	ND
	PFOA	7	101	2.24	1.94	0.87	0.94
	PFOS	8	101	16.19	7.19	0.44	0.91
	PFNA	8	101	0.80	0.94	1.18	ND
Needham et al. 2011	PFHxS	6	12	12.30	9.10	0.74	0.05
	PFOA	7	12	4.20	3.10	0.72	0.91
	PFOS	8	12	19.70	6.60	0.34	0.82
	PFNA	8	12	0.76	0.37	0.50	0.84
	PFDA	9	12	0.34	0.10	0.29	0.91

Table 3-2. Serum (or Plasma) Concentrations in Matched Human Maternal-Infant Pairs

Study	Perfluoro-alkyl	Perfluoroalkyl chain length	N	Maternal (ng/mL)	Cord (ng/mL)	Ratio[a]	r
Ode et al. 2013	PFOA	7	263	2.30	2.80	1.30	0.74
	PFOS	8	263	17.00	7.40	0.45	0.76
	PFNA	8	263	0.31	0.26	0.93	0.51
Porpora et al. 2013	PFOA	7	38	2.90	1.60	0.55	0.70
	PFOS	8	38	3.20	1.40	0.44	0.72
Yang et al. 2016a	PFHxS	6	50	0.064	0.033	0.52	0.80
	PFOA	7	50	1.24	1.03	0.83	0.93
	PFOS	8	50	2.98	1.23	0.41	0.88
	PFNA	8	50	0.55	0.35	0.64	0.89
	PFDA	9	50	0.56	0.22	0.39	0.92
	PFUnA	10	50	0.55	0.23	0.42	0.88
	PFDoDA	11	50	0.085	0.058	0.68	0.76
Yang et al. 2016b	PFHxS	6	157	0.53	0.26	0.43	0.68
	PFOA	7	157	1.74	1.32	0.71	0.81
	PFOS	8	157	4.23	1.52	0.36	0.63
	PFNA	8	157	0.46	0.23	0.49	0.70
	PFDA	9	157	0.37	0.13	0.35	0.65
	PFUnA	10	157	0.38	0.14	0.36	0.63
	PFDoDA	11	157	0.040	0.026	0.61	0.52

[a]Ratio of cord:maternal perfluoroalkyl level.

FOSA = perfluorooctane sulfonamide; ND = no data (detected but below limit of quantification); NR = not reported; PFBS = perfluorobutane sulfonic acid; PFDA = perfluorodecanoic acid; PFDoDA = perfluorododecanoic acid; PFHxS = perfluorohexane sulfonic acid; PFHpA = Perfluoroheptanoic acid; PFNA = perfluorononanoic acid; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid; PFTrDA = perfluorotridecanoic acid; PFUnA = perfluoroundecanoic acid

Studies in rats and mice provide further support for maternal-fetal transfer of perfluoroalkyls. Following gavage administration of 0.1–10 mg/kg/day PFOS to rats during gestation, PFOS was distributed to fetal serum, liver, and brain, with fetal concentrations increasing with maternal dose (Chang et al. 2009; Lau et al. 2003; Luebker et al. 2005a, 2005b; Thibodeaux et al. 2003). Levels in fetal serum and liver generally were similar and higher than in brain. Studies did not report on concentrations of PFOS in other fetal tissues. Paired fetal-maternal levels of PFOS were examined in rats following exposure (gavage) to potassium PFOS at doses of 0.1, 0.4, 1.6, or 3.2 mg/kg/day on GDs 0–20 (Luebker et al. 2005b). On GD 21, fetal:maternal serum ratios were 2.1, 1.7, 1.6, and 1.1 at doses of 0.1, 0.4, 1.6, and 3.2 mg/kg/day, respectively; these results suggest that fetal:maternal serum ratios varied inversely with dose. Fetal:maternal liver ratios (0.37–0.44) were similar across the dose range. In mice administered a single

gavage dose of 12.5 mg/kg [^{35}S]PFOS on GD 16, fetal organ:maternal blood ratios of ^{35}S on GD 18 were 2.8 for kidneys, 2.6 for liver, 2.3 for blood, 2.1 for lungs, and 1.2 for brain (Borg et al. 2010).

Maternal-fetal transfer of PFOA has also been studied in rats and mice (Das et al. 2008; Hinderliter et al. 2005). In rats, PFOA concentrations in amniotic fluid, placenta, and fetus (measured on days 10, 15, or 21 of gestation) increased with increasing maternal oral dose (3, 10, or 30 mg/kg/day, administered daily beginning on GD 4) (Hinderliter et al. 2005). Fetal plasma concentrations of PFOA measured on GD 21 were approximately 40% of maternal plasma concentration. Following gavage administration of 0.01, 1, or 5 mg/kg ammonium PFOA on GD 17 in mice, PFOA was detected in amniotic fluid and pup serum, with dose-dependent increases (Fenton et al. 2009). On PND 1, pup serum PFOA concentrations were approximately 1.7–2.0-fold greater than levels in maternal serum.

Following administration of ammonium PFBA (35, 175, or 350 mg/kg) to pregnant mice on GDs 0–17, fetal serum and liver levels of PFBA were determined on PND 1 (Das et al. 2008). The fetal:maternal serum ratio of PFBA was approximately 0.15 and did not vary with maternal dose. Fetal liver:serum ratios were 0.44, 0.75, and 0.78 at maternal doses of 35, 175, and 350 mg/kg, respectively. PFHxS was detected in fetal blood and in the liver of neonates following exposure of dams to potassium PFHxS (0.3, 1, 3, and 10 mg/kg) throughout gestation (Butenhoff et al. 2009a); concentrations in serum and liver increased with dose.

Maternal-infant Transfer. Perfluoroalkyls can be transferred to nursing infants (Barbarossa et al. 2013; Cariou et al. 2015; Fromme et al. 2010; Kärrman et al. 2007; Kim et al. 2011; Kuklenyik et al. 2004; Liu et al. 2011; Tao et al. 2008a, 2008b). Studies that measured perfluoroalkyls in maternal serum (or plasma) and breast milk in matched mother-infant pairs found highly variable correlations (Table 3-3). Relatively high correlations have been reported for PFOA (Kärrman et al. 2007; Liu et al. 2011). Transfer to breast milk appears to be a significant route of elimination of perfluoroalkyls during breastfeeding. Comparisons of serum concentrations of women who did or did not breastfeed their infants showed that breastfeeding significantly decreases maternal serum concentrations of PFOA, PFOS, PFHxS, and PFNA (Bjermo et al. 2013; Brantsaeter et al. 2013; Mondal et al. 2012, 2014; von Ehrenstein et al. 2009). The decrease was estimated to be 2–3% decrease per month of breastfeeding (Brantsaeter et al. 2013; Mondal et al. 2012, 2014). Concentrations of perfluoroalkyls in breast milk also decrease with breastfeeding duration (Tao et al. 2008b; Thomsen et al. 2010). Numerous perfluoroalkyls (including PFOS, PFOA, PFBS, PFHxS, PFNA, PFDA, PFDoDA, PFUnA, and FOSA) have been detected in breast milk samples in women in China, Korea, Japan, Malaysia, Cambodia, India, Korea, Vietnam, Indonesia,

Norway, Philippines, Sweden, and the United States (Forns et al. 2015; Fujii et al. 2012; Kang et al. 2016; Kärrman et al. 2007; Kim et al. 2011; Liu et al. 2010, 2011; Mondal et al. 2014; So et al. 2006b; Tao et al. 2008a). The mean concentrations for perfluoroalkyls in breast milk collected from 45 women in Massachusetts were 0.131 ng/mL (range of <0.032.0–617 ng/mL) for PFOS, 0.043.8 ng/mL (<0.0301–0.161 ng/mL) for PFOA, and 0.0145 ng/mL (<0.0120–0.0638 ng/mL) for PFHxS (Tao et al. 2008b). PFHpA, PFDA, PFUnA, PFDoDA, and PFBS were also detected in the breast milk; however, ≤4 samples had levels that exceeded the limit of quantitation. Serum concentrations in breastfed infants can be higher than maternal levels. Although cord:maternal serum ratios of PFOA, PFOS, and PFNA at birth are typically lower than 1 (see Table 3-2), infant serum levels increase several-fold during the first 6 months after birth (Fromme et al. 2010; Mondal et al. 2014; Post et al. 2012; Verner 2016a, 2016b). This increase is likely because breast milk concentrations of perfluoroalkyls and fluid intake per infant body weight are highest during this time period. Fromme et al. (2010) also showed increases in serum levels of PFNA in infants fed formula made with contaminated drinking water. Mogensen et al. (2015b) reported that following weaning, significant (<0.0001) decreases were observed in infant serum concentrations of PFOS, PFOA, and PFHxS.

Table 3-3. Matched Serum (or Plasma) and Breast Milk Concentrations in Humans

Study	Perfluoroalkyl	Perfluoroalkyl chain length	N	Serum (ng/mL)	Milk (ng/mL)	Ratio[a]	r
Cariou et al. 2015	PFHxS	6	9	2.28	0.026	0.011	0.36
	PFOA	7	10	1.22	0.041	0.034	0.72
	PFOS	8	19	3.62	0.040	0.011	0.85
Kärrman et al. 2007a	PFHxS	6	12	4.7	0.085	0.020	ND
	PFOA	7	12	3.8	0.49	0.120	0.88
	PFOS	8	12	20.7	0.20	0.010	0.83
	FOSA	8	12	0.24	0.013	0.070	ND
	PFNA	8	12	0.80	0.017	0.010	ND
Kim et al. 2011	PFHxS	6	20	0.89	0.007	0.008	NS
	PFOA	7	20	1.60	0.041	0.026	NS
	PFOS	8	20	5.60	0.061	0.011	0.60
	PFNA	8	20	0.79	<0.0088	ND	ND
	PFDA	9	20	0.36	<0.018	ND	ND
	PFUnA	10	20	1.60	<0.024	ND	ND

Table 3-3. Matched Serum (or Plasma) and Breast Milk Concentrations in Humans

Study	Perfluoroalkyl	Perfluoroalkyl chain length	N	Serum (ng/mL)	Milk (ng/mL)	Ratio[a]	r
Liu et al. 2011	PFHxS	6	50	0.08	ND	ND	ND
	PFOA	7	50	1.66	0.181	0.109	0.77
	PFOS	8	50	3.18	0.056	0.018	0.57
	PFNA	8	50	0.55	0.026	0.048	0.62
	PFDA	9	50	0.58	0.02	0.034	0.54
	PFUnA	10	50	0.56	0.026	0.046	0.44
	PFDoDA	11	50	0.08	ND	ND	ND
	PFTrDA	12	50	0.08	ND	ND	ND

[a]Milk to serum ratio.

FOSA = perfluorooctane sulfonamide; ND = no data (detected but below limit of quantification); NS = not significantly correlated; PFDA = perfluorodecanoic acid; PFDoDA = perfluorododecanoic acid; PFHxS = perfluorohexane sulfonic acid; PFNA = perfluorononanoic acid; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid; PFTrDA = perfluorotridecanoic acid; PFUnA = perfluoroundecanoic acid

Studies conducted in rats and mice provide further support for maternal-infant transfer of perfluoroalkyls through breast milk (Fenton et al. 2009; Hinderliter et al. 2005; Lau et al. 2003; Luebker et al. 2005a; Yu et al. 2009b). PFOA concentrations in breast milk of nursing rats increased with increasing maternal oral dose (3, 10, or 30 mg/kg/day, administered daily beginning on GD 4) (Hinderliter et al. 2005). Milk concentrations of PFOA measured on postpartum days 3, 7, 14, or 21 in rats were approximately 0.1 of maternal plasma concentration. In dams exposed to 0.1, 1, or 5 mg/kg PFOA by gavage on GD 17, a dose-dependent increase in PFOA concentrations in breast milk was observed on PND 2, with breast milk:serum ratios of approximately 0.15, 0.38, and 0.25 at 0.1, 1, and 5 mg/kg doses, respectively; milk/serum concentration ratios for PFOA ranged from 0.15 to 0.56 (Fenton et al. 2009). Following lactational exposure of control rat pups to PFOS in breast milk of dams treated with dietary PFOS (3.2 mg/kg diet; approximately equivalent to 0.33 mg/kg/day), pup serum and liver concentrations increased throughout the 35-day lactation period (Yu et al. 2009b). At PND 35, the pup liver:serum PFOS ratios were 2.55 and 2.43 in male and female pups, respectively. Results of a cross-foster study show that pups are exposed to PFOS through breast milk (Luebker et al. 2005a). Postnatal toxicity observed in cross-fostered pups that nursed from exposed dams provides additional evidence of maternal-infant transfer of PFOS in rats and mice (see Section 2.17).

Mechanisms of Distribution. Perfluoroalkyls in plasma bind to serum albumin and various other plasma proteins including gamma-globulin, alpha-globulin, alpha-2-macroglobulin, transferrin, and beta-lipoproteins (Bischel et al. 2011; Butenhoff et al. 2012d; Chen and Guo 2009; Han et al. 2003, 2005; Kerstner-Wood et al. 2003; Luo et al. 2012; Ohmori et al. 2003; Salvalaglio et al. 2010; Vanden Heuvel et al. 1992b; Wu et al. 2009; Ylinen and Auriola 1990; Zhang et al. 2009). The dissociation constant for albumin-bound PFOA in serum is approximately 0.4 mM (0.38 mM, ±0.04 SD for human serum albumin; 0.36 nM, ±0.08 SD for rat serum albumin) and involves 6–9 biding sites (Han et al. 2003). Noncovalent binding appears to be at the same sites as fatty acids (Chen and Guo 2009). Interactions between PFOS and human serum albumin include interaction of PFOS polar sulfonyl groups with albumin hydrophilic sites and interaction of perfluorinated groups with albumin hydrophobic sites (Luo et al. 2012).

Absorbed perfluoroalkyls distribute from plasma to soft tissues, with the highest extravascular concentrations achieved in liver. Mechanisms by which perfluoroalkyls enter the liver have not been elucidated and may involve interactions with organic anion transporters that function in the distribution of fatty acids or other organic anions (Andersen et al. 2008). PFOA appears to be a substrate for organic anion transporters in the luminal and basolateral membranes of renal tubular epithelial cells, which facilitates entry of PFOA into renal tubular cells (Kudo et al. 2002; Nakagawa et al. 2008; Vanden Heuvel et al. 1992b; Weaver et al. 2010). The subcellular distribution of PFOA is sex- and dose-dependent in rats (Han et al. 2005; Kudo et al. 2007) and the association with the membrane fraction of liver cells decreases with increasing dose (Kudo et al. 2007), consistent with limited capacity of membrane proteins that bind PFOA (e.g., membrane transport proteins). Intracellular PFOA binds to proteins; protein complexes formed have not been fully characterized. PFOA exhibits a low affinity for binding to rat kidney and urine alpha-2μ-globulin (dissociation constants 1.5 and >2 mM, respectively) (Han et al. 2004).

3.1.3 Metabolism

Results of available intraperitoneal and *in vitro* studies suggest that the perfluoroalkyls discussed in this profile are not metabolized and do not undergo chemical reactions in the body. The absence of significant metabolism is attributed to the high stability and low reactivity of carbon-fluorine bonds in perfluoroalkyls. Studies conducted in male and female rats did not detect fluorine metabolites in the urine, plasma, or liver following a single injection of 4–150 mg/kg PFOA or 5–50 mg/kg PFDA (Goecke et al. 1992; Vanden Heuvel et al. 1991b, 1991c; Ylinen and Auriola 1990). Following a single intraperitoneal dose of approximately 4 mg/kg of ^{14}C-PFOA, only parent compound was excreted in the

urine and bile (Vanden Heuvel et al. 1991c). PFOA was not metabolized when incubated with microsomal fractions of human or rat intestine, kidney, or liver homogenates (Kemper and Nabb 2005). Although no studies examining metabolism of other perfluoroalkyls, including PFOS, following inhalation, oral, or dermal exposure were identified, metabolism by these exposure routes is not anticipated.

3.1.4 Excretion

As noted in Section 3.1.3 (Metabolism), there is presently no evidence that perfluoroalkyls undergo metabolism. The absence of significant metabolism is attributed to the high stability and low reactivity of carbon-fluorine bonds in perfluoroalkyls. Therefore, route-specific differences in excretion patterns are not expected. Selected studies in which elimination half-lives rates (i.e., $t_{1/2}$) of perfluoroalkyls have been determined (see summaries in Table 3-5) show that, in general, elimination $t_{1/2}$ values are similar following intravenous, intraperitoneal, and oral exposures. Findings suggest that the route of absorption has no substantial effect of rates of elimination of absorbed perfluoroalkyls (Butenhoff et al. 2004c; Chang et al. 2008a; Kemper 2003; Kudo et al. 2002; Ohmori et al. 2003; Vanden Heuvel et al. 1991b; Ylinen et al. 1990). As discussed in this section, perfluoroalkyls are primarily eliminated in the urine, with smaller amounts eliminated in the feces, breast milk (see Section 3.1.2; Distribution, Maternal-fetal Transfer), and menstrual fluid. Perfluoroalkyls undergo biliary excretion, but substantial reabsorption occurs; therefore, biliary excretion does not represent a major elimination pathway. Perfluoroalkyls do not appear to be eliminated in sweat, as induction of perspiration by exercise or sauna does not alter clearance of PFOA, PFOA, PFHxA, or PFNA (Genuis et al. 2013). The elimination of perfluoroalkyls in menstrual fluid appears to contribute to sex differences in serum elimination rates (Wong et al. 2014, 2015; Zhang et al. 2013). Only free (unbound) perfluoroalkyls are available for redistribution, excretion, and renal reabsorption; the interaction of perfluoroalkyls with proteins plays a critical role in bioaccumulation, and the tissue environment highly favors protein bonding.

In humans, absorbed perfluoroalkyls are excreted in urine. Estimates of renal clearance of PFOA and PFOS from serum in humans ranged from 0.8 to 3.3 mL/day for PFOA (serum concentration range: 5–16 ng/mL) and 0.1–1.5 mL/day for PFOS (serum concentration range 9–49 ng/mL). These clearance values were <0.001% of glomerular filtration rate (Harada et al. 2005a). Assuming that 99% of the serum PFOA and PFOS was bound to albumin (see Section 3.1.2), <0.1% of filtered perfluoroalkyls were excreted in urine, suggesting extensive reabsorption of filtered PFOA and PFOS in the renal tubule. Renal clearance was not different in males and females. Mean renal clearances for PFOA were

2.12 mL/day (±0.80 SD, n=5) in males and 1.15 (±0.33 SD, n=5) in five females (mean age 22 and 23 years, respectively). Mean renal clearances for PFOS were 0.66 mL/day (±0.48 SD, n=5) in males and 0.91 (±0.56 SD, n=5) for females. Fujii et al. (2015a) reported renal clearances (mL/day/kg; mean±SD) for several perfluoroalkyls in humans (three males and five females), including PFOA (0.044±0.01), PFNA (0.038±0.01), PFDA (0.015±0.01), PFUnA (0.005±0.00), and PFDoDA (0.005±0.00). Zhang et al. (2013) reported renal clearances for several perfluoroalkyls (mL/day/kg) and found that clearance of PFOS was similar in younger females (≤50 years, 0.050 mL/day/kg, 95% CI 0.037–0.064) and a combined group of males and older females (grouped together since there were no significant differences in serum concentrations) (0.037 mL/day/kg, 95% CI 0.026–0.049). However, there appeared to be differences in renal clearance for PFOA; clearance rates were 0.30 mL/day/kg (95% CI 0.11–0.49) in young females and 0.77 mL/day/kg (95% CI 0.47–1.1) in the combined older women and all males group. Urinary excretion of perfluoroalkyls may show sex and age differences (Zhang et al. 2015b). Urinary excretion of PFOA as a fraction of estimated intake in male adults (n=29) was 31% (p=0.002) higher than in nonpregnant female adults (n=25). In addition, urinary excretion of PFOS was inversely correlated with age (r=0.334; p=0.015).

Absorbed PFOA and PFOS are also secreted into bile in humans, but the biliary pathway is not a major excretory pathway because PFOA and PFOS are reabsorbed after biliary secretion. Estimates of total body clearance, serum-to-urine clearance, and serum-to-bile clearance of PFOA and PFOS in humans are presented in Table 3-4 (Harada et al. 2007). Biliary clearances of PFOA and PFOS were 1.06 and 2.98 mL/kg body weight/day, respectively, and greatly exceeded total body clearance (0.150 and 0.106 mL/kg/day) and urinary clearance (0.030 and 0.015 mL/kg/day). Based on these estimates, approximately 89% of the PFOA secreted into bile and 97% of secreted PFOS was estimated to have been reabsorbed from the gastrointestinal tract. Fujii et al. (2015a) also reported that biliary clearances of several perfluoroalkyls (PFOA, PFNA, PFDA, PFUnA, PFDoDA) were much higher than total body clearance in humans, further supporting that perfluoroalkyls excreted in bile undergo extensive reabsorption.

Table 3-4. Excretory Clearance of PFOA and PFOS in Humans

Parameter	Units	PFOA	PFOS
Serum $t_{1/2}$[a]	day	1,387	1,971
Total clearance[b]	mL/kg/day	0.150	0.106
Urinary clearance[c]	mL/kg/day	0.030	0.150

Table 3-4. Excretory Clearance of PFOA and PFOS in Humans

Parameter	Units	PFOA	PFOS
Biliary clearance[d]	mL/kg/day	1.06	2.98
Reabsorbed from bile[e]	%	89	97

[a]Estimates from Olsen et al. (2005).
[b]$\ln(t_{1/2})$xVd, where Vd is the volume of distribution (300 mL/kg).
[c]Estimates from Harada et al. (2005a).
[d]Estimates from Harada et al. (2007).
[e]1-(Total-Urinary)/Biliary.

PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid

Source: Harada et al. (2007)

Studies conducted in nonhuman primates and rodents provide further evidence that urine is the major route of excretion of perfluoroalkyls, accounting for >93% of absorbed PFOA and PFOS (Benskin et al. 2009; Butenhoff et al. 2004c; Chang et al. 2008a, 2012; Chengelis et al. 2009a; Hanhijarvi et al. 1982, 1987; Hundley et al. 2006; Johnson and Ober 1979, 1980, 1999a, 1999b; Kemper 2003; Kudo et al. 2001; Olsen et al. 2009; Sundström et al. 2012; Vanden Heuvel et al. 1991b, 1991c). Studies conducted in rats have shown that PFDA, PFNA, PFOA, and PFHxA are secreted in bile and undergo extensive reabsorption from the gastrointestinal tract (Kudo et al. 2001; Vanden Heuvel et al. 1991b, 1991c). PFOS, PFHxS, and PFBS are excreted in feces following intravenous dosing of rats, suggesting that these perfluoroalkyls may also be secreted into bile (Chang et al. 2012; Johnson et al. 1984; Olsen et al. 2009; Sundström et al. 2012). The percentage of the dose excreted in the feces appears to vary with compound, 8–13% for PFOS, <0.5% for PFHxS, and 0.13–0.36% for PFBS. Renal clearances of PFOA from plasma in rats were approximately 0.032 mL/minute/kg body weight in male rats and 0.73 mL/minute/kg in female rats; plasma concentrations of PFOA during these measurements ranged from approximately 0.8 to 80 µg/mL (Kudo et al. 2002). In the latter study, approximately >95% of plasma PFOA was bound to high molecular weight protein and the glomerular filtration rate was approximately 10 mL/minute/kg; therefore, urinary excretion of PFOA was approximately 6% of the rate of glomerular filtration of PFOA in males and 146% in females. These estimates indicate that net renal tubular reabsorption of filtered PFOA occurred in male rats, whereas net renal tubular secretion of PFOA occurred in female rats (i.e., clearance of free PFOA in plasma > glomerular filtration rate). The pronounced sex difference in renal clearance of PFOA has been attributed to modulation of renal excretory transport of PFOA by testosterone and estradiol (Kudo et al. 2002; Vanden Heuvel et al. 1992a; see Section 3.1.5).

Rates of elimination of perfluoroalkyls vary substantially across chemical species and animal species, and show sex differences and age-dependencies within certain species. Table 3-5 summarizes estimates of the elimination $t_{1/2}$ for perfluoroalkyls in humans and experimental animals. In compiling the estimates presented in Table 3-5, preference was given to the terminal $t_{1/2}$ when multiple $t_{1/2}$ values were reported. The significance of the terminal $t_{1/2}$ is that it determines the time required for complete elimination of the perfluoroalkyl as well as the exposure duration required to achieve a steady state. Most of the $t_{1/2}$ values in Table 3-5 were estimated from analyses of data on declining serum concentrations of perfluoroalkyls after a single dose or following cessation of a period of repeated dosing. Estimates of the terminal $t_{1/2}$ based on serum concentrations can vary with the length of the observation period following the last dose and with the modeling approach used to estimate the $t_{1/2}$. Longer observation times are required to estimate the slowest phases of elimination. As a result, estimates of $t_{1/2}$ based on observation periods of 1–2 days can be much shorter than estimates for the same perfluoroalkyl based on observation periods of several weeks. Direct comparisons of $t_{1/2}$ values should be made with consideration of whether or not the observation periods were comparable. Differences in estimation methodology can also contribute to differences in $t_{1/2}$ values. Values reported in Table 3-5 are based on fitting data to single or multi-compartment models, or noncompartmental modeling of the data. While the terminal $t_{1/2}$ provides a metric for comparing times required for complete elimination and steady state, it does not always provide a measure of how rapidly the perfluoroalkyl is cleared from the body. A more useful metric for this is the systemic clearance (Cl_S), typically estimated from the absorbed dose (AD) and the area under the serum concentration curve (AUC_S):

$$Cl_S = \frac{AD}{AUC_S} \qquad \text{Eq. (3-3)}$$

Equation 3-3 will provide an accurate estimate of systemic clearance following an oral dose if the oral dose is completely absorbed. Accurate estimation of AUC_S also depends on fitness of the underlying model used to predict serum concentrations. Estimates of systemic clearance based on pharmacokinetics analyses of serum data from animal studies are presented in Table 3-6.

Table 3-5. Summary Elimination Half-Lives for Perfluoroalkyls Estimated in Humans and Experimental Animals

Species, age, and sex	Route	Dose	Exposure duration[a]	Elimination half-life[b]	Reference
PFOA—Human					
Human (n=26), adult, M (n=24) F (n=2)	NA	NA	NA	3.8 years (95% CI 3.1–4.4, GM 3.5)	Olsen et al. 2007a
Human (n=20) 15–50 years, M	NA	NA	NA	2.8 years (95% CI 2.4–3.4)	Li et al. 2018
Human (n=30) 15–50 years, F	NA	NA	NA	2.4 years (95% CI 2.0–3.0)	Li et al. 2018
Human (n=66), >50 years, M, F	NA	NA	NA	2.6 years (SE 0.4, GM 1.2)	Zhang et al. 2013
Human (n=20), ≤50 years, F	NA	NA	NA	2.1 years (SE 0.3, GM 1.5)	Zhang et al. 2013
Human (n=45), M, F	NA	NA	NA	3.9 years	Worley et al. 2017a
Human (n=5), 22±0.9, M	NA	NA	NA	2.3 years	Harada et al. 2005a
Human (n=5), 68±5, M	NA	NA	NA	2.6 years	Harada et al. 2005a
Human (n=5), 23±3, F	NA	NA	NA	3.5 years	Harada et al. 2005a
Human (n=5), 69±5, F	NA	NA	NA	2.9 years	Harada et al. 2005a
Human (n=200) 54±15, M, F	Oral	NA	NA	2.3 years (95% CI 2.1–2.4)	Bartell et al. 2010
Human (n=643), adult, M, F	Oral	NA	NA	2.9 years (<4 years) (95% CI 2.3–3.8) 10.1 years (>4 years)	Seals et al. 2011
Human (n=1,029), adult, M, F	Oral	NA	NA	8.5 years (<9 years) (95% CI 7.1–10.1)	Seals et al. 2011
Humans (n=17), adult, M, F	Oral	NA	NA	5.1 years (SD 1.7, GM 4.8)	Costa et al. 2009
Humans (n=6) adults, F	Inhalation	NA	NA	2.5 (range 1.8–3.1)	Gomis et a. 2016
PFOS—Human					
Human (n=26), adult, M (24) F (2)	NA	NA	NA	5.4 years (95% CI 3.9–6.9, GM 4.8)	Olsen et al. 2007a
Human (n≅1,000), >12–>80 years, M	NA	NA	NA	4.7 years	Wong et al. 2014

Table 3-5. Summary Elimination Half-Lives for Perfluoroalkyls Estimated in Humans and Experimental Animals

Species, age, and sex	Route	Dose	Exposure duration[a]	Elimination half-life[b]	Reference
Human (n≅1,000), >12–>80 years, F	NA	NA	NA	4.3 years (95% CI 4.1–4.5)	Wong et al. 2015
Human (n=66), >50 years, M, F	NA	NA	NA	27 years (SE 3.1, GM 18)	Zhang et al. 2013
Human (n=20), ≤50 years, F	NA	NA	NA	6.2 years (SE 0.5, GM 5.8)	Zhang et al. 2013
Human (n=45), M, F	NA	NA	NA	3.3 years	Worley et al. 2017a
Human (n=20) 15–50 years M	NA	NA	NA	4.6 years (95% CI 3.7–6.1)	Li et al. 2018
Human (n=30) 15–50 years, F	NA	NA	NA	3.1 years (95% CI 2.7–3.7)	Li et al. 2018
Human (n=5), 22±0.9, M	NA	NA	NA	4.9 years	Harada et al. 2005a
Human (n=5), 68±5, M	NA	NA	NA	7.4 years	Harada et al. 2005a
Human (n=5), 23±3, F	NA	NA	NA	4.5 years	Harada et al. 2005a
Human (n=5), 69±5, F	NA	NA	NA	4.6 years	Harada et al. 2005a
PFHxS—Human					
Human (n=26), adult, M (24), F (2)	NA	NA	NA	8.5 years (95% CI 6.4–10.6, GM 7.3)	Olsen et al. 2007a
Human (n=20), ≤50 years, F	NA	NA	NA	7.7 years (SE 0.6, GM 7.1)	Zhang et al. 2013
Human (n=20) 15–50 years, M	NA	NA	NA	7.4 years (95% CI 6.0–9.7)	Li et al. 2018
Human (n=30) 15–50 years F	NA	NA	NA	4.7 years (95% CI 3.9–5.9)	Li et al. 2018
Human (n=45), M, F	NA	NA	NA	15.5 years	Worley et al. 2017a
Human (n=66), >50 years, M, F	NA	NA	NA	35 years (SE 3.9, GM 25)	Zhang et al. 2013
PFBA—Human					
Human (n=3), adult, M	NA	NA	NA	81 hours (SD 41)	Chang et al. 2008b
Human (n=9), adult, M (7), F (2)	NA	NA	NA	72 hours (SD 38)	Chang et al. 2008b
PFBS—Human					
Human (n=6), adult M (5), F(1)				665 hours (SD 266)	Olsen et al. 2009

Table 3-5. Summary Elimination Half-Lives for Perfluoroalkyls Estimated in Humans and Experimental Animals

Species, age, and sex	Route	Dose	Exposure duration[a]	Elimination half-life[b]	Reference
PFNA—Human					
Human (n=66), >50 years, M, F	NA	NA	NA	4.3 years (SE 0.5, GM 3.2)	Zhang et al. 2013
Human (n=20), ≤50 years, F	NA	NA	NA	2.5 years (SE 0.6, GM 1.7)	Zhang et al. 2013
PFDA—Human					
Human (n=66), >50 years, M, F	NA	NA	NA	12 years (SE 1.5, GM 7.1)	Zhang et al. 2013
Human (n=20), ≤50 years, F	NA	NA	NA	4.5 years (SE 0.4, GM 4.0)	Zhang et al. 2013
PFUnA—Human					
Human (n=66), >50 years, M, F	NA	NA	NA	12 years (SE 2.0, GM 7.4)	Zhang et al. 2013
Human (n=20), ≤50 years, F	NA	NA	NA	4.5 years (SE 0.5, GM 4.0)	Zhang et al. 2013
PFHpA—Human					
Human (n=66), >50 years, M, F	NA	NA	NA	1.2 years (SE 0.2, GM 0.82)	Zhang et al. 2013
Human (n=20), ≤50 years, F	NA	NA	NA	1.5 years (SE 0.3, GM 1.0)	Zhang et al. 2013
PFOA—Nonhuman primate					
Cynomolgus monkey, adult, M	Oral	10 mg/kg/day	6 months	20.1 days	Butenhoff et al. 2004c
Cynomolgus monkey, adult, M	IV	10 mg/kg	1 day	20.9 days (SD 12.5)	Butenhoff et al. 2004c
Cynomolgus monkey, adult, F	IV	10 mg/kg	1 day	32.6 days (SD 8.0)	Butenhoff et al. 2004c
PFOS—Nonhuman primate					
Cynomolgus monkey, adult, M	Oral	0.15 mg/kg/day	6 months	170 days	Seacat et al. 2002
Cynomolgus monkey, adult, M	Oral	0.75 mg/kg/day	6 months	170 days	Seacat et al. 2002
Cynomolgus monkey, adult, F	Oral	0.15 mg/kg/day	6 months	170 days	Seacat et al. 2002
Cynomolgus monkey, adult, F	Oral	0.75 mg/kg/day	6 months	170 days	Seacat et al. 2002
Cynomolgus monkey, adult, M	IV	2 mg/kg	1 day	132 days (SE 7)	Chang et al. 2012
Cynomolgus monkey, adult, F	IV	2 mg/kg	1 day	110 days (SE 15)	Chang et al. 2012

Table 3-5. Summary Elimination Half-Lives for Perfluoroalkyls Estimated in Humans and Experimental Animals

Species, age, and sex	Route	Dose	Exposure duration[a]	Elimination half-life[b]	Reference
PFHxA—Nonhuman primate					
Cynomolgus monkey, adult, M	IV	10 mg/kg	1 day	5.3 days (SD 2.5)	Chengelis et al. 2009a
Cynomolgus monkey, adult, F	IV	10 mg/kg	1 day	2.4 days (SD 1.7)	Chengelis et al. 2009a
PFHxS—Nonhuman primate					
Cynomolgus monkey, adult, M	IV	10 mg/kg	1 day	141 days (SE 30.)	Sundström et al. 2012
Cynomolgus monkey, adult, F	IV	10 mg/kg	1 day	87 days (SE 27)	Sundström et al. 2012
PFBA—Nonhuman primate					
Cynomolgus monkey, adult, M	IV	10 mg/kg	1 day	40.3 hours (SD 2.4)	Chang et al. 2008b
Cynomolgus monkey, adult, F	IV	10 mg/kg	1 day	41.0 hours (SD 4.7)	Chang et al. 2008b
PFBS—Nonhuman primate					
Cynomolgus monkey, adult, M	IV	10 mg/kg	1 day	15.0 hours (SD 9.8)	Chengelis et al. 2009a
Cynomolgus monkey, adult, F	IV	10 mg/kg	1 day	8.0 hours (SD 2.0)	Chengelis et al. 2009a
Cynomolgus monkey, adult, M	IV	10 mg/kg	1 day	95.2 hours (SE 27.1)	Olsen et al. 2009
Cynomolgus monkey, adult, F	IV	10 mg/kg	1 day	83.2 hours (SE 41.9)	Olsen et al. 2009
PFOA—Rat					
Rat (CR), adult, M	Oral	11.4 mg/kg	1 day	115 hours	Johnson and Ober 1980
Rat (Sprague-Dawley), adult, M	Oral	0.1 mg/kg	1 day	202 hours (SD 38)	Kemper 2003
Rat (Sprague-Dawley), adult, M	Oral	1 mg/kg	1 day	138 hours (SD 32)	Kemper 2003
Rat (Sprague-Dawley), adult, M	Oral	1 mg/kg	1 day	44 hours	Kim et al. 2016b
Rat (Sprague-Dawley), adult, M	Oral	5 mg/kg	1 day	174 hours (SD 29)	Kemper 2003
Rat (Sprague-Dawley), adult, M	Oral	25 mg/kg	1 day	157 hours (SD 38)	Kemper 2003
Rat (Sprague-Dawley), adult, M	IV	1 mg/kg	1 day	185 hours (SD 19)	Kemper 2003
Rat (Sprague-Dawley), adult, M	IV	1 mg/kg	1 day	39 hours	Kim et al. 2016b
Rat (Sprague-Dawley), adult, M	Oral	0.4 mg/kg	1 day	322 hours (SD 38)	Benskin et al. 2009

Table 3-5. Summary Elimination Half-Lives for Perfluoroalkyls Estimated in Humans and Experimental Animals

Species, age, and sex	Route	Dose	Exposure duration[a]	Elimination half-life[b]	Reference
Rat (Sprague-Dawley), adult, M	Oral	0.022 mg/kg/day	12 weeks	218 hours (95% CL 127–792)	De Silva et al. 2009
Rat (Wistar), adult, M	IV	21.5 mg/kg	1 day	136 hours (SD 24)	Kudo et al. 2002
Rat (Wistar), adult, M	IV	20.1 mg/kg	1 day	135 hours (SD 29)	Ohmori et al. 2003
Rat (Sprague-Dawley), adult, M	IP	3.9 mg/kg	1 day	216 hours (SE 30.9)	Vanden Heuvel et al. 1991c
Rat (Wistar), adult, M	IP	50 mg/kg	1 day	105 hours	Ylinen et al. 1990
Rat (Sprague-Dawley), adult, F	Oral	0.1 mg/kg	1 day	3.2 hours (SD 0.9)	Kemper 2003
Rat (Sprague-Dawley), adult, F	Oral	1 mg/kg	1 day	3.5 hours (SD 1.1)	Kemper 2003
Rat (Sprague-Dawley), adult, F	Oral	1 mg/kg	1 day	3.6 hours	Kim et al. 2016b
Rat (Sprague-Dawley), adult, F	Oral	5 mg/kg	1 day	4.6 hours (SD 0.6)	Kemper 2003
Rat (Sprague-Dawley), adult, F	Oral	25 mg/kg	1 day	16.2 hours (SD 9.9)	Kemper 2003
Rat (Sprague-Dawley), adult, F	IV	1 mg/kg	1 day	2.8 hours (SD 0.5)	Kemper 2003
Rat (Sprague-Dawley), adult, F	IV	1 mg/kg	1 day	4.6 hours	Kim et al. 2016b
Rat (Wistar), adult, F	IV	21.5 mg/kg	1 day	1.9 hours (SD 0.7)	Kudo et al. 2002
Rat (Wistar), adult, F	IV	20.1 mg/kg	1 day	1.9 hours (SD 0.7)	Ohmori et al. 2003
Rat (Sprague-Dawley), adult, F	IP	3.9 mg/kg	1 day	2.9 hours (SE 0.2)	Vanden Heuvel et al. 1991c
Rat (Wistar), adult, F	IP	50 mg/kg	1 day	24 hours	Ylinen et al. 1990
PFOS—Rat					
Rat (Sprague-Dawley), adult, M	Oral	4.2 mg/kg	1 day	179 hours	Johnson and Ober 1979
Rat (Sprague-Dawley), adult, M	Oral	0.27 mg/kg	1 day	809 hours	Benskin et al. 2009
Rat (Sprague-Dawley), adult, M	Oral	0.023 mg/kg/day	12 weeks	1,968 hours (95% CL 1.584–2.568)	De Silva et al. 2009
Rat (Sprague-Dawley), adult, M	Oral	2 mg/kg	1 day	1,495 hours (SE 50)	Chang et al. 2012
Rat (Sprague-Dawley), adult, M	Oral	2 mg/kg	1 day	635 hours	Kim et al. 2016b
Rat (Sprague-Dawley), adult, M	Oral	15 mg/kg	1 day	1,707 hours (SE 270)	Chang et al. 2012

Table 3-5. Summary Elimination Half-Lives for Perfluoroalkyls Estimated in Humans and Experimental Animals

Species, age, and sex	Route	Dose	Exposure duration[a]	Elimination half-life[b]	Reference
Rat (Sprague-Dawley), adult, F	Oral	0.023 mg/kg/day	12 weeks	1,992 hours (95% CL 1,752–2,280)	De Silva et al. 2009
Rat (Sprague-Dawley), adult, F	Oral	2 mg/kg	1 day	919 hours (SE 56)	Chang et al. 2012
Rat (Sprague-Dawley), adult, F	Oral	2 mg/kg	1 day	564 hours	Kim et al. 2016b
Rat (Sprague-Dawley), adult, F	Oral	15 mg/kg	1 day	989 hours (SE 48)	Chang et al. 2012
Rat (Sprague-Dawley), adult, M	IV	2 mg/kg	1 day	689 hours	Kim et al. 2016b
Rat (Sprague-Dawley), adult, F	IV	2 mg/kg	1 day	595 hours	Kim et al. 2016b
FOSA—Rat					
Rat (Sprague-Dawley), adult, M	Oral	5.0 mg/kg	1 day	125 hours	Seacat and Luebker 2000
PFDA—Rat					
Rat (Sprague-Dawley), adult, M	IP	4.8 mg/kg	1 day	1,008 hours	Vanden Heuvel et al. 1991b
Rat (Wistar), adult, M	IV	25 mg/kg	1 day	958 hours (SD 207)	Ohmori et al. 2003
Rat (Wistar), adult, F	IV	25 mg/kg	1 day	1,406 hours (SD 140)	Ohmori et al. 2003
Rat (Sprague-Dawley), adult, F	IP	4.8 mg/kg	1 day	552 hours	Vanden Heuvel et al. 1991b
PFNA—Rat					
Rat (Sprague-Dawley), adult, M	Oral	0.2 mg/kg	1 day	974 hours	Benskin et al. 2009
Rat (Sprague-Dawley), adult, M	Oral	0.029 mg/kg/day	12 weeks	1,128 hours (95% CL 935–1,416)	De Silva et al. 2009
Rat (Sprague-Dawley), adult M	Oral	1, 3, or 10 mg/kg	1 day	734.4 hours	Tatum-Gibbs et al. 2011
Rat (Wistar), adult, M	IV	22.6 mg/kg	1 day	710 hours (SD 55)	Ohmori et al. 2003
Rat (Sprague-Dawley), adult, F	Oral	1, 3, or 10 mg/kg	1 day	33.6 hours	Tatum-Gibbs et al. 2011
Rat (Wistar), adult, F	IV	22.6 mg/kg	1 day	58.6 hours (SD 9.8)	Ohmori et al. 2003
PFHpA—Rat					
Rat (Wistar), adult, M	IV	17.7 mg/kg	1 day	2.4 hours (SD 1.2)	Ohmori et al. 2003
Rat (Wistar), adult, F	IV	17.7 mg/kg	1 day	1.2 hours (SD 0.2)	Ohmori et al. 2003

Table 3-5. Summary Elimination Half-Lives for Perfluoroalkyls Estimated in Humans and Experimental Animals

Species, age, and sex	Route	Dose	Exposure duration[a]	Elimination half-life[b]	Reference
PFHxA—Rat					
Rat (Sprague-Dawley), adult, M	IV	10 mg/kg	1 day	1.0 hour	Chengelis et al. 2009a
Rat (Sprague-Dawley), adult, M	Oral	50 mg/kg	1 day	2.2 hours	Chengelis et al. 2009a
Rat (Sprague-Dawley), adult, M	Oral	150 mg/kg	1 day	2.4 hours	Chengelis et al. 2009a
Rat (Sprague-Dawley), adult, M	Oral	300 mg/kg	1 day	2.5 hours	Chengelis et al. 2009a
Rat (Sprague-Dawley), adult, F	IV	10 mg/kg	1 day	0.42 hour	Chengelis et al. 2009a
Rat (Sprague-Dawley), adult, F	Oral	50 mg/kg	1 day	2.6 hours	Chengelis et al. 2009a
Rat (Sprague-Dawley), adult, F	Oral	150 mg/kg	1 day	2.2 hours	Chengelis et al. 2009a
Rat (Sprague-Dawley), adult, F	Oral	300 mg/kg	1 day	2.1 hours	Chengelis et al. 2009a
Rat (Sprague-Dawley), adult, M	Oral	2 mg/kg	1 day	1.7 hours (SD 0.6)	Gannon et al. 2011
Rat (Sprague-Dawley), adult, M	Oral	10 mg/kg	1 day	0.5 hours (SD 0.1)	Gannon et al. 2011
Rat (Sprague-Dawley), adult, F	Oral	2 mg/kg	1 day	1.5 hours (SD 0.2)	Gannon et al. 2011
Rat (Sprague-Dawley), adult, F	Oral	10 mg/kg	1 day	0.7 hours (SD 0.3)	Gannon et al. 2011
Rat (Sprague-Dawley), adult, M	Oral	50 mg/kg/day	26 days	2.0 hours	Kirkpatrick 2005
Rat (Sprague-Dawley), adult, M	Oral	150 mg/kg/day	26 days	2.1 hours	Kirkpatrick 2005
Rat (Sprague-Dawley), adult, M	Oral	300 mg/kg/day	26 days	2.9 hours	Kirkpatrick 2005
Rat (Sprague-Dawley), adult, F	Oral	50 mg/kg/day	26 days	1.9 hours	Kirkpatrick 2005
Rat (Sprague-Dawley), adult, F	Oral	150 mg/kg/day	26 days	2.2 hours	Kirkpatrick 2005
Rat (Sprague-Dawley), adult, F	Oral	300 mg/kg/day	26 days	3.0 hours	Kirkpatrick 2005
PFHxS—Rat					
Rat (Sprague-Dawley), adult, M	Oral	0.030 mg/kg	1 day	382 hours	Benskin et al. 2009
Rat (Sprague-Dawley), adult, M	Oral	4 mg/kg	1 day	645.6 hours	Kim et al. 2016b
Rat (Sprague-Dawley), adult, F	Oral	4 mg/kg	1 day	41.28 hours	Kim et al. 2016b

Table 3-5. Summary Elimination Half-Lives for Perfluoroalkyls Estimated in Humans and Experimental Animals

Species, age, and sex	Route	Dose	Exposure duration[a]	Elimination half-life[b]	Reference
Rat (Sprague-Dawley), adult, M	IV	4 mg/kg	1 day	496.8 hours	Kim et al. 2016b
Rat (Sprague-Dawley), adult, M	IV	10 mg/kg	1 day	688 hours (SE 14.4)	Sundström et al. 2012
Rat (Sprague-Dawley), adult, F	IV	4 mg/kg	1 day	21.12 hours	Kim et al. 2016b
Rat (Sprague-Dawley), adult, F	IV	10 mg/kg	1 day	39 hours (SE 1.9)	Sundström et al. 2012
PFBA—Rat					
Rat (Sprague-Dawley), adult, M	Oral	30 mg/kg	1 day	9.22 hours (SE 0.75)	Chang et al. 2008b
Rat (Sprague-Dawley), adult, M	IV	30 mg/kg	1 day	6.38 hours (SE 0.53)	Chang et al. 2008b
Rat (Sprague-Dawley), adult, F	Oral	30 mg/kg	1 day	1.76 hours (SE 0.26)	Chang et al. 2008b
Rat (Sprague-Dawley), adult, F	IV	30 mg/kg	1 day	1.03 hours (SE 0.03)	Chang et al. 2008b
PFBS—Rat					
Rat (Sprague-Dawley), adult, M	IV	10 mg/kg	1 day	2.1 hours	Chengelis et al. 2009a
Rat (Sprague-Dawley), Rat (SD), adult, M	IV	30 mg/kg	1 day	4.51 hours (SE 2,22)	Olsen et al. 2009
Rat (Sprague-Dawley), adult, M	Oral	30 mg/kg	1 day	4.68 hours (SE 0.07)	Olsen et al. 2009
Rat (Sprague-Dawley), adult, F	IV	10 mg/kg	1 day	0.64 hours	Chengelis et al. 2009a
Rat (Sprague-Dawley), adult, F	IV	30 mg/kg	1 day	3.96 hours (SE 0.21)	Olsen et al. 2009
Rat (Sprague-Dawley), adult, F	Oral	30 mg/kg	1 day	7.42 hours (SE 0.79)	Olsen et al. 2009
PFOS—Mouse					
Mouse (CD), adult, M	Oral	1 mg/kg	1 day	1,027 hours	Chang et al. 2012
Mouse (CD), adult, M	Oral	20 mg/kg	1 day	874 hours	Chang et al. 2012
Mouse (CD), adult, F	Oral	1 mg/kg	1 day	907 hours	Chang et al. 2012
Mouse (CD), adult, F	Oral	20 mg/kg	1 day	731 hours	Chang et al. 2012

Table 3-5. Summary Elimination Half-Lives for Perfluoroalkyls Estimated in Humans and Experimental Animals

Species, age, and sex	Route	Dose	Exposure duration[a]	Elimination half-life[b]	Reference
PFHxS—Mouse					
Mouse (CD), adult, M	Oral	1 mg/kg	1 day	732 hours	Sundström et al. 2012
Mouse (CD), adult, M	Oral	20 mg/kg	1 day	671 hours	Sundström et al. 2012
Mouse (CD), adult, F	Oral	1 mg/kg	1 day	597 hours	Sundström et al. 2012
Mouse (CD), adult, F	Oral	20 mg/kg	1 day	643 hours	Sundström et al. 2012
PFNA—Mouse					
Mouse (CD-1), adult, M	Oral	1 or 10 mg/kg	1 day	823.2–1,653.6 hours	Tatum-Gibbs et al. 2011
Mouse (CD-1), adult, F	Oral	1 or 10 mg/kg	1 day	619.2–1,641.6 hours	Tatum-Gibbs et al. 2011
PFBA—Mouse					
Mouse (CD1), adult, M	Oral	10 mg/kg	1 day	13.34 hours (SE 4.55)	Chang et al. 2008b
Mouse (CD1), adult, M	Oral	30 mg/kg	1 day	16.3 hours (SE 7.2)	Chang et al. 2008b
Mouse (CD1), adult, M	Oral	100 mg/kg	1 day	5.22 hours (SE 2.27)	Chang et al. 2008b
Mouse (CD1), adult, F	Oral	10 mg/kg	1 day	2.87 hours (SE 0.30)	Chang et al. 2008b
Mouse (CD1), adult, F	Oral	30 mg/kg	1 day	3.08 hours (SE 0.26)	Chang et al. 2008b
Mouse (CD1), adult, F	Oral	100 mg/kg	1 day	2.79 hours (SE 0.3)	Chang et al. 2008b
PFOS—Rabbit					
Rabbit (New Zealand), adult, F	Oral	0.085 mg/kg/day	102 days	87 days (SD 31)	Tarazona et al. 2016

[a]Exposure durations of 1 day indicate that a single dose was administered.
[b]Reported half-lives are arithmetic means for the terminal elimination phase if multiple elimination phases were observed.

CI = confidence interval; CL = confidence limit; F = female; GM = geometric mean; IP = intraperitoneal; IV = intravenous; M = male; NA = not applicable; PFBA = perfluorobutanoic acid; PFDA = perfluorodecanoic acid; PFHpA = perfluoroheptanoic acid; PFHxS = perfluorohexane sulfonic acid; PFNA = perfluorononanoic acid; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid; FOSA = perfluorooctane sulfonamide; SD = standard deviation; SE = standard error

Table 3-6. Summary Systemic Clearance for Perfluoroalkyls Estimated in Experimental Animals

Species, age, and sex	Route	Dose (mg/kg)	Exposure duration	Systemic clearance (mL/day/kg)[a]	Reference
PFOA—Nonhuman primate					
Cynomolgus monkey, adult, M	IV	10	1 day	12.4 (SD 7.4)	Butenhoff et al. 2004c
Cynomolgus monkey, adult, F	IV	10	1 day	5.3 (SD 3.3)	Butenhoff et al. 2004c
PFOS—Nonhuman primate					
Cynomolgus monkey, adult, M	IV	2	1 day	1.10 (SE 0.06)	Chang et al. 2012
Cynomolgus monkey, adult, F	IV	2	1 day	1.65 (SE 0.04)	Chang et al. 2012
PFHxA—Nonhuman primate					
Cynomolgus monkey, adult, M	IV	10	1 day	569	Chengelis et al. 2009a
Cynomolgus monkey, adult, F	IV	10	1 day	535	Chengelis et al. 2009a
PFHxS—Nonhuman primate					
Cynomolgus monkey, adult, M	IV	10	1 day	1.3 (SE 0.1)	Sundström et al. 2012
Cynomolgus monkey, adult, F	IV	10	1 day	1.9 (SE 0.4)	Sundström et al. 2012
PFBA—Nonhuman primate					
Cynomolgus monkey, adult, M	IV	10	1 day	2,371 (SE 293)	Chang et al. 2008a
Cynomolgus monkey, adult, F	IV	10	1 day	1,075 (SE 91)	Chang et al. 2008a
PFBS—Nonhuman primate					
Cynomolgus monkey, adult, M	IV	10	1 day	159	Chengelis et al. 2009a
Cynomolgus monkey, adult, F	IV	10	1 day	238	Chengelis et al. 2009a
Cynomolgus monkey, adult, M	IV	10	1 day	12,264 (SE 3384)	Olsen et al. 2009
Cynomolgus monkey, adult, F	IV	10	1 day	8,832 (SE 2880)	Olsen et al. 2009
PFOA—Rat					
Rat (Sprague-Dawley), adult, M	Oral	0.1	1 day	23.1 (SD 5.8)	Kemper 2003
Rat (Sprague-Dawley), adult, M	Oral	1	1 day	20.9 (SD 3.8)	Kemper 2003
Rat (Sprague-Dawley), adult, M	Oral	1	1 day	40.40 (SD 2.29)	Kim et al. 2016b

Table 3-6. Summary Systemic Clearance for Perfluoroalkyls Estimated in Experimental Animals

Species, age, and sex	Route	Dose (mg/kg)	Exposure duration	Systemic clearance (mL/day/kg)[a]	Reference
Rat (Sprague-Dawley), adult, M	Oral	5	1 day	20.4 (SD 5.0)	Kemper 2003
Rat (Sprague-Dawley), adult, M	Oral	25	1 day	27.1 (SD 7.4)	Kemper 2003
Rat (Sprague-Dawley), adult, M	IV	1	1 day	21.5 (SD 2.0)	Kemper 2003
Rat (Sprague-Dawley), adult, M	IV	1	1 day	47.39 (SD 3.40)	Kim et al. 2016b
Rat (Sprague-Dawley), adult, F	Oral	0.1	1 day	778 (SD 144)	Kemper 2003
Rat (Sprague-Dawley), adult, F	Oral	1	1 day	655 (SD 173)	Kemper 2003
Rat (Sprague-Dawley), adult, F	Oral	1	1 day	645.12 (SD 43.44)	Kim et al. 2016b
Rat (Sprague-Dawley), adult, F	Oral	5	1 day	1,164 (SD 118)	Kemper 2003
Rat (Sprague-Dawley), adult, F	Oral	25	1 day	842 (SD 166)	Kemper 2003
Rat (Sprague-Dawley), adult, F	IV	1	1 day	816 (SD 221)	Kemper 2003
Rat (Sprague-Dawley), adult, F	IV	1	1 day	612.84 (SD 32.54)	Kim et al. 2016b
Rat (Wistar), adult, M	IV	21.5	1 day	50.4 (SD 14.4)	Kudo et al. 2002
Rat (Wistar), adult, F	IV	21.5	1 day	2,233 (SD 805)	Kudo et al. 2002
Rat (Wistar), adult, M	IV	20.1	1 day	135 (SD 29)	Ohmori et al. 2003
Rat (Wistar), adult, F	IV	20.1	1 day	2,233 (SD 805)	Ohmori et al. 2003
PFOS—Rat					
Rat (Sprague-Dawley), adult, M	Oral	2	1 day	7.33 (SD 0.55)	Kim et al. 2016b
Rat (Sprague-Dawley), adult, M	Oral	2	1 day	11.3 (SE 0.56)	Chang et al. 2012
Rat (Sprague-Dawley), adult, M	Oral	15	1 day	4.9 (SE 0.52)	Chang et al. 2012
Rat (Sprague-Dawley), adult, M	IV	2	1 day	9.24 (SD 0.37)	Kim et al. 2016b
Rat (Sprague-Dawley), adult, F	Oral	2	1 day	8.52 (SD 0.37)	Kim et al. 2016b
Rat (Sprague-Dawley), adult, F	Oral	2	1 day	22.2 (SE 0.28)	Chang et al. 2012
Rat (Sprague-Dawley), adult, F	Oral	15	1 day	5.4 (SE 20)	Chang et al. 2012
Rat (Sprague-Dawley), adult, F	IV	2	1 day	9.82 (SD 0.21)	Kim et al. 2016b

Table 3-6. Summary Systemic Clearance for Perfluoroalkyls Estimated in Experimental Animals

Species, age, and sex	Route	Dose (mg/kg)	Exposure duration	Systemic clearance (mL/day/kg)[a]	Reference
PFDA—Rat					
Rat (Wistar), adult, M	IV	25	1 day	207 (SD 0.054)	Ohmori et al. 2003
Rat (Wistar), adult, F	IV	25	1 day	140 (SD 0.008)	Ohmori et al. 2003
PFNA—Rat					
Rat (Wistar), adult, M	IV	22.6	1 day	6.9 (SD 0.6)	Ohmori et al. 2003
Rat (Wistar), adult, F	IV	22.6	1 day	106 (SD 31)	Ohmori et al. 2003
PFHpA—Rat					
Rat (Wistar), adult, M	IV	17.7	1 day	1,604 (SD 558)	Ohmori et al. 2003
Rat (Wistar), adult, F	IV	17.7	1 day	3,071 (SD 781)	Ohmori et al. 2003
PFHxA—Rat					
Rat (Sprague-Dawley), adult, M	IV	10	1 day	2,784	Chengelis et al. 2009a
Rat (Sprague-Dawley), adult, F	IV	10	1 day	18,600	Chengelis et al. 2009a
PFHxS—Rat					
Rat (Sprague-Dawley), adult, M	Oral	4	1 day	7.15 (SD 0.06)	Kim et al. 2016b
Rat (Sprague-Dawley), adult, M	IV	4	1 day	9.01 (SD 0.05)	Kim et al. 2016b
Rat (Sprague-Dawley), adult, M	IV	10	1 day	6.7 (SE 0.06)	Sundström et al. 2012
Rat (Sprague-Dawley), adult, F	Oral	4	1 day	124.83 (SD 3.40)	Kim et al. 2016b
Rat (Sprague-Dawley), adult, F	IV	4	1 day	227.93 (SD 6.73)	Kim et al. 2016b
Rat (Sprague-Dawley), adult, F	IV	10	1 day	53.4 (SE 4.38)	Sundström et al. 2012
PFBA—Rat					
Rat (Sprague-Dawley), adult, M	IV	30	1 day	851 (SE 61)	Chang et al. 2008a
Rat (Sprague-Dawley), adult, F	IV	30	1 day	2,949 (SE 59)	Chang et al. 2008a
Rat (Sprague-Dawley), adult, M	Oral	30	1 day	494 (SE 29)	Chang et al. 2008a
Rat (Sprague-Dawley), adult, F	Oral	30	1 day	1,527 (SE 145)	Chang et al. 2008a
PFBS—Rat					
Rat (Sprague-Dawley), adult, M	IV	10	1 day	946	Chengelis et al. 2009a
Rat (Sprague-Dawley), adult, F	IV	10	1 day	7,464	Chengelis et al. 2009a

Table 3-6. Summary Systemic Clearance for Perfluoroalkyls Estimated in Experimental Animals

Species, age, and sex	Route	Dose (mg/kg)	Exposure duration	Systemic clearance (mL/day/kg)[a]	Reference
Rat (Sprague-Dawley), adult, M	IV	30	1 day	2,856 (SE 816)	Olsen et al. 2009
Rat (Sprague-Dawley), adult, F	IV	30	1 day	11,265 (SE 960)	Olsen et al. 2009
PFOA—Mouse					
Mouse (FVB/NJcl), adult, M	IV	0.13	1 day	14.2 (SD 8.4)	Fujii et al. 2015a, 2015b
Mouse (FVB/NJcl), adult, F	IV	0.13	1 day	11.8 (SD 6.1)	Fujii et al. 2015a, 2015b
Mouse (FVB/NJcl), adult, M	Oral	1.3	1 day	13.1 (SD 7.4)	Fujii et al. 2015a, 2015b
Mouse (FVB/NJcl), adult, F	Oral	1.3	1 day	9.0 (SD 1.9)	Fujii et al. 2015a, 2015b
PFOS—Mouse					
Mouse (CD), adult, M	Oral	1	1 day	4.7	Chang et al. 2012
Mouse (CD), adult, M	Oral	20	1 day	4.7	Chang et al. 2012
Mouse (CD), adult, F	Oral	1	1 day	5.0	Chang et al. 2012
Mouse (CD), adult, F	Oral	20	1 day	6.0	Chang et al. 2012
PFHxS—Mouse					
Mouse (CD), adult, M	Oral	1	1 day	2.9	Sundström et al. 2012
Mouse (CD), adult, M	Oral	20	1 day	4.8	Sundström et al. 2012
Mouse (CD), adult, F	Oral	1	1 day	2.7	Sundström et al. 2012
Mouse (CD), adult, F	Oral	20	1 day	3.8	Sundström et al. 2012
PFNA—Mouse					
Mouse (FVB/NJcl), adult, M	IV	0.14	1 day	3.9 (SD 1.9)	Fujii et al. 2015a, 2015b
Mouse (FVB/NJcl), adult, F	IV	0.14	1 day	5.1 (SD 2.3)	Fujii et al. 2015a, 2015b
Mouse (FVB/NJcl), adult, M	Oral	1.4	1 day	4.0 (SD 1.7)	Fujii et al. 2015a, 2015b
Mouse (FVB/NJcl), adult, F	Oral	1.4	1 day	2.4 (SD 1.0)	Fujii et al. 2015a, 2015b
PFDA—Mouse					
Mouse (FVB/NJcl), adult, M	IV	0.16	1 day	2.2 (SD 0.9)	Fujii et al. 2015a, 2015b
Mouse (FVB/NJcl), adult, F	IV	0.16	1 day	2.8 (SD 1.2)	Fujii et al. 2015a, 2015b
Mouse (FVB/NJcl), adult, M	Oral	1.6	1 day	3.9 (SD 1.8)	Fujii et al. 2015a, 2015b
Mouse (FVB/NJcl), adult, F	Oral	1.6	1 day	2.2 (SD 1.1)	Fujii et al. 2015a, 2015b

Table 3-6. Summary Systemic Clearance for Perfluoroalkyls Estimated in Experimental Animals

Species, age, and sex	Route	Dose (mg/kg)	Exposure duration	Systemic clearance (mL/day/kg)[a]	Reference
PFUnA—Mouse					
Mouse (FVB/NJcl), adult, M	IV	0.17	1 day	2.8 (SD 1.0)	Fujii et al. 2015a, 2015b
Mouse (FVB/NJcl), adult, F	IV	0.17	1 day	3.4 (SD 1.5)	Fujii et al. 2015a, 2015b
Mouse (FVB/NJcl), adult, M	Oral	1.7	1 day	5.7 (SD 2.6)	Fujii et al. 2015a, 2015b
Mouse (FVB/NJcl), adult, F	Oral	1.7	1 day	3.1 (SD 1.7)	Fujii et al. 2015a, 2015b
PFDoDA—Mouse					
Mouse (FVB/NJcl), adult, M	IV	0.19	1 day	4.4 (SD 1.6)	Fujii et al. 2015a, 2015b
Mouse (FVB/NJcl), adult, F	IV	0.19	1 day	4.8 (SD 2.4)	Fujii et al. 2015a, 2015b
Mouse (FVB/NJcl), adult, M	Oral	1.9	1 day	9.4 (SD 4.1)	Fujii et al. 2015a, 2015b
Mouse (FVB/NJcl), adult, F	Oral	1.9	1 day	5.2 (SD 3.2)	Fujii et al. 2015a, 2015b
PFBA—Mouse					
Mouse (CD1), adult, M	Oral	10	1 day	280 (SE 72)	Chang et al. 2008b
Mouse (CD1), adult, M	Oral	30	1 day	296 (SE 640)	Chang et al. 2008b
Mouse (CD1), adult, M	Oral	100	1 day	784 (SE 112)	Chang et al. 2008b
Mouse (CD1), adult, F	Oral	10	1 day	564 (SE 24)	Chang et al. 2008b
Mouse (CD1), adult, F	Oral	30	1 day	696 (SE 32)	Chang et al. 2008b
Mouse (CD1), adult, F	Oral	100	1 day	1,336 (SE 64)	Chang et al. 2008b

[a]As reported in units of mL/day/kg or converted from mL/hour (x24), mL/hour (x24/body weight) or mL/minute (x60x24).

CI = confidence interval; F = female; IV = intravenous; M = male; PFBA = perfluorobutanoic acid; PFBS = perfluorobutane sulfonic acid; PFDA = perfluorodecanoic acid; PFHpA = perfluoroheptanoic acid; PFHxA = perfluorohexanoic acid; PFHxS = perfluorohexane sulfonic acid; PFNA = perfluorononanoic acid; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid; SD = standard deviation; SE = standard error

Elimination of Perfluoroalkyls in Humans. Elimination $t_{1/2}$ values for PFOA, PFOS, PFHxS, PFBA, and PFBS have been estimated in humans (Bartell et al. 2010; Costa et al. 2009; Chang et al. 2008a; Glynn et al. 2012; Harada et al. 2005a; Li et al. 2018; Olsen et al. 2007a, 2009; Seals et al. 2011; Spliethoff et al. 2008; Yeung et al. 2013; Wong et al. 2014, 2015; Worley et al. 2017a; Zhang et al. 2013). Estimates in humans are based on measurements of the decline in serum perfluoroalkyl concentrations following cessation or an abrupt decrease in exposure, or on measurements of renal plasma clearance

from serum in a general population sample from Japan (Harada et al. 2005a). The latter clearance estimates were converted to $t_{1/2}$ values, for display in Table 3-5 as follows (Equations 3-4 and 3-5):

$$k_e = \frac{Cl}{V} \qquad \text{Eq. (3-4)}$$

$$t_{1/2} = \frac{\ln(2)}{k_e} \qquad \text{Eq. (3-5)}$$

where k_e is the elimination rate constant (e.g., day^{-1}), Cl is the renal plasma clearance (e.g., mL plasma/day/kg), and V is the plasma volume (L/kg), which is assumed to be 4.3% of body weight (ICRP 1981). In general, these studies show that longer chain length is associated with slower elimination rates. For example, the elimination $t_{1/2}$ for PFBA was estimated to be 70–80 hours (Chang et al. 2008a), whereas the $t_{1/2}$ values for PFHxS, PFOS, and PFOA range from 2 to 35 years (Bartell et al. 2010; Harada et al. 2005a; Li et al. 2018; Olsen et al. 2007a; Seals et al. 2011; Worley et al. 2017a; Zhang et al. 2013). Longer $t_{1/2}$ values for PFOA have been reported with longer monitoring follow-up times, which allow the detection of slower elimination phases of multiphasic elimination kinetics (Seals et al. 2011). Perfluoroalkyl sulfonates are eliminated more slowly in humans than corresponding carboxylates of the same chain length (Zhang et al. 2013). Analytical methods typically used to measure serum perfluoroalkyls do not discriminate between linear and branched isomers and, as a result, these studies estimate elimination rates for the isomer mixture. A study that compared elimination rates of isomers of PFOA found that linear isomers tend to be eliminated more slowly than branched isomers (Zhang et al. 2013), consistent with results of studies conducted in rats (Benskin et al. 2009; De Silva et al. 2009).

An analysis of serum PFOS data from NHANES indicated that $t_{1/2}$ in females may be shorter (4.3 years) compared to males (4.7 years; Wong et al. 2014, 2015). The NHANES data are cross-sectional and, therefore, the estimates of $t_{1/2}$ required fitting the data to age patterns of PFOS intake. An improved fit to the data for females was achieved when estimated losses of PFOS in menstrual fluids were considered, suggesting that menstrual loss of PFOS may account for some, but not all, of the sex difference in the elimination rate (Verner and Longnecker 2015; Wong et al. 2015). Li et al. (2018) also found apparent sex differences in PFOS elimination in male and female residents in Sweden exposed to contaminated drinking water. The estimated $t_{1/2}$ for PFOS were 4.6 years in males and 3.1 years in females. Zhang et al. (2013) estimated serum $t_{1/2}$ for various age and sex strata in a population of 86 individuals. Serum $t_{1/2}$ for PFOS was lower for PFOS in younger females (≤50 years, $t_{1/2}$ = 6.2 years±0.3 SE, n=66) compared to males and older females ($t_{1/2}$ =27 years±3.1 SE, n=20). Zhang et al. (2013) attributed the difference in serum $t_{1/2}$ to clearance in menstrual fluids. However, the estimated serum $t_{1/2}$ of 27 years is much higher than values calculated from other studies; Zhang et al. (2013) noted that the serum $t_{1/2}$ should be

considered as an upper limit estimate. Estimated $t_{1/2}$ for PFOA was not different in younger females (2.1±0.3 SE, n=20) compared to males and older females (2.6±0.4 SE, n=66). Declines in serum PFOA concentrations were observed in populations following initiation of activated carbon filtration of public water supplies that had been contaminated with PFOA (Bartell et al. 2010). The estimated mean serum $t_{1/2}$ for a group of 200 adults followed for 1 year after filtration was initiated was 2.3 years (95% CI 2.1–2.4). Elimination rates were not different in males and females. Serum PFOA concentration ranged from 16 to 1,200 ng/mL. A larger follow-up study measured serum PFOA concentrations in two populations of former residents (n=1,672) of the same water districts (Seals et al. 2011). In one population (n=643), the serum $t_{1/2}$ increased with increasing elapsed time since leaving the water district. The $t_{1/2}$ values were 2.9 years (95% CI 2.3–3.8) for elapsed time of <4 years and 10.1 years for elapsed time of >4 years. In a second population with an elapsed time since residence of <9 years, the $t_{1/2}$ was 8.5 years (95% CI 7.1–10.1). Elimination rates (based on the annual percent decrease in serum concentrations) were faster in males (27%) compared to females (18%) for the first 4 years post-exposure; however, no difference was evident between sexes when elapsed time from exposure was >4 years.

Bartell (2012) and Russell et al. (2015) point out that most studies examining PFOA elimination half-lives fail to account for ongoing background exposure, which could result in an overestimation of elimination half-lives. Bartell (2012) estimated that the bias from background exposure could resulted in 1–26% overestimation of calculated PFOA half-lives and that greater overestimations can occur for half-lives based on longer follow-up times. Russell et al. (2015) estimated that the bias was greatest in populations with serum PFOA levels closest to background levels. In a re-analysis of the Olsen et al. (2007) occupational exposure data, Russell et al. (2015) estimated that overestimation was approximately 1.2% in workers with initial serum concentrations >500 ng/mL (100 times higher than NHANES general population data) and 13% for workers with lower initial serum PFOA levels. Restricting the elimination half-life calculation to workers with initial serum PFOA levels of >500 ng/mL would result in a half-life of 3.0 years (Russell et al. 2015), compared to 3.8 years calculated for the whole cohort (Olsen et al. 2007).

Analysis of kinetics of serum PFOS concentrations in retired U.S. fluorochemical production workers (24 males, 2 females) yielded a mean serum elimination $t_{1/2}$ estimate of 5.4 years (95% CI 3.9–6.9; geometric mean: 4.8 years, 95% CI 4.0–5.8) in subjects whose serum PFOS concentrations ranged from 37 to 3,490 ng/mL (Olsen et al. 2007a). Estimates for the two females in the same study were 4.9 and 6.8 years. Estimates based on renal clearance of PFOS from serum in subjects from the general population of Japan ranged from 2.9 to 7.4 years; these subjects had serum PFOS concentrations that

ranged from 4 to 49 ng/mL (Harada et al. 2005a). Estimates in males (7.4, 2.9 years) were similar to females (4.5, 4.6 years). This same study measured serum PFHxS concentrations in retired U.S. fluorochemical production workers (24 males, 2 females) and yielded a mean estimate of 8.5 years (95% CI 6.4–10.6; geometric mean: 7.3 years, 95% CI 5.8–9.2) for the serum elimination $t_{1/2}$ in subjects whose serum PFHxS concentrations ranged from 10 to 1,295 ng/mL (Olsen et al. 2007a). Estimates for the two females in the same study were 12.2 and 13.3 years.

The elimination rate of PFBA was estimated in fluorochemical workers who may have been exposed to various PFBA precursors (Chang et al. 2008a). In three male workers, the estimated mean $t_{1/2}$ based on serum PFBA kinetics was 81 hours (±41 SD). In a larger study of nine workers (seven males, two females), the mean $t_{1/2}$ was 72 hours (±38 SD). Estimates for the two female subjects were 56 and 118 hours. The combined mean value for the 12 estimates was 75 hours (±38 SD). Olsen et al. (2009) estimated serum $t_{1/2}$ of PFBS in six fluorochemical workers. The mean $t_{1/2}$ was 27.4 days (±11.1 SD). The group included a single female whose $t_{1/2}$ was 45.7 days. Based on these observations, PFBA (chain length 3) and PFBS (chain length 4) are eliminated substantially faster in humans than perfluoroalkyls having longer carbon chain lengths, such as PFHxS (chain length 6), PFOA (chain length 7), and PFOS (chain length 8).

Temporal trends in perfluoroalkyl serum concentrations have also been used to estimate population halving times (Glynn et al. 2012; Olsen et al. 2012; Spleithoff et al. 2008; Yeung et al. 2013). Population halving times are influenced by temporal trends in intakes and may therefore not accurately reflect clearance. Population halving times for PFOS ranged from 4 to 5 years (Olsen et al. 2012; Spleithoff et al. 2008; Yeung et al. 2013). Glynn et al. (2012) monitored serum perfluoroalkyls in a population of pregnant women (n=413) in Sweden over the period 1996–2010. Halving times were 22 years (95% CI 16–38) for PFOA and 8.2 years (95% CI 6.3–12) for PFOS.

Elimination of Perfluoroalkyls in Nonhuman Primates. Elimination $t_{1/2}$ values and systemic clearances for PFOA, PFOS, PFHxA, PFHxS, PFBA, and PFBS have been estimated in Cynomolgus monkeys (Buttenoff et al. 2004c; Chang et al. 2012; Chengelis et al. 2009a; Olsen et al. 2009; Seacat et al. 2002; Sundström et al. 2012). Estimated terminal $t_{1/2}$ values were 20–30 days for PFOA, 100–170 days for PFOS, 90–140 days for PFHxS, 40 hours for PFBA and 8–95 hours for PFBS. Elimination of perfluoroalkyls in monkeys is multiphasic and, as a result, estimates of the terminal $t_{1/2}$ can vary with the duration of the observation period and assumptions made in modeling elimination kinetics (Chang et al. 2012; Chengelis et al. 2009a; Olsen et al. 2009; Sundström et al. 2012). For example, the $t_{1/2}$ values for

PFBS were 8 and 15 hours in female and male monkeys, respectively, when monkeys were monitored for 48 hours following a single intravenous dose (Chengelis et al. 2009a), whereas the $t_{1/2}$ values were 95 and 83 hours in male and female monkeys, respectively, when the monitoring period was extended to 14 days and a three-compartment model was used to estimate the terminal $t_{1/2}$ (Olsen et al. 2009). Studies in monkeys confirm general trends observed in humans that perfluoroalkyl sulfonates are more slowly eliminated than perfluoroalkyl carboxylates and that elimination of longer-chain perfluoroalkyls occurs more slowly than short-chain perfluoroalkyls. Systemic clearances were lower for PFOS, PFHxS, and PFBS compared to the corresponding carboxylates, PFOA, PFHxA, and PFBA (Table 3-6). Systemic clearances were similar in male and female monkeys (Table 3-6).

Elimination of Perfluoroalkyls in Rats. Elimination $t_{1/2}$ values and systemic clearances for PFOA, PFOS, FOSA, PFDA, PFNA, PFHpA, PFHxA, PFHxS, PFBA, and PFBS have been estimated in rats (Benskin et al. 2009; Chang et al. 2008b, 2012; Chengelis et al. 2009a; De Silva et al. 2009; Johnson and Ober 1979; Kemper 2003; Kim et al. 2016b; Kudo et al. 2002; Ohmori et al. 2003; Olsen et al. 2009; Seacat and Luebker 2000; Sundström et al. 2012; Vanden Heuvel et al. 1991b, 1991c; Ylinen et al. 1990). Consistent with observations made in humans and Cynomolgus monkeys, perfluoroalkyl sulfonates are more slowly eliminated than perfluoroalkyl carboxylates and short-chain perfluoroalkyls (e.g., PFBA, PFBS) are eliminated faster in rats than long-chain perfluoroalkyls (e.g., PFOA, PFOS, PFHxA, PFHxS); Tables 3-5 and 3-6. Linear PFOA isomers tend to be eliminated more slowly than branched isomers (Benskin et al. 2009; De Silva et al. 2009).

Elimination of perfluoroalkyls exhibits pronounced sex differences in rats, with faster elimination in females than in males (Benskin et al. 2009; Chang et al. 2008b; Chengelis et al. 2009a; Kemper 2003; Kim et al. 2016b; Kudo et al. 2002; Ohmori et al. 2003; Sundström et al. 2012; Tatim-Gibbs et al. 2011; Vanden Heuvel et al. 1991c; Ylinen et al. 1990). Estimates of systemic clearance for PFOA in male rats ranged from 20 to 50 mL/day/kg, whereas estimates for female rats ranged from 600 to 2,200 mL/day/kg (Kemper 2003; Kudo et al. 2002; Ohmori et al. 2003). Systemic clearances of PFOA, PFOS, PFNA, PFHxA, PFHxS, PFBA, and PFBS are also higher in female rats compared to male rats (Table 3-6). Pronounced sex difference in elimination rates in rats (faster elimination in females) was observed in rats following 30-minute nose-only exposures to aerosols (MMAD=1.9–2.1 μm) of 1–25 mg ammonium PFOA/m^3 (Hinderliter et al. 2006a). Plasma PFOA concentrations were not detectable 12 hours after exposure of female rats, and were approximately 90% of peak plasma concentrations 24 hours after the exposure in male rats. The slower elimination of PFOA in male rats resulted in steady-state plasma concentrations within 3 weeks of repeated exposures (6 hours/day, 5 days/week) in male rats, whereas in

female rats, daily periodic oscillations of plasma concentrations from peak to below detection occurred on each day of exposure. Steady-state plasma concentrations in male rats were approximately 10 times that of daily peak concentrations in female rats.

Pronounced dose dependence appears in the $t_{1/2}$ estimates for PFOA in female rats. With increasing dose, plasma elimination kinetics in female rats converts from monophasic to biphasic. Following an oral dose of PFOA of 0.1, 1, 5, or 25 mg/kg, the terminal $t_{1/2}$ values in female rats were 3.2, 3.5, 4.6, or 16.2 hours, respectively; no apparent dose dependence was observed in male rats over the same dose range (Kemper 2003). Dose-dependent elimination of PFOA has been attributed to a capacity-limited renal tubular secretion of PFOA in female rats (see discussion below on *Mechanisms of Excretion*). The divergence in elimination kinetics between male and female rats appears to be age-dependent, with faster elimination becoming evident in female rats after 30 days of age, consistent with the timing of sexual maturation and involvement of sex hormones in the modulation of the renal excretion of PFOA in rats (Hinderliter et al. 2006b).

Elimination of Perfluoroalkyls in Mice. Elimination $t_{1/2}$ values and systemic clearances for PFOS, PFHxS, and PFBA have been estimated in mice (Chang et al. 2008a, 2012; Sundström et al. 2012). Consistent with studies conducted in rats and monkeys, PFBA is eliminated more rapidly in mice than PFOS and PFHxS. Systemic clearances ranged from 5 to 6 mL/day/kg for PFOS (Chang et al. 2012), from 3 to 5 mL/day/kg for PFHxS (Sundström et al. 2012), and from 300 to 1,300 mL/day/kg for PFBA (Chang et al. 2008a). Sex differences in elimination in mice were observed for PFBA, but not PFOS or PFHxS. Systemic clearances of PFBA in female mice were approximately 2 times that of males (Chang et al. 2008a). Systemic clearance of PFBA in male and female mice appeared to be dependent on dose. Systemic clearance following a single oral dose of 100 mg PFBA/kg was approximately 2 times higher than the systemic clearance following a dose of 10 or 30 mg PFBA/kg. Possible explanations for the apparent dependence of clearance on dose are dose-dependent bioavailability or that the one-compartment model used to estimate elimination rates and serum AUC did not adequately fit the serum kinetics observed at the higher dose (Chang et al. 2008a). The latter could occur if renal tubular reabsorption of PFBA or plasma protein binding of PFBA is saturable in mice. Systemic clearance rates for PFOA were similar in male mice (13.1 mL/kg/day) and in female mice (9.0 mL/kg/day) (Fuji et al. 2015a, 2015b).

Elimination of Perfluoroalkyls in Other Species. Sex differences in elimination of PFOA have also been observed in hamsters; unlike the rat, male hamsters excreted absorbed PFOA more rapidly than female hamsters. Following a single gavage dose of 10 mg/kg as ammonium [^{14}C]PFOA, cumulative excretion

of ^{14}C in urine at 24 hours post-dosing was 96.4% of the dose in female rats and 8.7% in male rats; 24.6% and 84.5% in female and male hamsters, respectively; 4.1% in male and female mice; and 90.5 and 80.2% in female and male rabbits, respectively (Hundley et al. 2006).

Mechanisms of Excretion. Urinary excretion of perfluoroalkyls involves glomerular filtration and renal tubular secretion and reabsorption (for PFOA, see Harada et al. 2005a; Kudo et al. 2002; Ohmori et al. 2003). Glomerular filtration of PFOA is limited by extensive binding of PFOA to albumin and other high molecular weight proteins in plasma (Han et al. 2003, 2005; Ohmori et al. 2003; Kerstner-Wood et al. 2003; Vanden Heuvel et al. 1992a, 1992b; Ylinen and Auriola 1990). Elimination of PFOA and other perfluoroalkyls shows pronounced sex differences in rats, with slower elimination in males for PFOA, PFOS, PFNA, PFHxA, PFHxS, PFBA, and PFBS (Chang et al. 2008a, 2012; Chengelis et al. 2009a; Kemper 2003; Kudo et al. 2002; Ohmori et al. 2003; Sundström et al. 2012). The sex difference in PFOA elimination in rats is dependent on testosterone (Hinderliter et al. 2006b; Kudo et al. 2002; Vanden Heuvel et al. 1992a). The significantly slower elimination of PFOA in adult male rats compared to female rats has been attributed to sex hormone modulation of organic anion transporters in kidney. At similar doses administered to male and female rats, PFOA undergoes net tubular reabsorption in male rats (i.e., urinary excretion rate < rate of glomerular filtration of PFOA) and net tubular secretion in female rats (i.e., urinary excretion rate > rate of glomerular filtration of PFOA) (Harada et al. 2005a; Kudo et al. 2002; Ohmori et al. 2003). In rats, several transporters have been shown to have affinity for C7–C9 perfluoroalkyl carboxylates. The transporters, OAT1 and OAT3, located on the basolateral membrane of the renal proximal tubule, appear to participate in secretion of C7–C9 perfluoroalkyl carboxylates into the tubular fluid (Nakagawa et al. 2008; Weaver et al. 2010). The transporters, OATP1a1 (rat), OAT4 (human), and URAT1 (human), located on the apical membrane, appear to mediate reabsorption of C8–C10 perfluoroalkyl carboxylates from the tubular fluid (Katakura et al. 2007; Nakagawa et al. 2009; Weaver et al. 2010; Yang et al. 2009, 2010). In rats and mice, expression of OAT1, OAT3, and OATP1a1 is controlled by male sex hormones and shows higher activities in males (Buist and Klaassen 2004; Gotoh et al. 2002; Kobayashi et al. 2002; Li et al. 2002; Lu et al. 1996; Lubojevic et al. 2004). The slower elimination of PFOA (and other long-chain perfluoroalkyl carboxylates) in male rats has been attributed to OATP1a1 (Weaver et al. 2010; Yang et al. 2009). Higher activity of OATP1a1 in male rats results in higher reabsorptive transport and lower rates of urinary excretion. However, saturation of this transporter could result in an increase in urinary elimination of perfluoroalkyls due to decreased tubular reabsorption. This is consistent with the apparent plateau in plasma concentration with increasing dose observed in cancer patients treated with PFOA (Convertino et al. 2018). Affinities of OATP1a1 (rat), OAT4 (human), and URAT1 (human) are highest for C7–C10 perfluoroalkyl carboxylates (Weaver et al.

2010; Yang et al. 2009, 2010). Affinity of rat OATP1a1 is strongly correlated with total clearance in rats (r^2=0.98; Yang et al. 2009).

Although sex differences for elimination of perfluoroalkyls have been detected in laboratory animals, human monitoring studies have not consistently detected sex differences in elimination $t_{1/2}$ of perfluoroalkyls; this may reflect limitations in the studies, including numbers and age of subjects (Bartell et al. 2010; Seals et al. 2011; Wong et al. 2014, 2015; Zhang et al. 2013). Menstruation may contribute to faster elimination of PFOS in women (Wong et al. 2014, 2015; Zhang et al. 2013). The effect of menstruation or other variables related to menstruation appear to contribute to faster elimination in younger (≤50 years) women compared to men and older women (Zhang et al. 2013). Two studies have found evidence for elimination of PFOS being affected by menstruation (Wong et al. 2014, 2015; Zhang et al. 2013). The estimated $t_{1/2}$ for PFOA was not different in younger females compared to males and older females. Mechanisms by which menstruation could affect PFOS clearance are not understood. Bulk elimination of blood would be expected to affect serum clearance of both PFOS and PFOA; therefore, other mechanisms must contribute that discriminate between perfluoroalkyl species. A better metric than serum $t_{1/2}$ for evaluating sex differences in elimination for this would be systemic or renal clearance of the perfluoroalkyl. Harada et al. (2005a) measured renal clearance in a small sample of young adults (five males and five females, age 22–23 years) and found that renal clearance was not different in males and females. Zhang et al. (2013) estimated renal clearance of PFOA and PFOS in a population of younger females (≤50 years, n=20), older females (>50 years), younger males (≤50 years), and older males (>50 years) and did not find significant sex or age differences. Studies that measured systemic clearance in monkeys also have not found significant sex differences in systemic clearance of PFOA (Buttenoff et al. 2004c) or PFOS (Chang et al. 2012).

Studies conducted in rats have shown that PFDA, PFNA, PFOA, PFOS, and PFHxA are secreted in bile and undergo extensive reabsorption from the gastrointestinal tract (Johnson et al. 1984; Kudo et al. 2001; Vanden Heuvel et al. 1991b, 1991c). Biliary secretion rates of PFOA are similar in male and female rats when renal excretion is blocked by ligation of the kidneys (Vanden Heuvel et al. 1991a, 1991b). This lack of sex influence on biliary secretion (compared to the sex influence on renal clearance) may reflect a relative sex insensitivity of OAT2 (or other organic anion transporter) expression in liver, compared to kidney; the latter is approximately 7–8 times higher in adult female rats compared to male rats (Kudo et al. 2002).

3.1.5 Physiologically Based Pharmacokinetic (PBPK)/Pharmacodynamic (PD) Models

PBPK models use mathematical descriptions of the uptake and disposition of chemical substances to quantitatively describe the relationships among critical biological processes (Krishnan et al. 1994). PBPK models are also called biologically based tissue dosimetry models. PBPK models are increasingly used in risk assessments, primarily to predict the concentration of potentially toxic moieties of a chemical that will be delivered to any given target tissue following various combinations of route, dose level, and test species (Clewell and Andersen 1985). Physiologically based pharmacodynamic (PBPD) models use mathematical descriptions of the dose-response function to quantitatively describe the relationship between target tissue dose and toxic endpoints.

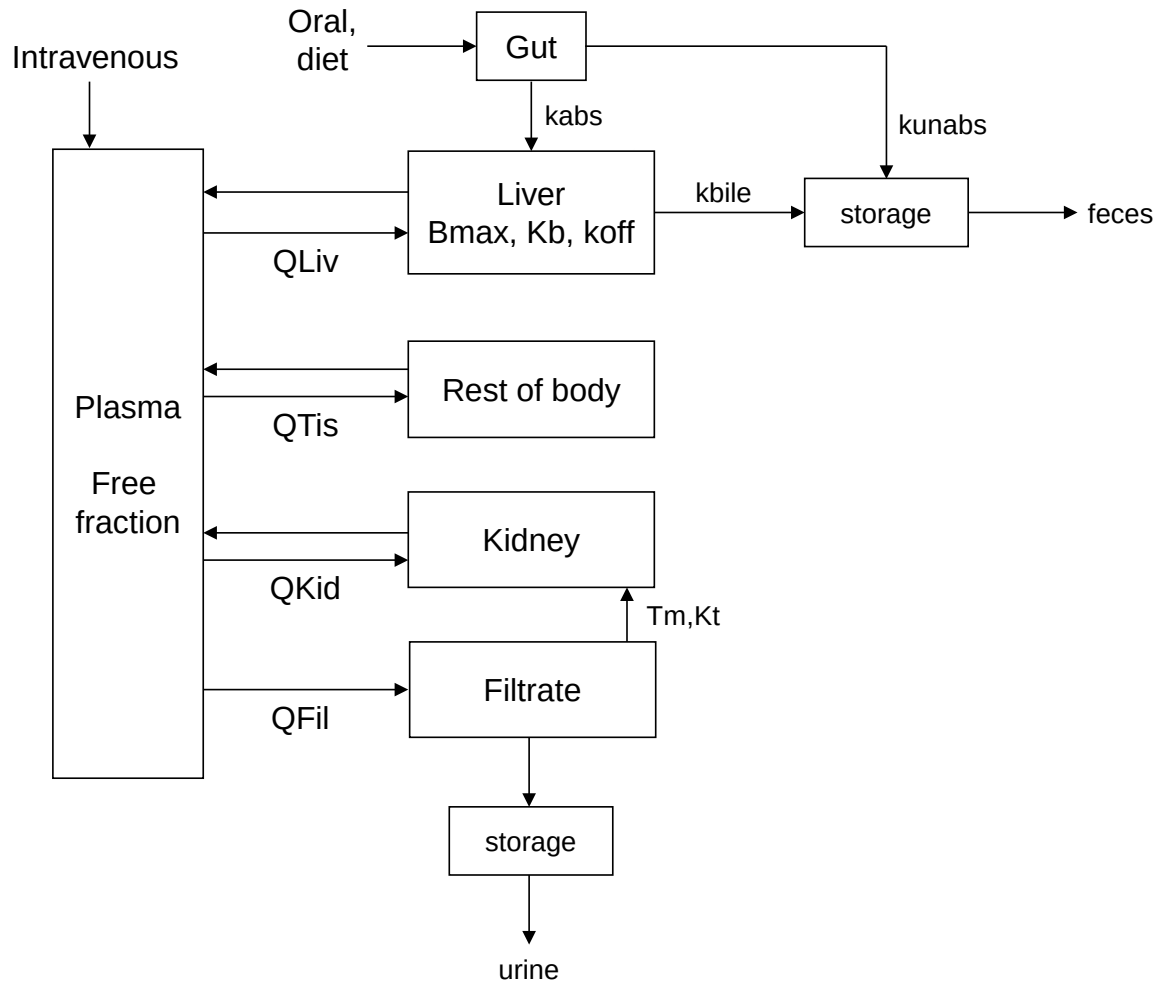
Several PBPK models of PFOA and PFOS have been reported. These include a human model for PFOA and PFOS (Fàbrega et al. 2014, 2016; Loccisano et al. 2011; Worley et al. 2017b), models for PFOA and PFOS in monkeys (Loccisano et al. 2011), models for PFOA and PFOS in rats (Harris and Barton 2008; Loccisano et al. 2012a, 2012b; Tan et al. 2008; Worley and Fisher 2015a. 2015b), and a model for PFOA in mice (Rodriguez et al. 2009). Models of PFOA and PFOS kinetics during gestation and lactation in rats and mice also have been reported (Loccisano et al. 2012a, 2012b; Rodriguez et al. 2009). Various empirical and compartmental models have also been reported (Hoffman et al. 2011; Lorber and Egeghy 2011; Lou et al. 2009; Thompson et al. 2010; Verner et al. 2016; Wambaugh et al. 2013; Wu et al. 2009). Tardiff et al. (2009) utilized a human pharmacokinetic model to estimate an average daily oral dose corresponding to a Reference Dose for PFOA plasma concentration in humans. Cheng and Ng (2017) developed a permeability-limited PBPK model for PFOA in male rats that could be used for *in vitro* to *in vivo* extrapolation. Kim et al. (2018) developed a PBPK model for PFHxS in rats and humans. PBPK models were not identified for other perfluoroalkyls examined in this profile. Given the toxicokinetic differences between compounds, the PFOA, PFOS, and PFHxS PBPK models may not be appropriate for other compounds.

3.1.5.1 Loccisano et al. (2012a, 2012b) Rat Models

Loccisano et al. (2012a) developed a model for simulating the kinetics of PFOA and PFOS in male and female rats. The model was based, in part, on a multi-compartmental model developed by Tan et al. (2008; Andersen et al. 2006). The female rat model (Loccisano et al. 2012a) was subsequently extended to include gestation and lactation (Loccisano et al. 2012b). The general structures of the models are depicted in Figures 3-3, 3-4, and 3-5. Complete lists of parameters and parameter values and the bases

for parameter values and evaluations of model predictions in comparison to observations are described in Loccisano et al. (2012a, 2012b).

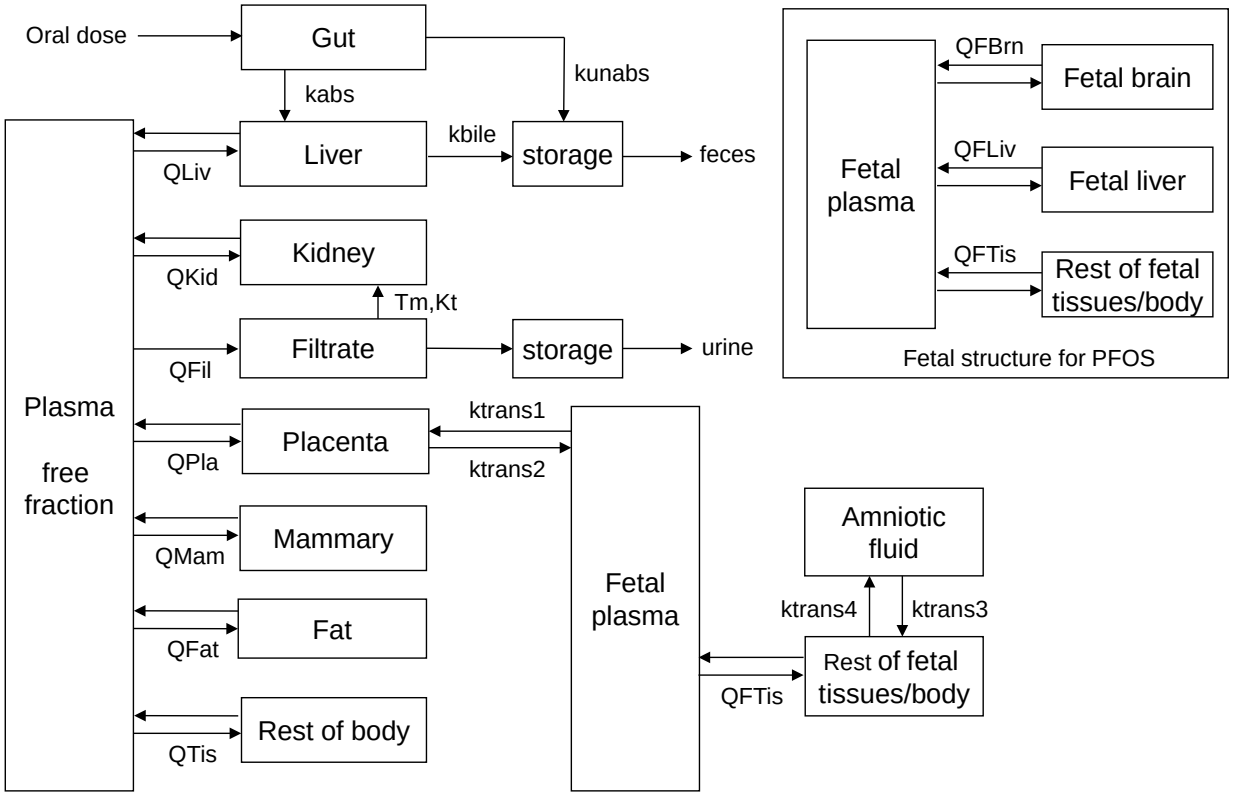
Figure 3-3. Structure of PBPK Model of PFOA and PFOS in the Rat



Bmax = liver binding capacity; kabs = first-order absorption rate constant; Kb = liver binding affinity constant; kbile = biliary excretion rate constant; Koff = liver binding dissociation constant; Kt = affinity constant; kunabs = rate of unabsorbed dose to appear in feces; PBPK = physiologically based pharmacokinetic; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid; QFil = clearance from plasma to glomerular filtrate; QKid = blood flow in and out of kidney; QLiv = blood flow in and out of liver; QTis = blood flow in and out of tissues; Tm = transporter maximum

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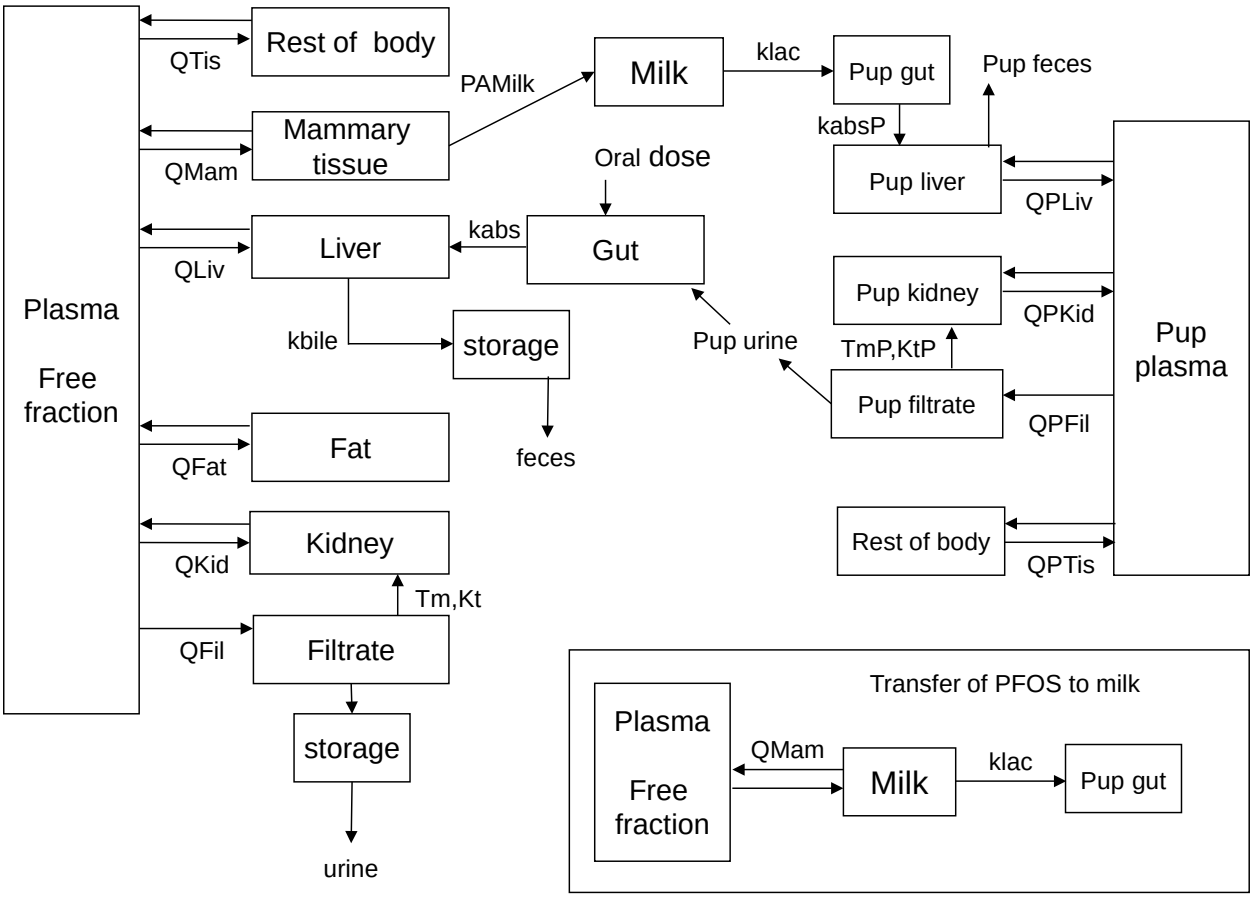
Figure 3-4. PBPK Model Structure for Simulating PFOA and PFOS Exposure During Gestation in the Rat (Dam, Left; Fetus, Right)



kabs = first-order absorption rate constant; kbile = biliary excretion rate constant; Kt = affinity constant; ktrans1/ktrans 2 = transfer between placenta and fetal plasma; ktrans3/ktrans4 = transfer between amniotic fluid and rest of the body; kunabs = rate of unabsorbed dose to appear in feces; PBPK = physiologically based pharmacokinetic; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid; QFat = blood flow in and out of fat; QFBrn = blood flow in and out of fetal brain; QFil = clearance from plasma to glomerular filtrate; QFLiv = blood flow in and out of fetal liver; QFTis = blood flow in and out of fetal tissue; QKid = blood flow in and out of kidney; QLiv = blood flow in and out of liver; QMam = blood flow in and out of mammary tissue; QPla = blood flow in and out of placenta; QTis = blood flow in and out of tissues; Tm = transporter maximum

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Figure 3-5. PBPK Model Structure for Simulating PFOA/PFOS Exposure During Lactation in the Rat (Dam, Left; Pup, Right)



kabs = first-order absorption rate constant; kabsP = pup first-order absorption rate constant; kbile = biliary excretion rate constant; klac = transfer to pup through milk; Kt = affinity constant; KtP = pup affinity constant; PAMilk = transfer from mammary tissue to liver; PBPK = physiologically based pharmacokinetic; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid; QFat = blood flow in and out of fat; QFil = clearance from plasma to glomerular filtrate; QKid = blood flow in and out of kidney; QLiv = blood flow in and out of liver; QMam = blood flow in and out of mammary tissue; QPFil = clearance from pup plasma to glomerular filtrate; QPKid = blood flow in and out of pup kidney; QPLiv = blood flow in and out of pup liver; QPTis = blood flow in and out of pup tissue; QTis = blood flow in and out of tissues; Tm = transporter maximum; TmP = pup transporter maximum

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The basic (i.e., adult nonpregnant rat) model includes compartments representing plasma (including a bound and free fraction), kidney and renal glomerular filtrate, liver, and a lumped compartment representing all other tissues. Two storage compartments are included in the model: one receives perfluoroalkyl from the gastrointestinal tract (unabsorbed) and liver (bile) and the other receives perfluoroalkyl from the glomerular filtrate. The storage compartments were included in the model to simulate time delays between elimination from plasma and appearance of perfluoroalkyl in feces or urine. Absorption from the gastrointestinal tract is simulated as the balance between first-order absorption and fecal excretion of unabsorbed chemical. Absorbed PFOA and PFOS are assumed to be delivered to the liver where saturable binding of PFOS (but not PFOA) to liver proteins occurs. Saturable binding of PFOS in liver was included to simulate the relatively long retention times of PFOS in liver that have been observed in rats. Exchanges between PFOA or PFOS in liver (free fraction), kidney, and other tissues with the free pool in plasma are assumed to be flow-limited (governed by blood flow) with equilibrium determined by the tissue:blood partition coefficient. PFOA and PFOS in plasma are simulated as instantaneous distributions into free and bound fractions. Extensive binding of PFOA and PFOS to plasma proteins has been demonstrated in various animal species including rats (see Section 3.1.2). For PFOA, the free fraction is assigned a constant of 4.5% in females and 0.6% in males. These values were optimized to fit observed kinetics of PFOA in plasma and urine of rats following intravenous and oral exposures (Loccisano et al. 2012a). Adequate fit to observed PFOS plasma kinetics following single doses of PFOS required introducing a time-dependence in binding of PFOS to protein (Loccisano et al. 2012a; Tan et al. 2008). The free fraction for PFOS in plasma decreases from an initial value (after dosing) of 2.2% to a minimum of 0.1% with a $t_{1/2}$ for the change of approximately 14 hours in a 0.25-kg rat ($k=0.035$ hours^{-1}/kg$^{-0.25}$). The relatively short $t_{1/2}$ for the change limits the effects of the time-dependent plasma kinetics over the first 1–2 days of dosing (including peak concentrations) and has no effect on longer-term kinetics or steady state. Although the time-dependence of the free fraction in plasma was needed to simulate short-term plasma PFOS kinetics in rats, the physiological mechanism for a dependence of plasma binding on the time following dosing (i.e., not on concentration of PFOS in plasma or some other dose surrogate) has not been established. Elimination of absorbed chemical occurs by biliary excretion and urinary excretion. Transfer from liver to feces (representing excretion following biliary transfer) is represented as a first-order process acting on the free fraction in liver. Excretion in urine is simulated as the balance between transfer from the free fraction to the glomerular filtrate and renal tubular reabsorption, which removes PFOA and PFOS from the glomerular filtrate and returns it to kidney tissue. Renal tubular reabsorption is simulated as a capacity-limited process with parameters T_m (µg/hour/kg body weight), representing the maximum rate of transport, and K_T (µg/L), representing affinity for the transporter (the concentration in the glomerular filtrate at which reabsorptive transport rate

is half of maximum). This representation of renal tubular reabsorption is used to simulate observed sex differences in elimination of PFOA from plasma, which have been attributed to higher reabsorptive capacity in male rats (see Section 3.1.4). Values for the maximum and affinity parameters for PFOA result in higher reabsorptive clearances from the glomerular filtrate (T_m/K_T=4.1) in male rats compared to female rats (T_m/K_T=0.045), and correspondingly lower urinary clearance of PFOA from plasma in male rats. Reabsorption parameters for PFOS are the same in both sexes and result in reabsorptive clearances that are approximately twice that of PFOA in female rats (T_m/K_T=7.2).

The basic rat model was extended to simulate gestation with inclusion of additional compartments representing adipose and mammary tissue in the dam, placenta, and fetus (Figure 3-4); Loccisano et al. 2012b). Transfer of PFOA and PFOS to the fetus is simulated as a flow-limited transfer to the placenta, with first-order exchange between the placenta and the free fraction in fetal plasma. The free fraction in fetal plasma is simulated as a constant fraction for PFOA and PFOS (i.e., no dependence on time as in the adult). Within the fetus, PFOA in the free fraction of plasma exchanges with a single lumped compartment representing the fetal body, which exchanges with PFOA in amniotic fluid. The fetal PFOS model subdivides fetal tissue into brain, liver, and a lumped compartment for other tissues, all of which undergo flow-limited exchanges with the free fraction of PFOS in fetal plasma. Binding of PFOA and PFOS in fetal liver is assumed to be negligible. Differences in the structure of the fetal models for PFOA and PFOS reflect the differences in the availability of data for estimating parameter values for the various compartments (e.g., perfluoroalkyl concentrations in amniotic fluid, liver).

The lactation model extends the dam portion of the gestational model to include milk and pup (Figure 3-5; Loccisano et al. 2012b). Transfer of PFOA to milk occurs through the mammary gland with flow-limited exchange between plasma and mammary tissue and diffusion into milk from mammary tissue. The model also includes transfer from the pup to the dam, which occurs during maternal stimulation of the neonatal pup to induce elimination and during pup grooming. Data on PFOS in mammary tissue of rodents were not available to establish parameters for a mammary tissue compartment; therefore, the mammary tissue compartment was left out of the PFOS model, and transfer of PFOS to milk is simulated as diffusion directly from plasma. The pup model includes compartments representing the free fraction in plasma, liver, kidney, glomerular filtrate, and a lumped compartment representing all other pup tissues. This structure is essentially identical to the nonpregnant rat model (Loccisano et al. 2012a) with a few differences. Absorption from the gastrointestinal tract is assumed to be complete in pups, and binding in pup liver is assumed to be negligible in pups. There are no storage compartments for biliary or glomerular filtrate perfluoroalkyl in the pup model. Sex differences in renal

tubular reabsorption of PFOA are assumed to develop in response to sexual maturation and, therefore, are not present during lactation (i.e., parameter values are allometrically scaled to pup body weight from the male rat values). Reabsorptive transport parameters for PFOS are allometrically scaled from the lactating dam. The liver/plasma partition coefficient for PFOS in the pups was set lower than that in the dam, based on observations in rats. All other parameters for PFOA and PFOS in the pup were the same or allometrically scaled from values for the dam.

Optimization of parameter values and evaluations of the rat models are described in Loccisano et al. (2012a, 2012b). Data sets utilized in developing and evaluating the nonpregnant rat models included single-dose intravenous and gavage studies and short-term feeding studies (Johnson and Ober 1979; Kemper 2003; Kudo et al. 2007; Perkins et al. 2004). Data used in development and evaluation of the gestation and lactation models included data from gestational and/or lactational exposure studies in rats (Chang et al. 2009; Hinderliter et al. 2005; Kuklenyik et al. 2004; Luebker et al. 2002, 2005a, 2005b; Thibodeaux et al. 2003).

Applications for Dosimetry Extrapolation and Risk Assessment. The wealth of data on pharmacokinetics of PFOA and PFOS in rats allowed an extensive evaluation of the rat models for predicting plasma urinary and liver PFOA and PFOS following single intravenous or single and repeated oral dosing. Inclusion of renal tubular reabsorption parameters in the model provided accurate simulations of sex differences in elimination rates of PFOA from plasma and excretion in urine, and differences in rates of elimination of PFOA and PFOS. The gestation model successfully predicted fetal plasma and liver PFOA and PFOS at the end (or near the end) of pregnancy. Consistent with observations, the model predicts higher fetal plasma concentrations and lower fetal liver concentrations of PFOS compared to maternal, and lower internal exposure (plasma concentrations) to PFOA in the fetus compared to maternal (fetal liver data were not available for PFOA). The lactation model successfully predicted PFOA and PFOS in pup plasma following dosing of the dam. Predicted plasma concentrations of PFOA in nursing pups were approximately 10–50% lower than maternal concentrations, whereas maternal and pup concentrations of PFOS were similar. The model could be used to estimate liver doses and corresponding plasma profiles resulting from single or repeated dosing of adult male or female rats, and maternal-fetal and maternal-pup transfer of PFOA and PFOS. The rat model was evaluated with data from a 14-week oral dosing study and has not been tested for longer exposures. Harris and Barton (2008) developed a PBPK model for PFOS in the rat and found that time adjustments that increased renal clearance and decreased the liver-plasma partition coefficient as a function of time and dose improved predictions of plasma and liver PFOS in adult rats exposed for a period of 105 weeks. Although the

Harris and Barton (2008) model is very different from the Loccisano et al. (2012a) model, these results suggest the possibility that clearance of PFOS may be age- and/or dose-dependent in rats. This may reflect age- or dose-related changes in kidney function, including tubular reabsorption or secretion of PFOS.

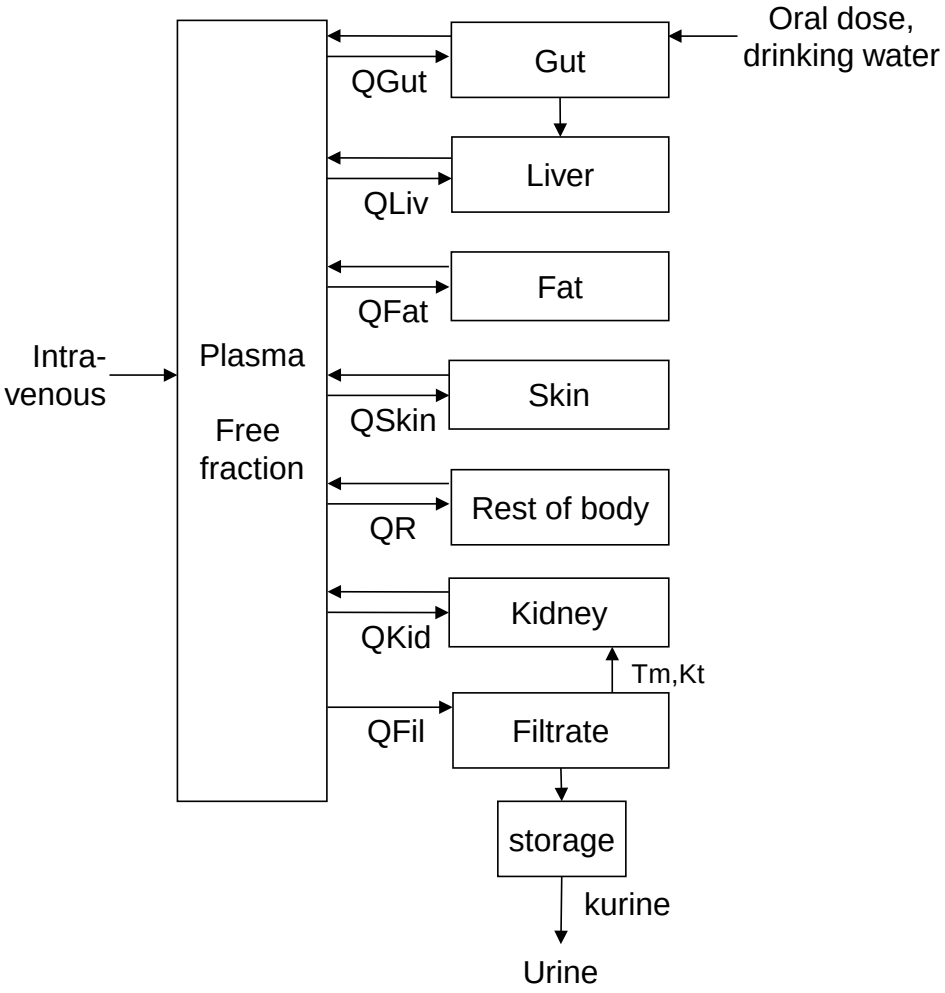
3.1.5.2 Loccisano et al. (2011, 2013) Monkey and Human Models

Loccisano et al. (2011) developed a model for simulating the kinetics of PFOA and PFOS in monkeys and humans. The human model described in Loccisano et al. (2011) was subsequently extended to include simulations of pregnancy and lactation (Loccisano et al. 2013). The monkey model was based, in part, on a multi-compartmental model developed by Tan et al. (2008; Andersen et al. 2006) for simulating the kinetics of plasma and urinary PFOA in monkeys. The structures of the monkey and human models are identical (Figure 3-6) and are very similar to the structure of the rat model (Loccisano et al. 2012a), with inclusion of compartments representing fat and skin, and absence of a storage compartment for biliary transfer. Complete lists of parameters and parameter values and the bases for parameter values and evaluations of model predictions in comparison to observations are reported in Loccisano et al. (2011).

Parameters in the monkey and human models differ in several ways from the rat model. The free fraction in plasma is represented as a constant for both PFOA and PFOS; time-dependency for PFOS in the rat model is absent in the monkey and human models. The parameters for renal tubular reabsorption of PFOA and PFOS are the same for males and females. This is consistent with the absence of evidence for a sex difference in elimination kinetics in monkeys (Butenhoff et al. 2002, 2004a; Seacat et al. 2002).

Values for the affinity constant (K_T) and maximum (T_m) for tubular reabsorption were optimized to plasma concentration kinetics in monkeys. The value for K_T in monkeys was used in the human model. The value for T_m for PFOA in humans was set to yield a plasma elimination $t_{1/2}$ of 2.3 or 3.8 years. The latter two values were derived from estimates of the serum $t_{1/2}$ in populations exposed to PFOA in drinking water (2.3 years; Bartell et al. 2010) or in retired fluorochemical workers (3.8 years; Olsen et al. 2007a). The value for T_m for PFOS in humans was set to yield a plasma elimination $t_{1/2}$ of 5.4 years, based on observations in retired fluorochemical workers (Olsen et al. 2007a). Binding of PFOA and PFOS in the liver was assumed to be negligible in monkeys and humans. Tissue-plasma partition coefficients used in both models were derived from observations in rodents and were the same in the monkey and human models.

Figure 3-6. Structure of PBPK Model for PFOA and PFOS in Monkeys and Humans



Kt = half-saturation constant; kurine = urinary elimination rate; PBPK = physiologically based pharmacokinetic; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid; QFat = blood flow in and out of fat; Qfil = clearance from plasma to glomerular filtrate; QGut = blood flow in and out of gut; QKid = blood flow in and out of kidney; QLiv = blood flow in and out of liver; QR = blood flow in and out of rest of body; QSkin = blood flow in and out of skin; Tm = transport maximum

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Optimization of parameter values and evaluation of the monkey and human models are described in Loccisano et al. (2011). Data sets utilized in developing and evaluating the monkey model included single-dose intravenous and oral studies and repeated-dose oral studies conducted in Cynomolgus monkeys (Butenhoff et al. 2004c; Noker and Gorman 2003; Seacat et al. 2002). Data used in evaluating the human model consisted of serum measurements in people who experienced environmental exposures (Emmett et al. 2006a; Hölzer et al. 2008; Steenland et al. 2009b), adult Red Cross donors (Olsen et al. 2003b, 2008), and retired fluorochemical workers (Olsen et al. 2007a). In general, PFOA and PFOS intakes and exposure durations were not known with certainty in these populations and, as a result, these data do not yield confident evaluations of the ability of the human model to predict intake-plasma level relationships. Follow-up monitoring after a cessation or decrease in exposure can provide data that allow evaluation of the ability of the model to accurately simulate elimination kinetics. Predicted declines in serum PFOA concentrations encompassed observed group mean declines when the T_m for renal tubular reabsorption was set to yield an elimination $t_{1/2}$ of 2.3 or 3.8 years. Group mean declines in serum PFOS were predicted reasonably well for some populations, but not all populations, when the T_m for renal tubular reabsorption was optimized to yield an elimination $t_{1/2}$ of 5.4 years.

The human pregnancy model includes additional compartments representing the free fractions in plasma, amniotic fluid, and a lumped compartment for fetal tissue (Loccisano et al. 2013). The same conceptual approach was used in the rat pregnancy model (Loccisano et al. 2012b, Figure 3-4). Rate constants for placental transfer were initially those from the rat model, adjusted to yield predicated maternal/fetal plasma ratios that agreed with observed maternal/fetal ratios in cord blood (Apelberg et al. 2007b; Fei et al. 2007; Midasch et al. 2007; Washino et al. 2009). Transfers from amniotic fluid to fetus were the same as those used in the rat model, as there were no data on which to base estimates for humans. The lactation model included additional compartments for mammary milk and a lumped compartment representing the infant. Transfer of PFOA to milk is simulated as flow-limited exchange between plasma and milk, governed by mammary tissue blood flow and a milk/plasma partition coefficient. This structure obviated the need to simulate mammary tissue kinetics, for which there were no data in humans. The milk/plasma partition coefficient was calibrated to yield predictions of observed milk/plasma ratios (Fromme et al. 2010; Kärrman et al. 2007). Transfer from maternal milk to infants is the product of the milk concentration and milk production rate (assumed to be equal to sucking rate). The pregnancy model was evaluated by comparing predicted maternal/fetal plasma ratios for PFOA and PFOS with observations from various human monitoring studies (Fei et al. 2007; Fromme et al. 2010; Hanssen et al. 2010; Inoue et al. 2004; Kim et al. 2011; Midasch et al. 2007; Monroy et al. 2008; Tittlemier et al. 2004). The lactation model was evaluated by comparing predicted maternal plasma/milk ratios for PFOA and PFOS

with observations from various human monitoring studies (Fromme et al. 2009; Kärrman et al. 2007; Liu et al. 2011). In general, most model predictions were within plus or minus 2-fold of observations.

Applications for Dosimetry Extrapolation and Risk Assessment. The model predicts plasma concentrations and tissue levels of PFOA and PFOS following intravenous or oral dosing. A skin compartment is included in the model, which may serve for simulating absorption and distribution following deposition onto the skin surface; however, the dermal absorption model was not evaluated in Loccisano et al. (2011). The human model was calibrated to predict $t_{1/2}$ values estimated for human populations (e.g., 2.3 or 3.8 years for PFOA, 5.4 years for PFOS). As a result, comparisons made between observed and predicted serum concentrations evaluate whether or not the populations actually exhibit the $t_{1/2}$ to which the model was calibrated, and not the validity of the model to predict the internal distribution of PFOA or PFOS. It is not currently possible to assess with confidence whether the human model can accurately predict doses to liver or any other tissues. Fábrega et al. (2014) applied the human adult model to estimate plasma concentrations and tissue levels of PFOA and PFOS in human autopsy samples. Exposure inputs to the model were intakes of PFOA and PFOS estimated from public water supply concentrations in the local area where the subjects had resided (Catalonia, Spain) and concentrations in local market basket foods (Domingo et al. 2012a, 2012b). The human model predicted levels of PFOA in plasma and liver that were approximately 10- and 5-fold higher, respectively, than observed. Predicted plasma levels of PFOS were approximately 2-fold higher than observed, and predicted levels of PFOS in kidney were approximately 25% of observed. Fábrega et al. (2014) explored alternative values for tissue/plasma partition coefficients, determined from human autopsy issues (Maestri et al. 2006). The adjusted partition coefficients improved predictions of observed tissue PFOA and PFOS levels. Although the model could be applied to predicting plasma concentrations of PFOA and PFOS or intakes associated with specific plasma concentrations (e.g., oral MRLs), it is not clear what advantages the model offers over simpler empirical or compartmental models similarly calibrated to predict the serum $t_{1/2}$. The monkey model has been more thoroughly evaluated for predicting plasma and urinary kinetics of PFOA and PFOS. This was possible because of the availability of more extensive experimental data on plasma and urine PFOA and PFOS following intravenous and oral (single and repeated) dosing in male and female monkeys. Nevertheless, data on internal distribution were not available to allow evaluation of how well the monkey model predicts doses to the liver or other tissues. Predictions of plasma PFOA and PFOS concentrations from the monkey (and human) model were highly sensitive to values assigned to the maximum rate for tubular reabsorption (T_m) and other parameters that govern urinary elimination of PFOA and PFOS (e.g., free fraction in plasma and glomerular filtration rate; Loccisano et al. 2011). Optimization of the monkey models relied heavily on adjusting these same parameters and, for the human

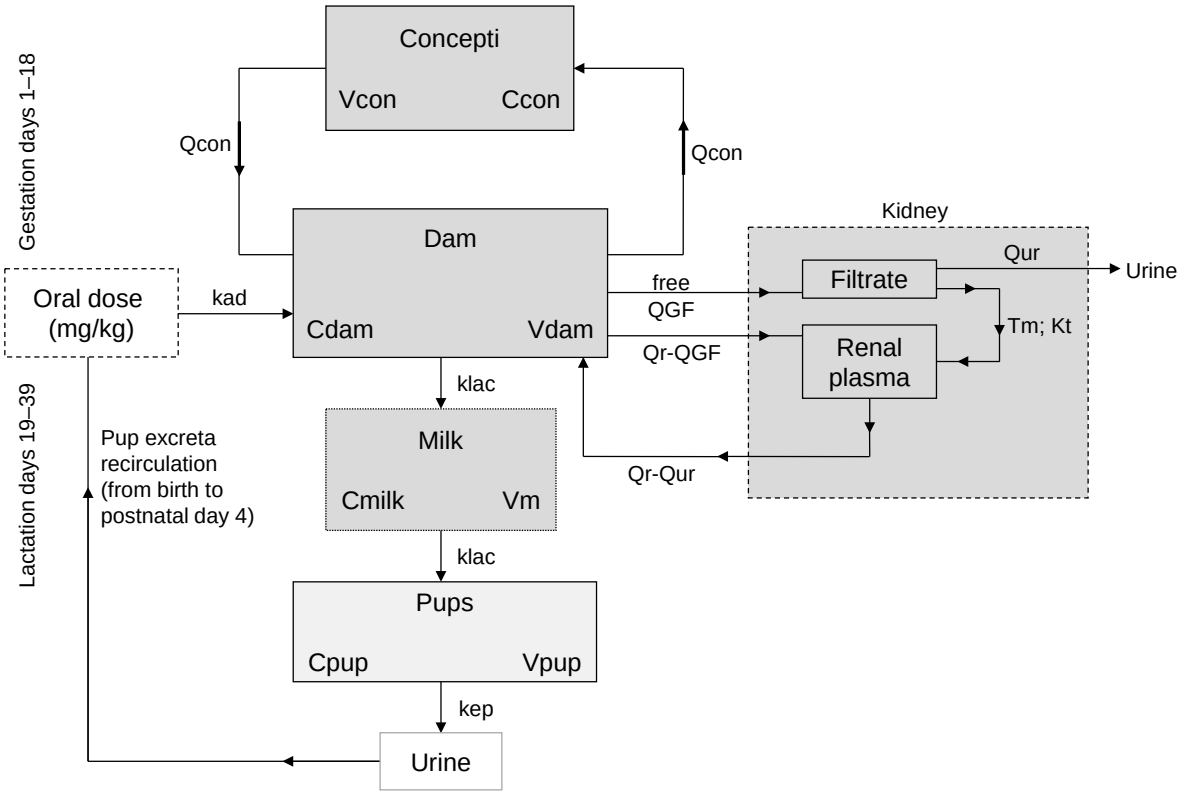
model, the target plasma elimination $t_{1/2}$ was achieved solely by adjusting T_m. Thus, despite the complexity of the models, their potential to accurately predict plasma elimination kinetics and, therefore, steady-state plasma concentrations and associated oral intakes, depends largely on how well they predict plasma clearance. If plasma clearance and the free-fraction in plasma can be reliably predicted empirically for the animal species of interest, then far simpler compartmental models can be used for dosimetry extrapolation of steady-state free plasma concentrations.

3.1.5.3 Rodriguez et al. (2009) Mouse Model

Rodriguez et al. (2009) developed a model for simulating the maternal-fetal and maternal-pup kinetics of PFOA in mice. The general structure of the model is depicted in Figure 3-7. Complete lists of parameters and parameter values and the bases for parameter values and evaluations of model predictions in comparison to observations are reported in Rodriguez et al. (2009). The maternal, fetal, and pup systems are simulated as single well-mixed compartments. Absorption from the gastrointestinal tract is simulated as first-order with complete absorption of the ingested dose. Elimination of absorbed PFOA from the maternal system is simulated as the balance between glomerular filtration and renal tubular reabsorption. The latter is represented as a saturable process with parameters T_m and K_T. Transfer to the fetus is flow-limited and governed by a fetus/maternal partition coefficient and placental blood flow. Transfer from the maternal system to the pup by lactation is simulated as first-order governed by a lactation transfer rate constant. Elimination of PFOA from the pup is first-order to urine. Data sets utilized in developing and evaluating the mouse model included oral gestational dosing studies.

Applications for Dosimetry Extrapolation and Risk Assessment. The model predicted observed concentrations of PFOA in maternal, fetal, and pup serum following oral gestational exposures to mice (Abbott et al. 2007; Lau et al. 2006; White et al. 2007). Residuals for predictions are presented, which provide a quantitative measure of how well the model predicted observations (Rodriguez et al. 2009). Similar to the rat, the mouse model predicts higher internal exposure (serum PFOA concentrations) in the maternal system compared to the fetus. It also predicts accelerated loss of PFOA from the maternal system during lactation. The model simulates the maternal, fetal, and pup systems as single compartments. Although this serves for simulating plasma concentrations (the main objective of the modeling effort), it does not allow for simulation of tissue levels of PFOA in the maternal system, fetus, or pup.

Figure 3-7. Renal Resorption Pharmacokinetic Model of Gestation and Lactation used in the Analysis of CD-1 Mice



Ccon = concentration in concepti; Cdam = concentration in dam; Cmilk = concentration in milk; Cpup = concentration in pup; kad = first-order absorption rate; kep = urinary excretion rate; klac = transfer rate via milk; Kt = half-saturation constant; Qcon = blood flow to and from placenta; QGF = glomerular filtrate; Qr = renal plasma flow; Qur = urine flow; Tm = transport maximum; Vcon = volume in concepti; Vdam = volume in dam; Vmilk = volume in milk; Vpup = volume in pup

Source: Rodriguez et al. 2009 (reproduced with permission of Elsevier Inc. in the format reuse in a government report via Copyright Clearance Center; Reproductive Toxicology by Reproductive Toxicology Center; Washington, DC)

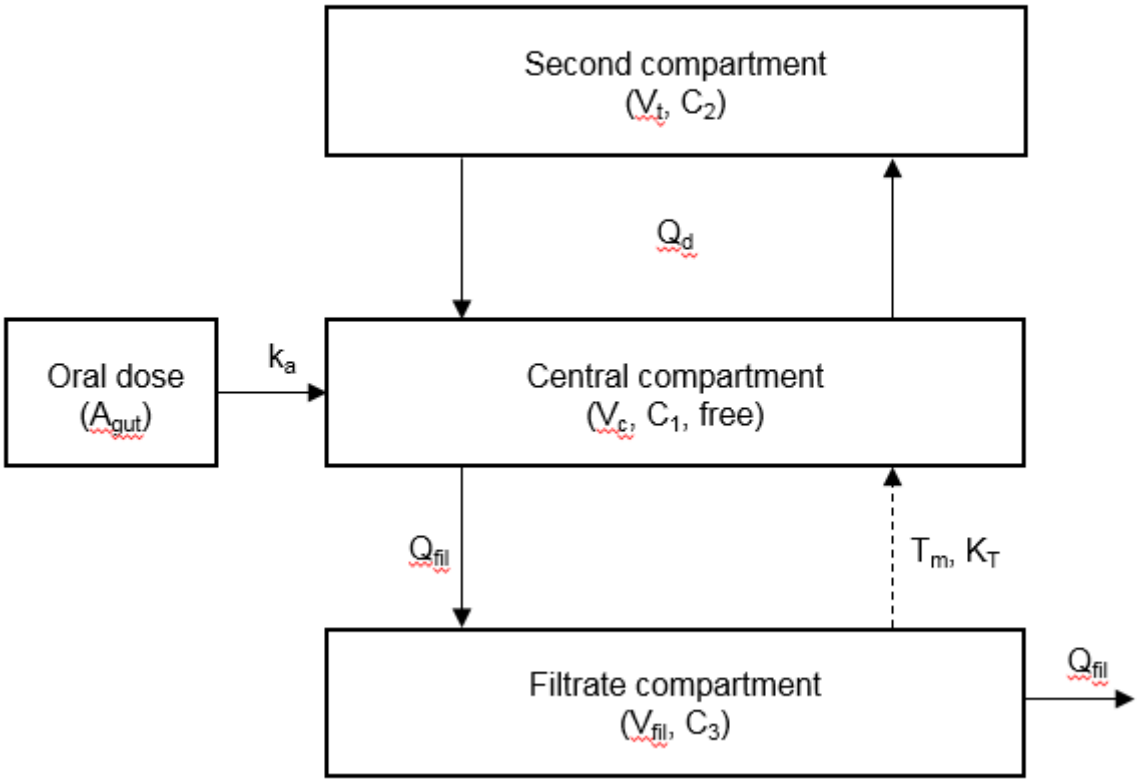
3.1.5.4. Wambaugh et al. 2013 (Andersen et al. 2006) Model

The Wambaugh et al. (2013) model is a three-compartment model based on the three-compartmental monkey model of Andersen et al. (2006). The structure of the two models are identical (Figure 3-8). Parameter values for the Wambaugh et al. (2013) model are presented in Table 3-7. The model includes a central compartment, a secondary distribution compartment, and a renal glomerular filtrate compartment. The central compartment (C1), which includes plasma, receives PFOA or PFOS from oral dosing (first-order k_a, $hour^{-1}$) and exchanges perfluoroalkyl with the secondary compartment (C2, which lumps all other tissues and distribution volumes into a single compartment) and with the glomerular filtrate (C3). A fraction of the perfluoroalkyl in C1 is free (Free) and available for exchange with C2 and C3. Exchanges between C1 and C2 are first order (k_{12}, k_{21}, $hour^{-1}$) with k_{21} assigned a value equal to the $R_{V2/V1}$, where $R_{V2/V1}$ is the ratio of the volumes of the two compartments (V_2/V_1). Transfer of perfluoroalkyl into the glomerular filtrate is first order and governed by the glomerular filtration rate (Q_{filc}, L/hour). Transfer for perfluoroalkyl from the glomerular filtrate to C1 (representing renal tubular reabsorption) is capacity limited (T_{maxc}, µmol/hr; K_T, µM). Perfluoroalkyl that is not reabsorbed is excreted.

Parameter values for the various species and strains were estimated from experimental pharmacokinetic data for each species and strain using Bayesian Markov Chain Monte Carlo (MCMC) analysis. Studies that provided data used to estimate parameter values are listed in Wambaugh et al. (2013). The parameter values shown in Table 3-7 are the mean values and posterior distributions (95% credible interval) from the MCMC analyses.

Applications for Dosimetry Extrapolation and Risk Assessment. Wambaugh et al. (2013) applied the model to predicting internal doses (mean and maximum serum concentrations and plasma AUC) for Benchmark Dose Software (BMDS) modeling and for comparing internal dosimetry from *in vivo* toxicity studies to estimates of potency (AC_{50}, maximum Efficacy) from *in vitro* studies. EPA applied the Wambaugh et al. (2013) model to deriving chronic oral reference doses (RfDs) for PFOA and PFOS (EPA 2016e, 2016f). The model was used to predict internal doses (time-integrated plasma PFOA or PFOS concentrations) achieved in toxicity studies conducted in various laboratory animal models (CD-1 mouse, C57Bl/6 mouse, Sprague-Dawley rat, Cynomolgus monkey). Plasma concentrations were then extrapolated to equivalent steady-state concentrations in humans using a model of first-order elimination of PFOA and PFOS from plasma. The same approach was used to derive MRLs for PFOA and PFOS (see Appendix A).

Figure 3-8. Andersen et al. (2006) Pharmacokinetic Model with Oral Absorption



A_{gut} is the amount of chemical in the gut; k_a is the first-order rate constant for absorption from the gut; Q_{fil} is the flow through the filtrate compartment; C_1, C_2, and C_3 are the chemical concentrations in the central, second, and filtrate compartments, respectively; V_c, V_t, and V_{fil} are the volumes of distribution of the central, second, and filtrate compartments; free is the free fraction of compound in the central compartment; Q_d is the flow between the central and second compartments; the saturable resorption process from the filtrate back into the central compartment is modeled with Michaelis-Menten kinetics, with a maximum rate $T_{maximum}$ and a half-maximum concentration K_T.

Reprinted from Wambaugh et al. (2013) by permission of Oxford University Press

Table 3-7. Estimated and Assumed Pharmacokinetic Parameters for the Modified Andersen et al. (2006) Model for PFOA and PFOS

		Parameter (units)										
Reference	Species	BW (kg)	Cardiac output[a] ($L/hour/kg^{0.74}$)	K_a ($hour^{-1}$)	V_{cc} (L/kg)	k_{12} ($hour^{-1}$)	$R_{V2:V1}$ (unitless)	T_{maxc} (μmole/hour)	K_T (μM)	Free (unitless)	Q_{filc} (L/hour)	V_{filc} (L/kg)
						PFOA[b]						
Lou et al. (2009)	Mouse: CD1 (F)	0.02	8.68	290 (0.6–73,000)	0.18 (0.16–2.0)	0.021 ($3.1x10^{-10}$ to $3.8x10^{4}$)	1.07 (0.26–5.84)	4.91 (1.75–2.96)	0.037 (0.0057–0.17)	0.011 (0.0026–0.051	0.077 (0.015–0.58)	$9.7x10^{-4}$ ($3.34x10^{-9}$–7.21)
Dewitt et al. (unpublished)	Mouse: C57Bl/6 (F)	0.02	8.68	340 (0.53–69,000)	0.17 (0.13–2.3)	0.35 (0.058–52)	53 (11–97)	2.7 (0.95–22)	0.12 (0.033–0.24)	0.034 (0.014–0.17)	0.017 (0.010–0.081)	$7.6x10^{-5}$ ($2.7x10^{-10}$–6.4)
Kemper (2003)	Rat: Sprague-Dawley (F)	0.20 (0.16–0.23)[c]	12.39	1.7 (1.1–3.1)	0.14 (0.11–0.17)	0.098 (0.039–0.27)	9.2 (3.4–28)	1.1 (0.25–9.6)	1.1 (0.27–4.5)	0.086 (0.031–0.23)	0.039 (0.014–0.13)	$2.6x10^{-5}$ ($2.9x10^{-10}$–28)
Kemper (2003)	Rat: Sprague-Dawley (M)	0.24 (0.21–0.28)[c]	12.39	1.1 (0.83–1.3)	0.15 (0.13–0.16)	0.028 (0.0096–0.08)	8.4 (3.1–23)	190 (5.5–50,000)	0.092 ($3.4x10^{-4}$–1.6)	0.08 (0.03–0.22)	0.22 (0.011–58)	0.0082 ($1.3x10^{-8}$–7.6)
Butenhoff et al. (2004b)	Monkey: Cynomolgus (M/F)	7 (m), 4.5 (f)	19.8	230 (0.27–73,000)	0.4 (0.29–0.55)	0.0011 ($2.4x10^{-10}$ to 3.5x104)	0.98 (0.25–3.8)	3.9 (0.65–9,700)	0.043 ($4.3x10^{-5}$–0.29	0.01 (0.0026–0.038)	0.15 (0.02–24)	0.0021 ($3.3x10^{-9}$–6.9)
		Parameter (units)										
Reference	Species	BW[d] (kg)	Cardiac output[e] ($L/hour/kg^{0.74}$)	K_a ($hour^{-1}$)	V_{cc} (L/kg)	k_{12} ($hour^{-1}$)	$R_{V2:V1}$ (unitless)	T_{maxc} (μmol/hour)	K_T (μM)	Free (unitless)	Q_{filc} (L/hour)	V_{filc} (L/kg)
						PFOS						
Chang et al. (2012)	Mouse: CD1 (F)	0.02	8.68	1.16 (0.617–42,400)	0.264 (0.24–0.286)	0.0093 (2.63e-10–38,900)	1.01 (0.251–4.06)	57.9 (0.671–32,000)	0.0109 ($1.44x10^{-5}$–1.45)	0.00963 (0.00238–0.0372)	0.439 (0.0125–307)	0.00142 ($4.4x10^{-10}$–6.2)
Chang et al. (2012)	Mouse: CD1 (M)	0.02	8.68	433.4 (0.51–803.8	0.292 (0.268–0.317	2,976 (2.8e-10–4.2e4)	1.29 (0.24–4.09)	1.1e4 (2.1–7.9e4)	381 ($2.6x10^{-5}$–2,900)	0.012 (0.0024–0.038)	27.59 (0.012–283)	0.51 ($3.5x10^{-10}$–6.09)
Chang et al. (2012)	Rat: Sprague-Dawley (F)	0.203	12.39	4.65 (3.02–1,980)	0.535 (0.49–0.581)	0.0124 (3.1e-10–46 800)	0.957 (0.238–3.62)	1,930 (4.11–83,400)	9.49 (0.00626–11,100)	0.00807 (0.00203–0.0291)	0.0666 (0.0107–8.95)	0.0185 ($8.2x10^{-7}$–7.34)
Chang et al. (2012)	Rat: Sprague-Dawley (M)	0.222	12.39	0.836 (0.522–1.51)	0.637 (0.593–0.68)	0.00524 (2.86e-10–43,200)	1.04 (0.256–4.01)	1.34e-06 (1.65e-10–44)	2.45 ($4.88x10^{-10}$–60 300)	0.00193 (0.000954–0.00249)	0.0122 (0.0101–0.025)	0.000194 ($1.48x10^{-9}$–5.51)
Seacat et al. (2002) and Chang et al. (2012)	Monkey: Cynomolgus (M/F)	3.42	19.8	132 (0.225–72,100)	0.303 (0.289–0.314)	0.00292 (2.59e-10–34,500)	1.03 (0.256–4.05)	15.5 (0.764–4,680)	0.00594 (2.34 $x10^{-5}$–0.0941)	0.0101 (0.00265–0.04)	0.198 (0.012–50.5)	0.0534 ($1.1x10^{-7}$–8.52)

[a]Cardiac outputs obtained from Davies and Morris (1993).
[b]Means and posterior distributions from the Bayesian Markov Chain Monte Carlo (MCMC) analysis (95% credible interval in parentheses) are reported.
[c]Estimated average body weight (BW) for species used except with Kemper (2003) study where individual rat weights were available and assumed to be constant.
[d]Average BW for species: individual-specific BWs.
[e]Cardiac outputs obtained from Davies and Morris (1993).

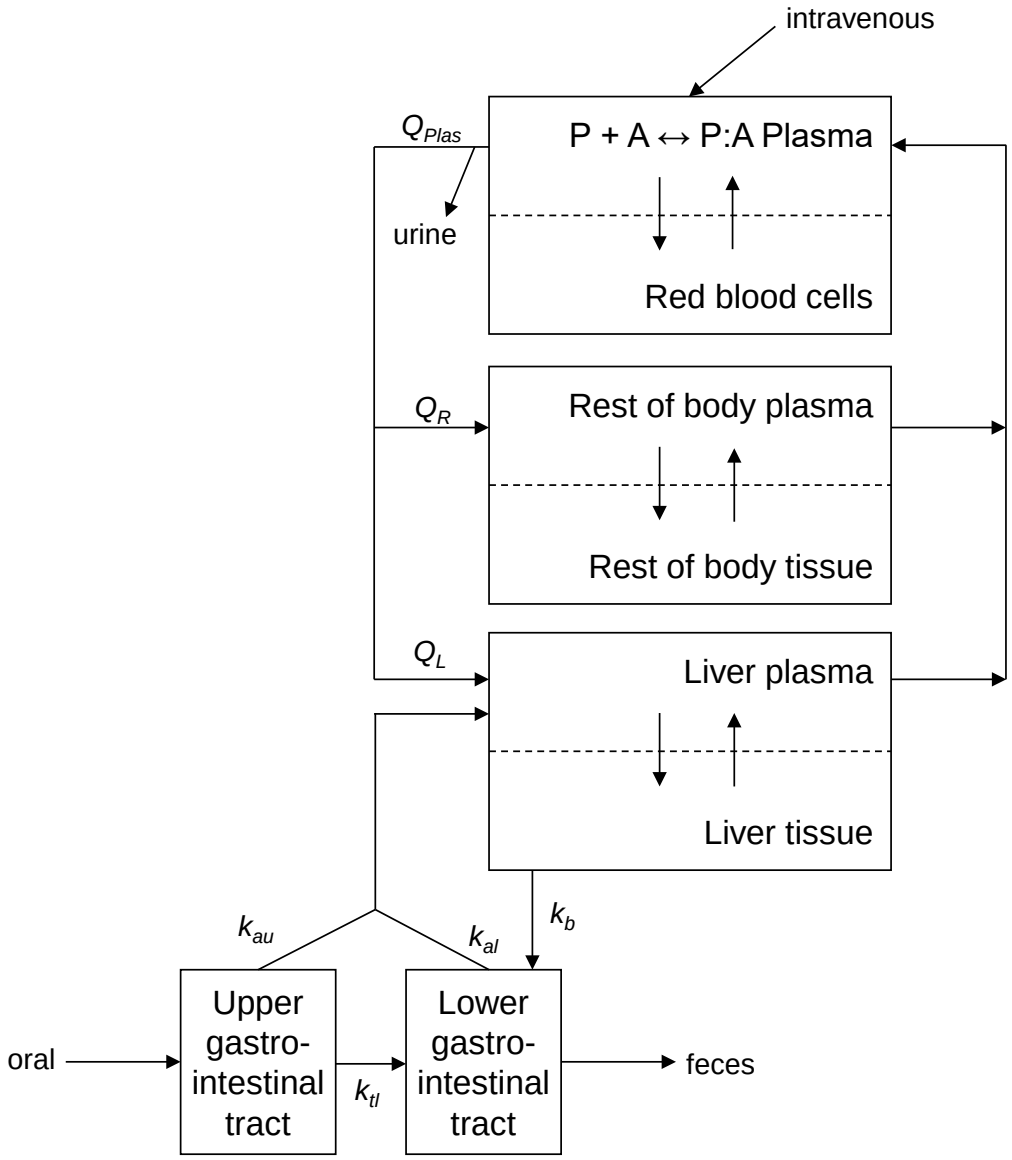
Source: Wambaugh et al. (2013)

3.1.5.5 Harris and Barton (2008) Rat Model

Harris and Barton (2008) developed a model for simulating PFOS kinetics in adult rats. The general structure of the model is depicted in Figure 3-9. Complete lists of parameters and parameter values and the bases for parameter values and evaluations of model predictions in comparison to observations are reported in Harris and Barton (2008). The model includes systemic compartments representing blood (including a bound and free fraction of plasma and red blood cells), liver, and a lumped compartment representing all other tissues. The gastrointestinal tract is simulated as separate compartments representing the upper and lower tracts. Absorption occurs from both the upper and lower tracts, with distinct first-order rate constants assigned to each. Biliary PFOS is transferred from liver to the lower tract. Absorbed PFOS is delivered to the liver where it enters plasma to be distributed to other tissues. Exchanges between PFOS in plasma and all tissues are assumed to be diffusion-limited, with the free pool in plasma participating in the exchange with red blood cells, and the total plasma pool exchanging with liver and all other tissues. Binding of PFOA to plasma albumin is assumed to be saturable, with a dissociation constant 10^{-7} M and a maximum capacity 4.1x 10^{-4} M. This is implemented by assigning bound PFOA to a subcompartment of plasma in which PFOA enters (binds) or exits (unbinds) at rates governed by binding *on* and *off* rates, respectively, that yield a dissociation constant of 10^{-7} M. Elimination of absorbed chemical occurs by biliary excretion and urinary excretion. Transfer from liver to the lower gastrointestinal tract (representing excretion following biliary transfer) is represented as a first-order process acting on the total amount of PFOS in liver. PFOA is transferred to urine from the free fraction of plasma at a rate governed by a urinary clearance parameter, which is assigned a value of 28% of renal plasma flow.

In evaluating performance of the model for simulating PFOS concentrations in a chronic rat feeding study, Harris and Barton (2008) found that the model predicted plasma and liver concentrations measured at 4 and 16 weeks, but over-predicted both at 104 weeks. Performance of the model was improved by having renal clearance increase and the liver/plasma partition coefficient decrease as a function of time (i.e., study duration). These results suggest the possibility that clearance of PFOS may be dependent on age and/or a metric of dose (e.g., cumulative internal dose). This may reflect age- or dose-related changes in kidney function, including tubular reabsorption or secretion of PFOS.

Figure 3-9. Conceptual Representation of a Physiologically Based Pharmacokinetic Model for PFOS Exposure in Rats



k_{al} = rate of absorption from the lower gastrointestinal tract; k_{au} = rate of absorption from the upper gastrointestinal tract; k_b = maximum rate of biliary elimination; k_{tl} = rate of transfer from upper-lower gastrointestinal tract; P:A = PFOS-bound albumin in plasma; PFOS = perfluorooctane sulfonic acid Q_L = plasma flow rate to the liver; Q_{Plas} = plasma flow rate by the heart; Q_R = plasma flow rate to the rest of body

Source: Harris and Barton 2008 (reproduced with permission of Elsevier Ireland Ltd. in the format reuse in a government report via Copyright Clearance Center; Toxicology Letters by European Societies of Toxicology)

Applications for Dosimetry Extrapolation and Risk Assessment. The model simulates kinetics of PFOS following oral or intravenous dosing in adult rats and includes several features that are different from other PBPK models of perfluoroalkyls. The Harris and Barton (2008) model includes a red cell compartment that allows predictions of whole-blood concentrations. The utility of this feature remains to be determined, since PFOS does not appreciably concentrate in red blood cells and PFOS (and other perfluoroalkyls) is typically monitored in the central compartment with measurements of plasma or serum concentrations. The model assumes that the total concentration of PFOS (not just the free concentration) in plasma is available for distribution to liver and other tissues, whereas other models assume that only the free pool in plasma exchanges with tissues. The practical consequence of this difference may not be significant in terms of the toxicokinetics of PFOS if the tissue/plasma partition coefficients in the various models were estimated based on the relevant perfluoroalkyl pool in plasma. However, without basing distribution kinetics on the free concentration, it is not possible for concentration-dependent free fraction to be modeled. The model assumes time-dependence in the liver uptake and urinary excretion of PFOS, which were needed to improve predictions of plasma and liver concentrations of PFOS during chronic exposures. Other rat models (Loccisano et al. 2012a) have not been similarly evaluated. A mechanistic understanding of the time-dependent changes in PFOS kinetics will be important for applications of these models for dosimetry extrapolation across exposure durations.

3.1.5.6 Worley and Fisher (2015a, 2015b) Rat Model

Worley and Fisher (2015a, 2015b) expanded the Loccisano et al. (2012a) adult rat model to include simulation of renal proximal tubule apical (tubule-lumen) and basolateral (tubule-plasma) PFOA transport. This configuration allowed the use of data from *in vitro* studies of kinetics of specific transporters thought to be involved in proximal tubular transport of PFOA in the parametrization of the model. The kidney compartment was expanded to include compartments representing the proximal tubule lumen (glomerular filtrate) and proximal tubule cells. In the model, transfer of PFOA to the tubule lumen is governed by the glomerular filtration rate, represented by a clearance parameter (L/hour/kg kidney). PFOA in the tubule lumen can undergo first-order transfer to urine or saturable transport into the tubule cell (Km, Vmax). PFOA in the tubule exchanges with PFOA in plasma by three mechanisms: saturable transport from plasma into the cell (Km, Vmax), first-order transport from the cell to plasma (kefflux), or bidirectional diffusion between the cell and plasma (kdif). Parameter values (Km, Vmax) for apical and basolateral transport of PFOA were derived from *in vitro* estimates for OATP1a1 (apical) and OAT1 and OAT3 (basolateral) (Nakagawa et al. 2008; Weaver et al. 2010; Yamada et al. 2007). These estimates were scaled to kidney proximal tubule cell mass (Hsu et al. 2014) and the mass-scaled estimates

of Vmax were adjusted with relative activity factors, which were calibrated to *in vivo* observations of plasma PFOA elimination kinetics in rats (Kemper 2003). Values for kefflux (proximal tubule cell to kidney plasma) and kdif (diffusion between kidney plasma and the tubule cell) were also calibrated with *in vivo* data (Kemper 2003; Kudo et al. 2007).

Calibration of the relative activity factor for apical and basolateral membrane transport of PFOA to serum observations made in male and female rats resulted in lower values for activity of both transporters in females compared to males. This resulted in the model predicting lower rates of reabsorptive transfer of filtered PFOA to plasma, and higher renal and systemic (plasma) clearance in females compared to males. Because proximal tubule transporters were assumed to be saturable, the model predicts an increase in clearance with increasing PFOA dose, with larger increases in clearance at lower doses in females compared to males. The model simulated the observed dose-dependent increase in serum clearance (decreasing serum $t_{1/2}$) and higher serum clearance of PFOA (lower $t_{1/2}$) in female rats compared to males (Kemper 2003).

3.1.5.7 Worley et al. (2017b) Human Model

Worley et al. (2017b) scaled and calibrated the Worley and Fisher (2015a, 2015b) rat model to simulate PFOA kinetics in humans exposed to PFOA in drinking water. Physiological parameters were allometrically scaled to the human. Tissue-plasma partition coefficients were derived from human autopsy data (kidney, liver) or studies of distribution of PFOA in rats (Fabrega et al. 2014; Kudo et al. 2007; Perez et al. 2013). Parameter values (Km, Vmax) for apical and basolateral transport of PFOA were derived from *in vitro* estimates for OAT4 (apical) and OAT1 and OAT3 (basolateral) (Nakagawa et al. 2008; Weaver et al. 2010; Yang et al. 2010; Yamada et al. 2007). These estimates were scaled to kidney proximal tubule cell mass (Hsu et al. 2014) and the mass-scaled estimates of Vmax were adjusted with relative activity factors. Parameters that control apical and basolateral transfers of PFOA in the proximal tubule and absorption in the gastrointestinal tract were calibrated against data on serum PFOA concentrations measured in people who drank water from a municipal water supply (Worley et al. 2017b). Model parameter values were adjusted to achieve agreement with geometric mean serum PFOA concentrations measured at two times separated by 6 years. The model was evaluated by comparing predicted and observed serum PFOA concentrations in populations exposed to PFOA in drinking water (Bartell et al. 2010; Emmett et al. 2006b; Steenland et al. 2009a, 2009b). A sensitivity analysis of the model identified that following biokinetic parameters that had standardized sensitivity coefficients >0.1: parameters controlling proximal tubule transport and urinary excretion, plasma-liver partition coefficient,

biliary excretion and protein binding. These parameters, along with drinking water consumption, were assigned probability distributions to conduct a Monte Carlo analysis of predicted serum PFOA predictions associated with exposures to PFOA in drinking water. The probabilistic model simulated interindividual variability in serum PFOA concentrations observed in exposed populations (Bartell et al. 2010; Emmett et al. 2006b; Steenland et al. 2009a, 2009b). These results suggest that that biokinetic variability, as well as exposure variability, may contribute to variability in serum PFOA concentrations observed in populations.

3.1.5.8 Fàbrega et al. (2014, 2016) Human Model

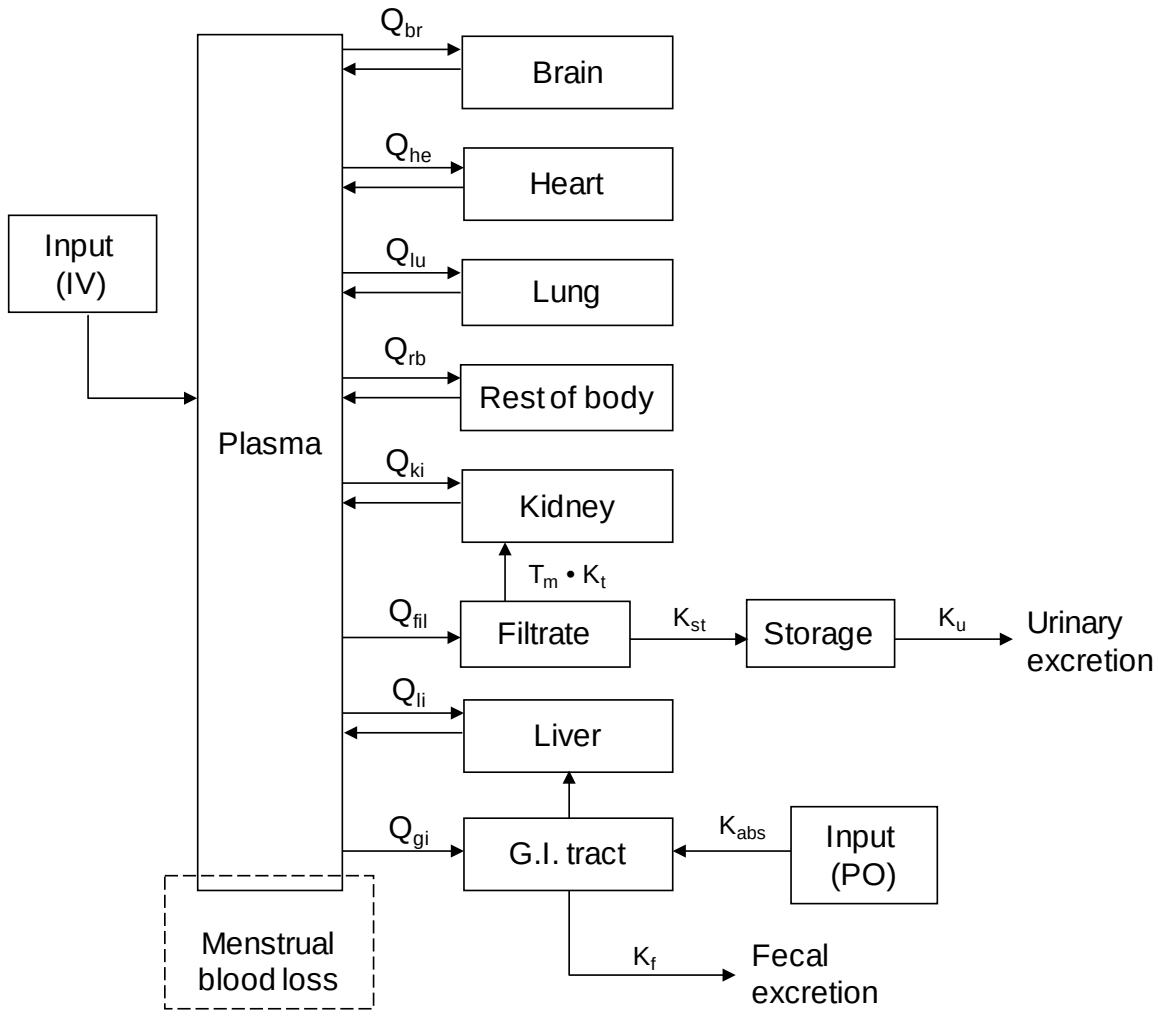
Fàbrega et al. (2014, 2016) modified the Loccisano et al. (2011, 2013) human models for PFOA and PFOS with inclusion of brain and lung compartments and removal of the skin compartment. Tissue-plasma partition coefficients were re-estimated using data from human cadavers (Maestri et al. 2006) in place of estimates based on rat data (Loccisano et al. 2011). The major differences in the partition coefficients for PFOA were lower values for liver in humans (1.03) compared to rats (2.20), higher values for fat in humans (0.47) compared to rats (0.04), and inclusion of partition coefficients for brain (0.17) and lung (1.27). For PFOS, the major differences in the partition coefficients were lower values for liver in humans (2.67) compared to rats (3.72) and higher values for fat in humans (0.33) compared to rats (0.14). Values for parameters that control urinary excretion (Tm and Km for reabsorptive transport from glomerular filtrate to kidney tissue) were recalibrated based on plasma concentration data (Ericson et al. 2007). Fàbrega et al. (2014) compared predictions to observed concentrations of PFOA and PFOS in cadaver samples (from Tarragona County, Spain) for constant intakes of 0.11 µg/day for PFOA or 0.13µg/day for PFOS. Better agreement with observations was achieved with partition coefficients based on cadaver data. Fàbrega et al. (2016) performed a quantitative uncertainty analysis of predictions of tissue PFOA and PFOS concentrations by assigning lognormal probability distributions to renal transport parameters, the unbound fraction in plasma, and intake. Probability distributions for PFOA and PFOS intakes were based on data from Domingo et al. (2012a, 2012b). Distributions for biokinetic parameters were established to achieve a coefficient of variation of 0.3 (Allen et al. 1996; Brochot et al. 2007; Sweeney et al. 2001). Observations of tissue PFOA and PFOS were within uncertainty bounds on predictions.

3.1.5.9 Kim et al. (2018) Rat and Human Model

Kim et al. (2018) developed a model for simulating the kinetics of PFHxS in rats and humans. The structures of the rat and human models are identical (Figure 3-10). Complete lists of parameters and parameter values and the bases for parameter values and evaluations of model predictions in comparison to observations are reported in Kim et al. (2018). The model includes compartments representing plasma (including a bound and free fraction), brain, gastrointestinal tract, heart, lung, kidney and renal glomerular filtrate, liver, and a lumped compartment representing all other tissues. A storage compartment receives PFHxS from the glomerular filtrate and is included in the model to simulate the time delay between elimination from plasma and appearance of PFHxS urine. Absorption from the gastrointestinal tract is simulated as the balance between first-order absorption and fecal excretion of unabsorbed PFHxS. Absorbed PFHxS is assumed to be delivered to the liver. Exchanges between PFHxS in tissues with the free pool in plasma are assumed to be flow-limited (governed by blood flow) with equilibrium determined by the tissue:plasma partition coefficient. Partition coefficients were estimated from the tissue:plasma concentration ratios measured in female and male rats 14 days after a single intravenous dose of PFHxS (0.5–10 mg/kg). Values for each sex were significantly different for brain, lung, liver, spleen, gastrointestinal tract, adipose, and skeletal muscle; in each case, male>female. The highest partition coefficient was in male liver (approximately 0.13), with the value for female being approximately half of the male value. PFHxS in plasma is simulated as instantaneous distributions into free and bound fractions. The free fraction was estimated from ultrafiltration studies of rat and human plasma. The free fraction was assigned a constant of 0.069% in female and 0.076% in male rats.

Elimination of absorbed PFHxS in the rat model occurs by fecal and urinary excretion. Fecal excretion of absorbed PFHxS is represented as flow-limited transfer from plasma to the gastrointestinal tract and first order transfer from the gastrointestinal tract to feces. Excretion in urine is simulated as the balance between transfer from the free fraction of plasma to the glomerular filtrate and renal tubular reabsorption, which removes PFHxS from the glomerular filtrate and returns it to kidney tissue. Renal tubular reabsorption is simulated as a capacity-limited process with parameters T_m (μg/hour), representing the maximum rate of transport, and K_T (μg/L), representing affinity for the transporter (the concentration in the glomerular filtrate at which reabsorptive transport rate is half of maximum). This representation of renal tubular reabsorption is used to simulate observed sex differences in elimination of PFHxS from

Figure 3-10. Structure of the PBPK Model for PFHxS in Rats and Humans*



*PFHxS can be resorbed into the kidney with transporter maximum (T_m) and transporter affinity constant (K_t). Ks indicates a rate constant; K_{st}, the rate constant to the storage compartment; K_u, the urinary elimination rate constant; K_f, the transfer rate constant from the G.I. tract to fecal elimination; and K_{abs}, the oral absorption rate constant. Q_s refers to the blood flows between plasma and tissues, except for Q_{fil}, which is a clearance from the plasma to the filtrate compartment. Menstrual blood loss (dotted square) is only applicable to female humans.

G.I. = gastrointestinal; IV = intravenous; PBPK = physiologically based pharmacokinetic; PFHxS = perfluorohexane sulfonic acid; PO = *per os*

Source: Reprinted by permission from Springer Nature, Kim et al. 2018

plasma, which have been attributed to higher reabsorptive capacity in male rats (see Section 3.1.4). Values for the maximum and affinity parameters for PFHxS result in higher reabsorptive clearances from the glomerular filtrate (T_m/K_T=5.2) in male rats compared to female rats (T_m/K_T=0.057), and correspondingly lower urinary clearance of PFHxS from plasma in male rats. Values for T_m and K_t in humans were assumed to be the same as those in rats. Tissue volumes and blood flows were assigned values based on various sources (Davies and Morris 1993; Igari et al. 1983). Glomerular filtrate volume and flow were assigned values from Loccisano et al. (2012a).

The rat model was calibrated and evaluated against data on plasma and tissue levels of PFHxS measured following a single intravenous (0.5–10 mg/kg) or gavage dose (1 or 4 mg/kg) of PFHxS (Kim et al. 2018). Temporal profiles of plasma PFHxS and cumulative urinary excretion following intravenous or oral dosing were within ±1 SD of observations. Predicted cumulative urinary excretion of PFHxS reproduced the observed sex differences in urinary excretion with slower excretion and higher plasma levels in males compared to females. Terminal levels of PFHxS in heart, kidney, liver, and lung predicted for 14 days following oral dosing were within the range of observed values.

The human model was developed from the rat model with the following attributes:

- Human tissue volumes and blood flows were assigned values based on various sources (Davies and Morris 1993; Igari et al. 1983).
- Glomerular filtrate volume and flow were assigned values from Loccisano et al. (2011).
- Values for the free fraction in human plasma were 0.023% in females and 0.025% in males, based on results from ultrafiltration studies.
- Sex-specific values for renal tubular reabsorption parameters, T_m and K_t, were assumed to be the same in rats and humans.
- First order rate constants were scaled by 0.25 power of body weight ($BW^{0.25}$).
- Loss of PFHxS in menstrual blood was included in the human female model. This is represented as a direct loss of loss of 42.5 mL blood (25 mL plasma) per month (Verner and Longnecker 2015).

Kim et al. (2018) does not report an evaluation of the human model.

Applications for Dosimetry Extrapolation and Risk Assessment. The rat and human models were applied interspecies dosimetry extrapolation of a rat NOAEL for PFHxS (1 mg/kg/day). The rationale for the rat NOAEL is described in Kim et al. (2018). The dosimetry extrapolation was applied to the rat

model to predict a steady-state plasma concentration of PFHxS corresponding to a chronic oral dose of 1 mg/kg/day (value not reported). The equivalent human dose was predicted from the human PBPK model as the daily dose required to achieve the same steady state concentration in the human.

3.1.6 Animal-to-Human Extrapolations

Interspecies differences in the toxicokinetics of perfluoroalkyls and possible differences in the mechanisms of toxicity have been found. The elimination rate for PFOA in female rats is approximately 45 times faster than in male rat, 150 times faster than in Cynomolgus monkeys, and approximately 5,000–9,000 times faster than in humans (Bartell et al. 2010; Butenhoff et al. 2004c; Kemper 2003; Olsen et al. 2007a). Elimination of PFOS in male rats is approximately 3 times faster than in Cynomolgus monkeys and approximately 40 times faster than in humans (Chang et al. 2012; De Silva et al. 2009; Olsen et al. 2007a; Seacat et al. 2002). These large differences in elimination rates imply that similar external PFOA or PFOS dosages (i.e., mg/kg/day) in rats, monkeys, or humans would be expected to result in substantially different steady-state internal doses (i.e., body burdens, serum concentrations) of these compounds in each species. In addition, exposure durations required to achieve steady state would be expected to be much longer in humans than in monkeys or rats. Assuming a terminal elimination $t_{1/2}$ of 1,400 days for PFOA in humans (Olsen et al. 2007a), a constant rate of intake for 17 years would be required to achieve 95% of steady state. Steady state (i.e., 95%) would be achieved in approximately 110 days in monkeys ($t_{1/2}$=25 days, Butenhoff et al. 2004c), 30 days in male rats ($t_{1/2}$=7 days; Kemper 2003), and 1 day in female rats ($t_{1/2}$=0.2 days; Kemper 2003). Using an internal dose metric such as serum perfluoroalkyl concentration and PBPK models that can account for these differences in elimination rates can decrease the uncertainty in extrapolating from animals to humans.

The mode of action for most health outcomes associated with perfluoroalkyl exposure has not been fully characterized in humans or laboratory animals. Some perfluoroalkyl-induced effects observed in rats and mice appear to be mediated through the PPARα-dependent and -independent mechanisms (see Section 2.20 for additional information). Interpretation of the relevance of the effects observed in laboratory animals is complicated since it is generally agreed that humans and nonhuman primates are refractory, or at least less responsive than rodents, to PPARα-mediated effects (Corton et al. 2014; Klaunig et al. 2003; Maloney and Waxman 1999). While studies in mice have identified specific effects that require PPARα activation, for example, postnatal viability (Abbott et al. 2007) and some immunological effects (Yang et al. 2002b), other effects such as hepatomegaly and antigen-specific antibody response (DeWitt et al. 2016) were reported to be PPARα-independent (Yang et al.

2002b). Therefore, further studies are needed to expand the knowledge regarding PPARα-dependent and -independent effects that would allow selection of an appropriate animal model for perfluoroalkyls toxicity. In the absence of data to the contrary, ATSDR assumes that the health effects observed in laboratory animals are relevant to humans. The exception is some of the hepatic effects observed in rodents; increases in liver weight and hepatocellular hypertrophy observed in rats and mice were considered adaptive and not relevant to humans (see Section 2.9 for details).

3.2 CHILDREN AND OTHER POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

This section discusses potential health effects from exposures during the period from conception to maturity at 18 years of age in humans. Potential effects on offspring resulting from exposures of parental germ cells are considered, as well as any indirect effects on the fetus and neonate resulting from maternal exposure during gestation and lactation. Children may be more or less susceptible than adults to health effects from exposure to hazardous substances and the relationship may change with developmental age.

This section also discusses unusually susceptible populations. A susceptible population may exhibit different or enhanced responses to certain chemicals than most persons exposed to the same level of these chemicals in the environment. Factors involved with increased susceptibility may include genetic makeup, age, health and nutritional status, and exposure to other toxic substances (e.g., cigarette smoke). These parameters can reduce detoxification or excretion or compromise organ function.

Populations at greater exposure risk to unusually high exposure levels to perfluoroalkyls are discussed in Section 5.7, Populations with Potentially High Exposures.

The possible association between serum perfluoroalkyl levels in children and health effects has been examined in participants of the C8 Health Project and in the general population. The studies examined a number of health effects including alterations in serum lipid levels, adverse renal outcomes, neurodevelopmental alterations, and reproductive development. Immunotoxicity has been examined in children in several general population studies. Additionally, a large number of studies have examined the possible association of elevated serum perfluoroalkyl levels and adverse birth outcomes.

Similar to adults, associations between serum PFOA and PFOS and serum cholesterol levels were observed in a study of over 12,000 children (Frisbee et al. 2010); an increased risk of high cholesterol was also observed in children with higher serum PFOA and PFOS levels. A smaller study of children (n=43)

living in the Mid-Ohio Valley did not find associations between serum PFOA levels and hematology parameters, total cholesterol and liver enzymes, indices of kidney function, or serum TSH levels (Emmett et al. 2006b). Another study of highly-exposed residents did not find any associations between serum PFOA levels in children aged 6–12 years and IQ, reading and math skills, language, memory, learning, or attention (Stein et al. 2013). Similarly, no association between serum PFOA, PFOS, or PFNA levels in children 5–18 years old and the likelihood of ADHD diagnosis was observed in a study of highly-exposed residents, although the study did find an increased risk associated with higher PFHxS levels (Stein and Savitz 2011). A general population study that utilized the NHANES data found an association between serum PFOA, PFOS, and PFHxS levels and the risk of ADHD diagnosis (as reported by the parent) (Hoffman et al. 2010). Another smaller-scale study found associations between serum PFOS, PFNA, PFDA, PFHxS, and FOSA and impulsivity; no association with PFOA was found (Gump et al. 2011). A study of children 8–18 years of age participating in the C8 studies found reduced odds of reaching puberty at higher serum PFOA levels (Lopez-Espinosa et al. 2011); however, the biological significance of the short delay (4–5 months) is not known.

Several studies have evaluated immunotoxicity in children and adolescents. These studies have found impaired antibody responses associated with serum PFOA, PFOS, PFHxS, and PFDA (Grandjean et al. 2012, 2017; Granum et al. 2013; Mogensen et al. 2015a; Stein et al. 2016a). An increased asthma diagnosis was also associated with serum PFOA levels (Dong et al. 2013; Humblet et al. 2014; Zhu et al. 2016). Marginal evidence of an association with asthma diagnosis was also found for PFOS, PFHxS, PFNA, PFDA, PFBS, and PFDoDA (Dong et al. 2013; Zhu et al. 2016), although some studies found no associations for these compounds (Humblet et al. 2014; Smit et al. 2015; Stein et al. 2016a).

Hines et al. (2009) showed that *in utero* exposure (GDs 1–17) to low levels of PFOA (0.01–0.3 mg/kg/day) resulted in increases in body weight gain in 10–40-week-old mice; by 18 months of age, the body weights in these mice were similar to controls. Increases in serum insulin and leptin levels were also observed in the mice exposed to 0.01 and 0.1 mg/kg/day. The study also compared body weight and body composition of *in utero* exposed mice (exposed on GDs 1–17) and adult exposed mice (exposed for 17 days starting at 8 weeks of age) and found that *in utero* exposure to 1 mg/kg/day resulted in significantly higher body weight, brown fat weight, and white fat weight; this was not observed in mice exposed to 5 mg/kg/day. The results of the study suggest that gestational exposure to low doses of PFOA may result in increased susceptibility to PFOA toxicity.

A number of studies of highly exposed residents and the general population have examined the potential associations between serum perfluoroalkyl levels and alterations in birth weight. Decreases in birth weight have been found to be associated with higher PFOA (Fei et al. 2007; Lee et al. 2013; Maisonet et al. 2012; Savitz et al. 2012b) or PFOS levels (Maisonet et al. 2012), but not with lower levels of perfluoroalkyls (Fei et al. 2007; Hamm et al. 2010; Inoue et al. 2004; Kim et al. 2011; Monroy et al. 2008; Washino et al. 2009; Whitworth et al. 2012b). The decreases in birth weight were small (<20 g or 0.7 ounces per 1 ng/mL). Additionally, no increases in the risk of low birth weight infants were found in highly exposed populations (Darrow et al. 2013; Nolan et al. 2009; Savitz et al. 2012b; Stein et al. 2009). No apparent alterations in the risk of birth defects were found in C8 Health Studies (Darrow et al. 2013; Savitz et al. 2012b; Stein et al. 2009) or in another study of these communities (Nolan et al. 2009).

The developmental toxicity of PFOA and PFOS has been investigated in a number of rat and mouse studies. The observed effects include PFOA- and PFOS-induced increases in prenatal losses and decreases in pup survival, decreases in pup body weight, and neurodevelopmental toxicity (Abbott et al. 2007; Albrecht et al. 2013; Case et al. 2001; Chen et al. 2012b; Era et al. 2009; Fuentes et al. 2006, 2007a, 2007b; Grasty et al. 2003; Hu et al. 2010; Johansson et al. 2008; Lau et al. 2003, 2006; Luebker et al. 2005a, 2005b; Onishchenko et al. 2011; Thibodeaux et al. 2003; White et al. 2007, 2009, 2011; Wolf et al. 2007; Xia et al. 2011; Yahia et al. 2008, 2010). Additionally, delays in mammary gland development were observed in mice exposed to PFOA (Macon et al. 2011; White et al. 2007, 2009, 2011). A limited number of developmental endpoints have been examined in rats and mice exposed to PFDA, PFHxS, or PFBA (Butenhoff et al. 2009a; Das et al. 2008; Harris and Birnbaum 1989; Johansson et al. 2008; Viberg et al. 2013). A more in-depth discussion of the developmental toxicity of perfluoroalkyls in animals is included in Section 2.17.

PFOA and PFOS, as well as other perfluoroalkyls, are valid biomarkers of exposure to these compounds in children, as they are in adults. No relevant studies were located regarding interactions of perfluoroalkyls with other chemicals in children or adults.

No studies examining increased susceptibility to the toxicity of perfluoroalkyls were identified. The available epidemiological data identify several potential targets of toxicity of perfluoroalkyls, and individuals with pre-existing conditions may be unusually susceptible. For example, it appears that exposure to PFOA or PFOS can result in increases in serum lipid levels, particularly cholesterol levels. Thus, an increase in serum cholesterol may result in a greater health impact in individuals with high levels of cholesterol or with other existing cardiovascular risk factors. Associations have been found between

PFOA and PFOS levels and an increased risk of hypertension/pre-eclampsia in pregnant women. The liver has been shown to be a sensitive target in a number of animal species and there is some indication that it is also a target in humans. Therefore, individuals with compromised liver function may represent a susceptible population. Likewise, individuals with a compromised immune system may have an increased risk of perfluoroalkyl-induced immunotoxicity.

3.3 BIOMARKERS OF EXPOSURE AND EFFECT

Biomarkers are broadly defined as indicators signaling events in biologic systems or samples. They have been classified as biomarkers of exposure, biomarkers of effect, and biomarkers of susceptibility (NAS/NRC 1989).

A biomarker of exposure is a xenobiotic substance or its metabolite(s) or the product of an interaction between a xenobiotic agent and some target molecule(s) or cell(s) that is measured within a compartment of an organism (NAS/NRC 1989). The preferred biomarkers of exposure are generally the substance itself, substance-specific metabolites in readily obtainable body fluid(s), or excreta. Biomarkers of exposure to perfluoroalkyls are discussed in Section 3.3.1. The National Report on Human Exposure to Environmental Chemicals provides an ongoing assessment of the exposure of a generalizable sample of the U.S. population to environmental chemicals using biomonitoring (see http://www.cdc.gov/exposurereport/). If available, biomonitoring data for perfluoroalkyls from this report are discussed in Section 5.6, General Population Exposure.

Biomarkers of effect are defined as any measurable biochemical, physiologic, or other alteration within an organism that (depending on magnitude) can be recognized as an established or potential health impairment or disease (NAS/NRC 1989). This definition encompasses biochemical or cellular signals of tissue dysfunction (e.g., increased liver enzyme activity or pathologic changes in female genital epithelial cells), as well as physiologic signs of dysfunction such as increased blood pressure or decreased lung capacity. Note that these markers are not often substance specific. They also may not be directly adverse, but can indicate potential health impairment (e.g., DNA adducts). Biomarkers of effect caused by perfluoroalkyls are discussed in Section 3.3.2.

A biomarker of susceptibility is an indicator of an inherent or acquired limitation of an organism's ability to respond to the challenge of exposure to a specific xenobiotic substance. It can be an intrinsic genetic or other characteristic or a preexisting disease that results in an increase in absorbed dose, a decrease in the

biologically effective dose, or a target tissue response. If biomarkers of susceptibility exist, they are discussed in Section 3.2, Children and Other Populations that are Unusually Susceptible.

3.3.1 Biomarkers of Exposure

Measurement of serum or whole-blood perfluoroalkyl concentrations is the standard accepted biomarker of perfluoroalkyl exposure in humans. Perfluoroalkyls have been detected in the serum of workers, residents living near perfluoroalkyl facilities, and the general population. As part of NHANES, CDC has been measuring serum levels of perfluoroalkyls in the U.S. general population since 1999. Of the 12 perfluoroalkyls examined in this toxicological profile, blood concentrations of 7 compounds (PFOA, PFOS, PFDA, PFHxS, PFNA, and PFUnA) were detected in enough subjects to allow for estimation of the geometric mean. As compared to the general population, serum PFOA and PFOS levels are much higher in individuals with occupational exposure to these compounds (Olsen et al. 2003a; Sakr et al. 2007a) and PFOA levels are much higher in individuals living near a PFOA manufacturing facility (Emmett et al. 2006a; Steenland et al. 2009a), suggesting that serum levels are a good biomarker of exposure. Due to the long half-life of some perfluoroalkyls, particularly PFOA and PFOS, elevated serum levels may not be indicative of recent exposure. Although elevated serum levels are likely to be indicative of exposure to the parent compound, their presence in blood can also indicate exposure to other perfluoroalkyls. For example, PFOS can be derived from metabolism of FOSA (Olsen et al. 2005; Seacat and Luebker 2000). PFOA can be derived from metabolism of 8-2 fluorotelomer alcohol (Fasano et al. 2006; Henderson and Smith 2007; Kudo et al. 2005; Nabb et al. 2007). Exposure of mice to 8–2 telomer alcohol also generated PFNA as a metabolite (Kudo et al. 2005). Most epidemiological studies measured serum perfluoroalkyl levels as a biomarker of exposure. In general, these studies provided a one-time serum perfluoroalkyl level, but lacked information on actual environmental exposure concentrations or doses, route of exposure, and exposure duration. The differences in elimination half-lives between perfluoroalkyls also confounds the interpretation of one-time measurements; the relative concentration of the perfluoroalkyls measured in serum may not be reflective of the actual mixture to which the individual was exposed.

Two studies have also evaluated the use of perfluoroalkyl levels in hair as a biomarker of exposure. In rats administered PFOA, PFOS, or PFNA in the drinking water for 90 days, significant correlations between hair perfluoroalkyl levels and serum and tissue (liver, heart, lung, kidney) levels were found, suggesting that hair perfluoroalkyl levels may be a reliable biomarker of exposure (Gao et al. 2015). A study in humans (Alves et al. 2015) has also found detectable levels of PFBA, PFHxA, PFOA, PFBS, and

PFHxS in hair samples, but PFHpA, PFNA, and PFOS were not detected in hair samples. The study did not evaluate the potential relationship between serum perfluoroalkyl levels and hair levels, which does not allow for an assessment of whether hair is a viable biomarker of exposure.

Urinary perfluoroalkyl levels have also been evaluated as a biomarker of exposure (Worley et al. 2017a). A study of highly exposed residents measured urinary PFOA, PFOS, PFNA, and PFHxS levels. With the exception of PFOA, the proportion of values below the detection limit was too high to calculate mean or median values. The study found a strong linear correlation between serum PFOA levels and urinary PFOA levels in men and a nonsignificant weak correlation between serum and urinary PFOA levels in women.

3.3.2 Biomarkers of Effect

There are no specific biomarkers of effect caused by perfluoroalkyls.

3.4 INTERACTIONS WITH OTHER CHEMICALS

There are limited data on the interactions of perfluoroalkyls with other chemicals. Particularly absent are studies examining toxicological and toxicokinetic interactions of a perfluoroalkyl with other perfluoroalkyls. Olestra decreased the absorption of PFOA from the gastrointestinal tract of mice (Jandacek et al. 2010). No additional information was located regarding interactions among chemicals of this class or between perfluoroalkyls and other chemicals. Both PFOA and PFOS (and many other diverse chemicals) can activate the PPARα, as well as other PPARs to a lesser extent (Takacs and Abbott 2007; Vanden Heuvel et al. 2006). Therefore, it is not unreasonable to speculate that interactions at the receptor level might occur; however, there are no experimental data to support or rule out this presumption. PPARα-independent mechanism are also involved in the toxicity of perfluoroalkyls and interactions between compounds are also likely to influence these mechanisms.

CHAPTER 4. CHEMICAL AND PHYSICAL INFORMATION

4.1 CHEMICAL IDENTITY

Information regarding the chemical identity of perfluoroalkyls is located in Table 4-1. This information includes synonyms, chemical formulas and structures, and identification numbers. The perfluoroalkyls discussed in this profile exist as linear and branched isomers depending upon the method of production (see Chapter 5) and the reported values for the physical-chemical properties are typically reflective of the mixtures rather than a single specific isomer.

4.2 PHYSICAL AND CHEMICAL PROPERTIES

Information regarding the physical and chemical properties of perfluoroalkyls is located in Table 4-2.

Perfluoroalkyls are very stable, owing to the strength of the carbon-fluorine bonds, the presence of the three electron pairs surrounding each fluorine atom, and the shielding of the carbon atoms by the fluorine atoms (3M 1999; Kissa 2001; Schultz et al. 2003). Perfluoroalkyl carboxylates and sulfonates are resistant to direct photolysis and reaction with acids, bases, oxidants, and reductants (3M 2000; EPA 2008a; OECD 2002, 2006a, 2007; Schultz et al. 2003).

APFO was shown to decompose starting at 196°C (Krusic and Roe 2004) and PFOA was shown to decompose rapidly in the presence of crushed borosilicate glass at 307°C (Krusic et al. 2005). 1-H perfluoroheptane and perfluoroheptene are noted degradation products.

Perfluoroalkyl carboxylates and sulfonates consist of a perfluorocarbon tail that is both hydrophobic and oleophobic and a charged end that is hydrophilic (3M 1999; de Vos et al. 2008; Kissa 2001; Schultz et al. 2003). This combination of hydrophobic and oleophobic characteristics makes these substances very useful as surfactants. The ability of these substances to repel oil, fat, and water has resulted in their use in surface protectants (Kissa 2001). Their ability to reduce the surface tension of aqueous systems to <20 mN/m has resulted in their use as wetting agents (Kissa 2001). Neutral or uncharged perfluoroalkyls or very long chain constituents are expected to form separate layers when mixed with hydrocarbons and water. Conversely, charged species, salts, and ionized species at relevant pH (i.e., PFOS, PFOA, PFHpA, PFNA) and short-chain species (i.e., PFBA, PFBS) have relatively good solubility in water and alcohol.

Table 4-1. Chemical Identity of Perfluoroalkyls

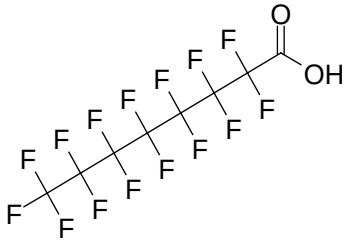
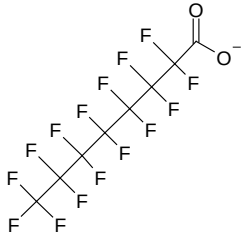
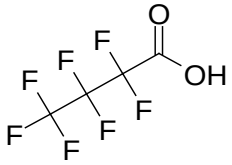
Characteristic	Information		
Chemical name	Perfluorooctanoic acid	Ammonium perfluorooctanoate	Perfluorobutanoic acid
Synonym(s)	PFOA; pentadecafluoro-1-octanoic acid; pentadecafluoro-n-octanoic acid; pentadecafluorooctanoic acid; perfluorocaprylic acid; perfluorooctanoic acid; perfluoroheptanecarboxylic acid; octanoic acid, 2,2,3,3,4,4,5,5,6,6,7,7,8,8, 8-pentadecafluoro-	APFO; ammonium pentadecafluorooctanoate; octanoic acid, 2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-pentadecafluoro-ammonium salt (1:1)	PFBA; heptafluoro-1-butanoic acid; heptafluorobutanoic acid; heptafluorobutyric acid; perfluorobutyric acid; perfluoropropanecarboxylic acid; 2,2,3,3,4,4,4-heptafluorobutanoic acid
Registered trade name(s)	No data	No data	No data
Chemical formula	$C_8HF_{15}O_2$	$C_8H_4F_{15}NO_2$	$C_4HF_7O_2$
Chemical structure			
CAS Registry Number	335-67-1	3825-26-1	375-22-4

Table 4-1. Chemical Identity of Perfluoroalkyls

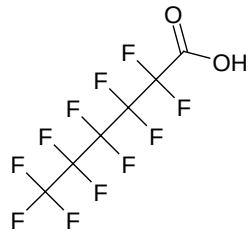
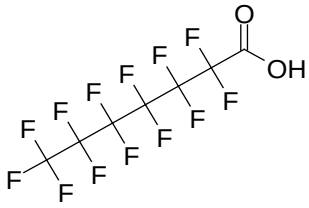
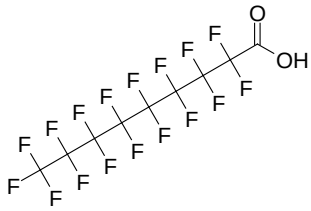
Characteristic	Information		
Chemical name	Perfluorohexanoic acid	Perfluoroheptanoic acid	Perfluorononanoic acid
Synonym(s)	PFHxA; undecafluoro-1-hexanoic acid; hexanoic acid, 2,2,3,3,4,4,5,5,6,6,6-undecafluoro-	PFHpA; perfluoro-n-heptanoic acid; tridecafluoro-1-heptanoic acid; heptanoic acid, 2,2,3,3,4,4,5,5,6,6,7,7,7-tridecafluoro-	PFNA; perfluoro-n-nonanoic acid; perfluorononan-1-oic acid; heptadecafluoro-nonanoic acid; nonanoic acid, 2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,9-heptadecafluoro-
Registered trade name(s)	No data	No data	No data
Chemical formula	$C_6HF_{11}O_2$	$C_7HF_{13}O_2$	$C_9HF_{17}O_2$
Chemical structure			
CAS Registry Number	307-24-4	375-85-9	375-95-1

Table 4-1. Chemical Identity of Perfluoroalkyls

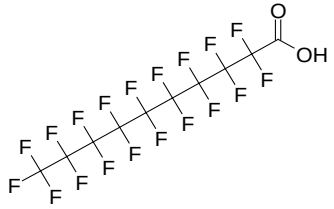
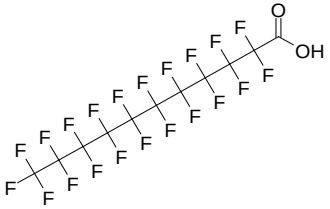
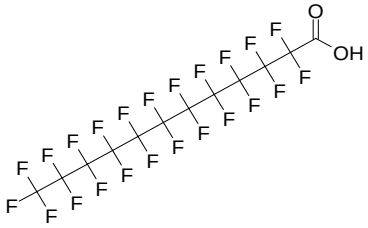
Characteristic	Information		
Chemical name	Perfluorodecanoic acid	Perfluoroundecanoic acid	Perfluorododecanoic acid
Synonym(s)	PFDA; PFDeA; Ndfda; nonadecafluoro-n-decanoic acid; nonadecafluorodecanoic acid; perfluoro-n-decanoic acid; decanoic acid, 2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-nonadecafluoro-	PFUnA; perfluoro-n-undecanoic acid; henicosafluoroundecanoic acid; 2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,11,11,11-heneicosafluoroundecanoic acid	PFDoDA; tricosafluorododecanoic acid; dodecanoic acid, 2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,11,11,12,12,12-tricosafluoro-
Registered trade name(s)	No data	No data	No data
Chemical formula	$C_{10}HF_{19}O_2$	$C_{11}HF_{21}O_2$	$C_{12}HF_{23}O_2$
Chemical structure			
CAS Registry Number	335-76-2	2058-94-8	307-55-1

Table 4-1. Chemical Identity of Perfluoroalkyls

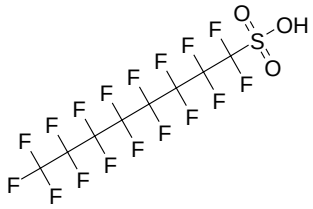
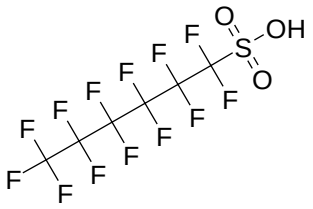
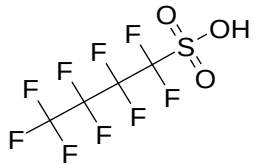
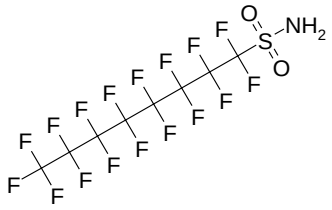
Characteristic	Information	
Chemical name	Perfluorooctane sulfonic acid	Perfluorohexane sulfonic acid
Synonym(s)	PFOS; 1-perfluorooctanesulfonic acid; heptadecafluoro-1-octanesulfonic acid; heptadecafluorooctan-1-sulphonic acid; perfluorooctane sulfonate; perfluorooctylsulfonic acid; 1-octanesulfonic acid, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluoro-	PFHxS; perfluorohexane-1-sulphonic acid; 1-hexanesulfonic acid, 1,1,2,2,3,3,4,4,5,5,6,6,6-tridecafluoro-; 1,1,2,2,3,3,4,4,5,5,6,6,6-tridecafluorohexane-1-sulfonic
Registered trade name(s)	No data	No data
Chemical formula	$C_8HF_{17}O_3S$	$C_6HF_{13}O_3S$
Chemical structure		
CAS Registry Number	1763-23-1	355-46-4

Table 4-1. Chemical Identity of Perfluoroalkyls

Characteristic	Information	
Chemical name	Perfluorobutane sulfonic acid	Perfluorooctanesulfonamide
Synonym(s)	PFBS; 1-perfluorobutanesulfonic acid; nonafluoro-1-butanesulfonic acid; nonafluorobutanesulfonic acid; pentyl perfluorobutanoate; 1,1,2,2,3,3,4,4,4-nonafluoro-1-butanesulfonic acid; 1,1,2,2,3,3,4,4,4-nonafluoro-butane-1-sulphonic acid; 1-butanesulfonic acid, nonafluoro- (6Cl,7Cl,8Cl)	FOSA; perfluorooctylsulfonamide; perfluoro-octanesulfonic acid amide; heptadecafluorooctane-sulphonamide; 1-octanesulfonamide, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluoro-
Registered trade name(s)	No data	No data
Chemical formula	$C_4HF_9O_3S$	$C_8H_2F_{17}NO_2S$
Chemical structure		
CAS Registry Number	375-73-5	754-91-6

CAS = Chemical Abstracts Services

Sources: Calafat et al. 2007a, 2007b; CAS 2008; ChemIDplus 2008, 2017; RTECS 2008

Table 4-2. Physical and Chemical Properties of Perfluoroalkyls

Property	PFOA	APFO	PFBA	PFHxA
Molecular weight	414.069[a]	431.1[b]	214.039[a]	314.06[c]
Color	White to off-white[d]	No data	No data	Colorless[d]
Physical state	Solid[e]	Solid[b]	Liquid[a]	Liquid[d]
Melting point	54.3°C[a]	Decomposition starts above 105°C[b]	-17.5°C[a]	No data
Boiling point	188°C[a]	No data	121°C[a]	168°C at 742 mm Hg[f]
Density at 20°C	1.8 g/cm^3[g]	No data	1.651 g/cm^3[a]	1.789[f]
Odor	No data	No data	No data	No data
Odor threshold:				
Water	No data	No data	No data	No data
Air	No data	No data	No data	No data
Solubility:				
Water	9.5x10^3 mg/L at 25°C[h] 2.29 x10^3 mg/L at 24°C[i] 3.3x10^3 mg/L at 25°C[j] 4.34x10^3 mg/L at 24.1°C[k]	>500 g/L[b]	2.14x10^5 mg/L at 25°C[l]	15,700 mg/L[m]
Organic solvents	No data	No data	Soluble in ethanol and toluene; insoluble in petroleum ether[a]	No data
Partition coefficients:				
Log K_{ow}	Not applicable[n]	No data	Not applicable[n]	Not applicable[n]
Log K_{oc}	1.69–2.36[o] 2.06[p]	K_{OC} 49–230[q]	2.17, average (n=7)[o]	2.06, average (n=7)[o]
pKa	-0.5[r] 0.5[s]	No data	0.08 (estimated)[t]	-0.16[m]
Vapor pressure	0.017 mm Hg at 20°C (extrapolated); 0.962 mm Hg at 59.25°C (measured)[u] 0.0316 mm Hg at 25°C[i]	0.0081 Pa at 20°C[b]	44 mm Hg at 56°C[h]	No data
Henry's law constant	0.362 Pa-m^3/mol[l]	No data	1.24Pa-m^3/mol[l]	No data
Autoignition temperature	Not applicable[v]	No data	Not applicable[v]	Not applicable[v]
Flashpoint	Not applicable[v]	No data	Not applicable[v]	Not applicable[v]
Flammability limits	Not applicable[u]	No data	Not applicable[u]	Not applicable[u]
Conversion factors	1 ppm=16.94 mg/m^3; 1 mg/m^3=0.059 ppm[w]	1 ppm=17.63 mg/m^3; 1 mg/m^3=0.057 ppm[w]	1 ppm=8.75 mg/m^3; 1 mg/m^3=0.11 ppm[w]	1 ppm=12.84 mg/m^3; 1 mg/m^3=0.078 ppm[w]
Explosive limits	Not applicable[v]	Not applicable[v]	Not applicable[v]	Not applicable[v]

Table 4-2. Physical and Chemical Properties of Perfluoroalkyls

Property	PFHpA	PFNA	PFDA	PFUnA
Molecular weight	364.06[u]	464.08[u]	514.084[t]	564.085[w]
Color	Beige[d]	No data	No data	No data
Physical state	Crystalline solid[d]	No data	No data	No data
Melting point	24–30°C[x]	No data	No data	97.9–100.3°C[x]
Boiling point	175°C at 742 mm Hg[h]	No data	219°C	No data
Density at 20°C	1.792 g/cm^3 [y]	No data	No data	No data
Odor	No data	No data	No data	No data
Odor threshold:				
Water	No data	No data	No data	No data
Air	No data	No data	No data	No data
Solubility:				
Water	4.37x10^5 mg/L at 25°C[l]	No data	No data	No data
Organic solvents	No data	No data	No data	No data
Partition coefficients:				
Log K_{ow}	Not applicable[n]	Not applicable[n]	Not applicable[n]	Not applicable[n]
Log K_{oc}	2.04, average (n=7)[o]	2.39[p]	2.79[p]	3.30[p]
pKa	-0.15 (estimated)[t] -2.29 (estimated)	-0.21 (estimated)[d]	-0.17 (estimated)[t]	-0.17 (estimated)[t]
Vapor pressure	4.6 mm Hg at 25°C[h] 0.133 at 25°C[d]	4.83x10^{-3} mm Hg at 20°C (extrapolated); 8.4 mm Hg at 99.63°C (measured)[t]	7.62x10^{-4} mm Hg at 20°C (extrapolated); 23.5 mm Hg at 129.56°C (measured)[aa]	3.44x10^{-4} mm Hg at 20°C (extrapolated); 4.62 mm Hg at 112.04°C (measured)[aa]
Henry's law constant at 25°C	0.573 Pa-m^3/mol[h]	No data	No data	No data
Autoignition temperature	Not applicable[v]	Not applicable[v]	Not applicable[v]	Not applicable[v]
Flashpoint	Not applicable[v]	Not applicable[v]	Not applicable[v]	Not applicable[v]
Flammability limits	Not applicable[v]	Not applicable[v]	Not applicable[v]	Not applicable[v]
Conversion factors	1 ppm=14.89 mg/m^3; 1 mg/m^3=0.067 ppm[w]	1 ppm=18.98 mg/m^3; 1 mg/m^3=0.053 ppm[w]	1 ppm=21.03 mg/m^3; 1 mg/m^3=0.048 ppm[w]	1 ppm=23.07 mg/m^3; 1 mg/m^3=0.043 ppm[w]
Explosive limits	Not applicable[v]	Not applicable[v]	Not applicable[v]	Not applicable[v]

Table 4-2. Physical and Chemical Properties of Perfluoroalkyls

Property	PFDoDA	PFOS	PFHxS
Molecular weight	614.1[c]	500.03[c]	400.12[c]
Color	No data	No data	No data
Physical state	No data	No data	No data
Melting point	No data	≥400°C (potassium salt)[z]	No data
Boiling point	No data	No data	No data
Density at 20°C	No data	No data	No data
Odor	No data	No data	No data
Odor threshold:			
Water	No data	No data	No data
Air	No data	No data	No data
Solubility:			
Water	No data	570 mg/L (potassium salt in pure water)[z]	No data
Organic solvents	No data	No data	No data
Partition coefficients:			
Log K_{ow}	Not applicable[j]	Not applicable[j]	Not applicable[j]
Log K_{oc}	No data	3.14, average (n=7)[o] 2.57[p]	2.28, average (n=7)[o]
pKa	-0.17 (estimated)[t]	0.14 (estimated)[t]	0.14 (estimated)[t]
Vapor pressure	5.11×10^{-60} mm Hg at 20°C (extrapolated)[z]	2.48×10^{-6} mm Hg at 20°C (potassium salt)[e]	No data
Henry's law constant at 25°C	No data	No data	No data
Autoignition temperature	Not applicable[v]	Not applicable[v]	Not applicable[v]
Flashpoint	Not applicable[v]	Not applicable[v]	Not applicable[v]
Flammability limits	Not applicable[v]	Not applicable[v]	Not applicable[v]
Conversion factors	1 ppm=25.12 mg/m^3; 1 mg/m^3=0.04 ppm[w]	1 ppm=20.45 mg/m^3; 1 mg/m^3=0.049 ppm[w]	1 ppm=16.36 mg/m^3; 1 mg/m^3=0.061 ppm[w]
Explosive limits	Not applicable[v]	Not applicable[v]	Not applicable[v]

Table 4-2. Physical and Chemical Properties of Perfluoroalkyls

Property	PFBS	FOSA
Molecular weight	300.1[c]	499.15[c]
Color	No data	No data
Physical state	No data	No data
Melting point	No data	No data
Boiling point	No data	No data
Density at 20°C	No data	No data
Odor	No data	No data
Odor threshold:		
Water	No data	No data
Air	No data	No data
Solubility:		
Water	No data	No data
Organic solvents	No data	No data
Partition coefficients:		
Log K_{ow}	Not applicable[n]	Not applicable[n]
Log K_{oc}	2.06 avg (n=7)[o]	No data
pKa	0.14 (estimated)[t]	6.24 (estimated)[t]
Vapor pressure	No data	No data
Henry's law constant	No data	No data
Autoignition temperature	Not applicable[v]	Not applicable[v]
Flashpoint	Not applicable[v]	Not applicable[v]
Flammability limits	Not applicable[v]	Not applicable[v]

Table 4-2. Physical and Chemical Properties of Perfluoroalkyls

Conversion factors	1 ppm=12.27 mg/m^3; 1 mg/m^3=0.081 ppm[u]	1 ppm=20.42 mg/m^3; 1 mg/m^3=0.049 ppm[w]
Explosive limits	Not applicable[u]	Not applicable[u]

[a]Lide 2005.
[b]EPA 2014.
[c]EPA 2008c.
[d]HSDB 2019.
[e]3M 2008c.
[f]Savu 1994a.
[g]Kroschwitz and Howe-Grant 1994.
[h]Kauck and Diesslin 1951.
[i]Bhhatarai and Gramatica 2011.
[j]Inoue et al. 2012.
[k]Rahman et al. 2014.
[l]Kwan 2001.
[m]Zhao et al. 2014.
[n]The log K_{ow} is not measurable since these substances are expected to form multiple layers in an octanol-water mixture (3M 1999, 2008c; EPA 2005a).
[o]McGuire et al. 2014.
[p]Higgins and Luthy 2006.
[q]Prevedouros et al. 2006.
[r]Goss 2008.
[s]Vierke et al. 2013.
[t]SPARC 2008.
[u]Kaiser et al. 2005.
[v]Perfluorocarboxylates and perfluorosulfonates are nonflammable (3M 1999, Kissa 2001, OECD 2007). However, they readily degrade via incineration (Krusic and Roe 2004; Krusic et al. 2005; Yamada et al. 2005).
[w]Calculated using molecular weight and the equation ppm=(X mg/m^3) x (24.45/molecular weight); mg/m^3=(X.ppm) x (molecular weight/24.45).
[x]ChemID Plus 2008.
[y]Kunleda and Shinoda 1976.
[z]Siegemund et al. 2015.
[aa]3M 2000.

APFO= ammonium perfluorooctanoate; FOSA = perfluorooctane sulfonamide; PFBA = perfluorobutanoic acid; PFBS = perfluorobutane sulfonic acid; PFDA = perfluorodecanoic acid; PFDoDA = perfluorododecanoic acid; PFHpA = perfluoroheptanoic acid; PFHxA = perfluorohexanoic acid; PFHxS = perfluorohexane sulfonic acid; PFNA = perfluorononanoic acid; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid; PFUnA = perfluoroundecanoic acid

The formation of an emulsified layer between the octanol and water interface makes the accurate measurement of properties such as log K_{ow} very difficult using conventional experimental techniques like the shake flask method or the slow stir technique (EPA 2005a, 3M 1999, 2008c). Xiang et al. (2018) used reverse-phase high-performance liquid chromatography (HPLC) to study the partitioning between the stationary column and mobile phase as a means of simulating the octanol-water partitioning process. As discussed in this study, acidic ionizable compounds are difficult to measure with this method since the pH of the mobile phase should be 2 log units lower than the pK_a of the chemical; however, extremely low pHs damage the stationary column. Therefore, a mobile phase over a range of pHs (1.09–5.00) was used to estimate the log D (sum of the ionized and unionized species in octanol/sum of the ionized and unionized species in water) over this pH range for 11 perfluoroalkylcarboxylic acids, including PFOA, and then the log D was converted to the neutral species log K_{ow}. The estimated log K_{ow} values of the perfluoroalkylcarboxylic acids (C4–C14) were in the range of 1.05 (PFBA) to 7.19 (perfluorotetra-decanoic acid).

Both the potential to form separate layers when mixed with hydrocarbons and water and the propensity for charged or ionized perfluoroalkyls to concentrate at interfaces make the measurement of the n-octanol water partition coefficient impractical (3M 1999; EPA 2005a).

The pKa range (Table 4-2) indicates that perfluoroalkyls will exist in anion form when in contact with water at environmental and physiologically relevant pHs. An estimated pKa of 6.24 indicates that FOSA will exist as both the anion and the neutral species (SPARC 2008). Perfluoroalkyl salts, such as APFO, will form the corresponding anions when dissolved in water. Prevedouros et al. (2006) reported a Krafft point of 22°C and critical micelle concentration of $3.7x10^3$ mg/L for the perfluorooctanoate anion (PFO). At temperatures above the Krafft point, the solubility of PFO is expected to increase abruptly due to the formation of micelles.

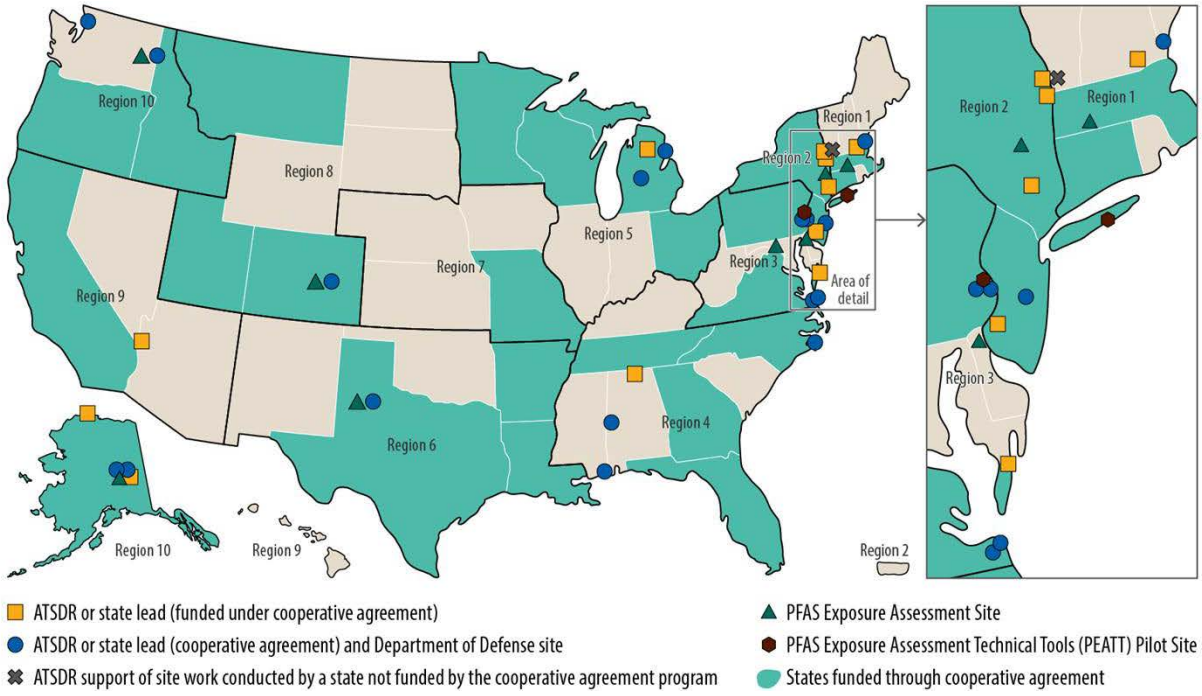
Vapor pressures at 25°C were extrapolated for PFOA, PFNA, PFDA, PFUnA, and PFDoDA using Antoine coefficients. Experimental vapor pressures were as follows: 0.962–724 mm Hg (59.25–190.80°C) for PFOA; 8.40–750 mm Hg (99.63–203.12°C) for PFNA; 23.5–750 mm Hg (129.56–218.88°C) for PFDA; 4.62–750 mm Hg (112.04–237.65°C) for PFUnA; and 6.42–750 mm Hg (127.58–247.36°C) for PFDoDA (Kaiser et al. 2005).

CHAPTER 5. POTENTIAL FOR HUMAN EXPOSURE

5.1 OVERVIEW

Perfluoroalkyls have been identified in at least 4 of the 1,854 hazardous waste sites that have been proposed for inclusion on the EPA National Priorities List (NPL) (ATSDR 2017). However, the number of sites in which substance perfluoroalkyls have been evaluated is not known. Figure 5-1 illustrates perfluorinated compound (PFAS) sites with ATSDR, state health department, EPA, or Department of Defense involvement.

Figure 5-1. Perfluorinated Compound (PFAS) Sites with ATSDR, State Health Department, U.S. Environmental Protection Agency, or Department of Defense Involvement



Source: ATSDR 2018

- The general population is exposed to the perfluoroalkyls through food and water ingestion, dust ingestion, inhalation exposure, and hand-to-mouth transfer of materials containing these substances.
- PFOA, PFOS, and their precursor substances are no longer produced or used in the United States or most other industrialized nations; however, these substances are persistent in the environment and exposure near highly contaminated sites may continue to occur.
- Serum levels of PFOA and PFOS in the general population of the United States have declined dramatically since 2000.

Perfluoroalkyls have been released to air, water, and soil in and around fluorochemical facilities located within the United States (3M 2007b, 2008a, 2008b; Barton et al. 2007; Davis et al. 2007; DuPont 2008; EPA 2008a; Post et al. 2013). Since the early 2000s, eight companies in the fluorochemical industry have been working in concert with the EPA to phase out the production and use of long-chain perfluoroalkyls and their precursors (3M 2008a; DuPont 2008; EPA 2007a, 2008a, 2016a). Perfluorocarboxylic acids containing seven or more perfluorinated carbon groups and perfluoroalkyl sulfonic acids containing six or more perfluorinated carbon units are considered long-chain substances. Perfluorinated carboxylic acids and sulfonic acids containing less than seven and six perfluorinated carbons, respectively, are considered short-chain substances. PFOA, PFOA precursors, and higher homologues have been phased out by the eight corporations in the perfluorotelomer/fluorotelomer industry (Arkema, Asahi, BASF [successor to Ciba], Clariant, Daikin, 3M/Dyneon, DuPont, and Solvay Solexis) as part of the EPA's PFOA Stewardship Program (DuPont 2008; EPA 2008a, 2016a). Industrial releases of these compounds in the United States have declined or have been totally eliminated based on company reports submitted to EPA (EPA 2008a, 2016a). It is noted that PFOA and PFOS may still be produced domestically, imported, and used by companies not participating in the PFOA Stewardship Program. PFOA and PFOS may also be present in imported articles. Although the United States and most industrialized nations have stopped producing PFOA and PFOS, China remains a major producer and user of both substances, and its production has increased as production in the rest of the world has declined (HAES 2017; Li et al. 2015; Lim et al. 2011).

Perfluoroalkyl carboxylic acids and sulfonic acids are expected to dissociate in the environment based on their low pKa values (Kissa 2001; SPARC 2008), and anions will not volatilize from water or soil surfaces (Prevedouros et al. 2006). The unique surfactant properties of these substances may prevent total dissociation of perfluoroalkyls in water (EPA 2005a; Kissa 2001; Prevedouros et al. 2006); therefore, some volatilization of perfluoroalkyls may occur since the neutral forms of these substances are considered to be volatile (Barton et al. 2007; EPA 2005a; Kim and Kannan 2007). Perfluoroalkyls have been detected in air both in the vapor phase and as adsorbed to particulates (Kim and Kannan 2007). Perfluoroalkyls are very stable compounds and are resistant to biodegradation, direct photolysis, atmospheric photooxidation, and hydrolysis (3M 2000; EPA 2008a; OECD 2002, 2007; Schultz et al. 2003). Perfluoroalkyls released to the atmosphere are expected to adsorb to particles and settle to the ground through wet or dry deposition (Barton et al. 2007; Hurley et al. 2004; Prevedouros et al. 2006). The chemical stability of perfluoroalkyls and the low volatility of these substances in ionic form indicate that perfluoroalkyls will be persistent in water and soil (3M 2000; Prevedouros et al. 2006). Soil

adsorption coefficient data as well as monitoring studies suggest that perfluoroalkyls such as PFOA are mobile in soil and can leach into groundwater (Davis et al. 2007; Prevedouros et al. 2006).

Perfluoroalkyls have been detected in environmental media and biota of the Arctic region and in other remote locations such as open ocean waters (Barber et al. 2007; Brown et al. 2018; Prevedouros et al. 2006; Wei et al. 2007a; Yamashita et al. 2005, 2008). Proposed source pathways include long-range atmospheric transport of precursor compounds followed by photooxidation to form perfluoroalkyls, direct long-range transport of perfluoroalkyls via oceanic currents, and transport of perfluoroalkyls in the form of marine aerosols (Armitage et al. 2006; Barber et al. 2007; Brown et al. 2018; Prevedouros et al. 2006; Wania 2007). Direct transport of perfluoroalkyls in the atmosphere has also been proposed as a source pathway since these substances have been detected in the vapor phase in outdoor air samples (CEMN 2008; Prevedouros et al. 2006). The actual source of perfluoroalkyls in remote locations is likely to be a combination of these pathways.

The highest concentrations of PFOA and PFOS were in apex predators, such as polar bears, which indicates that these substances biomagnify in food webs (de Vos et al. 2008; Houde et al. 2006b; Kannan et al. 2005; Kelly et al. 2007; Smithwick et al. 2005a, 2005b, 2006). The bioaccumulation potential of perfluoroalkyls is reported to increase with increasing chain length (de Vos et al. 2008; Furdui et al. 2007; Martin et al. 2004b). In living organisms, perfluoroalkyls bind to protein albumin in blood, liver, and eggs and do not accumulate in fat tissue (de Vos et al. 2008; Kissa 2001).

The levels of PFOA and PFOS in serum samples of U.S. residents have decreased appreciably since the phase out of these substances in the United States. The geometric mean serum levels of PFOS have declined over 84% from NHANES survey years 1999–2000 (30.4 ng/mL) to 2013–2014 (4.72 ng/mL) and the geometric mean serum levels of PFOA have declined 70% over the same temporal period, decreasing from 5.2 ng/mL in years 1999–2000 to 1.56 ng/mL for 2015–2016 (CDC 2018).

Mean concentrations of PFHpA, PFNA, PFDA, PFUnA, PFDoDA, PFBS, PFBA, and FOSA are generally <1 ng/mL (Calafat et al. 2006b, 2007a, 2007b; CDC 2015; De Silva and Mabury 2006; Kuklenyik et al. 2004; Olsen et al. 2003a, 2003b, 2004c, 2005, 2007a). Major PFOS exposure pathways proposed for the general population include food and water ingestion, dust ingestion, and hand-to-mouth transfer from mill- or home-treated carpets (Trudel et al. 2008). For PFOA, the major exposure pathways are proposed to be oral exposure resulting from general food and water ingestion, inhalation from impregnated clothes, and dust ingestion. While migration of residual PFOA in paper packaging and

wrapping into food is also a potential route of exposure (Trudel et al. 2008), polyfluoroalkyl phosphoric acids in food packaging can also be metabolized in the body to PFOA (D'eon and Mabury 2007; D'eon et al. 2009). Polyfluoroalkyl phosphoric acids are fluorinated surfactant substances used to greaseproof food-containing paper products. Biotransformation of the 8:2 polyfluoroalkyl phosphoric acid and the 8:2 fluorotelomer alcohol into PFOA has been demonstrated (D'eon et al. 2009). Based on these proposed exposure pathways, Trudel et al. (2008) estimated that adult uptake doses for high-exposure scenarios were approximately 30 and 47 ng/kg body weight/day for PFOS and PFOA, respectively. The estimated dosage for children under the age of 12 under a high-exposure scenario were estimated to be 101–219 and 65.2–128 ng/kg body weight/day for PFOS and PFOA, respectively (Trudel et al. 2008). It is noted that the Trudel et al. (2008) study used older monitoring data and thus, may not be an accurate reflection of current intakes. Estimated daily doses for the general population were also estimated by Vestergren et al. (2008) to range from 3.9 to 520 ng/kg body weight/day for PFOS and from 0.3 to 150 ng/kg body weight/day for PFOA. Infants and toddlers had the highest estimated dosages due to greater hand-to-mouth contact with treated carpeting, mouthing activities of clothes, and greater dust ingestion. While conversion of precursor compounds to PFOA and PFOS was generally considered as a minor contribution to the total exposure, under certain scenarios, it was estimated that up to 80% of the intake could be attributable to exposure to precursor substances followed by subsequent metabolism to PFOS or PFOA (Vestergren et al. 2008).

Perfluoroalkyls have been detected in human breast milk and umbilical cord blood. The reported maximum concentrations of PFOS and PFOA measured in human breast milk samples were 0.360–0.685 and 0.210–0.609 ng/mL, respectively (Kärrman et al. 2007; Llorca et al. 2010; So et al. 2006b; Völkel et al. 2008). Maximum concentrations of other perfluoroalkyls were <0.18 ng/mL (Kärrman et al. 2007). PFOS and PFOA have been detected in most umbilical cord blood samples with reported concentrations of 4.9–11.0 and 1.6–3.7 ng/mL, respectively (Apelberg et al. 2007a, 2007b; Fei et al. 2007; Inoue et al. 2004; Midasch et al. 2007). Other perfluoroalkyls have been detected less frequently, with maximum concentrations of <2.6 ng/mL.

Individuals who perform jobs that require frequent contact with perfluoroalkyl-containing products, such as individuals who install and treat carpets or firefighters, are expected to have occupational exposure to these substances. Individuals who work at fluorochemical facilities generally have had higher perfluoroalkyl serum levels than the general population based on exposures in the work environment (3M 2007b, 2008b, 2008c; Barton et al. 2006; Davis et al. 2007). Studies of individuals living near fluorochemical facilities indicate that drinking water is the major exposure pathway (Emmett et al. 2006a;

Hölzer et al. 2008; Wilhelm et al. 2009). 3M conducted an exposure assessment to estimate the cumulative exposure to PFOA due to activities at the Decatur, Alabama facility. On-site exposure to groundskeepers, maintenance workers, construction workers, and on-site trespassers were considered. Off-site exposures to anglers, boaters, and residential individuals were also estimated. Various plausible exposure scenarios were considered, and the highest PFOA exposure doses by receptor and pathway occurred for local residents from groundwater followed by residents consuming drinking water from the West Morgan/East Lawrence (WM/EL) public drinking water supply (3M 2008c).

5.2 PRODUCTION, IMPORT/EXPORT, USE, AND DISPOSAL

5.2.1 Production

Perfluoroalkyls have been manufactured industrially by electrochemical fluorination (ECF), fluorotelomer iodide oxidation, fluorotelomer olefin oxidation, and fluorotelomer iodide carboxylation (Prevedouros et al. 2006; Schultz et al. 2003). During the ECF process, an organic acyl or sulfonyl fluoride backbone structure is dissolved in a solution of aqueous hydrogen fluoride (Savu 1994b; Siegemund et al. 2005). A direct electrical current is then passed through the solution, which replaces all of the hydrogens on the molecule with fluorines. Perfluoroacyl fluorides produced by ECF are hydrolyzed to form the perfluorocarboxylic acid, which is then separated via distillation. This method was used extensively by 3M in the production of perfluoroalkylsulfonates such as PFOS (3M 1999; Hekster et al. 2003; Schultz et al. 2003).

$$R_hCOF + HF \longrightarrow R_fCOF + H_2 + \text{byproducts}$$

$$R_fCOF + H_2O \longrightarrow R_fCOOH + HF$$

Perfluoroalkanesulfonyl fluorides produced by ECF are hydrolyzed under alkaline conditions to form the corresponding salt (Savu 1994b; Siegemund et al. 2005). Acidification followed by distillation yields the anhydrous perfluoroalkanesulfonic acid.

$$R_hSO_2F + HF \longrightarrow R_fSO_2F + H_2$$

$$R_fSO_2F + KOH \longrightarrow R_fSO_3K + HF$$

$$R_fSO_3K + H_2SO_4 \longrightarrow R_fSO_3H + KHSO_4$$

Perfluorosulfonamide compounds, such as FOSA, can be formed by reacting the perfluoroalkanesulfonyl fluoride with a primary or secondary amine (3M 1999; Hekster et al. 2003; Siegemund et al. 2005).

The fluorotelomer iodide oxidation process was developed by DuPont and has served as the basis for their fluoropolymer production chemistry (Buck et al. 2011; Hekster et al. 2003; Savu 1994a; Siegemund et al. 2005). It begins with the preparation of pentafluoroiodoethane from tetrafluoroethene. Tetrafluoroethene is then added to this product at a molar ratio that gives a product of desired chain length. Finally, the product is oxidized to form the carboxylic acid. The process produces linear perfluorocarboxylic acids of even carbon numbers as illustrated below.

$$5C_2F_4 + IF_5 + 2I_2 \xrightarrow{\text{catalyst}} 5C_2F_5I$$

$$C_2F_5I + nC_2F_4 \longrightarrow C_2F_5(C_2F_4)_nI$$

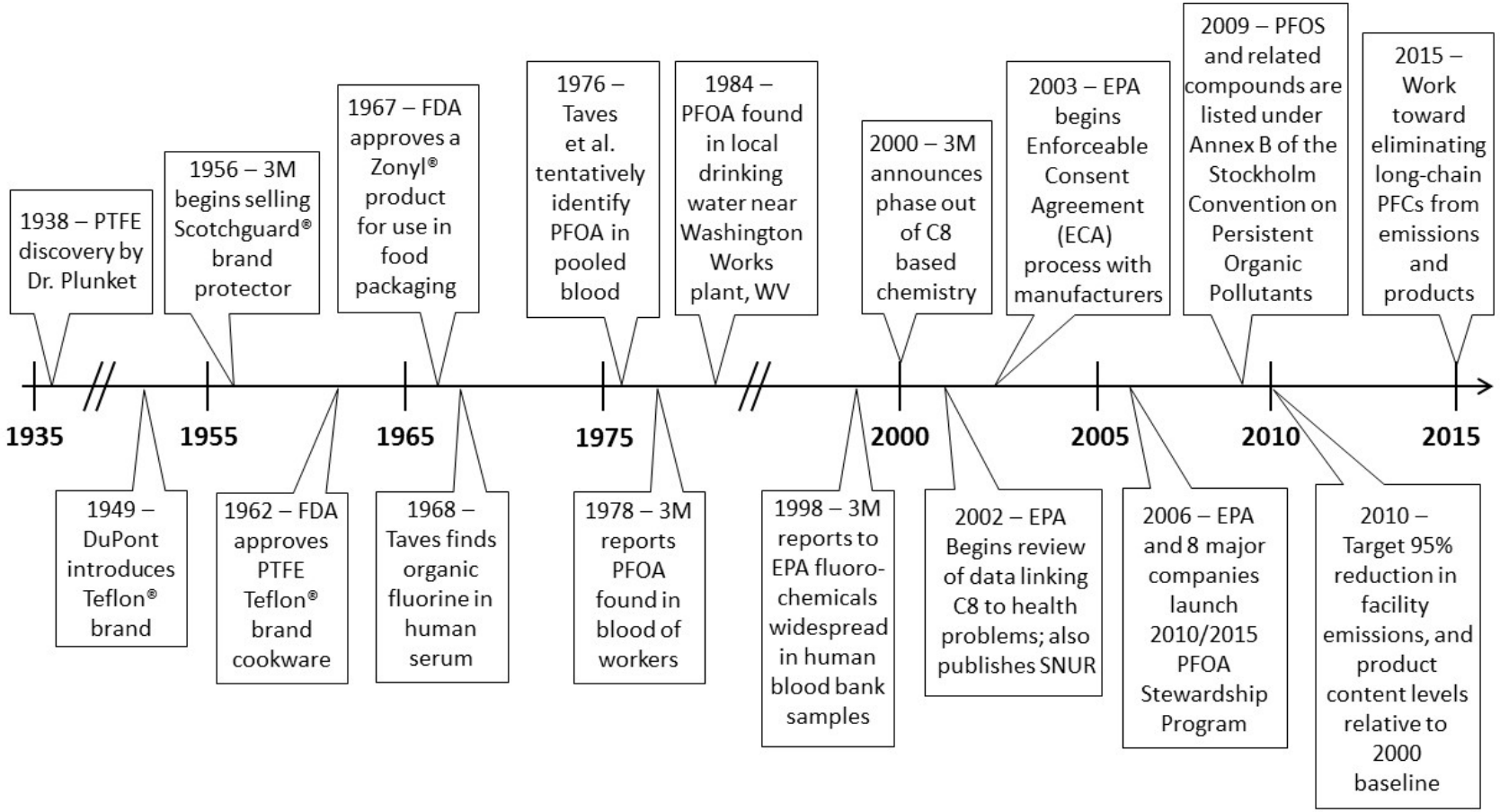
$$C_2F_5(C_2F_4)_nI \xrightarrow{SO_3} C_2F_5(C_2F_4)_{n-1}CF_2COOH$$

Fluorotelomer alcohols are created similarly, but the perfluoroalkyl iodide (telomer A) is reacted with ethylene to create $F(CF_2)_nCH_2CH_2I$ (telomer B), which is converted to the alcohol. The ECF process resulted in a mixture of linear and branched isomers, whereas the telomerization processes yielded predominantly linear products. It has been reported that the 3M ECF process resulted in approximately 70% linear and 30% branched isomers for PFOS and 78% linear and 22% branched isomers for PFOA (Benskin et al. 2009).

No information is available in the TRI database on facilities that manufacture, process, or otherwise use perfluoroalkyls because this class of substances is not required to be reported under Section 313 of the Emergency Planning and Community Right-to-Know Act (Title III of the Superfund Amendments and Reauthorization Act of 1986) (EPA 2005a, 2016g). The Chemical Data Reporting (CDR) rule, under the Toxic Substances Control Act (TSCA), requires manufacturers (including importers) to provide EPA with information on the production and use of chemicals in commerce in large quantities. Information on perfluoroalkyls can be found at (https://www.epa.gov/chemical-data-reporting/basic-information-chemical-data-reporting#what).

Perfluoroalkyls have been manufactured for their direct use in commercial products as well as for their use in industrial process streams. Two important chemicals that have resulted from manufacturing involving perfluoroalkyls, namely PFOS and PFOA, are of worldwide interest given their detection in multiple media in the environment. However, these substances and related long-chain perfluoroalkyls have been essentially phased out as a joint effort by EPA and industry (Lindstrom et al. 2011). The timeline for history of perfluorinated compound production, use, and phase out is presented in Figure 5-2.

Figure 5-2. Timeline of Important Events in the History of Polyfluorinated Compounds



EPA = U.S. Environmental Protection Agency; PFC = perfluorinated compound; PFOA = perfluorooctanoic acid; PTFE = polytetrafluoroethylene; SNUR = significant new use rule; WV = West Virginia

Source: Reprinted (adapted) with permission from Lindstrom et al. 2011 (Environ Sci Technol 45:7954-7961). Copyright 2011 American Chemical Society.

Given their unique properties, certain narrow exceptions exist for specific applications. Additionally, many of the substances that were used in the production of stain resistant or anti-sticking products that could break down into PFOA and PFOS have also been replaced.

The 3M Company was the principal worldwide manufacturer of PFOS and related chemicals. As a result of its phase-out decision in May 2000, 3M no longer manufactures perfluorooctanyl compounds (PFOA and PFOS). The company ceased manufacturing and using the vast majority of these compounds within approximately 2 years of the phase-out announcement, and ceased all manufacturing and the last significant use of this chemistry by the end of 2008 (3M 2008a; EPA 2007a). In 2000, EPA finalized the SNUR for 88 perfluoroalkyl sulfonate compounds, which requires manufacturers to notify EPA 90 days prior to commencing manufacture or import of these substances for a significant new use to allow time for evaluation (EPA 2002, 2007a, 2008a). The purpose of this rule was to limit future manufacturing and importation of these substances. According to EPA, the rule allowed for the continuation of a few limited, highly technical uses for which no alternatives are available, and which are characterized by very low volume, low exposure, and low releases. The SNUR was amended in 2007 to include 183 additional perfluoroalkyl sulfonate compounds (EPA 2007a, 2008a).

Included on the current list are PFOS, PFHxS, and FOSA. EPA believed that the perfluoroalkyl sulfonate compounds listed under the SNUR were no longer manufactured in the United States; however, during the comment period of the 2007 amendment, EPA learned of the ongoing use of tetraethylammonium perfluorooctanesulfonate as a fume/mist suppressant in metal finishing and plating baths (EPA 2007a). EPA has since excluded this from the list of significant uses. This rule has been amended again by the EPA to designate the processing, use, or importation of long-chain perfluoroalkyls as a significant new use if there are no current ongoing uses, or for uses that were scheduled to end December 31, 2015 (EPA 2015). As part of this amendment, EPA proposed to amend a SNUR for perfluoroalkyl sulfonate chemical substances that would make the exemption inapplicable for persons who import perfluoroalkyl sulfonate chemical substances as part of carpets or any articles that contain long-chain perfluoroalkyls.

In 2006, the eight major companies of the perfluoropolymer/fluorotelomer industry agreed to participate in EPA's PFOA Stewardship Program (EPA 2008a). All public documents and reports from the PFOA Stewardship Program may be reviewed at the EPA docket (EPA-HQ-OPPT-2006-0621). This program included voluntary commitments from these companies to reduce facility emissions and product content of PFOA and related chemicals on a global basis by 95% no later than 2010, and to work toward elimination of these substances in products by 2015. Progress reports have been submitted annually

beginning in 2007 and are available from the EPA PFOA Stewardship Program website. Data from these reports that list the content and percent reduction of PFOA, PFOA precursors, and higher PFOA homologues in products are listed in Table 5-1. Nonconfidential emission reports from 2014 and 2015 obtained from the EPA docket indicate that the companies have met the goals of the program. According to its 2015 emission report to EPA, Solvay Specialty Polymers (formerly Solvay Solexis) ceased using PFOA and related higher homologs in 2010 and ceased importation of products containing PFOA residuals in December 2015 (Jones 2016). DuPont does not use PFOA any longer in the emulsion polymerization of polytetrafluoroethylene (PTFE), replacing it with a processing aid consisting of the ammonium salt of 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)-propanoate, also known as GenX or hexafluoropropylene oxide dimer acid (HFPO-DA) (Wang et al. 2013b). (In 2013, DuPont separated its performance chemicals, titanium technologies, fluoroproducts division, and chemical solutions into a separate company named Chemours). Similarly, 3M and other manufacturers are using various poly- and perfluoroether acid salts in fluoropolymer manufacturing and have reformulated surface treatment products to employ short-chain substances that are not as bioaccumulative as the long-chain perfluoroalkyls. Wang et al. (2013b) provide a comprehensive review of the newer substances that manufacturers are using as replacements for legacy perfluoroalkyls in fluoropolymer manufacturing, metal plating, firefighting foams, and other miscellaneous uses such as food packaging materials. Although expected to be less, bioaccumulative modeling studies have suggested that short-chain homologues and other replacements are likely to be persistent and mobile in the environment (Gomis et al. 2015)

Table 5-1. Content (ppm) and Percent Reduction of PFOA, PFOA Homologues, or PFOA Precursors in Products from 2006 and 2013 U.S. Operations of Fluoropolymer/Fluorotelomer Companies

		Dispersions		Other fluoropolymers		Telomers	
Company	Chemicals	Content	Percent reduction[a]	Content	Percent reduction[a]	Content	Percent reduction[a]
2006 (EPA 2008a)							
Arkema, Inc.	PFOA and higher homologues	>500–1,000	0%	>70–150	30	Not applicable	Not applicable
	Precursors	Not applicable	Not applicable	Not applicable	Not applicable	Not applicable	Not applicable

Table 5-1. Content (ppm) and Percent Reduction of PFOA, PFOA Homologues, or PFOA Precursors in Products from 2006 and 2013 U.S. Operations of Fluoropolymer/Fluorotelomer Companies

		Dispersions		Other fluoropolymers		Telomers	
Company	Chemicals	Content	Percent reduction[a]	Content	Percent reduction[a]	Content	Percent reduction[a]
Asahi Glass Company	PFOA, PFOA salts, and higher homologues	500–1,570	12%	0.12	Not applicable	Not applicable	Not applicable
	Precursors	Not applicable	Not applicable	Not applicable	Not applicable	Not applicable	Not applicable
Ciba Specialty Chemicals Corporation	PFOA	0.05 kg	>99%	0.05 kg	>99%	0.05 kg	>99%
	Higher homologues	0.05 kg	>99%	0.05 kg	>99%	0.05 kg	>99%
	Precursors	0	>99%	0	>99%	0	>99%
Clariant International Ltd.	Not applicable	Not applicable	Not applicable	Not applicable	Not applicable	Not applicable	Not applicable
Daikin America, Inc.	PFOA	280	34%	2; 300	0%	0.28	72%
	Precursors and higher homologues	Not applicable	Not applicable	Not applicable	Not applicable	2,500	78%
E.I. DuPont de Nemours and Company	PFOA, PFOA salts	547	44%	69	80%	246 kg	50%
	Direct precursors	Not applicable	Not applicable	Not applicable	Not applicable	57 kg	14%
3M/Dyneon	PFOA	0	100%	Not reported	Not reported	Not applicable	Not applicable
Solvay Solexis	PFOA and PFOA salts	600–700	59%	Not applicable	Not applicable	Not applicable	Not applicable
	Higher homologues	Not applicable	Not applicable	170–200	0%	Not applicable	Not applicable
	Precursors	0	Not applicable	Not applicable	Not applicable	Not applicable	Not applicable
2013 (EPA 2016a)							
Arkema, Inc.	PFOA and higher homologues	Not applicable	100%	>25–75	96%	Not applicable	Not applicable
	Precursors	Not applicable	Not applicable	Not applicable	Not applicable	Not applicable	Not applicable
Asahi Glass Company	PFOA, PFOA salts, and higher homologues	0	100%	18	100%	Negligible	Not applicable
	Precursors	Not applicable	Not applicable	Not applicable	Not applicable	Not applicable	Not applicable

Table 5-1. Content (ppm) and Percent Reduction of PFOA, PFOA Homologues, or PFOA Precursors in Products from 2006 and 2013 U.S. Operations of Fluoropolymer/Fluorotelomer Companies

		Dispersions		Other fluoropolymers		Telomers	
Company	Chemicals	Content	Percent reduction[a]	Content	Percent reduction[a]	Content	Percent reduction[a]
BASF (Ciba Specialty Chemicals Corporation)	PFOA and higher homologues	Not applicable	100%	Not applicable	100%	Not applicable	100%
	Precursors	Not applicable	100%	Not applicable	100%	Not applicable	100%
Clariant International Ltd.	PFOA, PFOA salts, and higher homologues	None reported	Not applicable	None reported	Not applicable	<1 kg	Not applicable
	Precursors	None reported	Not applicable	None reported	Not applicable	<7 kg	Not applicable
Daikin America, Inc.	PFOA	None reported	100%	None reported	100%	None reported	100%
	Precursors and higher homologues	None reported	Not applicable	None reported	Not applicable	None reported	>99%
E.I. DuPont de Nemours and Company	PFOA and PFOA salts	1	99%	0	99%	9 kg	>99%
	Higher homologues	1	99%	0	99%	None reported	99%
	Precursors	None reported	None reported	None reported	None reported	None reported	98%
3M/Dyneon	PFOA, PFOA salts, and higher homologues	0	100%	0	Not applicable	0	No telomere production
	Precursors	No precursor production	No precursor production	No precursor production	No precursor production	No precursor production	No precursor production

Table 5-1. Content (ppm) and Percent Reduction of PFOA, PFOA Homologues, or PFOA Precursors in Products from 2006 and 2013 U.S. Operations of Fluoropolymer/Fluorotelomer Companies

		Dispersions		Other fluoropolymers		Telomers	
Company	Chemicals	Content	Percent reduction[a]	Content	Percent reduction[a]	Content	Percent reduction[a]
Solvay Solexis	PFOA, PFOA salts, and higher homologues	Not applicable	>99.999%	Not applicable	>99.999%	Not applicable	>99.999%
	Precursors	Not applicable	Not applicable	Not applicable	Not applicable	Not applicable	Not applicable

[a]Percent reduction in product content of these compounds from baseline year levels. The baseline year is the year nearest to the year 2000 for which company data are available.

PFOA precursors include: octane, 1,1,1,2,2,3,3,4,4,5,5,6,6,7,7, 8,8-heptadecafluoro-8-iodo- (CAS 507-63-1); 1-decanol, 3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-heptadecafluoro-(CAS 678-39-7); 1-decene, 3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-heptadecafluoro- (CAS 21652-58-4); 2-propenoic acid, 3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-heptadecafluorodecyl ester (CAS 27905-45-9); 2-propenoic acid, 2-methy 1-, 3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-heptadecafluorodecyl ester (CAS 1996-88-9); 2-decenoic acid, 3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-hexadecafluoro- (CAS 70887-84-2); and decanoic acid, 3,3,4,4,5,5,6,6,7, 7,8,8,9,9,10,10,10-heptadecafluoro- (CAS 27854-31-5).

Higher homologues include: dodecane, 1,1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,11,11,12,12-pentacosafluoro-12-iodo (CAS 307-60-8); decane, 1,1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10-heneicosafluoro-10-iodo- (CAS 423-62-1); nonanoic acid, heptadecafluoro- (CAS 375-95-1); decanoic acid, nonadecafluoro- (CAS 335-76-2); 1-Decanol, 3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-heptadecafluoro- (CAS 678-39-7); decane, 1,1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8-heptadecafluoro-10-iodo- (CAS 2043-53-0); dodecane, 1,1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10-heneicosafluoro-12-iodo- (CAS 2043-54-1); 2-propenoic acid, 3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-heptadecafluorodecyl ester (CAS 4980-53-4); and 2-propenoic acid, 2-methyl-, 3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,11,11,12,12,12-heneicosafluorododecyl ester (CAS 17741-60-5).

CAS = Chemical Abstracts Service; PFOA = perfluorooctanoic acid

China is one of the few remaining producers and consumers of PFOA and its salts, with a total of 480 metric tons produced from 2004 to 2012 (Li et al. 2015). China also continues to be a producer of PFOS. Growth in production volumes in China have coincided with decreases in production in the west. For example, China produced approximately 30 metric tons of PFOS in 2001; however, as 3M ceased production, China's production of PFOS increased to 91, 165, and 247 metric tons in 2004, 2005, and 2006, respectively (Lim et al. 2011). PFOA production in China was approximately 30 metric tons in 2004, but increased to approximately 90 metric tons in 2012 (Li et al. 2015). According to a report from the Hubei Academy of Environmental Sciences (HAES), China is planning to gradually phase out the production of some PFOS uses before 2019 and conduct a best available technology (BAT)/best environmental practice (BEP) analysis with the ultimate goal of completely phasing out the production and use of PFOS and potential precursors (HAES 2017).

Historical U.S. production volume data for PFOA, PFBA, and PFOS reported by manufacturers under the EPA Inventory Update Rule (IUR) are provided in Table 5-2. Production volume ranges for the ammonium salt of PFOA, APFO, are also listed. During the reporting year 2002, manufacturers reported that the production volumes were within the range of 10,000–500,000 pounds (6–227 metric tons) for PFOS and PFOA and within the range of 500,000–1,000,000 pounds (227–454 metric tons) for APFO (EPA 2008b). PFBA was reported as having a production volume within the range of 10,000–500,000 pounds (6–227 metric tons) during 1986; however, PFBA production volumes were not reported for subsequent years (EPA 2008b). None of the other perfluoroalkyls were listed in EPA's IUR database. Current U.S. production volume data for perfluoroalkyls are limited. The IUR database has been superseded by the Chemical Data Reporting (CDR) database. Data for 2012 indicated that PFOA was not imported into the United States, but any use or production volume data were reported as confidential business information. No data were located in the CDR for the other substances listed in Table 5-2. Nonconfidential emission reports from 2015 obtained from the EPA docket indicate that there is no current production of PFOA or PFOS in the United States.

Table 5-2. U.S. Production Volume Ranges for Perfluoroalkyls (1986–2002) Reported under the EPA Inventory Update Rule

Perfluoro-alkyl	Reporting year production volume range (pounds)				
	1986	1990	1994	1998	2002
PFOA	10,000–500,000	Not reported	10,000–500,000	10,000–500,000	10,000–500,000
APFO	10,000–500,000	10,000–500,000	10,000–500,000	10,000–500,000	500,000–1,000,000
PFBA	10,000–500,000	Not reported	Not reported	Not reported	Not reported
PFOS	Not reported	Not reported	10,000–500,000	Not reported	10,000–500,000

APFO = ammonium perfluorooctanoate; EPA = Environmental Protection Agency; PFBA = perfluorobutanoic acid; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid

Source: EPA 2008b

5.2.2 Import/Export

The SNURs cited in Section 5.2.1 severely limits the production, import, or export of the long-chain perfluoroalkyls. There were no reported imports of chemicals listed in Table 4-1 in 2012 from the CDR database. Production volumes and import volumes of the 8:2 fluorotelomer alcohol were listed as confidential business information by DuPont. Shaw Industries Group reported that they imported 37,478 pounds of 6:2 fluorotelomer alcohol into the United States in 2012, but DuPont declared both production volume and import volumes as confidential business information (EPA 2016d).

5.2.3 Use

Applications of perfluoroalkyls have made use of their unique surfactant properties (Schultz et al. 2003). The alkyl tails of perfluoroalkyls make these substances both hydrophobic (water-repelling) and oleophobic (oil-repelling) (3M 1999; Kissa 2001; Schultz et al. 2003). Because of these properties, perfluoroalkyls have been used extensively in surface coating and protectant formulations (Kissa 2001). Major applications have included protectants for paper and cardboard packaging products, carpets, leather products, and textiles that enhance water, grease, and soil repellency (Hekster et al. 2003; Schultz et al. 2003). These compounds have been widely used in industrial surfactants, emulsifiers, wetting agents, additives, and coatings as well (3M 1999; Schultz et al. 2003). Perfluoroalkyls have been used in fire-fighting foams since they are effective in extinguishing hydrocarbon fueled fires (Schultz et al. 2003). Perfluoroalkyls have also been used as processing aids in the manufacture of fluoropolymers such as nonstick coatings on cookware, membranes for clothing that are both waterproof and breathable, personal care products (such as dental floss, cosmetics, sunscreens), electrical wire casing, fire and chemical resistant tubing, and plumbing thread seal tape (DuPont 2008; EPA 2008a).

5.2.4 Disposal

Information concerning disposal of individual perfluoroalkyl products may be found on Material Safety Data Sheets (MSDS) or Safety Data Sheets (SDS) from the manufacturers of the chemicals. Two methods are generally recommended for the disposal of fluoropolymer dispersions. The first method involves precipitation, decanting, or filtering to separate solids from liquid waste. The dry solids are then disposed of in an approved industrial solid waste landfill or incinerated, while the liquid waste is discharged to a waste water treatment facility (Plastics Europe 2012). The second method involves incineration at temperatures >800°C using a scrubber to remove hydrogen fluoride (Plastics Europe 2012). According to perfluorochemical facility assessment reports, historical disposal of perfluoroalkyl containing waste has been through on- and off-site landfills, through sludge incorporation (subsurface injection), and through incineration (3M 2007b, 2008a; ATSDR 2005). Pilot scale studies in which carpet samples were incinerated using a rotary kiln incinerator indicated that most perfluoroalkyls were effectively destroyed in combustors (Lemieux et al. 2007). Similar conclusions were reached by Yamada et al. (2005) when studying the incineration of textiles and paper treated with fluorotelomer-based acrylic polymers. Incineration at conventional temperatures is a proven technology for treating wastes containing perfluoroalkyls.

5.3 RELEASES TO THE ENVIRONMENT

There is no information listed in EPA's Toxic Release Inventory (TRI) on releases of perfluoroalkyls to the environment from facilities manufacturing, processing, or otherwise using perfluoroalkyls because these releases are not required to be reported within this program (EPA 2005b, 2016g).

Perfluoroalkyls are man-made compounds that are not naturally occurring in the environment. Perfluoroalkyls such as PFOS and PFOA have been widely used in the manufacturing of many consumer products (Hekster et al. 2003; Schultz et al. 2003). These substances are still detected in both environmental and biological media around the world as well as in serum samples collected from the general population (Calafat et al. 2006b, 2007a, 2007b; CDC 2018, 2019; De Silva and Mabury 2006; Kuklenyik et al. 2004; Olsen et al. 2003b, 2003c, 2004b, 2004c, 2005, 2007a; Prevedouros et al. 2006).

In 2006, the eight major companies of the perfluoropolymer/perfluorotelomer industry agreed to participate in EPA's PFOA Stewardship Program (EPA 2008a). This included voluntary commitments from these companies to reduce facility emissions and product content of PFOA and related chemicals on a global basis by 95% no later than 2010, and to work toward elimination of these substances by 2015 (EPA 2008a). Data from 2007 and 2013 progress reports regarding releases of PFOA, PFOA precursors, and higher PFOA homologues to all media as well as percent reduction in releases are listed in Table 5-3.

Table 5-3. Reported Emissions of PFOA, PFOA Homologues, or PFOA Precursors in Products from the 2006 and 2013 U.S. Operations of Fluoropolymer/Fluorotelomer Companies

		Releases to all media from fluoro-telomer and telomer manufacturing		
Company	Chemicals	kg	kg of release/100 kg of product produced	Percent reduction in emissions[a]
		2006 Data (EPA 2008a)		
Arkema, Inc.	PFOA and higher homologues	>1,000–10,000	For fluorotelomer production: >0.1–1	22%
	Precursors	Not applicable	Not applicable	Not applicable
Asahi Glass Company	PFOA, PFOA salts, and higher homologues	4,922	For fluorotelomer production: <1	6%
	Precursors	Not applicable	Not applicable	Not applicable
BASF (Ciba Specialty Chemicals Corporation)	PFOA	0.05[b]		>99%
	Higher homologues	0.05[b]		>99%
	Precursors	0[b]		>99%

Table 5-3. Reported Emissions of PFOA, PFOA Homologues, or PFOA Precursors in Products from the 2006 and 2013 U.S. Operations of Fluoropolymer/Fluorotelomer Companies

		Releases to all media from fluorotelomer and telomer manufacturing		
Company	Chemicals	kg	kg of release/100 kg of product produced	Percent reduction in emissions[a]
Clariant International Ltd.	Not applicable	Not applicable	Not applicable	Not applicable
Daikin America, Inc.	PFOA	Confidential business information	For fluorotelomer production: 8.0×10^{-3}; for telomer production: 6.4×10^{-7}	94% for FP production; 92% for telomer production
	Precursors and higher homologues	Confidential business information	For production: 6.4×10^{-7}	22% for telomer production
E.I. DuPont de Nemours and Company	PFOA, PFOA salts	1,100	Not reported	98%
	Direct precursors	Confidential business information	Not reported	Confidential business information
3M/Dyneon	PFOA	0	0	100%
Solvay Solexis	PFOA and PFOA salts	Not applicable	Not applicable	Not applicable
	Higher homologues	>1,000–10,000	For fluorotelomer production: 0.161	28%
	Precursors	Not applicable	Not applicable	Not applicable
	2013 data (EPA 2016a)			
Arkema, Inc.	PFOA and higher homologues	>500–2,000	>0.001–0.005	91%
	Precursors	Not applicable	Not applicable	Not applicable
Asahi Glass Company	PFOA, PFOA salts, and higher homologues	0	Not applicable	100%
	Precursors	Not applicable	Not applicable	Not applicable
BASF (Ciba Specialty Chemicals Corporation)	PFOA	Not applicable	Not applicable	100%
	Precursors	Not applicable	Not applicable	100%
Clariant International Ltd.	PFOA, PFOA salts	Not applicable	Not applicable	Not applicable
	Precursors	Not applicable	Not applicable	Not applicable
Daikin America, Inc.	PFOA	0	0	100%

Table 5-3. Reported Emissions of PFOA, PFOA Homologues, or PFOA Precursors in Products from the 2006 and 2013 U.S. Operations of Fluoropolymer/Fluorotelomer Companies

		Releases to all media from fluoro-telomer and telomer manufacturing		
Company	Chemicals	kg	kg of release/100 kg of product produced	Percent reduction in emissions[a]
E.I. DuPont de Nemours and Company	Precursors and higher homologues	0	0	100%
	PFOA, PFOA salts	90	None reported	99.8%
	Higher homologues	None reported	None reported	None reported
	Precursors	Confidential business information	None reported	Confidential business information
3M/Dyneon	PFOA, PFOA salts, and higher homologues	0	0	100%
	Precursors	0	0	Not applicable
Solvay Solexis	PFOA, PFOA salts, and higher homologues	0	0	>99.999%
	Precursors	0	0	Not applicable

[a]Percent reduction in product content of these compounds from baseline year levels. The baseline year is the year nearest to the year 2000 for which company data are available.
[b]Total for emissions and product content

PFOA = perfluorooctanoic acid

While the United States and most industrialized countries around the world have ceased production of PFOS and PFOA, China is still a major producer of both substances (Li et al. 2015; Lim et al. 2011). Over the period from 2004 to 2012, it was estimated that 250 metric tons of PFOA were released to the environment from production in China (Li et al. 2015). Fluoropolymer manufacturing and processing was considered the dominant source of environmental releases, accounting for >80% of the total, while PFOA releases related to end use consumer products accounted for 6% of the total. Brazil produced 379 metric tons of N-EtFOSA for use in the pesticide, Sulfuramid, from 2004 and 2015, which contribute to PFOS releases to the environment (Wang et al. 2017).

Prevedouros et al. (2006) estimated the total global historical emissions of perfluoroalkyl carboxylates into the environment from both direct and indirect sources from the time period of 1951–2004. These data are provided in Table 5-4. Based on these estimations, direct emissions (3,200–6,900 metric tons) have far exceeded indirect emissions (30–350 metric tons). The largest direct emissions identified are

from industrial processes such as the manufacture of perfluoroalkyl carboxylates (470–900 metric tons), fluoropolymer manufacture (2,400–5,400 metric tons), and fluoropolymer processing (210–320 metric tons). Direct release of perfluoroalkyl carboxylates from use of aqueous firefighting foams and consumer and industrial products were estimated to be 50–100 and 40–200 metric tons, respectively. The largest indirect emissions identified were from perfluoroalkyl carboxylate residual impurities in perfluorooctyl-sulfonyl fluoride products (20–130 metric tons) and fluorotelomer-based precursor degradation (6–130 metric tons). Wang et al. (2014) expanded upon the work of Prevedouros et al. (2006) by considering additional emission sources of these substances and estimating emissions from 2003 to 2015 and projecting future emissions. These authors estimated emissions of 820–7,180 metric tons for 2003–2015 and projected between 20 and 6,420 metric tons for years 2016–2030. The estimates by Prevedouros et al. (2006) and Wang et al. (2014) contain a great degree of uncertainty as demonstrated by the wide range of values presented in the data. Wang et al. (2014) stated that uncertainty analysis using Monte Carlo methods is not possible because there is insufficient information available with respect to the range or distribution of the emissions. Instead, they introduced a scoring system to provide a qualitative description of the accuracy of the estimates that ranged from 0–1 (low uncertainty) to 2–3 (high uncertainty where estimates were based on crude assumptions or extrapolations).

Table 5-4. Global Historical PFCA Production and Emissions Estimates from 1951 to 2004[a]

Environmental input source	Historical time period (years)	Estimated total global historical PFCA emissions (tonnes)
Direct PFCA sources		
PFCA manufacture		
PFO/APFO	1951–2004	400–700
PFN/APFN	1975–2004	70–200
Total manufactured		470–900
Industrial and consumer uses		
Fluoropolymer manufacture (APFO)	1951–2004	2,000–4,000
Fluoropolymer dispersion processing (APFO)	1951–2004	200–300
Fluoropolymer manufacture (APFN)	1975–2004	400–1,400
Fluoropolymer processing (APFN)	1975–2004	10–20
Aqueous firefighting foams (AFFF)	1965–1974	50–100
Consumer and industrial products	1960–2000	40–200

Table 5-4. Global Historical PFCA Production and Emissions Estimates from 1951 to 2004[a]

Environmental input source	Historical time period (years)	Estimated total global historical PFCA emissions (tonnes)
Total direct		3,200–6,900
Indirect PFCA sources		
POSF-based products		
PFCA residual impurities[b]	1960–2002	20–130
POSF-based precursor degradation	1960–2002	1–30
POSF-based AFFF	1970–2002	3–30
Fluorotelomer-based products		
PFCA residual impurities[b]	1974–2004	0.3–30
Fluorotelomer-based precursor degradation	1974–2004	6–130
Fluorotelomer-based AFFF	1975–2004	<1
Total indirect		30–350
Total source emissions (direct and indirect)		3,200–7,300

[a]Low and high estimated values as well as the period of use/production for each source are based upon publicly available information cited in the text.
[b]Some authors classify residual impurities as a direct emission source rather than indirect emission source (Buck et al. 2011).

AFFF = aqueous firefighting foams; APFN = ammonium perfluorononanoate; APFO = ammonium perfluoro-octanoate; PFCA = perfluorinated carboxylic acid; PFN = perfluorononanoate; PFO = perfluorooctanoate; POSF = perfluorooctanesulfonyl fluoride

Source: Prevedouros et al. 2006

Wang et al. (2017) estimated the global emissions in 1958–2015 as 1,228–4,930 tonnes of PFOS and 1,230–8,738 tonnes of PFOS precursors, with most emissions occurring from 1958 to 2002. It was estimated that PFOS emissions from 2016 to 2030 will be 8–153 tonnes.

5.3.1 Air

There is no information listed in the TRI on releases of perfluoroalkyls to the atmosphere from facilities manufacturing, processing, or otherwise using perfluoroalkyls because these releases are not required to be reported (EPA 2005b, 2016g).

According to 3M, low levels of PFOA were released to air during manufacturing processes at the Decatur, Alabama facility until use of this substance ceased in 2004 (3M 2008b). This company states that there are currently no process-related air emissions of PFOA at this facility (3M 2008b). PFOA concentrations as high as 75,000–900,000 pg/m^3 were measured at the fence line of the DuPont Washington Works

facility near Parkersburg, West Virginia in 2004 (Barton et al. 2006; Davis et al. 2007; Prevedouros et al. 2006). High volume air samples collected at several monitoring stations near the Washington Works facility during nine events between August and October of 2005 contained PFOA at reported concentrations ranging from 10 to 75,900 pg/m^3 (EPA 2007b). The mean and median of these reported concentrations are 5,500 and 240 pg/m^3, respectively.

The presence of perfluoroalkyls in indoor air and dust indicates that perfluoroalkyl-containing consumer products such as treated carpets and textiles may be sources of release to air (Barber et al. 2007; Jahnke et al. 2007b; Kubwabo et al. 2005; Moriwaki et al. 2003; Prevedouros et al. 2006; Shoeib et al. 2004; Strynar and Lindstrom 2008). Perfluoroalkyls have also been identified on both indoor and outdoor window films (Gewurtz et al. 2009). Disposal of perfluoroalkyl-containing consumer products is also expected to be a source of release to air (Prevedouros et al. 2006). Harada et al. (2005a, 2006) proposed that automobiles may be a source of PFOA in urban air based on elevated levels measured near heavy traffic areas and the widespread use of this substance in automobile materials.

Perfluoroalkyl carboxylic acids and perfluoroalkyl sulfonic acids are formed by the atmospheric photooxidation of precursor compounds such as fluorotelomer alcohols and perfluoroalkyl sulfonamides (D'eon et al. 2006; Ellis et al. 2004; Martin et al. 2006; Wallington et al. 2006; Wania 2007). Perfluoroalkyl carboxylic acids including PFOA, PFNA, PFHpA, and PFBA were observed as products during a laboratory study involving the photooxidation of 4:2, 6:2, and 8:2 fluorotelomer alcohols (Ellis et al. 2003, 2004). D'eon et al. (2006) observed both perfluoroalkyl carboxylic acids and perfluorobutane sulfonate among products of the photooxidation of N-methyl perfluorobutane sulfonamidoethanol.

5.3.2 Water

There is no information listed in the TRI on releases of perfluoroalkyls to water from facilities manufacturing, processing, or otherwise using perfluoroalkyls because these releases are not required to be reported (EPA 2005b, 2016g).

There are a number of sources of perfluoroalkyl release to surface water and groundwater, including release from manufacturing sites, industrial use, use and disposal of perfluoroalkyl-containing consumer products, fire/crash training areas, waste water treatment facilities, and from the use of contaminated biosolids (3M 2008b; Clara et al. 2009; Davis et al. 2007; Eggen et al. 2010; EPA 2009a; Kelly and

Solem 2009; Moody and Field 1999; Moody et al. 2003; Sinclair and Kannan 2006; Prevedouros et al. 2006).

Waste water discharge was identified as a release pathway for APFO from the DuPont Washington Works facility in West Virginia (Davis et al. 2007). The average monthly concentrations of APFO measured in surface water from three outlets at the facility during 2007 and early 2008 ranged from 3.65 to 377 µg/L (EPA 2008d). Reported concentrations of APFO and PFOA measured in surface water from four separate outlets at this facility during the same period were 3–64 and 2.3–61 µg/L, respectively. During perfluorochemical operations at the 3M Cottage Grove facility in Minnesota, waste water treatment plant effluent containing perfluoroalkyls was discharged to the Mississippi River. Discharge into Bakers Creek from the waste water treatment plant at the 3M Decatur facility was a principal source of PFOA release from this facility (3M 2008b).

Elevated levels of perfluoroalkyls, such as PFOA, PFOS, and PFHxS, measured in groundwater near fire-training areas are attributed to the use of these substances in aqueous firefighting foams (Moody and Field 1999; Moody et al. 2003). The concentrations of these three perfluoroalkyls in groundwater near a military fire-training site in Michigan were 8–105, 4.0–110, and 9–120 µg/L, respectively (Moody et al. 2003). A study of landfill leachates showed that perfluoroalkyls were primarily distributed to the water phase of leachates, which could eventually contaminate ground water (Eggen et al. 2010). Lang et al. (2017) estimated that between 563 and 638 kg of perfluoroalkyl substances were released from landfill leachate in the United States in 2013.

Waste water treatment plants have been shown to be significant contributors to perfluoroalkyls contamination of surface and ground water (Clara et al. 2009; EPA 2009a; Kelly and Solem 2009; Loganathan et al. 2007; Sinclair and Kannan 2006; Yu et al. 2009c). Influent, effluent, and sludge samples from 28 public and private waste water treatment plants in Minnesota were analyzed for 13 perfluoroalkyls; detectable concentrations of perfluoroalkyls were found in several facilities, primarily urban treatment plants (Kelly and Solem 2009). Elevated levels of PFOS at one facility (1.51 µg/L in effluent) were attributed to a chrome plating facility using a surfactant containing fluorosulfonate to control hexavalent chromium emissions. Another study of chromium electroplating facilities in Chicago, Illinois and Cleveland, Ohio also found them to be significant sources of PFOS and other perfluoroalkyls in the environment (EPA 2009a). It was determined that perfluoroalkyls were being discharged from all 11 facilities at quantifiable levels and that PFOS was detected in waste water from 10 out of 11 facilities at levels of 0.0314–39 µg/L. PFOA and PFOS were detected in effluents of six waste water treatment

plants located in New York at levels of 0.058–1.05 and 0.003–0.068 µg/L, respectively (Sinclair and Kannan 2006). PFOS and PFOA were detected in effluents of two waste water treatment plants located in Singapore at levels of 0.0053–0.5609 and 0.0112–1.057 µg/L, respectively (Yu et al. 2009c). PFOA, PFOS, and several other perfluoroalkyls were detected in effluent samples of 21 waste water treatment plants and 9 industrial point sources; PFOA and PFOS were reportedly identified in the effluents of all of the facilities monitored at an average level of 0.060 µg/L for both substances (Clara et al. 2009).

Studies comparing perfluoroalkyl levels in influent and effluent from municipal waste water treatment facilities have found higher levels of some perfluoroalkyls, such as PFNA, PFOA, PFOS, and FOSA, in the effluent, as compared to the influent (Loganathan et al. 2007; Schultz et al. 2006b). For others, such as PFHxS and PFDA, waste water treatment resulted in lower concentrations or no change in the concentrations. Increases in perfluoroalkyl concentrations are likely due to the breakdown of perfluoroalkyl precursors such as polyfluoroalkyl phosphoric acids or fluorotelomer alcohols (D'eon et al. 2009; Gauthier and Mabury 2005; Wang et al. 2005a, 2005b).

A study of eight waste water treatment plants that discharge effluent to the San Francisco Bay found increases in PFBA and PFHxA levels between 2009 and 2014 and declines in longer-chain legacy substances (Houtz et al. 2016). Average concentrations of PFBA were 7.4±4.7 ng/L in 2009 and 16±5.8 ng/L in 2014 and the average concentration of PFHxA rose from 17±4.0 ng/L in 2009 to 25±5.1 ng/L in 2014. The average PFOA concentration decreased from 32±30 ng/L in 2009 to 21±13 ng/L in 2014 and the average PFOS concentration decreased from 24±32 ng/L in 2009 to 13±4.4 ng/L in 2014.

Land application of biosolids (treated sewage sludge) can also result in the release of perfluoroalkyls to surface and groundwater (Clark and Smith 2011; Lindstrom et al. 2011; Sepulvado et al. 2011). There appears to be some differences in the distribution of PFOA and PFOS in waste water effluent and biosolids, with higher levels of PFOA in waste water and higher PFOS levels in biosolids (Guo et al. 2010).

5.3.3 Soil

There is no information listed in the TRI on releases of perfluoroalkyls to soil from facilities manufacturing, processing, or otherwise using perfluoroalkyls because these releases are not required to be reported (EPA 2005b, 2016g).

Perfluoroalkyls can be inadvertently released to soils through the use of biosolids applied as fertilizer to help maintain productive agricultural soils and stimulate plant growth. PFOA and PFOS were detected in both biosolids and biosolid-amended soils (Sepulvado et al. 2011). Six samples of biosolids obtained from the Metropolitan Water Reclamation District of Greater Chicago had levels of PFOS and PFOA of 80–219 and 8–68 ng/g, respectively (Sepulvado et al. 2011). The mean sum (±SD) of all perfluoroalkyls in the biosolids was 433±121 ng/g, with PFOS being most prominent. Perfluoroalkyls can also be released into soil due to atmospheric transport and wet/dry deposition (Rankin et al. 2016; Strynar et al. 2012).

Liu et al. (2007) measured PFOA as a product of the biodegradation of 8:2 fluorotelomer alcohol in soil. This result, along with similar findings in activated sludge tests, indicates that biodegradation of fluorotelomer alcohols may result in the formation of perfluoroalkyl carboxylic acids in soil (Liu et al. 2007; Rankin et al. 2014; Wang et al. 2005a, 2005b).

5.4 ENVIRONMENTAL FATE

5.4.1 Transport and Partitioning

Air. Barton et al. (2007) investigated the atmospheric partitioning of PFOA during rain events near an industrial facility and concluded that this substance will be primarily adsorbed to particles in the air since PFOA was not detected in the vapor phase (detection limit of 0.2 ng/m^3). Concentrations of PFOA in raindrops and as particulates were 11.3–1,660 ng/L and 0.09–12.40 ng/m^3, respectively. The authors proposed that PFOA or APFO released into air from industrial facilities will be scavenged by atmospheric particles (including aqueous aerosols and raindrops) and dissociate to form the perfluorooctanoate anion. Although Barton et al. (2007) did not detect PFOA in the vapor phase during rain events, low concentrations (<0.12–3.16 pg/m^3) of vapor-phase perfluoroalkyls measured by Kim and Kannan (2007) in urban air provide evidence of a partitioning equilibrium. Wet and dry deposition are expected to be the principal removal mechanisms for perfluoroalkyl carboxylic acids and sulfonic acids in particulate form from the atmosphere. Residence times with respect to these processes are expected to be days to weeks (Barton et al. 2007; Hurley et al. 2004; Kim and Kannan 2007).

Long-range atmospheric transport of precursor compounds such as fluorotelomer alcohols and perfluoroalkyl sulfonamides followed by the atmospheric photooxidation of these substances to form perfluoroalkyl carboxylic acids and perfluoroalkyl sulfonic acids resulted in PFOA and PFOS

contamination in remote locations with no direct point sources for these compounds (Barber et al. 2007; D'eon et al. 2006; Dinglasan-Panlilio and Mabury 2006; Ellis et al. 2004; Martin et al. 2006; Simcik 2005; Small 2009; Wallington et al. 2006; Wania 2007). Fluorotelomer alcohols and perfluoroalkyl sulfonamides are volatile and possess long enough atmospheric residence times for long-range transport to occur (Barber et al. 2007; Yarwood et al. 2007). The presence of fluorotelomer alcohols and perfluoroalkyl sulfonamides in urban and Arctic air offers evidence of long-range atmospheric transport (Loewen et al. 2005; Shoeib et al. 2006; Stock et al. 2004). Photooxidation studies have demonstrated the conversion of these substances to perfluoroalkyl carboxylic acids and sulfonates. According to Young et al. (2007), the presence of perfluorodecanoic acid and perfluoroundecanoic acid in an Arctic ice cap indicates atmospheric oxidation of precursors as a source. Yeung et al. (2017) collected samples of snow and water from the Central Arctic region in 2012 and observed that perfluorinated alkyl substances were only detectable in ocean waters above a depth of 150 m. Atmospheric deposition from precursors was estimated to account for approximately 34–59% of the PFOA input to the ocean. Gawor et al. (2014) studied the distribution of fluorotelomer alcohols, fluorinated sulfonamides, and fluorinated sulfonamidoethanols in polar, remote, and urban regions of the world during four sampling campaigns covering the years 2005–2011. They observed higher levels of fluorotelomer alcohols as compared to the fluorinated sulfonamides and sulfonamidoethanols, with the 8:2 fluorotelomer alcohol being the predominant species in most samples.

Water. The pKa range of perfluoroalkyls indicates that these substances will exist primarily as the dissociated conjugate base (anion) when in contact with water at environmental pH (pH 5–9). Volatilization will not be an important environmental fate process when the substances exist as anions; however, under acidic conditions, undissociated perfluoroalkyls may volatilize into the atmosphere (Martin et al. 2006). Perfluoroalkyls may be transported to remote areas by direct oceanic advection of these substances (Armitage et al. 2006; Barber et al. 2007; Simcik 2005; Wania 2007; Yamashita et al. 2005, 2008). Perfluoroalkyls may also be transported over long distances in the form of marine aerosols (Barber et al. 2007; CEMN 2008; Prevedouros et al. 2006). This transport mechanism may be especially relevant since surfactants have been shown to accumulate in upper sea layers and at water surfaces (Prevedouros et al. 2006).

Perfluoroalkyls have been measured in invertebrates, fish, amphibians, reptiles, birds, bird eggs, and mammals located around the world (Brown et al. 2018; Dai et al. 2006; Giesy and Kannan 2001; Houde et al. 2005, 2006a, 2006b; Keller et al. 2005; Kannan et al. 2001a, 2001b, 2002a, 2002b, 2002c, 2002d, 2005, 2006; Sinclair et al. 2006; So et al. 2006a; Wang et al. 2008). The highest concentrations of PFOA

and PFOS in animals are measured in apex predators, such as polar bears, which indicates that these substances biomagnify in food webs (de Vos et al. 2008; Houde et al. 2006b; Kannan et al. 2005; Kelly et al. 2007). Loi et al. (2011) also provided evidence of the biomagnification potential of several perfluoroalkyl substances in tropical food webs. Evidence for biomagnification of chlorinated polyfluoroalkyl ether sulfonic acids, which have been used in the plating industry, has recently been reported (Liu et al. 2017; Shi et al. 2015). Table 5-5 shows levels of PFOA and PFOS measured in Arctic organisms. The bioaccumulation potential of perfluoroalkyls increases with increasing chain length from 4 to 8 carbon units and then declines with further increases in chain length (Conder et al. 2008; de Vos et al. 2008; Furdui et al. 2007; Martin et al. 2004b). In living organisms, perfluoroalkyls bind to protein albumin in blood, liver, and eggs and do not accumulate in fat tissue, which may explain why bioconcentration factors (BCFs) are lower than expected in aquatic organisms (de Vos et al. 2008; Kissa 2001). The Arctic Monitoring and Assessment Programme December 2017 report contains a large summary of environmental monitoring data for perfluoroalkyl substances in the Arctic (AMAP 2017). Temporal trends indicated declining residues of PFOA in the liver of ringed seals at several Arctic monitoring sites; however, PFNA and PFDA showed increasing levels at some locations and declining levels at others (AMPA 2017). Declining levels of PFOA and PFOS were observed for polar bears in Canadian Arctic and Greenland; however, total perfluoroalkyl levels remained relatively constant as precursor substances appear to continue to be transported and degraded in the Arctic food web. Moreover, newer perfluorinated substances such as perfluoroethylcyclohexane sulfonate (used in hydraulic fluids) and perfluorobutane sulfonamide (a PFBS precursor) have been detected in some environmental media.

Table 5-5. Biological Monitoring of PFOA and PFOS in the Arctic

	Concentration (ng/g)		
Location and organism	PFOA	PFOS	Reference
Northeastern Canada, 1996–2002; wet weight[a]			Tomy et al. 2004
Zooplankton (n=5)	2.6	1.8	
Clams (n=5)	ND	0.28	
Shrimp (n=7)	0.17	0.35	
Arctic cod (n=6)	0.16	1.3	
Redfish (n=7)	1.2	1.4	
Walrus (n=5)	0.34	2.4	
Narwhal (n=5)	0.9	10.9	
Beluga (n=5)	1.6	12.6	
Black-legged kittiwake (n=4)	ND	10.0	
Glaucous gulls (n=5)	0.14	20.2	

Table 5-5. Biological Monitoring of PFOA and PFOS in the Arctic

Location and organism	Concentration (ng/g) PFOA	PFOS	Reference
Northern Canada, 1992–2002[a]			Martin et al. 2004a
Polar bear (n=7)	8.6	3,100	
Arctic fox (n=10)	<2	250	
Ringed seal (n=9)	<2	16	
Mink (n=10)	<2	8.7	
Common loon (n=5)	<2	20	
Northern fulmar (n=5)	<2	1.3	
Black guillemot (n=5)	<2	ND	
White sucker (n=3)	<2	7.6	
Brook trout (n=2)	<2	39	
Lake whitefish (n=2)	<2	12	
Lake trout (n=1)	<2	31	
Northern pike (n=1)	<2	5.7	
Arctic sculpin (n=1)	<2	12	
Northwestern Canada, 2004			Powley et al. 2008
Zooplankton (n=3)	ND	ND–0.2	
Arctic cod (n=5)	ND	0.3–0.7	
Ringed seal (n=5)		2.5–8.6	
Bearded seal (n=1)	ND	1.3	
Northern Norway; ng/g wet weight[a]			Verreault et al. 2005, 2007
Herring gull eggs	<0.091–0.652	21.4–42.2	
Glaucous gulls			
Eggs (n=10)	<0.70	104	
Plasma (n=20)	<0.70–0.74	134	
Nanavut, Canada			Butt et al. 2007a, 2007b
Thick-billed murres	<MDL[b]–0.16	<0.40–0.76	
Northern fulmars	<MDL[b]–0.09	<0.40–0.60	
Ringed seals	<0.85–6.2	2–20	
Northern Canada, 2002–2005			Butt et al. 2008
Ringed seal livers (n=110)	<0.7–13.9	0.89–189	
Greenland			Bossi et al. 2005
Ringed seals	<1.2	12.5–95.6	
North American and European Arctic, 1999–2002			Smithwick et al. 2005a
Polar bears (n>72)	<2.3–57.1	263–6,340	
Greenland, 1999–2001			Smithwick et al. 2005b
Polar bears (n=29)[a]	10	2,470	
Greenland, 1972–2002			Smithwick et al. 2006
Polar bears	1.6–4.4	120–1,400	
Arviat, 2010–2012			AMAP 2017
Ringed seal liver (n=28)	0.62±0.6	No data	

Table 5-5. Biological Monitoring of PFOA and PFOS in the Arctic

Location and organism	Concentration (ng/g) PFOA	PFOS	Reference
Pangnirtung, 2009–2011			AMAP 2017
Ringed seal liver (n=7)	0.22±0.15	No data	
Resolute, 2010–2013			AMAP 2017
Ringed seal liver (n=46)	0.23±0.16	No data	
Sachs Harbor, 2011–2013			AMAP 2017
Ringed seal liver (n=29)	0.54±0.36	No data	
Ulukhaktok, 2010–2013			AMAP 2017
Ringed seal liver (n=9)	1.4±0.11	No data	

[a]Reported as mean values
[b]Minimum detection limits for study analytes ranged from 0.03 to 2.3 ng/g. To calculate means, concentrations less than the MDL were replaced with a random value that was less than half the MDL.

MDL = minimum detection limit; ND = not detected; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid

Sediment and Soil. K_{oc} values of 17–230 measured for PFOA in soils of various organic carbon content indicate that PFOA will be mobile in soil and will not adsorb to suspended solids and sediment in the water column (Davis et al. 2007; Prevedouros et al. 2006); rather, it tends to remain in solution and migrate with groundwater (Davis et al. 2007). This is supported by the presence of PFOA in groundwater at the Decatur, Cottage Grove, and Washington Works fluorochemical industrial facilities (3M 2007b, 2008b; Davis et al. 2007). Other sources of perfluoroalkyls in soil include air emissions followed by atmospheric deposition to soils and subsequent leaching (Davis et al. 2007). Low volatility, high water solubility (9,500 mg/L at 25°C), and low sorption to solids indicate that the perfluorooctanoate anion will accumulate in surface waters, especially oceans (Armitage et al. 2006; Kauck and Diesslin 1951; Prevedouros et al. 2006; Wania 2007). McGuire et al. (2014) reported field-based K_{oc} values for several perfluoroalkyl substances (see Table 4-2).

Perfluoroalkyls can be taken up by plants in contaminated soils. Laboratory studies have suggested that short-chain perfluoroalkyls such as PFBA are more concentrated in edible portions of plants when compared to longer carbon chain substances such as PFOA or PFOS (Blaine et al. 2013, 2014a, 2014b; MDH 2014). Yoo et al. (2011) studied the accumulation of perfluoroalkyl carboxylic acids, perfluorosulfonic acids, and fluorotelomer alcohols in grass samples collected near Decatur, Alabama and calculated the grass-soil accumulation factor (GSAF), which is the concentration of perfluoroalkyl in grass divided by the concentration of perfluoroalkyl in soil. The shortest chain compounds had the largest GSAFs, and accumulation factors decreased rapidly with chain length. The mean (±SD) GSAF values

were 3.4±2.6, 0.90±0.66, 0.25±0.23, 0.12±0.08, 0.10±0.08, 0.11±0.09, and 0.10±0.09 for PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnA, and PFDoDA, respectively. The GSAF for PFOS was 0.07±0.04. Increasing salinity and temperature was shown to increase uptake and transport from the roots into the shoots by the wheat plants grown in hydroponic systems spiked with perfluorocarboxylic acids (Zhao et al. 2016). Transport into the shoots also increased with decreasing carbon chain length. Concentrations in the shoots of the wheat plants increased in the following order: PFBA > PFHpA > PFOA > PFDoDA. Stahl et al. (2009) demonstrated that both PFOA and PFOS were taken up from soil by five cultivated plants (spring wheat, oats, potatoes, maize, and perennial ryegrass) and the amount of uptake was generally linearly dependent upon the concentration of PFOA or PFOS spiked to the soil. Lysimeter studies suggested that PFOA and short-chain perfluoroalkyls such as PFBA pass through the soil and get taken up by plants more quickly as compared to PFOS (Stahl et al. 2013).

Krippner et al. (2014) analyzed the uptake of perfluoroalkyl substances with chain length of C4–C10 over a pH range of 5–7. They observed that short-chain perfluoroalkyls partition predominantly and at higher concentrations to the shoot. Longer-chain substances such as PFOA, PFNA, and PFDA, as well as PFHxS and PFOS, accumulated at higher concentrations in the roots of maize plants. Maize grown in soil pots containing perfluoroalkyls only accumulated compounds with a chain length ≤C8 in the kernels (Krippner et al. 2015).

Other Media. Data are not available regarding the transport and partitioning of perfluoroalkyls in other media.

5.4.2 Transformation and Degradation

Perfluoroalkyls are considered to be environmentally persistent chemicals (EPA 2008a; OECD 2002, 2007; Schultz et al. 2003). The carbon atoms of the perfluoroalkyl chain are protected from attack by the shielding effect of the fluorine atoms; furthermore, environmental degradation processes generally do not possess the energy needed to break apart the strong fluorine-carbon bonds (3M 2000; Hekster et al. 2003; Schultz et al. 2003). Perfluoroalkyls are resistant to biodegradation, direct photolysis, atmospheric photooxidation, and hydrolysis (OECD 2002, 2007; Prevedouros et al. 2006).

Air. Although transport and partitioning information indicates that air will not be a sink for perfluoroalkyls in the environment, low concentrations of perfluoroalkyl carboxylic acids, sulfonic acids, and sulfonamides have been measured in air both in the vapor phase and as bound to particulates (Barton

et al. 2007; Kim and Kannan 2007). Available information indicates that photodegradation will not compete with wet deposition as an atmospheric removal process for perfluoroalkyls (Barton et al. 2007; Hurley et al. 2004; Prevedouros et al. 2006). However, photooxidation may be an important degradation mechanism for perfluoroalkyl sulfonamides (D'eon et al. 2006; Martin et al. 2006).

PFOA does not absorb UV light at environmentally relevant wavelengths (>290 nm); Hori et al. (2004a) reported a weak absorption band for PFOA that ranged from 220 to 270 nm. Based on the measured absorption wavelength of PFOA, perfluoroalkyl carboxylic acids are not expected to undergo direct photolysis. Following irradiation of the potassium salt of PFOS with light of wavelength 290–800 nm for 67–167 hours, it was concluded that there was no evidence of direct photolysis of PFOS under any of the test conditions (OECD 2002). Based on these test results for PFOS, perfluoroalkyl sulfonic acids are not expected to undergo direct photolysis in the atmosphere.

A measured photooxidation rate constant is not available for PFOA. Hurley et al. (2004) measured the reaction of short-chain (C1–C4) perfluoroalkyl carboxylic acids with photochemically generated hydroxyl radicals. The proposed mechanism begins with abstraction of the carboxyl hydrogen, which is followed by the removal of the carboxyl group and generation of a perfluoroalkyl radical. Finally, the perfluoroalkyl chain is broken down one carbon atom at a time through an unzipping sequence. The same rate constant, 1.69×10^{-13} cm^3/molecule-second, was measured for the photooxidation of the C2, C3, and C4 molecules, indicating that the chain length may have little effect on the reactivity of perfluoroalkyls with hydroxyl radical. According to the authors, this rate constant corresponds to a half-life of 130 days. Based on the data for the short-chain structures, the authors concluded that atmospheric photooxidation of perfluoroalkyl carboxylic acids is not expected to compete with wet and dry deposition, which is predicted to occur on a time scale of the order of 10 days.

Atmospheric photooxidation data are not available for perfluoroalkyl sulfonic acids. Atmospheric photooxidation studies involving n-methyl perfluorobutane sulfonamidoethanol (Me-FBSE) and n-ethyl perfluorobutanesulfonamide (Et-FBSA) indicate possible mechanisms for the reaction of these substances with atmospheric hydroxyl radicals (D'eon et al. 2006; Martin et al. 2006). Products observed from the photooxidation of these compounds indicate the following pathways: removal of an alkyl from the amide (cleavage of the N-C bond); removal of the amido group (cleavage of the S-N bond); and removal of the sulfonamido group (cleavage of the S-C bond) (D'eon et al. 2006; Martin et al. 2006). The last two pathways indicate that FOSA may be photooxidized through removal of the amido or sulfonamido group. The third pathway, cleavage of the S-C bond, also indicates a photooxidation mechanism for

perfluoroalkyl sulfonic acids. Martin et al. (2006) proposes an unzipping sequence for the perfluoroalkyl chain following removal of the sulfonyl group.

Measured rate constants for the reaction of Me-FBSE and Et-FBSA with atmospheric hydroxyl radicals are $5.8x10^{-12}$ and $3.74x10^{-13}$ cm^3/molecule-second, respectively (D'eon et al. 2006; Martin et al. 2006). Atmospheric half-lives calculated using these rate constants were 2 days for Me-FBSE and 20–50 days for Et-FBSA.

Water. PFOS and PFOA are expected to be stable to hydrolysis in the environment based on half-lives of 41 and 92 years, respectively, calculated from experimental hydrolysis data that were measured at pH 5, 7, and 9 (OECD 2002, 2006b). Based on the data for PFOS and PFOA, hydrolysis is not expected to be an important degradation process for perfluorinated carboxylates and sulfonates in the environment. Hydrolysis data were not located for perfluoroalkyl sulfonamides.

Available information indicates that perfluoroalkyls are resistant to aerobic biodegradation. PFOA and PFNA were not biodegraded using an Organisation for Economic Co-operation and Development (OECD) guideline (301F) manometric respirometry screening test for ready biodegradability; 0% of the theoretical oxygen demand was reached after 28 days (Stasinakis et al. 2008). Meesters and Schröder (2004) reported that PFOA and PFOS were not degraded from an initial concentration of 5 mg/L in aerobic sewage sludge in a laboratory scale reactor.

Substances such as fluorotelomer alcohols and perfluoroalkyl sulfonamides are degraded to other substances such as PFOA and PFOS in water and can be considered a source of these substances in the environment (Liu et al. 2007).

Sediment and Soil. Data are not available regarding the transformation and degradation of perfluoroalkyls in sediment and soil. Based on the chemical stability of these substances and their resistance to biodegradation in screening tests, environmental degradation processes are not expected to be important removal mechanisms for perfluoroalkyls in sediment and soil (3M 2000; EPA 2008a; Hekster et al. 2003; OECD 2002, 2007; Prevedouros et al. 2006; Schultz et al. 2003).

Substances such as fluorotelomer alcohols and perfluoroalkyl sulfonamides are degraded to other substances such as PFOA and PFOS in soil and sediment and can be considered a source of these substances in the environment (Liu et al. 2007; Washington and Jenkins 2015; Washington et al. 2015).

Other Media. Data are not available regarding the transformation and degradation of perfluoroalkyls in other media.

5.5 LEVELS IN THE ENVIRONMENT

Reliable evaluation of the potential for human exposure to perfluoroalkyls depends, in part, on the reliability of supporting analytical data from environmental samples and biological specimens. Concentrations of perfluoroalkyls in unpolluted atmospheres and in pristine surface waters are often so low as to be near the limits of current analytical methods. In reviewing data on perfluoroalkyls levels monitored or estimated in the environment, it should also be noted that the amount of chemical identified analytically is not necessarily equivalent to the amount that is bioavailable.

Table 5-6 shows the limits of detection typically achieved by analytical analysis in environmental media. An overview summary of the range of concentrations detected in environmental media is presented in Table 5-7.

Detections of perfluoroalkyls in air, water, and soil at NPL sites are summarized in Table 5-8.

Table 5-6. Lowest Limit of Detection Based on Standards[a]

Media	Detection limit	Reference
Air	0.1 pg/m^3	Harada et al. 2006
Drinking water	0.5–6.5 ng/L	EPA 2009c (Method 537)
Surface water and groundwater	0.2 ng/L	Nakayama et al. 2007
Soil	0.11–0.75 µg/kg (median reporting limits)	Anderson et al. 2016
Sediment	0.21–1.2 µg/kg (median reporting limits)	Anderson et al. 2016
Whole blood Serum	0.1–2 ng/mL 0.082–0.2 ng/mL	Kärrman et al. 2005 CDC 2015

[a]Detection limits based on using appropriate preparation and analytics. These limits may not be possible in all situations.

Table 5-7. Summary of Environmental Levels of Perfluoroalkyls[a]

Media	Low	High[b]	For more information
Outdoor air (pg/m^3)			Table 5-9
PFOA	1.22	900,000	
PFOS	0.46	9.8	
Indoor air (pg/m^3)			Table 5-10
PFOA		4.4 (mean)	
PFOS		<47.4 (mean)	
Dust (ng/g)			Table 5-11
PFOA	<2.29	9,818	
PFOS	<4.56	18,071	
Surface water (ppb)			Tables 5-12, 5-14, 5-17
PFOA	0.00051	598	
PFOS	<0.00008	8,970	
Groundwater (ppb)			Tables 5-14, 5-17
PFOA	0.083	619	
PFOS	0.0404	4,300	
Drinking water (ppb)			EPA 2010
PFOA	<0.0023	~100	
Ocean water (pg/L)			Table 5-15
PFOA	88	192,000	
PFOS	8	57,700	
Food (ppb)			Fromme et al. 2007b
PFOA	0.025	118.29	
PFOS	0.025	1.03	
Soil (ppb)			Tables 5-16, 5-17
PFOA	<0.00017	140	
PFOS	0.0002	9700	

[a]For PFOA or PFOS only.
[b]High levels are representative of monitoring data at localized contaminated sites and are not reflective of background environmental levels.

Table 5-8. Perfluoroalkyls Levels in Water, Soil, and Air of National Priorities List (NPL) Sites

Medium	Median[a]	Geometric mean[a]	Geometric standard deviation[a]	Number of quantitative measurements	NPL sites
PFOA					
Water (ppb)	0.35	0.25	6,064	5	4
Soil (ppb)	18,050	18,050	1,000	2	2
Air (ppbv)			No data		
PFOS					
Water (ppb)	0.91	0.35	9,089	4	3
Soil (ppb)	108,000	108,000	1,000	2	2
Air (ppbv)			No data		

Table 5-8. Perfluoroalkyls Levels in Water, Soil, and Air of National Priorities List (NPL) Sites

Medium	Median[a]	Geometric mean[a]	Geometric standard deviation[a]	Number of quantitative measurements	NPL sites
PFBA					
Water (ppb)	2.15	1.03	28,192	3	3
Soil (ppb)	1,600	1,600	1,000	2	2
Air (ppbv)			No data		
PFBS					
Water (ppb)	0.05	0.02	6,770	2	2
Soil (ppb)	224	224	1,000	2	2
Air (ppbv)			No data		
PFHpA					
Water (ppb)	0.07	0.04	3,169	3	2
Soil (ppb)	1,275	1,275	1,000	2	2
Air (ppbv)			No data		
PFHxA					
Water (ppb)	0.25	0.10	8,444	2	2
Soil (ppb)	1,175	1,175	1,000	2	2
Air (ppbv)			No data		
PFHxS					
Water (ppb)	0.26	1.12	52,496	4	3
Soil (ppb)	5,585	5,585	1,000	2	2
Air (ppbv)			No data		
PFNA					
Water (ppb)			No data		
Soil (ppb)	27.2	27.2	1,000	2	2
Air (ppbv)			No data		
PFPeA					
Water (ppb)	0.18	0.11	3,465	3	3
Soil (ppb)	178	178	1,000	2	2
Air (ppbv)			No data		

[a]Concentrations found in ATSDR site documents from 1981 to 2017 for 1,854 NPL sites (ATSDR 2017). Maximum concentrations were abstracted for types of environmental media for which exposure is likely. Pathways do not necessarily involve exposure or levels of concern.

5.5.1 Air

Perfluoroalkyl levels have been measured in outdoor air at locations in the United States, Europe, Japan, and over the Atlantic Ocean (Barber et al. 2007; Barton et al. 2006; Harada et al. 2005a, 2006; Kim and Kannan 2007). Concentrations reported in these studies are provided in Table 5-9.

Mean PFOA levels ranged from 1.54 to 15.2 pg/m^3 in air samples collected in the urban locations in Albany, New York; Fukuchiyama, Japan; and Morioka, Japan and in the rural locations in Kjeller, Norway and Mace Head, Ireland. Higher mean concentrations (101–552 pg/m^3) were measured at the urban locations in Oyamazaki, Japan and Manchester, United Kingdom, and semirural locations in Hazelrigg, United Kingdom. Maximum reported concentrations at Oyamazaki and Hazelrigg were 919 and 828 pg/m^3, respectively. The authors attributed the elevated concentrations at the Hazelrigg location to emissions from a fluoropolymer production plant located 20 km upwind of this semirural community.

Elevated levels of PFOA were observed in air samples collected along the fence line of the DuPont Washington Works fluoropolymer manufacturing facility, which is located near Parkersburg, West Virginia, in the Ohio River valley (Barton et al. 2006).

Table 5-9. Concentrations of Perfluoroalkyl in Outdoor Air

Location	Mean (range) concentration (pg/m^3)			Reference
	PFOA	PFHpA	PFNA	
Urban				
Albany, New York				
Gas phase (n=8)	3.16 (1.89–6.53)	0.26 (0.13–0.42)	0.21 (0.16–0.31)	Kim and Kannan 2007
Particulate phase (n=8)	2.03 (0.76–4.19)	0.37 (<0.12–0.81)	0.13 (<0.12–0.40)	Kim and Kannan 2007
Oyamazaki, Japan (n=12)	262.7 (72–919); 3,412.8 ng/g in dust	—	—	Harada et al. 2005b
Fukuchiyama, Japan	15.2; 314 ng/g in dust	—	—	Harada et al. 2006
Morioka, Japan (n=8)	2.0 (1.59–2.58)	—	—	Harada et al. 2005b
Manchester, United Kingdom (n=2,1)[a]	341, 15.7	8.2, 0.2	<26.6, 0.8	Barber et al. 2007

Table 5-9. Concentrations of Perfluoroalkyl in Outdoor Air

Rural				
Kjeller, Norway (n=2)	1.54	0.87	0.12	Barber et al. 2007
Mace Head, Ireland (n=4)	8.9	<0.001	<3.3	Barber et al. 2007
Hazelrigg, United Kingdom (semi-rural) (n=10)	101, 552[b,c]	1.6, 14.4[b]	0.9	Barber et al. 2007
Marine air				
Near Europe (northwest) (n=3)	1.22 (0.5–2.0)	<0.6 (ND–<0.6)	0.3 (ND–0.5)	Jahnke et al. 2007a
Near Africa (east coast) (n=5)	<0.5 (ND–0.7)	ND	<0.2 (ND–0.3)	Jahnke et al. 2007a
Source dominated				
DuPont Washington Works Facility; Parkersburg, West Virginia (n=28)	430,000 (75,000–900,000)[d]	—	—	Barton et al. 2006
DuPont Washington Works Facility; Parkersburg, West Virginia (n=90)	5,500 (10–75,900)	—	—	EPA 2007b
	Mean (range) concentration (pg/m^3)			
Location	PFDA	PFUnA	PFDoDA	Reference
Urban				
Albany, New York				
Gas phase (n=8)	0.63 (0.24–1.56)	<0.12 (ND–0.16)	0.27 (0.14–0.43)	Kim and Kannan 2007
Particulate phase (n=8)	0.27 (0.13–0.49)	ND	0.12 (<0.12–0.38)	Kim and Kannan 2007
Oyamazaki, Japan (n=12)	—	—	—	Harada et al. 2005b
Fukuchiyama, Japan	—	—	—	Harada et al. 2006
Morioka, Japan (n=8)	—	—	—	Harada et al. 2005b
Manchester, United Kingdom (n=2,1)[a]	5.4, <0.8	<0.01, <0.4	<0.01, <0.01	Barber et al. 2007
Rural				
Kjeller, Norway (n=2)	<0.15	<0.12	<0.12	Barber et al. 2007
Mace Head, Ireland (n=4)	<2.8	<0.002	<0.003	Barber et al. 2007
Hazelrigg, United Kingdom (semi-rural) (n=10)	1.0, 8.3[b]	0.7	<0.01	Barber et al. 2007

Table 5-9. Concentrations of Perfluoroalkyl in Outdoor Air

Marine air					
Near Europe (northwest) (n=3)	<0.6 (ND–0.6)	ND		<0.14 (ND–0.17)	Jahnke et al. 2007a
Near Africa (east coast) (n=5)	ND	0.03 (ND–0.2)		ND	Jahnke et al. 2007a
Source dominated					
DuPont Washington Works Facility; Parkersburg, West Virginia (n=28)	—	—		—	Barton et al. 2006
DuPont Washington Works Facility; Parkersburg, West Virginia (n=90)	—	—		—	EPA 2007b
	Mean (range) concentration (pg/m^3)				
Location	PFOS	PFBS	PFHxS	FOSA	Reference
Urban					
Albany, New York					
Gas phase (n=8)	1.70 (0.94–3.0)	—	0.31 (0.13–0.44)	0.67 (0.22–2.26)	Kim and Kannan 2007
Particulate phase (n=8)	0.64 (0.35–1.16)	—	<0.12	0.29 (<0.12–0.79)	Kim and Kannan 2007
Oyamazaki, Japan (n=12)	5.2 (2.51–-9.80); 72.2 ng/g in dust	—	—	—	Harada et al. 2005b
Fukuchiyama, Japan	2.2; 46.0 ng/g in dust	—	—	—	Harada et al. 2006
Morioka, Japan (n=8)	0.7 (0.46–1.19)	—	—	—	Harada et al. 2005b
Manchester, United Kingdom (n=2,1)[a]	46, 7.1	2.2, <1.6	1.0, 0.1	<1.6, <0.2	Barber et al. 2007
Rural					
Kjeller, Norway (n=2)	1.0	<0.09	0.05	0.78	Barber et al. 2007
Mace Head, Ireland (n=4)	<1.8	<1.0	0.07	<0.56	Barber et al. 2007
Hazelrigg, United Kingdom (semi-rural) (n=10)	1.6	2.6	0.04	0.2	Barber et al. 2007
Marine air					
Near Europe (north west) (n=3)	1.36 (0.4–2.5)	ND	0.12 (0.02–0.3)	ND	Jahnke et al. 2007a
Near Africa (east coast) (n=5)	0.544 (0.05–1.9)	ND	0.013 (ND–0.05)	ND	Jahnke et al. 2007a

Table 5-9. Concentrations of Perfluoroalkyl in Outdoor Air

Source dominated					
DuPont Washington Works Facility; Parkersburg, West Virginia (n=28)	—	—	—	—	Barton et al. 2006
DuPont Washington Works Facility; Parkersburg, West Virginia (n=90)	—	—	—	—	EPA 2007b

[a]Mean values were reported for separate sampling sessions.
[b]The second concentration reported was measured during an earlier sampling session (n=2).
[c]A maximum PFOA concentration of 828 pg/m^3 was measured in air at Hazelrigg, United Kingdom.
[d]Average and range of concentrations in 6 out of 28 samples that contained PFOA.

"—" indicates no available data; FOSA = perfluorooctane sulfonamide; ND = not detected; PFBS = perfluorobutane sulfonic acid; PFDA = perfluorodecanoic acid; PFDoDA = perfluorododecanoic acid; PFHpA = perfluoroheptanoic acid; PFHxS = perfluorohexane sulfonic acid; PFNA = perfluorononanoic acid; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid; PFUnA = perfluoroundecanoic acid

The reported concentrations in these samples ranged from 75,000 to 900,000 pg/m^3. The highest concentrations were measured at locations downwind of the facility. High volume air samples collected at several monitoring stations near the Washington Works facility contained PFOA at reported concentrations ranging from 10 to 75,900 pg/m^3 (EPA 2007b). The mean and median of these reported concentrations are 5,500 and 240 pg/m^3, respectively.

PFOS was detected above quantitation limits in most of the studies, but concentrations were generally below 5 pg/m^3. A concentration of 46 pg/m^3 was reported in samples from Manchester, United Kingdom. Reported concentrations of other perfluoroalkyls (PFHpA, PFNA, PFDA, PFUnA, PFDoDA, PFBS, PFHxS, and FOSA) were generally <1 pg/m^3 in these studies. PFHpA was detected at slightly higher concentrations (8.2 and 14.4 pg/m^3) at Manchester and Hazelrigg, United Kingdom, respectively.

Jahnke et al. (2007a) collected eight marine air samples during a cruise between Germany and South Africa (53°N to 33°S). Perfluoroalkyl concentrations steadily declined as the sampling moved further from Europe and toward less industrialized regions. Only PFOS was detected in the two samples collected over the Atlantic Ocean east of southern Africa.

Measurements of perfluoroalkyls in snow samples collected from Canadian Arctic ice caps suggest that volatile precursors, such as fluorinated telomer alcohols, may oxidize in the atmosphere at these locations (Young et al. 2007). Reported concentrations in these snow samples were 2.6–86 pg/L for PFOS, 12–147 pg/L for PFOA, 5.0–246 pg/L for PFNA, <8–22 pg/L for PFDA, and <6–27 pg/L for PFUnA.

The concentration of PFOS measured in rainwater collected during a rain event in Winnipeg, Manitoba was 0.59 ng/L (Loewen et al. 2005). PFOA, PFNA, PFDA, PFUnA, and PFDoDA were not detected in the rainwater. Reported method detection limits for these compounds were 7.2, 3.7, 1.7, 1.2, and 1.1 ng/L, respectively.

Studies of perfluoroalkyl concentrations in indoor environments are available. The reported mean concentrations of perfluoroalkyls measured in four indoor air samples collected from Tromso, Norway (Barber et al. 2007) are presented in Table 5-10.

Table 5-10. Concentrations of Perfluoroalkyl in Indoor Air[a]

	Mean concentration (pg/m^3)
PFOA	4.4
PFHpA	0.8
PFNA	2.7
PFDA	3.4
PFUnA	<1.3
PFDoDA	1.2
PFOS	<47.4
PFBS	<0.5
PFHxS	<4.1
FOSA	2.8

[a]Samples (n=4) collected in Tromso, Norway in May–June 2005.

FOSA = perfluorooctane sulfonamide; PFBS = perfluorobutane sulfonic acid; PFDA = perfluorodecanoic acid; PFDoDA = perfluorododecanoic acid; PFHpA = perfluoroheptanoic acid; PFHxS = perfluorohexane sulfonic acid; PFNA = perfluorononanoic acid; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid; PFUnA = perfluoroundecanoic acid

Source: Barber et al. 2007

Several studies have measured perfluoroalkyl levels in indoor dust samples (Table 5-11). Kubwabo et al. (2005) measured the concentrations of selected perfluoroalkyls in dust samples from 67 Canadian homes. PFOA, PFOS, and PFHxS were each detected in 37, 33, and 15% of these samples, respectively (detection limits of 2.29, 4.56, and 4.56 ng/g, respectively). FOSA was only detected above 0.99 ng/g in 10% of the samples and PFBS was not detected in any of the samples. Moriwaki et al. (2003) measured PFOS and PFOA concentrations in vacuum cleaner dust samples collected from 16 Japanese homes. PFOS and PFOA were detected in every sample with reported concentrations of 11–140 and 69–380 ng/g, respectively, in 15 of the 16 samples. One of the samples contained 2,500 ng/g PFOS and 3,700 ng/g PFOA. The geometric means of PFOA and PFOS in pooled indoor air sample meta data analysis were

reported as 37.34 and 38.91 ng/g, respectively (Mitro et al. 2016). Precursor substances present in dust can be biotransformed to perfluoroalkyl substances and may be a further source of human exposure. Fluorotelomer alcohols were detected in hotel dust in China at levels ranging from 24.8 to 678 ng/g (Yao et al. 2018). Makey et al. (2017) reported precursor substances such as dipolyfluoroalkyl phosphates (diPAPs), fluorotelomer alcohols, perfluorooctyl sulfonamides, and sulfonamidoethanols in airborne and dust samples.

Table 5-11. Concentrations of Perfluoroalkyls in Indoor Dust

	Concentration (ng/g): mean (range); median			
Location	PFOA	PFHpA	PFNA	Reference
Ottawa, Canada (n=67)	106.00 (<2.29–1,234); 19.72[a]	—	—	Kubwabo et al. 2005
Japan (n=16)	380 (70–3,700); 165	—	—	Moriwaki et al. 2003
North Carolina and Ohio (n=112)	296 (<10.2–1,960); 142[b]	109 (<12.5–1,150); 50.2[b]	22.1 (<11.3–263); 7.99[b]	Strynar and Lindstrom 2008
United Kingdom, Australia, German, and United States (n=39)[c]	96.5 (9,818)	97.3 (5,195)	<LOQ (832)	Kato et al. 2009a
Stockholm, Sweden[d]		—	—	Björklund et al. 2009
Houses (n=10)	54 (15–98)			
Apartments (n=38)	93 (17–850)			
Daycare centers (n=10)	41 (31–110)			
Offices (n=10)	70 (14–510)			
Cars (n=5)	33 (12–96)			

	Concentration (ng/g): mean (range) median			
Location	PFDA	PFUnA	PFDoDA	Reference
Ottawa, Canada (n=67)	—	—	—	Kubwabo et al. 2005
Japan (n=16)	—	—	—	Moriwaki et al. 2003
North Carolina and Ohio (n=112)	15.5 (<9.40–267); 6.65[b]	30.4 (<10.7–588); 7.57[b]	18.0 (<11.0–520); 7.78[b]	Strynar and Lindstrom 2008
United Kingdom, Australia, German, and United States (n=39)[c]	<LOQ (1,965)	<LOQ (732)	<LOQ (1,048)	Kato et al. 2009a
Stockholm, Sweden[d]	—	—	—	

	Concentration (ng/g): mean (range); median				
Location	PFOS	PFBS	PFHxS	FOSA	Reference
Ottawa, Canada (n=67)	443.68 (<4.56–5,065); 37.8[a]	ND[a]	391.96 (<4.56–4,305); 23.1[a]	<0.99[a]	Kubwabo et al. 2005
Japan (n=16)	200 (11–2,500); 24.5	—	—	—	Moriwaki et al. 2003
North Carolina and Ohio (n=112)	761 (<8.93–12,100); 201[b]	41.7 (<12.5–1,150); 9.11[b]	874 (<12.9–35,700); 45.5[b]	—	Strynar and Lindstrom 2008

Table 5-11. Concentrations of Perfluoroalkyls in Indoor Dust

United Kingdom, Australia, German, and United States (n=39)[c]	479.6 (18,071)	359.0 (7,718)	185.5 (43,765)	<LOQ (18.4)	Kato et al. 2009a
Stockholm, Sweden[d]		—	—	—	Björklund et al. 2009
Houses (n=10)	39 (15–120)				
Apartments (n=38)	85 (8–1,100)				
Daycare centers (n=10)	31 (23–65)				
Offices (n=10)	110 (29–490)				
Cars (n=5)	12 (8–33)				

[a]Method detection limits (MDL) and percent below MDL are as follows: PFOA (2.29 ng/g, 37%), PFOS (4.56 ng/g, 33%), PFBS (1.38 ng/g, 100%), PFHxS (4.56, 15%), and FOSA (0.99 ng/g, 90%).
[b]LOQ and percent above LOQ are as follows: PFHpA (12.5 ng/g, 74.1%), PFOA (10.2 ng/g, 96.4%), PFNA (11.3 ng/g, 42.9%), PFDA (9.40 ng/g, 30.4%), PFUnA (10.7 ng/g, 36.6%), PFDoDA (11.0 ng/g, 18.7%), PFOS (8.93 ng/g, 94.6%), PFHxS (12.9 ng/g, 77.7%), PFBS (12.5 ng/g, 33.0%). Values below the LOQ were assigned a value of LOQ/1.412 when calculating the median and mean.
[c]Reported values are the 50th percentile and (maximum) values; all minimum values were <LOQ.
[d]Reported values are the median and range.

"—" indicates no available data; FOSA = perfluorooctane sulfonamide; LOQ = limit of quantification; ND = not detected; PFBS = perfluorobutane sulfonic acid; PFDA = perfluorodecanoic acid; PFDoDA = perfluorododecanoic acid; PFHpA = perfluoroheptanoic acid; PFHxS = perfluorohexane sulfonic acid; PFNA = perfluorononanoic acid; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid; PFUnA = perfluorundecanoic acid

Strynar and Lindstrom (2008) measured perfluoroalkyl levels in 112 indoor dust samples collected from homes and daycare centers in North Carolina and Ohio. These authors detected PFHpA, PFOA, PFNA, PFDA, PFUnA, PFDoDA, PFOS, PFHxS, and PFBS. PFOS and PFOA were detected in 94.6 and 96.4% of the samples, respectively. Maximum detections in the samples were as high as 12,100 ng/g for PFOS and 35,700 ng/g for PFHxS. Household dust samples collected from the United Kingdom, Australia, Germany, and the United States showed the presence of perfluoroalkyls (Kato et al. 2009a). PFOS, PFBS, and PFHxS were detected in 74.4, 92.3, and 79.5% of the samples, respectively, whereas PFOA, PFNA, and PFDA were detected in 64.1, 25.6, and 38.5% of the samples, respectively. Björklund et al. (2009) measured PFOA and PFOS in dust samples collected in houses, apartments, daycare centers, offices, and cars in Sweden. PFOA and PFOS were detected in 100 and 79% of the apartment samples and in 100 and 60% of the car samples. The authors concluded that while dietary intake was the major PFOA/PFOS exposure pathway for adults and toddlers in the general population, dust ingestion could become an important pathway under a worst-case scenario (e.g., high dust ingestion and maximum dust levels).

Perfluoroalkyls in indoor and outdoor environments in urban, suburban, and rural locations near Toronto, Ontario, Canada were assessed using window films for passive sampling. The sum of perfluoroalkyls concentrations on outdoor window films ranged from 0.04 to 0.75 pg/cm^2 in winter and from 0.04 to

0.92 pg/cm^2 in summer, with higher values found in urban and suburban locations than in rural locations. Indoors, concentrations on window film ranged from less than the detection limit (which ranged from 0.033 to 0.06 pg/cm^2) to 2.1 pg/cm^2 in winter and from 0.08 to 4.3 pg/cm^2 in summer, although there were no distinct trends between urban and rural for indoor concentrations (Gewurtz et al. 2009).

5.5.2 Water

PFOS and PFOA have been widely detected in surface water samples collected from various rivers, lakes, and streams in the United States (Boulanger et al. 2004; DRBC 2013; Kannan et al. 2005; Kim and Kannan 2007; Nakayama et al. 2007; Simcik and Dorweiler 2005; Sinclair et al. 2004, 2006). Levels of these substances in surface water appear to be declining since the phase out of these two substances. Zhang et al. (2016) measured surface water levels of perfluoroalkyls in 2014 at 37 sites across the northeastern United States. Detectable levels of PFOA and PFNA were found at all sites, and PFHxS, PFOS, and PFDA were detectable at >90% of the sites. The respective maximum concentrations of PFOA, PFOS, PFHxS, and PFNA were 56 ng/L measured in the Passaic River, New Jersey, 27.5 ng/L measured in the Woonasquatucket River, Rhode Island, 43 ng/L measured at Mill Cove, Rhode Island, and 14 ng/L measured at Mill Cove, Rhode Island (Zhang et al. 2016).

Less data are available regarding the concentrations of other perfluoroalkyls in surface water. PFHpA and PFHxS were commonly detected in the few studies that analyzed surface water for these compounds (DRBC 2013; Kim and Kannan 2007; Nakayama et al. 2007; Simcik and Dorweiler 2005). Concentrations of PFOA and PFOS measured in surface water are presented in Table 5-12 and other perfluoroalkyls are summarized in Table 5-13. Maximum concentrations of PFOS, PFOA, PFHpA, PFNA, PFDA, PFUnA, PFDoDA, PFBS, and PFHxS measured in surface water collected from the Cape Fear Basin, North Carolina were 287, 132, 329, 194, 120, 52.1, 4.46, 9.41, and 35.1 ng/L, respectively (Nakayama et al. 2007). Much higher concentrations of PFOS (198–1,090 ng/L) have been measured in Onondaga Lake in Syracuse, New York (Sinclair et al. 2006). Onondaga Lake is a Superfund site that has become contaminated through industrial activity along its banks.

Table 5-12. Concentrations of PFOA and PFOS in Surface Water (ng/L)

	Concentration		
Location	PFOA	PFOS	Reference
Great Lakes			Boulanger et al. 2004
Lake Ontario (n=8)	15–70	6–121	

Table 5-12. Concentrations of PFOA and PFOS in Surface Water (ng/L)

Location	Concentration		Reference
	PFOA	PFOS	
Lake Erie (n=8)	21–47	11–39	
New York State waters			Sinclair et al. 2006
Lake Ontario (n=13)	18–34	2.9–30	
Niagara River (n=3)	18–22	3.3–6.7	
Lake Erie (n=3)	13–27	2.8–5.5	
Finger Lakes (n=13)	11–20	1.3–2.6	
Onondaga Lake (n=3)	39–64	198–1,090 (median=756)	
Oneida Lake (n=1)	19	3.5	
Erie Canal (n=3)	25–59	5.7–13	
Hudson River (n=8)	22–173 (median=35)	1.5–3.4	
Lake Champlain (n=4)	10–46	0.8–7.7	
Albany, New York			Kim and Kannan 2007
Lake water (n=11)	3.27–15.8 (median=7.20)	ND–9.30 (median=2.88)	
Surface water runoff (n=14)	0.51–29.3 (median=3.80)	<0.25–14.6 (median=0.81)	
New York/New Jersey/Rhode Island metropolitan region	56 (maximum) 3.5 (median)	27.5 (maximum) 0.96 (median)	Zhang et al. 2016
Michigan water regions			Sinclair et al. 2004
Detroit (n=10)	<8–16.14	<0.08–6.13	
Flint (n=4)	<8–23.01	1.50–12.31	
Saginaw Bay (n=5)	<8–24.08	3.10–12.69	
Northeastern Michigan (n=2)	<8	0.87–6.34	
Upper Peninsula (n=7)	<8–13.77	<0.8–3.09	
Northwestern Michigan (n=2)	11.96	<0.8–4.48	
Western Michigan (n=6)	<8–15.17	<0.8–5.32	
Southwestern Michigan (n=5)	8.74–35.86	7.22–29.26	
Lansing (n=3)	<8–13.37	1.04–4.96	
Minnesota Waters and Lake Michigan			Simcik and Dorweiler 2005
Remote (n=4)			
Loiten	0.7	ND	
Little Trout	0.3	1.2	
Nipisiquit	0.1	ND	
Tettegouche	0.5	0.2	
Urban (n=4)			
Calhoun	20	47	
Lake Harriet	3.5	21	
Lake of the Isles	0.5	2.4	

Table 5-12. Concentrations of PFOA and PFOS in Surface Water (ng/L)

Location	Concentration		Reference
	PFOA	PFOS	
Minnesota River	1.2	9	
Lake Michigan (n=4)	<0.6–0.5	1–3.2	
Cape Fear Basin, North Carolina			Nakayama et al. 2007
80 Sites (n=100)			
Mean	43.4	31.2	
Median	12.6	28.9	
Minimum	ND	<1	
Maximum	287	132	
Percent not detected[a]	7.6	0	
Raisin and St. Clair Rivers, Michigan			Kannan et al. 2005
Raisin River	14.7	3.5	
St. Clair River (n=3)	4.0–5.0	1.9–3.9	
Conasauga River, Georgia	253–1,150	192–318	Konwick et al. 2008
Dalton, Georgia	49.9–299	15.8–120	Konwick et al. 2008
Tidal Delaware River (six locations)			DRBC 2013
2007	3.54–75.40	2.70–8.42	
2008	3.99–48	3.53–11.7	
2009	3.29–27.7	2.86–7.97	
Several rivers in Japan	0.1–67,000	0.3–59	Harada and Koizumi 2009
Lake Victoria Gulf, Kenya	0.4–96.4 (rivers) 0.4–11.6 (lakes)	<0.4–13.23 (rivers); <0.4–2.53 (lakes)	Orata et al. 2009
River Po, Italy	1–1,270	1–25	Loos et al. 2008

[a]Detection limit is 0.05 ng/L

ND = not detected; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid

Table 5-13. Concentrations of Other Perfluoroalkyls in Surface Water

Location (reference)[a]	Concentration (ng/L)							
	PFHpA	PFNA	PFDA	PFUnA	PFDoDA	PFBS	PFHxS	FOSA
Great Lakes (Boulanger et al. 2004)								
Lake Ontario (n=8)	—	—	—	—	—	—	—	—
Lake Erie (n=8)	—	—	—	—	—	—	—	—
New York State waters (Sinclair et al. 2006)								
Onondaga Lake (n=3)	—	—	—	—	—	—	4.2–8.5	—

Table 5-13. Concentrations of Other Perfluoroalkyls in Surface Water

Location (reference)[a]	Concentration (ng/L)							
	PFHpA	PFNA	PFDA	PFUnA	PFDoDA	PFBS	PFHxS	FOSA
Erie Canal (n=3)	—	—	—	—	—	—	2.5–5.6	—
Other lakes and rivers	—	—	—	—	—	—	0.5–2.8	—
Albany, New York (Kim and Kannan 2007)								
Lake water (n=11)	1.15–12.7	ND–3.51	0.25–3.58	ND–1.45	ND–<0.25	—	<0.25–4.05	ND–0.47
Surface water runoff (n=14)	<0.25–6.44	<0.25–5.90	ND–8.39	ND–1.99	ND–1.60	—	ND–13.5	ND–2.14
New York/New Jersey/Rhode Island metropolitan region (Zhang et al. 2016)								
	48.2 (max); 0.9 (median)	14 (max); 0.4 (median)	5.8 (max); 0.2 (median)	1.9 (max); 0.1 (median)	2.6 (max); 0.0 (median)	6.2 (max); 0.4 (median)	43 (max); 0.7 (median)	—
Minnesota waters and Lake Michigan (Simcik and Dorweiler 2005)								
Remote (n=4)								
Loiten	10	ND	ND	—	—	—	—	—
Little Trout	4.8	ND	ND	—	—	—	—	—
Nipisiquit	0.9	<0.3	ND	—	—	—	—	—
Tettegouche	3.1	ND	ND	—	—	—	—	—
Urban (n=4)					—	—	—	—
Calhoun	11	0.6	0.5	—	—	—	—	—
Lake Harriet	2.6	ND	ND	—	—	—	—	—
Lake of the Isles	0.4	ND	ND	—	—	—	—	—
Minnesota River	0.7	1.9	ND	—	—	—	—	—
Lake Michigan (n=4)	<0.6–4.1	<0.6–3.1	ND	—	—	—	—	—
Cape Fear Basin, North Carolina (Nakayama et al. 2007)								
80 Sites (n=100)								
Mean	38.7	33.6	22.1	10.4	2.17	2.58	7.29	—
Median	14.8	5.70	13.2	5.67	1.95	2.46	5.66	—
Maximum	329	194	120	52.1	4.46	9.41	35.1	—
Percent not detected[b]	32.9	10.1	15.2	17.7	53.2	38.0	45.6	—
Raisin and St. Clair Rivers, Michigan (Kannan et al. 2005)								
Raisin River	—	—	—	—	—	—	<1	<10
St. Clair River (n=3)	—	—	—	—	—	—	<1	<10

Table 5-13. Concentrations of Other Perfluoroalkyls in Surface Water

Location (reference)[a]	Concentration (ng/L)							
	PFHpA	PFNA	PFDA	PFUnA	PFDoDA	PFBS	PFHxS	FOSA
Delaware River (6 locations) (DRBC 2013)								
2007	ND–24.30	1.71–976	ND-9.97	ND-26	ND	—	ND-4.48	ND
2008	2.03–16.3	3.24–650	ND-5.25	ND-11.8	ND	—	BD-3.55	ND
2009	1.14–47.4	1.65–546	ND-1.75	ND-8.3	ND	—	ND-3.62	ND

[a]See Table 5-12 for numbers of samples collected at these locations.
[b]Detection limit = 0.05 ng/L.

"—" indicates no available data; ND = not detected; FOSA = perfluorooctane sulfonamide; PFBS = perfluorobutane sulfonic acid; PFDA = perfluorodecanoic acid; PFDoDA = perfluorododecanoic acid; PFHpA = perfluoroheptanoic acid; PFHxS = perfluorohexane sulfonic acid; PFNA = perfluorononanoic acid; PFUnA = perfluoroundecanoic acid

Higher levels of perfluoroalkyls are expected in surface water and groundwater surrounding perfluorochemical industrial facilities. Paustenbach et al. (2007) estimated PFOA concentrations in environmental media for communities located near the DuPont Washington Works chemical manufacturing facility. From this analysis, the authors concluded that much of the PFOA detected in groundwater near the facility was attributed to deposition to soil surfaces following atmospheric emissions from the plant followed by subsequent leaching into groundwater. DuPont entered into an agreement with the EPA to collect monitoring data for PFOA in the Ohio River around the DuPont Washington Works facility. In its final phase III assessment, it was reported that levels of PFOA downstream from the facility in 2011 were about an order of magnitude lower than when monitoring began in 2002, while levels upstream essentially remained unchanged (URS 2012). The maximum concentration measured in 2011 was 200 ng/L and was obtained from a monitoring location adjacent to the site.

PFOA, PFOS, PFBA, PFHxS, and PFBS have been detected in the municipal drinking water of communities located near the 3M Cottage Grove fluorochemical facility (ATSDR 2008). Xiao et al. (2015) summarized soil and groundwater monitoring data of select perfluoroalkyls near this facility. Groundwater samples for PFBA, PFHxA, PFOA, PFHxS, and PFOS in wells surrounding the site obtained from 2009 to 2013 showed little or no change in concentration over this time period, even though 3M had stopped producing perfluoroalkyls at this facility in 2002. Levels in groundwater were shown to decrease exponentially with distance from the source. A measured PFOA concentration of approximately 20,000 ng/L was observed from a well near the historical unlined disposal site of this

facility, but levels decreased to <100 ng/L measured at a distance of 1.4 km away. Some other examples are provided in Table 5-14.

Table 5-14. Concentrations of Perfluoroalkyls in Surface Water and Groundwater at Fluorochemical Industrial Facilities

Location	Percent detection and concentration (µg/L)					Reference
	PFOA	PFBA	PFOS	PFHxS	PFBS	
DuPont Washington Works Facility, West Virginia						
Groundwater						
Borings (n=18)						Davis et al. 2007
Percent detected	89%[a]	—	—	—	—	
Minimum	0.0912[a]	—	—	—	—	
Maximum	78[a]	—	—	—	—	
Wells (n=14)			—	—	—	Davis et al. 2007
Percent detected	100%[a]	—	—	—	—	
Minimum	0.081[a]	—	—	—	—	
Maximum	37.1[a]	—	—	—	—	
Wells (n=3)			—	—	—	EPA 2008d
Percent detected	100%	—	—	—	—	
Minimum	2.8	—	—	—	—	
Maximum	100	—	—	—	—	
Surface water						
Outlets (n=4)						EPA 2008d
Percent detected	100%	—	—	—	—	
Minimum	2.3	—	—	—	—	
Maximum	61	—	—	—	—	
3M Cottage Grove Facility, Minnesota						
Groundwater						
Wells (n=1–7)						3M 2007b
Percent detected	100%	100%	100%	100%	100%	
Minimum	24.6	23.3	26.0	6.47	2.11	
Maximum	619	318	26.0	40.0	26.1	
Surface water						
East and West Cove (n=3–9)						3M 2007b
Percent detected	100%	100%	100%	100%	78%	
Minimum	0.172	0.803	0.227	0.0936	0.304	
Maximum	2.79	1.01	3.12	4.58	9.69	
Mississippi River Shoreline (n=52–80)						3M 2007b
Percent detected	60%	52%	43%	28%	56%	
Maximum	0.760	6.92	0.539	1.04	3.05	

Table 5-14. Concentrations of Perfluoroalkyls in Surface Water and Groundwater at Fluorochemical Industrial Facilities

	Percent detection and concentration (µg/L)					
Location	PFOA	PFBA	PFOS	PFHxS	PFBS	Reference
Mississippi River Transect (n=34–44)						3M 2007b
Percent detected	14%	12%	0%	0%	0%	
Maximum	0.0501	0.0530	ND	ND	ND	
3M Decatur Facility, Alabama						
Groundwater						
Off-site groundwater (n=18)						3M 2008c
Percent detected	94%	—	—	—	—	
Mean	1.87	—	—	—	—	
Range	0.083–19.8	—	—	—	—	
Off-site groundwater						3M 2010
Range			0.0404–2.41	0.0396–0.622	0.0615–0.480	
Groundwater range (n=51)	0.149–6.41	0.0104–1.26	0.012–0.151	0.0127–0.0875	0.0101–0.0766	Lindstrom et al. 2011
Surface water						
On-site surface water (n=7)						3M 2008c
Percent detected	100%	—	—	—	—	
Median	2.66	—	—	—	—	
Range	0.32–127	—	—	—	—	
Off-site surface water (n=60)						3M 2008c
Percent detected	98%	—	—	—	—	
Range	0.026–27.7	—	—	—	—	
Tennessee River						Hansen et al. 2002
Upstream of facility (n=19)						
Percent detected	0%	—	100%	—	—	
Range	<25	—	16.8–52.6	—	—	
Downstream of facility (n=21)				—	—	
Percent detected	0%	—	100%	—	—	
Median	355	—	107	—	—	
Range	<25–598	—	30.3–144	—	—	

[a]Analyte was reported as APFO.

"—" indicates no available data; APFO = ammonium perfluorooctanoate; ND = not detected; PFBA = perfluorobutanoic acid; PFBS = perfluorobutane sulfonic acid; PFHxS = perfluorohexane sulfonic acid; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid

Groundwater sampling of PFOA and PFOS was conducted at 401 military installations (DoD 2018). The PFOS/PFOA levels exceeded EPA's lifetime health advisories of 70 ppt at 22% of the installations (9/64 Army, 40/127 Navy/U.S. Marine Corp, 39/203 Air Force, and 2/7 Defense Logistics Agency

installations). The PFOS/PFOA levels exceeded the EPA lifetime health advisories in 1,621 of the 2,668 groundwater wells sampled (61%).

Yamashita et al. (2005) measured PFOA, PFOS, PFNA, and PFHxS concentrations in ocean water collected from locations in the Atlantic Ocean, Pacific Ocean, and areas near China, Korea, and Japan. These concentrations are listed in Table 5-15. Wei et al. (2007a) measured perfluoroalkyl concentrations in surface seawaters from the western Pacific Ocean, Indian Ocean, and near-Antarctic region. PFOS and PFOA were detected in 60 and 40% of the samples, respectively, with maximum concentrations of 71.7 and 441.6 pg/L, respectively. Concentrations of other perfluoroalkyls (PFHxS, PFBS, PFDoDA, PFDA, PFNA, PFHpA) were generally below detection in most samples, with the exceptions being in samples collected near Shanghai, the Philippines, and Indonesia. Maximum concentrations of these perfluoroalkyls ranged from 3.1 to 70.2 pg/L near Shanghai. PFOA, PFOS, and other perfluoroalkyl species were monitored in waste water effluents and 20 rivers located in Japan (Murakami et al. 2008). Perfluoroalkyls were ubiquitous in the river water samples, with concentrations of PFOA as large as 0.054–0.192 µg/L in seven of the river samples with low waste water effluent sources.

Table 5-15. Concentrations of PFOA and PFOS in Ocean Water[a]

	Concentration (pg/L)			
Location	PFOA	PFOS	PFNA	PFHxS
North Atlantic (n=9)	160–338	8.6–36	15–36	4.1–6.1
Mid Atlantic (n=7)	100–439	37–73	—	2.6–12
Central to Eastern Pacific (n=14)	15–62	1.1–20	1.0–16	0.1–1.6
Western Pacific (n=2)	136–142	54–78	—	2.2–[a]2.8
Tokyo Bay (n=8)	1,800–192,000	338–57,700	163–71,000	17–5,600
Offshore Japan (n=4)	137–1,060	40–75	—	3.0–6.1
Coastal Hong Kong (n=12)	673–5,450	70–2,600	22–207	<5–311
Coastal China (n=14)	243–15,300	23–9,680	2.0–692	<5–1,360
Coastal Korea (n=10)	239–11,350	39–2,530	15–518	<5–1,390
Sulu Sea (n=5)	88–510	<17–109	—	<0.2
South China Sea (n=2)	160–420	8–113	—	<0.2

[a]Includes samples of coastal and open ocean water.

"—" indicates no available data; PFHxS = perfluorohexane sulfonic acid; PFNA = perfluorononanoic acid; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid

Source: Yamashita et al. 2005

The presence of PFOA and PFOS in surface water in the United States indicates that drinking water taken from these sources may contain detectable levels of these substances. The most vulnerable drinking water systems are those in close proximity to sites that are contaminated with perfluoroalkyls. PFOA was detected in 12 out of 13 samples collected from four municipal drinking water treatment plants that draw water from the Tennessee River and are located downstream from the 3M Decatur Facility in Alabama. Reported concentrations ranged from 0.025 to 0.16 μg/L (3M 2008c). PFOA was not detected in any samples collected from a fifth plant located upstream of the 3M Decatur facility (3M 2008c). Sampling conducted in October 2015 continued to show a presence of perfluoroalkyls in water samples collected at nine sites along the Tennessee River near Decatur, with maximum PFOS, PFBS, and PFOA levels reported as 0.220, 0.160, and 0.120 μg/L, respectively (Newton et al. 2017). Waterways adjacent to areas where aqueous film forming foams (AFFFs) used to fight fires have been shown to have high levels of perfluoroalkyls. For example, a PFOS concentration of 2,210 μg/L was detected in the Etobicoke Creek, Canada following the use of AFFF at the Toronto Pearson Airport (D'agostino and Mabury 2017). The vertical distribution of 15 perfluoroalkyl substances was studied in concrete samples obtained from a location in which AFFFs were used in firefighter training (Baduel et al. 2015). At the surface of the concrete pad, PFOS was observed to be the dominant substance measured; however, shorter-chain compounds were observed to a depth of 12 cm from the surface, suggesting vertical transport of the shorter-chain compounds and the potential for movement into groundwater.

Hu et al. (2016) presented geospatial monitoring data for six perfluoroalkyls (PFBS, PFHxS, PFHpA, PFOA, PFOS, and PFNA) in U.S. drinking water from information contained in the EPA third Unregulated Contaminant Monitoring Rule (UCMR 3) program. Frequency of detection and concentrations in drinking water was correlated with proximity to industrial facilities using perfluoroalkyls, military fire training areas, and the number of waste water treatment plants. Note that UCMR 3 required all large water systems (4,120 PWSs, serving >10,000 people) and a representative sample of 800 small water systems (serving ≤10,000 people) to monitor for PFOA and PFOS from 2013 through 2015.

It was reported that 66 public drinking water systems that serve 6 million U.S. residents had at least one sample that exceeded the current EPA health advisory level of 0.07 μg/L for PFOA and PFOS (Hu et al. 2016). The dataset used by Hu et al. (2016) has since been updated by the EPA (EPA 2017). The most recent report dated January 2017 showed that PFOS, PFOA, PFNA, PFHxS, PFHpA, and PFBS were detected above their respective minimal reporting level in 95, 117, 14, 55, 86, and 8 out of 4,920 public water systems (EPA 2017). PFOA and PFOS were identified above the health advisory level of

0.07 μg/L in 13 and 46 out of the 4,920 public water supplies, respectively (EPA 2017). It is possible that combined concentrations of PFOA and PFOS may exceed the advisory level while individual concentrations may still be below 0.07 μg/L.

Based on a memorandum of understanding with the EPA, DuPont began collecting water monitoring data of both public and private wells near the Washington Works chemical plant. The quarterly reports and monitoring data affiliated with these reports may be obtained from the regulations.gov portal (http://www.regulations.gov). In samples of water collected at 17 public water facilities from 2002 to 2009 in West Virginia and Ohio, PFOA levels ranged from below the detection limit (0.0023 μg/L) to nearly 100 μg/L in a few test wells in Little Hocking, Ohio (EPA 2010). In the final phase III summary report, PFOA concentrations were reported to range from below the detection limit to 0.79 μg/L in 34 wells located approximately 3 miles upstream and 82 miles downstream from the facility (URS 2012).

Rumsby et al. (2009) reviewed the presence of PFOS and PFOA in drinking waters worldwide and discussed treatment methods for removing these substances from public water supplies. Conventional waste water treatment does not always efficiently remove perfluoroalkyls, and effluent may contain higher levels of some perfluoroalkyls than influent due to degradation of precursor substances during the treatment process (Schultz et al. 2006a, 2006b). While granulated activated carbon and reverse osmosis followed by nanofiltration have been shown to be effective methods of removing perfluoroalkyls, conventional methods such as chlorination, ozonolysis, and slow sand filtration may not be as effective. As a consequence, public drinking water systems impacted by effluent from waste water treatment plants often contain higher levels of perfluoroalkyls than systems that are not impacted by waste water treatment plant effluent. Quinones and Snyder (2009) analyzed raw and finished water at seven different public water systems in the United States for the presence of perfluoroalkyls. Water systems that were heavily impacted by waste water treatment plant effluents had greater frequency and higher levels of perfluoroalkyls when compared to water systems that were not highly impacted by waste water treatment plants. For example, no perfluoroalkyls were detected in either influent or finished water from a public water system in Aurora, Colorado with no impact from waste water treatment plant effluent; however, PFHxA, PFOA, PFNA, PFDA PFUnA, PFHxS, and PFOS were detected in all samples of a Los Angeles, California public water system that was highly impacted by waste water effluent. Perfluoroalkyls were commonly detected in the influent and effluent of 10 waste water treatment plants across the United States (Schultz et al. 2006a). PFBS was detected in 100% of both influent and effluent samples of the 10 plants, while other perfluoroalkyls like PFOA, PFOS, PFNA, PFHxA, PFHpA, and PFHxS were detected in 80% of the influent and effluent samples at the 10 plants. In a national study of

10 perfluoroalkyls in raw and treated drinking water of France, Boiteux et al. (2012) observed that several perfluoroalkyl carboxylic acids had greater concentrations in treated water than the raw water. In eight drinking water treatment plants, PFBA, PFHxA, and PFHpA were not detected in raw water, but were detected in treated water, indicating that these substances were released from saturated activated carbon used to treat raw waters or were formed by the degradation of precursor substances. Perfluoroalkyl sulfonates appeared to be removed more efficiently than the carboxylates. PFHxS, PFBS, and PFOS were detected less frequently in treated water as compared to raw water influent. These three compounds comprised 53% of the total concentration of perfluoroalkyls in the raw water samples, but only 37% of the total concentration of the perfluoroalkyls in the treated water. The summed concentration of 10 perfluoroalkyls was analyzed in the raw water and treated water of two drinking water treatment plants downstream from a fluoropolymer manufacturing facility located in France (Dauchy et al. 2012). The total concentration of perfluoroalkyls in the raw water at four sampling locations of the first plant ranged from 0.140 to 0.287 μg/L, while the summed concentration in the treated water was 0.179 μg/L. The total concentration of the 10 perfluoroalkyls in raw water at the second plant was 0.132 μg/L, while the total concentration in the treated water was 0.130 μg/L. Levels of PFHxA were greater in the treated water than the raw water at three of the four raw water sampling points, and levels of PFNA were greater in treated water than raw water at all sampling points of the first plant, but were slightly lower in the treated water of the second drinking water plant even though both systems used simple chlorination to treat the water.

PFOA was detected in 65% of the public drinking water systems tested in New Jersey in 2006 at concentrations ranging from 0.005 to 0.039 μg/L (Post et al. 2009). In a follow-up study conducted in 2009, PFOA was detected in 57% of raw water samples from 29 additional public drinking water systems in New Jersey at a maximum concentration of 0.100 μg/L (Post et al. 2013). Nine other perfluoroalkyls were also tested for, with PFOS and PFNA being the most frequently detected compounds (30% detection frequency each) after PFOA. PFOA and PFOS were detected in tap water from 21 cities located in China at concentrations of <0.0001–0.0459 and <0.0001–0.0148 μg/L, respectively (Jin et al. 2009). Mak et al. (2009) published a study comparing detections of perfluoroalkyls including PFOA and PFOS in tap water collected in China, Japan, India, Canada, and the United States. PFOA and PFOS were the predominant species measured, accounting for 40–50% of the total perfluoroalkyls present in water, with the exception of certain location of India where PFOS or PFOA may not have been present or were present at low levels.

Gellrich et al. (2013) analyzed 119 samples of mineral water, 26 samples of tap water, 18 spring water samples, and 14 raw water samples from Germany for the presence of perfluoroalkyls. Perfluoroalkyls were detected in 58% of all of the samples tested, with the greatest summed total concentration observed in tap water at 0.0427 μg/L. The maximum concentration of individual perfluoroalkyls occurring in bottled water, spring water, untreated water, and tap water were observed for PFBS (0.0133 μg/L), PFOA (0.0074 μg/L), PFBS (0.010 μg/L), and PFHxS (0.0121 μg/L), respectively. Perfluoroalkyls were widely detected in drinking water samples collected in 2008 at 40 different locations of Catalonia, Spain (Ericson et al. 2009). Median concentrations ranged from 0.00002 μg/L (FOSA) to 0.00098 μg/L (PFOA). The most frequently detected compounds were PFOS and PFHxS, which were detected in 35 and 31 samples, respectively. PFOS, PFOA, and PFHxS were detected in all samples collected in a study of drinking water contamination of perfluoroalkyls in Rio de Janeiro, Brazil (Quinete et al. 2009). Concentration ranges were 0.00058–0.00670 μg/L (PFOS), 0.00035–0.00282 μg/L (PFOA), and 0.00015–0.001 μg/L (PFHxS) respectively.

Perfluoropolyethers (PFPEs) such as perfluoroether carboxylic and sulfonic acids containing one or more ether oxygens in the carbon backbone are expected to be less persistent than the legacy substances. However, hexafluoropropylene oxide trimer and dimer acid (HFPO-TA and HFPO-DA) were detected in downstream water samples near a fluoropolymer facility in China at levels of 5.2–68.5 μg/L (Pan et al. 2017). Pan et al. (2018) also provided data of HFPO-DA and HFPO-TA in surface waters in China, United Kingdom, United States, Sweden, Germany, Netherlands, and South Korea. The substances were frequently detected in surface waters in all countries with median levels of 0.00095 μg/L (HFPO-DA) and 0.00021 μg/L (HFPO-TA). Gebbink et al. (2017) also reported that the GenX (HFPO-DA) was detected in all sampling sites downstream from a fluoropolymer facility in the Netherlands and at three out of four drinking water facilities located near the facility. Short-chain perfluoroalkyl compounds and legacy perfluoroalkyls were analyzed for in 97 drinking water samples from Canada and other nations in 2015–2016 (Kabore et al. 2018). PFOA and PFOS levels did not exceed 0.005 μg/L in any of the 97 samples; however, high detection frequencies ranging from 64 to 92% were observed in tap water for some short-chain perfluoroalkyls.

5.5.3 Sediment and Soil

Concentrations of perfluoroalkyls in soils are expected to be greater in the vicinity of fluorochemical plants that produced or used these substances as processing aids in the manufacture of fluoropolymers than in the environment at large. Levels of some perfluoroalkyls measured in soil and sediment

surrounding perfluorochemical industrial facilities are listed in Table 5-16. PFOA was detected in most soil and sediment samples collected on- and off-site at the 3M Decatur facility in Alabama in monitoring studies conducted between October 2004 and December 2006. Maximum soil concentrations were as high as 14,750 ng/g on-site and 7.85 ng/g off-site, and maximum sediment concentrations were as high as 347 ng/g on-site and 2,385 ng/g off-site (3M 2008c). The highest levels of PFOA were measured in soil from on-site fields formerly amended with PFOA-containing sludge. In its final project report for this location, six on-site soil samples were analyzed in December 2012 for the presence of PFOA, PFOS, PFBS, and PFHxS. Average levels were 3.86–3,890 ng/g (PFOS), 3.56–270 ng/g (PFHxS), 0.423–64.8 ng/g (PFBS), and 17.0–1,410 ng/g (PFOA) (3M 2012).

Table 5-16. Concentrations of Perfluoroalkyls in Soil and Sediment at Fluorochemical Industrial Facilities

	Percent detection and concentration (ng/g)					
Location	PFOA	PFBA	PFOS	PFHxS	PFBS	Reference
DuPont Washington Works Facility, West Virginia						
Soil						
Boring samples (n=22)						Davis et al. 2007
Percent detected	36%[a]	—	—	—	—	
Minimum	<0.17[a]	—	—	—	—	
Maximum	170[a]	—	—	—	—	
3M Cottage Grove Facility, Minnesota						
Soil						
Boring samples (n=50–108)						3M 2007b
Percent detected	100%	—	95%	90%	60%	
Maximum	21,800	—	104,000	3,470	139	
Fire training area (n=8–11)						3M 2007b
Percent detected	91%	82%	100%	100%	73%	
Maximum	262	11.5	2,948	62.2	24.6	
Sediment						
East and West Cove (n=21–28)						3M 2007b
Percent detected	100%	93%	100%	96%	65%	
Minimum	0.764	ND	40.0	ND	ND	
Maximum	1,845	94.6	65,450	126	9.14	
Mississippi River shoreline (n=84–92)						3M 2007b
Percent detected	70%	44%	80%	28%	29%	
Maximum	341	124	79.0	11.5	29.4	
Mississippi River transect (n=38–40)						3M 2007b
Percent detected	18%	0%	82%	0%	0%	
Maximum	1.09	ND	3.16	ND	ND	

Table 5-16. Concentrations of Perfluoroalkyls in Soil and Sediment at Fluorochemical Industrial Facilities

	Percent detection and concentration (ng/g)					
Location	PFOA	PFBA	PFOS	PFHxS	PFBS	Reference
3M Decatur Facility, Alabama						
Soil						
On-site former sludge incorporation area (n=357)						3M 2008c
Percent detected	99%	—	—	—	—	
Mean	885–929					
Range	2.91–14,750	—	—	—	—	
On-site background (n=18)						3M 2008c
Percent detected	100%	—	—	—	—	
Mean	3.53–4.1					
Range	1.61–6.03	—	—	—	—	
Off-site soil (n=23)						3M 2008c
Percent detected	100%	—	—	—	—	
Mean	3.68–4.6					
Range	0.72–7.85	—	—	—	—	
Sediment						
On-site sediment (n=8)						3M 2008c
Percent detected	88%	—	—	—	—	
Median	16.8					
Range	1.64–347	—	—	—	—	
Off-site sediment (n=30)						3M 2008c
Percent detected	93%	—	—	—	—	
Range	0.39–2,385	—	—	—	—	
3M Decatur Facility, Alabama December 2012						
On-site former sludge incorporation area (n=6)						3M 2012
Percent detected	100%	—	100%	86%	86%	
Mean	17.0–1,410	—	3.86–3,890	3.56–270	0.423–64.8	
3M Cottage Grove Facility, Minnesota						
Surface soil along U.S. Highway 10 near facility						Xiao et al. 2015
Percent detected	100%	—	100%	—	—	
Median	8.0		12.2			
Range	5.5–125.7		0.2–28.2			

[a]Analyte was reported as APFO.

"—" indicates no available data; APFO = ammonium perfluorooctanoate; ND = not detected; PFBA = perfluorobutanoic acid; PFBS = perfluorobutane sulfonic acid; PFHxS = perfluorohexane sulfonic acid; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid

PFOA, PFOS, and PFHxS were detected in 90–100% of soil samples collected from a former tar neutralization area, a former sludge disposal area, a former solids burn pit area, a former waste water treatment plant area, and a former fire training area at the 3M Cottage Grove facility in Minnesota (3M 2007b). PFBS was detected in 60–73% of these samples. Maximum concentrations for these substances were 21,800, 104,000, 3,470, and 139 ng/g, respectively. Levels of PFBA were only reported for soil in the fire training area; it was detected in 9 out of 11 samples from this location at 0.306–11.5 ng/g. The percent detection of these compounds in sediment from the East and West Cove sites was similar to that in soil. Maximum concentrations of PFOA and PFOS were 1,845 and 65,450 ng/g, respectively. These perfluoroalkyls were also analyzed in Mississippi River sediment near the Cottage Grove Facility. Levels of these compounds were much greater along the facility shoreline compared to levels in transect samples collected at points crossing the river. Maximum shoreline concentrations for PFOA, PFBA, PFOS, PFHxS, and PFBS were 341, 124, 79.0, 11.5, and 29.4 ng/g, respectively. PFHxS, PFBS, and PFBA were not detected in any of the transect samples, and PFOA was found in only 18%. Although the maximum concentration of PFOS was 3.16 ng/g, it was still detected in 82% of the transect samples.

PFOA and PFOS were detected in all surface soils (top 10 cm) samples collected at 28 sites in September and October of 2012 along U.S. Highway 10 running from Cottage Grove, Minnesota (where the former 3M perfluoroalkyl manufacturing facility was located) to Big Lake, Minnesota (Xiao et al. 2015). Measured levels of PFOS and PFOA ranged from 0.2 to 28.2 and from 5.5 to 125.7 ng/g, respectively. Subsurface soils up to a depth of 65 cm were collected at four sites as well. Levels of PFOA and PFOS generally increased with increasing depth at each of the locations, suggesting a downward movement of the contaminants and the potential to contaminate groundwater.

The use of aqueous firefighting foams at fire training areas of military installations has resulted in widespread contamination of perfluoroalkyls in the soil and groundwater at these facilities. Monitoring data obtained from 40 sites at 10 U.S. military installations in the continental United States and Alaska were collected for several perfluoroalkyls (Anderson et al. 2016). These data are summarized in Table 5-17.

Table 5-17. Summary of Perfluoroalkyls Detected in Soil, Sediment, Surface Water, and Groundwater at 10 Military Installations[a]

Compound	Parameter	Surface soil (µg/kg)	Subsurface soil (µg/kg)	Sediment (µg/kg)	Surface water (µg/L)	Groundwater (µg/L)
PFBA	DF	38.46	29.81	24.24	84.00	85.51
	Median	1.00	0.960	1.70	0.076	0.180
	Maximum	31.0	14.0	140	110	64.0
PFBS	DF	35.16	34.62	39.39	80.00	78.26
	Median	0.775	1.30	0.710	0.106	0.200
	Maximum	52.0	79.0	340	317	110
PFHxA	DF	70.33	65.38	63.64	96.00	94.20
	Median	1.75	1.04	1.70	0.320	0.820
	Maximum	51.0	140	710	292	120
PFHxS	DF	76.92	59.62	72.73	88.00	94.93
	Median	5.70	4.40	9.10	0.710	0.870
	Maximum	1,300	520	2,700	815	290
PFHpA	DF	59.34	45.19	48.48	84.00	85.51
	Median	0.705	0.660	1.07	0.099	0.235
	Maximum	11.4	17.0	130	57.0	75.0
PFOA	DF	79.12	48.08	66.67	88.00	89.86
	Median	1.45	1.55	2.45	0.382	0.405
	Maximum	58.0	140	950	210	250
FOSA	DF	64.84	29.81	75.76	52.00	48.55
	Median	1.20	0.470	1.30	0.014	0.032
	Maximum	620	160	380	15.0	12.0
PFOS	DF	98.90	78.85	93.94	96.00	84.06
	Median	52.5	11.5	31.0	2.17	4.22
	Maximum	9,700	1,700	190,000	8,970	4,300
PFNA	DF	71.43	14.42	12.12	36.00	46.38
	Median	1.30	1.50	1.10	0.096	0.105
	Maximum	23.0	6.49	59.0	10.0	3.00
PFDA	DF	67.03	12.50	48.48	52.00	34.78
	Median	0.980	1.40	1.90	0.067	0.023
	Maximum	15.0	9.40	59.0	3.20	1.80
PFUnA	DF	45.05	9.62	24.24	20.00	8.70
	Median	0.798	1.15	160	0.021	0.025
	Maximum	10.0	2.00	14.0	0.210	0.086

Table 5-17. Summary of Perfluoroalkyls Detected in Soil, Sediment, Surface Water, and Groundwater at 10 Military Installations[a]

Compound	Parameter	Surface soil (µg/kg)	Subsurface soil (µg/kg)	Sediment (µg/kg)	Surface water (µg/L)	Groundwater (µg/L)
PFDoDA	DF	21.98	6.73	45.45	20.00	4.35
	Median	1.95	2.40	2.80	0.058	0.022
	Maximum	18.0	5.10	84.0	0.071	0.062

[a]Water concentrations are ppb (µg/L); soil and sediment levels are ppb (µg/kg).

DF = detection frequency as a percentage; FOSA = perfluorooctane sulfonamide; PFBA = perfluorobutanoic acid; PFBS = perfluorobutane sulfonic acid; PFDoDA = perfluorododecanoic acid; PFDA = perfluoro-n-decanoic acid; PFHpA = perfluoroheptanoic acid; PFHxA = perfluorohexanoic acid; PFHxS = perfluorohexane sulfonic acid; PFNA = perfluorononanoic acid; PFOS = perfluorooctane sulfonic acid; PFUnA = perfluoroundecanoic acid

Source: Anderson et al. (2016)

Perfluoroalkyls have been detected in soils that were amended with biosolids (Sepulvado et al. 2011). Several perfluoroalkyls were detected in biosolid-amended soils, with PFOS being the predominant compound with levels ranging from 5.5 to 483 ng/g, depending upon the loading rate.

5.5.4 Other Media

In a study conducted by the Food and Drug Administration (FDA) of 91 food samples collected during the 2017 Total Diet Study, PFOS was detected in 10 meat/seafood samples; the levels ranged from 0.134 ng/g in a boiled frankfurter to 0.865 ng/g in baked tilapia (FDA 2019). PFBA was detected in one sample of raw/frozen pineapple (0.068 ng/g). PFOA, PFHxS, PFNA, PFDA, PFBS, and PFHxA levels were below the lower limit of quantitation. It is important to note that FDA states that the sample size is limited and cannot be used to draw definitive conclusions. Levels of PFOS, PFOA, PFBS, PFHxS, PFHxA, PFHpA, PFDA, PFNA, and PFDoDA were analyzed in 31 food items collected from 5 grocery stores located in Texas in 2009 (Schecter et al. 2010). PFOA was the most frequently detected item (detected in 17 of 31 of the food samples), with levels ranging from 0.07 ng/g in potatoes to 1.80 ng/g in olive oil. PFOS, PFHxA, PFHpA, PFNA, PFDA, and PFDoDA were not detected in any samples. PFBS and PFHxS were detected in cod at 0.12 and 0.07 ng/g, respectively. The data for PFOA are summarized in Table 5-18. Several studies have evaluated the levels of perfluoroalkyls in fish from lakes and rivers in the United States; these data are summarized in Table 5-19.

Table 5-18. Detections of PFOA in 31 U.S. Food Items

Food	PFOA concentration in ng/g (LOD for non-detects)
Hamburger	0.15
Bacon	0.24
Sliced turkey	ND (0.02)
Sausage	0.09
Ham	0.02
Sliced chicken breast	0.02
Roast beef	ND (0.02)
Canned chili	0.02
Salmon	0.23
Canned tuna	ND (0.05)
Fresh catfish fillet	0.30
Tilapia	0.10
Cod	0.10
Canned sardines	0.19
Frozen fish sticks	0.21
Butter	1.07
American cheese	ND (0.04)
Other cheese	ND (0.04)
Whole milk	ND (0.02)
Ice cream	ND (0.03)
Frozen yogurt	ND (0.02)
Whole milk yogurt	ND (0.02)
Cream cheese	ND (0.03)
Eggs	ND (0.04)
Olive oil	1.80
Canola oil	ND (0.05)
Margarine	0.19
Cereals	ND (0.04)
Apples	ND (0.02)
Potatoes	0.07
Peanut butter	0.10

LOD = limit of detection; ND = not detected; PFOA = perfluorooctanoic acid

Source: Schecter et al. 2010

Table 5-19. Detections of Perfluoroalkyls in Fish from U.S. Lakes and Rivers

Type of seafood (location)	Perfluoroalkyl concentration in ng/g										Reference
	PFOA	PFOS	PFHxS	PFNA	PFDA	PFUnA	PFHpA	PFBS	PFDoDA	FOSA	
Lake trout (Lake Superior)	1.1	4.8	<0.01–0.43	1.0	0.72	0.90	<0.02–0.87		0.37	0.25	Furdui et al. 2007
Lake trout (Lake Superior)	<0.42	2.3	<0.10	0.70	0.39	1.1			0.97		DeSilva et al. 2011
Lake trout (Lake Michigan)	4.4	16	<0.01–0.87	0.57	0.76	0.74	<0.02–0.97		0.41	0.99	Furdui et al. 2007
Lake trout (Lake Huron)	1.6	39	<0.01–6.2	2.8	2.2	2.7	<0.02–1.43		0.88	1.6	Furdui et al. 2007
Lake trout (Lake Huron)	<0.42	17	<0.10	1.4	1.3	1.8	NE		0.74		DeSilva et al. 2011
Lake trout (Lake Erie)	1.6	121	<0.01–1.2	2.9	4.9	3.5	<0.02–0.71		0.97	2.1	Furdui et al. 2007
Lake trout (Eastern Lake Erie)	<0.42	96	1.4	2.6	6.1	5.7	NE		2.0		DeSilva et al. 2011
Walleye (Western Lake Erie)	0.50	1.1	<0.10	1.2	3.6	3.1	NE		1.1		DeSilva et al. 2011
Lake trout (Lake Ontario)	1.5	46	0.65	1.1	1.8	1.6	<0.02–1.39		0.70	0.82	Furdui et al. 2007
Lake trout (Lake Ontario)	0.88	2.5	0.70	0.90	1.4	2.	0.64		0.32		DeSilva et al. 2011
Mixture of whole fish (Missouri River)	<1.00	84.7	1.89	0.43	0.25	<1.00	1.53	<0.40	0.49		Ye et al. 2008
Mixture of whole fish (Mississippi River)	<0.20	83.1	0.42	0.78	1.24	3.38	0.27	<0.20	<0.40		Ye et al. 2008
Mixture of whole fish (Ohio River)	<1.00	147	0.52	1.03	3.88	6.57	<4.00	<0.40	1.72		Ye et al. 2008
Smallmouth bass (Raisin River)	<2	2.0–41.3	<1							<1–4.1	Kannan e al. 2005

Table 5-19. Detections of Perfluoroalkyls in Fish from U.S. Lakes and Rivers

Type of seafood (location)	Perfluoroalkyl concentration in ng/g										Reference
	PFOA	PFOS	PFHxS	PFNA	PFDA	PFUnA	PFHpA	PFBS	PFDoDA	FOSA	
Smallmouth bass (St Clair River)	<2	<2–2.7	<1							1.1–6.3	Kannan e al. 2005
Smallmouth bass (Calumet River)	<2	2.5–7.6	<1							<1	Kannan e al. 2005
Carp[a] (Saginaw Bay)	<36	124	<34							<19	Kannan e al. 2005
Carp[a] (Saginaw Bay)		120									Giesy and Kannan 2001
Lake whitefish[a] (Michigan waters)		130									Giesy and Kannan 2001
Chinook salmon[a] (Michigan waters)		110									Giesy and Kannan 2001
Brown trout[a] (Michigan waters)		<6–46									Giesy and Kannan 2001
Bluegill (St Croix River, Minnesota)		2.87			<LOQ	<LOQ			<LOQ		Delinsky et al. 2009
Bluegill (Lake Calhoun, Minnesota)		272			5.82	4.18			4.72		Delinsky et al. 2009
Bluegill (Haw River, North Carolina)		29.8			10.3	26.9			7.25		Delinsky et al. 2009
Mixture of fish fillet (Zumbrol Lake, Minnesota)		52.4			3.24						Delinsky et al. 2010
Mixture of fish fillet (McCarrons Lake, Minnesota)		47.3			1.97						Delinsky et al. 2010

Table 5-19. Detections of Perfluoroalkyls in Fish from U.S. Lakes and Rivers

Type of seafood (location)	Perfluoroalkyl concentration in ng/g										Reference
	PFOA	PFOS	PFHxS	PFNA	PFDA	PFUnA	PFHpA	PFBS	PFDoDA	FOSA	
Mixture of fish fillet (Pickerel Lake, Minnesota)		10.0			1.23						Delinsky et al. 2010
Mixture of fish fillet (Carlos Lake, Minnesota)		12.3									Delinsky et al. 2010
Mixture of fish fillet (other Minnesota lakes)		1.08–5.13									Delinsky et al. 2010
Mixture of fish fillet (Mississippi River sites, Minnesota)		3.06–20.00	0.47		2.94–15.0	2.13–6.72			3.74–4.42		Delinsky et al. 2010

[a]Perfluoroalkyl levels measured in muscle.

Grayed cells indicate that study did not evaluate compound; FOSA = perfluorooctane sulfonamide; LOQ = limit of quantification; NE = not evaluated; PFBS = perfluorobutane sulfonic acid; PFDoDA = perfluorododecanoic acid; PFDA = perfluorodecanoic acid; PFHpA = perfluoroheptanoic acid; PFHxS = perfluorohexane sulfonic acid; PFNA = perfluorononanoic acid; PFOS = perfluorooctane sulfonic acid; PFUnA = perfluoroundecanoic acid

Concentrations of perfluoroalkyls have been reported in foods sampled in Canada, the United Kingdom, and Germany (Food Standards Agency 2006; Fromme et al. 2007b; Tittlemier et al. 2007). Perfluoroalkyls were detected in only 9 out of 54 food composites collected during Canadian Total Diet studies from 1992 to 2004 (Tittlemier et al. 2007). PFOS was detected in beef steak, ground beef, luncheon meats, marine fish, freshwater fish, and microwave popcorn at concentrations ranging from 0.98 to 2.7 ng/g, wet weight. PFOA was detected in roast beef, pizza, and microwave popcorn at 0.74–3.6 ng/g, wet weight. PFHpA was detected in pizza and microwave popcorn at 1.5–2.0 ng/g, wet weight. PFNA was detected only in beef steak at 4.5 ng/g, wet weight. PFDA, PFUnA, and PFDoDA were analyzed for but not detected in any of the food composites. During the U.K. Food Standards Agency Total Diet Study, PFOS was detected in eggs, sugars and preserves, potatoes, and canned vegetables at 1, 1, 10, and 2 μg/kg, respectively (Food Standards Agency 2006). PFOA was detected only in potatoes at 1 μg/kg. Neither substance was detected in the bread, miscellaneous cereals, carcass meats, offal, meat products, poultry, fish, oils and fats, green vegetables, other vegetables, fresh fruit, fruit products, beverages, milk, dairy products, or nuts categories. Fromme et al. (2007b) detected PFOS, PFOA, and PFHxS in 33, 45, and 3%, respectively, of 214 daily duplicate food portions for 31 adults in the city of Munich, Germany. Concentrations were 0.025–1.03 ng/g fresh weight for PFOS, 0.025–118.29 ng/g fresh weight for PFOA, and 0.05–3.03 ng/g fresh weight for PFHxS. Reported 90th percentile values were 0.11 and 0.21 ng/g fresh weight for PFOS and PFOA, respectively (Fromme et al. 2007b).

The temporal trend of perfluoroalkyl residues in eggs, milk, and farmed rainbow trout from Sweden were studied from 1999 to 2010 (Johansson et al. 2014). Over this period, the mean annual decreases in levels of PFOS were 18 and 31% in rainbow trout and eggs, respectively. The mean annual decreases of PFOA and PFHxS were 12 and 11%, respectively, in eggs. The detection frequency of PFOA and PFHxS was too low in milk samples to assess changes in levels over the time period, and decreases in the levels of PFOS were found to be not statistically significant over the temporal period. The mean annual decrease in levels of PFHxS in rainbow trout was 4.3% annually.

Elevated levels of PFOS were measured in water and fish samples obtained from 2009 and 2012 at six sampling locations along the Welland River and Lake Niapenco in Ontario, Canada (Gewurtz et al. 2014). These locations were downstream from the Hamilton International airport where PFOS containing AFFF was used until the mid-1990s at a firefighting training facility at the airport. PFOS concentrations were generally highest in benthic feeding fish collected at the sampling locations nearest to the airport. The maximum level of PFOS was observed in common carp collected at a site near the airport at a concentration of 2,300 ng/g. Maximum levels of PFOS in smallmouth bass, largemouth bass, and

channel catfish were 640, 450, and 430 ng/g, respectively. Freshwater fish and seafood obtained from China had detectable levels of nine perfluoroalkyls, with PFOS being detected in 62% of the samples at a concentration range of <0.10–26.2 ng/g and PFOA detected in 70% of the samples at levels of <0.10–1.99 ng/g (Zhang et al. 2011).

PFOA was detected in the packaging paper of two microwave popcorn bags at 0.3–4.7 ng/cm^2 uncooked and 0.5–4.3 ng/cm^2 cooked (Sinclair et al. 2007). The mean mass of PFOA in the gas phase of popcorn vapors following popping was 16–17 ng/cm^2. PFHpA, PFNA, PFDA, PFUnA, and PFDoDA were detected in one of the bags at 0.4–3.2 ng/cm^2 uncooked and 0.5–4.3 ng/cm^2 cooked; however, these perfluoroalkyls were not detected (<0.2 ng/cm^2) in the second bag. Begley et al. (2005) measured PFOA concentrations of 6–290 μg/kg in microwave popcorn bags. These authors also tested a hamburger wrapper, sandwich wrapper, French fry box, and soak-proof paper plates and did not find PFOA above the detection limit in these products. The concentration of PFOA measured in undiluted perfluoro paper coating formulations ranged from 88,000 to 160,000 μg/kg (Begley et al. 2005).

A study of perfluorinated and polyfluorinated substances in food packaging from U.S. fast food restaurants found perfluoroalkyl carboxylates (such as PFOA and PFHxA), perfluoroalkyl sulfonates (such as PFBS), fluorotelomer sulfonates, and unknown polyfluorinated compounds (Schaider et al. 2017). PFOA was detected in 6 of the 20 samples collected in 2014–2015. Fluorotelomer alcohols that can degrade or metabolize to perfluoroalkyl carboxylic acids such as PFOA continue to be detected in food packaging materials. Yuan et al. (2016) analyzed 69 food contact materials (paper tableware, paper cups, cupcake cups, paper boxes, paper bags, and microwave popcorn bags) produced in China and 25 materials (paper tableware, microwave popcorn bags, and paper cups) produced in the United States. The median concentration of total fluorotelomer alcohols in food contact materials produced in China (sum of 6:2, 8:2, 10:2, 12:2, 14:2, 16:2, and 18:2 fluorotelomer alcohol) ranged from 2 to 18,200 ng/g, with the highest levels observed in microwave popcorn bags. The detection frequencies of 6:2, 8:2, 10:2, 12:2, 14:2, 16:2, and 18:2 fluorotelomer alcohol in all food contact materials were reported as 38, 65, 77, 70, 58, 35, and 30%, respectively. The only fluorotelomer detected in paper tableware produced in the United States was 6:2 fluorotelomer alcohol, with a detection frequency of 11%; however, all fluorotelomer alcohols, with the exception of 18:2 fluorotelomer alcohol, were detected in microwave popcorn bags from the United States, although the levels were much lower than those produced in China. Table 5-20 shows the median concentration of the fluorotelomer alcohols in microwave popcorn bags produced in the United States versus those produced in China. In contrast to the products produced in China, the predominant fluorotelomer alcohol currently detected in food contact materials produced in the

United States appears to be 6:2 fluorotelomer alcohol. The authors concluded that the results of the PFOA Stewardship Program had effectively reduced the usage of long-chain fluorotelomer alcohols in the United States; however, they were still widely present in products produced in China.

Table 5-20. Fluorotelomer Alcohols Detected in Microwaveable Popcorn Bags Produced in China and the United States

Fluorotelomer alcohol	Median concentration of FTOH in microwave popcorn bag produced in China (ng/g)	Median concentration of FTOH in microwave popcorn bag produced in the United States (ng/g)
6:2 FTOH	80	485
8:2 FTOH	4,810	1.36
10:2 FTOH	6,700	0.73
12:2 FTOH	5,650	0.55
14:2 FTOH	384	0.12
16:2 FTOH	61	<MQL
18:2 FTOH	7.5	Not detected

FTOH = fluorotelomer alcohol; MQL = method quantitation limit

Source: Yuan et al. 2016

Washburn et al. (2005) measured the concentration of the perfluorooctanoate anion in fluorotelomer-treated consumer articles as well as the fluorotelomer formulations used for the treatments. PFOA was detected in mill-treated carpeting (0.2–0.6 mg/kg), carpet-care solution-treated carpeting (0.2–2 mg/kg), treated apparel (<0.02–1.4 mg/kg), treated home textiles (<0.02–1.4 mg/kg), industrial floor waxes and wax removers (0.0005–0.06 mg/kg), latex paint (0.02–0.08 mg/kg), and home and office cleaners (0.005–0.05 mg/kg). The concentrations of PFOA measured in the formulations used for these applications were 30–80, 1–50, <1–40, <1–40, 5–120, 50–150, and 50–150 mg/L, respectively. PFOA was not detected in treated upholstery (<0.034 mg/kg), treated technical textiles (<0.034 mg/kg), treated nonwoven medical garments (<0.034 mg/kg), or stone, tile, and wood sealants (<0.1 mg/kg).

Liu et al. (2014) measured levels of perfluoroalkyls in 35 consumer products that are typically used indoors, such as treated home textiles, food contact paper, carpet care products, and floor waxes. All products were obtained from retail stores in the United States between March 2007 and September 2011. The general trend was that these products contained decreasing quantities of perfluorocarboxylic acids, including PFOA, over the temporal period studied; however, there was an increase in use of PFBS, presumably as a replacement of PFOS in consumer products. Levels of perfluoroalkyls were analyzed in 115 random samples of consumer products obtained in Germany in 2010, including textiles, carpets,

cleaning and impregnating agents, leathers, food contact materials, baking and sandwich paper, and ski waxes (Kotthoff et al. 2015). Cleaning agents and some baking and sandwich papers had nondetectable or negligible amounts of perfluoroalkyls; however, PFOA and PFOS were frequently detected in outdoor textiles, ski wax, leather products, gloves, awning cloths, nanosprays, impregnation sprays, and food contact materials. Cleaning agents, nanosprays, and impregnation sprays tended to have the highest level of fluorotelomer alcohols. EPA (2009b) analyzed 116 articles of commerce obtained from retail outlets in the United States from March 2007 and May 2008 for the presence of perfluorocarboxylic acids ranging in carbon number from C5 (perfluoropentanoic acid) to C12 (perfluorododecanoic acid). Total C5–C12 perfluorocarboxylic acid levels ranged from below the detection limit (1.5 ng/g) to 47,100 ng/g, with levels of PFOA ranging from below the detection limit to 6,750 ng/g.

PTFE is a fluoropolymer used in applications such as nonstick cookware coatings and plumbing sealant tape. In the past, PFOA had been used as a processing aid in the emulsion polymerization of PTFE (DuPont 2008). PFOA was largely removed from the fluoropolymer material during the baking and curing step of nonstick cookware coatings in a high temperature oven; however, residual PFOA could be found in the final coatings (DuPont 2008). Begley et al. (2005) measured PFOA concentrations of 4–75 μg/kg in PTFE cookware, 3 μg/kg in PTFE-based dental floss, 4 μg/kg in PTFE-based dental tape, and 1,800 μg/kg in PTFE film/sealant tape. PFOA was not detected in tubing made of a fluoro-ethylene-propene copolymer (Begley et al. 2005).

Studies have been conducted that investigated the release of PFOA from PTFE cookware when heated. Sinclair et al. (2007) reported PFOA release concentrations ranging from 19 to 287 pg/cm^2 measured using four new nonstick frying pans. These concentrations were measured at normal cooking temperatures within the range of 180–229°C. PFOA was detected in water (7 and 75 ng) boiled for 10 minutes in two out of five non-stick pans (Sinclair et al. 2007). PFOA was not found above the detection limit (0.1 ng/cm^2) during 40 extraction tests on PTFE cookware using an ethanol/water mixture (Washburn et al. 2005). Likewise, Powley et al. (2005) conducted extraction texts on commercial fluoropolymer-treated cookware using water and water/ethanol mixtures at 100 and 125°C. Under simulated cooking conditions, PFOA was not identified above the detection limit of 100 pg/cm^2. Begley et al. (2005) reported that additional PFOA was not generated in the PTFE coating of three empty pans heated to 320°C (DuPont 2008). According to DuPont, the non-stick coating on a pan may begin to deteriorate if the pan is accidentally heated above 348°C, which is well above the maximum recommended cooking temperature of 260°C (DuPont 2008). Although it is possible for an unattended

empty pan to reach these high temperatures, overheating non-stick cookware is expected to be prevented in most cases because food oils begin to generate smoke around 190°C (Begley et al. 2005).

A comprehensive study that examined 116 articles of commerce (AOC) found perfluorocarboxylic acids, including PFOA, in many commercially available substances, such as carpet care products and waxes (EPA 2009b). Levels of PFOA ranged from nondetectable to 6,750 ng/g, and levels of total perfluorocarboxylic acids (the sum of C5–C12 acids) ranged from nondetectable to 47,100 ng/g. Perfluoroalkyls, including PFOA, have been detected at low levels in personal care products such as cosmetics and sunscreens (Fujii et al. 2013).

5.6 GENERAL POPULATION EXPOSURE

Levels of perfluoroalkyls have been measured in indoor air, outdoor air, dust, food, surface water, and various consumer products. Possible exposure pathways have been proposed; however, the relative importance of these pathways, including their association with the accumulation of perfluoroalkyls in blood, remains unclear (Apelberg et al. 2007b; Begley et al. 2005; Calafat et al. 2006b; Trudel et al. 2008; Washburn et al. 2005). For populations that have elevated levels of perfluoroalkyls in water supplies, the primary route of exposure is expected to be ingestion of contaminated drinking water.

Trudel et al. (2008) provides a thorough analysis of general population exposure to PFOS and PFOA based on the available information and proposes the following possible exposure pathways: food and water consumption, ingestion of house dust, hand-to-mouth transfer from treated carpets, migration into food from PFOA-containing paper or cardboard, inhalation of indoor and ambient air, and inhalation of impregnation spray aerosols. Other pathways proposed to be less significant included oral exposure from hand-to-mouth contact with clothes and upholstery, migration into food prepared with PTFE-coated cookware, dermal exposure from wearing treated clothes, deposition of spray droplets on skin while using impregnation sprays, skin contact with treated carpet and upholstery, and deposition of dust onto skin (Trudel et al. 2008). The strong correlation between PFOA and PFOS concentrations in human serum samples indicates that common exposure pathways for these two substances are possible (Calafat et al. 2007a).

In order to estimate human uptake and the major pathways for human exposure to PFOS and PFOA, reported levels of these compounds in various environmental media, including food and consumer products, were analyzed with respect to product use patterns, personal activity patterns, and personal

intake rates (Trudel et al. 2008). For PFOS, the major exposure pathways in a high-exposure scenario were proposed to be food and water ingestion, dust ingestion, and hand-to-mouth transfer from mill-treated carpets. Relative contributions of these pathways to the total uptake of PFOS in adults were estimated to be approximately 80, 15, and 5%, respectively (Trudel et al. 2008). For PFOA, the major exposure pathways in a high-exposure scenario were proposed to be oral exposure resulting from migration from paper packaging and wrapping into food, general food and water ingestion, inhalation from impregnated clothes, and dust ingestion. Relative contributions of these pathways to the total uptake of PFOA in adults were estimated to be approximately 60, 15, 15, and 10%, respectively (Trudel et al. 2008). Major exposure pathways for the intermediate and low exposure scenarios were proposed to be through food and drinking water (PFOA and PFOS) and ingestion of house dust (PFOA only). Based on these proposed exposure pathways, adult uptake doses estimated for low, medium, and high exposure scenarios were approximately 7, 15, and 30 ng/kg body weight/day, respectively, for PFOS and approximately 0.4, 2.5, and 41–47 ng/kg body weight/day, respectively, for PFOA (Trudel et al. 2008). The estimated uptake values were similar for men and women. Trudel et al. (2008) used older monitoring data and the estimated intakes may not be reflective of current exposure since there has been a downward trend in PFOA and PFOS exposure.

Fromme et al. (2009) assessed human exposure to perfluoroalkyls for adults in the general population of western countries. Based on measurements of indoor and outdoor air, house dust, drinking water, and dietary PFOS and PFOA levels, the investigators estimated average daily exposure levels of 1.6 ng/kg body weight/day for PFOS and 2.9 ng/kg body weight/day for PFOA. Upper daily exposure levels were determined to be 8.8 ng/kg body weight/day for PFOS and 12.6 ng/kg body weight/day for PFOA. The investigators concluded that the oral route, especially diet, was the primary route of exposure to perfluoroalkyls (Fromme et al. 2007a, 2007b, 2009).

As a group of compounds, perfluoroalkyls appear to be ubiquitous in human blood based on the widespread detection of these substances in human serum samples (Byrne et al. 2017; Calafat et al. 2006b, 2007a, 2007b; De Silva and Mabury 2006; Kuklenyik et al. 2004; Olsen et al. 2003b, 2003c, 2004b, 2004c, 2005, 2007a). Tables 5-21 and 5-22 list concentrations of perfluoroalkyls measured in serum samples collected from a representative sample of the general population in the United States. Most studies have reported that PFOA and PFOS levels have been detected in over 90% of subjects (Calafat et al. 2006b, 2007a, 2007b; Olsen et al. 2003b, 2007b, 2004c, 2005, 2008). PFHxS, PFNA, and PFDA are also typically detected in over 90% of the subjects (Calafat et al. 2006b, 2007a, 2017a, 2007b; Kuklenyik et al. 2004; Olsen et al. 2017a, 2017b).

Table 5-21. Concentrations of PFOA and PFOS in Human Serum Collected in the United States

Location	Detection and concentration (ng/mL [ppb])[a] PFOA	PFOS	Reference
U.S. Residents—NHANES			
1999–2000 (n=1,562)			Calafat et al. 2007a
Percent >LOD	100%	100%	
LOD	0.1	0.2	
Geometric mean	5.2	30.4	
95th percentile	11.9	75.6	
2003–2004 (n=2,094)			Calafat et al. 2007b
Percent >LOD	99.7%	99.9%	
LOD	0.1	0.4	
Geometric mean	3.95	20.7	
95th percentile	9.80	54.6	
2005–2006 (n=2,120)			
Percent >LOD	NR	NR	CDC 2018
LOD	0.1	0.2	
Geometric mean	3.92	17.1	
95th percentile	11.3	47.5	
2007–2008 (n=2,100)			CDC 2018
Percent >LOD	NR	NR	
LOD	0.1	0.2	
Geometric mean	4.12	13.2	
95th percentile	9.60	40.5	
2009–2010 (n=2,233)			CDC 2018
Percent >LOD	NR	NR	
LOD	0.1	0.2	
Geometric mean	3.07	9.32	
95th percentile	7.50	32.0	
2011–2012 (n=1,904)			CDC 2018
Percent >LOD	NR	NR	
LOD	0.1	0.2	
Geometric mean	2.08	6.31	
95th percentile	5.68	21.7	
2013–2014 (n=2,165)			CDC 2018
Percent >LOD	NR	NR	
LOD	0.1	0.2	
Geometric mean	1.94	4.99	
95th percentile	5.57	18.5	

Table 5-21. Concentrations of PFOA and PFOS in Human Serum Collected in the United States

	Detection and concentration (ng/mL [ppb])[a]		
Location	PFOA	PFOS	Reference
2015–2016 (n=1,993)			CDC 2019
Percent >LOD	NR	NR	
LOD	0.1	0.2	
Geometric mean	1.56	4.72	
95th percentile	4.17	18.3	
U.S. blood donors			
2000–2001 (n=645)			Olsen et al. 2003b
Percent >LLOQ[b]	92%	99.8%	
Geometric mean	4.6	34.9	
95th percentile[c]	12.1	88.5	
Maximum	52.3	1,656.0	
2006 (n=600)			Olsen et al. 2008, 2017b
Geometric mean	3.44	14.5	
95th percentile	7.9	31.5	
2010 (n=600)			Olsen et al. 2017b
Geometric mean	2.44	8.3	
95th percentile	5.6	21.8	
2015 (n=616)			Olsen et al. 2017b
Geometric mean	1.09	4.3	
95th percentile	3.2	8.6	
U.S. Regional			
Minneapolis-St. Paul blood donors (plasma)			
2005 (n=40)			Olsen et al. 2007b
Percent >LLOQ	95%	100%	
LLOQ	NR	3.4	
Geometric mean	2.2	15.1	
75th percentile	3.5	20.2	
Maximum	4.7	36.9	
Atlanta, Georgia			
2003 (n=20)			Kuklenyik et al. 2004
Percent >LOD	100%	100%	
LOD	0.1	0.4	
Mean	4.9	55.8	
Minimum	0.2	3.6	
Maximum	10.4	164.0	

Table 5-21. Concentrations of PFOA and PFOS in Human Serum Collected in the United States

Location	Detection and concentration (ng/mL [ppb])[a] PFOA	PFOS	Reference
Seattle, Washington elderly individuals			
No date reported (n=238)			Olsen et al. 2004c
Percent >LLOQ[b]	99.2%	99.5%	
Geometric mean	4.2	31.0	
95th percentile[b]	9.7	84.1	
Maximum	16.7	175.0	
Washington County, Maryland			
1974 (n=178)			Olsen et al. 2005
Percent >LLOQ	71%	100%	
LLOQ	1.9	3.9	
Geometric mean	2.1	30.1	
75th percentile	3.0	40.2	
1989 (n=178)			Olsen et al. 2005
Percent >LLOQ	99%[o]	100%[p]	
Geometric mean	5.5	33.3	
LLOQ	1.9	3.9	
75th percentile	6.7	44.0	
Pease Tradeport, Portsmouth, New Hampshire			
2015–2016 (n=1,578)			NH HHS 2016
Percent >LLOQ	99.2	99.8	
LOD	0.1	0.1	
Geometric mean	8.59	3.09	
95th percentile	8.28–8.91	2.99–3.19	
St. Lawrence Island, Alaska			
2013–2014 (n=85)			Byrne et al. 2017
Percent >LOD	92	99	
50th percentile	1.01	4.55	
95th percentile	2.14	12.32	

[a]"Less than" values indicate that the concentration was reported as below the LOD or LLOQ. For cases where samples had concentrations below the limit of detection or lower limit of quantification, a value between zero and the LOD or LLOQ was assigned when calculating the mean concentration.
[b]Experimental LLOQs not determined.
[c]Reported as bias-corrected estimates.
[d]LLOQ, LOQ, or LOD not reported.

CI = confidence interval; LLOQ = lower limit of quantification; LOD = limit of detection; LOQ = limit of quantification; NR = not reported; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid

Table 5-22. Concentrations of Other Perfluoroalkyls in Human Serum Collected in the United States

	Detection and concentration (ng/mL [ppb])[a]							
Sample population	PFHpA	PFNA	PFDA	PFUnA	PDoDA	PFBS	PFHxS	FOSA
U.S. Residents NHANES								
1999–2000 (n=1,562) (Calafat et al. 2007a)								
Percent >LOD	10%	95%	25%	12%	<1%		100%	100%
LOD	0.4	0.1	0.2	0.2	0.2		0.1	0.05
Geometric mean	<0.4	0.5	<0.2	<0.2	<0.2		2.1	0.4
95th percentile	NR	1.7	0.5	NR	NR		8.7	1.4
2003–2004 (n=2,094) (Calafat et al. 2007b)								
Percent >LOD	6.2%	98.8%	31.3%	9.7%	<0.1%	<0.4%	98.3%	22.2%
LOD	0.3	0.1	0.3	0.3	1.0	0.4	0.3	0.2
Geometric mean	<0.3	1.0	<0.3	<0.3	<1.0	<0.4	1.9	<0.2
95th percentile	0.4	3.2	0.8	0.6	<1.0	<0.4	8.3	0.2
2005–2006 (n=2,120) (CDC 2018)								
Percent >LOD	NR	NR	NR	NR	NR	NR	NR	NR
LOD	0.4	0.1	0.2	0.2	0.2	0.1	0.1	0.1
Geometric mean	—	1.09	0.355	—	—	—	1.67	—
95th percentile	0.700	3.60	1.50	0.700	<LOD	0.1	8.30	0.300
2007–2008 (n=2,100) (CDC 2018)								
Percent >LOD	NR	NR	NR	NR	NR	NR	NR	NR
LOD	0.4	0.082	0.2	0.2	0.2	0.1	0.1	0.1
Geometric mean	—	1.22	0.286	—	—	—	1.95	—
95th percentile	0.500	3.28	0.900	0.600	<LOD	<LOD	9.80	<LOD
2009–2010 (n=2,233) (CDC 2018)								
Percent >LOD	NR	NR	NR	NR	NR	NR	NR	NR
LOD	0.1	0.082	0.1	0.1	0.1	0.1	0.1	0.1
Geometric mean	—	1.26	0.279	0.172	—	—	1.66	—
95th percentile	0.200	3.77	0.900	0.900	<LOD	<LOD	6.90	<LOD
2011–2012 (n=1,904) (CDC 2018)								
Percent >LOD	NR	NR	NR	NR	NR	NR	NR	NR
LOD	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Geometric mean	—	0.881	0.199	—	—	—	1.28	—
95th percentile	0.220	2.54	0.690	0.620	0.140	<LOD	5.44	<LOD

Table 5-22. Concentrations of Other Perfluoroalkyls in Human Serum Collected in the United States

	Detection and concentration (ng/mL [ppb])[a]							
Sample population	PFHpA	PFNA	PFDA	PFUnA	PDoDA	PFBS	PFHxS	FOSA
2013–2014(n=2,168) (CDC 2018)								
Percent >LOD	NR	NR	NR	NR	NR	NR	NR	NR
LOD	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Geometric mean	—	0.675	0.185	—	—	—	1.35	NR
95th percentile	0.200	2.00	0.700	0.500	0.200	<LOD	5.60	NR
2015–2016(n=1,993) (CDC 2019)								
Percent >LOD		NR	NR	NR	NR		NR	
LOD		0.1	0.1	0.1	0.1		0.1	
Geometric mean		0.577	0.154	—	—		1.18	
95th percentile		1.90	0.700	0.400	<LOD		4.90	
U.S. blood donors								
2000–2001 (n=645) (Olsen et al. 2003b, 2017a, 2017b)								
Percent >LLOQ[c]	62%	100%	97.7%	93.8%	22.6%		52%	2%
Geometric mean	0.13	0.57	0.16	0.10	NR		1.9	NR
95th percentile[b]	0.5	1.4	0.4	0.3	0.04		9.5	NR
2006 (n=600) (Olsen et al. 2008, 2017a, 2017b)								
Percent>LLOQ	62%	100%	99.8%	99.8%	68%	1.2%	95.7%	
LLOQ[d]	<0.5	NR	<0.05	<0.05	<0.05	<0.5	<0.5	
Geometric mean	0.09	0.97	0.34	0.18	0.04	LLOQ	1.52	
95th percentile	0.4	2.2	0.8	0.5	0.07	LLOQ	5.7	
2010 (n=600) (Olsen et al. 2017a, 2017b)								
Percent>LLOQ	79.7%	100%	100%	99.8%	46.2%	22.5%	95.5%	
LLOQ[d]	<0.05	NR	NR	<0.025	<0.025	<1.0	<0.05	
Geometric mean	0.05	0.83	0.27	0.14	0.03	LLOQ	1.34	
95th percentile	0.2	2.3	0.8	0.5	0.06	0.3	5.3	
2015 (n=616) (Olsen et al. 2017a, 2017b)								
Percent>LLOQ	3.3%	100%	96.9%	47.9%	0.6%	8.4%	99.7%	0%
LLOQ[d]	<0.09	<0.093	<0.093	<0.466	<0.932	<0.047	<0.04	<0.093
Geometric mean	NR	0.43	0.15	NR	NR	LLOQ	0.87	LLOQ
95th percentile	0.16	1.1	0.49	0.25	LLOQ	0.02	3.5	LLOQ

Table 5-22. Concentrations of Other Perfluoroalkyls in Human Serum Collected in the United States

	Detection and concentration (ng/mL [ppb])[a]							
Sample population	PFHpA	PFNA	PFDA	PFUnA	PDoDA	PFBS	PFHxS	FOSA
U.S. Regional								
Midwestern United States (De Silva and Mabury 2006)								
2004–2005 (n=16)								
Percent detected[e]		100%	100%	13%	0%			
Mean		0.77	0.17	NR	NA			
Maximum		1.2	0.25	0.067	NA			
Atlanta, Georgia (Kuklenyik et al. 2004)								
2003 (n=20)								
Percent >LOD	10%	100%	75%	85%	10%		100%	75%
LOD	0.3	0.1	0.3	0.3	1		0.3	0.2
Mean[b]	<0.3	2.6	0.7	0.8	<1		3.9	0.34
Maximum	8.5	3.9	1.2	1.4	1.6		11.2	0.7
Seattle, Washington; elderly population (Olsen et al. 2004c)								
No date reported (n=238)								
Percent >LLOQ[c]							76%	"Few"
Geometric mean							2.2	NR
95th percentile[b]							8.3	NR
Maximum							40.3	NR
Washington County, Maryland (Olsen et al. 2005)								
1974 (n=178)								
Percent >LLOQ							63%	
LLOQ								
Geometric mean							1.5	
75th percentile							2.5	
1989 (n=178)								
Percent >LLOQ							82%	
LLOQ							1.4	
Geometric mean							2.5	
75th percentile							1.6	

Table 5-22. Concentrations of Other Perfluoroalkyls in Human Serum Collected in the United States

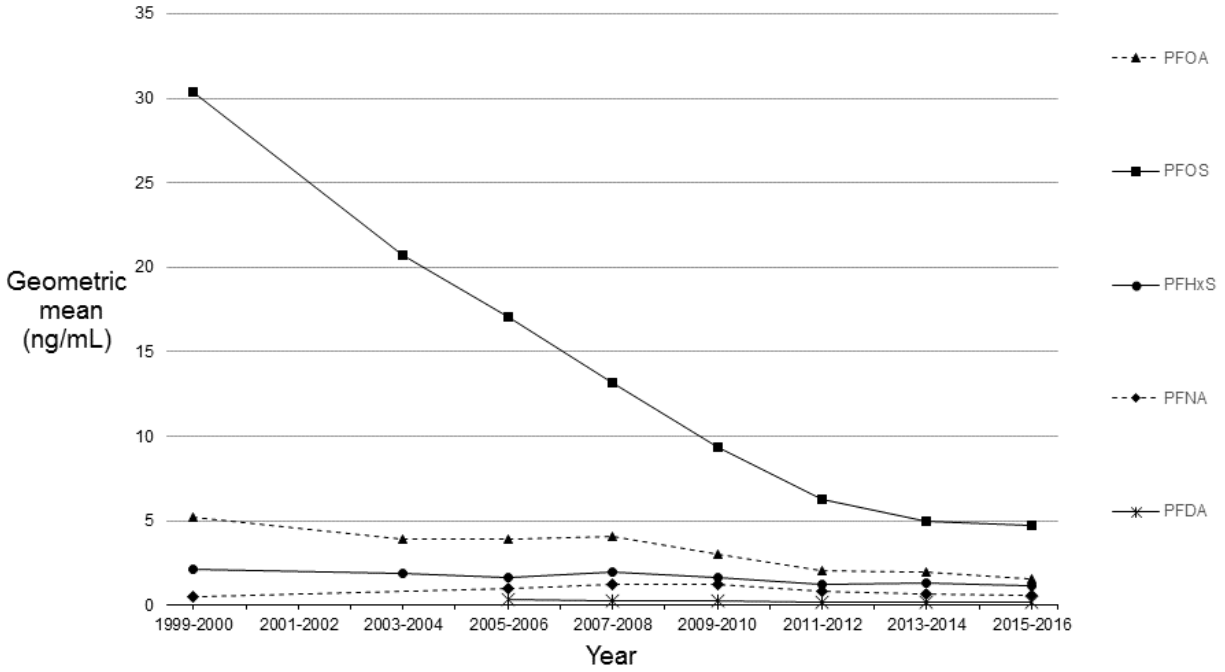
	Detection and concentration (ng/mL [ppb])[a]							
Sample population	PFHpA	PFNA	PFDA	PFUnA	PDoDA	PFBS	PFHxS	FOSA
Pease Tradeport, Portsmouth, New Hampshire (NH HHS 2016)								
2015–2016 (n=1,578)								
Percent >LOD	0.9	85.2	42.1	30.0	4.7	20.0	94.2	2.0
LOD	NA	0.1	0.1	0.1	NA	NA	0.1	0.1
Geometric mean	0.07	0.73	0.22	0.19	0.08	0.04	4.12	0.13
95th percentile	0.07	0.70–0.75	0.21–0.23	0.18–0.19	0.08	0.04	3.92–4.33	0.12–0.14
St. Lawrence Island, Alaska (Byrne et al. 2017)								
2013–2014 (n=85)								
Percent >LOD		99	39	72			32	
50th percentile		2.21	<LOD	0.72			<LOD	
95th percentile		7.35	1.06	1.72			2.74	

[a]"Less than" values indicate that the concentration was reported as below the LOD or LLOQ. For cases where samples had concentrations below the LOD or LLOQ, a value between zero and the LOD or LLOQ was assigned when calculating the mean concentration.
[b]Reported as bias-corrected estimates.
[c]Experimental LLOQs not determined.
[d]Highest LLOQ listed.
[e]LOD not reported.

Grayed cells indicate that study did not evaluate compound; "—" indicates no available data;
FOSA = perfluorooctane sulfonamide; LLOQ = lower limit of quantification; LOD = limit of detection; NA = not applicable; NR = not reported; PFBS = perfluorobutane sulfonic acid; PFDA = perfluorodecanoic acid; PFDoDA = perfluorododecanoic acid; PFHpA = perfluoroheptanoic acid; PFHxS = perfluorohexane sulfonic acid; PFNA = perfluorononanoic acid; PFUnA = perfluoroundecanoic acid

As illustrated in Figure 5-3, there has been a clear trend in decreasing serum levels of both PFOS and PFOA in the general population of the United States since 2000 as these substances were phased out; from 1999–2000 to 2015–2015 (CDC 2018, 2019), the geometric mean serum PFOS and PFOA levels in the general population have declined approximately 84 and 70%, respectively. Serum concentrations for PFHxS and PFDA (Figure 5-3) have also been declining over time, whereas serum PFNA concentrations increased from 1999–2000 to 2009–2010 and then started to decrease (Figure 5-3).

Figure 5-3. Geometric Mean Concentrations of PFOA, PFOS, PFHxS, PFNA, and PFDA in U.S. Residents from 1999 to 2016



NHANES = National Health and Nutrition Examination Survey; PFDA = perfluorodecanoic acid; PFHxS = perfluorohexane sulfonic acid; PFNA = perfluorononanoic acid; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid

Source: CDC 2018

Serial studies of American Red Cross blood donors also allow for temporal evaluations of serum perfluoroalkyl levels. Olsen et al. (2008) reported a nearly 60% decline in PFOS blood levels when comparing data from 2001 to 2006; from 2006 to 2015, the serum PFOS levels dropped another 70% (Olsen et al. 2017b). From 2000 to 2015, the PFOS levels dropped 88%. Serum PFOA levels among the blood donors decreased 76% from 2000 to 2015 (Olsen et al. 2003b, 2017b). Geometric mean serum PFHxS levels went from 1.9 ng/mL in 2000 to 0.87 ng/mL in 2015, a 54% decrease (Olsen et al. 2003b, 2017a, 2017b). From 2006 to 2015, both serum PFNA and PFDA levels decreased by 56% (Olsen et al. 2017a, 2017b).

Several studies have evaluated sex- and age-related differences in serum perfluoroalkyl levels in the general population. In an analysis of NHANES data from 1999 to 2008, Kato et al. (2011) found that males had significantly higher levels of PFOA, PFOS, and PFNA than females and that PFOS levels increased with age, especially in females. Fu et al. (2014b) analyzed the effects of sex and age on levels of perfluoroalkyls in a study of 133 (79 male, 54 female) participants. In general, higher levels of PFOA,

PFOS, PFNA, and PFDA were observed in male subjects; however, differences were only statistically significant for PFOA and PFDA. Sex differences in other perfluoroalkyls were not observed. For both male and female subjects, increasing levels of PFOA, PFNA, and PFOS were positively correlated with increasing age. Age- and sex-specific differences have been incorporated into a regression model developed by Jain (2015), which uses measured serum PFOA and PFOS levels to predict total serum perfluoroalkyl levels.

A small number of studies have measured serum perfluoroalkyl levels in U.S. children (Tables 5-23 and 5-24). NHANES has included adolescents (ages 12–19 years) in the 1999–2000 through 2013–2014 surveys (Calafat et al. 2007a, 2007b; CDC 2018). Analysis of NHANES data from younger children were conducted for the 2001–2002 survey (ages 6–11 years) (Kato et al. 2009b) and 2013–2014 survey (ages 3–5 and 6–11 years) (CDC 2018). Pinney et al. (2014) measured serum PFOA, PFOS, PFHxS, PFNA, and PFDA levels in girls (ages 6–8 years) living in Cincinnati, Ohio and San Francisco, California; and Olsen et al. (2004b) measured PFOA, PFOS, PFHxS, and FOSA in the serum of children ages 2–12 years from various locations in the United States who were diagnosed with group A streptococcal infections. Serum levels of several perfluoroalkyls were examined in children exposed to the World Trade Center (WTC) disaster (n=123) and a sociodemographically-matched comparison group (n=185) (Trasande et al. 2017). Children exposed during the WTC disaster were identified from the WTC Health Registry (WTCHR). All participants were ≤8 years of age on September 11, 2001. Blood serum levels of PFOA, PFOS, PFHxS, PFNA, PFDA, and PFUnA were collected during 2014–2016. For all perfluoroalkyls, serum levels in WTCHR children were significantly ($p<0.01$) higher than in matched controls, with percentage increases above control as follows: PFOA 29%; PFOS 34%; PFHxS 26%; PFNA 24%; PFDA 27%; and 200% PFUnA.

Geometric mean serum perfluoroalkyl levels in adolescents (ages 12–19 years) included in NHANES are similar to geometric mean concentrations reported for adults (Calafat et al. 2007a, 2007b; CDC 2018). For example, geometric mean concentrations of PFOA and PFOS measured during the 1999–2000 and 2003–2004 NHANES surveys were 3.9–5.5 and 19.3–29.1 ng/mL, respectively, in adolescent serum and 3.9–5.2 and 20.7–30.4 ng/mL, respectively, in serum of the total population. The most recent NHANES survey (2013–2014) also included serum levels for children aged 3–5 and 6–11 years; the geometric mean concentrations in the younger children were similar to those in adolescents and the total population.

Olsen et al. (2004b) also found that the geometric mean serum PFOA, PFOS, PFHxS, and PFNA levels in older children (12–19 years of age) were similar to those measured in adults (Olsen et al. 2003b).

However, estimated 95th percentile values of PFHxS measured in children were noted to be higher than values estimated for adults. Olsen et al. (2004b) reported bias-corrected 95th percentile estimates of 65 ng/mL for PFHxS in the serum of children ages 2–12 years. This value is higher than bias-corrected 95th percentile estimates of 9.5 and 8.3 ng/mL based on PFHxS measurements in the serum of adult blood donors (Olsen et al. 2003b) and elderly individuals (Olsen et al. 2004c), respectively (see Table 5-22). The difference is less extreme in the NHANES data, with PFHxS 95th percentile values of 12.9–13.1 ng/mL reported for children compared to values of 8.3–8.7 ng/mL reported for the total population. Reasons for the observed differences of PFHxS levels in childhood serum samples compared to adult samples have not been determined. Olsen et al. (2004b) stated that different exposure and activity patterns between children and adults should be considered. For example, children may have a higher exposure than adults to PFHxS, a substance that has been used in carpet treatment applications, since they are lower to the ground and have increased contact with carpeted floors (Calafat et al. 2007a; Olsen et al. 2004b).

Table 5-23. Percent Detection and Levels of PFOA and PFOS in Children's Serum

	Detection and concentration (ng/mL [ppb])[a]		
Location	PFOA	PFOS	Reference
U.S. Adolescents—NHANES (ages 12–19)			
1999–2000 (n=543)			Calafat et al. 2007a
Percent >LOD	100%	100%	
LOD	0.1	0.2	
Geometric mean	5.5	29.1	
95th percentile	11.2	56.8	
2003–2004 (n=640)			Calafat et al. 2007b
Percent >LOD	99.7%[b]	99.9%[b]	
LOD	0.1	0.4	
Geometric mean	3.9	19.3	
95th percentile	8.6	42.2	
2005–2006 (n=640)			CDC 2018
Percent >LOD	NR	NR	
LOD	0.1	0.2	
Geometric mean	3.59	15.0	
95th percentile	8.40	38.5	
2007–2009 (n=357)			CDC 2018
Percent >LOD	NR	NR	
LOD	0.1	0.2	
Geometric mean	3.91	11.3	
95th percentile	7.30	28.0	

Table 5-23. Percent Detection and Levels of PFOA and PFOS in Children's Serum

Location	Detection and concentration (ng/mL [ppb])[a] PFOA	PFOS	Reference
2009–2010 (n=364)			CDC 2013
Percent >LOD	NR	NR	
LOD	0.1	0.2	
Geometric mean	2.74	6.84	
95th percentile	5.00	18.1	
2011–2012 (n=344)			CDC 2018
Percent >LOD	NR	NR	
LOD	0.1	0.2	
Geometric mean	1.80	4.16	
95th percentile	3.59	10.8	
2013–2014 (n=401)			CDC 2018
Percent >LOD	NR	NR	
LOD	0.1	0.2	
Geometric mean	1.66	3.54	
95th percentile	3.47	9.30	
2015–2016 (n=353)			CDC 2019
Percent >LOD	NR	NR	
LOD	0.1	0.2	
Geometric mean	1.25	2.94	
95th percentile	2.47	6.60	
U.S. Children—NHANES			
2001–2002; ages 6–11 (n=936)			Kato et al. 2009b
Least square mean	6.1–7.6	30.45–42.45	
2013–2014; ages 3–5 (n=181)			
Percent >LOD	100%	100%	Ye et al. 2018a; CDC 2018
LOD	0.1	0.1	
Geometric mean	2.00	3.38	
95th percentile	5.58	8.82	
2013–2014; ages 6–11 (n=458)			
Percent >LOD	100%	100%	Ye et al. 2018a; CDC 2018
LOD	0.1	0.1	
Geometric mean	1.89	4.15	
95th percentile	3.84	12.4	
U.S. Regional			
Girls ages 6–8; Cincinnati, Ohio (n=353)			Pinney et al. 2014
Percent >LOD[c]	99.7%	99.7%	
Geometric mean	7.8	13.2	
Median	7.3	13.6	

Table 5-23. Percent Detection and Levels of PFOA and PFOS in Children's Serum

Location	Detection and concentration (ng/mL [ppb])[a] PFOA	PFOS	Reference
Girls ages 6–8; San Francisco, California (n=351)			Pinney et al. 2014
Percent >LOD[c]	100%	100%	
Geometric mean	5.7	13.2	
Median	5.8	12.5	
Children ages 6–10; Project Viva, Boston Massachusetts 2007–2010 (n=653)			Harris et al. 2017
Percent >LOD	99.5%	99.5%	
Geometric mean	4.2	6.2	
90th percentile	7.9	13.7	
Children ages 2–12; 23 states and District of Columbia 1994–1995 (n=598)			Olsen et al. 2004b
Percent >LLOQ[c]	96%	100%	
Geometric mean	4.9	37.5	
95th percentile[d]	10	89	
Children ages 1–19; West Virginia and Ohio 2005–2006 (n=4,943)			Mondal et al. 2012
Geometric mean	31.2	19.2	
90th percentile	201	36.8	
Children (DOB: 9/11/1993–9/10/2001); New York City, WTCHR 2014–2016 (n=123)			Trasande et al. 2017
Percent >LOD	100%	100%	
Median	1.81	3.72	
Children (DOB: 9/11/1993–9/10/2001); New York City, not eligible for WTCHR 2014–2016 (n=185)			Trasande et al. 2017
Percent >LOD	100%	100%	
Median	1.39	2.78	

[a]"Less than" values indicate that the concentration was reported as below the LOD or LLOQ. For cases where samples had concentrations below the LOD or LLOQ, a value between zero and the LOD or LLOQ was assigned when calculating the mean concentration.
[b]Percent detection for the adolescent age group was not specified for the 2003–2004 NHANES samples. Percentages listed here are for the total sample population.
[c]LOD or LLOQ not reported.

"—" indicates no available data; DOB = date of birth; LLOQ = lower limit of quantification; LOD = limit of detection; LOQ = limit of quantification; NR = not reported; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid; WTCHR = World Trade Center Health Registry

Table 5-24. Percent Detection and Levels of Other Perfluoroalkyls in Children's Serum

Sample population	Detection and concentration (ng/mL [ppb])[a]							
	PFHpA	PFNA	PFDA	PFUnA	PFDoDA	PFBS	PFHxS	FOSA
U.S. Adolescents NHANES (ages 12–19)								
1999–2000 (n=543) (Calafat et al. 2007a)								
Percent >LOD	10%[b]	96%	15%	12%[b]	<1%[b]		100%	100%
Geometric mean	—	0.5	<0.2	—	—		2.7	0.4
95th percentile	—	1.1	0.5	—	—		12.9	1.5
2003–2004 (n=640) (Calafat et al. 2007b)								
Percent >LOD	6.2%[b]	98.8%[b]	31.3%[b]	9.7%[b]	<0.1%[b]	<0.4%[b]	98.3%[a]	22.2%[b]
Geometric mean	<0.3	0.9	<0.3	<0.3	<1.0	<0.4	2.4	<0.2
95th percentile	0.5	2.7	0.7	<0.3	<1.0	<0.4	13.1	0.3
2005–2006 (n=640) (CDC 2018)								
Percent >LOD	NR	NR	NR	NR	NR	NR	NR	NR
Geometric mean	—	0.929	0.295	—	—	—	2.09	—
95th percentile	1.10	2.70	0.800	0.500	<LOD	0.100	14.1	0.300
2007–2008 (n=357) (CDC 2018)								
Percent >LOD	NR	NR	NR	NR	NR	NR	NR	NR
Geometric mean	—	1.16	0.231	—	—	—	2.40	—
95th percentile	0.600	2.54	0.800	0.300	<LOD	<LOD	15.9	0.300
2009–2010 (n=364) (CDC 2018)								
Percent >LOD	NR	NR	NR	NR	NR	NR	NR	NR
Geometric mean	—	1.10	0.220	—	—	—	2.03	—
95th percentile	0.400	2.62	0.600	0.400	<LOD	<LOD	12.3	<LOD
2011–2012 (n=344) (CDC 2018)								
Percent >LOD	NR	NR	NR	NR	NR	NR	NR	NR
Geometric mean	—	0.741	0.146	—	—	—	1.28	—
95th percentile	0.190	2.06	0.360	0.250	<LOD	<LOD	6.45	<LOD
2013–2014 (n=402) (CDC 2018)								
Percent >LOD	NR	NR	NR	NR	NR	NR	NR	NR
Geometric mean	—	0.599	0.136	—	—	—	1.27	—
95th percentile	0.200	2.00	0.400	0.200	0.200	<LOD	6.30	<LOD
2015–2016 (n=353) (CDC 2019)								
Percent >LOD		NR	NR	NR	NR		NR	
Geometric mean		0.481	—	—	—		0.918	
95th percentile		1.20	0.300	0.200	<LOD		3.10	
U.S. Children NHANES								
2001–2002; ages 6–11 (n=936) (Kato et al. 2009b)								
Least square mean		0.7-1.2						
2013–2014; ages 3–5 (n=181) (Ye et al. 2018a, 2018b; CDC 2018)								
Percent >LOD	27%	100%	50%	27%	0%	1%	100%	7%
LOD	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Geometric mean	<LOD	0.764	<LOD	<LOD	<LOD	<LOD	0.715	<LOD
95th percentile	0.310	3.49	0.370	0.370	<LOD	<LOD	1.62	0.110

Table 5-24. Percent Detection and Levels of Other Perfluoroalkyls in Children's Serum

Sample population	Detection and concentration (ng/mL [ppb])[a]							
	PFHpA	PFNA	PFDA	PFUnA	PFDoDA	PFBS	PFHxS	FOSA
2013–2014; ages 6–11 (n=458) (Ye et al. 2018a, 2018b; CDC 2018)								
Percent >LOD	15%	100%	46%	28%	0%	7%	100%	2%
LOD	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Geometric mean	<LOD	0.809	<LOD	<LOD	<LOD	<LOD	0.913	<LOD
95th percentile	0.170	3.19	0.350	0.250	<LOD	0.130	4.14	<LOD
U.S. Regional								
Girls ages 6–8; Cincinnati, Ohio (n=353) (Pinney et al. 2014)								
Percent >LOD[c]		99.9	75.8				99.7	19
Geometric mean		1.4	0.3				5.1	<LOD
Median		1.4	0.3				5.2	<LOD
Girls ages 6–8; San Francisco, California (n=351) (Pinney et al. 2014)								
Percent >LOD[c]		100	78.7				100	10
Geometric mean		1.7	0.3				3.0	<LOD
Median		1.6	0.3				2.3	<LOD
Children ages 6–10; Project Viva, Boston Massachusetts 2007–2010 (n=653) (Harris et al. 2017)								
Percent >LOD[c]		99.5%	88.2%				99.5%	0.9%
Geometric mean		1.7	0.3				2.2	<LOD
90th percentile		3.8	0.6				7.0	<LOD
Children ages 2–12; 23 states and District of Columbia 1994–1995 (n=598) (Olsen et al. 2004b)								
Percent >LLOQ							78%	14%
LLOQ							NR	1.0
Geometric mean							4.5	<2.0
95th percentile[b]							65	<2.0
Children (DOB: 9/11/1993–9/10/2001); New York City, WTCHR 2014–2016 (n=123) (Trasande et al. 2017)								
Percent >LOD	99.7[d]	75[d]	53[d]				100	
Median	0.61	0.14	0.12				0.67	
Children (DOB: 9/11/1993–9/10/2001); New York City, not eligible for WTCHR 2014-2015 (n=185) (Trasande et al. 2017)								
Percent >LOD	99.7[d]	75[d]	53[d]				100	
Median	0.49	0.11	0.04				0.53	

[a]"Less than" values indicate that the concentration was reported as below the LOD or LLOQ. For cases where samples had concentrations below the LOD or LLOQ, a value between zero and the LOD or LLOQ was assigned when calculating the mean concentration.
[b]Percent detection for the adolescent age group was not specified for these samples. Percentages listed here are for the total sample population.
[c]LOD or LLOQ not reported.
[d]%>LOD reported for combined WTCHR and non-WTCHR groups.

Gray cells indicate that chemical was not evaluated; "—" indicates no available data; DOB = date of birth; FOSA = perfluorooctane sulfonamide; LLOQ = lower limit of quantification; LOD = limit of detection; LOQ = limit of quantification; NC = not calculated; ND = no data; NR = not reported; PFBS = perfluorobutane sulfonic acid; PFDA = perfluorodecanoic acid; PFDoDA = perfluorododecanoic acid; PFHpA = perfluoroheptanoic acid; PFHxS = perfluorohexane sulfonic acid; PFNA = perfluorononanoic acid; PFUnA = perfluoroundecanoic acid

When estimating PFOS and PFOA uptake doses for children, Trudel et al. (2008) assumed the same exposure pathways for children as were proposed for adults, but considered exposure from hand-to-mouth transfer from treated carpets to be much larger in children. This pathway was estimated to contribute 40–60% of the total uptake of both PFOS and PFOA in infants (0–1 years), toddlers (1–4 years), and children (5–11 years) in the high-exposure scenario. Exposure via human breast milk was included in the food consumption pathway for infants. Exposure via mouthing of clothes, carpet, and upholstery was also considered for children <12 years old; however, this was considered to be a minor pathway of exposure. PFOS uptake doses estimated for the low-, medium-, and high-exposure scenarios were 18.1–219 ng/kg body weight/day for infants, 14.8–201 ng/kg body weight/day for toddlers, and 9.7–101 ng/kg body weight/day for children. PFOA uptake doses estimated for the low-, medium-, and high-exposure scenarios were 2.2–121 ng/kg body weight/day for infants, 1.2–128 ng/kg body weight/day for toddlers, and 0.8–65.2 ng/kg body weight/day for children. In contrast to the estimates for children under age 12, relative exposure pathways and uptake doses estimated for teenagers (12–20 years old) were approximately the same as for adults.

Perfluoroalkyls have been measured human breast milk and umbilical cord blood; reported concentrations are listed in Tables 5-25 and 5-26. Measurements of perfluoroalkyls in amniotic fluid, meconium, neonatal blood, or other tissues have not been located.

Table 5-25. Percent Detection and Levels of PFOA and PFOS in Umbilical Cord Blood and Breast Milk

Location	Detection and concentration (ng/mL [ppb])[a]		Reference
	PFOA	PFOS	
Umbilical cord blood			
San Francisco, California			Morello-Frosch et al. 2016
Percent >LOD[c]	56%	100%	
Geometric mean	–	2.27	
95th percentile	1.68	4.35	

Table 5-25. Percent Detection and Levels of PFOA and PFOS in Umbilical Cord Blood and Breast Milk

Location	Detection and concentration (ng/mL [ppb])[a] PFOA	PFOS	Reference
Baltimore THREE Study			Apelberg et al. 2007a, 2007b
Cord serum (n=299)			
Percent >LOD	100%	99%	
LOD	0.1–0.2	0.2	
Geometric mean	1.6	4.9	
Minimum	0.3	<0.2	
Maximum	7.1	34.8	
Maternal serum (n=293)			
Median	1.4–1.6	4.1–5.0	
Germany			Midasch et al. 2007
Cord plasma (n=11)			
Percent detected	100%	100%	
LOQ	0.5	0.5	
Median	3.4	7.3	
Maternal plasma (n=11)			
Percent detected	100%	100%	
LOQ	0.5	0.5	
Median	2.6	13.0	
Spain			Manzano-Salgado et al. 2015
Cord serum (n=66)			
Percent >LOD	100%	100%	
Minimum	0.60	0.53	
Maximum	10.56	4.71	
Maternal serum (n=53)			
Percent >LOD	100%	100%	
Minimum	0.86	1.17	
Maximum	14.54	23.14	
Danish National Birth Cohort			Fei et al. 2007
Cord blood (n=50)			
Percent >LLOQ	98%	100%	
LLOQ	1.0	1.0	
Mean	3.7	11.0	
Maternal blood (n=200)			
Percent >LLOQ	98%	100%	
LLOQ	1.0	1.0	
Mean	4.5	29.9	

Table 5-25. Percent Detection and Levels of PFOA and PFOS in Umbilical Cord Blood and Breast Milk

Location	Detection and concentration (ng/mL [ppb])[a]		Reference
	PFOA	PFOS	
Japan			Inoue et al. 2004
Cord serum (n=15)			
Percent >LOD	0%	100%	
LOD	0.5	0.5	
Range		1.6–5.3	
Maternal serum (n=15)			
Percent >LOD	20%	100%	
LOD	0.5	0.5	
Range	0.5–2.3	4.9–17.6	
Breast milk			
Massachusetts (n=45)			Tao et al. 2008b
Milk			
Percent >LOQ[b]	89%	96%	
Mean	0.0438	0.131	
Minimum	<0.0301	<0.032	
Maximum	0.161	0.617	
Sweden (n=12)			Kärrman et al. 2007
Milk			
Percent >LOD	8%[c]	100%	
LOD	0.01	0.005	
Mean	–	0.201	
Range	<0.209–0.492	0.060–0.470	
Maternal serum			
Percent >LOD	100%	100%	
LOD	0.01	0.005	
Mean	3.8	20.7	
Range	2.4–5.3	8.2–48.0	
Sweden (n=20)			Sundstrom et al. 2011
Milk			
LOD	0.005	0.005	
1997 Mean	0.138	0.237	
2007 Mean	0.086	0.122	
Norway (n=9)			Thomsen et al. 2010
Milk			
LOD	0.008	0.003	
Median	0.05	0.11	
Range	0.016–0.19	0.028–0.36	

Table 5-25. Percent Detection and Levels of PFOA and PFOS in Umbilical Cord Blood and Breast Milk

	Detection and concentration (ng/mL [ppb])[a]		
Location	PFOA	PFOS	Reference
China (n=19)			So et al. 2006b
Percent >LOD	100%	100%	
LOD	0.021–0.027	0.001–0.0036	
Range	0.047–0.210	0.045–0.360	
Middle East, including Jordan (n=19)			Al-sheyab et al. 2015
Percent >LOQ	100%	94%	
LOQ	0.01	0.01	
Mean	0.14	0.035	
Range	0.024–1.22	0.006–0.18	
France (n=48)			Antignac et al. 2013
Percent >LOD	90%	98%	
LOD	0.05	0.05	
Mean	0.082	0.092	
Range	<LOD-0.22	<LOD-0.33	
France (n=61)			Cariou et al. 2015
Percent >LOD	77%	82%	
LOD	0.05	0.04	
Mean	0.041	0.04	
Range	<LOD-0.31	<LOD-0.376	
Belgium			Croes et al. 2012
Percent >LOD	100%	100%	
LOD	0.01	0.01	
Mean	0.08	0.13	
Italy (n=49)			Guerranti et al. 2013
Percent >LOD	2% (1 sample)	27%	
LOD	0.5[e]	0.5[d]	
Mean	8.04 [e]	0.85[e]	
Italy			Barbarossa et al. 2013
Primiparous (n=21)			
Percent >LOQ	81%	90%	
LOQ	0.024	0.015	
Mean	0.076	0.057	
Range	<LOQ–-0.24	<LOQ–0.29	
Multiparous (n=16)			
Percent >LOQ	69%	62%	
LOQ	0.024	0.015	
Mean	0.043	0.036	
Range	<LOQ–0.1	<LOQ–0.12	

Table 5-25. Percent Detection and Levels of PFOA and PFOS in Umbilical Cord Blood and Breast Milk

Location	Detection and concentration (ng/mL [ppb])[a]		Reference
	PFOA	PFOS	
Korea			Kang et al. 2016
Percent >LOD	98%	98%	
LOD	0.019	0.007	
Median	0.072	0.05	
Czech Republic (n=50)			Lankova et al. 2013
Percent >LOQ	100%	100%	
LOQ	0.006	0.005	
Mean	0.05	0.033	
Range	0.012–0.13	0.007–0.11	
Germany/Hungary (n=70)			Völkel et al. 2008
Percent >LOQ	16%	100%	
Minimum	<0.200	0.028	
Maximum	0.460	0.639	

[a]"Less than" values indicate that the concentration was reported as below the LOD or LLOQ. For cases where samples had concentrations below the LOD or LLOQ, a value between zero and the LOD or LLOQ was assigned when calculating the mean concentration.
[b]LOD or LLOQ not reported.
[c]Reported as bias-corrected estimates.
[d]ng/g wet weight.

"—" indicates no available data; DOB = date of birth; LLOQ = lower limit of quantification; LOD = limit of detection; LOQ = limit of quantification; NR = not reported; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid; WTCHR = World Trade Center Health Registry

Table 5-26. Percent Detection and Levels of Other Perfluoroalkyls in Children's Umbilical Cord Blood and Breast Milk

Sample population	Detection and concentration (ng/mL [ppb])[a]							
	PFHpA	PFNA	PFDA	PFUnA	PFDoDA	PFBS	PFHxS	FOSA
Umbilical cord blood								
San Francisco, California (Morello-Frosch et al. 2016) (n=65)								
Percent >LOD[c]	56%	97%	9%	84%	0%	25%		91%
Geometric mean	—	0.29	—	0.03	—	—		0.02
95th percentile	0.23	0.93	0.49	0.16	—	0.03		0.10
Baltimore THREE Study (Apelberg et al. 2007a, 2007b)								
Cord serum (n=299)								
Percent >LOD	2%		24%	34%	5%	3%		26%
LOD	0.4		0.2	0.2	0.2	0.1		0.05
Minimum	<0.4		<0.2	<0.2	<0.2	<0.1		<0.05
Maximum	2.6		1.1	1.9	1.7	0.2		0.8

Table 5-26. Percent Detection and Levels of Other Perfluoroalkyls in Children's Umbilical Cord Blood and Breast Milk

Sample population	Detection and concentration (ng/mL [ppb])[a]							
	PFHpA	PFNA	PFDA	PFUnA	PFDoDA	PFBS	PFHxS	FOSA
Japan (Inoue et al. 2004)								
Cord serum (n=15)								
Percent >LOD								0%
LOD								1.0
Maternal serum (n=15)								
Percent >LOD								0%
LOD								1.0
Spain (Manzano-Salgado et al. 2015)								
Cord blood (n=66)								
Percent >LOD		100%					88%	
Minimum		0.13					0.05	
Maximum		2.24					1.93	
Maternal serum (n=53)								
Percent >LOD		100%					96%	
Minimum		0.20					0.05	
Maximum		5.37					2.53	
Breast milk								
Massachusetts (n=45) (Tao et al. 2008b)								
Milk								
Percent >LOQ[b]	<1%	64%	<1%	<1%	<1%	<1%	51%	
Mean	NR	0.01726	NR	NR	NR	NR	0.0145	
Minimum	<0.010	<0.0052	<0.00772	<0.00499	<0.00440	<0.0100	<0.0120	
Maximum	0.0234	0.0184	0.0111	0.00884	0.00974	0.0198	63.8	
France (n=49) (Antignac et al. 2013)								
Percent >LOD	2%	2%					100%	
LOD	0.05	0.05					NR	
Mean	—	—					0.049	
Minimum	<LOD	<LOD					0.040	
Maximum	0.074	0.064					0.066	
France (n=61) (Cariou et al. 2015)								
Percent >LOD		0%					15%	
LOD		0.05					0.03	
Mean		0.014					0.026	
Minimum		<LOD					<LOD	
Maximum		<LOD					0.217	
Belgium (n=40) (Croes et al. 2012)								
Percent >LOD		42%					20%	
LOD		0.01					0.01	
Mean		—					—	

Table 5-26. Percent Detection and Levels of Other Perfluoroalkyls in Children's Umbilical Cord Blood and Breast Milk

Sample population	Detection and concentration (ng/mL [ppb])[a]							
	PFHpA	PFNA	PFDA	PFUnA	PFDoDA	PFBS	PFHxS	FOSA
Sweden (n=12) (Kärrman et al. 2007a)								
Milk								
Percent >LOD		17%	0%	0%			100%	67%
LOD		0.005	0.008	0.005			0.01	0.007
Mean		0.017	—	—			0.085	0.013
Range		<0.005–0.020	—	—			0.031–0.172	<0.007–0.030
Maternal serum								
Percent >LOD		100%	100%	100%			100%	75%
LOD		0.005	0.008	0.005			0.01	0.007
Mean		0.80	0.53	0.40			4.7	0.24
Range		0.43–2.5	0.27–1.8	0.20–1.5			1.8–11.8	0.16–0.19
China (n=19) (So et al. 2006b)								
Percent >LOD	37%	100%	100%	100%		11%	100%	
LOD	0.005–0.010	0.001–0.010	0.0011–0.0025	0.0022–0.0050		0.001–0.005	0.001–0.010	
Range	<0.005–0.0067	0.01–0.062	0.0038–0.011	0.0091–0.056		<0.001–0.0025	0.004–0.10	

[a]"Less than" values indicate that the concentration was reported as below the LOD or LLOQ. For cases where samples had concentrations below the LOD or LLOQ, a value between zero and the LOD or LLOQ was assigned when calculating the mean concentration.
[b]LOD or LLOQ not reported.

Gray cells indicate that chemical was not evaluated; "—" indicates no available data; DOB = date of birth; FOSA = perfluorooctane sulfonamide; LLOQ = lower limit of quantification; LOD = limit of detection; LOQ = limit of quantification; ND = no data; NR = not reported; PFBS = perfluorobutane sulfonic acid; PFDA = perfluorodecanoic acid; PFDoDA = perfluorododecanoic acid; PFHpA = perfluoroheptanoic acid; PFHxS = perfluorohexane sulfonic acid; PFNA = perfluorononanoic acid; PFUnA = perfluoroundecanoic acid

Tao et al. (2008b) measured perfluoroalkyl concentrations in 45 human breast milk samples collected from Massachusetts. PFOS, PFOA, PFHxS, and PFNA were each detected in 96, 89, 51, and 64% of the samples, respectively, with median concentrations of 106, 36.1, 12.1, and 6.97 pg/mL, respectively. PFHpA, PFDA, PFUnA, PFDoDA, and PFBS were each detected in <1% of the samples. Perfluoroalkyls have also been measured in the human breast milk of individuals from Sweden, China, and Germany/ Hungary (Kärrman et al. 2007; So et al. 2006b; Völkel et al. 2008). PFOS was detected in all samples, while detection of PFOA ranged from 8 to 100% in these studies. The reported maximum concentrations of PFOS and PFOA measured in human breast milk samples collected during these studies were 0.360–0.639 and 0.210–0.490 ng/mL, respectively (Kärrman et al. 2007; So et al. 2006b; Völkel et al. 2008). Other perfluoroalkyls detected in human breast milk included PFHpA, PFNA, PFDA, PFUnA, PFBS,

PFHxS, and FOSA. Maximum concentrations of these compounds were reported to be <0.18 ng/mL (Kärrman et al. 2007).

The presence of perfluoroalkyls in umbilical cord blood indicates that these substances can cross the placental barrier resulting in the exposure of babies *in utero* (Apelberg et al. 2007a, 2007b; Fei et al. 2007; Inoue et al. 2004; Midasch et al. 2007). In most studies, PFOS and PFOA have been detected in most umbilical cord blood samples with reported maximum (or 95th percentile) concentrations of 5.3–34.8 and 1.68–7.1 ng/mL, respectively (Apelberg et al. 2007a, 2007b; Fei et al. 2007; Inoue et al. 2004; Midasch et al. 2007; Morello-Frosch et al. 2016). Inoue et al. (2004) did not detect PFOA in 15 cord blood samples from Japan; however, this compound was only detected in the maternal serum of three mothers. Apelberg et al. (2007a) also reported concentrations of other perfluoroalkyls measured in 299 cord serum samples collected during the Baltimore THREE Study. Of these compounds, PFDA, PFUnA, and FOSA were detected most frequently (24, 34, and 26%, respectively). Maximum concentrations in these samples ranged from 1.1 to 1.9 ng/mL. PFHpA, PFDoDA, and PFBS were each detected in <6% of the samples, with maximum concentrations ranging from 0.2 to 2.6 ng/mL. Manzano-Salgado et al. (2015) studied the potential transfer of perfluoroalkyls from mothers to their children during pregnancy. Maternal blood and cord serum were collected from 66 mother-child pairs and analyzed for the presence of perfluoroalkyls. A positive correlation was found between maternal plasma and maternal serum with cord serum levels, and the authors concluded that either maternal plasma or maternal serum could be used as a method to estimate fetal exposure to perfluoroalkyls. Median concentrations of PFOS and PFOA were 6.18 and 2.85 ng/mL, respectively, in maternal plasma and 6.99 and 2.97 ng/mL, respectively, in maternal serum. PFOS and PFOA levels in cord serum were 1.86 and 1.90 ng/mL, respectively. A biomonitoring survey of 1,533 pregnant females in Denmark from 2008 to 2013 showed decreasing levels of most perfluoroalkyls in the females' blood during this time period (Bjerregaard-Olesen et al. 2016). The results of this study showed that serum levels of PFHxS, PFOS, PFOA, PFNA, and PFDA decreased at a rate of 7.0, 9.3, 9.1, 6.2, and 6.3 per year, respectively. Morello-Frosch et al. (2016) measured the levels of perfluoroalkyls in 77 maternal and 65 paired umbilical cord blood samples from pregnant females and newborn children in San Francisco, California. Perfluoroalkyls, including PFOA and PFOS, were widely detected; however, concentrations in cord blood or serum were typically equal to or lower than maternal blood levels.

5.7 POPULATIONS WITH POTENTIALLY HIGH EXPOSURES

Potentially high exposures to perfluoroalkyls can occur in the following population categories: perfluoroalkyl production and manufacturing workers, communities located near fluorochemical facilities, and individuals with prolonged use of perfluoroalkyl-containing products. Workers at perfluoroalkyl production and manufacturing facilities and community members living near these facilities may have higher exposure to perfluoroalkyls than the general population based on elevated concentrations of these substances measured in air, soil, sediment, surface water, groundwater, and vegetation surrounding these facilities (3M 2007b, 2008b, 2008c; Barton et al. 2006; Davis et al. 2007; Olsen 2015). Additionally, children may be at risk for higher potential exposure due to hand-to-mouth transfer of chemicals from dust and the ingestion of dirt (Shoeib et al. 2004; Trudel et al. 2008). Levels of perfluoroalkyls measured in the blood of production and manufacturing workers at several facilities are listed in Table 5-27. The serum PFOA, PFOS, and PFHxS levels in workers were frequently 100–1,000 times higher than in the general population. 3M estimated PFOA doses for various on-site exposure scenarios based on monitoring information collected at the Decatur Facility in Alabama (3M 2008c). Occupational exposure scenarios included groundskeeper/maintenance worker and construction/utility worker exposed to on-site soils, surface water, and sediment. According to 3M, estimated on-site exposure to PFOA ranged from $3.2x10^{-6}$ to 2.4 ng/kg/day, with the highest estimated exposure corresponding to construction/utility workers engaged in projects involving contact with soil from an on-site field. Chang et al. (2008a) measured concentrations of PFBA in the serum of 127 former employees and 50 current employees of the 3M Cottage Grove Facility in Minnesota. PFBA serum concentrations were below the detection limit in 73.2% of the former employees and 68.0% of the current employees. Only 4% of the serum samples contained PFBA above 2 ng/mL, with maximum concentrations of 6.2 ng/mL for the former employees and 2.2 ng/mL for the current employees.

Table 5-27. Concentrations of PFOA, PFOS, and PFHxS in Human Serum for Occupationally Exposed Individuals

	Mean concentration (ng/mL [ppb])			
Location	PFOA	PFOS	PFHxS	Reference
3M Decatur, Alabama				
1993 (n=111)	0.00–80,000 (range)			Olsen et al. 1998b
1995 (n=80)	0.00–114,100 (range)			Olsen et al. 1998b
1995 (n=90)		2,440		Olsen et al. 1999
1997 (n=84)		1,960		Olsen et al. 1999
1998 (n=126)	1,536	1,505	345	Olsen 2015
2000 (n=215)	11,900	1,400		Olsen et al. 2003a

Table 5-27. Concentrations of PFOA, PFOS, and PFHxS in Human Serum for Occupationally Exposed Individuals

Location	Mean concentration (ng/mL [ppb])			Reference
	PFOA	PFOS	PFHxS	
2000 (n=263)	1,780	1,320	250	Olsen 2015
1999–2004 (n=26 retired workers)				Olsen et al. 2007a
Initial	691	799	290	
Final	262	403	182	
3M Cottage Grove, Minnesota				
1993 (n=111)	5,000 (1,100 geometric mean)			Olsen et al. 2000; Olsen 2015
1995 (n=80)	6,800 (1,200 geometric mean)			Olsen et al. 2000; Olsen 2015
1997 (n=74)	6,400 (1,300 geometric mean)			Olsen et al. 2000; Olsen 2015
2000 (n=122)	4,630 (810 geometric mean)	860		Olsen and Zobel 2007; Olsen 2015
DuPont Washington Works, Ohio				
1979-2004				Woskie et al. 2012
All workers (n=1308 workers, 2125 samples)	2,050			
Direct PFOA exposure (n=170 workers; 541 samples)	5,470			
Direct PFOA exposure (n=96 workers; 208 samples)	2,530			
2004–2005				Emmett et al. 2006a
Substantial occupational exposure (n=18)	824			
2004				Sakr et al. 2007b
Current occupational exposure (n=259)	494 (median)			
Intermittent current occupational exposure (n=160)	176 (median)			
Past occupational exposure (n=264)	195 (median)			
3M Antwerp, Belgium				
1995 (n=88)		1,930		Olsen et al. 1999
1997 (n=65)		1,480		Olsen et al. 1999
2000 (n=206)	1,030	960		Olsen et al. 2003a
2000 (n=258)	840	800	170	Olsen 2015

Table 5-27. Concentrations of PFOA, PFOS, and PFHxS in Human Serum for Occupationally Exposed Individuals

Location	Mean concentration (ng/mL [ppb]) PFOA	PFOS	PFHxS	Reference
Miteni, Trissino, Italy				
2007				
Current occupational exposure (n=39)	5,710[c] (200–47,040)			Costa et al. 2009
Former occupational exposure (n=11)	4,430[c] (530–18,660)			Costa et al. 2009
2000 (n=25)	18,800			Costa et al. 2009
2001 (n=42)	19,700			Costa et al. 2009
2002 (n=46)	19,300			Costa et al. 2009
2003 (n=41)	13,700			Costa et al. 2009
2004 (n=34)	11,400			Costa et al. 2009
2006 (n=49)	10,800			Costa et al. 2009
2007 (n=50)	11,600			Costa et al. 2009

[a]Data include results from three retirees from the 3M plant in Cottage Grove, Minnesota.

Gray cells indicate that chemical was not evaluated; PFHxS = perfluorohexane sulfonic acid; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid

Individuals who performed jobs that require frequent and/or prolonged contact with perfluoroalkyl containing products, such as firefighters, waste handlers, individuals who install and treat carpets, or individuals with prolonged use of ski wax may also have higher occupational exposure to perfluoroalkyls. Some firefighting foams contain perfluoroalkyls, and firefighters who use these products have been shown to have greater exposures as compared to the general population. Dobraca et al. (2015) compared perfluoroalkyl serum levels of a group of firefighters in California to an adult population from the NHANES survey. Levels of PFOA and PFOS were only slightly higher in the firefighter group (geometric means 3.75 and 12.50 ng/mL, respectively) when compared to adult males in the 2009–2010 NHANES general population survey (3.61 and 12.13 ng/mL, respectively); however, PFDA serum concentrations of firefighters were up to 3 times greater than the NHANES comparison group for the 25^{th}–95^{th} percentiles (50^{th} percentile in firefighters; 0.72 ng/mL compared to 0.30 ng/mL) and the geometric mean (0.90 ng/mL compared to 0.30 ng/mL). In a small-scale study of 37 firefighters participating in the C8 Health Project, significantly (adjusted for age, water district, household income, and smoking) higher levels of PFOA and PFHxS were found in the firefighters compared to 5,373 male participants with other jobs (Jin et al. 2011). Geometric mean PFOA and PFHxS levels were 37.59 and 4.77 ng/mL, respectively, in the firefighters and 31.59 and 3.62 ng/mL, respectively, in the other

participants. No significant differences in PFOS or PFNA levels were found between the groups. A biomonitoring study of 149 firefighters in Australia showed that 100% of serum samples collected had detectable levels of PFOA, PFOS, PFHxS, and PFNA (Rotander et al. 2015). Serum levels of PFHxS were found to be approximately 10–15 times higher than levels found in the general population of Australia and Canada, while PFOS levels in the firefighters were approximately 6–10 times greater than the general population of these nations.

Elevated serum levels of PFOA, PFNA, PFHxA, PFHpA, PFDA, and PFUnA have been found in professional ski waxes containing fluorotelomers; the perfluoroalkyls were likely formed via fluorotelomer metabolism (Olsen 2015). Christensen et al. (2016) conducted a biomonitoring study of perfluoroalkyls on male fishermen from Wisconsin ≥50 years old with a history of sport fish consumption. Increasing age and lower BMI were generally associated with higher levels of the perfluoroalkyls; however, there were only weak correlations observed between amounts of fish consumption and perfluoroalkyl levels, with the exception of PFDA. Levels of PFOA, PFNA, and PFHxS in the blood of the male anglers were similar to the levels for a subset of the NHANES 2011–2012 survey (non-Hispanic white males ≥50 years old); however, levels of PFOS and PFDA were approximately 2 times greater in the anglers as compared to the NHANES survey subgroup. The median and 95th percentile concentrations of PFOS in the anglers were 19.00 and 54.00 ng/mL, respectively, as compared to 10.33 and 25.83 ng/mL, respectively, in the NHANES study group. The median and 95th percentile concentrations of PFDA in the anglers were 0.52 and 1.90 ng/mL, respectively, as compared to 0.23 and 0.53 ng/mL in the NHANES study group. Family members of occupationally exposed workers have been shown to have higher exposure to perfluoroalkyls via dust transfer as compared to family members of nonoccupationally exposed workers (Fu et al. 2015).

PFOA, PFOS, PFBA, PFBS, PFNA, and PFHxS have been detected in the municipal drinking water and private wells of some communities located near fluorochemical facilities (3M 2008c; ATSDR 2008; Emmett et al. 2006a; Hoffman et al. 2011; Hölzer et al. 2008; Post et al. 2013; Steenland et al. 2009a; Wilhelm et al. 2009). Emmett et al. (2006a) compared PFOA serum levels to various types of exposure for individuals living in the Little Hocking community (near DuPont's Washington Works facility) and concluded that residential water source was the primary determinant of serum PFOA at this location. These authors reported that the mean human serum PFOA level was 105 times higher than the residential drinking water level. Median serum PFOA levels were 371 ng/mL in residents for whom this was the only residential water source and 71 ng/mL in those who used bottled, cistern, or spring water. Increased serum PFOA was associated with increasing number of drinks of tap water daily and also with increasing

use of water for making soups and stews and in-home canning of fruits and vegetables. Use of a carbon water filter reduced PFOA levels by about 25%. In a follow-up study, 231 study participants in the Little Hocking Water District were evaluated 15 months later with 88% using bottled water exclusively; 8% had made other changes to their ingestion of residential water including use of activated carbon water filters. PFOA levels had decreased an average of 26% from the initial levels (Emmett et al. 2009). Similarly, Bartell et al. (2010) found that serum levels of PFOA declined significantly following the implementation of GAC filtration of the public water supply. The average decrease in serum PFOA levels for Lubeck, West Virginia residents primarily consuming public water at home (n=130) was 26% 1 year after treatment began. Similar trends were reported for residents of Little Hocking, Ohio. The average decrease in PFOA serum levels for residents primarily consuming public water (n=39) was about 11% 6 months after treatment began.

Median PFOA serum levels for residents currently residing in six water districts located in the mid-Ohio Valley near the Washington Works facility ranged from 12.1 to 224.1 ng/mL, while the median concentration ranged from 10.5 to 33.7 ng/mL for residents who previously worked or resided in these districts (Steenland et al. 2009a). Former employees at the chemical plant had much higher levels (median=75 ng/mL) than people who had not worked at the plant (median=24 ng/mL), but lower levels than those who continued to be employed at the plant during the monitoring period (median=148 ng/mL). Another study of this community reported a median serum PFOA concentration of 24.3 ng/mL for 45,276 non-occupationally exposed individuals in 2005–2006 (Shin et al. 2011a). This was about 8 times greater than the median concentration (3.20 ng/mL) of 2,120 residents of the general population taken from the NHANES data for 2005–2006 (CDC 2018).

A study of residents consuming drinking water from the Ohio River and the Ohio River Aquifer reported elevated median PFOA levels of 13.8 ng/mL in blood samples collected in 1991–1993 from 139 residents (Herrick et al. 2017). In samples collected in 2011–2013 (n=133), the serum PFOA levels dropped to 4.3 ng/mL. The serum PFOS levels in samples collected in 1994–1996 (median of 32.2 ng/mL, n=189) were similar to levels in the general population (Herrick et al. 2017); similar to the decline observed in the general population, median serum PFOS levels were 6.3 ng/mL in 2011–2013.

The Minnesota Department of Health conducted a biomonitoring study of 196 residents living in Washington county, east of the Minneapolis-St. Paul metropolitan area; private and municipal drinking water wells were shown to be contaminated with perfluoroalkyls (MDH 2009). In 2008–2009, geometric mean serum PFOA, PFOS, and PFHxS levels were 15.4, 35.9, and 8.4 ng/mL, respectively; these values

were higher than levels reported in NHANES (Landsteiner et al. 2014; MDH 2009). The investigators noted that the geometric mean serum concentrations of these three substances were greater in residents obtaining drinking water from municipal water supplies as compared to residents with private wells (Landsteiner et al. 2014). Monitoring efforts also conducted in Washington County, Minnesota near the 3M Cottage Grove Facility revealed widespread contamination of PFBA in the groundwater in 2006 (ATSDR 2008). PFBA was detected in 28% of the community members' serum samples; the 75th percentile serum concentration for PFBA was 0.135 ng/mL and the maximum concentration was 8.5 ng/mL (MDH 2000).

ATSDR performed an exposure investigation for residents of Decatur, Alabama following an accidental release of perfluoroalkyls into the Decatur waste water treatment plant (ATSDR 2013). A group of 155 residents had their blood tested for levels of eight perfluoroalkyls. Serum levels for PFNA, PFDA, and FOSA were lower or similar to levels of the general population when compared to the NHANES results. Elevated serum PFOA, PFOS, and PFHxS levels were observed; geometric mean values were 16.3, 39.8, and 6.4 ng/mL, respectively. Residents who used the West Morgan/East Lawrence public water supply had significantly higher geometric mean serum levels of PFOA (17.59 ng/mL) and PFHxS (6.68 ng/mL) as compared to the geometric mean for a similar demographic group from the NHANES survey. Serum perfluoroalkyl levels in the residents were shown to be much lower than levels found in occupationally exposed individuals who regularly worked with these substances (ATSDR 2013).

Additional blood serum levels of PFOA and PFOS for residents in selected areas of Ohio, West Virginia, New Jersey, and Minnesota whose residential source of drinking water may have been contaminated are available from the EPA docket on PFOA and related perfluoroalkyls (EPA-HQ-OPPT-2003-0012) (Bilott 2004, 2005a, 2005b, 2007).

The Emmett et al. (2006a) and Steenland et al. (2009a) studies of the community near the Washington Works facility and the 3M (2008c) study of the Decatur Facility in Alabama found age-related differences in serum PFOA levels. In a comparison of serum PFOA levels in various age groups of residents serviced by the Little Hocking Water Association district, Emmett et al. (2006a) found that 2–5-year-old children had a higher serum PFOA (median 600 ng/mL) compared with residents in all other age groups (median 321 ng/mL) except for the group aged >60 years, whose levels were similar to those in young children. Several factors may have contributed to the observed high levels in children: infants and young children proportionally drink more water per kg of body weight than adults; children (and also the elderly) tend to spend more time at home with exclusive use of residential water than other age groups; and trans-

placental and breast milk exposures could also contribute to levels in children. In the Steenland et al. (2009a) study, PFOA serum levels tended to be highest for children aged 0–9 years and persons >50 years old. The serum levels of the 69,030 residents participating in this study categorized by age are provided in Table 5-28. 3M (2008c) estimated doses for various off-site exposure scenarios based on monitoring information collected at the Decatur Facility. Exposure scenarios include local children and adult residents exposed to PFOA in off-site soils, groundwater, municipal water, fish from the Tennessee River, and surface water and sediments in the Tennessee River. According to 3M, estimated off-site exposure of local residents to PFOA ranged from 0.011 to 260 ng/kg/day, with the highest estimated exposure corresponding to children whose source of drinking water was groundwater adjacent to the southern side of the facility.

Table 5-28. Blood Serum Levels for 69,030 Current and Former Residents of Six Water Districts in the Mid-Ohio Valley (2005–2006)

Age (years)	Number (percentage of total)	Median perfluorooctanoic acid (PFOA) level (ng/mL)
0–9	4,915 (7.1)	32.8
10–19	9,658 (14.0)	26.6
20–29	10,073 (14.6)	21.0
30–39	10,547 (15.3)	22.7
40–49	12,113 (17.6)	28.0
50–59	10,515 (15.2)	33.6
60–69	6,881 (10)	42.9
≥70	4,328 (6.3)	40.1

Source: Steenland et al. 2009a

CHAPTER 6. ADEQUACY OF THE DATABASE

Section 104(i)(5) of CERCLA, as amended, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of perfluoroalkyls is available. Where adequate information is not available, ATSDR, in conjunction with NTP, is required to assure the initiation of a program of research designed to determine the adverse health effects (and techniques for developing methods to determine such health effects) of perfluoroalkyls.

Data needs are defined as substance-specific informational needs that, if met, would reduce the uncertainties of human health risk assessment. This definition should not be interpreted to mean that all data needs discussed in this section must be filled. In the future, the identified data needs will be evaluated and prioritized, and a substance-specific research agenda will be proposed.

6.1 Existing Information on Health Effects

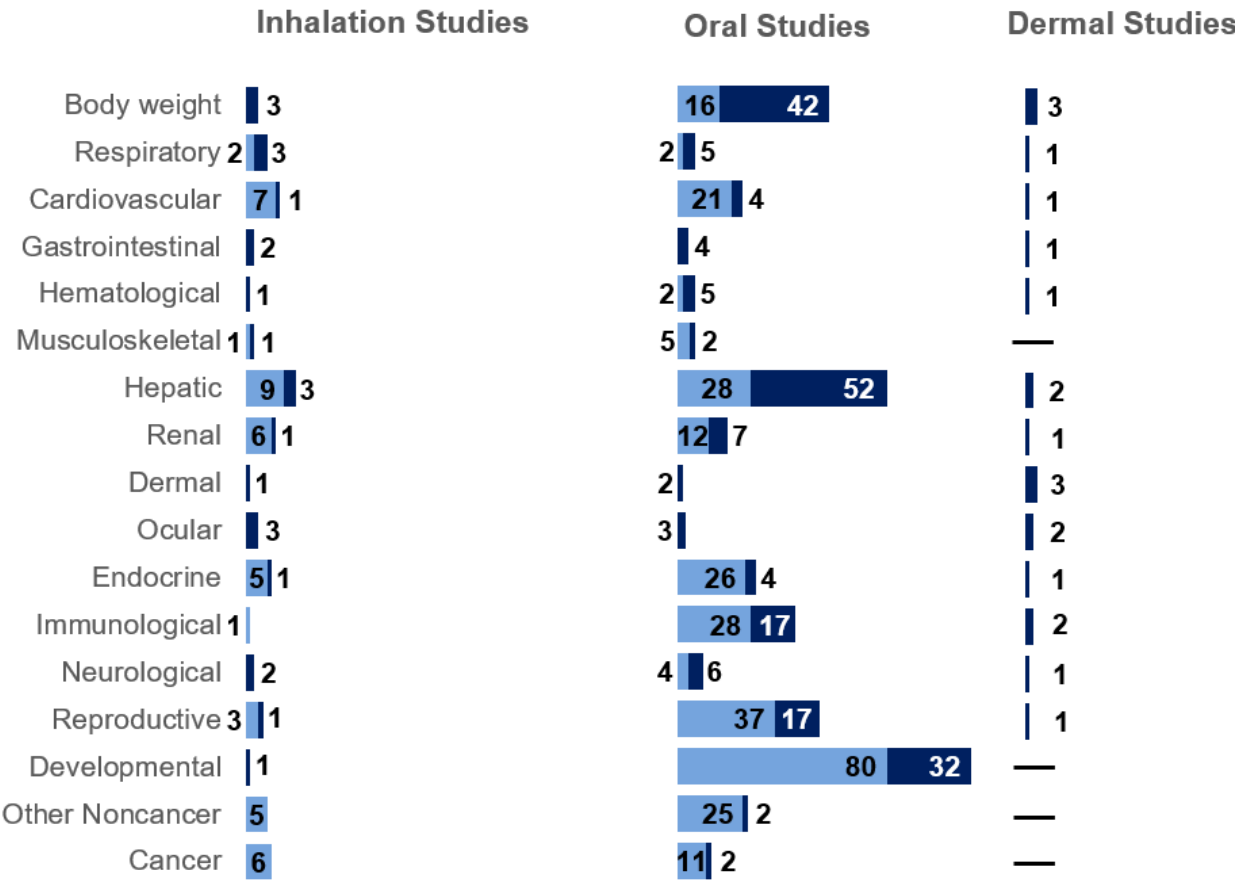
Studies evaluating the health effects of inhalation, oral, and dermal exposure of humans and animals to PFOA, PFOS, and other perfluoroalkyls that are discussed in Chapter 2 are summarized in Figures 6-1, 6-2, and 6-3, respectively. The purpose of these figures is to illustrate the information concerning the health effects of perfluoroalkyls. The number of human and animal studies examining each endpoint is indicated regardless of whether an effect was found and the quality of the study or studies.

As illustrated in Figures 6-1, 6-2, and 6-3, most of the data on the toxicity of PFOA, PFOS, and other perfluoroalkyls come from epidemiological studies in humans; oral exposure is the assumed route of exposure for the epidemiological studies. The epidemiology database consists of health evaluations of subjects exposed in occupational settings (primarily PFOA and PFOS), highly exposed residents living near a PFOA facility, and studies of the general population. The most commonly examined endpoints in the epidemiological studies were developmental, hepatic, reproductive, and immunological effects.

Figure 6-1. Summary of Existing Health Effects Studies on PFOA by Route and Endpoint*

Potential body weight, hepatic, and developmental effects were the most studied endpoints

The majority of the studies examined oral exposure in **humans** (versus **animals**)

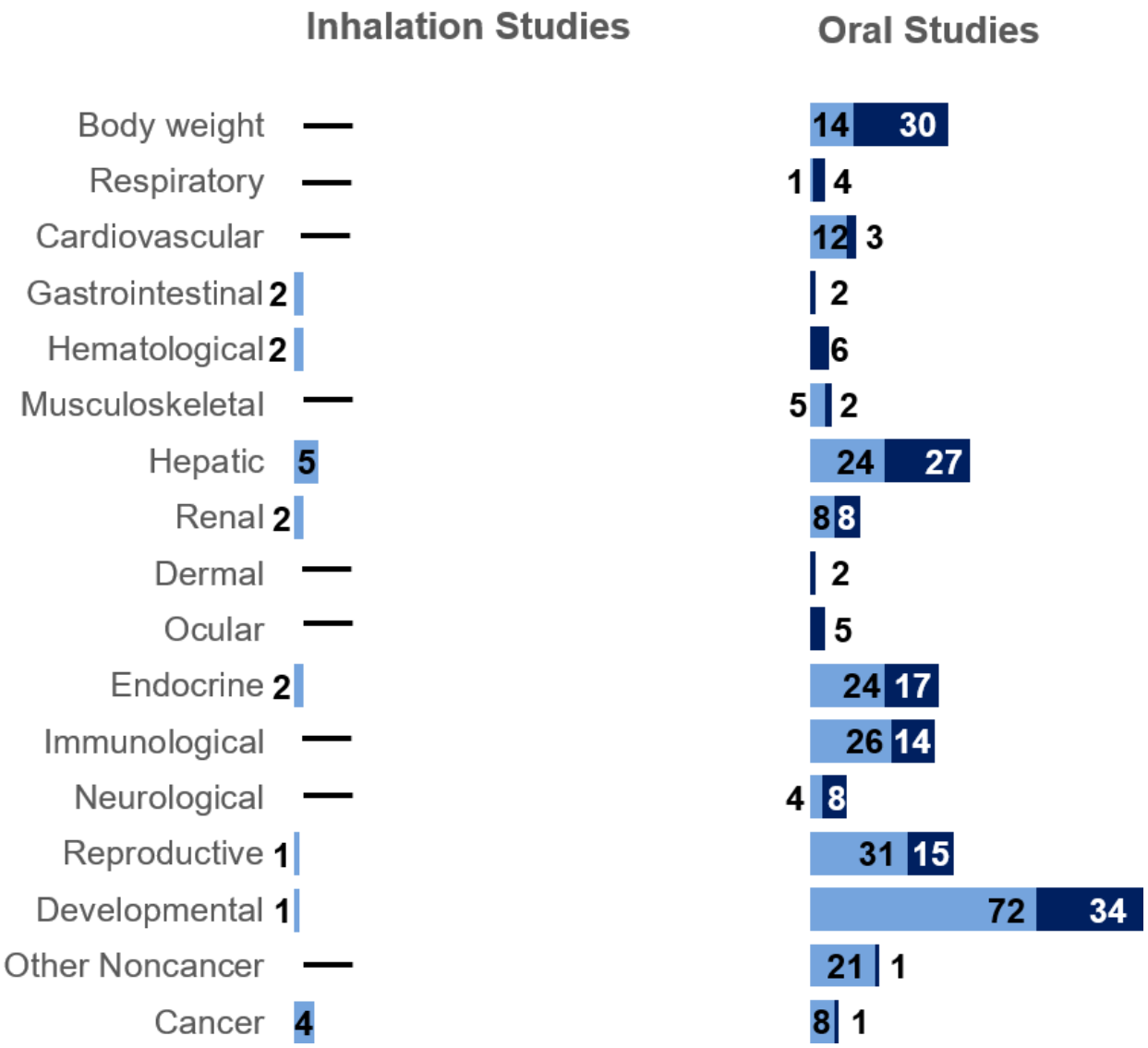


*Oral exposure was the presumed route of exposure for human studies involving environmental exposure. Human and animal studies may have examined more than one endpoint. A "—" indicates that no studies are available.

Figure 6-2. Summary of Existing Health Effects Studies on PFOS by Route and Endpoint*

Potential developmental, hepatic, and reproductive effects were the most studied endpoints

The studies examined oral exposure in **humans** (versus **animals**)

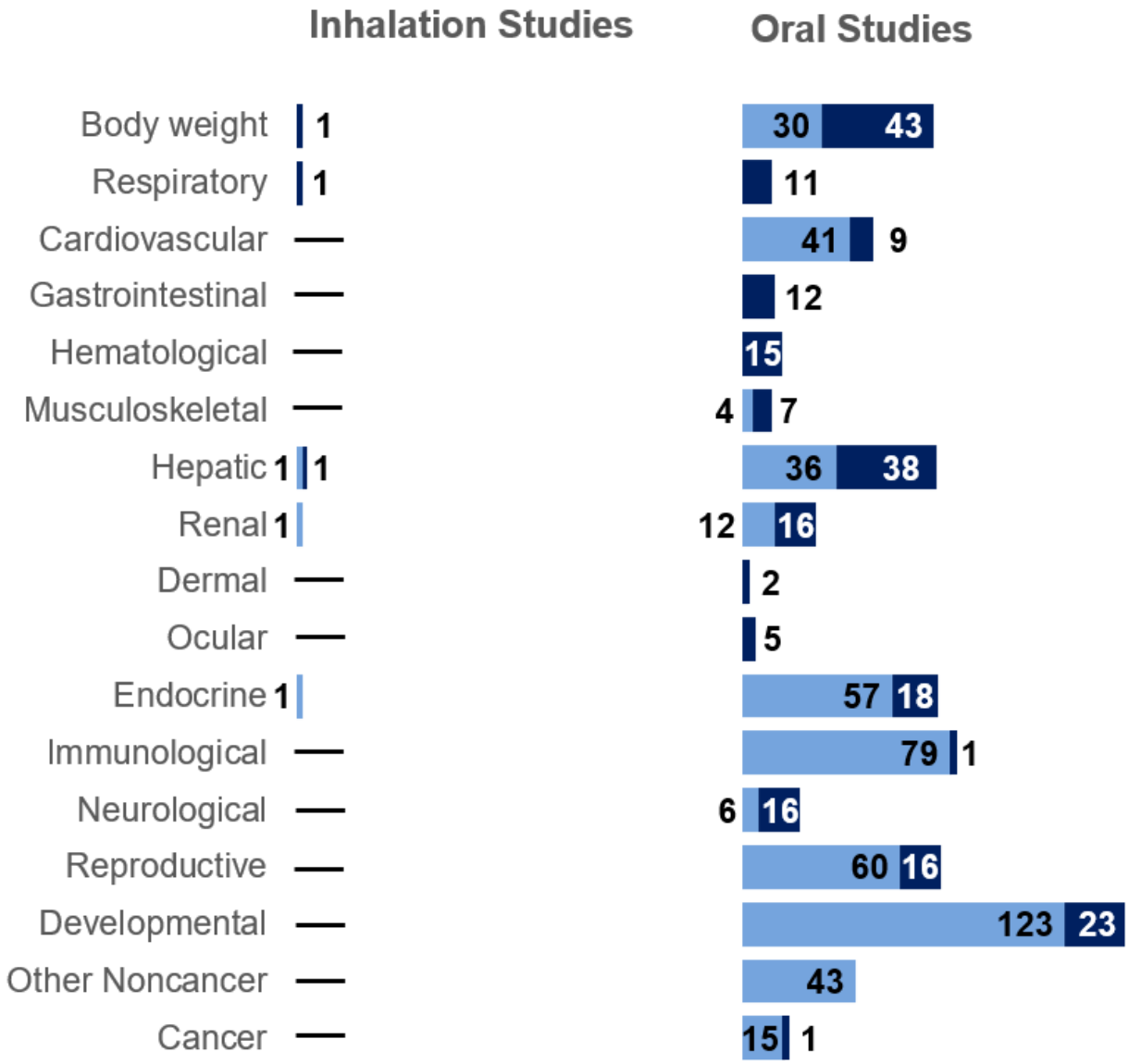


*Oral exposure was the presumed route of exposure for human studies involving environmental exposure. Human and animal studies may have examined more than one endpoint. A "—" indicates that no studies are available.

Figure 6-3. Summary of Existing Health Effects Studies on Other Perfluoroalkyls by Route and Endpoint*

Potential hepatic, immunological, and developmental effects were the most studied endpoints

The majority of the studies examined oral exposure in **humans** (versus **animals**)



*Oral exposure is the presumed route of exposure for human studies involving environmental exposure. Most human studies examined multiple perfluoroalkyls. Human and animal studies may have examined more than one endpoint. A "—" indicates that no studies are available. Includes data for PFBA, PFHxA, PFHpA, PFNA, PFDA, PFUnA, PFBS, PFHxS, PFDoDA, and FOSA.

Most of the information regarding the effects of perfluoroalkyls in animals has been derived from oral studies; considerably less information is available from inhalation and dermal exposure studies. PFOA and PFOS have been the most extensively studied members of this class of chemicals, and oral administration has been the preferred route of exposure in animal studies. Information regarding other perfluoroalkyls covered in this profile is limited to acute-duration oral studies with PFHxS, PFNA, PFDA, PFBA, PFDoDA, PFHxA, and FOSA; intermediate-duration oral studies with PFHxS, PFNA, PFDA, PFUnA, PFBS, PFBA, PFDoDA, and PFHxA; and a chronic-duration oral study with PFHxA. An acute-duration-inhalation study with PFNA is also available. The most commonly examined endpoints were hepatic, body weight, developmental, reproductive, and immunological effects.

6.2 Identification of Data Needs

Missing information in Figures 6-1, 6-2, and 6-3 should not be interpreted as a "data need." A data need, as defined in ATSDR's *Decision Guide for Identifying Substance-Specific Data Needs Related to Toxicological Profiles* (ATSDR 1989), is substance-specific information necessary to conduct comprehensive public health assessments. Generally, ATSDR defines a data gap more broadly as any substance-specific information missing from the scientific literature.

Acute-Duration MRLs. The available acute inhalation database for PFOA was considered inadequate for derivation of an MRL due to lack of measured serum PFOA levels in the available animal studies and the lack of PBPK model parameters that could be used to predict serum levels. The inhalation database for PFNA was not considered adequate due to the limited endpoints examined and the short exposure duration of the only available study. No inhalation data were available for PFOS or the other perfluoroalkyls. A number of studies have evaluated the acute toxicity of PFOA and PFOS following oral exposure and have identified several sensitive targets of toxicity. Smaller numbers of studies evaluated potential sensitive targets of acute toxicity for PFNA and PFDA. However, toxicokinetic differences between humans and laboratory animals, particularly the relative short half-life in rodents compared to humans, preclude derivation of an acute MRL for these compounds. For other perfluoroalkyls (PFHxS, PFBA, PFDoDA, FOSA), the available studies were not considered adequate for identification of critical targets; did not examine sensitive targets that were identified for other perfluoroalkyls, such as developmental and immunological endpoints; or involved a single exposure. No acute oral data were identified for PFUnA, PFHpA, or PFBS. Research is needed to develop a PBPK model that would allow for extrapolation from rodents to humans. Additionally, toxicity studies are needed for most perfluoroalkyls to identify critical targets of toxicity and/or establish dose-response relationships. These

studies should examine developmental and reproductive endpoints that have been established as the most sensitive targets of toxicity for PFOA and PFOS.

Intermediate-Duration MRLs. No intermediate-duration inhalation studies were identified for perfluoroalkyls. Oral studies suggest that developmental and immune effects are the most sensitive targets of toxicity; similar effects are likely to occur following inhalation exposure because perfluoroalkyls are not metabolized. Inhalation studies are needed to establish dose-response relationships and to establish whether the respiratory tract is a sensitive target of toxicity. The intermediate-duration oral databases were considered adequate for derivation of MRLs for PFOA and PFOS. The MRL for PFOA is based on altered bone development measured in mature mice exposed *in utero*. The principal study only tested one PFOA dose level and only examined long bones; additional studies utilizing several dose levels, examining other types of bone, and testing a second species would provide support for the MRL. A modifying factor was used for PFOS due to the lack of PBPK modeling parameters; additional studies are needed that would allow for predicting steady-state serum PFOS levels for immunotoxicity studies in laboratory animals. An important decision made in the derivation of intermediate oral MRLs was to use the time-weighted average serum concentrations (C_{TWA}) of PFOA or PFOS as the basis for extrapolations of the dose-response PODs from animal studies to human equivalent doses (HEDs). However, the available data on the toxicity of PFOA and PFOS do not provide convincing evidence that toxicity outcomes are more likely to be determined by C_{TWA} rather than C_{max}. Since the PBPK model used for dosimetry modeling of the animal studies predicted that C_{TWA} is lower than C_{max} in the principal studies for the MRLs, selection of C_{TWA} as the internal dose metric results in lower values for MRLs. Therefore, use of C_{TWA}, rather than C_{max} is a health-protective decision that might be more adequately evaluated with additional studies that evaluate associations between C_{max}, C_{TWA}, and toxicity outcome responses.

Intermediate-duration MRLs were also derived for PFHxS and PFNA; however, these were based on marginal databases and additional dose-response studies are needed to support the basis of the MRL. The databases were not considered adequate for PFUnA, PFBS, PFBA, or PFDoDA due to the lack of studies examining potential sensitive targets (developmental and/or immune effects). No intermediate-duration oral studies are available for PFDA, PFHpA, or FOSA. Intermediate-duration oral studies are needed for these seven perfluoroalkyls to provide information on sensitive targets and establish dose-response relationships. These studies should include measurement of serum perfluoroalkyl levels, which would allow for estimating HEDs.

An additional uncertainty in the MRLs was the confidence in the elimination half-lives for perfluoroalkyls used in the empirical model for calculating HEDs. Estimated half-lives for PFOA and PFOS vary and contributors to this variability have not been completely characterized. The empirical model used to calculate HEDs is linear; therefore, the change in the HED is approximately proportional to the change in the half-life. A halving of the half-life would result in a doubling of the HED. Studies that can improve confidence in the half-life estimates would increase confidence in MRLs.

Chronic-Duration MRLs. The lack of chronic-duration inhalation studies for perfluoroalkyls precluded derivation of chronic MRLs. Chronic toxicity studies examining a wide range of endpoints are needed to identify the most sensitive target and establish concentration-response relationships. A small number of chronic duration oral studies have been identified in laboratory animals. Four studies examined the chronic toxicity of PFOA, PFOS, or PFHxA. These studies were not considered suitable for derivation of MRLs because they did not evaluate immunotoxicity which was a sensitive target following shorter term exposures. Studies examining this potentially sensitive endpoint are needed to identify the most sensitive target following chronic exposure.

Health Effects. Over 600 studies have evaluated the toxicity of perfluoroalkyls; epidemiological studies account for over 400 of the toxicity studies. Evidence from epidemiological studies suggest associations between perfluoroalkyl exposure and several health outcomes including liver damage, increases in serum lipids, thyroid disease, immune effects, reproductive toxicity, and developmental toxicity. The primary health effects observed in laboratory animals are liver, developmental, and immune toxicity. Although a large number of studies evaluating health effects are available, there is a need for additional studies to address data gaps. Future laboratory animal studies should include measurement of serum perfluoroalkyl levels, as this would provide valuable information for comparing effects observed in laboratory animals to effects observed in humans.

Hepatic Effects. Evidence from acute, intermediate, and/or chronic oral studies in rats, mice, and monkeys indicates that the liver is a sensitive target of PFOA, PFOS, PFHxS, PFNA, PFDA, PFUnA, PFBA, PFBS, PFDoDA, and PFHpA toxicity. The effects observed in rodents differ from those observed in humans. In humans, exposure to PFOA, PFOS, PFNA, and PFDA appear to result in increases in serum lipid levels, particularly total cholesterol levels. However, animal studies have found decreases in serum lipid levels associated with exposure to most perfluoroalkyls. It is not known if the species differences are due to different mechanisms of toxicity or differences in

exposure levels (serum levels observed in animal studies are orders of magnitude higher than those in human studies).

Immune Effects: Epidemiological data suggest an association between PFOA, PFOS, PFHxS, and PFDA and decreased antibody response to vaccines. This is supported by acute- and intermediate-duration studies of PFOA and PFOS in laboratory animals. There is also evidence of immunotoxicity following a single injection of PFNA; some of the immune effects persisted 4 weeks post-exposure. Shorter-term studies are needed for other perfluoroalkyls. In addition, chronic-duration studies evaluating immune endpoints, particularly immunosuppression, for all perfluoroalkyls would allow for identification of the critical targets of toxicity.

Reproductive Effects. Decreases in mammary gland development have been demonstrated in several PFOA mouse studies. The effect levels observed in these studies are very low, although there is some indication that at lower doses, the changes in mammary gland development do not affect lactation. Additional studies are needed to evaluate the adversity of these alterations. This endpoint has not been evaluated for other perfluoroalkyls and studies are needed to determine whether it is also a sensitive effect for these compounds.

Developmental Effects. Based on the results of laboratory animal studies, developmental endpoints are targets of PFOA, PFOS, PFHxS, PFNA, PFDA, PFUnA, and PFBA toxicity following acute- and/or intermediate-duration oral exposure. Studies are needed to evaluate potential developmental effects for PFHxS following intermediate-duration oral exposure. Additionally, cross-fostering studies would provide information that could be used to evaluate the health impact of lactational exposure to perfluoroalkyls. Epidemiological studies in children suggest altered responses to vaccination; two animal studies have evaluated immune effects following perinatal exposure to PFOA and PFOS, but data are lacking for other perfluoroalkyls.

Potential Interactions between Perfluoroalkyls. A common limitation of the epidemiological data is co-exposure to multiple perfluoroalkyls. There are limited data on possible interactions between perfluoroalkyls and possible effects on toxicity and toxicokinetics. Animal studies examining the possible interactions between perfluoroalkyls would be useful for interpreting the epidemiological study results; this is especially important since humans are typically exposed to multiple perfluoroalkyls and many of them are likely to have similar mechanisms of action.

Mechanisms of Toxicity. Many of the effects observed in rodents, particularly liver and developmental effects, involve the activation of PPARα; humans and nonhuman primates are less responsive to PPARα agonists than rats and mice. However, the results of studies in PPARα-null mice suggest that PPARα-independent mechanisms also play a role in the liver, immunological, and developmental toxicity. Additional studies are needed on the mechanisms of toxicity to assess whether the effects observed in laboratory animals are relevant to humans. Mechanistic studies would also provide support for the critical effects used to derive the MRLs for PFOA, PFOS, PFHxS, and PFNA.

Epidemiology and Human Dosimetry Studies. As previously mentioned, information is available regarding the effects of exposure to perfluoroalkyls in humans derived from health evaluations of subjects exposed in occupational settings, residents living near a PFOA manufacturing facility with high levels of PFOA in the drinking water, and the general population. Although many studies found statistically significant associations between serum perfluoroalkyl levels and the occurrence of an adverse health effect, the findings were not consistent across studies. Interpretation of the human data is limited by the reliance of cross-sectional studies, which do not establish causality, and the lack of exposure data. Studies on serum lipids suggest that the dose-response curve is steeper at lower concentrations and flattens out at higher serum perfluoroalkyl concentrations (Steenland et al. 2010a); additional studies that could be used to establish dose-response relationships would be valuable. Mechanistic studies examining the association between perfluoroalkyl exposure and serum lipid levels would also provide valuable insight. Clarification of the significance and dose-response relationships for other observed effects is also needed. Longitudinal studies examining a wide range of endpoints would be useful for identifying critical targets of toxicity in humans exposed to perfluoroalkyls. The available human studies have identified some potential targets of toxicity; however, cause-and-effect relationships have not been established for any of the effects, and the effects have not been consistently found in all studies. Mechanistic studies would be useful for establishing causality. When possible, health assessments should include subjects of different race/ethnicity and age to determine potential race/ethnicity- and age-based susceptibilities. Another limitation of the epidemiological studies is co-exposure to other perfluoroalkyls; studies that statistically controlled for co-exposure to other pollutants would decrease this uncertainty. As noted previously, there is a need for studies evaluating potential interactions between perfluoroalkyls.

Biomarkers of Exposure and Effect. Data are available regarding levels of perfluoroalkyls in serum from the general population, highly exposed residents, and perfluoroalkyl workers. Information is needed regarding the toxicokinetics (see also below) of perfluoroalkyls in humans to be able to relate levels of

these compounds in serum to exposure to specific perfluoroalkyls; data on matched serum and urine samples would be valuable. Also needed is further information on the relationship between serum and liver concentrations of perfluoroalkyls in humans.

Absorption, Distribution, Metabolism, and Excretion. Several epidemiological studies have examined the kinetics of serum perfluoroalkyl concentrations following a change in environmental or occupational exposure, from which estimates of terminal elimination half-lives in adults are available for PFOA, PFOS, PFHxS, PFBA, and PFBS. Other studies provide data on the renal clearances of PFOA and PFOS, binding of PFOA, PFOS, and PFHxS to human plasma protein, tissue levels (primarily blood, maternal and fetal cord serum, and breast milk). Data on other aspects of the toxicokinetics of perfluoroalkyls in humans are not available and could serve to improve predictions of internal dosimetry associated with exposures to perfluoroalkyls (bioavailability, kinetics of tissue distribution and elimination, binding in tissues, external-internal dose relationships, all aspects of toxicokinetics in children and aging populations).

Toxicokinetics of perfluoroalkyls have been studied much more extensively in rodents (rats and mice) and less extensively in Cynomolgus monkeys; however, a number of data gaps have been identified:

- Absorption studies; oral absorption data are available for PFOA, PFOS, and PFBA, but are more limited for other perfluoroalkyls and for other exposure routes. Studies elucidating the mechanisms of pulmonary and gastrointestinal absorption are also needed.

- Studies have shown that elimination kinetics, and therefore, internal dose-external dose relationships, are dependent on structure, including the terminal acid group (carboxylate or sulfonate), carbon chain length, and carbon chain branching. These structural features affect plasma and tissue protein binding, renal and biliary clearances, tissue levels, maternal-fetal transfer, and lactational transfer of perfluoroalkyls. Studies examining differences between perfluoroalkyls would be useful for extrapolating health effects and toxicokinetic data across compounds.

- Toxicokinetic studies have found sex- and dose-dependent subcellular distribution of PFOA in rats. Further studies on the mechanisms for dose-dependency, characterization of subcellular binding proteins, and mechanistic linkages between subcellular distribution and toxicity of perfluoroalkyls are needed.

- The distribution and elimination of PFOA and PFOS are greatly influenced by binding interactions with albumin and other high molecular weight plasma proteins; available data suggest that binding to plasma proteins, as well as the volume of distribution, may be sex- and species-specific. Interactions with albumin have been partially characterized to the extent that binding capacity and affinity constants have been estimated, but the rates of association and dissociation have not been reported.

- Liver uptake and renal clearance of PFOS also appeared to be time-dependent in a PBPK model used to predict plasma and liver in concentrations of PFOS in a chronic rat study (Harris and Barton et al. 2008). Mechanisms underlying these time dependencies have not been elucidated.

Comparative Toxicokinetics. Toxicokinetic studies conducted in various rodent species (mice, rats, hamsters, rabbits) and in Cynomolgus monkeys have revealed profound species and sex differences as well as dose dependencies in the tissue distribution and elimination kinetics of PFOA and PFOS. Studies conducted in rats have revealed contributing mechanisms for sex differences in elimination of PFOA; slower elimination of PFOA in male rats compared to female rats has been attributed to sex hormone-modulated renal tubular transport of PFOA that results in markedly lower renal clearance of PFOA in the sexually mature male rat (see Section 3.5.1, Excretion). Sex differences in elimination of PFOA have also been observed in hamsters; unlike the rat, male hamsters excreted absorbed PFOA more rapidly than female hamsters. Sex differences in elimination of PFOA have not been observed in other rodent species, in Cynomolgus monkeys, or in limited observations made in humans. Sex differences in elimination rates of perfluoroalkyls in humans have not been demonstrated in population studies of serum elimination kinetics or renal clearance. Although the few studies that estimated elimination half-lives or renal clearances in male and female humans have not found significant sex differences, these outcomes may reflect the relatively low serum concentrations in these subjects compared with studies that were conducted in nonhuman primates and rodents (i.e., sex differences in elimination may vary with dose and/or plasma concentration). Additionally, the failure to account for the influence of reduced estrogen levels (in postmenopausal women) and reduced testosterone levels (in older males) in occupational and/or site-related epidemiological studies may also account for the lack of findings of sex-related differences.

Children's Susceptibility. Data needs relating to both prenatal and childhood exposures, and developmental effects expressed either prenatally or during childhood, are discussed in the Health Effects subsection above. It is not known whether children are more or less susceptible than adults to the effects of exposure to perfluoroalkyls because there are no studies that specifically addressed this question. Several studies have examined the possible associations between perfluoroalkyl exposure and health outcomes in children living in an area with high PFOA contamination and in the general population. Although some studies have found statistically significant associations, they are not adequate for establishing causality. Follow-up studies of the C8 population could allow for a longitudinal assessment of health effects in children and would be useful in determining whether the observed effects are due to perfluoroalkyl exposure. Toxicokinetics information in children is needed. Half-life studies have been conducted in adults; there is the need to understand if these are applicable to children. There are no

studies that have examined whether young animals are more or less susceptible than adults to perfluoroalkyls toxicity. Additional information on this issue would be useful.

Physical and Chemical Properties. Perfluoroalkyls have unique and complex physical and chemical properties (Kissa 2001; Schultz et al. 2003). Sources are available that provide helpful insights into the structural aspects and surfactant nature of these substances; however, many of the properties are still not well understood (CEMN 2008; Kissa 2001; Schultz et al. 2003). In general, specific properties such as physical state, melting point, boiling point, density, solubility, vapor pressure, micelle formation, and acid dissociation in water have not been determined or are not well described for these compounds. Measurements of these endpoints are needed. Information regarding the potential association of these species in water would be useful. Where determination of a particular endpoint is not possible, a thorough description of the physical and chemical properties as they relate to that endpoint would be helpful. Perfluoroalkyls discussed in this profile exist as a mixture of linear and branched isomers. Isomer-specific data would also be useful for the various physical-chemical properties. Wang et al. (2013b) identified several of the fluorinated compounds that are currently being used by major manufacturers as alternatives to PFOA and PFOS. These compounds are being used as processing aids in the emulsion polymerization of PTFE and other polymers as well as surface treatment uses, metal plating uses, firefighting foams, and other miscellaneous uses such as food contact materials. A data need exists to determine the physical and chemical properties of these replacement substances.

The production, use, import, and export of perfluoroalkyls have changed dramatically since 2000. Most nations no longer produce or use PFOS or PFOA (China is a notable exception). Major fluoropolymer manufacturers in the United States have altered their chemical processes to use alternative fluorinated substances in their production processes. Information regarding the production, import, and export volumes of these substances is needed.

Recommended methods for the disposal of perfluoroalkyls have not been located. In the past, perfluoroalkyl-containing waste has been disposed of in on- and off-site landfills, through sludge incorporation, and through incineration (3M 2007b, 2008b; ATSDR 2005). New disposal methods that avoid release of these substances into the open environment and prevent contamination of nearby soil, sediment, and groundwater should be developed. The eventual breakdown of fluorotelomer-based polymers with the eventual release of substances such as PFOA is not well understood. Early researchers have concluded that the half-life for this process is >1,000 years; however, more recent data suggest much shorter time scale of 1–2 decades (Rankin et al. 2014; Washington and Jenkins 2015; Washington et al.

2009, 2014, 2015). Additional studies on the potential release of perfluoroalkyls from the eventual degradation of fluoropolymers in landfills would be useful.

Environmental Fate. Perfluoroalkyls are very stable compounds and are resistant to biodegradation, direct photolysis, atmospheric photooxidation, and hydrolysis (3M 2000; EPA 2008a; OECD 2002, 2007; Schultz et al. 2003). The chemical stability of perfluoroalkyls and the low volatility of these substances in ionic form indicate that perfluoroalkyls will be persistent in water and soil (3M 2000; Prevedouros et al. 2006). K_{oc} values ranging from 17 to 230 indicate that PFOA will be mobile in soil and can leach into groundwater (Davis et al. 2007; Prevedouros et al. 2006). Environmental fate and potential pathways of PFOA exposure at and near the DuPont Washington Works site have been discussed (Small 2009). Wang et al. (2013b) identified several of the fluorinated compounds that are currently being used by major manufacturers as alternatives to PFOA and PFOS. Environmental fate and toxicity research of newer replacement substances is ongoing (De Silva et al. 2016; Gomis et al. 2018; Kabore et al. 2018).

Bioavailability from Environmental Media. Perfluoroalkyls are widely detected in humans and animals, indicating that several of these substances are bioavailable. The bioaccumulation potential of perfluoroalkyls is reported to increase with increasing chain length (de Vos et al. 2008; Furdui et al. 2007; Martin et al. 2004b). In living organisms, perfluoroalkyls bind to protein albumin in blood, liver, and eggs and do not accumulate in fat tissue (de Vos et al. 2008; Kissa 2001). The mechanism of perfluoroalkyl uptake in animals is not fully understood; additional studies would be helpful (de Vos et al. 2008). Perfluoroalkyls discussed in this profile exist as a mixture of linear and branched isomers. Data regarding the bioavailability of branched versus linear substances would be useful. A data need exists to determine the bioavailability of the replacement substances identified in Wang et al. (2013b) used in place of PFOA and PFOS.

Food Chain Bioaccumulation. High levels of certain perfluoroalkyls in animals have been measured in apex predators, such as polar bears, which indicates that some perfluoroalkyls possess the ability to bioaccumulate (de Vos et al. 2008; Houde et al. 2006a; Kannan et al. 2005; Smithwick et al. 2005a, 2005b, 2006). Perfluoroalkyl sulfonates with carbon chain length lower than 8 tend to bioaccumulate less than PFOS. Ongoing monitoring of perfluoroalkyl levels in animals may help to determine whether efforts to phase out these substances will have had an effect on their biomagnification. A data need exists to determine the bioaccumulation potential of the new replacement substances used in place of PFOA and PFOS.

Exposure Levels in Environmental Media. Reliable monitoring data for the levels of perfluoroalkyls in contaminated media at hazardous waste sites are needed so that the information obtained on levels of perfluoroalkyls in the environment can be used in combination with the known body burden of perfluoroalkyls to assess the potential risk of adverse health effects in populations living in the vicinity of hazardous waste sites.

Concentrations of perfluoroalkyls have been measured in surface water from several locations across the United States (Boulanger et al. 2004; Kannan et al. 2005; Kim and Kannan 2007; Nakayama et al. 2007; Simcik and Dorweiler 2005; Sinclair et al. 2004, 2006). Continued monitoring for perfluoroalkyls in surface water would be useful. Data are available regarding levels of perfluoroalkyls in outdoor air, indoor air, indoor dust, food, food packaging, and consumer products (3M 2001; Barber et al. 2007; Begley et al. 2005; Food Standards Agency 2006; Fromme et al. 2007b; Harada et al. 2005b, 2006; Jogsten et al. 2009; Kim and Kannan 2007; Kubwabo et al. 2005; Moriwaki et al. 2003; Tittlemier et al. 2007; Washburn et al. 2005). Comprehensive studies monitoring for perfluoroalkyls in these matrices within the United States are needed. Elevated concentrations of perfluoroalkyls have been measured in air, water, soil, and sediment near fluorochemical industrial facilities (3M 2007b, 2008b, 2008c; Barton et al. 2006; Davis et al. 2007; Hansen et al. 2002). Additional research is needed to evaluate how soil physical and chemical properties influence the bioavailability of perfluoroalkyls. Continued monitoring for perfluoroalkyls in these matrices are needed to assess exposure of individuals working at these locations and individuals who live near these facilities. A data need also exists to perform environmental monitoring of the replacement substances identified in Wang et al. (2013b) used in place of PFOA and PFOS, particularly near manufacturing locations.

Exposure Levels in Humans. Trudel et al. (2008) provided a thorough assessment of the exposure of the general population to PFOS and PFOA. 3M (2008b) provided an assessment of exposure of individuals to PFOA on-site at a fluoropolymer facility. Uptake values and exposure pathways determined in these studies should be examined further. Conclusions made in these assessments are expected to be adjusted as future monitoring data are made available. Large-scale monitoring of perfluoroalkyls in human serum in the United States is ongoing (Calafat et al. 2006a). Future results of human monitoring studies would be useful for assessing human exposure to these substances over time. The results of these studies can be examined for correlations between human perfluoroalkyl levels and the phasing out of perfluoroalkyls by companies of the fluorochemical industry. Levels of perfluoroalkyls in human urine have been reported (Jurado-Sanchez et al. 2014). Higher exposure levels for individuals who reside in areas where substances such as PFOA contaminated both public and private water supplies

have been documented (Emmett et al. 2006a, 2009). Continued biomonitoring of legacy compounds such as PFOA and PFOS as well as other perfluoroalkyls is needed.

This information is necessary for assessing the need to conduct health studies on these populations.

Exposures of Children. Trudel et al. (2008) provided a thorough assessment of the exposure of children to PFOS and PFOA. These conclusions should be reexamined with respect to future biomonitoring data when they become available. Data are available regarding the levels of perfluoroalkyls in young children (Kato et al. 2009b; Olsen et al. 2004b; Toms et al. 2009). NHANES monitoring data for 2013–2014 for children of ages 3–11 years have recently been released (CDC 2018; Ye et al. 2018a). Data provided from these efforts will be useful in assessing the exposure of young children to perfluoroalkyls.

Concentrations of perfluoroalkyls have been measured in human breast milk and cord blood (Apelberg et al. 2007a, 2007b; Fei et al. 2007; Inoue et al. 2004; Kärrman et al. 2007; Midasch et al. 2007; So et al. 2006b; Völkel et al. 2008). Additional monitoring for perfluoroalkyls in these media would be useful. Continued biomonitoring of legacy compounds such as PFOA and PFOS as well as replacement substances is needed.

6.3 Ongoing Studies

A number of federal agencies are sponsoring ongoing studies; a list of these studies are available at https://www.atsdr.cdc.gov/pfas/PFAS-health-effects.html.

CHAPTER 7. REGULATIONS AND GUIDELINES

Pertinent international and national regulations, advisories, and guidelines regarding perfluoroalkyls in air, water, and other media are summarized in Table 7-1. This table is not an exhaustive list, and current regulations should be verified by the appropriate regulatory agency. A list of some select state drinking water regulations/guidelines or health-based values are summarized in Table 7-2.

ATSDR develops MRLs, which are substance-specific guidelines intended to serve as screening levels by ATSDR health assessors and other responders to identify contaminants and potential health effects that may be of concern at hazardous waste sites. See Section 1.3 and Appendix A for detailed information on the MRLs for perfluoroalkyls.

Table 7-1. Regulations and Guidelines Applicable to Perfluoroalkyls

Agency	Description	Information	Reference
	Air		
EPA	RfC	No data	IRIS 2018
WHO	Air quality guidelines	No data	WHO 2010
	Water & Food		
EPA	Drinking water standards and health advisories		EPA 2018
	DWEL		
	PFOA	0.00037 mg/L	EPA 2016e
	PFOS	0.00037 mg/L	EPA 2016f
	Lifetime Health Advisory		
	PFOA	0.07 µg/L	EPA 2016e
	PFOS	0.07 µg/L	EPA 2016f
	National primary drinking water regulations	No data	EPA 2009d
	RfD	No data	IRIS 2018
	PFOA	$2x10^{-5}$ mg/kg/day	EPA 2016e
	PFOS	$2x10^{-5}$ mg/kg/day	EPA 2016f
WHO	Drinking water quality guidelines	No data	WHO 2017
FDA	Substances added to food	No data[a]	FDA 2018
	Cancer		
ACGIH	Carcinogenicity classification		
	APFO	A3[b]	ACGIH 2001
HHS	Carcinogenicity classification	No data	NTP 2016a
EPA	Carcinogenicity classification	No data	IRIS 2018
	PFOA	Suggestive evidence for carcinogenic potential	EPA 2016e

Table 7-1. Regulations and Guidelines Applicable to Perfluoroalkyls

Agency	Description	Information	Reference
	PFOS	Suggestive evidence for carcinogenic potential	EPA 2016f
	10^{-6} Cancer risk		
	PFOA	0.5 µg/L	EPA 2016e
IARC	Carcinogenicity classification		IARC 2017
	PFOA	Group 2B[c]	
	Occupational		
ACGIH	TLV-TWA		
	APFO	0.01 mg/m^3 [d]	ACGIH 2001
OSHA	PEL (8-hour TWA) for general industry, shipyards and construction	No data	OSHA 2018b 29 CFR 1910.1000, Table Z-1
	PEL (8-hour TWA) for shipyards and construction	No data	OSHA 2018a 29 CFR 1915.1000, Table Z
	PEL (8-hour TWA) for construction	No data	OSHA 2018c 29 CFR 1926.55, Appendix A
NIOSH	REL (up to 10-hour TWA)	No data	NIOSH 2016
	Emergency Criteria		
EPA	AEGLs-air	No data	EPA 2016b
DOE	PACs-air		DOE 2018b
	PFOA		
	PAC-1[c]	1.1 mg/m^3	
	PAC-2[c]	12 mg/m^3	
	PAC-3[c]	75 mg/m^3	
	PFBA		
	PAC-1[c]	0.5 mg/m^3	
	PAC-2[c]	5.5 mg/m^3	
	PAC-3[c]	33 mg/m^3	

[a]The Substances Added to Food inventory replaces EAFUS and contains the following types of ingredients: food and color additives listed in FDA regulations, flavoring substances evaluated by FEMA or JECFA, GRAS substances listed in FDA regulations, substances approved for specific uses in food prior to September 6, 1958, substances that are listed in FDA regulations as prohibited in food, delisted color additives, and some substances "no longer FEMA GRAS".
[b]A3: confirmed animal carcinogen with unknown relevance to humans.
[c]Group 2B: possibly carcinogenic to humans.
[d]Skin notation.
[e]Definitions of PAC terminology are available from DOE (2018a).

ACGIH = American Conference of Governmental Industrial Hygienists; AEGL = acute exposure guideline level; AIHA = American Industrial Hygiene Association; APFO = ammonium perfluorooctanoate; CFR = Code of Federal Regulations; DOE = Department of Energy; DWEL = Drinking Water Equivalent Level; EAFUS = Everything Added to Food in the United States; EPA = Environmental Protection Agency; FAO = Food and Agriculture Organization of the United Nations; FDA = Food and Drug Administration; FEMA = Federal Emergency Management Agency; GRAS = generally recognized as safe; HHS = Department of Health and Human Services; IARC = International Agency for Research on Cancer; IRIS = Integrated Risk Information System; JEFCA = Joint FAO/WHO Expert Committee on Food Additives; NIOSH = National Institute for Occupational Safety and Health; NTP = National

Table 7-1. Regulations and Guidelines Applicable to Perfluoroalkyls

Agency	Description	Information	Reference

Toxicology Program; OSHA = Occupational Safety and Health Administration; PAC = Protective Action Criteria; PEL = permissible exposure limit; PFBA = perfluorobutanoic acid; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid; REL = recommended exposure limit; RfC = inhalation reference concentration; RfD = oral reference dose; TLV = threshold limit values; TWA = time-weighted average; WHO = World Health Organization

Table 7-2. Select State Drinking Water Guidelines for Perfluoroalkyls[a]

	Value (ppb or µg/L)						
Value type	PFOA	PFOS	PFBS	PFBA	PFNA	PFHxS	Reference
Connecticut							
Drinking water action level	0.07[b]	0.07[b]	ND	ND	0.07[b]	0.07[b]	Connecticut DPH 2016
Maine							
Maximum exposure guideline for drinking water	0.07[c]	0.07[c]	ND	ND	ND	ND	MECDC 2016
Massachusetts							
Drinking water guidelines	0.07[b]	0.07b[a]	ND	ND	0.07[b]	0.07[b]	MassDEP 2018
Michigan							
Residential and nonresidential drinking water criteria	0.07[d]	0.07[d]	ND	ND	ND	ND	Michigan DEQ 2018a
Human noncancer drinking water value	0.42	0.011	ND	ND	ND	ND	Michigan DEQ 2016
Minnesota							
Health risk limit[e]							MDH 2019
Short-term	0.035	ND	ND	7	ND	ND	
Subchronic	0.035	ND	9	7	ND	ND	
Chronic	0.035	0.3	7	7	ND	ND	
Health-based value[e]							
Short-term	ND	0.015	3	ND	ND	0.047	
Subchronic	ND	0.015	3	ND	ND	0.047	
Chronic	ND	0.015	2	ND	ND	0.047	
Nevada							
Basic comparison level	0.667	0.667	667	ND	ND	ND	NDEP 2017
New Jersey							
Health-based chronic maximum contaminant level	0.014 (recommend-ation)	0.013 (recommend-ation)	ND	ND	0.013	ND	DWQI 2018a, 2017, 2018b, 2015

Table 7-2. Select State Drinking Water Guidelines for Perfluoroalkyls[a]

	Value (ppb or µg/L)						
Value type	PFOA	PFOS	PFBS	PFBA	PFNA	PFHxS	Reference
North Carolina							
Interim maximum allowable concentration in groundwater	2	ND	ND	ND	ND	ND	NC DEQ 2013, NCDENR 2012
Vermont							
Drinking water health advisory	0.02[b]	0.02[b]	ND	ND	0.02[b]	0.02[b]	Vermont DOH 2018

[a]Current as of September 2018.
[b]Value applies to the sum of PFOA, PFOS, PFHxS, PFHpA, and PFNA concentrations.
[c]MECDC notes that according to the EPA lifetime health advisory for PFOA and PFOS, when both PFOS and PFOA are present in drinking water, the combined levels are not to exceed 0.07 ppb.
[d]Value applies to the sum of PFOA and PFOS groundwater concentrations (Michigan DEQ 2018b).
[e]Health risk limits are rule values and health-based values are guidance values. Guidance is developed in-between rulemaking and may update an older rule. As a result, both rules and guidance values may be available for a contaminant (dual guidance) and the two values may be different (MDH 2014).

DEQ = Department of Environmental Quality; DOH = Department of Health; DPH = Department of Public Health; DWQI = Drinking Water Quality Institute; MassDEP = Massachusetts Department of Environmental Protection; MECDC = Maine Center for Disease Control and Prevention; MDH = Minnesota Department of Health; NCDENR = North Carolina Department of Environment and Natural Resources; ND = no data; NDEP = Nevada Division of Environmental Protection; PFBA = perfluorobutanoic acid; PFBS = perfluorobutane sulfonic acid; PFHpA = perfluoroheptanoic acid; PFHxS = perfluorohexane sulfonic acid; PFNA = perfluorononanoic acid; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid

CHAPTER 8. REFERENCES

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APPENDIX A. ATSDR MINIMAL RISK LEVEL WORKSHEETS

MRLs are derived when reliable and sufficient data exist to identify the target organ(s) of effect or the most sensitive health effect(s) for a specific duration for a given route of exposure. An MRL is an estimate of the daily human exposure to a hazardous substance that is likely to be without appreciable risk of adverse noncancer health effects over a specified route and duration of exposure. MRLs are based on noncancer health effects only; cancer effects are not considered. These substance-specific estimates, which are intended to serve as screening levels, are used by ATSDR health assessors to identify contaminants and potential health effects that may be of concern at hazardous waste sites. It is important to note that MRLs are not intended to define clean-up or action levels.

ATSDR uses the POD/uncertainty factor approach to derive MRLs. Potential PODs are NOAELs, LOAELs, or the BMDL. They are below levels that might cause adverse health effects in the people most sensitive to such chemical-induced effects. MRLs are derived for acute (1–14 days), intermediate (15–364 days), and chronic (≥365 days) durations and for the oral and inhalation routes of exposure. Currently, MRLs for the dermal route of exposure are not derived because ATSDR has not yet identified a method suitable for this route of exposure. MRLs are generally based on the most sensitive substance-induced endpoint considered to be of relevance to humans. Serious health effects (such as irreparable damage to the liver or kidneys, or birth defects) are not used as a basis for establishing MRLs. Exposure to a level above the MRL does not mean that adverse health effects will occur.

MRLs are intended only to serve as a screening tool to help public health professionals decide where to look more closely. They may also be viewed as a mechanism to identify those hazardous waste sites that are not expected to cause adverse health effects. Most MRLs contain a degree of uncertainty because of the lack of precise toxicological information on the people who might be most sensitive (e.g., infants, elderly, nutritionally or immunologically compromised) to the effects of hazardous substances. ATSDR uses a conservative (i.e., protective) approach to address this uncertainty consistent with the public health principle of prevention. Although human data are preferred, MRLs often must be based on animal studies because relevant human studies are lacking. In the absence of evidence to the contrary, ATSDR assumes that humans are more sensitive to the effects of hazardous substance than animals and that certain persons may be particularly sensitive. Thus, the resulting MRL may be as much as 100-fold below levels that have been shown to be nontoxic in laboratory animals. ATSDR utilizes uncertainty factors to account for uncertainties associated with extrapolating from: (1) a LOAEL to a NOAEL; (2) extrapolating from animals to humans; and (3) to account for human variability (Chou et al. 1998; Pohl and Abadin 1995).

Default values of 10 are used for each of these categories of uncertainty factors; a value of 1 can be used if complete certainty exists for a particular uncertainty factor category. A partial uncertainty factor of 3 can be used when chemical-specific data decreases the uncertainty. On a case-by-case basis, ATSDR also utilizes modifying factors to account for MRL-specific database deficiencies.

Proposed MRLs undergo a rigorous review process: Health Effects/MRL Workgroup reviews within the Division of Toxicology and Human Health Sciences, expert panel peer reviews, and agency-wide MRL Workgroup reviews, with participation from other federal agencies and comments from the public. They are subject to change as new information becomes available concomitant with updating the toxicological profiles. Thus, MRLs in the most recent toxicological profiles supersede previously published MRLs. For additional information regarding MRLs, please contact the Division of Toxicology and Human Health Sciences, Agency for Toxic Substances and Disease Registry, 1600 Clifton Road NE, Mailstop S102-1, Atlanta, Georgia 30329-4027.

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INTRODUCTION

Overview of Epidemiological Studies

A large number of epidemiological studies have evaluated a wide range of potential health outcomes resulting from exposure to perfluoroalkyls, particularly PFOA and PFOS. The epidemiological studies fall into three broad categories: occupational exposure primarily to airborne PFOA and PFOS, exposure to PFOA-contaminated drinking water by residents living near a PFOA production facility, and general population exposure to background levels of perfluoroalkyls. Most of the occupational exposure studies were conducted in workers at four facilities in Minnesota, Alabama, West Virginia, and the Netherlands. Studies of the highly-exposed residents primarily come from several large-scale studies (C8 Health Project, C8 Health Study) of Mid-Ohio Valley residents living near the Washington Works facility in West Virginia who were exposed to high levels of PFOA in the drinking water. General population studies primarily utilized data collected in NHANES in the United States and several large-scale health studies conducted in Europe.

Most of the epidemiological studies lack environmental monitoring data and there is a potential for multiple sources of exposure (inhalation and oral). However, the majority of the epidemiological studies used serum perfluoroalkyl levels as a biomarker of exposure. One limitation of the C8 Health Studies is that they used blood samples collected in 2005–2006. However, the facility started using PFOA in the 1950s and peak usage was in the 1990s and by 2003, there was an 87% decline in PFOA emissions, as compared to 1999 levels (Emmett et al. 2006a). Therefore, serum PFOA levels measured in 2005–2006 likely do not represent earlier higher exposures, which may have contributed to observed health outcomes. As an alternative to using older serum PFOA levels, several C8 Health Studies estimated serum levels based on data on the release of PFOA from the facility and pharmacokinetic modeling. Of the three categories of subjects examined in the epidemiological studies, workers have the highest potential exposure to perfluoroalkyls, followed by the highly-exposed residents in the Mid-Ohio Valley (referred to as community exposure), and then the general population. In one study of workers at the Washington Works facility in West Virginia, the average serum PFOA level in 2001–2004 was 1,000 ng/mL (Sakr et al. 2007a); the mean PFOA level in community residents (without occupational exposure) near this facility was 423 ng/mL in 2004–2005 (Emmett et al. 2006a). By comparison, the geometric mean concentration of PFOA in the U.S. population was 3.92 ng/mL in 2005–2006 (CDC 2013).

Identification of Adverse Health Effects Based on Epidemiological Studies. Although a large number of epidemiological studies have examined the potential of perfluoroalkyls to induce adverse health effects, most of the studies were cross-sectional in design and do not establish causality. Epidemiological studies have found statistically significant associations between serum perfluoroalkyl levels and several health effects, although the results were not consistent across studies. Many of the studies reported dose-related trends, but these trends were not as apparent when comparing across studies; some effects were observed in populations with background PFOA levels but not in populations with high serum PFOA levels. Given the inconsistencies, ATSDR evaluated whether the preponderance of the data supported an association between perfluoroalkyl exposure and a particular health effect, taking into consideration the consistency of the findings across studies, the quality of the studies, dose-response, and plausibility. It should be noted that although the data may provide strong evidence for an association, it does not imply that the observed effect is biologically relevant because the magnitude of the change is within the normal limits or not indicative of an adverse health outcome. Plausibility depends primarily on experimental toxicology studies that establish a biological mechanism for the observed effects.

Using this approach, the available epidemiological data identify several potential health hazards of PFOA, PFOS, PFHxS, PFNA, and PFDA in humans as listed below.

PFOA

- Pregnancy-induced hypertension/pre-eclampsia
- Increases in serum hepatic enzymes, particularly alanine aminotransferase, and decreases in serum bilirubin levels
- Increases in serum lipids, particularly total cholesterol and LDL cholesterol
- Decreased antibody response to vaccines
- Small (<20 g or 0.7 ounces per 1 ng/mL increase in blood perfluoroalkyl level) decreases in birth weight

PFOS

- Pregnancy-induced hypertension/pre-eclampsia
- Liver damage, as evidenced by increases in serum enzymes and decreases in serum bilirubin levels
- Increases in serum lipids, particularly total cholesterol and LDL cholesterol
- Decreased antibody response to vaccines
- Small (<20 g or 0.7 ounces per 1 ng/mL increase in blood perfluoroalkyl level) decreases in birth weight

PFHxS

- Liver damage, as evidenced by increases in serum enzymes and decreases in serum bilirubin levels
- Decreased antibody response to vaccines

PFNA

- Increases in serum lipids, particularly total cholesterol and LDL cholesterol
- Decreased antibody response to vaccines (based on limited evidence)

PFDA

- Increases in serum lipids, particularly total cholesterol and LDL cholesterol
- Decreased antibody response to vaccines

Limitations of Epidemiological Data. There are sufficient epidemiological data to identify possible sensitive targets for many of the perfluoroalkyls; however, there are two major limitations to establishing dose-response relationships for these effects and using the epidemiological studies to derive MRLs: accurate identification of environmental exposure levels producing increased risk for adverse effects (exposure estimates and routes of exposure) and likely co-exposure to mixtures of perfluoroalkyls. Other limitations include the cross-sectional design of the majority of epidemiological studies and the potential that reverse causality contributes to the observed associations.

Uncertainty in Exposure Estimates. In general, epidemiological studies provide a one-time serum perfluoroalkyl concentration, but lack information on actual environmental exposure concentration or doses, routes of exposure, and exposure duration. Although serum perfluoroalkyl levels provide reliable information on recent exposure (weeks to years, depending on the elimination $t_{1/2}$ for the perfluoroalkyl), they likely do not reflect historical exposure levels or exposure levels at the onset of the effect. This is especially true for occupational exposure cohorts where past exposure levels were higher before industrial hygiene improved and in the C8 community studies since peak PFOA levels in drinking water occurred at least 10 years prior to the onset of the studies. Additionally, data from NHANES suggest that some

perfluoroalkyl (PFOA, PFOS, PFHxS, and PFDA) levels are declining in the general population; for example, the geometric mean serum levels of PFOA and PFOS declined from 5.2 and 30.4 ng/mL, respectively, in 1999–2000 to 1.56 and 4.72 ng/mL in 2015–2016. In contrast, levels of PFNA have increased during that time frame; the geometric mean went from 0.5 ng/mL in 1999–2000 to 1.26 ng/mL in 2009–2010 and then decreased to 0.675 ng/mL in 2015–2016. Most studies do not provide adequate information to determine whether perfluoroalkyl levels reflect a steady state and relatively constant exposure, since most designs only include a single measurement. An added uncertainty occurs in studies that used maternal serum levels as the biomarker of exposure for effects in children or for effects on fertility.

It is assumed that workers were primarily exposed via inhalation; however, oral exposure may have also contributed to the total perfluoroalkyl body burden, particularly since workers frequently lived in communities with elevated levels of PFOA in the drinking water. It has been determined that drinking water was the primary source of perfluoroalkyls in residents living near a PFOA facility (Emmett et al. 2006a); however, it is likely that airborne PFOA contributed to overall body burden. Drinking water is the likely primary route of exposure for the general population.

Uncertainty due to Co-Exposure to Other Perfluoroalkyls. Based on NHANES data, the U.S. general population is exposed to a variety of perfluoroalkyls. A number of studies reported a high degree of correlation between different perfluoroalkyls; however, most studies did not control for exposure to other perfluoroalkyls. Given that many of the perfluoroalkyls have similar targets of toxicity and possible mechanisms of action, it is likely that several perfluoroalkyls contributed to the observed effects. The potential interactions between different perfluoroalkyls have not been fully elucidated.

In summary, the epidemiological databases for several perfluoroalkyls provide valuable information on hazard identification; however, uncertainties regarding doses associated with adverse effects and possible interactions between compounds preclude use of these data to derive MRLs.

Overview of Laboratory Animal Studies

Laboratory animal studies are available for 11 perfluoroalkyls (no data were located for PFHpA); however, more than 70% of the studies examined PFOA and/or PFOS. The laboratory animal studies primarily involved oral exposure and examined a wide range of potential health outcomes. The primary health effects observed in laboratory animals were liver toxicity, developmental toxicity, and immune toxicity. Other effects typically observed at higher doses included weight loss, histological alterations in reproductive tissues, and histological alterations in the thyroid gland. The sensitive targets of toxicity identified in the laboratory animals are similar to those observed in epidemiological studies.

Limitations of Laboratory Animal Studies for Derivation of MRLs. Use of controlled animal studies eliminates the uncertainties regarding effective doses and co-exposure to other perfluoroalkyls. However, there are uncertainties associated with derivation of MRLs based on animal studies, in part, because of large interspecies differences in the toxicokinetics of perfluoroalkyls for which mechanisms are not completely understood. Available information on the toxicokinetics of perfluoroalkyls in humans, nonhuman primates, and various rodent species indicate that elimination rates (and very likely elimination mechanisms and hormonal regulation of these mechanisms) vary substantially across chemical species (i.e., carbon chain length) and animal species (i.e., slower in humans compared to nonhuman primates and rodents), and show pronounced sex differences within certain species (e.g., faster elimination in female rats). As a result, there is some uncertainty associated with extrapolation of external dose-response relationships from animals to humans. Several PBPK models of PFOA and PFOS have been reported that simulate the substantial differences in pharmacokinetics of these compounds between humans and nonhuman primates or between humans and rats. These include human models for PFOA and PFOS (see

Section 3.1.5). An additional uncertainty in the animal data is the relevance of effects associated with activation of PPARα. Many of the effects observed in rodents, particularly liver and developmental effects, involve the activation of PPARα; humans and nonhuman primates are less responsive to PPARα agonists than rats and mice. However, studies in PPARα-null mice suggest that PPARα-independent mechanisms also play a role in the liver, immunological, and developmental toxicity.

MRL Approach

The following approach was used for derivation of MRLs:

- Identify sensitive endpoints from epidemiological studies
- Identify laboratory animal studies that have evaluated dose-response relationships for toxicity targets identified in epidemiological studies
- Estimate a POD using animal serum perfluoroalkyl levels for sensitive endpoints
- Calculate HEDs using the assumption that a serum concentration resulting in an effect in a laboratory animal would also result in an effect in humans. An empirical pharmacokinetic model was used to estimate a human dose associated with this serum concentration for PFOA and PFOS. Measured serum concentrations in laboratory animal studies were used to calculate the HEDs for PFHxS and PFNA.
- Apply appropriate uncertainty factors informed by comparison of the POD to serum perfluoroalkyl levels reported in epidemiological studies

Rationale for Internal Dose Metric Used in Dosimetry Extrapolation. The time-weighted average serum concentration (C_{TWA}) was selected as the internal dose metric for dose-response modeling and dosimetry extrapolation. The C_{TWA} was used rather than the maximum concentration (C_{max}) for the following reasons:

- C_{TWA} provides a better representation of the history of exposure in the principal studies selected for the MRLs for PFOA (Koskela et al. 2016) and PFOS (Luebker et al. 2005a). The relatively slow elimination of PFOA and PFOS predicted in mice and rats results in a build-up of serum concentrations during the exposure duration. As a result, the C_{max} is predicted to occur soon after the last dose in these studies (based on the Wambaugh et al. 2013 model).

- The assumption that must be accepted to justify using the C_{max} is that only the last dose of PFOA or PFOS, which results in the C_{max}, determines the toxicity outcome, and the earlier exposure history contributes only by building up the levels to the C_{max}.

- The available data on the toxicity of PFOA and PFOS do not provide convincing evidence that toxicity outcomes are more likely to be determined by C_{max} rather than the exposure history, represented by C_{TWA}.

- Given that C_{max} is predicted to exceed C_{TWA} in the principal studies (based on the Wambaugh et al. 2013 model), the resulting HED that achieves a steady-state serum concentration equal to the C_{max} would be larger than the corresponding HED based on C_{TWA}. In the absence of strong evidence for C_{max} being a more appropriate dose metric than C_{TWA}, use of C_{TWA} for dosimetry extrapolation is an appropriate health-protective assumption in the derivation of the MRL.

Predicting Mean Serum PFOA and PFOS Concentrations in Laboratory Animals. TWA serum concentrations corresponding to external doses (mg/kg/day) and exposure durations (days) were predicted with a pharmacokinetic (PK) model for the animal species, strain, and sex used in the studies (Wambaugh et al. 2013). The Wambaugh et al. (2013) model was selected over other available

pharmacokinetic models for the following reasons: (1) it provided a single model structure (parameters) for simulating kinetics of PFOA and PFOS; (2) Wambaugh et al. (2013) derived parameter values for sex-specific species and strains used in the candidate principal studies for the oral MRLs (female and male CD1 mouse, C57BL/6 mouse, Sprague-Dawley rat, cynomolgus monkey); and (3) models were calibrated using Bayesian parameter estimation based on multiple PFOA (n=6) and PFOS (n=2) pharmacokinetics studies for specific species, strains, and sexes; and were then evaluated by comparing predicted and observed serum concentrations from toxicology studies performed on the same species, strains, and sex. Predicted and observed terminal serum concentrations for PFOA and PFOS agreed within a factor of 2, showed strong linear correlation, and distributed symmetrically along the line of identity, suggesting minimal bias in predictions across species, strains, and sexes.

The TWA serum concentration was calculated as follows (Equation A-1):

$$C_{TWA} = \frac{C_{AUC}}{ED} \quad \text{Eq. (A-1)}$$

where C_{TWA} is the predicted TWA serum concentration (mg/L), C_{AUC} is the predicted area under the curve (AUC) of the serum concentration-time profile for the exposure (mg hour/L), and *ED* is the exposure duration (hour). Gavage studies were simulated as a single dose (e.g., gavage) given once every 24 hours. Daily drinking water exposures were simulated as a 12-hour period of dosing followed by 12 hours with no dosing. This assumes that the animals consumed water during a 12-hour active period and received the total daily dose during this 12-hour period. The dosing interval was 0.1 hour.

The Wambaugh et al. (2013) model was originally implemented in R (v2.10.0) and was migrated to MATLAB (vR2016) for calculations of MRLs. Wambaugh et al. (2013) reported mean and confidence limits for parameter values estimated from a Bayesian Markov Chain Monte Carlo (MCMC) analysis. The posterior means were used as point estimates for parameters in the MATLAB version. Function of the point estimate implementation in MATLAB was verified by comparing predictions of C_{AUC} obtained from the MATLAB version with predictions from the MCMC analysis reported in EPA (2016e, 2016f). This comparison for PFOA included a total of 18 predictions of C_{AUC} for female CD-1 mice (Lau et al. 2006; Wolf et al. 2007), female C57Bl6 mice (DeWitt et al. 2008), and male Sprague-Dawley rats (Butenhoff et al. 2004b). The r^2 for MATLAB vs R predictions of C_{AUC} was 0.99 and the average relative percent difference (MATLAB-R) was 2.8% (range: -6.6–13.5). The comparison for PFOS included a total of 28 predictions of C_{AUC} for female CD-1 mice (Lau et al. 2003), female Sprague-Dawley rats (Butenhoff et al. 2009b; Lau et al. 2003; Luebker et al. 2005a, 2005b), and male and female Cynomolgus monkeys (Seacat et al. 2002). The r^2 for MATLAB vs R predictions of C_{AUC} was 1.00 and the average relative percent difference (MATLAB-R) was 4.6% (range: -11–20).

Estimating TWA Serum PFHxS and PFNA Concentrations in Laboratory Animals. Because a PK model for predicting the TWA serum concentrations was not identified for PFHxS and PFNA, a TWA serum concentration was estimated from measured serum concentrations. ATSDR estimated the TWA values from the areas under the curve calculated using the trapezoid rule. Since most studies did not report pre-exposure levels, serum concentrations in the control group were used as the baseline concentration.

Estimating HEDs for Perfluoroalkyls. The serum concentration PODs identified from the laboratory animal data were converted to an equivalent dose in humans, which is defined as the continuous ingestion dose (mg/kg/day) that would result in steady-state serum concentrations of perfluoroalkyl equal to the serum concentration (µg/mL) selected as the POD. Although human PBPK models for PFOA and PFOS have been reported, the simpler empirical model was selected for deriving

HEDs for the following reasons. Human PBPK models have not been validated with observations made in humans for which individual exposures were known with sufficient certainty to evaluate confidence in predicting dose-serum concentration relationships. Calibration and validation of human PBPK models have relied on comparing predicted and observed declines in serum concentrations following declines or cessation of exposure (Fàbrega et al. 2014, 2016; Loccisano et al. 2011; Worley et al. 2017b). This approach validates the ability of the models to predict serum concentration half-lives, which can be estimated directly from the observation data and represented in the empirical model. However, it does not validate the ability of the models to predict serum concentration in association with known exposures. Worley et al. (2017b) reported good agreement between the distribution of observed and predicted serum concentrations of PFOA within a study population, when assumptions about variability in exposure and biokinetics were incorporated into the simulations.

The relationship between perfluoroalkyl external dosage (mg/kg/day) and steady-state serum concentration (C_{ss}, mg/L) in humans was estimated assuming a single-compartment first-order model in which elimination kinetics are adequately represented by observed serum elimination $t_{1/2}$ values for the specific perfluoroalkyl, an assumed apparent volume of distribution (V_d, L/kg) and gastrointestinal absorption fraction. In the first-order single-compartment model, continuous exposure will result in a steady-state body burden (BB_{SS}, mg/kg) for PFOA or PFOS, which will be distributed in a single volume of distribution to yield a steady-state serum concentration (Equation A-2):

$$C_{SS} = \frac{BB_{SS}}{V_d} \quad \text{Eq. (A-2)}$$

At steady state, the rate of first-order elimination rate (a constant fraction of the body burden, k_e per day) will equal the absorbed dosage (D_{ss}, mg/kg/day) adjusted for gastrointestinal absorption (AF) (Equation A-3):

$$D_{SS} \cdot AF = BB_{SS} \cdot k_e \quad \text{Eq. (A-3)}$$

Rearrangement of Equation A-3 allows calculation of the steady-state body burden corresponding to a given external dosage (Equation A-4):

$$BB_{SS} = \frac{D_{SS} \cdot AF}{k_e} \quad \text{Eq. (A-4)}$$

The relationship between the elimination rate constant (k_e, day^{-1}) and the elimination half-life ($t_{1/2}$, day), is given in Equation A-5:

$$k_e = \frac{\ln(2)}{t_{1/2}} \quad \text{Eq. (A-5)}$$

Combining Equations A-2 and A-3 yields an expression relating the external steady-state dosage and steady-state serum concentration (Equation A-6):

$$D_{SS} = \frac{C_{SS} \cdot k_e \cdot V_d}{AF} \quad \text{Eq. (A-6)}$$

The above estimates of C_{SS}/D_{SS} are sensitive to the input parameters, $t_{1/2}$, AF, and V_d. The empirical model used to calculate HEDs is linear; therefore, the change in the HED is approximately proportional to the change in the half-life. A halving of the half-life would result in a doubling of the HED.

PFOA and PFOS. Several studies have estimated PFOA and PFOS half-lives ($t_{1/2}$) in workers (Costa et al. 2009; Olsen et al. 2007a) or highly exposed residents (Bartell et al. 2010). Estimates of the half-lives based on Olsen et al. (2007a) were derived from longitudinal measurements of serum concentrations of PFOA and PFOS in a group of fluorochemical production workers (24 males, 2 females); the estimated half-lives were 3.8 years (95% confidence limit [CL] 3.1–4.4) and 5.4 years (95% CL 3.9–6.9), respectively. Costa et al. (2009) reported a half-life for PFOA of 5.1 years (SD 1.7) for a group of workers (n=16) following their cessation of PFOA production work. A longitudinal study by Bartell et al. (2010) followed serum PFOA concentrations in 200 subjects recruited from the Lubeck Public Service District and Little Hocking Water Association and followed for a period of 6–12 months after mitigation of exposures from drinking water. The estimated half-life for PFOA was 2.3 years (95% CL 2.1–2.4). A fourth study estimated half-lives in a cross-sectional study of residents served by the Lubeck Public Service District and Little Hocking Water Association (Seals et al. 2011). The estimated half-lives ranged from 2.9 to 10.1 years (1,059–3,687 days) for PFOA. Results from the longitudinal studies are shown in Table A-1. For the MRL calculations, the PFOA half-life estimated by Olsen et al. (2007a) was selected over the half-life estimated by Bartell et al. (2010) because the Olsen et al. (2007a) study had a longer follow-up time (>5 years compared to 6–12 months) and estimates of the terminal half-life appear to increase with longer follow-ups because slower kinetics make a larger contribution to the terminal half-life (Seals et al. 2011). This may reflect a larger contribution of slower kinetics or ongoing exposure to the terminal half-life observable with longer follow-ups (Worley et al. 2017a) or other factors such as differences in the age-distribution of the populations studied. The decision to use the longer half-life from Olsen et al. (2007a) is also health protective in that a longer half-life would result in higher predicted serum concentrations for a given intake and, therefore, lower HEDs for a given serum concentration POD. Estimates of the half-life for PFOA and PFOS are most applicable to serum concentrations within the above ranges and would be less certain if applied to serum concentrations substantially below or above these ranges. Serum concentrations during the 5-year observation period in the Olsen et al. (2007a) study are provided in Table A-2.

Table A-1. Half-Life PFOA and PFOS Levels in Humans

PFOA $t_{1/2}$ (days)	PFOS $t_{1/2}$ (days)	Exposure type	Number	Source
1,378	1,976	Occupational	26	Olsen et al. (2007a)
1,862	NA	Occupational	16	Costa et al. (2009)
840	NA	Environmental	200	Bartell et al. (2010)

NA = not available; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid

Table A-2. Serum PFOA and PFOS Concentrations Measured in Fluorochemical Production Workers

	PFOA (ppb)	PFOS (ppb)
Initial	408 (72, 5,100)	626 (145, 3,490)
Final	148 (17, 2,435)	295 (37, 1,740)

PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid

Source: Olsen et al. 2007a

Estimates of volume of distribution (V_d) are based on non-compartmental modeling of serum concentration kinetics in monkeys and are assumed to be applicable to humans at the above serum concentrations (Table A-3).

Table A-3. Apparent Volume of Distribution for PFOA and PFOS

PFOA V_d (L/kg)	PFOS V_d (L/kg)	Source
0.18 (male) 0.20 (female)	NA NA	Butenhoff et al. (2004c)
NA NA	0.20 (male) 0.27 (female)	Chang et al. (2012)
0.3	0.3	Harada et al. (2005a)

NA = not applicable; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid

Numerous studies conducted in various animal models provide evidence for approximately complete absorption of oral doses of PFOA and PFOS (i.e., AF≈1, see Section 3.1.1).

PFHxS. For PFHxS, the estimate of the elimination t½ was derived from longitudinal measurements of serum concentrations of PFHxS in a group of retired fluorochemical production workers (24 males and 2 females) observed for a 5-year period; the estimated half-life was 8.5 years (3,109 days) (Olsen et al. 2007a). The range of initial serum concentrations was 16–1,295 ng/mL (mean of 290 ng/mL), and the final concentrations ranged from 10 to 791 ng/mL (mean of 182 ng/mL). Estimates of the t½ for PFHxS are most applicable to serum concentrations within the above ranges and would be less certain if applied to serum concentrations substantially below or above these ranges.

Estimates of volume of distribution (V_d) are based on non-compartmental modeling of serum concentration kinetics in monkeys and are assumed to be applicable to humans at the above serum concentrations. Sundström et al. (2012) estimated the apparent Vd for PFHxS at 0.287 L/kg for male Cynomolgus monkeys and at 0.213 L/kg for female Cynomolgus monkeys.

Few studies have been conducted in animals that provide estimates for a gastrointestinal absorption factor of oral doses of PFHxS. Sundström et al. (2012), based on comparison of the AUC for oral and intravenous administration, estimated an oral absorption fraction for PFHxS (administered as a single 10 mg/kg dose) of 50% in female rats. However, as the authors point out, this estimate may not be reliable due to the short (24 hours) observation period (Sundström et al. 2012) and that "female C_{max} values did not differ significantly between the oral and IV doses, and T_{max} after oral dosing was estimated to be at approximately 30 min." These latter observations suggest approximately complete bioavailability. The AUC for male rats following oral exposure was not available, and the AUC after

intravenous administration was done in only one male rat (Sundström et al. 2012). A study conducted by Kim et al. (2016) in rats estimated an approximately 100% oral bioavailability based on the C_{max} value and AUC comparison between oral and intravenous doses. Therefore, an absorption fraction (AF) of 1 was used for PFHxS.

PFNA. For PFNA, the elimination half-life estimates were derived by paired blood and urine samples (n=86) from Chinese adults in a study that measured the concentrations of a number of perfluoroalkyls, including PFNA (Zhang et al. 2013). The participants were first divided into four groups; young females (age ≤50 years, n=20), older females (>50 years, n=19), young males (≤50 years, n=32), and older males (>50 years, n=15). The group of young females had significantly lower levels of perfluoroalkyls than the other groups; therefore, the three other groups were combined. The lower perfluoroalkyl levels were likely due to the elimination via menstrual bleeding, pregnancy, and lactation. The estimated arithmetic mean elimination half-lives for the young female group and the combined male and older female group for PFNA were 2.5 and 4.3 years (913 and 1,570 days), respectively.

Toxicokinetics parameters for perfluorocarboxylic acids, among them PFOA and PFNA analogs, were investigated in rats by Ohmori et al. (2003). The authors estimated that the V_d values in steady state were not much different between the perfluorocarboxylic acids and between the sexes. Based on this, the estimated volume of distribution for PFNA in humans will be assumed to be the same for PFOA, 0.2 L/kg.

There are no studies on absorption of PFNA in humans. In rodents, oral absorption occurs rapidly as indicated by its presence in the serum of rodents soon after oral administration (Tatum-Gibbs et al. 2011). Therefore, based on animal studies of PFNA and other perfluorocarboxylic acid analogs, as well as sufficient findings of PFNA and other perfluorocarboxylic acids in human blood, it can be assumed that PFNA is well absorbed after oral exposure; therefore, an AF of 1 was used.

Model Input Parameters. The first-order one-compartment model input parameters ($t_{1/2}$, V_d, and AF) are provided in Table A-4.

Table A-4. First Order One-Compartment Model Parameters

Parameter	PFOA	PFOS	PFHxS	PFNA
Serum elimination half-life[a]; $t_{1/2}$ (day)	1,400[a]	2,000[a]	3,100[a]	900[b]
Serum elimination rate constant[c], k_e (day^{-1})	4.95×10^{-4}	3.47×10^{-4}	2.23×10^{-4}	7.59×10^{-4}
Gastrointestinal absorption fraction[d], AF	1	1	1	1
Apparent volume of distribution, V_d (L/kg)	0.2[e]	0.2[e]	0.287[f]	0.2[e]

[a]Estimates from Olsen et al. (2007a).
[b]Estimates from Zhang et al. (2013) for young females.
[c]Calculated using Equation 5.
[d]Based on studies in rodents and nonhuman primates.
[e]Estimates based on studies in nonhuman primates (Butenhoff et al. 2004c; Chang et al. 2012; Harada et al. 2005a).
[f]Estimates based on studies in nonhuman male primates (Sundström et al. 2012).

PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid; PFHxS = perfluorohexane sulfonic acid; PFNA = perfluorononanoic acid

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Perfluorooctanoic acid (PFOA)
CAS Numbers: 335-67-1
Date: March 2020
Profile Status: Final
Route: Inhalation
Duration: Acute

MRL Summary: There are insufficient data for derivation of an acute-duration inhalation MRL for PFOA.

Rationale for Not Deriving an MRL: Derivation of an inhalation MRL was precluded because inhalation-specific PBPK/pharmacokinetic model parameters are not available for PFOA and none of the studies reported serum PFOA concentrations.

Four studies have examined the acute toxicity of airborne PFOA in laboratory animals (Griffith and Long 1980; Kennedy et al. 1986; Staples et al. 1984). The observed effects included excessive salivation and eye and nose irritation in rats exposed to 18,600 mg/m^3 for 1 hour (Griffith and Long 1980), weight loss and pulmonary edema in rats exposed to 380 mg/m^3 for 4 hours (Kennedy et al. 1986), weight loss in rats exposed nose-only to 84 mg/m^3 6 hours/day, 5 days/week for 2 weeks (Kennedy et al. 1986), and decreases in maternal weight gain at 10 mg/m^3 and maternal deaths and decreases in neonatal body weight at 25 mg/m^3 in rats exposed 6 hours/day on GDs 6–15 (Staples et al. 1984). The 2-week study also reported increases in liver weight and hepatocellular hypertrophy in rats exposed to 7.6 mg/m^3.

Agency Contacts (Chemical Managers): Melanie Buser

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Perfluorooctanoic acid (PFOA)
CAS Numbers: 335-67-1
Date: March 2020
Profile Status: Final
Route: Inhalation
Duration: Intermediate

MRL Summary: There are insufficient data for derivation of an intermediate-duration inhalation MRL for PFOA.

Rationale for Not Deriving an MRL: No intermediate-duration inhalation studies in laboratory animals were identified for PFOA.

Agency Contacts (Chemical Managers): Melanie Buser

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Perfluorooctanoic acid (PFOA)
CAS Numbers: 335-67-1
Date: March 2020
Profile Status: Final
Route: Inhalation
Duration: Chronic

MRL Summary: There are insufficient data for derivation of a chronic-duration inhalation MRL for PFOA.

Rationale for Not Deriving an MRL: No chronic-duration inhalation studies in laboratory animals were identified for PFOA.

Agency Contacts (Chemical Managers): Melanie Buser

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Perfluorooctanoic acid (PFOA)
CAS Numbers: 335-67-1
Date: March 2020
Profile Status: Final
Route: Oral
Duration: Acute

MRL Summary: There are insufficient data for derivation of an acute-duration oral MRL for PFOA.

Rationale for Not Deriving an MRL: An acute-duration oral MRL cannot be derived for PFOA because the modeling approach used for estimating HEDs cannot be used to estimate acute human exposure where the exposure duration of 14 days is 1% of the elimination half-life in humans.

Acute-duration oral studies are available in rats and mice and provide information on body weight, hepatic, immunological, reproductive, and developmental effects. The liver effects consisted of increases in liver weight, hepatocellular hypertrophy, and/or decreases in serum cholesterol and triglycerides in rats and mice exposed to ≥1 mg/kg/day (Cook et al. 1992; Elcombe et al. 2010; Haughom and Spydevold 1992; Ikeda et al. 1985; Kawashima et al. 1995; Kennedy 1987; Liu et al. 1996; Pastoor et al. 1987; Iwai and Yamashita 2006; Permadi et al. 1992, 1993; Vetvicka and Vetvickova 2013; White et al. 2009; Wolf et al. 2007; Xie et al. 2003; Yang et al. 2000, 2001, 2002b). Consistent with the Hall et al. (2012) criteria (see Section 2.9 for a discussion of the criteria), the liver weight increases and hypertrophy observed in rats and mice were not considered relevant to human risk assessment. Although there is uncertainty regarding the exact, and possibly multiple, mechanism(s) for these liver effects, peroxisome proliferation is a likely contributor, a mechanism that cannot be reliably extrapolated to humans (Hall et al. 2012). Therefore, increases in liver weight and hepatocellular hypertrophy, and alterations in serum lipid levels observed in rats and mice, in the absence of other degenerative lesions, were not considered appropriate endpoints for deriving MRLs.

The immunological effects consisted of impaired responses to T-dependent antigens, such as sRBCs, altered antibody response, and decreases in spleen and thymus weights at 11.5 mg/kg/day and higher (DeWitt et al. 2009; Vetvicka and Vetvickova 2013; Yang et al. 2001, 2002a). Information on the potential reproductive toxicity of PFOA is limited to three studies that reported increases in serum estradiol levels in rats exposed to ≥2 mg/kg/day for 14 days (Biegel et al. 1995; Cook et al. 1992; Liu et al. 1996). A number of studies have evaluated the developmental toxicity of PFOA. In the only acute-duration developmental toxicity study in rats, no alterations in fetal body weight or malformations were observed at 100 mg/kg/day (Staples et al. 1984). Mice appear to be more sensitive to PFOA's developmental toxicity; observed effects include decreases in litter weight (Hu et al. 2010), decreases in pup body weight (White et al. 2007, 2009; Wolf et al. 2007), alterations in spontaneous activity (Johansson et al. 2008), increases in resorbed embryos (Chen et al. 2017b), and delays in mammary gland development (White et al. 2007, 2009; Wolf et al. 2007). The lowest LOAEL for developmental effects in mice was 0.5 mg/kg/day for decreased litter weight. A list of the NOAEL and LOAEL values for the immunological, reproductive, and developmental effects is presented in Table A-5.

Table A-5. Summary of the Adverse Effects Observed in Laboratory Animals Following Acute-Duration Oral Exposure

Species and exposure duration	NOAEL (mg/kg/day)	LOAEL[a] (mg/kg/day)	Effect	Reference
Immunological				
Mouse 10 days		11.5	Decreased spleen and thymus weights	Yang et al. 2001
Mouse 10 days	7.5	15	Altered response to sRBC	DeWitt et al. 2009
Mouse 7 days		20	Altered response to sRBC, decreased antibody formation	Vetvicka and Vetvickova 2013
Mouse 7 days		24	Decreased response to horse red blood cells	Yang et al. 2002a
Reproductive				
Rat 14 days	0.2	2	2-Fold increase in serum estradiol levels	Liu et al. 1996
Mouse GDs 1–7		2.5	Decrease in the number of corpora lutea	Chen et al. 2017b
Rat 14 days	1	10	63% increase in serum estradiol levels	Cook et al. 1992
Rat 14 days		25	184% increase in serum estradiol levels	Biegel et al. 1995
Developmental				
Mouse GDs 6–17		0.5	Decreased litter weight on PND 2	Hu et al. 2010
Mouse PND 10		0.58	Decreased spontaneous behavior and altered response to cholinergic stimulant	Johansson et al. 2008
Mouse GDs 8–17 or 12–17		5	Altered mammary gland development, decreased pup body weight on PND 20	White et al. 2007
Mouse GDs 8–17		5	Delayed mammary gland development, decreased pup body weight on PND 20	White et al. 2007
Mouse Various GDs		5	Delayed mammary gland development; decreased pup body weight at weaning	White et al. 2009 Wolf et al. 2007

[a]LOAELs are for less serious effects.

GD = gestation day; LOAEL = lowest-observed-adverse-effect level; NOAEL = no-observed-adverse-effect level; PND = postnatal day; sRBC = sheep red blood cell

The lowest LOAEL values were identified for developmental effects; Hu et al. (2010) identified a LOAEL of 0.5 mg/kg/day for decreases in litter weight on PND 2 and Johansson et al. (2008) identified a LOAEL of 0.58 mg/kg for decreases in spontaneous behavior (locomotion and total activity) and decreased response to a cholinergic stimulant in adult mice exposed to PFOA on PND 10. Neither study identified NOAEL values.

Agency Contacts (Chemical Managers): Melanie Buser

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Perfluorooctanoic acid (PFOA)
CAS Numbers: 335-67-1
Date: March 2020
Profile Status: Final
Route: Oral
Duration: Intermediate
MRL: $3x10^{-6}$ mg/kg/day
Critical Effect: Skeletal alterations in adult offspring
Reference: Koskela et al. 2016
Point of Departure: 0.000821 mg/kg/day
Uncertainty Factor: 300
LSE Graph Key: 63
Species: Mouse

MRL Summary: An intermediate-duration oral MRL of $3x10^{-6}$ mg/kg/day was derived for PFOA based on skeletal alterations at 13 and 17 months of age in the offspring of mice fed a diet containing PFOA on GD 1 through GD 21 (Koskela et al. 2016). The MRL is based on a HED LOAEL of 0.000821 mg/kg/day and a total uncertainty factor of 300 (10 for use of a LOAEL, 3 for extrapolation from animals to humans with dosimetric adjustments, and 10 for human variability).

Selection of the Critical Effect: Intermediate-duration oral studies of PFOA in animals indicate that the liver, immune system, reproductive system, and the developing organism are the primary targets of toxicity because adverse outcomes were observed at lower doses than other effects and have been consistently observed across studies. A summary of the lower LOAEL values (and associated NOAEL values) for these tissues/systems is presented in Table A-6; given the large number of studies, this table is limited to studies that identified LOAEL values of ≤4 mg/kg/day. Although these studies identified the lowest LOAEL values, not all were considered suitable as the basis of an intermediate-duration oral MRL.

Exposure to low levels of PFOA results in increases in liver weight, hepatocellular hypertrophy, and decreases in serum lipids in rats, mice, and monkeys exposed to PFOA for intermediate durations. The increases in liver weight, hepatocellular hypertrophy, and alterations in serum lipid levels observed in the rodents are likely adaptive responses to peroxisome proliferation and are not considered relevant for human risk assessment (Hall et al. 2012). Consistent with the Hall et al. (2012) criteria, the increases in liver weight and hepatocellular hypertrophy, in the absence of other degenerative alterations, were not considered adverse. Although there is uncertainty regarding the exact, and possibly multiple, mechanism(s) for these liver effects, peroxisome proliferation is a likely contributor, a mechanism that cannot be reliably extrapolated to humans (Hall et al. 2012). Therefore, increases in liver weight, hepatocellular hypertrophy, and alterations in serum lipid levels, in the absence of other degenerative lesions, were not considered appropriate endpoints for deriving MRLs.

A small number of animal studies have reported degenerative lesions, lesions to specialty cells, bile duct lesions, or inflammation; these endpoints were considered relevant for human risk assessment (Butenhoff et al. 2004b; Cui et al. 2009; Loveless et al. 2008). The lowest LOAEL for adverse liver effects was 0.96 mg/kg/day for increased liver weight, hepatocellular hypertrophy, and focal necrosis in mice exposed for 28 days (Loveless et al. 2008). *In utero* exposure has also resulted in liver effects in offspring (Filgo et al. 2015a; Quist et al. 2015a); the lowest maternal LOAEL identified in these studies was

0.01 mg/kg/day (Quist et al. 2015a). Because the Quist et al. (2015a) study did not provide incidence data for the reported inflammation, this study was not considered suitable for derivation of an MRL. Hepatic effects consisting of increases in absolute liver weight at ≥3 mg/kg/day and increases in serum triglyceride levels at 30/20 mg/kg/day have also been observed in monkeys administered capsules containing PFOA (Butenhoff et al. 2002); no histological alterations were observed in surviving animals, but hepatocellular degeneration and necrosis was noted in a monkey sacrificed early due to morbidity. The small number of animals examined and early deaths at several dose levels precludes using this study as the basis of an MRL.

Two studies examining the immunotoxicity of PFOA following intermediate-duration oral exposure found decreases in antigen-specific antibody responses in mice exposed for 15 days (DeWitt et al. 2008, 2016); the lowest LOAEL was 1.88 mg/kg/day (DeWitt et al. 2016). Reproductive and developmental toxicity studies have identified very low LOAELs of ≥0.0024 mg/kg/day for delays in mammary gland development in dams and offspring (Macon et al. 2011; Tucker et al. 2015; White et al. 2011). However, the mammary gland effect did not result in an adverse effect on lactational support at maternal doses as high as 1 mg/kg/day, based on normal growth and survival in F2 pups (White et al. 2011). Given that milk production was adequate to support growth, the biological significance of the delayed development of the mammary gland observed at very low doses is uncertain and was not considered a suitable basis for the MRL. Other developmental effects include increases in locomotor activity (Cheng et al. 2013; Goulding et al. 2017; Onishchenko et al. 2011; Sobolewski et al. 2014) at ≥0.1 mg/kg/day, reduced ossification of proximal phalanges and early preputial separation and delayed vaginal opening at ≥1 mg/kg/day (Lau et al. 2006; Yang et al. 2009), altered long bone morphology and decreased bone mineral density in 13- and 17-month-old mice following *in utero* exposure to 0.3 mg/kg/day (Koskela et al. 2016), decreases in pup survival at ≥0.6 mg/kg/day (Abbott et al. 2007; Albrecht et al. 2013), decreases in the number of successful births at ≥3 mg/kg/day (Ngo et al. 2014), and reduced neonatal weight gain and delayed eye opening at ≥3 mg/kg/day (Wolf et al. 2007).

Table A-6. Summary of the Adverse Effects Observed in Laboratory Animals Following Intermediate-Duration Oral Exposure

Species and exposure duration	NOAEL (mg/kg/day)	LOAEL[a] (mg/kg/day)	Effect	Reference
Hepatic				
Mouse GDs 1–17		0.01	Hepatocellular hypertrophy and periportal inflammation in offspring	Quist et al. 2015a, 2015b
Mouse 28 days	0.29	0.96	Moderate to severe hepatocellular hypertrophy and focal necrosis	Loveless et al. 2008
Mouse GDs 1–17 (examined at 18 months of age)	0.3	1	Increased severity of chronic inflammation in liver	Filgo et al. 2015a, 2015b
Rat 70–90 days	1	3	Increased liver weight, hepatocellular hypertrophy and necrosis	Butenhoff et al. 2004b
Monkey 26 weeks		3	Increased absolute liver weight	Butenhoff et al. 2002

Table A-6. Summary of the Adverse Effects Observed in Laboratory Animals Following Intermediate-Duration Oral Exposure

Species and exposure duration	NOAEL (mg/kg/day)	LOAEL[a] (mg/kg/day)	Effect	Reference
Immunological				
Mouse 15 days	0.94	1.88	Reduced antibody response	DeWitt et al. 2016
Mouse 15 days	1.88	3.75	Reduced sRBC response	DeWitt et al. 2008
Reproductive				
Mouse GDs 1–17		0.0024	Delayed mammary gland development in dams (3-generation study)	White et al. 2011
Mouse GDs 1–17		1	Delayed mammary gland development in dams (single-generation study)	White et al. 2011
Developmental				
Mouse GD 7–PND 22		0.0024	Impaired development of mammary glands	White et al. 2011
Mouse GDs 10–17		0.01	Impaired development of mammary glands	Macon et al. 2011
Mouse GDs 1–17		0.01	Impaired development of mammary glands	Tucker et al. 2015
Mouse GD 7–PND 21		0.1	Neurodevelopmental	Sobolewski et al. 2014
Mouse GDs 1–21		0.3	Altered exploratory behavior in adult offspring; increased global activity in males	Onishchenko et al. 2011
Mouse GDs 1–21		0.3	Skeletal alterations in mature offspring	Koskela et al. 2016
Mouse GDs 1–17		0.3	Impaired development of mammary glands	Macon et al. 2011
Mouse GDs 1–17	0.3	0.6 (SLOAEL)	Decreased pup survival	Abbott et al. 2007
Mouse GDs 1–17		1	Reduced ossification of proximal phalanges and advanced preputial separation	Lau et al. 2006
Mouse 4 weeks starting at PND 21		1	Delayed vaginal opening	Yang et al. 2009
Mouse GDs 1–17	0.3	1	Increased ambulatory activity	Goulding et al. 2017
Rat GD 1–PND 21		1.6	Neurodevelopmental	Cheng et al. 2013
Mouse GDs 1–17	0.1	3	Decreased number of successful births	Ngo et al. 2014

Table A-6. Summary of the Adverse Effects Observed in Laboratory Animals Following Intermediate-Duration Oral Exposure

Species and exposure duration	NOAEL (mg/kg/day)	LOAEL[a] (mg/kg/day)	Effect	Reference
Mouse GDs 1–17		3	Reduced pups per litter on PND 20	Albrecht et al. 2013
Mouse GDs 1–17		3	Reduced weight gain, delayed eye opening	Wolf et al. 2007

[a]Unless otherwise noted, LOAELs are for less serious effects.

GD = gestation day; LOAEL = lowest-observed-adverse-effect level; NOAEL = no-observed-adverse-effect level; PND = postnatal day; SLOAEL = serious LOAEL; sRBC = sheep red blood cell

Selection of the Principal Study: As outlined in the MRL approach section, serum PFOA levels were predicted for the administered doses for most of the studies listed in Table A-6. Mean serum PFOA levels could not be predicted for four studies because pharmacokinetic model parameters were not available for Wistar rats (Cheng et al. 2013), male CD-1 mice (Loveless et al. 2008), or 129S1/SvlmJ wild-type mice (Abbott et al. 2007; Albrecht et al. 2013). A summary of the predicted serum PFOA levels is presented in Table A-7.

Table A-7. Summary of the Predicted TWA Serum PFOA levels in Laboratory Animals Following Intermediate-Duration Oral Exposure

Species and exposure duration	Dose (mg/kg/day)	Predicted TWA serum PFOA (µg/mL)	Effect	Reference
Hepatic				
CD Mouse 28 days	0.29 0.96 9.6	Not calculated	Moderate to severe hepatocellular hypertrophy and focal necrosis at 0.96 mg/kg/day	Loveless et al. 2008
129/Sv Mouse GDs 1–17	0.01 0.1 0.3 1 5	0.423 4.21 12.5 39.2 102	Increased severity of chronic inflammation in liver of offspring aged 18 months at 1 mg/kg/day	Filgo et al. 2015a, 2015b
Sprague-Dawley Rat 70–90 days	1 3 10 30	60.4 136 222 242	Increased liver weight, hepatocellular hypertrophy and necrosis at 10 mg/kg/day	Butenhoff et al. 2004b
Cynomolgus Monkey 6 months	3 10 20/30	68.5 93.8 113	Increased liver weight at 3 mg/kg/day	Butenhoff et al. 2002

Table A-7. Summary of the Predicted TWA Serum PFOA levels in Laboratory Animals Following Intermediate-Duration Oral Exposure

Species and exposure duration	Dose (mg/kg/day)	Predicted TWA serum PFOA (µg/mL)	Effect	Reference
Immunological				
C58BL/6N Mouse 15 days	0.94	21.4	Reduced antibody response at 1.88 mg/kg/day	DeWitt et al. 2016
	1.88	42.5		
	3.75	58.4		
	7.5	83.5		
C57BL/6N Mouse 15 days	0.94	21.4	Reduced sRBC response at 3.75 mg/kg/day	DeWitt et al. 2008
	1.88	42.5		
	3.75	58.4		
	7.5	83.5		
	15	109		
	30	149		
Developmental				
C57BL/6 Mouse GD 7–PND 21	0.1	2.23	Neurodevelopmental effects (increased horizontal and vertical activity and decreased resting activity) at 0.1 mg/kg/day	Sobolewski et al. 2014
C57BL/6 Mouse GDs 1–21	0.3	8.29	Skeletal alterations at 0.3 mg/kg/day	Koskela et al. 2016
C57BL/6 Mouse GDs 1–21	0.3	8.29	Neurodevelopmental (decreased number of inactive periods, altered novelty induced activity) at 0.3 mg/kg/day	Onishchenko et al. 2011
129S1/SvlmJ Mouse GDs 1–17	0.1	Not calculated[a]	Decreased pup survival at 0.6 mg/kg/day	Abbott et al. 2007
	0.3			
	0.6			
	1			
	3			
	5			
	10			
	20			
CD-1 Mouse GDs 1–17	1	39.2	Reduced ossification of proximal phalanges and advanced preputial separation at 1 mg/kg/day	Lau et al. 2006
	3	83.6		
	5	102		
	10	125		
	20	155		
	40	205		

Table A-7. Summary of the Predicted TWA Serum PFOA levels in Laboratory Animals Following Intermediate-Duration Oral Exposure

Species and exposure duration	Dose (mg/kg/day)	Predicted TWA serum PFOA (µg/mL)	Effect	Reference
Mouse 4 weeks starting at PND 21	1 5 10	Not calculated	Delayed vaginal opening	Yang et al. 2009
Mouse GDs 1–17	0.1 0.3 1	4.21 12.5 39.2	Increased ambulatory activity at 1 mg/kg/day	Goulding et al. 2017
Wistar Rat GD 1–PND 21	1.6	Not calculated	Neurodevelopmental effects (increased locomotor activity in males and decreased activity in females) at 1.6 mg/kg/day	Cheng et al. 2013
C57BL/6J Mouse GDs 1–17	0.1 3	2.43 62.0	Decreased number of successful births at 3 mg/kg/day	Ngo et al. 2014
SV/129 Mouse GDs 1–17	3	Not calculated[b]	Reduced pups per litter on PND 20 at 3 mg/kg/day	Albrecht et al. 2013
CD-1 Mouse GDs 1–17	3 5	84.8 102	Reduced weight gain, delayed eye opening at 3 mg/kg/day	Wolf et al. 2007

[a]Reported serum PFOA concentrations at weaning for the dams that did not have pups which survived to weaning were 4.4, 10.4, 17.4, 26.3, 76.6, 72.4, and 68.2 µg/mL in the 0.1, 0.3, 0.5, 1, 5, 10, and 20 mg/kg/day group, respectively.
[b]Reported serum PFOA concentration was 17 µg/mL for dams treated with 1.6 mg/kg/day.

GD = gestation day; PFOA = perfluorooctanoic acid; PND = postnatal day; sRBC = sheep red blood cell; TWA = time-weighted average

Selection of the Point of Departure for the MRL: The NOAEL/LOAEL and the benchmark dose (BMD) approaches were utilized to identify potential PODs for derivation of the intermediate-duration oral MRL for PFOA. The only datasets with predicted TWA serum PFOA levels amenable to BMD modeling were from the DeWitt et al. (2008, 2016) immunotoxicity studies and Lau et al. (2006) developmental toxicity study. The Sobolewski et al. (2014), Onishchenko et al. (2011), Koskela et al. (2016), Ngo et al. (2014), and Wolf et al. (2007) studies were not considered for BMD modeling because only one or two PFOA doses were tested. No adequate BMD models adequately fit the data from the Lau et al. (2006) study. Adequate fit was found for the DeWitt et al. (2008, 2016) studies; the BMD modeling results are presented at the end of this section.

HEDs were calculated for each potential PODs (NOAEL, LOAEL, or BMD value) identified in laboratory animal studies using the first order single-compartment model previously discussed and the assumption that humans would have similar effects as the laboratory animal at a given serum concentration. The HEDs for each POD are presented in Table A-8. The potential POD_{HED} values were divided by an uncertainty factor to calculate candidate MRLs; these values are also presented in Table A-8. The candidate MRLs range from $7.4x10^{-7}$ mg/kg/day for neurodevelopmental effects in mice

(Sobolewski et al. 2014) to $4.5x10^{-4}$ mg/kg/day for liver effects in male rats (Butenhoff et al. 2004b). The lowest LOAEL (expressed as predicted serum concentration) was identified in the Sobolewski et al. (2014) study, which found neurodevelopmental effects in mouse offspring at predicted serum PFOA concentration of 2.23 µg/mL. However, this study was not considered suitable as the basis of the MRL because the subroute and vehicle used for the controls (peanut oil with anisole administered via gavage) were different from the PFOA group (PFOA dissolved in water and added to diet). Rather, the Onishchenko et al. (2011) and Koskela et al. (2016) studies, which identified the second lowest LOAEL (serum PFOA concentration) of 8.29 µg/mL were considered. In the Onishchenko et al. (2011) study, circadian activity was assessed using a TrafficCage in which all animals in the group were placed in a single cage and activity was measured. Thus, activity was only measured on a group basis and it is possible that one animal could skew the results. Thus, this study was not considered a suitable basis for an MRL.

Table A-8. Summary of Potential Points of Departures (PODs) and Human Equivalent Doses (HEDs) for Intermediate-Duration Oral MRL for PFOA

	Predicted serum concentrations (µg/mL)				
Endpoint (reference)	NOAEL or BMDL	LOAEL	POD_{HED}[a] (mg/kg/day)	Total UF	Candidate MRLs (mg/kg/day)
Neurodevelopmental effects (increased horizontal and vertical activity and decreased resting activity) in mice (Sobolewski et al. 2014)		2.23	0.000221	300[b]	$7.4x10^{-7}$
Neurodevelopmental effects (decreased number of inactive periods, altered novelty induced activity) in mice (Onishchenko et al. 2011)		8.29	0.000821	300[b]	$2.7x10^{-6}$
Skeletal alterations in mice (Koskela et al. 2016)		8.29	0.000821	300[b]	$2.7x10^{-6}$
Decreased number of successful births in mice (Ngo et al. 2014)	2.43	62.0	0.000241	30[c]	$8.0x10^{-6}$
Reduced ossification of proximal phalanges and advanced preputial separation in mice (Lau et al. 2006)		39.2	0.00388	300[b]	$1.3x10^{-5}$
Increased ambulatory activity (Goulding et al. 2017)	12.5	39.2	0.00124	30[c]	$4.1x10^{-5}$
Reduced weight gain and delayed eye opening (Wolf et al. 2007)		84.8	0.00840	300[b]	$2.8x10^{-5}$
Reduced response to dinitrophenyl-ficoll (DNP) antigen in female mice (DeWitt et al. 2016)	12.23 ($BMDL_{1SD}$)		0.00121	30[c]	$4.0x10^{-5}$
Increased severity of chronic inflammation in liver of offspring aged 18 months (Filgo et al. 2015a, 2015b)	12.5	39.2	0.00124	30[c]	$4.1x10^{-5}$

Table A-8. Summary of Potential Points of Departures (PODs) and Human Equivalent Doses (HEDs) for Intermediate-Duration Oral MRL for PFOA

Endpoint (reference)	Predicted serum concentrations (µg/mL) NOAEL or BMDL	LOAEL	POD_{HED}[a] (mg/kg/day)	Total UF	Candidate MRLs (mg/kg/day)
Reduced response to sRBC in female mice (DeWitt et al. 2008)	33.49 ($BMDL_{1SD}$)		0.00332	30[c]	$1.1x10^{-4}$
Increased liver weight, hepatocellular hypertrophy, and necrosis in male rats (Butenhoff et al. 2004b)	136	222	0.0135	30[c]	$4.5x10^{-4}$

[a]HED calculated using Equation A-6 where C_{ss} is the serum concentration associated with the NOAEL or BMDL or the LOAEL if there was no NOAEL or BMDL, $K_e=4.95x10^{-4}$; $V_d=0.2$, and AF=1.
[b]UF of 10 for extrapolation from a LOAEL, 3 for extrapolation from animals to humans with dosimetric adjustments, and UF of 10 for human variability.
[c]UF of 3 for extrapolation from animals to humans with dosimetric adjustments, and 10 for human variability.

BMDL = lower confidence limit on the BMD; HED = human equivalent dose; LOAEL = lowest-observed-adverse-effect level; MRL = Minimal Risk Level; NOAEL = no-observed-adverse-effect level; POD = point of departure; sRBC = sheep red blood cell; UF = uncertainty factor

Summary of the Principal Study:

Koskela A, Finnila MA, Korkalainen M, et al. 2016. Effects of developmental exposure to perfluorooctanoic acid (PFOA) on long bone morphology and bone cell differentiation. Toxicol Appl Pharmacol 301:14-21.

Pregnant C57BL/6/Bk1 mice were exposed to PFOA (96% pure) in food at dose levels of 0 mg/kg/day (n=10) or 0.3 mg/kg/day (n=6) from GD 1 throughout pregnancy (presumed GD 21). PFOA was dissolved in ethanol and applied to palatable food in volumes adjusted according to individual body weights to provide 0.3 mg/kg/day, followed by evaporation of ethanol; controls received food with ethanol applied and then evaporated. It is noted that litter mates of these offspring were examined for neurobehavioral effects in a study conducted by Onishchenko et al. (2011). Groups of five female offspring were sacrificed at either 13 or 17 months of age. The following parameters were used to assess toxicity: body weight and morphometric/biochemical properties in bone (femurs and tibias) of offspring.

As reported in Onishchenko et al. (2011), no differences in dams weight gain, litter size or sex ratio, or pup body weight or brain weight at birth were observed; significant increases in pup liver weight was observed in the PFOA group. Offspring body weight was significantly higher in comparison with controls at 13 and 17 months of age (9.9 and 7.8%, respectively). In 17-month-old offspring, there was a 6.8% increase in periosteal area of the femoral cortical bone and increases in the peri- and endosteal perimeters (3.2 and 5.2%, respectively) and the marrow area (10.0%); an increase in medullary area was also observed. There were no differences in femoral cortical bone area or femoral mineral density. In the tibia, the total area inside the periosteal envelope and the periosteal perimeter were increased (4.9 and 3.5%, respectively). Although the investigators noted in the text that tibial medullary areas were "essentially the same between groups," data in Figure 2 of the paper show a significant increase at 17 months. Significant decreases in tibial mineral density were observed at 13 and 17 months. There

were no significant differences in the tibial medullary area or the endosteal perimeter. There was a trend for increasing maximum force (Fmax); however, the effect was not statistically significant. There were no significant effects on any other measured biochemical parameter in the femur or tibia (stiffness, maximum energy, absorption). Concentrations of PFOA in the femurs and tibias of treated animals were significantly higher (4–5 times) than controls at 13 and 17 months. Koskela et al. (2016) suggested that the PFOA-induced increase in body weight gain may have indirectly affected bone homeostasis, but noted that *in vitro* data provide evidence of a direct effect on osteoblasts and osteoclasts.

Strengths and Weaknesses: The Koskela et al. (2016) study has a number of strengths including examination of several measures of bone status tested at different ages, measurement of bone PFOA levels, and tests to evaluate potential mechanisms of action. To evaluate whether developmental exposure resulted in bone damage in mature animals, the study evaluated bone morphology (periosteal, cortical, and medullary areas and bone mineral density) and bone biomechanical properties (stiffness, maximum force, and maximum energy); all tests were conducted on femur and tibia bone. Measurement at two ages (13 and 17 months) allowed for an evaluation of whether the effect of PFOA on bone changed as the animals aged. The companion *in vitro* study of osteoclasts and osteoblasts provided mechanistic support for the *in vivo* findings. Additionally, the *in vitro* study evaluated four PFOA concentrations and found concentration-related differences.

There are several study limitations that affect the interpretation of the study results; these include the small number of animals tested, use of only one PFOA dose level, inadequate reporting of dietary PFOA levels, and lack of measured serum PFOA levels. Tests of potential alterations in bone mineral density and bone biomechanical properties were only evaluated in 5–6 female offspring per group; however, support for the finding comes from the consistency of the findings at 13 and 17 months of age. The use of only one PFOA dose level does not allow for the establishment of dose-response relationships. This study limitation is mitigated by the extensive intermediate-duration oral exposure database, which allows for an overall assessment of dose-response. The dams were exposed to PFOA dissolved in alcohol and sprayed onto the food pellets. The study did not measure the amount of residual alcohol or the actual amount of PFOA on the food pellets. Koskela et al. (2016) measured PFOA levels in the tibias and femurs but did not measure serum PFOA levels. ATSDR estimated the TWA serum PFOA concentrations using the Wambaugh et al. (2013) model. The lack of measured serum PFOA levels did not allow for validation of whether the model accurately predicted serum levels; the model was validated using data from other intermediate-duration PFOA studies in rats and mice.

Calculation of Internal Dosimetric: TWA serum PFOA concentrations corresponding to external doses and exposure durations were predicted from a pharmacokinetic model (Wambaugh et al. 2013) using animal species-, strain-, and sex-specific parameters (see MRL approach section for details).

Human Equivalent Dose: HEDs were calculated based on the assumption that humans would have similar effects as the laboratory animal at a given serum concentration. HEDs that would result in steady-state serum concentrations of PFOA equal to the serum concentration selected as the POD were calculated using the first-order single-compartment model (see MRL approach section for details).

Uncertainty Factor: The $LOAEL_{HED}$ is divided by a total uncertainty factor of 300:

- 10 for the use of a LOAEL
- 3 for extrapolation from animals to humans with dosimetric adjustment
- 10 for human variability

$MRL = LOAEL_{HED} \div UFs$
$0.000821 \text{ mg/kg/day} \div (10 \times 3 \times 10) = 3 \times 10^{-6} \text{ mg/kg/day}$

Other Additional Studies or Pertinent Information that Lend Support to this MRL: *In vitro* studies conducted by Kosela et al. (2016) found that at lower concentrations (0.1–10 µM), PFOA stimulated osteoblast differentiation, as evidenced by increased osteocalcin mRNA expression and increased calcium secretion. At higher PFOA levels (>100 µM), osteocalcin expression and calcium secretion were decreased. Lower concentrations of PFOA (0.1–1.0 µM) also increased the number of osteoclasts and increased resorption activity; as with osteoblasts, decreased activity was observed at >100 µM PFOA concentrations.

Epidemiological studies have not evaluated the potential association between serum PFOA levels and impaired development of bone. A small number of studies in adults have examined potential associations with osteoarthritis risk. Innes et al. (2011) reported an elevated risk of physician diagnosed osteoarthritis among adults under 55 years of age with serum PFOA concentrations >13.6 ng/mL; the OR (95% CI) was 1.22 (1.02–1.45) among participants with serum PFOA in the second quartile (13.6–28.0 ng/mL). In a study of NHANES participants, Khalil et al. (2016) found an elevated risk of osteoarthritis among women, OR of 1.84 (1.17–2.90); the mean serum PFOA concentration was 3.7 ng/mL. This study also found in inverse association between serum PFOA and femur neck mineral density in women, but not in men. A second study of NHANES participants also found an elevated risk of osteoarthritis in women with serum PFOA levels of >5.89 ng/mL, OR of 1.98 (1.24–3.19) (Uhl et al. 2013). When segregated by age, an association was found in younger women (20–49 years of age), OR of 4.95 (1.27–19.4), but not among older women (50–84 years of age), OR of 1.33 (0.82–1.16) (Uhl et al. 2013). No association between estimated cumulative exposure to PFOA and the risk of osteoarthritis was observed in an occupational study in which 80% of the cohort was male (Steenland et al. 2015). A discussion of the other findings from epidemiological studies is presented in the MRL introduction section.

Benchmark Dose Modeling: BMD modeling was conducted for the DeWitt et al. (2008) and DeWitt et al. (2016) immunotoxicity studies. Using predicted TWA serum PFOA levels as the internal dosimetric, the IgM response data (summarized in Tables A-9 and A-10) were fit to all available continuous models in EPA's Benchmark Dose Software (BMDS, version 2.6.0). The following procedure for fitting continuous data was used: the simplest model (linear) was first applied to the data while assuming constant variance; if the data were consistent with the assumption of constant variance ($p \geq 0.1$), then the fit of the linear model to the means was evaluated and the polynomial, power, and Hill models were fit to the data while assuming constant variance. Adequate model fit was judged by three criteria: goodness-of-fit p-value ($p > 0.1$), visual inspection of the dose-response curve, and scaled residual at the data point (except the control) closest to the predefined benchmark dose response (BMR). Among all of the models providing adequate fit to the data, the lowest BMDL (the lower limit of a one-sided 95% CI on the BMD) was selected as a reasonably conservative POD when differences between the BMDLs estimated from these models are >2–3-fold; otherwise, the BMDL from the model with the lowest Akaike's information criterion (AIC) was chosen. If the test for constant variance was negative, the linear model was run again while applying the power model integrated into the BMDS to account for nonhomogenous variance. If the nonhomogenous variance model provided an adequate fit ($p \geq 0.1$) to the variance data, then the fit of the linear model to the means was evaluated and the polynomial, power, and Hill models were fit to the data and evaluated while the variance model was applied. Model fit and POD selection proceeded as described earlier. For both datasets, a BMR of 1 SD change from the control was used.

Table A-9. T-Cell Independent IgM Antibody Response in C57BL/6N Female Mice Immunized with Sheep Red Blood Cells

Number of animals per group	Administered dose (mg/kg/day)	Predicted TWA serum PFOA concentration (µg/mL)	IgM antibody titers[a] [mean serum IgM titer (log_2) to reach 0.5 OD]	SE[a]
8	0	0	7.28	0.13
8	0.94	21.4	7.39	0.07
8	1.88	42.5	7.08	0.10
8	3.75	58.4	6.75[b]	0.09
8	7.5	83.5	6.61[b]	0.12

[a]Data taken from Figure 3-C using GrabIt.
[b]Statistically different from controls ($p<0.05$).

PFOA = perfluorooctanoic acid; SE = standard error; TWA = time-weighted-average

Source: DeWitt et al. 2008

Table A-10. T-Cell Independent IgM Antibody Response In C57BL/6 Female Mice Immunized with Dinitrophenyl-Ficoll

Number of animals per group	Administered dose (mg/kg/day)	Predicted TWA serum PFOA concentration (µg/mL)	T-cell independent IgM antibody response[a] [mean serum titer (log_2) to reach 0.5 OD]	SD[a]
8	0	0	11.38	0.56
8	0.94	21.4	11.01	1.11
8	1.88	42.5	9.67[b]	1.34
8	3.75	58.4	9.81[b]	1.46
8	7.5	83.5	9.62[b]	0.97

[a]Data taken from Figure 3b using GrabIt.
[b]Statistically different from controls ($p<0.05$).

PFOA = perfluorooctanoic acid; SE = standard error; TWA = time-weighted-average

Source: DeWitt et al. 2016

The results of the BMD analysis of the DeWitt et al. (2008) and DeWitt et al. (2016) datasets are presented in Tables A-11 and A-12. For the DeWitt et al. (2008) data, the Hill model with constant variance provided the best fit to the IgM response data, as judged by the model with the lowest AIC since the range of BMDL values were sufficiently close; the fit of this model is presented in Figure A-1. For the DeWitt et al. (2016) IgM response data, constant variance models provided adequate fit; since the estimated BMDL values were not sufficiently close, the model with the lowest BMDL (Exponential 4) was selected; the fit of this model is presented in Figure A-2.

Table A-11. T-Cell Independent IgM Antibody Response in C57BL/6N Female Mice Immunized With Sheep Red Blood Cells Using Predicted TWA Serum PFOA as the Dose Metric (DeWitt et al. 2008)

Model	Test for significant difference p-value[a]	Variance p-value[b]	Means p-value[b]	Scaled residuals[c] Dose below BMC	Scaled residuals[c] Dose above BMC	Scaled residuals[c] Overall largest	AIC	BMD_{1SD} (ng/mL)	$BMDL_{1SD}$ (ng/mL)
Constant variance									
Exponential (model 2)[d]	<0.0001	0.46	0.08	1.73	0.72	1.73	-50.41	ND	ND
Exponential (model 3)[d]	<0.0001	0.46	0.10	0.16	-1.30	1.33	-50.61	42.55	26.37
Exponential (model 4)[d]	<0.0001	0.46	0.08	1.73	0.72	1.73	-50.41	ND	ND
Exponential (model 5)[d]	<0.0001	0.46	0.36	-0.14	0.09	0.67	-52.34	44.11	33.33
Hill[d,e]	**<0.0001**	**0.46**	**0.41**	**-0.05**	**0.06**	**0.60**	**-52.47**	**43.57**	**33.49**
Linear[f]	<0.0001	0.46	0.09	1.69	0.67	1.69	-50.66	ND	ND
Polynomial (2-degree)[f]	<0.0001	0.46	0.08	0.10	-1.41	1.43	-50.00	ND	ND
Polynomial (3-degree)[f]	<0.0001	0.46	0.08	0.10	-1.41	1.43	-50.00	ND	ND
Polynomial (4-degree)[f]	<0.0001	0.46	0.08	0.10	-1.41	1.43	-50.00	ND	ND
Power[d]	<0.0001	0.46	0.10	0.16	-1.32	1.35	-50.49	42.62	26.23

[a]Values >0.05 fail to meet conventional goodness-of-fit criteria.
[b]Values <0.10 fail to meet conventional goodness-of-fit criteria.
[c]Scaled residuals at doses immediately below and above the benchmark dose; also the largest residual at any dose.
[d]Power restricted to ≥1.
[e]Selected model. Constant variance model provided adequate fit to variance data. With constant variance model applied, the only models that provided adequate fit to the means were the Exponential 3 and 5, Hill, and Power models. BMDLs for models providing adequate fit were considered to be sufficiently close (differed by <2–3-fold); therefore, the model with the lowest AIC was selected (Hill).
[f]Coefficients restricted to be negative.

AIC = Akaike Information Criterion; BMD = maximum likelihood estimate of the exposure concentration associated with the selected benchmark response; BMDL = 95% lower confidence limit on the BMD (subscripts denote benchmark response: i.e., $_{10}$ = exposure concentration associated with 10% extra risk); ND = not determined, model did not provide adequate fit; PFOA = perfluorooctanoic acid; SD = standard deviation; TWA = time-weighted average

Figure A-1. Predicted (Hill Model with Constant Variance, 1 Standard Deviation Benchmark Response) and Observed IgM Response Using Predicted TWA Serum PFOA as the Dose Metric

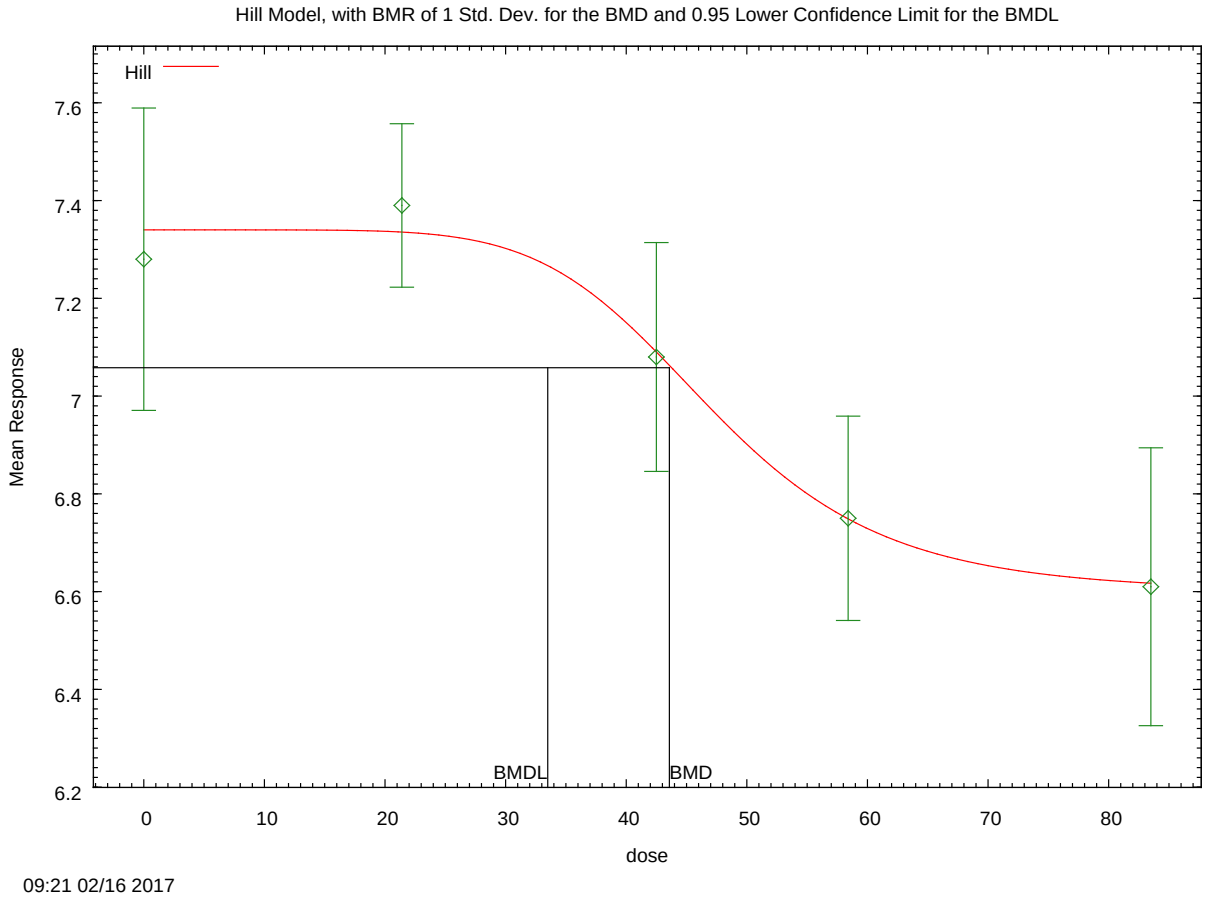


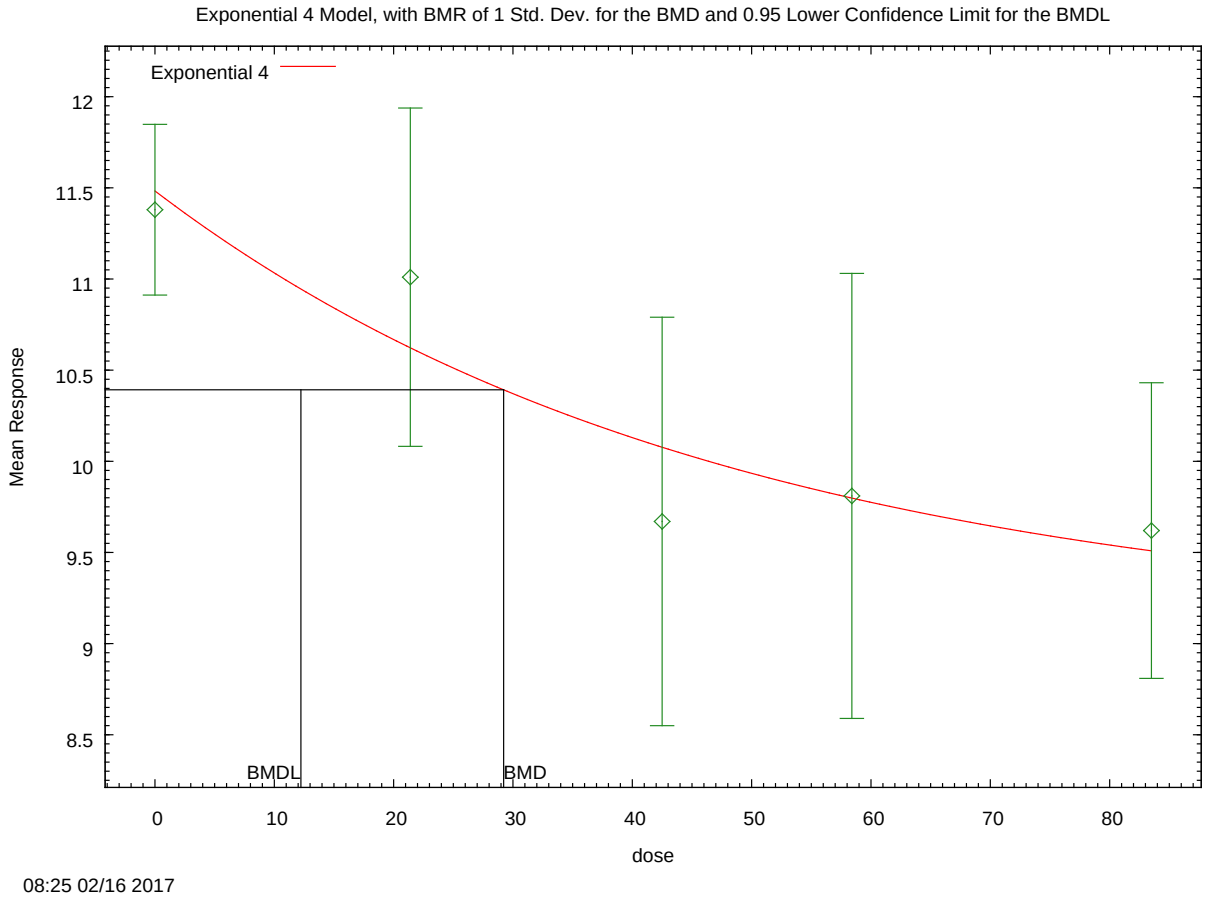
Table A-12. T-Cell Independent IgM Antibody Response In C57BL/6 Female Mice Immunized With Dinitrophenyl-Ficoll Using Predicted TWA Serum PFOA as the Dose Metric (DeWitt et al. 2016)

Model	Test for significant difference p-value[a]	Variance p-value[b]	Means p-value[b]	Scaled residuals[c] Dose below BMC	Scaled residuals[c] Dose above BMC	Scaled residuals[c] Overall largest	AIC	BMD_{1SD} (ng/mL)	$BMDL_{1SD}$ (ng/mL)
Constant variance									
Exponential (model 2)[d]	0.0029	0.12	0.34	-1.47	-0.17	-1.47	54.00	45.00	29.56
Exponential (model 3)[d]	0.0029	0.12	0.34	-1.47	-0.17	-1.47	54.00	45.00	29.56
Exponential (model 4)[d,e]	**0.0029**	**0.12**	**0.31**	**1.01**	**-1.06**	**-1.06**	**54.97**	**29.22**	**12.23**
Exponential (model 5)[d]	0.0029	0.12	0.71	0.00	-0.08	0.29	54.76	26.62	18.75
Hill[d]	0.0029	0.12	0.93	0.00	-0.08	0.29	52.76	23.66	19.11
Linear[f]	0.0029	0.12	0.30	-1.52	-0.23	-1.52	54.27	47.96	32.63
Polynomial (2-degree)[f]	0.0029	0.12	0.30	-1.52	-0.23	-1.52	54.27	47.96	32.63
Polynomial (3-degree)[f]	0.0029	0.12	0.30	-1.52	-0.23	-1.52	54.27	47.96	32.63
Polynomial (4-degree)[f]	0.0029	0.12	0.30	-1.52	-0.23	-1.52	54.27	47.96	32.63
Power[d]	0.0029	0.12	0.30	-1.52	-0.23	-1.52	54.27	47.96	32.63

[a]Values >0.05 fail to meet conventional goodness-of-fit criteria.
[b]Values <0.10 fail to meet conventional goodness-of-fit criteria.
[c]Scaled residuals at doses immediately below and above the benchmark dose; also the largest residual at any dose.
[d]Power restricted to ≥1.
[e]Selected model. Constant variance model provided adequate fit to variance data. With constant variance model applied, all models provided adequate fit to the means. BMDLs for models providing adequate fit were not considered to be sufficiently close (differed by >2-fold, but <3-fold). In order to remain conservative, the model with the lowest BMDL was selected (Exponential 4).
[f]Coefficients restricted to be negative.

AIC = Akaike Information Criterion; BMD = maximum likelihood estimate of the exposure concentration associated with the selected benchmark response; BMDL = 95% lower confidence limit on the BMD (subscripts denote benchmark response: i.e., $_{10}$ = exposure concentration associated with 10% extra risk); NA = not applicable (BMDL computation failed); SD = standard deviation; TWA = time-weighted average

Figure A-2. Predicted (Exponential 4 Model with Constant Variance, 1 Standard Deviation Benchmark Response) and Observed IgM Response Using Predicted TWA Serum PFOA as the Dose Metric



Agency Contacts (Chemical Managers): Melanie Buser

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Perfluorooctanoic acid (PFOA)
CAS Numbers: 335-67-1
Date: March 2020
Profile Status: Final
Route: Oral
Duration: Chronic

MRL Summary: ATSDR did not identify an adequate study with an exposure duration of ≥365 days. Although adequate data are available for intermediate-duration exposure, ATSDR does not extrapolate across exposure duration.

Rationale for Not Deriving an MRL: The chronic oral animal database for PFOA is limited to dietary exposure studies in male and female rats (3M 1983; Butenhoff et al. 2012c) or male rats (Biegel et al. 2001). The lowest LOAEL identified in the Butenhoff et al. (2012c; 3M 1983) study was 1.5 mg/kg/day for inflammation of salivary gland in male rats exposed to PFOA in the diet for 2 years. At 15 mg/kg/day, hepatocellular necrosis was observed after 1 year of exposure and vascular mineralization was observed in the testes. In the Biegel et al. (2001) study, exposure to 13.6 mg/kg/day PFOA in the diet for 2 years resulted in decreases in body weight gain, increases in Leydig cell hyperplasia, and pancreatic acinar cell hyperplasia in male rats.

The chronic-duration database for PFOA was not considered adequate for MRL derivation due to uncertainty in the selection of the critical effect. The Butenhoff et al. (2012c) study identified the salivary gland as the most sensitive target, but these alterations were only observed in males and may have been due to an antemortem viral infection. Intermediate-duration oral studies have suggested that the immune system is a sensitive target of toxicity in mice; however, potential alterations in immune function have not been investigated in chronic-duration studies; the Butenhoff et al. (2012c) study did conduct histological examinations of immune tissues.

Agency Contacts (Chemical Managers): Melanie Buser

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Perfluorooctane sulfonic acid (PFOS)
CAS Numbers: 1763-23-1
Date: March 2020
Profile Status: Final
Route: Inhalation
Duration: Acute

MRL Summary: There are insufficient data for derivation of an acute-duration inhalation MRL for PFOS.

Rationale for Not Deriving an MRL: No inhalation studies in laboratory animals were identified for PFOS.

Agency Contacts (Chemical Managers): Melanie Buser

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Perfluorooctane sulfonic acid (PFOS)
CAS Numbers: 1763-23-1
Date: March 2020
Profile Status: Final
Route: Inhalation
Duration: Intermediate

MRL Summary: There are insufficient data for derivation of an intermediate-duration inhalation MRL for PFOS.

Rationale for Not Deriving an MRL: No inhalation studies in laboratory animals were identified for PFOS.

Agency Contacts (Chemical Managers): Melanie Buser

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Perfluorooctane sulfonic acid (PFOS)
CAS Numbers: 1763-23-1
Date: March 2020
Profile Status: Final
Route: Inhalation
Duration: Chronic

MRL Summary: There are insufficient data for derivation of a chronic-duration inhalation MRL for PFOS.

Rationale for Not Deriving an MRL: No inhalation studies in laboratory animals were identified for PFOS.

Agency Contacts (Chemical Managers): Melanie Buser

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Perfluorooctane sulfonic acid (PFOS)
CAS Numbers: 1763-23-1
Date: March 2020
Profile Status: Final
Route: Oral
Duration: Acute

MRL Summary: There are insufficient data for derivation of an acute-duration oral MRL for PFOS.

Rationale for Not Deriving an MRL: An acute-duration oral MRL for PFOS cannot be derived because the modeling approach used for estimating HEDs cannot be used to estimate acute human exposure where the exposure duration of 14 days is <1% of the PFOS elimination half-life in humans.

A number of studies have examined the toxicity of PFOS in laboratory animals following acute-duration exposure. The available data suggest that the liver, developing organism, and immune system are sensitive targets. The liver effects consisted of decreases in serum lipids, increases in liver weight, and hepatocellular hypertrophy (Elcombe et al. 2012a, 2012b; Era et al. 2009; Fuentes et al. 2006; Haughom and Spydevold 1992; Vetvicka and Vetvickova 2013; Wan et al. 2011); using the Hall et al. (2012) criteria (see Section 2.9 for a discussion of the criteria) for assessing the adversity of liver alterations for peroxisome proliferators, these effects were not considered relevant for human risk assessment. Although there is uncertainty regarding the exact, and possibly multiple, mechanism(s) for these liver effects, peroxisome proliferation is a likely contributor, a mechanism that cannot be reliably extrapolated to humans (Hall et al. 2012). Therefore, increases in liver weight, hepatocellular hypertrophy, and alterations in serum lipid levels observed in rats and mice, in the absence of other degenerative lesions, were not considered appropriate endpoints for deriving MRLs. A decrease in serum HDL cholesterol levels was also observed in male and female monkeys receiving three doses of 13.3 or 14 mg/kg/day (TWA dose in males and females, respectively) over 315 days (Chang et al. 2017).

Immunological effects included altered responses to sRBC and decreased IgM antibody formation in response to antigen exposure in mice (Vetvicka and Vetvickova 2013; Zheng et al. 2009). Developmental effects consisted of decreases in neonatal survival (Abbott et al. 2009; Grasty et al. 2003), increases in post-implantation losses (Lee et al. 2015a), decreases in fetal body weight (Case et al. 2001; Era et al. 2009; Fuentes et al. 2007b; Lee et al. 2015a), increases in malformations (Era et al. 2009), and alterations in motor activity (Hallgren et al. 2015; Johansson et al. 2008). The lowest LOAEL identified was 0.5 mg/kg/day for increased post-implantation losses in mice (Lee et al. 2015a). A summary of the adverse effect levels for the immunological and developmental effects are presented in Table A-13.

Table A-13. Summary of the Adverse Effects Observed in Laboratory Animals Following Acute-Duration Oral Exposure to PFOS

Species and exposure duration	NOAEL (mg/kg/day)	LOAEL[a] (mg/kg/day)	Effect	Reference
Immunological				
Mouse 7 days		5	Impaired response to T-cell mitogens; suppressed response to sRBC	Zheng et al. 2009
Mouse 7 days		20	Inhibition of T lymphocyte proliferation in response to sRBC; decreased phagocytosis by peripheral blood cells and NK cell activity; decreased IgM antibody formation in response to OVA	Vetvicka and Vetvickova 2013
Developmental				
Mouse GDs 11–16		0.5	Increased post-implantation losses	Lee et al. 2015a
Mouse Once		0.75	Decreased motor activity	Johansson et al. 2008
Rabbit GDs 6–20	1	2.5	Decreased fetal body weight	Case et al. 2001
Mouse GDs 15–18		4.5	Decreased number of live pups per litter on PND 15	Abbott et al. 2009
Mouse GDs 12–18		6	Reduced pup body weight on PNDs 4 and 8	Fuentes et al. 2007b
Mouse Once		11.3	Altered spontaneous behavior in pups	Hallgren et al. 2015
Rat GDs 19–20		25 (SLOAEL)	Decreased neonatal survival	Grasty et al. 2003
Rat GDs 2–5, 6–9, 10–13, 14–17, or 17–20		25 (SLOAEL)	Decreased neonatal survival	Grasty et al. 2003
Mouse GDs 11–15		50	Cleft palate and reduced fetal body weight	Era et al. 2009

[a]Unless otherwise noted, the LOAEL is for a less serious effect.

GD = gestation day; LOAEL = lowest-observed-adverse-effect level; NK = natural killer; NOAEL = no-observed-adverse-effect level; perfluorooctane sulfonic acid; PND = postnatal day; SLOAEL = LOAEL for a serious effect; sRBC = sheep red blood cell

Agency Contacts (Chemical Managers): Melanie Buser

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Perfluorooctane sulfonic acid (PFOS)
CAS Numbers: 1763-23-1
Date: March 2020
Profile Status: Final
Route: Oral
Duration: Intermediate
MRL: $2x10^{-6}$ mg/kg/day
Critical Effect: Delayed eye opening and decreased pup body weight
Reference: Luebker et al. 2005a
Point of Departure: 0.000515 mg/kg/day
Uncertainty Factor: 30
Modifying Factor: 10
LSE Graph Key: 35
Species: Rat

MRL Summary: An intermediate-duration oral MRL of $2x10^{-6}$ mg/kg/day was derived for PFOS based on delayed eye opening and transient decrease in F2 body weight during lactation in the offspring of rats administered PFOS via gavage in a 2-generation study (Luebker et al. 2005a). The MRL is based on a HED NOAEL of 0.000515 mg/kg/day and a total uncertainty factor of 30 (3 for extrapolation from animals to humans with dosimetric adjustments and 10 for human variability) and a modifying factor of 10 for concern that immunotoxicity may be a more sensitive endpoint than developmental toxicity.

Selection of the Critical Effect: Intermediate-duration studies in monkeys, rats, and mice have identified several sensitive targets of PFOS toxicity including the liver, nervous system, immune system, and the developing organism; adverse outcomes occurred in these tissues at lower doses than other effects. The lowest LOAEL and NOAEL values for these outcomes are presented in Table A-14; given the large number of intermediate-duration studies, this table was limited to studies which identified LOAEL values of ≤3 mg/kg/day. The liver effects observed in monkeys, rats, and mice included increases in liver weight, decreases in serum lipids, hepatocellular degeneration, and focal necrosis (Cui et al. 2009; Curran et al. 2008; Elcombe et al. 2012a; Lefebvre et al. 2008; Seacat et al. 2002, 2003; Thibodeaux et al. 2003; Wan et al. 2011; Yahia et al. 2008). In the absence of degenerative changes such as necrosis, the liver hypertrophy observed in rodent studies was not considered relevant to human risk assessment (Hall et al. 2012). Although there is uncertainty regarding the exact, and possibly multiple, mechanism(s) for these liver effects, peroxisome proliferation is a likely contributor, a mechanism that cannot be reliably extrapolated to humans (Hall et al. 2012). Therefore, increases in liver weight, hepatocellular hypertrophy, and alterations in serum lipid levels observed in rats and mice, in the absence of other degenerative lesions, were not considered appropriate endpoints for deriving MRLs.

Several studies have examined potential neurological endpoints and found overt signs of neurotoxicity (cachexia, lethargy, and tonic convulsions in response to stimuli) in rats exposed to 5 or 8.5 mg/kg/day (Cui et al. 2009; Kawamoto et al. 2011) and impaired spatial learning and memory in mice exposed to 2.15 mg/kg/day (Long et al. 2013). Four studies have evaluated the immune response of PFOS exposed mice following exposure to an antigen (sRBC) or a virus (Dong et al. 2009, 2011; Guruge et al. 2009; Peden-Adams et al. 2008). Although the studies have consistently reported adverse effects, there is considerable overlap in LOAEL values. Peden-Adams et al. (2008) identified the lowest LOAEL of 0.00166 mg/kg/day with a NOAEL of 0.000166 mg/kg/day for a suppressed response to sRBC in mice administered PFOS for 28 days. Longer duration studies (Dong et al. 2009, 2011) have identified NOAEL values (0.0083 and 0.0167 mg/kg/day) in mice exposed to PFOS for 60 days that are higher than

the LOAELs identified in the Peden-Adams et al. (2008) study. It is noted that the studies used different mouse strains (B6C3F1 in the Peden-Adams study and C57BL/6N in the Dong studies), which may account for this difference. A variety of developmental effects have been observed in rats and mice; these include increases in postnatal mortality (Chen et al. 2012b; Lau et al. 2003; Luebker et al. 2005a, 2005b; Xia et al. 2011; Yahia et al. 2008), neurodevelopmental alterations (locomotor activity and impaired learning) (Butenhoff et al. 2009b; Onishchenko et al. 2011; Wang et al. 2015c), developmental delays (Lau et al. 2003; Luebker et al. 2005a), and malformations and anomalies (sternal defects and cleft palate) (Era et al. 2009; Thibodeaux et al. 2003; Yahia et al. 2008). The Wang et al. (2015c) study showed that decreases in spatial learning were observed in rats exposed *in utero* and in rat pups exposed postnatally (PND 7). Other effects that occur at similar doses include decreases in body weight (Lefebvre et al. 2008; Luebker et al. 2005a, 2005b; Seacat et al. 2002) and alterations in thyroid hormone levels (decreases in T3 and T4 levels and increases in TSH levels) (Curran et al. 2008; Luebker et al. 2005b; Thibodeaux et al. 2003).

The most sensitive targets of PFOS toxicity in laboratory animals are similar to those identified in longer term epidemiological studies. These effects include liver damage and increases in serum lipids, decreased antibody response to vaccines, and small decreases in birth weight; epidemiological studies have not consistently found neurological effects to be associated with serum PFOS levels.

Table A-14. Summary of the Adverse Effects Observed in Laboratory Animals Following Intermediate-Duration Oral Exposure to PFOS

Species and exposure duration	NOAEL (mg/kg/day)	LOAEL[a] (mg/kg/day)	Effect	Reference
Hepatic				
Monkey 26 weeks	0.15	0.75	Increased liver weight, decreased serum cholesterol, hepatocellular hypertrophy, lipid vacuolation	Seacat et al. 2002
Neurological				
Mouse 3 months	0.43	2.15	Impaired spatial learning and memory	Long et al. 2013
Immunological				
Mouse 28 days	0.00016	0.00166	Suppressed response to sRBC	Peden-Adams et al. 2008
Mouse 21 days	0.005	0.025	Decreased resistance to influenza virus	Guruge et al. 2009
Mouse 60 days	0.0083	0.083	Impaired response to sRBC	Dong et al. 2009
Mouse 60 days	0.0167	0.083	Impaired response to sRBC	Dong et al. 2011
Developmental				
Mouse GDs 1–21		0.3	Decreased locomotion, muscle strength, motor coordination in adult offspring	Onishchenko et al. 2011
Rat 84 days	0.1	0.4	Delayed eye opening	Luebker et al. 2005a
Rat 67 days		0.4	Decreased pup weight	Luebker et al. 2005b

Table A-14. Summary of the Adverse Effects Observed in Laboratory Animals Following Intermediate-Duration Oral Exposure to PFOS

Species and exposure duration	NOAEL (mg/kg/day)	LOAEL[a] (mg/kg/day)	Effect	Reference
Rat GD 1–PND 1		0.8	Decreased spatial learning	Wang et al. 2015c
Rat GD 0–PND 20	0.3	1	Increased locomotor activity and concurrent failure to habituate to test environment in male pups on PND 17	Butenhoff et al. 2009b
Mouse GDs 1–17		1	Delayed eye opening	Lau et al. 2003
Mouse GDs 0–17		1	Increased sternal defects	Yahia et al. 2008
Rat GDs 1–21	0.1	2	Increased postnatal mortality and severe lung histopathology	Chen et al. 2012b
Rat GDs 2–21	0.6	2	Increased neonatal mortality	Xia et al. 2011

[a]LOAELs for less serious health effects.

GD = gestation day; LOAEL = lowest-observed-adverse-effect level; NK = natural killer; NOAEL = no-observed-adverse-effect level; PFOS = perfluorooctane sulfonic acid; PND = postnatal day; sRBC = sheep red blood cell

Selection of the Principal Study: Using the Wambaugh et al. (2013) pharmacokinetic model, TWA serum concentrations corresponding to external doses (mg/kg/day) and exposure durations (days) were predicted for the studies listed in Table A-14. Pharmacokinetic model parameters were not available for C57BL/6N mice, B6C3F1 mice, or Wistar rats, which precluded predicting TWA serum concentrations for the Long et al. (2013), Dong et al. (2009, 2011), Guruge et al. (2009), Peden-Adams et al. (2008), Wang et al. (2015c), Onishchenko et al. (2011), and Yahia et al. (2008) studies. The predicted serum PFOS levels for each administered dose is presented in Table A-15.

Table A-15. Summary of the Predicted TWA Serum PFOS levels in Laboratory Animals Following Intermediate-Duration Oral Exposure

Species and exposure duration	Dose (mg/kg/day)	Predicted TWA serum PFOS (µg/mL)	Effect	Reference
Hepatic				
Cynomolgus Monkey 26 weeks	0.03 (males)	7.81	Increased liver weight, decreased serum cholesterol, hepatocellular hypertrophy, mild bile stasis, lipid vacuolation at 0.75 mg/kg/day	Seacat et al. 2002
	0.15 (males)	37.8		
	0.75 (males)	150		
	0.03 (females)	7.72		
	0.15 (females)	37.6		
	0.75 (females)	146		

Table A-15. Summary of the Predicted TWA Serum PFOS levels in Laboratory Animals Following Intermediate-Duration Oral Exposure

Species and exposure duration	Dose (mg/kg/day)	Predicted TWA serum PFOS (µg/mL)	Effect	Reference
Neurological				
C57BL/6 Mouse 3 months	0.43 2.15 10.75	Not calculated	Impaired spatial learning and memory at 2.15 mg/kg/day	Long et al. 2013
Immunological				
B6C3F1 Mouse 28 days	0.00016 0.00166 0.00331 0.0166 0.0331	Not calculated[a]	Suppressed response to sRBC at 0.00166 mg/kg/day	Peden-Adams et al. 2008
B6C3F1 Mouse 21 days	0.005 0.025	Not calculated[a]	Decreased resistance to influenza virus at 0.025 mg/kg/day	Guruge et al. 2009
C57BL/6N Mouse 60 days	0.0083 0.083 0.41667 0.8333 2.0833	Not calculated[a]	Impaired response to sRBC at 0.083 mg/kg/day	Dong et al. 2009
C57BL/6N Mouse 60 days	0.0083 0.0167 0.083 0.41667 0.8333	Not calculated[a]	Impaired response to sRBC at 0.083 mg/kg/day	Dong et al. 2011
Developmental				
C57BL/6 Mouse GDs 1–21	0.3	Not calculated	Decreased locomotion, muscle strength, motor coordination in adult offspring at 0.3 mg/kg/day	Onishchenko et al. 2011
Sprague-Dawley Rat 84 days	0.1 0.4 1.6 3.2	7.43 29.7 119 238	Delayed eye opening in F1 pups and transient decrease in F2 pup body weight during lactation at 0.4 mg/kg/day	Luebker et al. 2005a
Sprague-Dawley Rat 67 days	0.4 0.8 1 1.2 1.6 3.2	24.1 48.1 60.1 72.2 96.2 120	Decreased pup weight per litter at birth and on LD 5 at 0.4 mg/kg/day	Luebker et al. 2005b

Table A-15. Summary of the Predicted TWA Serum PFOS levels in Laboratory Animals Following Intermediate-Duration Oral Exposure

Species and exposure duration	Dose (mg/kg/day)	Predicted TWA serum PFOS (µg/mL)	Effect	Reference
Wistar Rat GD 1–PND 1	0.8	Not calculated[b]	Decreased spatial learning at 0.8 mg/kg/day	Wang et al. 2015c
Sprague-Dawley Rat GD 0–PND 20	0.1 0.3 1	3.75 11.3 37.5	Increased locomotor activity and concurrent failure to habituate to test environment in male pups on PND 17 at 1 mg/kg/day	Butenhoff et al. 2009b
CD-1 Mouse GDs 1–17	1 5 10 15 20	31.9 146 216 244 260	Delayed eye opening at 1 mg/kg/day	Lau et al. 2003
ICR Mouse GDs 0–17	1	Not calculated	Increased sternal defects at 1 mg/kg/day	Yahia et al. 2008
Sprague-Dawley Rat GDs 1–21	0.1 2	2.01 40.1	Increased postnatal mortality and severe lung histopathology at 2 mg/kg/day	Chen et al. 2012b
Sprague-Dawley Rat GDs 2–21	0.1 0.6 2	1.92 11.5 38.3	Increased neonatal mortality at 2 mg/kg/day	Xia et al. 2011

[a]See Table A-17 for measured serum PFOS concentrations.
[b]Reported serum PFOS concentrations of 25.7 and 64.3 µg/mL in dams on PND 7 and 35, respectively.

GD = gestation day; LD = lactation day; PFOS = perfluorooctane sulfonic acid; PND = postnatal day; sRBC = sheep red blood cell; TWA = time-weighted average

Selection of the Point of Departure for the MRL: None of the studies with predicted serum PFOS levels had datasets that were amenable for BMD modeling; thus, the NOAEL/LOAEL approach was used to identify PODs for derivation of the intermediate-duration MRL for PFOS. A summary of the PODs is presented in Table A-16. HEDs were calculated for each potential POD (NOAEL or LOAEL) identified in laboratory animal studies using the first-order single-compartment model previously discussed and the assumption that humans would have similar effects as the laboratory animal at a given serum concentration. The HEDs for each POD are presented in Table A-16. The potential POD_{HED} values were divided by a total uncertainty factor to calculate candidate MRLs; these values are also presented in Table A-16. The lowest administered doses associated with adverse effects were found in the immunotoxicity studies conducted by Dong et al. (2009, 2011), Guruge et al. (2009), and Peden-Adams et al. (2008). These data could not be considered as PODs because TWA serum PFOS values could not be predicted due to the lack of pharmacokinetic model parameters for the two mouse strains tested. Although there is considerable overlap between the LOAEL for IgM response to sRBC (0.00166 mg/kg/day) identified in the Peden-Adams et al. (2008) 28-day study and the NOAELs for IgM

response to sRBC (0.0083 and 0.0167 mg/kg/day) identified in the Dong et al. (2009, 2011) 60-day studies, the data do suggest that immunotoxicity could occur at <0.3 mg/kg/day (the lowest LOAEL identified in developmental toxicity studies).

Table A-16. Summary of Potential Points of Departures Human Equivalent Doses (POD_{HED}) for Intermediate-Duration Oral MRL for PFOS

Endpoint (reference)	Predicted serum concentrations (µg/mL) NOAEL	LOAEL	POD_{HED}[a] (mg/kg/day)	Total UF	Candidate MRLs (mg/kg/day)
Increased rat pup mortality and lung histopathology (Chen et al. 2012b)	2.01	40.1	0.000139	300[b]	$4.6x10^{-7}$
Decreased rat pup weight at birth and on PND 4 (Luebker et al. 2005b)		24.1	0.00167	3,000[c]	$5.6x10^{-7}$
Delayed eye opening in mouse pups (Lau et al. 2003)		31.9	0.00221	3,000[c]	$7.4x10^{-7}$
Neurodevelopmental effects in male rat pups (Butenhoff et al. 2009b)	11.3	37.5	0.000780	300[b]	$2.6x10^{-6}$
Delayed eye opening and decreased F2 rat pup body weight (Luebker et al. 2005a)	7.43	29.7	0.000515	300[b]	$1.7x10^{-6}$
Increased neonatal mortality in rat pups (Xia et al. 2011)	11.5	38.3	0.000797	300[b]	$2.7x10^{-6}$
Hepatic effects in monkeys (Seacat et al. 2002)	37.8	150	0.00262	300[b]	$8.7x10^{-6}$

[a]HED calculated using Equation A-6 where C_{ss} is the serum concentration associated with the NOAEL or BMDL or the LOAEL if there was no NOAEL or BMDL, $K_e=3.74x10^{-4}$; $V_d=0.2$, and AF=1.
[b]UFs of 3 for extrapolation from animals to humans with dosimetric adjustments and 10 for human variability and modifying factor (MF) of 10 for concern that immunotoxicity may be a more sensitive endpoint than developmental toxicity.
[c]UF of 10 for extrapolation from a LOAEL, 3 for extrapolation from animals to humans with dosimetric adjustments, and UF of 10 for human variability and MF of 10 for concern that immunotoxicity may be a more sensitive endpoint than developmental toxicity.

BMDL = lower limit on the benchmark dose; LOAEL = lowest-observed-adverse-effect level; MRL = Minimal Risk Level; NOAEL = no-observed-adverse-effect level; PFOS = perfluorooctane sulfonic acid; PND = postnatal day; UF = uncertainty factor

The serum PFOS concentrations predicted to occur at the lowest LOAEL values were 24.1, 29.7, and 31.9 µg/mL identified in the Luebker et al. (2005b), Luebker et al. (2005a), and Lau et al. (2003) studies; decreases in pup body weight and delays in eye opening were observed at these levels. Luebker et al. (2005a) was the only study that identified a NOAEL for these effects. The predicted serum concentration for this NOAEL dose was selected as the basis for the MRL.

Summary of the Principal Study:

Luebker DJ, Case MT, York RG, et al. 2005a. Two-generation reproduction and cross-foster studies of perfluorooctanesulfonate (PFOS) in rats. Toxicol 215:126-148.

Groups of Sprague-Dawley rats (P generation) (35/sex/dose level) were administered PFOS (86.9% pure) by gavage in deionized water with 2% Tween-80 at doses of 0, 0.1, 0.4, 1.6, or 3.2 mg/kg/day for 6 weeks before mating and until sacrifice (after mating for males, GD 10 for some females, and PND 21 for the remaining females). Body weight and feed consumption were evaluated during the dosing period. Prior to mating, 15 females per dose group were evaluated for estrous cycling. Ten females/dose group were sacrificed on GD 10 and the remaining females were allowed to give birth (F1 generation). Parental rats sacrificed on GD 10 were examined for number of corpora lutea, implantations, and viable and non-viable fetuses. Body weight of F1 was evaluated during lactation; also, F1 rats were assessed for developmental landmarks during lactation. On PND 22, the F1 rats were started on the same diet as the parental rats. At approximately PND 90, F1 were mated to produce the F2 generation. F1 males and females were killed as the P generation. F1 females and males were evaluated for vaginal patency and preputial separation, respectively. At age 24 days, F1 rats were administered three neurobehavioral tests (learning, memory retention, and avoidance memory). At the age of 70 days, F1 were administered three different neurobehavioral tests (neuromuscular coordination, swimming ability, learning, and memory). PFOS was analyzed in liver and blood from parental females and in liver from F1on PND 21; and in liver and serum from parental males after mating and after 42–56 days of dosing.

There were no deaths in parental males or females and no clinical signs in parental males. High-dose parental males had significantly reduced terminal body weight (11% reduction). Absolute and relative food consumption was reduced during treatment in males by less than 10%. Parental females at 0.4 mg/kg/day and higher had localized areas of partial alopecia. Body weight of parental females in the 3.2 mg/kg/day group was significantly lower during cohabitation and gestation (11% reduced). Absolute and relative food consumption were significantly reduced in 3.2 mg/kg/day parental females during premating and gestation (>15%) and in 1.6 mg/kg/day parental females during lactation. Administration of PFOS did not affect any mating or fertility parameter. Estrous cycling was not affected. Examination of parental females sacrificed on GD 10 showed no significant effect on numbers of corpora lutea or implantations or viable and non-viable fetuses. Significant delivery observations for 3.2 mg/kg/day parental females included reduced number of implantations per delivered litter, decreased gestational length, increased number of dams with all pups dying on PNDs 1–4 (also at 1.6 mg/kg/day). Observation of F1 pups during PNDs 1–21 showed significantly reduced weight and decreased viability (≥1.6 mg/kg/day). Examination of dead F1 pups did not reveal a cause of death; no labored breathing was noted in pups at birth. Developmental delays were noted at 1.6 mg/kg/day (pinna unfolding, surface righting, and air righting), and 0.4 mg/kg/day (eye opening). The investigators noted that the delay in eye opening was not considered an adverse outcome but did not provide a rationale for this conclusion. Follow-up observations of 0.1 and 0.4 mg/kg/day offspring showed no alterations in body weight or food consumption, including F1 females during gestation and lactation. Sexual maturation was not affected in F1 males or females; no effects were noted in the neurobehavioral tests. Reproductive performance of F1 were not affected. Viability of F2 pups during PNDs 1–21 was not affected. F2 pup weight was significantly reduced at 0.4 mg/kg/day on PND 7 (13%) and PND 14 (9.6%). The investigators noted that the decrease in pup weight was not considered toxicologically relevant and may have been due to minimally larger live litter sizes, as compared to the control group, and that there were no differences on PND 21. ATSDR notes that there were no significant differences in pup body weight between the control group and the 0.4 mg/kg/day group on PND 4 prior to culling and after culling, and considers the delay in eye opening to be toxicologically relevant. Serum and liver PFOS increased with dose.

Strengths and Weaknesses: The Luebker et al. (2005a) study is a well-designed 2-generation study evaluating a number of reproductive and developmental endpoints in adequate number of animals. The study was designed to evaluate four PFOS dose levels administered prior to mating and during mating, gestation, and lactation across two generations. The test included a number of parameters to assess reproductive performance (mating, estrous cycling, and fertility), reproductive outcomes (gestation length, number of implantation sites, stillbirths), and neonatal toxicity (survival and body weight). The experiment also included a cross-foster study, which allowed for the evaluation of whether neonatal effects were due to maternal care/maternal toxicity or to a direct effect on the pups. An additional strength of the study is that it evaluated several endpoints (e.g., lung morphology and lung glycogen stores) that could elucidate the mechanisms of action for fetal deaths. Luebker et al. (2005a) measured serum and liver PFOS levels, which allowed for validation of the Wambaugh et al. (2013) model's predicted serum TWA PFOS level. Although the study was designed to evaluate four PFOS dose levels, high mortality in the F1 offspring at the two highest dose levels resulted in a discontinuation of these dose levels, which limits the amount of data that can be used to establish dose-response relationships.

Calculations of Internal Dosimetric: TWA serum PFOS concentrations corresponding to external doses and exposure durations were predicted from a pharmacokinetic model (Wambaugh et al. 2013) using animal species-, strain-, and sex-specific parameters (see MRL approach section for details).

Human Equivalent Dose: HEDs were calculated based on the assumption that humans would have similar effects as the laboratory animal at a given serum concentration. HEDs that would result in steady-state serum concentrations of PFOS equal to the serum concentration selected as the POD were calculated using the first order single-compartment model (see MRL approach section for details).

Uncertainty Factor and Modifying Factor: The $NOAEL_{HED}$ is divided by a total uncertainty factor (UF) of 30 and modifying factor (MF) of 10:

- 3 UF for extrapolation from animals to humans with dosimetric adjustment
- 10 UF for human variability
- 10 MF for concern that immunotoxicity may be a more sensitive endpoint of PFOS toxicity than developmental toxicity

$MRL = NOAEL_{HED} \div (UFs \times MF)$
$0.000515 \text{ mg/kg/day} \div ((3 \times 10) \times 10) = 2 \times 10^{-6} \text{ mg/kg/day}$

Although pharmacokinetic model parameters were not available for the strain/sex of the animals tested in the immunotoxicity studies, most of the studies did provide measured serum PFOS levels. The serum PFOS levels at the NOAEL and LOAEL doses are presented in Table A-17. The measured serum PFOS levels associated with altered immune responses are approximately 1–10 times lower than the serum concentration predicted to occur at the NOAEL dose. These data suggest that immunotoxicity may be a more sensitive effect than developmental toxicity.

Table A-17. Measured Serum PFOS Levels at the NOAEL and LOAEL Doses for Immunological Effects

Effect, species and exposure duration		Dose (mg/kg/day)	Measured mean serum PFOS (µg/mL)	Reference
Impaired response to sRBC in mice exposed for 60 days	NOAEL	0.0083	0.674	Dong et al. 2009
	LOAEL	0.083	7.132	

Table A-17. Measured Serum PFOS Levels at the NOAEL and LOAEL Doses for Immunological Effects

Effect, species and exposure duration		Dose (mg/kg/day)	Measured mean serum PFOS (µg/mL)	Reference
Impaired response to sRBC in mice exposed for 60 days	NOAEL	0.0167	2.36	Dong et al. 2011
	LOAEL	0.083	10.75	
Decreased resistance to influenza virus in mice exposed for 21 days	NOAEL	0.005	0.189	Guruge et al. 2009
	LOAEL	0.025	0.670	
Suppressed response to sRBC in mice exposed for 28 days	NOAEL	0.00016	0.0178	Peden-Adams et al. 2008
	LOAEL	0.00166	0.0915	

PFOS = perfluorooctane sulfonic acid; sRBC = sheep red blood cell

A candidate MRL was calculated using the NOAEL of 0.0167 mg/kg/day identified in the Dong et al. (2011). This study was selected over the other immunotoxicity studies because it identified the highest NOAEL for immunotoxicity and it had the longest exposure duration; the Peden-Adams et al. (2008) was not selected because the LOAEL of 0.00166 mg/kg/day is not supported by the other three studies. A TWA concentration was estimated using a similar approach described for PFHxS and PFNA in the MRL approach section. The estimated TWA concentration was 1.2 µg/mL for the 0.0167 mg/kg/day; this estimated TWA concentration was used to calculate a HED of 0.000083 mg/kg/day. A candidate MRL of $3x10^{-6}$ was calculated using an uncertainty factor of 30 (3 for extrapolation from animals to humans using dosimetric adjustments and 10 for human variability). This MRL is similar to the MRL calculated from the Luebker et al. (2005a) study and lends support to using the additional modifying factor of 10 to account for the lack of pharmacokinetic modeling parameters for the mouse strains tested for immunotoxicity.

Other Additional Studies or Pertinent Information that Lend Support to this MRL: A discussion of the findings from epidemiological studies is presented in the MRL introduction section.

Agency Contacts (Chemical Managers): Melanie Buser

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Perfluorooctane sulfonic acid (PFOS)
CAS Numbers: 1763-23-1
Date: March 2020
Profile Status: Final
Route: Oral
Duration: Chronic

MRL Summary: ATSDR did not identify an adequate study with an exposure duration of ≥365 days. Although adequate data are available for intermediate-duration exposure, ATSDR does not extrapolate across exposure duration.

Rationale for Not Deriving an MRL: Immune function was not examined following chronic-duration oral exposure in laboratory animal studies; the only chronic-duration oral study (Butenhoff et al. 2012b; Thomford 2002b), did not find histological alterations in immune tissues (lymph nodes, spleen, and thymus) in rats at doses as high as 1.04 mg/kg/day. Impaired immune function was the most sensitive endpoint in intermediate-duration mouse studies. Given the concern that immunotoxicity may occur at lower doses than liver toxicity, a chronic-duration oral MRL for PFOS is not recommended at this time.

One study has evaluated the chronic toxicity of PFOS in laboratory animals. Histological alterations in the liver were the primary effects observed in rats exposed to PFOS in the diet for 2 years (Butenhoff et al. 2012b; Thomford 2002b). Centrilobular hepatocellular hypertrophy was observed in rats exposed to ≥0.1 mg/kg/day. At 1.04 mg/kg/day, increases in the incidence of single cell necrosis and cystic degeneration were observed in the liver. Decreases in body weight were observed at 1.04 mg/kg/day in female rats. Thus, the 1.04 mg/kg/day dose was identified as the lowest LOAEL for this study. Epidemiological data (Dalsager et al. 2016; Dong et al. 2013; Fei et al. 2010; Grandjean et al. 2012, 2016; Granum et al. 2013; Kielsen et al. 2016; Mogensen et al. 2015a; Stein et al. 2016a; Zhu et al. 2016) suggest that the immune system is a sensitive target of PFOS toxicity following long-term exposures, which is supported by intermediate-duration PFOS laboratory animal studies (Dong et al. 2009, 2011; Guruge et al. 2009; Peden-Adams et al. 2008).

Agency Contacts (Chemical Managers): Melanie Buser

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Perfluorohexane sulfonic acid (PFHxS)
CAS Numbers: 355-46-4
Date: March 2020
Profile Status: Final
Route: Inhalation
Duration: Acute

MRL Summary: There are insufficient data for derivation of an acute-duration inhalation MRL for PFHxS.

Rationale for Not Deriving an MRL: No inhalation studies in laboratory animals were identified for PFHxS.

Agency Contacts (Chemical Managers): Melanie Buser

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Perfluorohexane sulfonic acid (PFHxS)
CAS Numbers: 355-46-4
Date: March 2020
Profile Status: Final
Route: Inhalation
Duration: Intermediate

MRL Summary: There are insufficient data for derivation of an intermediate-duration inhalation MRL for PFHxS.

Rationale for Not Deriving an MRL: No inhalation studies in laboratory animals were identified for PFHxS.

Agency Contacts (Chemical Managers): Melanie Buser

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Perfluorohexane sulfonic acid (PFHxS)
CAS Numbers: 355-46-4
Date: March 2020
Profile Status: Final
Route: Inhalation
Duration: Chronic

MRL Summary: There are insufficient data for derivation of a chronic-duration inhalation MRL for PFHxS.

Rationale for Not Deriving an MRL: No inhalation studies in laboratory animals were identified for PFHxS.

Agency Contacts (Chemical Managers): Melanie Buser

APPENDIX A

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Perfluorohexane sulfonic acid (PFHxS)
CAS Numbers: 355-46-4
Date: March 2020
Profile Status: Final
Route: Oral
Duration: Acute

MRL Summary: There are insufficient data for derivation of an acute-duration oral MRL for PFHxS.

Rationale for Not Deriving an MRL: The acute oral database for PFHxS was not considered adequate for derivation of an MRL due to the short duration of the only available study and the lack of pharmacokinetic model parameters for calculating an HED.

In the only available study of PFHxS in laboratory animals, Viberg et al. (2013) reported altered spontaneous behavior and habituation in adult mice administered a single gavage dose of 9.2 mg/kg/day PFHxS on PND 10; no alterations were observed at 6.1 mg/kg/day. This single exposure study was not considered adequate as the basis of an acute-duration MRL for PFHxS due to the uncertainty of whether an MRL based on this study would be protective for repeated exposures or for other potential sensitive endpoints, such as immunotoxicity.

For perfluoroalkyls, ATSDR has used the approach of predicting TWA serum perfluoroalkyl levels in laboratory animals and calculating HEDs for these serum concentrations. For PFOA and PFOS, the Wambaugh et al. (2013) pharmacokinetic model was utilized for predicting the TWA serum perfluoroalkyl concentrations. However, strain-, sex-, and compound-specific model parameters are not available for other perfluoroalkyls, thus precluding deriving MRLs for other perfluoroalkyls. Other approaches such as “read across” (i.e., using data for a particular endpoint from one chemical to predict the same endpoint for another chemical that has similar chemical structure or mechanisms of action) or equivalency factors were considered for the other perfluoroalkyls; however, there are limited data available that would allow for comparison of the toxicity and toxicokinetic properties of different perfluoroalkyls. Peters and Gonzalez (2011) noted that the toxic equivalency factor approach would not be suitable for perfluoroalkyls because the current data suggest that the toxicity of these compounds appear to be mediated by multiple receptors, including PPARα, CAR, and PXR, and that there may be species differences in the response mediated by different receptors. Additionally, available data suggest that there are qualitative differences in the toxicities of various perfluoroalkyls.

Agency Contacts (Chemical Managers): Melanie Buser

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Perfluorohexane sulfonic acid (PFHxS)
CAS Numbers: 355-46-4
Date: March 2020
Profile Status: Final
Route: Oral
Duration: Intermediate
MRL: $2x10^{-5}$ mg/kg/day
Critical Effect: Thyroid follicular epithelial hypertrophy/hyperplasia
Reference: Butenhoff et al. 2009a
Point of Departure: 0.0047 mg/kg/day
Uncertainty Factor: 30
Modifying Factor: 10
LSE Graph Key: 33
Species: Rat

MRL Summary: An intermediate-duration oral MRL of $2x10^{-5}$ mg/kg/day was derived for PFHxS based on thyroid follicular epithelial hypertrophy/hyperplasia in adult male rats administered via gavage PFHxS for a minimum of 42 days (Butenhoff et al. 2009a). The MRL is based on a HED NOAEL of 0.0047 mg/kg/day and a total uncertainty factor of 30 (3 for extrapolation from animals to humans with dosimetric adjustments and 10 for human variability) and a modifying factor of 10 for database limitations.

Selection of the Critical Effect: Four intermediate-duration studies in laboratory animals have been identified for PFHxS. In a developmental toxicity study, increased incidences of thyroid follicular cells hypertrophy/hyperplasia were observed in F0 male rats administered ≥3 mg/kg/day (Butenhoff et al. 2009a). Increased liver weight and centrilobular hepatocellular hypertrophy were also observed in the males at ≥3 mg/kg/day. Consistent with the Hall et al. (2012), the liver effects were not considered a relevant endpoint for humans. Although there is uncertainty regarding the exact, and possibly multiple, mechanism(s) for these liver effects, peroxisome proliferation is a likely contributor, a mechanism that cannot be reliably extrapolated to humans (Hall et al. 2012). Therefore, increases in liver weight and hepatic lipid levels and alterations in serum lipid levels observed in rats and mice, in the absence of other degenerative lesions, were not considered appropriate endpoints for deriving MRLs.

No reproductive or developmental effects were reported in the Butenhoff et al. (2009a) study. A second developmental toxicity study reported decreases in serum thyroxine levels in rat dams and pups administered 5 mg/kg/day PFHxS on GDs 7–22 (Ramhøj et al. 2018); no alterations in pup birth weight or weight gain were observed. In a 1-generation reproductive/developmental toxicity study, decreases in the number of pups per litter were observed in the offspring of mice administered 1 mg/kg/day (Chang et al. 2018). At 3 mg/kg/day, single cell necrosis and microvascular fatty changes were observed at 3 mg/kg/day. Liver effects (decreases in serum lipids, increases in hepatic triglyceride levels, and increases in liver weight) were also observed in mice exposed to 6 mg/kg/day PFHxS in the diet for 4–6 weeks (Bijland et al. 2011) and mice administered 0.3 mg/kg/day (Chang et al. 2018). Using the Hall et al. (2012) criteria (see Section 2.9 for a discussion of the criteria), the liver effects were not considered relevant for human risk assessment. Thus, the lowest LOAEL identified in intermediate-duration studies was 3 mg/kg/day for thyroid effects.

There is some uncertainty regarding the selection of thyroid alterations as the critical effect. Butenhoff et al. (2009a) suggested that the histological alterations in the thyroid may be secondary to the liver effects

(hepatocellular hypertrophy). The alteration may be due to binding competition between PFHxS and thyroid hormones and possible induction of thyroid hormone metabolism by the liver. Ramhøj et al. (2018) reported decreases in serum T4 levels but did not evaluate possible thyroid gland histological alterations. The Chang et al. (2018) 1-generation reproduction study did not find alterations in serum TSH levels in mice.

A limited number of epidemiological studies have examined potential thyroid effects. Two epidemiological studies have examined thyroid disease associated with PFHxS exposure (Chan et al. 2011; Wen et al. 2013); one study found increased risk of subclinical hypothyroidism and subclinical hyperthyroidism among women (Wen et al. 2013) and the second study did not find an increased risk of hypothyroxinemia (Chan et al. 2011). The small number of studies precludes evaluating the possible association between PFHxS exposure and thyroid disease in humans. A meta-analysis of epidemiological data (Kim et al. 2018) found an inverse correlation between serum PFHxS levels and total T4 levels in the general population; there was no correlation among pregnant women. No associations were found for free T4, total T3, or TSH.

Species-related differences in thyroid parameters between rats and humans also add to the uncertainty. Some differences include higher rate of T4 production in rats than in humans, and very low levels of thyroxine binding globulin compared to high levels in humans, and sex-related differences in serum TSH levels (higher levels in males compared to females) in rats, but not in humans (Choksi et al. 2003). It is not known if these species differences would influence the relative toxicity of PFHxS.

Selection of the Principal Study: Since the liver effects were not considered relevant to humans, the lowest LOAEL identified for PFHxS was 1 mg/kg/day for decreases in the number of pups per litter identified in the Chang et al. (2018) study. The investigators noted that the toxicological significance of this alteration was uncertain because there was no clear dose-response and no alterations in the number of implantation sites, number of viable pups, or pup to implant ratios. Thus, the Butenhoff et al. (2009a) study, which reported thyroid effects in male rats at LOAEL of 3 mg/kg/day, with a NOAEL of 1 mg/kg/day, was selected as the principal study.

Summary of the Principal Study:

Butenhoff JL, Chang SC, Ehresman DJ, et al. 2009a. Evaluation of potential reproductive and developmental toxicity of potassium perfluorohexanesulfonate in Sprague Dawley rats. Reprod Toxicol 27:331-341. (Results from this study are also reported in Hoberman and York 2003.)

The reproductive/developmental effects of PFHxS were studied in Sprague-Dawley rats (15/sex/group). Doses of 0, 0.3, 1, 3, or 10 mg/kg/day PFHxS were administered by gavage in an aqueous vehicle. Male rats were dosed beginning 14 days before cohabitation and continued until 1 day before sacrifice (a minimum of 42 days). Females were dosed beginning 14 days before cohabitation and continued until 1 day before sacrifice on PND 21 or GD 25 (rats that did not deliver a litter). Endpoints evaluated included: body weight, food consumption, estrous cycling, functional observational battery (FOB; tests of autonomic function, reactivity and sensitivity, excitability, gait and sensorimotor coordination, grip strength, and clinical signs), hematology and clinical chemistry, gross necropsy, organ weights, histopathology, and sperm evaluations. At parturition, litters were evaluated for size and viability; weight of the pups was also recorded. Pups were sacrificed on PND 22.

The following are findings for male F0 rats. Treatment with PFHxS did not affect survival and did not induce clinical signs that could be attributed to the chemical. Terminal body weight in the 10 mg/kg/day groups was approximately 6% lower than controls. Food consumption was not affected. Necropsy did not reveal any treatment-related changes. Histopathological effects were restricted to the liver and

thyroid of males treated with 3 and 10 mg/kg/day. Liver effects consisted of minimal to moderate hypertrophy of centrilobular hepatocytes. The affected hepatocytes were enlarged with an increased amount of dense eosinophilic granular cytoplasm. In the thyroid, the changes consisted of hypertrophy and/or hyperplasia of follicular cells. These effects could have been associated with the liver effects.

Significant organ weight changes consisted of increased absolute and relative liver weight at 3 and 10 mg/kg/day and decreased heart/brain weight at 10 mg/kg/day. Significant hematology changes consisted of decreased hemoglobin at 1 mg/kg/day, decreased red cell count and hematocrit at 3 mg/kg/day, and increased prothrombin time at 0.3 mg/kg/day. Increases in albumin, BUN, alkaline phosphatase, calcium, and albumin/globulin ratio were seen at 10 mg/kg/day. The investigators noted that the alterations in prothrombin time were slight and did not follow a specific trend and the values were within the normal range. There were no significant effects on the FOB or on motor activity and no significant effects on sperm parameters. There were no significant effects in any parameter monitored in F0 females or in pups. Treatment with PFHxS had no significant effect on the gross or microscopic morphology of the spleen, thymus, or lymph nodes. There were no significant effects on sex organ weights or gross or microscopic lesions in the reproductive organs of males and females. Fertility was not affected by treatment with PFHxS and there were no significant effects on sperm parameters. Estrous cycling was not affected by dosing with PFHxS. Treatment with PFHxS did not significantly affect any of the developmental parameters evaluated including gestation length, number of dams delivering litters, averages for implantation sites per delivered litter, number of dams with stillborn pups, number of dams with no live pups, dams with all pups dying, number of pups surviving per litter, sex ratios, litter size, or pup weight. Also, necropsy of the pups showed no treatment-related effects, and pup liver weight was not affected. Treatment with PFHxS had no significant effect on the FOB or motor activity. The battery tested autonomic functions, reactivity and sensitivity to stimuli, excitability, gait and sensorimotor coordination, limb grip strength, and abnormal clinical signs.

Strengths and Weaknesses: The Butenhoff et al. (2009a) study is a well-designed study evaluating male and female reproductive endpoints and developmental endpoints. An adequate number of animals were exposed to three PFHxS dose levels. An additional strength of the study is the inclusion of parameters that evaluated potential neurobehavioral effects, hematological and clinical chemistry parameters, and histopathological examination of the liver and thyroid. Measurement of serum PFHxS levels allowed for estimation of a TWA serum concentration that could be used to calculate a HED. One weakness of the study is that thyroid hormone levels were not measured; these data could have been useful in evaluating the observed histological alterations in the thyroid gland.

Selection of the Point of Departure for the MRL: The HED of the NOAEL of 1 mg/kg/day identified in the Butenhoff et al. (2009a) developmental toxicity study was selected as the POD for the MRL. A TWA serum PFHxS concentration of 73.22 µg/mL was estimated for the adult males exposed to 1 mg/kg/day (Butenhoff et al. 2009a).

Human Equivalent Dose: The HED was calculated based on the assumption that humans would have similar effects as the laboratory animal at a given serum concentration. HEDs that would result in steady-state serum concentrations of PFHxS equal to the estimated TWA serum concentration selected as the POD were calculated using the first-order single-compartment model (see MRL approach section for details). The HED was calculated using Equation A-6 where C_{ss} is 73.22 µg/mL, $K_e=2.23x10^{-4}$; $V_d=0.287$, and AF=1. The $NOAEL_{HED}$ is 0.0047 mg/kg/day

Uncertainty Factor and Modifying Factor: The $NOAEL_{HED}$ is divided by a total uncertainty factor (UF) of 30 and a modifying factor (MF) of 10:

- 3 UF for extrapolation from animals to humans with dosimetric adjustment
- 10 UF for human variability

- 10 MF for database limitations to account for small number of studies examining the toxicity of PFHxS following intermediate-duration exposure and the limited scope of these studies in particular studies examining immunotoxicity, a sensitive endpoint for other perfluoroalkyls.

  $MRL = NOAEL_{HED} \div (UFs \times MF)$
  $0.0047 \text{ mg/kg/day} \div ((10 \times 3) \times 10) = 2x10^{-5} \text{ mg/kg/day}$

Other Additional Studies or Pertinent Information that Lend Support to this MRL: A discussion of the findings from epidemiological studies is presented in the MRL introduction section. An empirical steady state model was used to estimate the HED from a POD based on a 42-day exposure of adult rats. The resulting HED is lower than the daily 42-day human dose that would be expected to achieve the POD serum concentration.

Agency Contacts (Chemical Managers): Melanie Buser

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Perfluorohexane sulfonic acid (PFHxS)
CAS Numbers: 355-46-4
Date: March 2020
Profile Status: Final
Route: Oral
Duration: Chronic

MRL Summary: No chronic duration studies were identified for PFHxS. Although adequate data are available for intermediate-duration exposure, ATSDR does not extrapolate across exposure duration.

Rationale for Not Deriving an MRL: No chronic-duration oral studies in laboratory animals were identified for PFHxS.

Agency Contacts (Chemical Managers): Melanie Buser

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Perfluorononanoic acid (PFNA)
CAS Numbers: 375-95-1
Date: March 2020
Profile Status: Final
Route: Inhalation
Duration: Acute

MRL Summary: There are insufficient data for derivation of an acute-duration inhalation MRL for PFNA.

Rationale for Not Deriving an MRL: The only available inhalation exposure study for PFNA (Kinney et al. 1989) was not considered suitable for derivation of an inhalation MRL due to its lack of histopathological examination and short exposure duration.

In the only available inhalation exposure study for PFNA, Kinney et al. (1989) noted labored breathing in rats during and after a 4-hour nose-only exposure to 590 mg/m^3 exposure; the study also reported an increase in relative liver weight 5 days after exposure to $\geq$67 mg/m^3.

Agency Contacts (Chemical Managers): Melanie Buser

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Perfluorononanoic acid (PFNA)
CAS Numbers: 375-95-1
Date: March 2020
Profile Status: Final
Route: Inhalation
Duration: Intermediate

MRL Summary: There are insufficient data for derivation of an intermediate-duration inhalation MRL for PFNA.

Rationale for Not Deriving an MRL: No intermediate-duration inhalation studies in laboratory animals were identified for PFNA.

Agency Contacts (Chemical Managers): Melanie Buser

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Perfluorononanoic acid (PFNA)
CAS Numbers: 375-95-1
Date: March 2020
Profile Status: Final
Route: Inhalation
Duration: Chronic

MRL Summary: There are insufficient data for derivation of a chronic-duration inhalation MRL for PFNA.

Rationale for Not Deriving an MRL: No chronic-duration inhalation studies in laboratory animals were identified for PFNA.

Agency Contacts (Chemical Managers): Melanie Buser

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Perfluorononanoic acid (PFNA)
CAS Numbers: 375-95-1
Date: March 2020
Profile Status: Final
Route: Oral
Duration: Acute

MRL Summary: There are insufficient data for derivation of an acute-duration oral MRL for PFNA.

Rationale for Not Deriving an MRL: An acute-duration oral MRL cannot be derived for PFNA because the study identifying the lowest dose for a non-hepatic effect (Fang et al. 2009) did not measure serum PFNA levels, which are needed for estimating an HED.

A number of studies examined the toxicity of PFNA in rats and mice exposed for acute durations. These studies reported immune, liver, and body weight effects. Immune effects included increases in thymus weight in rats at 1 mg/kg/day (Fang et al. 2009), decreases in thymus and spleen weights in rats at 3 mg/kg/day (Fang et al. 2009, 2010), and an alteration in splenic lymphocyte phenotypes in mice at 1 mg/kg/day (Fang et al. 2008). In the only study examining immune function, no alterations in splenic lymphocyte response to ConA were observed at doses as high as 5 mg/kg/day in mice (Fang et al. 2008). Liver effects included increases in hepatic lipid levels at ≥0.2 mg/kg/day (Wang et al. 2015a), increases in liver weights at ≥0.2 mg/kg/day (Wang et al. 2015a; Kennedy 1987), serum lipid levels at ≥1 mg/kg/day (Fang et al. 2012a, 2012b), hepatocellular vacuolation at 5 mg/kg/day (Fang et al. 2012b), and increases in serum aminotransferases at 5 mg/kg/day (Wang et al. 2015a). Although there is uncertainty regarding the exact, and possibly multiple, mechanism(s) for these liver effects, peroxisome proliferation is a likely contributor, a mechanism that cannot be reliably extrapolated to humans (Hall et al. 2012). Therefore, increases in liver weight and hepatic lipid levels and alterations in serum lipid levels observed in rats and mice, in the absence of other degenerative lesions, were not considered appropriate endpoints for deriving MRLs.

Decreases in body weight were observed in rats and mice administered 5 mg/kg/day (Hadrup et al. 2016; Wang et al. 2015a).

Agency Contacts (Chemical Managers): Melanie Buser

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Perfluorononanoic acid (PFNA)
CAS Numbers: 375-95-1
Date: March 2020
Profile Status: Final
Route: Oral
Duration: Intermediate
MRL: $3x10^{-6}$ mg/kg/day
Critical Effect: Decreased body weight and developmental delays
Reference: Das et al. 2015
Point of Departure: 0.001 mg/kg/day
Uncertainty Factor: 30
Modifying Factor: 10
LSE Graph Key: 39
Species: Mouse

MRL Summary: An intermediate-duration oral MRL of $3x10^{-6}$ mg/kg/day was derived for PFNA based on decreased body weight gain and developmental delays in the offspring of mice administered via gavage PFNA on GDs 1–17 (Das et al. 2015). The MRL is based on a HED NOAEL of 0.001 mg/kg/day and a total uncertainty factor of 30 (3 for extrapolation from animals to humans with dosimetric adjustments and 10 for human variability), and a modifying factor of 10 for database limitations.

Selection of the Critical Effect: The intermediate-duration database consists of three developmental toxicity studies in rats and mice and a 90-day study in mice. The lowest LOAEL for developmental toxicity was 1.1 mg/kg/day in mice administered PFNA on GDs 1–18; at this dose, decreases in litter size and pup survival were observed (Wolf et al. 2010). At higher doses (2–5 mg/kg/day), decreases in pup body weight, delays in postnatal development (Das et al. 2015; Rogers et al. 2014; Wolf et al. 2010), increases in pup systolic blood pressure (Rogers et al. 2014), and reduced nephron endowment (Rogers et al. 2014) were observed. A study of PPARα knockout mice did not find alterations pup body weight or postnatal development at 2 mg/kg/day (Wolf et al. 2010). In the 90-day study, decreased sperm motility, viability, and number; degenerative changes in seminiferous tubules; and decreased litter size (males mated to unexposed females) were observed at 0.5 mg/kg/day; no changes were observed at 0.2 mg/kg/day (Singh and Singh 2018). A summary of the observed effects is presented in Table A-18.

Table A-18. Summary of the Adverse Effects Observed in Laboratory Animals Following Intermediate-Duration Oral Exposure to PFNA

Species and exposure duration	Dose (mg/kg/day)	Effect	Reference
Parkes mouse 90 days	0.2	No effects reported	Singh and Singh 2018
	0.5	Decreased sperm motility, viability, and number; degenerative changes in seminiferous tubules; decreased litter size	

Table A-18. Summary of the Adverse Effects Observed in Laboratory Animals Following Intermediate-Duration Oral Exposure to PFNA

Species and exposure duration	Dose (mg/kg/day)	Effect	Reference
129S1/svlm mouse GDs 1–18 (offspring followed until PND 21)	0.83	No effects reported	Wolf et al. 2010
	1.1	Decreased litter size and pup survival	
	1.5	No effects reported	
	2.0	Decreased number of live pups per litter and decreased pup body weight gain	
CD-1 mouse GDs 1–17 (offspring followed until PND 287)	1	No effects reported	Das et al. 2015
	3	Decreased body weight gain and delayed eye opening, preputial separation, and vaginal opening	
	5	Decreased postnatal survival, 80% mortality between PND 2 and 10	
	10	Full litter resorption	
Sprague-Dawley rat GDs 1–20 offspring followed through PND 434)	5	Decreased birth weight, increased blood pressure at 10 weeks of age; reduced nephron endowment	Rogers et al. 2014

GD = gestation day; PFNA = perfluorononanoic acid; PND = postnatal day

Selection of the Principal Study: The lowest LOAEL was 0.5 mg/kg/day identified in the Singh and Singh (2018) reproductive toxicity study. However, this study could not be used to derive an MRL for PFNA because the investigators did not measure serum PFNA levels. Developmental toxicity, including decreases in pup survival, developmental delays, and decreases in birth weight have been observed in three studies. A comparison of the estimated TWA serum PFNA levels (Table A-19) for the Wolf et al. (2010) and Das et al. (2015) studies (measured serum levels were not available from the Rogers et al. 2014 study) showed that the lowest LOAEL for developmental effects was 10.9 µg/mL (Das et al. 2015); this study reported a NOAEL of 6.8 µg/mL. Thus, the Das et al. (2015) study was selected as the principal study for the MRL.

Table A-19. Summary of Estimated TWA Serum PFNA levels in Laboratory Animals Following Intermediate-Duration Oral Exposure

Species and exposure duration	Dose (mg/kg/day)	Estimated TWA serum PFNA (µg/mL)	Effect	Reference
Parkes mouse 90 days	0.2	Not calculated	No effects reported	Singh and Singh 2018
	0.5		Decreased sperm motility, viability, and number; degenerative changes in seminiferous tubules; decreased litter size	

Table A-19. Summary of Estimated TWA Serum PFNA levels in Laboratory Animals Following Intermediate-Duration Oral Exposure

Species and exposure duration	Dose (mg/kg/day)	Estimated TWA serum PFNA (µg/mL)	Effect	Reference
129S1/svlm mouse GDs 1–18 (offspring followed until PND 21)	0.83	4.47	No effects reported	Wolf et al. 2010
	1.1	11.6	Decreased litter size and pup survival	
	1.5	10.5	No effects reported	
	2.0	17.6	Decreased number of live pups per litter and decreased pup body weight gain	
CD-1 mouse GDs 1–17 (offspring followed until PND 287	1	6.8	No effects reported	Das et al. 2015
	3	10.9	Decreased body weight gain and delayed eye opening, preputial separation, and vaginal opening	
	5	39.7	Decreased postnatal survival, 80% mortality between PND 2 and 10	
	10	NA	Full litter resorption	
Sprague-Dawley rat GDs 1–20 (offspring followed through PND 434)	5	Not calculated	Decreased birth weight, increased blood pressure at 10 weeks of age; reduced nephron endowment	Rogers et al. 2014

GD = gestation day; PFNA perfluorononanoic acid; PND = postnatal day

Summary of the Principal Study:

Das KP, Grey BE, Rosen MB, et al. 2015. Developmental toxicity of perfluorononanoic acid in mice. Reprod Toxicol 51:133-144.

Groups of 8–10 timed-pregnant female CD-1 mice were administered via gavage 0, 1, 3, 5, or 10 mg/kg/day PNFA at a dosing volume of 10 ml/kg body weight in deionized water on GDs 1–17. On GD 17, selected mice from each group were sacrificed for maternal and fetal examination, while the remaining mice were allowed to give birth. Pups were observed for postnatal survival up to PND 24 as well as growth and development up to PND 287. The following parameters were used to assess toxicity: clinical observations, maternal body weight, pup body weight (pre- and postnatal), organ weights (liver, gravid uterus weight), number of implantation sites, percent of live fetuses, percent of prenatal loss per litter, and morphological changes (eye opening, vaginal opening, preputial separation).

Maternal weight loss beginning on GD 8 was observed at 10 mg/kg/day; on GD 13, the 10 mg/kg/day group weighed approximately 30% less than controls. The 10 mg/kg/day group was terminated on GD 13. Significant increases in full litter resorptions occurred at 10 mg/kg/day (7/7 compared to 2/8 in controls). There were no adverse effects on pregnancy outcome following *in utero* exposure to 5 mg/kg. Statistically significant dose-related increases in absolute and relative liver weights were observed in dams in the 1, 3, and 5 mg/kg/day groups examined on GD 17, as well as in dams examined on post-weaning day 28. There were no effects on the number of implants, number of live fetuses, or fetal body weight. Relative and absolute fetal liver weight were significantly increased; however, the increase did not appear to be dose-related. Visceral and skeletal examination of fetuses revealed no treatment-related

effects. Increases in postnatal deaths were observed in the 5 mg/kg/day offspring between PND 2 and 10; postnatal survival was approximately 20% on PND 10. Weight gain was significantly reduced in pups from the 3 and 5 mg/kg dose groups from PND 1 to 24. The changes in males were dose-related and persisted from PND 25 to 287. Weight reduction in females was less substantial in comparison with males and returned to control levels by 7 weeks of age. Relative pup liver weights were significantly increased at all doses up to PND 24 and at 3 and 5 mg/kg/day on PND 42. No significant effects on liver weight were detectable by PND 70. Postnatal development (eye opening, preputial separation, and vaginal opening) was significantly delayed (by 2–7 days) at 3 and 5 mg/kg/day.

The serum PFNA levels (means±standard error of the mean) in the pregnant dams (measured at term) were 0.015±0.003, 13.67±1.45, 21.85±3.17, and 79.48±22.69 µg/mL in the 0. 1, 3, and 5 mg/kg/day groups (serum concentrations were provided to ATSDR by C. Lau).

Strengths and Weaknesses: Das et al. (2015) is a well-designed developmental toxicity study in mice. One strength of the study is that it included evaluation of potential anomalies in fetuses, as well as monitoring postnatal growth and development through PND 70. An additional strength of the study is the inclusion of serum and liver PFNA measurements, which allow for cross-species evaluations. Inclusion of measurement of the expression of genes related to PPARα, CAR, and PXR provides valuable mechanisms-of-action data. Although the study tested four PFNA dose levels, increases in maternal morbidity at 10 mg/kg/day and pup lethality at 5 mg/kg/day limited the data available to establish dose-response relationships.

Selection of the Point of Departure for the MRL: The HED of the NOAEL of 1 mg/kg/day identified in the Das et al. (2015) developmental toxicity study was selected as the POD for the MRL. A TWA serum PFNA concentration was estimated for dams using the serum concentration in the control group (0.015 µg/mL) as the baseline concentrations and the terminal concentration for the 1 mg/kg/day group (13.67 µg/mL) resulting in an estimated TWA serum concentration of 6.8 µg/mL.

Human Equivalent Dose: The HED was calculated based on the assumption that humans would have similar effects as the laboratory animal at a given serum concentration. HEDs that would result in steady-state serum concentrations of PFNA equal to the estimated TWA serum concentration selected as the POD were calculated using the first-order single-compartment model (see MRL approach section for details). The HED was calculated using Equation A-6 where C_{ss} is 6.8 µg/mL, $K_e=7.59x10^{-4}$; $V_d=0.2$, and AF=1. The K_e was calculated using the 2.5-year elimination half-life in young women; this value was selected over the 4.3-year value for the combined group of males and older females because the MRL is based on a developmental toxicity study. The $NOAEL_{HED}$ is 0.001 mg/kg/day.

Uncertainty Factor and Modifying Factor: The $NOAEL_{HED}$ is divided by a total uncertainty factor (UF) of 30 and modifying factor (MF) of 10:

- 3 UF for extrapolation from animals to humans with dosimetric adjustment
- 10 UF for human variability
- 10 MF for database limitations to account for small number of studies examining the toxicity of PFNA following intermediate-duration exposure and the limited scope of these studies. The available data suggest that reproductive toxicity may be a more sensitive endpoint than developmental toxicity; however, this endpoint could not be used to derive the MRL because the Singh and Singh (2018) study did not measure serum PFNA levels. Additionally, intermediate-duration studies for other perfluoroalkyls suggest that immune function is a sensitive target of toxicity; however, this potential endpoint has not been examined in intermediate-duration PFNA studies.

  $MRL = NOAEL_{HED} \div (UFs \times MF)$

0.001 mg/kg/day ÷ ((10 x 3) x 10) = $3x10^{-6}$ mg/kg/day

Other Additional Studies or Pertinent Information that Lend Support to this MRL: A discussion of the findings from epidemiological studies is presented in the MRL introduction section.

Agency Contacts (Chemical Managers): Melanie Buser

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Perfluorononanoic acid (PFNA)
CAS Numbers: 375-95-1
Date: March 2020
Profile Status: Final
Route: Oral
Duration: Chronic

MRL Summary: No chronic-duration studies were identified for PFNA. Although adequate data are available for intermediate-duration exposure, ATSDR does not extrapolate across exposure duration.

Rationale for Not Deriving an MRL: No chronic-duration oral studies in laboratory animals were identified for PFNA.

Agency Contacts (Chemical Managers): Melanie Buser

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Perfluorodecanoic acid (PFDA)
CAS Numbers: 335-76-2
Date: March 2020
Profile Status: Final
Route: Inhalation
Duration: Acute

MRL Summary: There are insufficient data for derivation of an acute-duration inhalation MRL for PFDA.

Rationale for Not Deriving an MRL: No inhalation studies in laboratory animals were identified for PFDA.

Agency Contacts (Chemical Managers): Melanie Buser

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Perfluorodecanoic acid (PFDA)
CAS Numbers: 335-76-2
Date: March 2020
Profile Status: Final
Route: Inhalation
Duration: Intermediate

MRL Summary: There are insufficient data for derivation of an intermediate-duration inhalation MRL for PFDA.

Rationale for Not Deriving an MRL: No inhalation studies in laboratory animals were identified for PFDA.

Agency Contacts (Chemical Managers): Melanie Buser

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Perfluorodecanoic acid (PFDA)
CAS Numbers: 335-76-2
Date: March 2020
Profile Status: Final
Route: Inhalation
Duration: Chronic

MRL Summary: There are insufficient data for derivation of a chronic-duration inhalation MRL for PFDA.

Rationale for Not Deriving an MRL: No inhalation studies in laboratory animals were identified for PFDA.

Agency Contacts (Chemical Managers): Melanie Buser

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Perfluorodecanoic acid (PFDA)
CAS Numbers: 335-76-2
Date: March 2020
Profile Status: Final
Route: Oral
Duration: Acute

MRL Summary: There are insufficient data for derivation of an acute-duration oral MRL for PFDA.

Rationale for Not Deriving an MRL: The available acute oral database for PFDA was not considered adequate for derivation of an MRL because the study identifying the lowest adverse effect level did not measure serum PFDA levels, which are needed to estimate HEDs.

Several laboratory animal studies have examined the acute oral toxicity of PFDA; most were limited in scope. The lowest LOAEL was 1 mg/kg/day for decreases in fetal weight in mice administered PFDA on GDs 6–15 (Harris and Birnbaum 1989). At 12.8 mg/kg/day, decreases in the number of live fetuses per litter were observed; maternal weight loss was also observed at this dose level (Harris and Birnbaum 1989). Another developmental toxicity study did not report alterations in performance on neurobehavioral tests in 2–4-month-old mice administered 10.8 mg/kg/day PFDA on PND 10 (Johansson et al. 2008). Other effects observed in acute exposure studies include decreases in maternal weight gain at 6.4 mg/kg/day (Harris and Birnbaum 1989), weight loss at ≥9.5 mg/kg/day in rats (Kawashima et al. 1995) and mice (Harris and Birnbaum 1989; Permadi et al. 1992, 1993), increases in T3 and T4 levels in mice at 80 mg/kg/day (Harris et al. 1989), decreases in spleen weight in mice at 80 mg/kg/day (Harris et al. 1989), and atrophy and lymphoid depletion in thymus and spleen in mice at 160 mg/kg/day (Harris et al. 1989). Liver effects included increases in liver weight at ≥2.4 mg/kg/day (Brewster and Birnbaum 1989; Harris et al. 1989; Kawashima et al. 1995; Permadi et al. 1992, 1993), increases in hepatic lipid levels at ≥9.5 mg/kg/day (Brewster and Birnbaum 1989; Kawashima et al. 1995), and hepatocellular hypertrophy at ≥20 mg/kg/day (Harris et al. 1989). Although there is uncertainty regarding the exact, and possibly multiple, mechanism(s) for these liver effects, peroxisome proliferation is a likely contributor, a mechanism that cannot be reliably extrapolated to humans (Hall et al. 2012). Therefore, increases in liver weight and hepatic lipid levels and alterations in serum lipid levels observed in rats and mice, in the absence of other degenerative lesions, were not considered appropriate endpoints for deriving MRLs.

To derive MRLs for perfluoroalkyls, ATSDR used the approach of predicting TWA serum perfluoroalkyl levels in laboratory animals or measured serum perfluoroalkyl levels and calculating HEDs for these serum concentrations. For PFOA and PFOS, the Wambaugh et al. (2013) pharmacokinetic model was utilized for predicting the TWA serum perfluoroalkyl concentrations. However, strain-, sex-, and compound-specific model parameters are not available for other perfluoroalkyls. The Harris and Birnbaum (1989) study, which identified the lowest adverse effect level, did not measure maternal serum PFDA levels. Thus, HEDs could not be calculated using animal serum PFDA levels. Other approaches such as "read across" or equivalency factors were considered; however, there are limited data available that would allow for comparison of the toxicity and toxicokinetic properties of different perfluoroalkyls. Peters and Gonzalez (2011) noted that the toxic equivalency factor approach would not be suitable for perfluoroalkyls because the current data suggest that the toxicity of these compounds appear to be mediated by multiple receptors, including PPARα, CAR, and PXR, and that there may be species differences in the response mediated by different receptors. Additionally, available data suggest that there are qualitative differences in the toxicities of various perfluoroalkyls.

Agency Contacts (Chemical Managers): Melanie Buser

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Perfluorodecanoic acid (PFDA)
CAS Numbers: 335-76-2
Date: March 2020
Profile Status: Final
Route: Oral
Duration: Intermediate

MRL Summary: There are insufficient data for derivation of an intermediate-duration oral MRL for PFDA.

Rationale for Not Deriving an MRL: Two studies conducted by Frawley et al. (2018) evaluated the intermediate-duration toxicity of PFDA. In a 28-day study in rats, increases in mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration and decreases in phagocytosis by fixed tissue macrophages in the liver were observed in female rats administered 0.25 mg/kg/day PFDA. At 0.5 mg/kg/day, single cell necrosis was observed. In the second study, decreases in splenic T-cells and macrophages were observed in mice administered 1.25 mg/kg PFDA once a week for 4 weeks.

The Frawley et al. (2018) study was not considered for the principal study since it did not measure serum PFDA levels.

Agency Contacts (Chemical Managers): Melanie Buser

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Perfluorodecanoic acid (PFDA)
CAS Numbers: 335-76-2
Date: March 2020
Profile Status: Final
Route: Oral
Duration: Chronic

MRL Summary: There are insufficient data for derivation of a chronic-duration oral MRL for PFDA.

Rationale for Not Deriving an MRL: No chronic-duration oral studies in laboratory animals were identified for PFDA.

Agency Contacts (Chemical Managers): Melanie Buser

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Perfluoroundecanoic acid (PFUnA)
CAS Numbers: 2058-94-8
Date: March 2020
Profile Status: Final
Route: Inhalation
Duration: Acute

MRL Summary: There are insufficient data for derivation of an acute-duration inhalation MRL for PFUnA.

Rationale for Not Deriving an MRL: No inhalation studies in laboratory animals were identified for PFUnA.

Agency Contacts (Chemical Managers): Melanie Buser

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Perfluoroundecanoic acid (PFUnA)
CAS Numbers: 2058-94-8
Date: March 2020
Profile Status: Final
Route: Inhalation
Duration: Intermediate

MRL Summary: There are insufficient data for derivation of an intermediate-duration inhalation MRL for PFUnA.

Rationale for Not Deriving an MRL: No inhalation studies in laboratory animals were identified for PFUnA.

Agency Contacts (Chemical Managers): Melanie Buser

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Perfluoroundecanoic acid (PFUnA)
CAS Numbers: 2058-94-8
Date: March 2020
Profile Status: Final
Route: Inhalation
Duration: Chronic

MRL Summary: There are insufficient data for derivation of a chronic-duration inhalation MRL for PFUnA.

Rationale for Not Deriving an MRL: No inhalation studies in laboratory animals were identified for PFUnA.

Agency Contacts (Chemical Managers): Melanie Buser

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Perfluoroundecanoic acid (PFUnA)
CAS Numbers: 2058-94-8
Date: March 2020
Profile Status: Final
Route: Oral
Duration: Acute

MRL Summary: There are insufficient data for derivation of an acute-duration oral MRL for PFUnA.

Rationale for Not Deriving an MRL: No acute-duration oral studies in laboratory animals were identified for PFUnA.

Agency Contacts (Chemical Managers): Melanie Buser

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Perfluoroundecanoic acid (PFUnA)
CAS Numbers: 2058-94-8
Date: March 2020
Profile Status: Final
Route: Oral
Duration: Intermediate

MRL Summary: There are insufficient data for derivation of an intermediate-duration oral MRL for PFUnA.

Rationale for Not Deriving an MRL: Intermediate-duration oral database was considered inadequate for derivation of an MRL for PFUnA because the only available study did not measure serum PFUnA levels, which are needed to calculated HEDs (see MRL approach in Appendix A introduction).

One study was identified that examined the oral toxicity of PFUnA in laboratory animals. In this study, decreases in body weight, hematological alterations, increases in liver weight, and centrilobular hypertrophy were observed in rat dams administered 1.0 mg/kg/day for 41–46 days (Takahashi et al. 2014). The study also found decreases in pup body weight on PNDs 0 and 4 at 1.0 mg/kg/day.

Agency Contacts (Chemical Managers): Melanie Buser

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Perfluoroundecanoic acid (PFUnA)
CAS Numbers: 2058-94-8
Date: March 2020
Profile Status: Final
Route: Oral
Duration: Chronic

MRL Summary: There are insufficient data for derivation of a chronic-duration oral MRL for PFUnA.

Rationale for Not Deriving an MRL: No chronic-duration oral studies in laboratory animals were identified for PFUnA.

Agency Contacts (Chemical Managers): Melanie Buser

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Perfluoroheptanoic acid (PFHpA)
CAS Numbers: 375-85-9
Date: March 2020
Profile Status: Final
Route: Inhalation
Duration: Acute

MRL Summary: There are insufficient data for derivation of an acute-duration inhalation MRL for PFHpA.

Rationale for Not Deriving an MRL: No inhalation studies in laboratory animals were identified for PFHpA.

Agency Contacts (Chemical Managers): Melanie Buser

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Perfluoroheptanoic acid (PFHpA)
CAS Numbers: 375-85-9
Date: March 2020
Profile Status: Final
Route: Inhalation
Duration: Intermediate

MRL Summary: There are insufficient data for derivation of an intermediate-duration inhalation MRL for PFHpA.

Rationale for Not Deriving an MRL: No inhalation studies in laboratory animals were identified for PFHpA.

Agency Contacts (Chemical Managers): Melanie Buser

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Perfluoroheptanoic acid (PFHpA)
CAS Numbers: 375-85-9
Date: March 2020
Profile Status: Final
Route: Inhalation
Duration: Chronic

MRL Summary: There are insufficient data for derivation of a chronic-duration inhalation MRL for PFHpA.

Rationale for Not Deriving an MRL: No inhalation studies in laboratory animals were identified for PFHpA.

Agency Contacts (Chemical Managers): Melanie Buser

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Perfluoroheptanoic acid (PFHpA)
CAS Numbers: 375-85-9
Date: March 2020
Profile Status: Final
Route: Oral
Duration: Acute

MRL Summary: There are insufficient data for derivation of an acute-duration oral MRL for PFHpA.

Rationale for Not Deriving an MRL: No oral studies in laboratory animals were identified for PFHpA.

Agency Contacts (Chemical Managers): Melanie Buser

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Perfluoroheptanoic acid (PFHpA)
CAS Numbers: 375-85-9
Date: March 2020
Profile Status: Final
Route: Oral
Duration: Intermediate

MRL Summary: There are insufficient data for derivation of an intermediate-duration oral MRL for PFHpA.

Rationale for Not Deriving an MRL: No oral studies in laboratory animals were identified for PFHpA.

Agency Contacts (Chemical Managers): Melanie Buser

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Perfluoroheptanoic acid (PFHpA)
CAS Numbers: 375-85-9
Date: March 2020
Profile Status: Final
Route: Oral
Duration: Chronic

MRL Summary: There are insufficient data for derivation of a chronic-duration oral MRL for PFHpA.

Rationale for Not Deriving an MRL: No oral studies in laboratory animals were identified for PFHpA.

Agency Contacts (Chemical Managers): Melanie Buser

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Perfluorobutane sulfonic acid (PFBS)
CAS Numbers: 375-73-5
Date: March 2020
Profile Status: Final
Route: Inhalation
Duration: Acute

MRL Summary: There are insufficient data for derivation of an acute-duration inhalation MRL for PFBS.

Rationale for Not Deriving an MRL: No inhalation studies in laboratory animals were identified for PFBS.

Agency Contacts (Chemical Managers): Melanie Buser

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Perfluorobutane sulfonic acid (PFBS)
CAS Numbers: 375-73-5
Date: March 2020
Profile Status: Final
Route: Inhalation
Duration: Intermediate

MRL Summary: There are insufficient data for derivation of an intermediate-duration inhalation MRL for PFBS.

Rationale for Not Deriving an MRL: No inhalation studies in laboratory animals were identified for PFBS.

Agency Contacts (Chemical Managers): Melanie Buser

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Perfluorobutane sulfonic acid (PFBS)
CAS Numbers: 375-73-5
Date: March 2020
Profile Status: Final
Route: Inhalation
Duration: Chronic

MRL Summary: There are insufficient data for derivation of a chronic-duration inhalation MRL for PFBS.

Rationale for Not Deriving an MRL: No inhalation studies in laboratory animals were identified for PFBS.

Agency Contacts (Chemical Managers): Melanie Buser

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Perfluorobutane sulfonic acid (PFBS)
CAS Numbers: 375-73-5
Date: March 2020
Profile Status: Final
Route: Oral
Duration: Acute

MRL Summary: There are insufficient data for derivation of an acute-duration oral MRL for PFBS.

Rationale for Not Deriving an MRL: No acute-duration oral studies in laboratory animals were identified for PFBS.

Agency Contacts (Chemical Managers): Melanie Buser

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Perfluorobutane sulfonic acid (PFBS)
CAS Numbers: 375-73-5
Date: March 2020
Profile Status: Final
Route: Oral
Duration: Intermediate

MRL Summary: There are insufficient data for derivation of an intermediate-duration oral MRL for PFBS.

Rationale for Not Deriving an MRL: Several studies have evaluated the toxicity of PFBS following intermediate-duration oral exposure and have identified several targets of toxicity. However, none of these studies included measurement of serum PFBS levels that are needed to calculate a HED and MRL derivation.

Limited data available on the toxicity of PFBS in laboratory animals have identified the liver, kidneys, stomach, and hematological systems and the developing organism as targets of toxicity. Decreases in hemoglobin and hematocrit levels were observed in male rats administered 200 mg/kg/day PFBS for 90 days (Lieder et al. 2009a); decreases in erythrocyte levels were observed at 600 mg/kg/day. Administration of 600 mg/kg/day for 90 days also resulted in tubular and ductal papillary epithelial hyperplasia in the kidneys and necrosis and hyperplasia/hyperkeratosis in the forestomach (Lieder et al. 2009a). Effects in the liver consisted of decreases in plasma triglyceride levels in mice exposed to 30 mg/kg/day for 4–6 weeks (Bijland et al. 2011), increases in absolute and relative liver weight in male rats administered 300 mg/kg/day for at least 70 days (Lieder et al. 2009b) or 900 mg/kg/day for 28 days (3M 2001), and hepatocellular hypertrophy in rats administered 1,000 mg/kg/day in a 2-generation study (Lieder et al. 2009b). In general, no biologically relevant alterations in performance on FOB tests or motor activity tests were observed in rats administered 900 mg/kg/day PFBS for 28 days (3M 2001) or 600 mg/kg/day for 90 days (Lieder et al. 2009a).

Decreases in fetal body weight were observed in two studies involving administration of PFBS to rats on GDs 6–20 (York 2002, 2003a); one study reported a LOAEL of 1,000 mg/kg/day (York 2002) and the other a LOAEL of 2,000 mg/kg/day with a NOAEL of 1,000 mg/kg/day (York 2003a). In a developmental toxicity mouse study, decreases in pup body weight, developmental delays (eye opening and vaginal opening), impaired development of the reproductive system (delay in first estrous, decreases in ovarian follicles, decreases in uterine endometrial and myometrial thickness), and decreases in total T4 and T3 and increases in TSH were observed in the offspring of mice administered 200 mg/kg/day PFBS (Feng et al. 2017). Decreases in maternal total T4, free T4, and total T3 and increases in TSH were observed at 200 mg/kg/day.

Agency Contacts (Chemical Managers): Melanie Buser

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Perfluorobutane sulfonic acid (PFBS)
CAS Numbers: 375-73-5
Date: March 2020
Profile Status: Final
Route: Oral
Duration: Chronic

MRL Summary: There are insufficient data for derivation of a chronic-duration oral MRL for PFBS.

Rationale for Not Deriving an MRL: No chronic-duration oral studies in laboratory animals were identified for PFBS.

Agency Contacts (Chemical Managers): Melanie Buser

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Perfluorobutanoic acid (PFBA)
CAS Numbers: 375-22-4
Date: March 2020
Profile Status: Final
Route: Inhalation
Duration: Acute

MRL Summary: There are insufficient data for derivation of an acute-duration inhalation MRL for PFBA.

Rationale for Not Deriving an MRL: No inhalation studies in laboratory animals were identified for PFBA.

Agency Contacts (Chemical Managers): Melanie Buser

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Perfluorobutanoic acid (PFBA)
CAS Numbers: 375-22-4
Date: March 2020
Profile Status: Final
Route: Inhalation
Duration: Intermediate

MRL Summary: There are insufficient data for derivation of an intermediate-duration inhalation MRL for PFBA.

Rationale for Not Deriving an MRL: No inhalation studies in laboratory animals were identified for PFBA.

Agency Contacts (Chemical Managers): Melanie Buser

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Perfluorobutanoic acid (PFBA)
CAS Numbers: 375-22-4
Date: March 2020
Profile Status: Final
Route: Inhalation
Duration: Chronic

MRL Summary: There are insufficient data for derivation of a chronic-duration inhalation MRL for PFBA.

Rationale for Not Deriving an MRL: No inhalation studies in laboratory animals were identified for PFBA.

Agency Contacts (Chemical Managers): Melanie Buser

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Perfluorobutanoic acid (PFBA)
CAS Numbers: 375-22-4
Date: March 2020
Profile Status: Final
Route: Oral
Duration: Acute

MRL Summary: There are insufficient data for derivation of an acute-duration oral MRL for PFBA.

Rationale for Not Deriving an MRL: Laboratory animal studies for other perfluoroalkyls have identified immunotoxicity and developmental toxicity as sensitive endpoints following acute-duration oral exposure; these potential targets have not been investigated for PFBA. Thus, the database was considered inadequate for identifying a critical endpoint and evaluating dose-response relationships.

Three studies have examined the acute toxicity of PFBA in laboratory animals for a limited number of potential endpoints. Ikeda et al. (1985) reported that administration of approximately 20 mg/kg/day PFBA in the diet to male rats for 2 weeks did not significantly affect relative liver weight, but increased catalase activity in liver homogenates by 42% and induced peroxisome proliferation, as assessed by electron microscopy. In a similar study, dietary administration of approximately 78 mg/kg/day PFBA to male mice for 10 days induced a 63% increase in absolute liver weight (Permadi et al. 1992). The increase in liver weight was accompanied by changes in enzymes involved in drug metabolism and/or in deactivation of reactive oxygen species; however, PFBA did not have a significant effect on parameters of peroxisomal fatty acid β-oxidation (Permadi et al. 1993). In a more comprehensive study, no significant effect on a wide range of endpoints including body and organ weights, hematology and clinical chemistry, and histopathology were observed in rats administered 184 mg/kg/day for 5 days (3M 2007a).

Agency Contacts (Chemical Managers): Melanie Buser

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Perfluorobutanoic acid (PFBA)
CAS Numbers: 375-22-4
Date: March 2020
Profile Status: Final
Route: Oral
Duration: Intermediate

MRL Summary: There are insufficient data for derivation of an intermediate-duration oral MRL for PFBA.

Rationale for Not Deriving an MRL: The available intermediate-duration database was not considered adequate for derivation of an MRL. Although the available studies have examined potentially sensitive endpoints and developmental toxicity and both studies measured serum PFBA levels, the database is missing a reliable estimate of elimination half-life in humans. Chang et al. (2008b) reported serum half-lives in small groups of subjects (<10 subjects); only 2 of the subjects were females. Because developmental toxicity is one of the more sensitive endpoints, data from females is needed in order to estimate the HED.

The intermediate-duration oral database for PFBA consists of a developmental study in mice (Das et al. 2008) and 28- and 90-day gavage studies in rats (Butenhoff et al. 2012a; van Otterdijk 2007a, 2007b). In the developmental study, PFBA administered to pregnant mice on GDs 1–17 did not affect newborn weight gain or viability (Das et al. 2008). The most sensitive response was a delay in eye opening in the pups at maternal doses of PFBA of 35 mg/kg/day. In the 28- and 90-day studies, hyperplasia/hypertrophy of the follicular epithelium of the thyroid and hepatocellular hypertrophy were observed at ≥30 mg/kg/day (Butenhoff et al. 2012a; van Otterdijk 2007a, 2007b). In addition, the 90-day study reported hematological alterations in male rats dosed with 30 mg/kg/day PFBA. The NOAEL for these effects was 6 mg/kg/day.

Agency Contacts (Chemical Managers): Melanie Buser

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Perfluorobutanoic acid (PFBA)
CAS Numbers: 375-22-4
Date: March 2020
Profile Status: Final
Route: Oral
Duration: Chronic

MRL Summary: There are insufficient data for derivation of a chronic-duration oral MRL for PFBA.

Rationale for Not Deriving an MRL: No chronic-duration oral studies in laboratory animals were identified for PFBA.

Agency Contacts (Chemical Managers): Melanie Buser

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Perfluorododecanoic acid (PFDoDA)
CAS Numbers: 307-55-1
Date: March 2020
Profile Status: Final
Route: Inhalation
Duration: Acute

MRL Summary: There are insufficient data for derivation of an acute-duration inhalation MRL for PFDoDA.

Rationale for Not Deriving an MRL: No inhalation studies in laboratory animals were identified for PFDoDA.

Agency Contacts (Chemical Managers): Melanie Buser

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Perfluorododecanoic acid (PFDoDA)
CAS Numbers: 307-55-1
Date: March 2020
Profile Status: Final
Route: Inhalation
Duration: Intermediate

MRL Summary: There are insufficient data for derivation of an intermediate-duration inhalation MRL for PFDoDA.

Rationale for Not Deriving an MRL: No inhalation studies in laboratory animals were identified for PFDoDA.

Agency Contacts (Chemical Managers): Melanie Buser

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Perfluorododecanoic acid (PFDoDA)
CAS Numbers: 307-55-1
Date: March 2020
Profile Status: Final
Route: Inhalation
Duration: Chronic

MRL Summary: There are insufficient data for derivation of a chronic-duration inhalation MRL for PFDoDA.

Rationale for Not Deriving an MRL: No inhalation studies in laboratory animals were identified for PFDoDA.

Agency Contacts (Chemical Managers): Melanie Buser

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Perfluorododecanoic acid (PFDoDA)
CAS Numbers: 307-55-1
Date: March 2020
Profile Status: Final
Route: Oral
Duration: Acute

MRL Summary: There are insufficient data for derivation of an acute-duration oral MRL for PFDoDA.

Rationale for Not Deriving an MRL: The database was considered inadequate for derivation of an MRL.

Two studies have examined the acute-oral toxicity of PFDoDA. Shi et al. (2007) reported decreases in body weight and decreases in serum testosterone and estradiol levels in rats following a 14-day gavage administration of 5 mg/kg/day (Shi et al. 2007). The study also reported an increase in serum cholesterol levels at 10 mg/kg/day. In the second study, Zhang et al. (2008) found increases in liver weight and hepatic triglyceride and cholesterol levels in rats administered via gavage ≥5 mg/kg/day for 14 days; these liver effects were not considered relevant to humans. Given the limited number of endpoints examined in Shi et al. (2007) this study, including the lack of histopathological examination, this study was not considered suitable for the derivation of an MRL.

Agency Contacts (Chemical Managers): Melanie Buser

APPENDIX A

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Perfluorododecanoic acid (PFDoDA)
CAS Numbers: 307-55-1
Date: March 2020
Profile Status: Final
Route: Oral
Duration: Intermediate

MRL Summary: There are insufficient data for derivation of an intermediate-duration oral MRL for PFDoDA.

Rationale for Not Deriving an MRL: Three intermediate-duration studies examined the oral toxicity of PFDoDA. Decreases in serum estradiol and increases in serum cholesterol were observed in pubertal females exposed to 3 mg/kg/day PFDoDA on PNDs 24–72 (Shi et al. 2009b). In a second study by this group, decreases in serum testosterone levels were observed in male rats administered 0.2 mg/kg/day PFDoDA for 110 days (Shi et al. 2009a). In a one-generation reproductive/developmental toxicity study, increases in maternal deaths were observed in rats administered 2.5 mg/kg/day PFDoDA prior to mating and throughout gestation, and lactation days 1–5 (Kato et al. 2015). Other effects observed in the male and female parental animals administered 2.5 mg/kg/day included decreases in body weight, decreases in mean corpuscular volume and reticulocytes, single cell hepatocellular necrosis (females only), pancreatic interstitial edema (females only), atrophy of the thymic cortex (females only), atrophy of the adrenal cortex (males only), decrease forelimb grip strength (males only), hemorrhage at the implantation site, and continuous diestrus in unmated females (Kato et al. 2015). The study also reported decreases in pup body weight in the only litter with live pups.

The Shi et al. (2009a) study identified the lowest LOAEL of 0.5 mg/kg/day. However, this study was not considered suitable for MRL derivation because it examined a limited number of endpoints (body weight and reproductive toxicity in males). The Kato et al. (2015) study examined a wide range of endpoints, but effects were only observed at a lethal dose.

Agency Contacts (Chemical Managers): Melanie Buser

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Perfluorododecanoic acid (PFDoDA)
CAS Numbers: 307-55-1
Date: March 2020
Profile Status: Final
Route: Oral
Duration: Chronic

MRL Summary: There are insufficient data for derivation of a chronic-duration oral MRL for PFDoDA.

Rationale for Not Deriving an MRL: No chronic-duration oral studies in laboratory animals were identified for PFDoDA.

Agency Contacts (Chemical Managers): Melanie Buser

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Perfluorooctanesulfonamide (FOSA)
CAS Numbers: 754-91-6
Date: March 2020
Profile Status: Final
Route: Inhalation
Duration: Acute

MRL Summary: There are insufficient data for derivation of an acute-duration inhalation MRL for FOSA.

Rationale for Not Deriving an MRL: No inhalation studies in laboratory animals were identified for FOSA.

Agency Contacts (Chemical Managers): Melanie Buser

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Perfluorooctanesulfonamide (FOSA)
CAS Numbers: 754-91-6
Date: March 2020
Profile Status: Final
Route: Inhalation
Duration: Intermediate

MRL Summary: There are insufficient data for derivation of an intermediate-duration inhalation MRL for FOSA.

Rationale for Not Deriving an MRL: No inhalation studies in laboratory animals were identified for FOSA.

Agency Contacts (Chemical Managers): Melanie Buser

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Perfluorooctanesulfonamide (FOSA)
CAS Numbers: 754-91-6
Date: March 2020
Profile Status: Final
Route: Inhalation
Duration: Chronic

MRL Summary: There are insufficient data for derivation of a chronic-duration inhalation MRL for FOSA.

Rationale for Not Deriving an MRL: No inhalation studies in laboratory animals were identified for FOSA.

Agency Contacts (Chemical Managers): Melanie Buser

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Perfluorooctanesulfonamide (FOSA)
CAS Numbers: 754-91-6
Date: March 2020
Profile Status: Final
Route: Oral
Duration: Acute

MRL Summary: There are insufficient data for derivation of an acute-duration oral MRL for FOSA.

Rationale for Not Deriving an MRL: The acute-duration database for FOSA was not considered adequate for identifying critical targets of toxicity because the Seacat and Luebker (2000) study only examined a limited number of potential endpoints and the potential developmental and immunological effects (sensitive targets for other perfluoroalkyls) were not examined.

One laboratory animal study evaluated the acute oral toxicity of FOSA. Seacat and Luebker (2000) did not find alterations in body weight or liver weight in rats administered a single dose of 5 mg/kg/day FOSA.

Agency Contacts (Chemical Managers): Melanie Buser

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Perfluorooctanesulfonamide (FOSA)
CAS Numbers: 754-91-6
Date: March 2020
Profile Status: Final
Route: Oral
Duration: Intermediate

MRL Summary: There are insufficient data for derivation of an intermediate-duration oral MRL for FOSA.

Rationale for Not Deriving an MRL: No intermediate-duration oral studies in laboratory animals were identified for FOSA.

Agency Contacts (Chemical Managers): Melanie Buser

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Perfluorooctanesulfonamide (FOSA)
CAS Numbers: 754-91-6
Date: March 2020
Profile Status: Final
Route: Oral
Duration: Chronic

MRL Summary: There are insufficient data for derivation of a chronic-duration oral MRL for FOSA.

Rationale for Not Deriving an MRL: No chronic-duration oral studies in laboratory animals were identified for FOSA.

Agency Contacts (Chemical Managers): Melanie Buser

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Perfluorohexanoic acid (PFHxA)
CAS Numbers: 307-24-4
Date: March 2020
Profile Status: Final
Route: Inhalation
Duration: Acute

MRL Summary: There are insufficient data for derivation of an acute-duration inhalation MRL for PFHxA.

Rationale for Not Deriving an MRL: No acute-duration inhalation studies in laboratory animals were identified for PFHxA.

Agency Contacts (Chemical Managers): Melanie Buser

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Perfluorohexanoic acid (PFHxA)
CAS Numbers: 307-24-4
Date: March 2020
Profile Status: Final
Route: Inhalation
Duration: Intermediate

MRL Summary: There are insufficient data for derivation of an intermediate-duration inhalation MRL for PFHxA.

Rationale for Not Deriving an MRL: No intermediate-duration inhalation studies in laboratory animals were identified for PFHxA.

Agency Contacts (Chemical Managers): Melanie Buser

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Perfluorohexanoic acid (PFHxA)
CAS Numbers: 307-24-4
Date: March 2020
Profile Status: Final
Route: Inhalation
Duration: Chronic

MRL Summary: There are insufficient data for derivation of a chronic-duration inhalation MRL for PFHxA.

Rationale for Not Deriving an MRL: No chronic-duration inhalation studies in laboratory animals were identified for PFHxA.

Agency Contacts (Chemical Managers): Melanie Buser

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Perfluorohexanoic acid (PFHxA)
CAS Numbers: 307-24-4
Date: March 2020
Profile Status: Final
Route: Oral
Duration: Acute

MRL Summary: There are insufficient data for derivation of an acute-duration oral MRL for PFHxA.

Rationale for Not Deriving an MRL: The acute database for PFHxA was not considered adequate for derivation of an MRL because serum PFHxA levels at the lowest LOAEL were below the detection limit and an elimination half-life has not been estimated for humans; both of these toxicokinetic parameters are needed to estimate HEDs.

Two developmental toxicity studies conducted by Iwai and Hoberman (2014) examined the acute toxicity of PFHxA following gavage administration. Increases in stillborn pups and decreases in pup body weight were observed at 175 mg/kg/day; no effects were observed at 35 mg/kg/day. In the second study, decreases in birth weight and delayed eye opening was observed at 350 mg/kg/day; the NOAEL was 100 mg/kg/day. These studies were not considered adequate for derivation of an MRL because the measured serum PFHxA levels at 35 and 175 mg/kg/day groups were below the limit of detection. Additionally, the elimination half-life has not been estimated in humans.

Agency Contacts (Chemical Managers): Melanie Buser

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Perfluorohexanoic acid (PFHxA)
CAS Numbers: 307-24-4
Date: March 2020
Profile Status: Final
Route: Oral
Duration: Intermediate

MRL Summary: There are insufficient data for derivation of an intermediate-duration oral MRL for PFHxA.

Rationale for Not Deriving an MRL: Five studies have evaluated the intermediate-duration toxicity of PFHxA in rats. A summary of the adverse effects observed in these studies is presented in Table A-20. These studies identify several targets of toxicity including the respiratory tract, erythrocytes, thyroid, thymus, and developing organism; the lowest LOAEL is 100 mg/kg/day for nasal lesions and decrease in body weight gain in males. None of the available studies evaluated immune function, which has been identified as a sensitive target in intermediate oral studies for other perfluoroalkyls.

The data are considered inadequate for MRL derivation because an elimination half-life has not been estimated in humans. Thus, a HED cannot be calculated and an MRL cannot be derived.

Table A-20. Summary of the Adverse Effects Observed in Laboratory Animals Following Intermediate-Duration Oral Exposure to PFHxA

Species and exposure duration	Dose (mg/kg/day)	Effect	Reference
Rat 92–93 days (GW) 30 M, 30 F	20	No effects observed	Loveless et al. 2009
	100	Degeneration/atrophy of nasal olfactory epithelium	
	500	Respiratory metaplasia, decreased RBC, hemoglobin, and hematocrit; increased reticulocytes; thyroid follicular epithelial hypertrophy	
Rat 110–120 days (premating, gestation, lactation) (GW) 20 M, 20 F	20	No effect observed	Loveless et al. 2009
	100	Decreased weight gain in males	
	500	Decreased maternal weight gain, decreased pup body weight during lactation period	
Rat 90 days (GW) 10 M, 10 F	10	No effects observed	Chengelis et al. 2009b
	50	No effects observed	
	200	Slight decrease in RBC, hemoglobin, and hematocrit and increase in reticulocytes	

Table A-20. Summary of the Adverse Effects Observed in Laboratory Animals Following Intermediate-Duration Oral Exposure to PFHxA

Species and exposure duration	Dose (mg/kg/day)	Effect	Reference
Rat 32–44 days (GW) 10–15 M, 10–15 F	50	No effects observed	Kirkpatrick 2005
	150	Decreased hemoglobin levels (males only)	
	315 (TWA dose)	Decreased hemoglobin levels (males only), increased reticulocyte levels (males only), thymic atrophy (females only)	
Rat GDs 1–20 (GW) 22 F	20	No effect observed	Loveless et al. 2009
	100	No effect observed	
	500	Decreased maternal weight gain; decreased fetal body weight	

F = female(s); GD = gestation day; (GW) = gavage in water; M = male(s); RBC = red blood cell; TWA = time-weighted average

Agency Contacts (Chemical Managers): Melanie Buser

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Perfluorohexanoic acid (PFHxA)
CAS Numbers: 307-24-4
Date: March 2020
Profile Status: Final
Route: Oral
Duration: Chronic

MRL Summary: There are insufficient data for derivation of a chronic-duration oral MRL for PFHxA.

Rationale for Not Deriving an MRL: The chronic duration oral database for PFHxA is not considered adequate for derivation of a chronic MRL because the only study available did not measure serum PFHxA levels and elimination half-life data are not available for humans. These toxicokinetic data are needed to derive HEDs.

One study has evaluated the chronic oral toxicity of PFHxA in laboratory animals (Klaunig et al. 2015). Exposure to female rats to 200 mg/kg/day resulted in hematological alterations (decreases in red blood cells and hemoglobin levels and increases in reticulocyte counts), renal effects (tubular degeneration, necrosis, increased urine volume and reduced specific gravity), and liver effects (necrosis); no adverse alterations were observed at 30 mg/kg/day or at 100 mg/kg/day in males. This study was not considered suitable for derivation of an MRL because serum PFHxA levels were not measured. Additionally, an elimination half-life has not been estimated in humans.

Agency Contacts (Chemical Managers): Melanie Buser

APPENDIX B. LITERATURE SEARCH FRAMEWORK FOR PERFLUOROALKYLS

The objective of the toxicological profile is to evaluate the potential for human exposure and the potential health hazards associated with inhalation, oral, or dermal/ocular exposure to perfluoroalkyls.

B.1 LITERATURE SEARCH AND SCREEN

A literature search and screen was conducted to identify studies examining health effects, toxicokinetics, mechanisms of action, susceptible populations, biomarkers, chemical interactions, physical and chemical properties, production, use, environmental fate, environmental releases, and environmental and biological monitoring data for perfluoroalkyls. ATSDR primarily focused on peer-reviewed articles without publication date or language restrictions. Non-peer-reviewed studies that were considered relevant to the assessment of the health effects of perfluoroalkyls have undergone peer review by at least three ATSDR-selected experts who have been screened for conflict of interest. The inclusion criteria used to identify relevant studies examining the health effects of perfluoroalkyls are presented in Table B-1.

Table B-1. Inclusion Criteria for the Literature Search and Screen

- Health Effects
  - Species
    - Human
    - Laboratory mammals
  - Route of exposure
    - Inhalation
    - Oral
    - Dermal (or ocular)
    - Parenteral (these studies will be considered supporting data)
  - Health outcome
    - Death
    - Systemic effects
    - Body weight effects
    - Respiratory effects
    - Cardiovascular effects
    - Gastrointestinal effects
    - Hematological effects
    - Musculoskeletal effects
    - Hepatic effects
    - Renal effects
    - Dermal effects
    - Ocular effects
    - Endocrine effects
    - Immunological effects
    - Neurological effects
    - Reproductive effects
    - Developmental effects

Table B-1. Inclusion Criteria for the Literature Search and Screen

Other noncancer effects
Cancer
Toxicokinetics
Absorption
Distribution
Metabolism
Excretion
PBPK models
Biomarkers
Biomarkers of exposure
Biomarkers of effect
Interactions with other chemicals
Potential for human exposure
Releases to the environment
Air
Water
Soil
Environmental fate
Transport and partitioning
Transformation and degradation
Environmental monitoring
Air
Water
Sediment and soil
Other media
Biomonitoring
General populations
Occupation populations

B.1.1 Literature Search

The current literature search was intended to update the draft toxicological profile for perfluoroalkyls released for public comment in 2015. The following main databases were searched in March 2008, September/October 2013, May 2016, and September 2018:

- PubMed
- National Library of Medicine's TOXLINE
- Scientific and Technical Information Network's TOXCENTER

The search strategy used the chemical names, Chemical Abstracts Service (CAS) numbers, synonyms, and Medical Subject Headings (MeSH) terms for perfluoroalkyls. The query strings used for the literature search are presented in Table B-2.

The search was augmented by searching the Toxic Substances Control Act Test Submissions (TSCATS), NTP website, and National Institute of Health Research Portfolio Online Reporting Tools Expenditures and Results (NIH RePORTER) databases using the queries presented in Table B-3. Additional databases were searched in the creation of various tables and figures, such as the TRI Explorer, the Substance priority list (SPL) resource page, and other items as needed. Regulations applicable to perfluoroalkyls were identified by searching international and U.S. agency websites and documents.

Review articles were identified and used for the purpose of providing background information and identifying additional references. ATSDR also identified reports from the grey literature, which included unpublished research reports, technical reports from government agencies, conference proceedings and abstracts, and theses and dissertations.

Table B-2. Database Query Strings Post Public Comment Searches

Database search date	Query string
PubMed	
9/11/2018	((1763-23-1[rn] OR 2058-94-8[rn] OR 2355-31-9[rn] OR 2991-50-6[rn] OR 307-55-1[rn] OR 335-67-1[rn] OR 335-76-2[rn] OR 355-46-4[rn] OR 375-22-4[rn] OR 375-73-5[rn] OR 375-85-9[rn] OR 375-95-1[rn] OR 754-91-6[rn] OR "perfluorododecanoic acid"[nm] OR "perfluorobutanesulfonic acid"[nm] OR "1,1,2,2,3,3,4,4,4-Nonafluoro-1-butanesulfonic acid"[tw] OR "1,1,2,2,3,3,4,4,4-Nonafluorobutane-1-sulphonic acid"[tw] OR "1,1,2,2,3,3,4,4,5,5,6,6,6-Tridecafluorohexane-1-sulfonic acid"[tw] OR "1-Perfluorobutanesulfonic acid"[tw] OR "2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,9-heptadecafluoro-Nonanoic acid"[tw] OR "2-(N-Ethyl-perfluorooctane sulfonamido) acetic acid"[tw] OR "2-(N-Methyl-perfluorooctane sulfonamido) acetic acid"[tw] OR "C11-PFA"[tw] OR "et-pfosa-acoh"[tw] OR "Glycine, N-ethyl-N-((1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluorooctyl)sulfonyl)-"[tw] OR "Henicosafluoroundecanoic acid"[tw] OR "heptadecafluoro-1-octane sulfonic acid"[tw] OR "Heptadecafluoro-1-octanesulfonic acid"[tw] OR "heptadecafluorooctane sulfonic acid"[tw] OR "Heptadecafluorooctane-1-sulphonic acid"[tw] OR "Heptadecafluorooctanesulphonamide"[tw] OR "Heptafluoro-1-butanoic acid"[tw] OR "Heptafluorobutanoic acid"[tw] OR "Heptafluorobutyric acid"[tw] OR "me-pfosa-acoh"[tw] OR "N-Ethyl-N-((heptadecafluorooctyl)sulphonyl)glycine"[tw] OR "Ndfda"[tw] OR "Nonadecafluoro-n-decanoic acid"[tw] OR "Nonadecafluorodecanoic acid"[tw] OR "Nonafluoro-1-butanesulfonic acid"[tw] OR "Nonafluorobutanesulfonic acid"[tw] OR "Pentadecafluoro-1-octanoic acid"[tw] OR "Pentadecafluoro-n-octanoic acid"[tw] OR "Pentadecafluorooctanoic acid"[tw] OR "Pentyl perfluorobutanoate"[tw] OR "Perfluoro-n-decanoic acid"[tw] OR "Perfluoro-n-heptanoic acid"[tw] OR "Perfluoro-n-nonanoic acid"[tw] OR "Perfluoro-n-undecanoic acid"[tw] OR "Perfluorobutane sulfonic acid"[tw] OR "Perfluorobutanesulfonic acid"[tw] OR "Perfluorobutanoic acid"[tw] OR "Perfluorobutyric acid"[tw] OR "Perfluorocaprylic acid"[tw] OR "Perfluoroctanoic acid"[tw] OR "Perfluoroctylsulfonamide"[tw] OR "Perfluorodecanoic acid"[tw] OR "Perfluorododecanoic acid"[tw] OR "Perfluorododecanoic acid "[tw] OR "Perfluoroheptanecarboxylic acid"[tw] OR "Perfluoroheptanoic acid"[tw] OR "Perfluorohexane sulfonic acid"[tw] OR "Perfluorohexane-1-sulphonic acid"[tw] OR "perfluorohexanesulfonate"[tw] OR "perfluorohexanesulfonic acid"[tw] OR "Perfluorolauric acid"[tw] OR "Perfluorononan-1-oic acid"[tw] OR "Perfluorononanoic acid"[tw] OR "Perfluorooctane sulfonamide"[tw] OR "Perfluorooctane sulfonate"[tw] OR "Perfluorooctane sulfonic acid "[tw] OR "perfluorooctane sulphonic acid"[tw] OR "Perfluorooctanesulfonamide"[tw] OR "Perfluorooctanesulfonate"[tw] OR "perfluorooctanesulfonic acid"[tw] OR "Perfluorooctanesulfonic acid amide"[tw] OR "Perfluorooctanoic acid"[tw] OR "Perfluorooctylsulfonic acid"[tw] OR "Perfluoropropanecarboxylic acid"[tw] OR "Perfluoroundecanoic acid"[tw] OR "pfbus"[tw] OR "PFDA"[tw] OR "pfdea"[tw] OR "pfdoa"[tw] OR "Pfhpa"[tw] OR "PFHS cpd"[tw] OR "pfhxs"[tw] OR "pfna"[tw] OR "PFOA"[tw] OR "PFOS"[tw] OR "pfsoa"[tw] OR "Pfua"[tw] OR "Tricosafluorododecanoic acid"[tw] OR "Tridecafluoro-1-heptanoic acid"[tw] OR "Tridecafluoroheptanoic acid"[tw]) AND (2015/05/01:3000[dp] OR 2016/05/01:3000[mhda] OR 2016/05/01:3000[crdat] OR 2016/05/01:3000[edat])) AND (to[sh] OR po[sh] OR ae[sh] OR pk[sh] OR ai[sh] OR ci[sh] OR bl[sh] OR cf[sh] OR ur[sh] OR "pharmacology"[sh:noexp] OR "environmental exposure"[mh] OR "endocrine system"[mh] OR "hormones, hormone substitutes, and hormone antagonists"[mh] OR "endocrine disruptors"[mh] OR "Computational biology"[mh] OR "Medical Informatics"[mh] OR Genomics[mh] OR Genome[mh] OR Proteomics[mh] OR Proteome[mh] OR

Table B-2. Database Query Strings Post Public Comment Searches

Database search date	Query string
	Metabolomics[mh] OR Metabolome[mh] OR Genes[mh] OR "Gene expression"[mh] OR Phenotype[mh] OR genetics[mh] OR genotype[mh] OR Transcriptome[mh] OR ("Systems Biology"[mh] AND ("Environmental Exposure"[mh] OR "Epidemiological Monitoring"[mh] OR analysis[sh])) OR "Transcription, Genetic "[mh] OR "Reverse transcription"[mh] OR "Transcriptional activation"[mh] OR "Transcription factors"[mh] OR ("biosynthesis"[sh] AND (RNA[mh] OR DNA[mh])) OR "RNA, Messenger"[mh] OR "RNA, Transfer"[mh] OR "peptide biosynthesis"[mh] OR "protein biosynthesis"[mh] OR "Reverse Transcriptase Polymerase Chain Reaction"[mh] OR "Base Sequence"[mh] OR "Trans-activators"[mh] OR "Gene Expression Profiling"[mh] OR cancer[sb] OR "pharmacology"[sh:noexp] OR toxicokinetics[mh:noexp] OR (me[sh] AND ("humans"[mh] OR "animals"[mh]))) (("FC-143 surfactant"[nm] OR 307-24-4[rn] OR 3825-26-1[rn] OR "Hexanoic acid, 2,2,3,3,4,4,5,5,6,6,6-undecafluoro-"[tw] OR "Hexanoic acid, undecafluoro-"[tw] OR "Perfluorohexanoic acid"[tw] OR "Undecafluoro-1-hexanoic acid"[tw] OR "Undecafluorohexanoic acid"[tw] OR "Ammonium pentadecafluorooctanoate"[tw] OR "Ammonium perfluorocaprilate"[tw] OR "Ammonium perfluorocaprylate"[tw] OR "Ammonium perfluorooctanoate"[tw] OR "FC 143"[tw] OR "Octanoic acid, 2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-pentadecafluoro-, ammonium salt"[tw] OR "Octanoic acid, pentadecafluoro-, ammonium salt"[tw] OR "Pentadecafluoro-1-octanoic acid, ammonium salt"[tw] OR "Pentadecafluorooctanoic acid, ammonium salt"[tw] OR "Perfluoroammonium octanoate"[tw] OR "Perfluorooctanoic acid, ammonium salt"[tw]) NOT (1763-23-1[rn] OR 2058-94-8[rn] OR 2355-31-9[rn] OR 2991-50-6[rn] OR 307-55-1[rn] OR 335-67-1[rn] OR 335-76-2[rn] OR 355-46-4[rn] OR 375-22-4[rn] OR 375-73-5[rn] OR 375-85-9[rn] OR 375-95-1[rn] OR 754-91-6[rn] OR "perfluorododecanoic acid"[nm] OR "perfluorobutanesulfonic acid"[nm] OR "1,1,2,2,3,3,4,4,4-Nonafluoro-1-butanesulfonic acid"[tw] OR "1,1,2,2,3,3,4,4,4-Nonafluorobutane-1-sulphonic acid"[tw] OR "1,1,2,2,3,3,4,4,5,5,6,6,6-Tridecafluorohexane-1-sulfonic acid"[tw] OR "1-Perfluorobutanesulfonic acid"[tw] OR "2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,9-heptadecafluoro-Nonanoic acid"[tw] OR "2-(N-Ethyl-perfluorooctane sulfonamido) acetic acid"[tw] OR "2-(N-Methyl-perfluorooctane sulfonamido) acetic acid"[tw] OR "C11-PFA"[tw] OR "et-pfosa-acoh"[tw] OR "Glycine, N-ethyl-N-((1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluorooctyl)sulfonyl)-"[tw] OR "Henicosafluoroundecanoic acid"[tw] OR "heptadecafluoro-1-octane sulfonic acid"[tw] OR "Heptadecafluoro-1-octanesulfonic acid"[tw] OR "heptadecafluorooctane sulfonic acid"[tw] OR "Heptadecafluorooctane-1-sulphonic acid"[tw] OR "Heptadecafluorooctanesulphonamide"[tw] OR "Heptafluoro-1-butanoic acid"[tw] OR "Heptafluorobutanoic acid"[tw] OR "Heptafluorobutyric acid"[tw] OR "me-pfosa-acoh"[tw] OR "N-Ethyl-N-((heptadecafluorooctyl)sulphonyl)glycine"[tw] OR "Ndfda"[tw] OR "Nonadecafluoro-n-decanoic acid"[tw] OR "Nonadecafluorodecanoic acid"[tw] OR "Nonafluoro-1-butanesulfonic acid"[tw] OR "Nonafluorobutanesulfonic acid"[tw] OR "Pentadecafluoro-1-octanoic acid"[tw] OR "Pentadecafluoro-n-octanoic acid"[tw] OR "Pentadecafluorooctanoic acid"[tw] OR "Pentyl perfluorobutanoate"[tw] OR "Perfluoro-n-decanoic acid"[tw] OR "Perfluoro-n-heptanoic acid"[tw] OR "Perfluoro-n-nonanoic acid"[tw] OR "Perfluoro-n-undecanoic acid"[tw] OR "Perfluorobutane sulfonic acid"[tw] OR "Perfluorobutanesulfonic acid"[tw] OR "Perfluorobutanoic acid"[tw] OR "Perfluorobutyric acid"[tw] OR "Perfluorocaprylic acid"[tw] OR "Perfluoroctanoic acid"[tw] OR "Perfluoroctylsulfonamide"[tw] OR "Perfluorodecanoic acid"[tw] OR "Perfluorododecanoic acid"[tw] OR "Perfluorododecanoic acid "[tw] OR "Perfluoroheptanecarboxylic acid"[tw] OR "Perfluoroheptanoic acid"[tw] OR "Perfluorohexane sulfonic acid"[tw] OR "Perfluorohexane-1-sulphonic acid"[tw] OR "perfluorohexanesulfonate"[tw] OR "perfluorohexanesulfonic acid"[tw] OR "Perfluorolauric acid"[tw] OR "Perfluorononan-1-oic acid"[tw] OR "Perfluorononanoic acid"[tw] OR "Perfluorooctane sulfonamide"[tw] OR "Perfluorooctane sulfonate"[tw] OR "Perfluorooctane sulfonic acid "[tw] OR "perfluorooctane sulphonic acid"[tw] OR "Perfluorooctanesulfonamide"[tw] OR "Perfluorooctanesulfonate"[tw] OR "perfluorooctanesulfonic acid"[tw] OR "Perfluorooctanesulfonic acid amide"[tw] OR "Perfluorooctanoic acid"[tw] OR "Perfluorooctylsulfonic acid"[tw] OR "Perfluoropropanecarboxylic acid"[tw] OR "Perfluoroundecanoic acid"[tw] OR "pfbus"[tw] OR "PFDA"[tw] OR "pfdea"[tw] OR "pfdoa"[tw] OR "Pfhpa"[tw] OR "PFHS cpd"[tw] OR "pfhxs"[tw] OR "pfna"[tw] OR "PFOA"[tw] OR "PFOS"[tw] OR "pfsoa"[tw] OR "Pfua"[tw] OR "Tricosafluorododecanoic acid"[tw] OR "Tridecafluoro-1-heptanoic acid"[tw] OR "Tridecafluoroheptanoic acid"[tw])) AND (to[sh] OR po[sh] OR ae[sh] OR pk[sh] OR ai[sh] OR ci[sh] OR bl[sh] OR cf[sh] OR ur[sh] OR "pharmacology"[sh:noexp] OR "environmental exposure"[mh] OR "endocrine system"[mh] OR "hormones, hormone substitutes, and hormone antagonists"[mh] OR

Table B-2. Database Query Strings Post Public Comment Searches

Database search date	Query string
	"endocrine disruptors"[mh] OR "Computational biology"[mh] OR "Medical Informatics"[mh] OR Genomics[mh] OR Genome[mh] OR Proteomics[mh] OR Proteome[mh] OR Metabolomics[mh] OR Metabolome[mh] OR Genes[mh] OR "Gene expression"[mh] OR Phenotype[mh] OR genetics[mh] OR genotype[mh] OR Transcriptome[mh] OR ("Systems Biology"[mh] AND ("Environmental Exposure"[mh] OR "Epidemiological Monitoring"[mh] OR analysis[sh])) OR "Transcription, Genetic "[mh] OR "Reverse transcription"[mh] OR "Transcriptional activation"[mh] OR "Transcription factors"[mh] OR ("biosynthesis"[sh] AND (RNA[mh] OR DNA[mh])) OR "RNA, Messenger"[mh] OR "RNA, Transfer"[mh] OR "peptide biosynthesis"[mh] OR "protein biosynthesis"[mh] OR "Reverse Transcriptase Polymerase Chain Reaction"[mh] OR "Base Sequence"[mh] OR "Trans-activators"[mh] OR "Gene Expression Profiling"[mh] OR cancer[sb] OR "pharmacology"[sh:noexp] OR toxicokinetics[mh:noexp] OR (me[sh] AND ("humans"[mh] OR "animals"[mh]))))
05/24/2016	(((((("1,1,2,2,3,3,4,4,4-Nonafluoro-1-butanesulfonic acid"[tw] OR "1,1,2,2,3,3,4,4,4-Nonafluorobutane-1-sulphonic acid"[tw] OR "1,1,2,2,3,3,4,4,5,5,6,6,6-Tridecafluorohexane-1-sulfonic acid"[tw] OR "1-Perfluorobutanesulfonic acid"[tw] OR "2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,9-heptadecafluoro-Nonanoic acid"[tw] OR "2-(N-Ethyl-perfluorooctane sulfonamido) acetic acid"[tw] OR "2-(N-Methyl-perfluorooctane sulfonamido) acetic acid"[tw] OR "C11-PFA"[tw] OR "et-pfosa-acoh"[tw] OR "Glycine, N-ethyl-N-((1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluorooctyl)sulfonyl)-"[tw] OR "Henicosafluoroundecanoic acid"[tw] OR "heptadecafluoro-1-octane sulfonic acid"[tw] OR "Heptadecafluoro-1-octanesulfonic acid"[tw] OR "heptadecafluorooctane sulfonic acid"[tw] OR "Heptadecafluorooctane-1-sulphonic acid"[tw] OR "Heptadecafluorooctanesulphonamide"[tw] OR "Heptafluoro-1-butanoic acid"[tw] OR "Heptafluorobutanoic acid"[tw] OR "Heptafluorobutyric acid"[tw] OR "me-pfosa-acoh"[tw] OR "N-Ethyl-N-((heptadecafluorooctyl)sulphonyl)glycine"[tw] OR "Ndfda"[tw] OR "Nonadecafluoro-n-decanoic acid"[tw] OR "Nonadecafluorodecanoic acid"[tw] OR "Nonafluoro-1-butanesulfonic acid"[tw] OR "Nonafluorobutanesulfonic acid"[tw] OR "Pentadecafluoro-1-octanoic acid"[tw] OR "Pentadecafluoro-n-octanoic acid"[tw] OR "Pentadecafluorooctanoic acid"[tw] OR "Pentyl perfluorobutanoate"[tw] OR "Perfluoro-n-decanoic acid"[tw] OR "Perfluoro-n-heptanoic acid"[tw] OR "Perfluoro-n-nonanoic acid"[tw] OR "Perfluoro-n-undecanoic acid"[tw] OR "Perfluorobutane sulfonic acid"[tw] OR "Perfluorobutanesulfonic acid"[tw] OR "Perfluorobutanoic acid"[tw] OR "Perfluorobutyric acid"[tw] OR "Perfluorocaprylic acid"[tw] OR "Perfluoroctanoic acid"[tw] OR "Perfluoroctylsulfonamide"[tw] OR "Perfluorodecanoic acid"[tw] OR "Perfluorododecanoic acid "[tw] OR "Perfluorododecanoic acid"[tw] OR "Perfluoroheptanecarboxylic acid"[tw] OR "Perfluoroheptanoic acid"[tw] OR "Perfluorohexane sulfonic acid"[tw] OR "Perfluorohexane-1-sulphonic acid"[tw] OR "perfluorohexanesulfonate"[tw] OR "perfluorohexanesulfonic acid"[tw] OR "Perfluorolauric acid"[tw] OR "Perfluorononan-1-oic acid"[tw] OR "Perfluorononanoic acid"[tw] OR "Perfluorooctane sulfonamide"[tw] OR "Perfluorooctane sulfonate"[tw] OR "Perfluorooctane sulfonic acid "[tw] OR "perfluorooctane sulphonic acid"[tw] OR "Perfluorooctanesulfonamide"[tw] OR "Perfluorooctanesulfonate"[tw] OR "Perfluorooctanesulfonic acid amide"[tw] OR "perfluorooctanesulfonic acid"[tw] OR "Perfluorooctanoic acid"[tw] OR "Perfluorooctylsulfonic acid"[tw] OR "Perfluoropropanecarboxylic acid"[tw] OR "Perfluoroundecanoic acid"[tw] OR "pfbus"[tw] OR "PFDA"[tw] OR "pfdea"[tw] OR "pfdoa"[tw] OR "Pfhpa"[tw] OR "PFHS cpd"[tw] OR "pfhxs"[tw] OR "pfna"[tw] OR "PFOA"[tw] OR "PFOS"[tw] OR "pfsoa"[tw] OR "Pfua"[tw] OR "Tricosafluorododecanoic acid"[tw] OR "Tridecafluoro-1-heptanoic acid"[tw] OR "Tridecafluoroheptanoic acid"[tw]) AND (2013/09/01:3000[crdat] OR 2013/09/01:3000[edat])) NOT medline[sb]))))) OR ((((("1,1,2,2,3,3,4,4,4-Nonafluoro-1-butanesulfonic acid"[tw] OR "1,1,2,2,3,3,4,4,4-Nonafluorobutane-1-sulphonic acid"[tw] OR "1,1,2,2,3,3,4,4,5,5,6,6,6-Tridecafluorohexane-1-sulfonic acid"[tw] OR "1-Perfluorobutanesulfonic acid"[tw] OR "2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,9-heptadecafluoro-Nonanoic acid"[tw] OR "2-(N-Ethyl-perfluorooctane sulfonamido) acetic acid"[tw] OR "2-(N-Methyl-perfluorooctane sulfonamido) acetic acid"[tw] OR "C11-PFA"[tw] OR "et-pfosa-acoh"[tw] OR "Glycine, N-ethyl-N-((1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluorooctyl)sulfonyl)-"[tw] OR "Henicosafluoroundecanoic acid"[tw] OR "heptadecafluoro-1-octane sulfonic acid"[tw] OR "Heptadecafluoro-1-octanesulfonic acid"[tw] OR "heptadecafluorooctane sulfonic acid"[tw] OR "Heptadecafluorooctane-1-sulphonic acid"[tw] OR "Heptadecafluorooctanesulphonamide"[tw] OR "Heptafluoro-1-butanoic acid"[tw] OR "Heptafluorobutanoic acid"[tw] OR "Heptafluorobutyric acid"[tw] OR "me-pfosa-acoh"[tw] OR "N-Ethyl-N-((heptadecafluorooctyl)sulphonyl)glycine"[tw] OR "Ndfda"[tw] OR "Nonadecafluoro-n-decanoic acid"[tw] OR "Nonadecafluorodecanoic acid"[tw] OR "Nonafluoro-1-butanesulfonic acid"[tw] OR "Nonafluorobutanesulfonic acid"[tw] OR "Pentadecafluoro-1-octanoic

Table B-2. Database Query Strings Post Public Comment Searches

Database search date	Query string
	acid"[tw] OR "Pentadecafluoro-n-octanoic acid"[tw] OR "Pentadecafluorooctanoic acid"[tw] OR "Pentyl perfluorobutanoate"[tw] OR "Perfluoro-n-decanoic acid"[tw] OR "Perfluoro-n-heptanoic acid"[tw] OR "Perfluoro-n-nonanoic acid"[tw] OR "Perfluoro-n-undecanoic acid"[tw] OR "Perfluorobutane sulfonic acid"[tw] OR "Perfluorobutanesulfonic acid"[tw] OR "Perfluorobutanoic acid"[tw] OR "Perfluorobutyric acid"[tw] OR "Perfluorocaprylic acid"[tw] OR "Perfluorooctanoic acid"[tw] OR "Perfluorooctylsulfonamide"[tw] OR "Perfluorodecanoic acid"[tw] OR "Perfluorododecanoic acid "[tw] OR "Perfluorododecanoic acid"[tw] OR "Perfluoroheptanecarboxylic acid"[tw] OR "Perfluoroheptanoic acid"[tw] OR "Perfluorohexane sulfonic acid"[tw] OR "Perfluorohexane-1-sulphonic acid"[tw] OR "perfluorohexanesulfonate"[tw] OR "perfluorohexanesulfonic acid"[tw] OR "Perfluorolauric acid"[tw] OR "Perfluorononan-1-oic acid"[tw] OR "Perfluorononanoic acid"[tw] OR "Perfluorooctane sulfonamide"[tw] OR "Perfluorooctane sulfonate"[tw] OR "Perfluorooctane sulfonic acid "[tw] OR "perfluorooctane sulphonic acid"[tw] OR "Perfluorooctanesulfonamide"[tw] OR "Perfluorooctanesulfonate"[tw] OR "Perfluorooctanesulfonic acid amide"[tw] OR "perfluorooctanesulfonic acid"[tw] OR "Perfluorooctanoic acid"[tw] OR "Perfluorooctylsulfonic acid"[tw] OR "Perfluoropropanecarboxylic acid"[tw] OR "Perfluoroundecanoic acid"[tw] OR "pfbus"[tw] OR "PFDA"[tw] OR "pfdea"[tw] OR "pfdoa"[tw] OR "Pfhpa"[tw] OR "PFHS cpd"[tw] OR "pfhxs"[tw] OR "pfna"[tw] OR "PFOA"[tw] OR "PFOS"[tw] OR "pfsoa"[tw] OR "Pfua"[tw] OR "Tricosafluorododecanoic acid"[tw] OR "Tridecafluoro-1-heptanoic acid"[tw] OR "Tridecafluoroheptanoic acid"[tw] OR 1763-23-1[rn] OR 2058-94-8[rn] OR 2355-31-9[rn] OR 2991-50-6[rn] OR 307-55-1[rn] OR 335-67-1[rn] OR 335-76-2[rn] OR 355-46-4[rn] OR 375-22-4[rn] OR 375-73-5[rn] OR 375-85-9[rn] OR 375-95-1[rn] OR 754-91-6[rn]) AND (2013/09/01:3000[mhda] OR 2013/09/01:3000[crdat] OR 2013/09/01:3000[edat])) AND (to[sh] OR po[sh] OR ae[sh] OR pk[sh] OR ai[sh] OR ci[sh] OR bl[sh] OR cf[sh] OR ur[sh] OR "pharmacology"[sh:noexp] OR "environmental exposure"[mh] OR "endocrine system"[mh] OR "hormones, hormone substitutes, and hormone antagonists"[mh] OR "endocrine disruptors"[mh] OR "Computational biology"[mh] OR "Medical Informatics"[mh] OR Genomics[mh] OR Genome[mh] OR Proteomics[mh] OR Proteome[mh] OR Metabolomics[mh] OR Metabolome[mh] OR Genes[mh] OR "Gene expression"[mh] OR Phenotype[mh] OR genetics[mh] OR genotype[mh] OR Transcriptome[mh] OR ("Systems Biology"[mh] AND ("Environmental Exposure"[mh] OR "Epidemiological Monitoring"[mh] OR analysis[sh])) OR "Transcription, Genetic "[mh] OR "Reverse transcription"[mh] OR "Transcriptional activation"[mh] OR "Transcription factors"[mh] OR ("biosynthesis"[sh] AND (RNA[mh] OR DNA[mh])) OR "RNA, Messenger"[mh] OR "RNA, Transfer"[mh] OR "peptide biosynthesis"[mh] OR "protein biosynthesis"[mh] OR "Reverse Transcriptase Polymerase Chain Reaction"[mh] OR "Base Sequence"[mh] OR "Trans-activators"[mh] OR "Gene Expression Profiling"[mh] OR cancer[sb] OR (me[sh] AND ("humans"[mh] OR "animals"[mh]))))
10/03/2013	("Computational biology"[mh] OR "Medical Informatics"[mh] OR Genomics[mh] OR Genome[mh] OR Proteomics[mh] OR Proteome[mh] OR Metabolomics[mh] OR Metabolome[mh] OR Genes[mh] OR "Gene expression"[mh] OR Phenotype[mh] OR genetics[mh] OR genotype[mh] OR Transcriptome[mh] OR ("Systems Biology"[mh] AND ("Environmental Exposure"[mh] OR "Epidemiological Monitoring"[mh] OR analysis[sh])) OR "Transcription, Genetic "[mh] OR "Reverse transcription"[mh] OR "Transcriptional activation"[mh] OR "Transcription factors"[mh] OR ("biosynthesis"[sh] AND (RNA[mh] OR DNA[mh])) OR " RNA, Messenger "[mh] OR " RNA, Transfer"[mh] OR "peptide biosynthesis"[mh] OR "protein biosynthesis"[mh] OR "Reverse Transcriptase Polymerase Chain Reaction"[mh] OR "Base Sequence"[mh] OR "Trans-activators"[mh] OR "Gene Expression Profiling"[mh]) AND ((335-67-1[rn] OR 1763-23-1[rn] OR 355-46-4[rn] OR 2991-50-6[rn] OR 2355-31-9[rn] OR 335-76-2[rn] OR 375-73-5[rn] OR 375-85-9[rn] OR 375-95-1[rn] OR 754-91-6[rn] OR 2058-94-8[rn] OR 307-55-1[rn] OR 375-22-4[rn] OR 80AM718FML[rn]) AND 2007/05/01:2013/10/03[dp])
09/19/2013	(((335-67-1[rn] OR 1763-23-1[rn] OR 355-46-4[rn] OR 2991-50-6[rn] OR 2355-31-9[rn] OR 335-76-2[rn] OR 375-73-5[rn] OR 375-85-9[rn] OR 375-95-1[rn] OR 754-91-6[rn] OR 2058-94-8[rn] OR 307-55-1[rn] OR 375-22-4[rn] OR 80AM718FML[rn]) AND 2007/05/01:2013/09/19[dp]) AND (((Caprylates/metabolism[MeSH Terms] OR Fluorocarbons/metabolism[MeSH Terms] OR "Alkanesulfonic Acids/metabolism"[MeSH Terms] OR "Sulfonic Acids/metabolism"[MeSH Terms] OR "Decanoic Acids/metabolism"[MeSH Terms] OR "Heptanoic Acids/metabolism"[MeSH Terms] OR "Hydrocarbons, Fluorinated/metabolism"[MeSH Terms] OR "Fatty Acids/metabolism"[MeSH Terms]

Table B-2. Database Query Strings Post Public Comment Searches

Database search date	Query string
	OR Sulfonamides/metabolism[MeSH Terms]) AND ("humans"[MeSH Terms] OR "animals"[MeSH Terms])) OR ((Caprylates[MeSH Terms] OR Fluorocarbons[MeSH Terms] OR "Alkanesulfonic Acids"[MeSH Terms] OR "Sulfonic Acids"[MeSH Terms] OR "Decanoic Acids"[MeSH Terms] OR "Heptanoic Acids"[MeSH Terms] OR "Hydrocarbons, Fluorinated"[MeSH Terms] OR "Fatty Acids"[MeSH Terms] OR Sulfonamides[MeSH Terms]) AND (Endocrine System[mh] OR Hormones[mh] OR Endocrine disruptors[mh])) OR ((Caprylates[MeSH Terms] OR Fluorocarbons[MeSH Terms] OR "Alkanesulfonic Acids"[MeSH Terms] OR "Sulfonic Acids"[MeSH Terms] OR "Decanoic Acids"[MeSH Terms] OR "Heptanoic Acids"[MeSH Terms] OR "Hydrocarbons, Fluorinated"[MeSH Terms] OR "Fatty Acids"[MeSH Terms] OR Sulfonamides[MeSH Terms]) AND "environmental exposure"[MeSH Terms]) OR ((Caprylates[MeSH Terms] OR Fluorocarbons[MeSH Terms] OR "Alkanesulfonic Acids"[MeSH Terms] OR "Sulfonic Acids"[MeSH Terms] OR "Decanoic Acids"[MeSH Terms] OR "Heptanoic Acids"[MeSH Terms] OR "Hydrocarbons, Fluorinated"[MeSH Terms] OR "Fatty Acids"[MeSH Terms] OR Sulfonamides[MeSH Terms]) AND "chemically induced"[MeSH Subheading]) OR ((((((((((("caprylates/adverse effects"[MeSH Terms] OR "caprylates/antagonists and inhibitors"[MeSH Terms] OR "caprylates/blood"[MeSH Terms] OR "caprylates/cerebrospinal fluid"[MeSH Terms] OR "caprylates/pharmacokinetics"[MeSH Terms] OR "caprylates/poisoning"[MeSH Terms] OR "caprylates/toxicity"[MeSH Terms] OR "caprylates/urine"[MeSH Terms])))) OR (("fluorocarbons/adverse effects"[MeSH Terms] OR "fluorocarbons/antagonists and inhibitors"[MeSH Terms] OR "fluorocarbons/blood"[MeSH Terms] OR "fluorocarbons/pharmacokinetics"[MeSH Terms] OR "fluorocarbons/poisoning"[MeSH Terms] OR "fluorocarbons/toxicity"[MeSH Terms] OR "fluorocarbons/urine"[MeSH Terms]))) OR (("alkanesulfonic acids/adverse effects"[MeSH Terms] OR "alkanesulfonic acids/antagonists and inhibitors"[MeSH Terms] OR "alkanesulfonic acids/blood"[MeSH Terms] OR "alkanesulfonic acids/cerebrospinal fluid"[MeSH Terms] OR "alkanesulfonic acids/pharmacokinetics"[MeSH Terms] OR "alkanesulfonic acids/poisoning"[MeSH Terms] OR "alkanesulfonic acids/toxicity"[MeSH Terms] OR "alkanesulfonic acids/urine"[MeSH Terms]))) OR (("sulfonic acids/adverse effects"[MeSH Terms] OR "sulfonic acids/antagonists and inhibitors"[MeSH Terms] OR "sulfonic acids/blood"[MeSH Terms] OR "sulfonic acids/cerebrospinal fluid"[MeSH Terms] OR "sulfonic acids/pharmacokinetics"[MeSH Terms] OR "sulfonic acids/poisoning"[MeSH Terms] OR "sulfonic acids/toxicity"[MeSH Terms] OR "sulfonic acids/urine"[MeSH Terms]))) OR (("decanoic acids/adverse effects"[MeSH Terms] OR "decanoic acids/antagonists and inhibitors"[MeSH Terms] OR "decanoic acids/blood"[MeSH Terms] OR "decanoic acids/pharmacokinetics"[MeSH Terms] OR "decanoic acids/poisoning"[MeSH Terms] OR "decanoic acids/toxicity"[MeSH Terms] OR "decanoic acids/urine"[MeSH Terms]))) OR (("heptanoic acids/adverse effects"[MeSH Terms] OR "heptanoic acids/antagonists and inhibitors"[MeSH Terms] OR "heptanoic acids/blood"[MeSH Terms] OR "heptanoic acids/cerebrospinal fluid"[MeSH Terms] OR "heptanoic acids/pharmacokinetics"[MeSH Terms] OR "heptanoic acids/poisoning"[MeSH Terms] OR "heptanoic acids/toxicity"[MeSH Terms] OR "heptanoic acids/urine"[MeSH Terms]))) OR (("hydrocarbons, fluorinated/adverse effects"[MeSH Terms] OR "hydrocarbons, fluorinated/antagonists and inhibitors"[MeSH Terms] OR "hydrocarbons, fluorinated/blood"[MeSH Terms] OR "hydrocarbons, fluorinated/cerebrospinal fluid"[MeSH Terms] OR "hydrocarbons, fluorinated/pharmacokinetics"[MeSH Terms] OR "hydrocarbons, fluorinated/toxicity"[MeSH Terms] OR "hydrocarbons, fluorinated/urine"[MeSH Terms]))) OR (("fatty acids/adverse effects"[MeSH Terms] OR "fatty acids/antagonists and inhibitors"[MeSH Terms] OR "fatty acids/blood"[MeSH Terms] OR "fatty acids/cerebrospinal fluid"[MeSH Terms] OR "fatty acids/pharmacokinetics"[MeSH Terms] OR "fatty acids/poisoning"[MeSH Terms] OR "fatty acids/toxicity"[MeSH Terms] OR "fatty acids/urine"[MeSH Terms]))) OR (("sulfonamides/adverse effects"[MeSH Terms] OR "sulfonamides/antagonists and inhibitors"[MeSH Terms] OR "sulfonamides/blood"[MeSH Terms] OR "sulfonamides/cerebrospinal fluid"[MeSH Terms] OR "sulfonamides/pharmacokinetics"[MeSH Terms] OR "sulfonamides/poisoning"[MeSH Terms] OR "sulfonamides/toxicity"[MeSH Terms] OR "sulfonamides/urine"[MeSH Terms])))))) OR (("Perfluorooctanoic acid"[tw] OR "Pentadecafluoro-1-octanoic acid"[tw] OR "Pentadecafluoro-n-octanoic acid"[tw] OR "Pentadecafluorooctanoic acid"[tw] OR "Perfluorocaprylic acid"[tw] OR "Perfluoroctanoic acid"[tw] OR "Perfluoroheptanecarboxylic acid"[tw] OR "Perfluorooctanoic acid"[tw] OR "Pentadecafluorooctanoic acid"[tw] OR "Perfluorooctanoic acid"[tw] OR "Perfluorooctane sulfonic acid "[tw] OR "Heptadecafluoro-1-octanesulfonic acid"[tw] OR "heptadecafluoro-1-octane sulfonic acid"[tw] OR "Heptadecafluorooctane-1-sulphonic acid"[tw] OR "heptadecafluorooctane sulfonic

Table B-2. Database Query Strings Post Public Comment Searches

Database search date	Query string
	acid"[tw] OR "Perfluorooctane sulfonate"[tw] OR "Perfluorooctylsulfonic acid"[tw] OR "perfluorooctane sulphonic acid"[tw] OR "perfluorooctanesulfonic acid"[tw] OR "Perfluorooctanesulfonate"[tw] OR "Heptadecafluorooctane-1-sulphonic acid1-Perfluorooctanesulfonic acid"[tw] OR "Perfluorohexane sulfonic acid"[tw] OR "pfhxs"[tw] OR "perfluorohexanesulfonic acid"[tw] OR "perfluorohexanesulfonate"[tw] OR "1,1,2,2,3,3,4,4,5,5,6,6,6-Tridecafluorohexane-1-sulfonic acid"[tw] OR "Perfluorohexane-1-sulphonic acid"[tw] OR "PFHS cpd"[tw] OR "2-(N-Ethyl-perfluorooctane sulfonamido) acetic acid"[tw] OR "et-pfosa-acoh"[tw] OR "N-Ethyl-N-((heptadecafluorooctyl)sulphonyl)glycine"[tw] OR "Glycine, N-ethyl-N-((1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluorooctyl)sulfonyl)-"[tw] OR "2-(N-Methyl-perfluorooctane sulfonamido) acetic acid"[tw] OR "me-pfosa-acoh"[tw] OR "Perfluorodecanoic acid"[tw] OR "Nonadecafluoro-n-decanoic acid"[tw] OR "Nonadecafluorodecanoic acid"[tw] OR "Perfluoro-N-decanoic acid"[tw] OR "Perfluoro-n-decanoic acid"[tw] OR "Perfluorodecanoic acid"[tw] OR "Nonadecafluorodecanoic acid"[tw] OR "Perfluoro-N-decanoic acid"[tw] OR "Perfluorobutane sulfonic acid"[tw] OR "Perfluorobutanesulfonic acid"[tw] OR "1,1,2,2,3,3,4,4,4-Nonafluoro-1-butanesulfonic acid"[tw] OR "1-Perfluorobutanesulfonic acid"[tw] OR "Nonafluoro-1-butanesulfonic acid"[tw] OR "Nonafluorobutanesulfonic acid"[tw] OR "Pentyl perfluorobutanoate"[tw] OR "1,1,2,2,3,3,4,4,4-Nonafluorobutane-1-sulphonic acid"[tw] OR "Nonafluoro-1-butanesulfonic acid"[tw] OR "Perfluoroheptanoic acid"[tw] OR "Tridecafluoro-1-heptanoic acid"[tw] OR "Perfluoro-n-heptanoic acid"[tw] OR "Perfluoroheptanoic acid"[tw] OR "Tridecafluoroheptanoic acid"[tw] OR "Perfluorononanoic acid"[tw] OR "Perfluoro-n-nonanoic acid"[tw] OR "2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,9-heptadecafluoro-Nonanoic acid"[tw] OR "Perfluorononan-1-oic acid"[tw] OR "Perfluorooctane sulfonamide"[tw] OR "Perfluorooctanesulfonamide"[tw] OR "Perfluoroctylsulfonamide"[tw] OR "Perfluorooctanesulfonic acid amide"[tw] OR "Heptadecafluorooctanesulphonamide"[tw] OR "Perfluoroundecanoic acid"[tw] OR "Perfluoro-n-undecanoic acid"[tw] OR "Henicosafluoroundecanoic acid"[tw] OR "Perfluorododecanoic acid "[tw] OR "Perfluorododecanoic acid"[tw] OR "Tricosafluorododecanoic acid"[tw] OR "Perfluorolauric acid"[tw] OR "Perfluorobutyric acid"[tw] OR "Heptafluorobutyric acid"[tw] OR "Heptafluoro-1-butanoic acid"[tw] OR "Heptafluorobutanoic acid"[tw] OR "Heptafluorobutyric acid"[tw] OR "Perfluorobutanoic acid"[tw] OR "Perfluoropropanecarboxylic acid"[tw]) NOT medline[sb]) OR (("PFOA"[tw] OR "PFOS"[tw] OR "Pfua"[tw] OR "pfdoa"[tw] OR "C11-PFA"[tw] OR "pfsoa"[tw] OR "pfna"[tw] OR "Pfhpa"[tw] OR "pfbus"[tw] OR "PFDA"[tw] OR "pfdea"[tw] OR "Ndfda"[tw]) NOT medline[sb])
Toxcenter	
9/11/2018	FILE 'TOXCENTER' ENTERED AT 15:57:25 ON 11 SEP 2018 CHARGED TO COST=EH011.10.LB.01.05 L1 7667 SEA FILE=TOXCENTER 1763-23-1 OR 2058-94-8 OR 2355-31-9 OR 2991-50-6 OR 307-55-1 OR 335-67-1 OR 335-76-2 OR 355-46-4 OR 375-22-4 OR 375-73-5 OR 375-85-9 OR 375-95-1 OR 754-91-6 L2 1413 SEA FILE=TOXCENTER 307-24-4 OR 3825-26-1 L4 189 SEA FILE=TOXCENTER L2 NOT L1 L5 7856 SEA FILE=TOXCENTER L1 OR L2 L6 7800 SEA FILE=TOXCENTER L5 NOT TSCATS/FS L7 7460 SEA FILE=TOXCENTER L6 NOT PATENT/DT ACTIVATE TOXQUERY/Q --------- L8 QUE (CHRONIC OR IMMUNOTOX? OR NEUROTOX? OR TOXICOKIN? OR BIOMARKER? OR NEUROLOG?) L9 QUE (PHARMACOKIN? OR SUBCHRONIC OR PBPK OR EPIDEMIOLOGY/ST,CT, IT) L10 QUE (ACUTE OR SUBACUTE OR LD50# OR LD(W)50 OR LC50# OR LC(W)50) L11 QUE (TOXICITY OR ADVERSE OR POISONING)/ST,CT,IT L12 QUE (INHAL? OR PULMON? OR NASAL? OR LUNG? OR RESPIR?) L13 QUE ((OCCUPATION? OR WORKPLACE? OR WORKER?) AND EXPOS?) L14 QUE (ORAL OR ORALLY OR INGEST? OR GAVAGE? OR DIET OR DIETS OR DIETARY OR DRINKING(W)WATER?)

Table B-2. Database Query Strings Post Public Comment Searches

Database search date	Query string
	L15 QUE (MAXIMUM AND CONCENTRATION? AND (ALLOWABLE OR PERMISSIBLE))
	L16 QUE (ABORT? OR ABNORMALIT? OR EMBRYO? OR CLEFT? OR FETUS?)
	L17 QUE (FOETUS? OR FETAL? OR FOETAL? OR FERTIL? OR MALFORM? OR OVUM?)
	L18 QUE (OVA OR OVARY OR PLACENTA? OR PREGNAN? OR PRENATAL?)
	L19 QUE (PERINATAL? OR POSTNATAL? OR REPRODUC? OR STERIL? OR TERATOGEN?)
	L20 QUE (SPERM OR SPERMAC? OR SPERMAG? OR SPERMATI? OR SPERMAS? OR SPERMATOB? OR SPERMATOC? OR SPERMATOG?)
	L21 QUE (SPERMATOI? OR SPERMATOL? OR SPERMATOR? OR SPERMATOX? OR SPERMATOZ? OR SPERMATU? OR SPERMI? OR SPERMO?)
	L22 QUE (NEONAT? OR NEWBORN? OR DEVELOPMENT OR DEVELOPMENTAL?)
	L23 QUE (ENDOCRIN? AND DISRUPT?)
	L24 QUE (ZYGOTE? OR CHILD OR CHILDREN OR ADOLESCEN? OR INFANT?)
	L25 QUE (WEAN? OR OFFSPRING OR AGE(W)FACTOR?)
	L26 QUE (DERMAL? OR DERMIS OR SKIN OR EPIDERM? OR CUTANEOUS?)
	L27 QUE (CARCINOG? OR COCARCINOG? OR CANCER? OR PRECANCER? OR NEOPLAS?)
	L28 QUE (TUMOR? OR TUMOUR? OR ONCOGEN? OR LYMPHOMA? OR CARCINOM?)
	L29 QUE (GENETOX? OR GENOTOX? OR MUTAGEN? OR GENETIC(W)TOXIC?)
	L30 QUE (NEPHROTOX? OR HEPATOTOX?)
	L31 QUE (ENDOCRIN? OR ESTROGEN? OR ANDROGEN? OR HORMON?)
	L32 QUE (OCCUPATION? OR WORKER? OR WORKPLACE? OR EPIDEM?)
	L33 QUE L8 OR L9 OR L10 OR L11 OR L12 OR L13 OR L14 OR L15 OR L16 OR L17 OR L18 OR L19 OR L20 OR L21 OR L22 OR L23 OR L24 OR L25 OR L26 OR L27 OR L28 OR L29 OR L30 OR L31 OR L32
	L34 QUE (RAT OR RATS OR MOUSE OR MICE OR GUINEA(W)PIG? OR MURIDAE OR DOG OR DOGS OR RABBIT? OR HAMSTER? OR PIG OR PIGS OR SWINE OR PORCINE OR MONKEY? OR MACAQUE?)
	L35 QUE (MARMOSET? OR FERRET? OR GERBIL? OR RODENT? OR LAGOMORPHA OR BABOON? OR CANINE OR CAT OR CATS OR FELINE OR MURINE)
	L36 QUE L33 OR L34 OR L35
	L37 QUE (HUMAN OR HUMANS OR HOMINIDAE OR MAMMALS OR MAMMAL? OR PRIMATES OR PRIMATE?)
	L38 QUE L36 OR L37

	L39 5371 SEA FILE=TOXCENTER L7 AND L38
	L40 1566 SEA FILE=TOXCENTER L39 AND MEDLINE/FS
	L41 1116 SEA FILE=TOXCENTER L39 AND BIOSIS/FS
	L42 2649 SEA FILE=TOXCENTER L39 AND CAPLUS/FS
	L43 40 SEA FILE=TOXCENTER L39 NOT (L40 OR L41 OR L42)
	L44 3543 DUP REM L40 L41 L43 L42 (1828 DUPLICATES REMOVED)
	ANSWERS '1-3543' FROM FILE TOXCENTER
	L*** DEL 1566 S L39 AND MEDLINE/FS
	L*** DEL 1566 S L39 AND MEDLINE/FS
	L45 1566 SEA FILE=TOXCENTER L44
	L*** DEL 1116 S L39 AND BIOSIS/FS
	L*** DEL 1116 S L39 AND BIOSIS/FS
	L46 594 SEA FILE=TOXCENTER L44
	L*** DEL 2649 S L39 AND CAPLUS/FS
	L*** DEL 2649 S L39 AND CAPLUS/FS
	L47 1350 SEA FILE=TOXCENTER L44
	L*** DEL 40 S L39 NOT (L40 OR L41 OR L42)
	L*** DEL 40 S L39 NOT (L40 OR L41 OR L42)
	L48 33 SEA FILE=TOXCENTER L44

Table B-2. Database Query Strings Post Public Comment Searches

Database search date	Query string
	L49 865 SEA FILE=TOXCENTER (L45 OR L46 OR L47 OR L48) AND (ED>20160401 OR PY>2015) L*** DEL 1566 S L39 AND MEDLINE/FS L*** DEL 1566 S L39 AND MEDLINE/FS L50 1566 SEA FILE=TOXCENTER L44 L*** DEL 1116 S L39 AND BIOSIS/FS L*** DEL 1116 S L39 AND BIOSIS/FS L51 594 SEA FILE=TOXCENTER L44 L*** DEL 2649 S L39 AND CAPLUS/FS L*** DEL 2649 S L39 AND CAPLUS/FS L52 1350 SEA FILE=TOXCENTER L44 L*** DEL 40 S L39 NOT (L40 OR L41 OR L42) L*** DEL 40 S L39 NOT (L40 OR L41 OR L42) L53 33 SEA FILE=TOXCENTER L44 L54 2678 SEA FILE=TOXCENTER (L50 OR L51 OR L52 OR L53) NOT L49 L55 43 SEA FILE=TOXCENTER L54 AND L4 L56 908 SEA FILE=TOXCENTER L49 OR L55 SAVE TEMP L56 PFOA/Q D SCAN L56
05/25/2016	FILE 'TOXCENTER' ENTERED AT 08:37:55 ON 25 MAY 2016 L1 5994 SEA 335-67-1 OR 1763-23-1 OR 355-46-4 OR 2991-50-6 OR 2355-31-9 OR 335-76-2 OR 375-73-5 OR 375-85-9 OR 375-95-1 OR 754-91-6 OR 2058-94-8 OR 307-55-1 OR 375-22-4 L2 5967 SEA L1 NOT TSCATS/FS L3 5731 SEA L2 NOT PATENT/DT L4 1847 SEA L3 AND ED>=20130701 ACT TOXQUERY/Q L5 QUE (CHRONIC OR IMMUNOTOX? OR NEUROTOX? OR TOXICOKIN? OR BIOMARKER? OR NEUROLOG?) L6 QUE (PHARMACOKIN? OR SUBCHRONIC OR PBPK OR EPIDEMIOLOGY/ST,CT, IT) L7 QUE (ACUTE OR SUBACUTE OR LD50# OR LD(W)50 OR LC50# OR LC(W)50) L8 QUE (TOXICITY OR ADVERSE OR POISONING)/ST,CT,IT L9 QUE (INHAL? OR PULMON? OR NASAL? OR LUNG? OR RESPIR?) L10 QUE ((OCCUPATION? OR WORKPLACE? OR WORKER?) AND EXPOS?) L11 QUE (ORAL OR ORALLY OR INGEST? OR GAVAGE? OR DIET OR DIETS OR DIETARY OR DRINKING(W)WATER?) L12 QUE (MAXIMUM AND CONCENTRATION? AND (ALLOWABLE OR PERMISSIBLE)) L13 QUE (ABORT? OR ABNORMALIT? OR EMBRYO? OR CLEFT? OR FETUS?) L14 QUE (FOETUS? OR FETAL? OR FOETAL? OR FERTIL? OR MALFORM? OR OVUM?) L15 QUE (OVA OR OVARY OR PLACENTA? OR PREGNAN? OR PRENATAL?) L16 QUE (PERINATAL? OR POSTNATAL? OR REPRODUC? OR STERIL? OR TERATOGEN?) L17 QUE (SPERM OR SPERMAC? OR SPERMAG? OR SPERMATI? OR SPERMAS? OR SPERMATOB? OR SPERMATOC? OR SPERMATOG?) L18 QUE (SPERMATOI? OR SPERMATOL? OR SPERMATOR? OR SPERMATOX? OR SPERMATOZ? OR SPERMATU? OR SPERMI? OR SPERMO?) L19 QUE (NEONAT? OR NEWBORN? OR DEVELOPMENT OR DEVELOPMENTAL?) L20 QUE (ENDOCRIN? AND DISRUPT?) L21 QUE (ZYGOTE? OR CHILD OR CHILDREN OR ADOLESCEN? OR INFANT?) L22 QUE (WEAN? OR OFFSPRING OR AGE(W)FACTOR?) L23 QUE (DERMAL? OR DERMIS OR SKIN OR EPIDERM? OR CUTANEOUS?) L24 QUE (CARCINOG? OR COCARCINOG? OR CANCER? OR PRECANCER? OR NEOPLAS?)

Table B-2. Database Query Strings Post Public Comment Searches

Database search date	Query string
	L25 QUE (TUMOR? OR TUMOUR? OR ONCOGEN? OR LYMPHOMA? OR CARCINOM?)
	L26 QUE (GENETOX? OR GENOTOX? OR MUTAGEN? OR GENETIC(W)TOXIC?)
	L27 QUE (NEPHROTOX? OR HEPATOTOX?)
	L28 QUE (ENDOCRIN? OR ESTROGEN? OR ANDROGEN? OR HORMON?)
	L29 QUE (OCCUPATION? OR WORKER? OR WORKPLACE? OR EPIDEM?)
	L30 QUE L5 OR L6 OR L7 OR L8 OR L9 OR L10 OR L11 OR L12 OR L13 OR L14 OR L15 OR L16 OR L17 OR L18 OR L19 OR L20 OR L21 OR L22 OR L23 OR L24 OR L25 OR L26 OR L27 OR L28 OR L29
	L31 QUE (RAT OR RATS OR MOUSE OR MICE OR GUINEA(W)PIG? OR MURIDAE OR DOG OR DOGS OR RABBIT? OR HAMSTER? OR PIG OR PIGS OR SWINE OR PORCINE OR MONKEY? OR MACAQUE?)
	L32 QUE (MARMOSET? OR FERRET? OR GERBIL? OR RODENT? OR LAGOMORPHA OR BABOON? OR CANINE OR CAT OR CATS OR FELINE OR MURINE)
	L33 QUE L30 OR L31 OR L32
	L34 QUE (NONHUMAN MAMMALS)/ORGN
	L35 QUE L33 OR L34
	L36 QUE (HUMAN OR HUMANS OR HOMINIDAE OR MAMMALS OR MAMMAL? OR PRIMATES OR PRIMATE?)
	L37 QUE L35 OR L36
	L38 1318 SEA L4 AND L37
	L39 1148 SEA L4 AND L30
	L40 356 SEA L38 AND MEDLINE/FS
	L41 297 SEA L38 AND BIOSIS/FS
	L42 664 SEA L38 AND CAPLUS/FS
	L43 1 SEA L38 NOT (MEDLINE/FS OR BIOSIS/FS OR CAPLUS/FS)
	L44 931 DUP REM L40 L41 L43 L42 (387 DUPLICATES REMOVED)
	L*** DEL 356 S L38 AND MEDLINE/FS
	L*** DEL 356 S L38 AND MEDLINE/FS
	L45 356 SEA L44
	L*** DEL 297 S L38 AND BIOSIS/FS
	L*** DEL 297 S L38 AND BIOSIS/FS
	L46 190 SEA L44
	L*** DEL 664 S L38 AND CAPLUS/FS
	L*** DEL 664 S L38 AND CAPLUS/FS
	L47 385 SEA L44
	L48 575 SEA (L45 OR L46 OR L47) NOT MEDLINE/FS
	D SCAN L48
09/19/2013	FILE 'TOXCENTER' ENTERED AT 09:10:51 ON 19 SEP 2013
	L1 3993 SEA 335-67-1 OR 1763-23-1 OR 355-46-4 OR 2991-50-6 OR 2355-31-9 OR 335-76-2 OR 375-73-5 OR 375-85-9 OR 375-95-1 OR 754-91-6 OR 2058-94-8 OR 307-55-1 OR 375-22-4
	L2 3966 SEA L1 NOT TSCATS/FS
	L3 3782 SEA L2 NOT PATENT/DT
	L4 2796 SEA L3 AND PY>2006
	ACTIVATE TOXBROAD/Q
	L5 QUE (CHRONIC OR IMMUNOTOX? OR NEUROTOX? OR TOXICOKIN? OR BIOMARKER? OR NEUROLOG?)
	L6 QUE (PHARMACOKIN? OR SUBCHRONIC OR PBPK OR EPIDEMIOLOGY/ST,CT)
	L7 QUE (ACUTE OR SUBACUTE OR LD50 OR LC50)
	L8 QUE (TOXICITY OR ADVERSE OR POISONING)/ST,CT
	L9 QUE (INHAL? OR PULMON? OR NASAL? OR LUNG? OR RESPIR?)
	L10 QUE (VAPOR? OR VAPOUR? OR AEROSOL?)
	L11 QUE ((OCCUPATION? OR WORKPLACE? OR WORKER?) AND EXPOS?)
	L12 QUE (ORAL OR ORALLY OR INGEST? OR GAVAGE? OR DIET? OR DRINKING(W)WATER?)
	L13 QUE (MAXIMUM AND CONCENTRATION? AND (ALLOWABLE OR PERMISSIBLE))

Table B-2. Database Query Strings Post Public Comment Searches

Database search date	Query string
	L14 QUE (ABORT? OR ABNORMALIT? OR EMBRYO? OR CLEFT? OR FETUS?)
	L15 QUE (FOETUS? OR FETAL? OR FOETAL? OR FERTIL? OR MALFORM? OR OVUM?)
	L16 QUE (OVA OR OVARY OR PLACENTA? OR PREGNAN? OR PRENATAL?)
	L17 QUE (PERINATAL? OR POSTNATAL? OR REPRODUC? OR STERIL? OR TERATOGEN?)
	L18 QUE (SPERM? OR NEONAT? OR NEWBORN? OR DEVELOPMENT OR DEVELOPMEN TAL?)
	L19 QUE (ENDOCRIN? AND DISRUPT?)
	L20 QUE (ZYGOTE? OR CHILD OR CHILDREN OR ADOLESCEN? OR INFANT?)
	L21 QUE (WEAN? OR OFFSPRING OR AGE(W)FACTOR?)
	L22 QUE (DERMAL? OR DERMIS OR SKIN OR EPIDERM? OR CUTANEOUS?)
	L23 QUE (CARCINOG? OR COCARCINOG? OR CANCER? OR PRECANCER? OR NEOPLAS?)
	L24 QUE (TUMOR? OR TUMOUR? OR ONCOGEN? OR LYMPHOMA? OR CARCINOM?)
	L25 QUE (GENETOX? OR GENOTOX? OR MUTAGEN?)
	L26 QUE GENETIC(W)TOXIC?
	L27 QUE L5 OR L6 OR L7 OR L9 OR L10 OR L11 OR L12 OR L13 OR L14 OR L15 OR L16 OR L17 OR L18 OR L19 OR L20 OR L21
	L28 QUE L27 OR L22 OR L23 OR L24 OR L25 OR L26
	L29 QUE L28 OR L8
	L30 QUE NEPHROTOX? OR HEPATOTOX? OR ENDOCRIN? OR ESTROGEN? OR ANDROGEN? OR HORMON?
	L31 QUE L29 OR L30
	L32 QUE RAT OR RATS OR MOUSE OR MICE OR GUINEA PIG OR MURIDAE OR DOG OR DOGS OR RABBIT? OR HAMSTER? OR PIG OR PIGS OR SWINE OR PORCINE OR GOAT OR GOATS OR SHEEP OR MONKEY? OR MACAQUE?
	L33 QUE MARMOSET? OR FERRET? OR GERBIL? OR HAMSTER? OR RODENT? OR LAGOMORPHA OR BABOON? OR BOVINE OR CANINE OR CAT OR CATS OR FELINE OR PIGEON?
	L34 QUE OCCUPATION? OR WORKER? OR WORKPLACE? OR EPIDEM?
	L35 QUE L31 OR L32 OR L33 OR L34
	L36 QUE NONHUMAN MAMMALS/ORGN
	L37 QUE L35 OR L36
	L38 QUE HUMAN? OR HOMINIDAE OR MAMMAL? OR PRIMATE?
	L39 QUE L37 OR L38
	L40 2012 SEA L4 AND L39
	L41 619 SEA L40 AND MEDLINE/FS
	L42 417 SEA L40 AND BIOSIS/FS
	L43 975 SEA L40 AND CAPLUS/FS
	L44 1 SEA L40 NOT (MEDLINE/FS OR BIOSIS/FS OR CAPLUS/FS)
	L45 1308 DUP REM L41 L42 L44 L43 (704 DUPLICATES REMOVED)
	L*** DEL 619 S L40 AND MEDLINE/FS
	L*** DEL 619 S L40 AND MEDLINE/FS
	L46 619 SEA L45
	L*** DEL 417 S L40 AND BIOSIS/FS
	L*** DEL 417 S L40 AND BIOSIS/FS
	L47 217 SEA L45
	L*** DEL 975 S L40 AND CAPLUS/FS
	L*** DEL 975 S L40 AND CAPLUS/FS
	L48 471 SEA L45
	L*** DEL 1 S L40 NOT (MEDLINE/FS OR BIOSIS/FS OR CAPLUS/FS)
	L*** DEL 1 S L40 NOT (MEDLINE/FS OR BIOSIS/FS OR CAPLUS/FS)
	L49 1 SEA L45
	L50 689 SEA (L46 OR L47 OR L48 OR L49) NOT MEDLINE/FS

Table B-2. Database Query Strings Post Public Comment Searches

Database search date	Query string
	SAVE TEMP L50 PERFLUOROALKYLS/A PFOA/A L51 217 SEA L50 AND BIOSIS/FS L52 471 SEA L50 AND CAPLUS/FS L53 220 SEA L52 AND 4-?/CC L54 1 SEA L50 NOT (MEDLINE/FS OR BIOSIS/FS OR CAPLUS/FS) L55 438 SEA L51 OR L53 OR L54 L56 689 SEA L55 OR L52 D SCAN L55
ToxLine	
9/11/2018	(1763-23-1 [rn] OR 2058-94-8 [rn] OR 2355-31-9 [rn] OR 2991-50-6 [rn] OR 307-55-1 [rn] OR 335-67-1 [rn] OR 335-76-2 [rn] OR 355-46-4 [rn] OR 375-22-4 [rn] OR 375-73-5 [rn] OR 375-85-9 [rn] OR 375-95-1 [rn] OR 754-91-6 [rn]) AND 2015:2017 [yr] AND (ANEUPL [org] OR BIOSIS [org] OR CIS [org] OR DART [org] OR EMIC [org] OR EPIDEM [org] OR FEDRIP [org] OR HEEP [org] OR HMTC [org] OR IPA [org] OR RISKLINE [org] OR MTGABS [org] OR NIOSH [org] OR NTIS [org] OR PESTAB [org] OR PPBIB [org]) AND NOT PubMed [org] AND NOT pubdart [org] ("perfluoro n heptanoic acid" OR "perfluoro n nonanoic acid" OR "perfluoro n undecanoic acid" OR "perfluorobutane sulfonic acid" OR "perfluorobutanesulfonic acid" OR "perfluorobutanoic acid" OR "perfluorobutyric acid" OR "perfluorocaprylic acid" OR "perfluorooctanoic acid" OR "perfluorooctylsulfonamide" OR "perfluorodecanoic acid" OR "perfluorododecanoic acid" OR "perfluorododecanoic acid " OR "perfluoroheptanecarboxylic acid" OR "perfluoroheptanoic acid" OR "perfluorohexane sulfonic acid" OR "perfluorohexane 1 sulphonic acid" OR "perfluorohexanesulfonate" OR "perfluorohexanesulfonic acid" OR "perfluorolauric acid" OR "perfluorononan 1 oic acid" OR "perfluorononanoic acid" OR "perfluorooctane sulfonamide" OR "perfluorooctane sulfonate" OR "perfluorooctane sulfonic acid " OR "perfluorooctane sulphonic acid" OR "perfluorooctanesulfonamide" OR "perfluorooctanesulfonate" OR "perfluorooctanesulfonic acid" OR "perfluorooctanesulfonic acid amide" OR "perfluorooctanoic acid" OR "perfluorooctylsulfonic acid" OR "perfluoropropanecarboxylic acid" OR "perfluoroundecanoic acid" OR "pfbus" OR "pfda" OR "pfdea" OR "pfdoa" OR "pfhpa" OR "pfhs cpd" OR "pfhxs" OR "pfna" OR "pfoa" OR "pfos" OR "pfsoa" OR "pfua" OR "tricosafluorododecanoic acid" OR "tridecafluoro 1 heptanoic acid" OR "tridecafluoroheptanoic acid") AND 2015:2017 [yr] AND (ANEUPL [org] OR BIOSIS [org] OR CIS [org] OR DART [org] OR EMIC [org] OR EPIDEM [org] OR FEDRIP [org] OR HEEP [org] OR HMTC [org] OR IPA [org] OR RISKLINE [org] OR MTGABS [org] OR NIOSH [org] OR NTIS [org] OR PESTAB [org] OR PPBIB [org]) AND NOT PubMed [org] AND NOT pubdart [org] ("1 1 2 2 3 3 4 4 4 nonafluoro 1 butanesulfonic acid" OR "1 1 2 2 3 3 4 4 4 nonafluorobutane 1 sulphonic acid" OR "1 1 2 2 3 3 4 4 5 5 6 6 6 tridecafluorohexane 1 sulfonic acid" OR "1 perfluorobutanesulfonic acid" OR "2 2 3 3 4 4 5 5 6 6 7 7 8 8 9 9 9 heptadecafluoro nonanoic acid" OR "2 (n ethyl perfluorooctane sulfonamido) acetic acid" OR "2 (n methyl perfluorooctane sulfonamido) acetic acid" OR "c11 pfa" OR "et pfosa acoh" OR "glycine n ethyl n ((1 1 2 2 3 3 4 4 5 5 6 6 7 7 8 8 8 heptadecafluorooctyl) sulfonyl) " OR "henicosafluoroundecanoic acid" OR "heptadecafluoro 1 octane sulfonic acid" OR "heptadecafluoro 1 octanesulfonic acid" OR "heptadecafluorooctane sulfonic acid" OR "heptadecafluorooctane 1 sulphonic acid" OR "heptadecafluorooctanesulphonamide" OR "heptafluoro 1 butanoic acid" OR "heptafluorobutanoic acid" OR "heptafluorobutyric acid" OR "me pfosa acoh" OR "n ethyl n ((heptadecafluorooctyl) sulphonyl) glycine" OR "ndfda" OR "nonadecafluoro n decanoic acid" OR "nonadecafluorodecanoic acid" OR "nonafluoro 1 butanesulfonic acid" OR "nonafluorobutanesulfonic acid" OR "pentadecafluoro 1 octanoic acid" OR "pentadecafluoro n octanoic acid" OR "pentadecafluorooctanoic acid" OR "pentyl perfluorobutanoate" OR "perfluoro n decanoic acid") AND 2015:2017 [yr] AND (ANEUPL [org] OR BIOSIS [org] OR CIS [org] OR DART [org] OR EMIC [org] OR EPIDEM [org] OR FEDRIP [org] OR HEEP [org] OR HMTC [org] OR IPA [org] OR RISKLINE [org] OR MTGABS [org] OR NIOSH [org] OR NTIS [org] OR PESTAB [org] OR PPBIB [org]) AND NOT PubMed [org] AND NOT pubdart [org] (307-24-4 [rn] OR 3825-26-1 [rn] OR "hexanoic acid 2 2 3 3 4 4 5 5 6 6 6-undecafluoro-" OR "hexanoic acid undecafluoro-" OR "perfluorohexanoic acid" OR "undecafluoro-1-hexanoic acid" OR "undecafluorohexanoic acid" OR "ammonium pentadecafluorooctanoate" OR "ammonium

Table B-2. Database Query Strings Post Public Comment Searches

Database search date	Query string
	perfluorocaprilate" OR "ammonium perfluorocaprylate" OR "ammonium perfluorooctanoate" OR "fc 143" OR "octanoic acid 2 2 3 3 4 4 5 5 6 6 7 7 8 8 8-pentadecafluoro- ammonium salt" OR "octanoic acid pentadecafluoro- ammonium salt" OR "pentadecafluoro-1-octanoic acid ammonium salt" OR "pentadecafluorooctanoic acid ammonium salt" OR "perfluoroammonium octanoate" OR "perfluorooctanoic acid ammonium salt") AND 1900:2017 [yr] AND (ANEUPL [org] OR BIOSIS [org] OR CIS [org] OR DART [org] OR EMIC [org] OR EPIDEM [org] OR FEDRIP [org] OR HEEP [org] OR HMTC [org] OR IPA [org] OR RISKLINE [org] OR MTGABS [org] OR NIOSH [org] OR NTIS [org] OR PESTAB [org] OR PPBIB [org]) AND NOT PubMed [org] AND NOT pubdart [org]
05/24/2016	((("C11-PFA" OR "et-pfosa-acoh" OR "Henicosafluoroundecanoic acid" OR "heptadecafluoro-1-octane sulfonic acid" OR "Heptadecafluoro-1-octanesulfonic acid" OR "heptadecafluorooctane sulfonic acid" OR "Heptadecafluorooctane-1-sulphonic acid" OR "Heptadecafluorooctane-1-sulphonic acid1-Perfluorooctanesulfonic acid" OR "Heptadecafluorooctanesulphonamide" OR "Heptafluoro-1-butanoic acid" OR "Heptafluorobutanoic acid" OR "Heptafluorobutyric acid" OR "me-pfosa-acoh" OR "N-Ethyl-N-((heptadecafluorooctyl)sulphonyl)glycine" OR "Ndfda" OR "Nonadecafluoro-n-decanoic acid" OR "Nonadecafluorodecanoic acid" OR "Nonafluoro-1-butanesulfonic acid" OR "Nonafluorobutanesulfonic acid" OR "Pentadecafluoro-1-octanoic acid" OR "Pentadecafluoro-n-octanoic acid" OR "Pentadecafluorooctanoic acid" OR "Pentyl perfluorobutanoate" OR "Perfluoro-n-decanoic acid" OR "Perfluoro-n-heptanoic acid" OR "Perfluoro-n-nonanoic acid" OR "Perfluoro-n-undecanoic acid" OR "Perfluorobutane sulfonic acid" OR "Perfluorobutanesulfonic acid" OR "Perfluorobutanoic acid" OR "Perfluorobutyric acid" OR "Perfluorocaprylic acid" OR "Perfluorooctanoic acid" OR "Perfluorooctylsulfonamide" OR "Perfluorodecanoic acid" OR "Perfluorododecanoic acid " OR "Perfluorododecanoic acid" OR "Perfluoroheptanecarboxylic acid" OR "Perfluoroheptanoic acid" OR "Perfluorohexane sulfonic acid" OR "Perfluorohexane-1-sulphonic acid" OR "perfluorohexanesulfonate" OR "perfluorohexanesulfonic acid" OR "Perfluorolauric acid" OR "Perfluorononan-1-oic acid" OR "Perfluorononanoic acid" OR "Perfluorooctane sulfonamide" OR "Perfluorooctane sulfonate" OR "Perfluorooctane sulfonic acid " OR "perfluorooctane sulphonic acid" OR "Perfluorooctanesulfonamide" OR "Perfluorooctanesulfonate" OR "Perfluorooctanesulfonic acid amide" OR "perfluorooctanesulfonic acid" OR "Perfluorooctanoic acid" OR "Perfluorooctylsulfonic acid" OR "Perfluoropropanecarboxylic acid" OR "Perfluoroundecanoic acid" OR "pfbus" OR "PFDA" OR "pfdea" OR "pfdoa" OR "Pfhpa" OR "PFHS cpd" OR "pfhxs" OR "pfna" OR "PFOA" OR "PFOS" OR "pfsoa" OR "Pfua" OR "Tricosafluorododecanoic acid" OR "Tridecafluoro-1-heptanoic acid" OR "Tridecafluoroheptanoic acid") AND (ANEUPL [org] OR BIOSIS [org] OR CIS [org] OR DART [org] OR EMIC [org] OR EPIDEM [org] OR HEEP [org] OR HMTC [org] OR IPA [org] OR RISKLINE [org] OR MTGABS [org] OR NIOSH [org] OR NTIS [org] OR PESTAB [org] OR PPBIB [org]) OR ("1763-23-1" OR "2058-94-8" OR "2355-31-9" OR "2991-50-6" OR "307-55-1" OR "335-67-1" OR "335-76-2" OR "355-46-4" OR "375-22-4" OR "375-73-5" OR "375-85-9" OR "375-95-1" OR "754-91-6") AND (ANEUPL [org] OR BIOSIS [org] OR CIS [org] OR DART [org] OR EMIC [org] OR EPIDEM [org] OR HEEP [org] OR HMTC [org] OR IPA [org] OR RISKLINE [org] OR MTGABS [org] OR NIOSH [org] OR NTIS [org] OR PESTAB [org] OR PPBIB [org]))) AND 2013:2016 [yr] ("1,1,2,2,3,3,4,4,4-Nonafluoro-1-butanesulfonic acid" OR "1,1,2,2,3,3,4,4,4-Nonafluorobutane-1-sulphonic acid" OR "1,1,2,2,3,3,4,4,5,5,6,6,6-Tridecafluorohexane-1-sulfonic acid" OR "1-Perfluorobutanesulfonic acid" OR "2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,9-heptadecafluoro-Nonanoic acid" OR "2-(N-Ethyl-perfluorooctane sulfonamido) acetic acid" OR "2-(N-Methyl-perfluorooctane sulfonamido) acetic acid" OR "Glycine, N-ethyl-N-((1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluorooctyl)sulfonyl)-") AND 2013:2016 [yr] AND (ANEUPL [org] OR BIOSIS [org] OR CIS [org] OR DART [org] OR EMIC [org] OR EPIDEM [org] OR HEEP [org] OR HMTC [org] OR IPA [org] OR RISKLINE [org] OR MTGABS [org] OR NIOSH [org] OR NTIS [org] OR PESTAB [org] OR PPBIB [org])
09/18/2013	("perfluorooctanoic acid" OR "pentadecafluoro 1 octanoic acid" OR "pentadecafluoro n octanoic acid" OR "pentadecafluorooctanoic acid" OR "perfluorocaprylic acid" OR "perfluoroctanoic acid" OR "perfluoroheptanecarboxylic acid" OR "perfluorooctanoic acid" OR "pentadecafluorooctanoic acid" OR "perfluorooctanoic acid" OR "perfluorooctane sulfonic acid " OR "heptadecafluoro 1 octanesulfonic acid" OR "heptadecafluoro 1 octane sulfonic acid" OR "heptadecafluorooctane 1 sulphonic acid" OR "heptadecafluorooctane sulfonic acid" OR "perfluorooctane sulfonate" OR "perfluorooctylsulfonic acid" OR "perfluorooctane sulphonic acid" OR "perfluorooctanesulfonic acid"

Table B-2. Database Query Strings Post Public Comment Searches

Database search date	Query string
	OR "perfluorooctanesulfonate" OR "heptadecafluorooctane 1 sulphonic acid1 perfluorooctanesulfonic acid" OR "perfluorohexane sulfonic acid pfhxs " OR "perfluorohexanesulfonic acid" OR "perfluorohexanesulfonate" OR "perfluorohexane 1 sulphonic acid" OR "pfhs cpd" OR "2 (n ethyl perfluorooctane sulfonamido) acetic acid" OR "et pfosa acoh" OR "n ethyl n ((heptadecafluorooctyl) sulphonyl) glycine" OR "2 (n methyl perfluorooctane sulfonamido) acetic acid" OR "me pfosa acoh" OR "perfluorodecanoic acid" OR "nonadecafluoro n decanoic acid" OR "nonadecafluorodecanoic acid" OR "perfluoro n decanoic acid" OR "perfluoro n decanoic acid" OR "perfluorodecanoic acid" OR "nonadecafluorodecanoic acid" OR "perfluoro n decanoic acid" OR "perfluorobutane sulfonic acid" OR "perfluorobutanesulfonic acid" OR OR "pentyl perfluorobutanoate" OR "nonafluoro 1 butanesulfonic acid" OR "perfluoroheptanoic acid" OR "tridecafluoro 1 heptanoic acid" OR "perfluoro n heptanoic acid" OR "perfluoroheptanoic acid" OR "tridecafluoroheptanoic acid" OR "perfluorononanoic acid" OR "perfluoro n nonanoic acid" OR "perfluorononan 1 oic acid" OR "perfluorooctane sulfonamide" OR "perfluorooctanesulfonamide" OR "perfluoroctylsulfonamide" OR "perfluorooctanesulfonic acid amide" OR "heptadecafluorooctanesulphonamide" OR "perfluoroundecanoic acid" OR "perfluoro n undecanoic acid" OR "hennone) AND 2007:2013 [yr] AND (ANEUPL [org] OR BIOSIS [org] OR CIS [org] OR NIH RePORTER [org] OR DART [org] OR EMIC [org] OR EPIDEM [org] OR FEDRIP [org] OR HEEP [org] OR HMTC [org] OR IPA [org] OR RISKLINE [org] OR MTGABS [org] OR NIOSH [org] OR NTIS [org] OR PESTAB [org] OR PPBIB [org]) NOT PubMed [org] NOT pubdart [org]

Table B-3. Strategies to Augment the Literature Search

Source	Query and number screened when available
TSCATS[a]	
9/11/2018 5/23/2016 9/18/2013	Compounds searched: 1763-23-1; 2058-94-8; 2355-31-9; 2991-50-6; 307-55-1; 335-67-1; 335-76-2; 355-46-4; 375-22-4; 375-73-5; 375-85-9; 375-95-1; 754-91-6; 307-24-4; 3825-26-1
NTP	
9/11/2018	Content types: Reports & Publications; Systematic Reviews; ROC Profiles, Reviews or Candidates; Testing Status "1763-23-1" "2058-94-8" "2355-31-9" "2991-50-6" "307-55-1" "335-67-1" "335-76-2" "355-46-4" "375-22-4" "375-73-5" "375-85-9" "375-95-1" "754-91-6" "Henicosafluoroundecanoic acid" "heptadecafluoro-1-octane sulfonic acid" "Heptadecafluoro-1-octanesulfonic acid" "heptadecafluorooctane sulfonic acid" "Heptadecafluorooctane-1-sulphonic acid" "Heptadecafluorooctanesulphonamide" "Heptafluoro-1-butanoic acid" "Heptafluorobutanoic acid" "Heptafluorobutyric acid" "me-pfosa-acoh" "N-Ethyl-N-((heptadecafluorooctyl)sulphonyl)glycine" "Ndfda" "Nonadecafluoro-n-decanoic acid" "Nonadecafluorodecanoic acid" "Nonafluoro-1-butanesulfonic acid" "Nonafluorobutanesulfonic acid" "Pentadecafluoro-1-octanoic acid" "Pentadecafluoro-n-octanoic acid" "Pentadecafluorooctanoic acid" "Pentyl perfluorobutanoate" "Perfluoro-n-decanoic acid" "Perfluoro-n-heptanoic acid" "Perfluoro-n-nonanoic acid" "Perfluoro-n-undecanoic acid" "Perfluorobutane sulfonic acid" "Perfluorobutanesulfonic acid" "Perfluorobutanoic acid" "Perfluorobutyric acid" "Perfluorocaprylic acid" "Perfluoroctanoic acid" "Perfluoroctylsulfonamide" "Perfluorodecanoic acid" "Perfluorododecanoic acid" "Perfluorododecanoic acid " "Perfluoroheptanecarboxylic acid" "Perfluoroheptanoic acid" "Perfluorohexane sulfonic acid" "Perfluorohexane-1-sulphonic acid" "perfluorohexanesulfonate" "perfluorohexanesulfonic acid" "Perfluorolauric acid" "Perfluorononan-1-oic acid" "Perfluorononanoic acid" "Perfluorooctane sulfonamide" "Perfluorooctane sulfonate" "Perfluorooctane sulfonic acid " "perfluorooctane sulphonic acid" "Perfluorooctanesulfonamide" "Perfluorooctanesulfonate" "perfluorooctanesulfonic acid" "Perfluorooctanesulfonic acid amide" "Perfluorooctanoic acid" "Perfluorooctylsulfonic acid" "Perfluoropropanecarboxylic acid" "Perfluoroundecanoic acid" "pfbus" "PFDA" "pfdea" "pfdoa" "Pfhpa" "PFHS cpd" "pfhxs" "pfna" "PFOA" "PFOS" "pfsoa"

Table B-3. Strategies to Augment the Literature Search

Source	Query and number screened when available
	"Pfua" "Tricosafluorododecanoic acid" "Tridecafluoro-1-heptanoic acid" "Tridecafluoroheptanoic acid" "307-24-4" "3825-26-1" "Perfluorohexanoic acid" "Undecafluoro-1-hexanoic acid" "Undecafluorohexanoic acid" "Ammonium pentadecafluorooctanoate" "Ammonium perfluorocaprilate" "Ammonium perfluorocaprylate" "Ammonium perfluorooctanoate" "FC 143" "Octanoic acid, pentadecafluoro-, ammonium salt" "Pentadecafluoro-1-octanoic acid, ammonium salt" "Pentadecafluorooctanoic acid, ammonium salt" "Perfluoroammonium octanoate" "Perfluorooctanoic acid, ammonium salt"
5/23/2016	"335-67-1" OR "1763-23-1" OR "355-46-4" OR "2991-50-6" OR "2355-31-9" OR "335-76-2" OR "375-73-5" OR "375-85-9" OR "375-95-1" OR "754-91-6" OR "2058-94-8" OR "307-55-1" OR "375-22-4" OR "Perfluorooctanoic acid" OR "PFOA" OR "Pentadecafluoro-1-octanoic acid" OR "Pentadecafluorooctanoic acid" OR "Perfluoroctanoic acid" OR "Perfluorooctane sulfonic acid" OR "Heptadecafluorooctane-1-sulphonic acid" OR "PFOS" OR "Perfluorooctane sulfonate" OR "Perfluorooctylsulfonic acid" OR "perfluorooctane sulphonic acid" OR "perfluorooctanesulfonic acid" OR "Perfluorooctanesulfonate" OR "Heptadecafluorooctane-1-sulphonic acid" OR "1,1,2,2,3,3,4,4,5,5,6,6,6-Tridecafluorohexane-1-sulfonic acid" OR "Perfluorohexane sulfonic acid" OR "Perfluorohexane-1-sulphonic acid" OR "perfluorohexanesulfonate" OR "perfluorohexanesulfonic acid" OR "pfhxs" OR "Ndfda" OR "Nonadecafluorodecanoic acid" OR "Nonadecafluoro-n-decanoic acid" OR "Perfluorodecanoic acid" OR "Perfluoro-n-decanoic acid" OR "PFDA" OR "pfdea" OR "1-Perfluorobutanesulfonic acid" OR "Nonafluoro-1-butanesulfonic acid" OR "Perfluorobutane sulfonic acid" OR "Perfluorobutanesulfonic acid" OR "pfbus" OR "Perfluorononanoic acid" OR "pfna" OR "Perfluorooctanesulfonamide" OR "Perfluorododecanoic acid" OR "pfdoa" OR "Heptafluorobutyric acid" OR "Perfluorobutanoic acid" OR "Perfluorobutyric acid" Screened: 146 hits
	"1,1,2,2,3,3,4,4,4-Nonafluoro-1-butanesulfonic acid" OR "1,1,2,2,3,3,4,4,4-Nonafluorobutane-1-sulphonic acid" OR "Nonafluorobutanesulfonic acid" OR "Pentyl perfluorobutanoate" OR "Pentadecafluoro-n-octanoic acid" OR "Perfluorocaprylic acid" OR "Perfluoroheptanecarboxylic acid" OR "Heptadecafluoro-1-octanesulfonic acid" OR "heptadecafluoro-1-octane sulfonic acid" OR "heptadecafluorooctane sulfonic acid" OR "1-Perfluorooctanesulfonic acid" OR "PFHS cpd" OR "2-(N-Ethyl-perfluorooctane sulfonamido) acetic acid" OR "et-pfosa-acoh" OR "N-Ethyl-N-((heptadecafluorooctyl)sulphonyl)glycine" OR "Glycine, N-ethyl-N-((1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluorooctyl)sulfonyl)-" OR "2-(N-Methyl-perfluorooctane sulfonamido) acetic acid" OR "me-pfosa-acoh" OR "Perfluoroheptanoic acid" OR "Perfluoro-n-heptanoic acid" OR "Pfhpa" OR "Tridecafluoro-1-heptanoic acid" OR "Tridecafluoroheptanoic acid" OR "2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,9-heptadecafluoro-Nonanoic acid" OR "Perfluoro-n-nonanoic acid" OR "Perfluorononan-1-oic acid" OR "Heptadecafluorooctanesulphonamide" OR "Perfluoroctylsulfonamide" OR "Perfluorooctane sulfonamide" OR "Perfluorooctanesulfonic acid amide" OR "Pfsoa" OR "C11-PFA" OR "Henicosafluoroundecanoic acid" OR "Perfluoro-n-undecanoic acid" OR "Perfluoroundecanoic acid" OR "Pfua" OR "Perfluorolauric acid" OR "Tricosafluorododecanoic acid" OR "Heptafluoro-1-butanoic acid" OR "Heptafluorobutanoic acid" OR "Perfluoropropanecarboxylic acid" Screened: 0 hits

Table B-3. Strategies to Augment the Literature Search

Source	Query and number screened when available
NIH RePORTER	
2/28/2017	Text Search: "1,1,2,2,3,3,4,4,4-Nonafluoro-1-butanesulfonic acid" OR "1,1,2,2,3,3,4,4,4-Nonafluorobutane-1-sulphonic acid" OR "1,1,2,2,3,3,4,4,5,5,6,6,6-Tridecafluorohexane-1-sulfonic acid" OR "1-Perfluorobutanesulfonic acid" OR "2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,9-heptadecafluoro-Nonanoic acid" OR "2-(N-Ethyl-perfluorooctane sulfonamido) acetic acid" OR "2-(N-Methyl-perfluorooctane sulfonamido) acetic acid" OR "C11-PFA" OR "et-pfosa-acoh" OR "Glycine, N-ethyl-N-((1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluorooctyl)sulfonyl)-" OR "Henicosafluoroundecanoic acid" OR "heptadecafluoro-1-octane sulfonic acid" OR "Heptadecafluoro-1-octanesulfonic acid" OR "heptadecafluorooctane sulfonic acid" OR "Heptadecafluorooctane-1-sulphonic acid" OR "Heptadecafluorooctanesulphonamide" OR "Heptafluoro-1-butanoic acid" OR "Heptafluorobutanoic acid" OR "Heptafluorobutyric acid" OR "me-pfosa-acoh" OR "N-Ethyl-N-((heptadecafluorooctyl)sulphonyl)glycine" OR "Ndfda" OR "Nonadecafluoro-n-decanoic acid" OR "Nonadecafluorodecanoic acid" OR "Nonafluoro-1-butanesulfonic acid" OR "Nonafluorobutanesulfonic acid" OR "Pentadecafluoro-1-octanoic acid" OR "Pentadecafluoro-n-octanoic acid" OR "Pentadecafluorooctanoic acid" OR "Pentyl perfluorobutanoate" OR "Perfluoro-n-decanoic acid" OR "Perfluoro-n-heptanoic acid" OR "Perfluoro-n-nonanoic acid" OR "Perfluoro-n-undecanoic acid" OR "Perfluorobutane sulfonic acid" OR "Perfluorobutanesulfonic acid" OR "Perfluorobutanoic acid" OR "Perfluorobutyric acid" OR "Perfluorocaprylic acid" OR "Perfluoroctanoic acid" OR "Perfluoroctylsulfonamide" OR "Perfluorodecanoic acid" OR "Perfluorododecanoic acid" OR "Perfluorododecanoic acid" OR "Perfluoroheptanecarboxylic acid" OR "Perfluoroheptanoic acid" OR "Perfluorohexane sulfonic acid" OR "Perfluorohexane-1-sulphonic acid" OR "perfluorohexanesulfonate" OR "perfluorohexanesulfonic acid" OR "Perfluorolauric acid" OR "Perfluorononan-1-oic acid" OR "Perfluorononanoic acid" OR "Perfluorooctane sulfonamide" OR "Perfluorooctane sulfonate" OR "Perfluorooctane sulfonic acid" OR "perfluorooctane sulphonic acid" OR "Perfluorooctanesulfonamide" OR "Perfluorooctanesulfonate" OR "Perfluorooctanesulfonic acid amide" OR "perfluorooctanesulfonic acid" OR "Perfluorooctanoic acid" OR "Perfluorooctylsulfonic acid" OR "Perfluoropropanecarboxylic acid" OR "Perfluoroundecanoic acid" OR "pfbus" OR "PFDA" OR "pfdea" OR "pfdoa" OR "Pfhpa" OR "PFHS cpd" OR "pfhxs" OR "pfna" OR "PFOA" OR "PFOS" OR "pfsoa" OR "Pfua" OR "Tricosafluorododecanoic acid" OR "Tridecafluoro-1-heptanoic acid" OR "Tridecafluoroheptanoic acid" (Advanced), Search in: Projects Admin IC: All, Fiscal Year: Active Projects, 2017, 2016, 2015, 2014, 2013, 2012 Screened: 80
4/7/2014	Compounds searched: 335-67-1; 1763-23-1; 355-46-4; 2991-50-6; 2355-31-9; 335-76-2; 375-73-5; 375-85-9; 375-95-1; 754-91-6; 2058-94-8; 307-55-1; 375-22-4 Screened: 82 hits
Other	Identified throughout the assessment process

[a]Several versions of the TSCATS database were searched, as needed, by CASRN including TSCATS1 via Toxline (no date limit), TSCATS2 via https://yosemite.epa.gov/oppts/epatscat8.nsf/ReportSearch?OpenForm (date restricted by EPA receipt date), and TSCATS via CDAT (date restricted by 'Mail Received Date Range'), as well as google for recent TSCA submissions.

The September 2018 results were:

- Number of records identified from PubMed, TOXLINE, and TOXCENTER (after duplicate removal): 941
- Number of records identified from other strategies: 153
- Total number of records to undergo literature screening: 1,094

B.1.2 Literature Screening

A two-step process was used to screen the literature search to identify relevant studies on perfluoroalkyls:

- Title and abstract screen
- Full text screen

Title and Abstract Screen. Within the reference library, titles and abstracts were screened manually for relevance. Studies that were considered relevant (see Table B-1 for inclusion criteria) were moved to the second step of the literature screening process. Studies were excluded when the title and abstract clearly indicated that the study was not relevant to the toxicological profile.

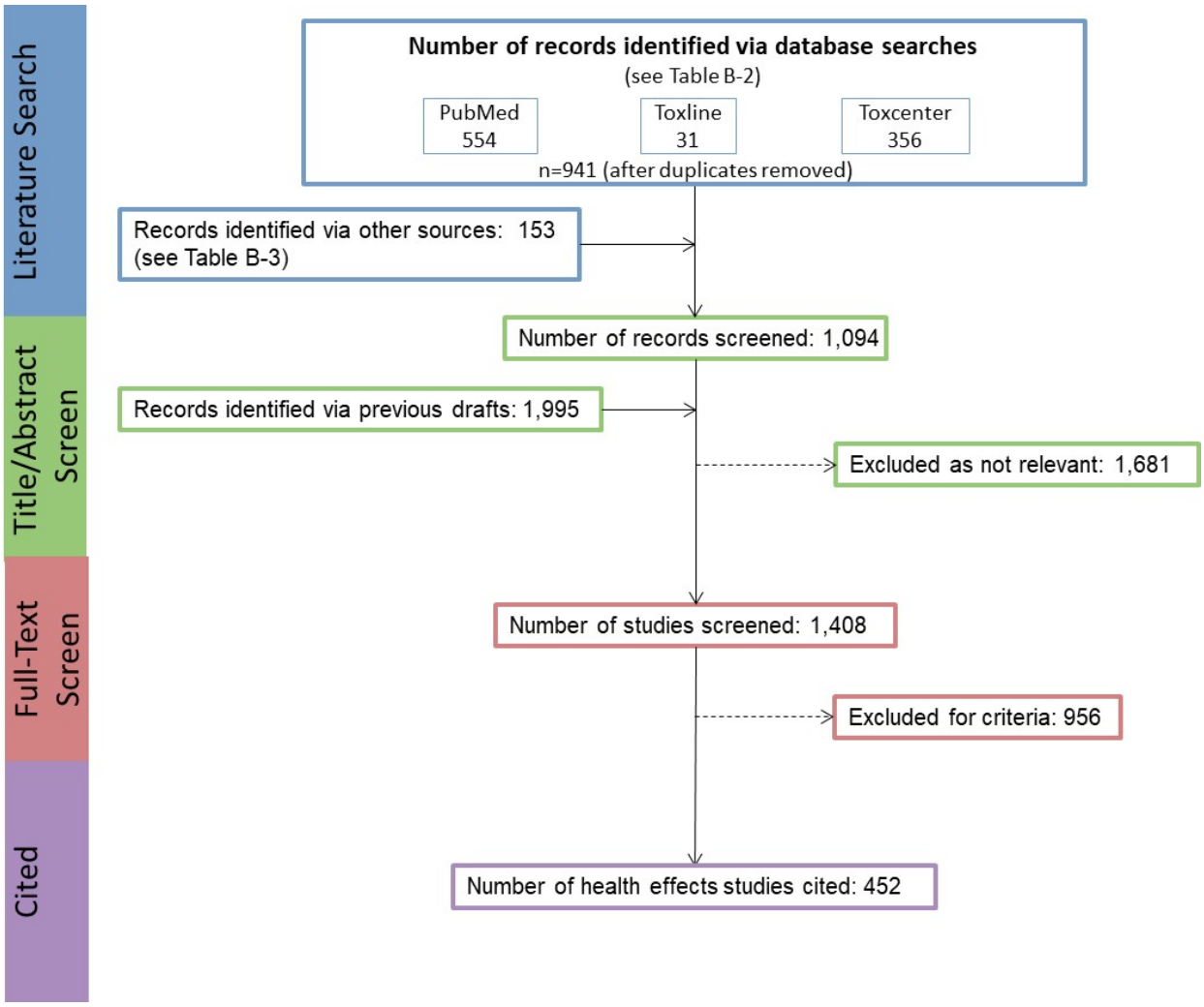
- Number of titles and abstracts screened: 1,094
- Number of studies considered relevant and moved to the next step: 1,408

Full Text Screen. The second step in the literature screening process was a full text review of individual studies considered relevant in the title and abstract screen step. Each study was reviewed to determine whether it was relevant for inclusion in the toxicological profile.

- Number of studies undergoing full text review: 1,408
- Total number of health effects studies cited in the profile: 452

A summary of the results of the literature search and screening is presented in Figure B-1.

Figure B-1. September 2018 Literature Search Results and Screen for Perfluoroalkyls



APPENDIX C. USER'S GUIDE

Chapter 1. Relevance to Public Health

This chapter provides an overview of U.S. exposures, a summary of health effects based on evaluations of existing toxicologic, epidemiologic, and toxicokinetic information, and an overview of the minimal risk levels. This is designed to present interpretive, weight-of-evidence discussions for human health endpoints by addressing the following questions:

1. What effects are known to occur in humans?

2. What effects observed in animals are likely to be of concern to humans?

3. What exposure conditions are likely to be of concern to humans, especially around hazardous waste sites?

Minimal Risk Levels (MRLs)

Where sufficient toxicologic information is available, ATSDR derives MRLs for inhalation and oral routes of entry at each duration of exposure (acute, intermediate, and chronic). These MRLs are not meant to support regulatory action, but to acquaint health professionals with exposure levels at which adverse health effects are not expected to occur in humans.

MRLs should help physicians and public health officials determine the safety of a community living near a hazardous substance emission, given the concentration of a contaminant in air or the estimated daily dose in water. MRLs are based largely on toxicological studies in animals and on reports of human occupational exposure.

MRL users should be familiar with the toxicologic information on which the number is based. Section 1.2, Summary of Health Effects, contains basic information known about the substance. Other sections, such as Section 3.2 Children and Other Populations that are Unusually Susceptible and Section 3.4 Interactions with Other Substances, provide important supplemental information.

MRL users should also understand the MRL derivation methodology. MRLs are derived using a modified version of the risk assessment methodology that the Environmental Protection Agency (EPA) provides (Barnes and Dourson 1988) to determine reference doses (RfDs) for lifetime exposure.

To derive an MRL, ATSDR generally selects the most sensitive endpoint which, in its best judgement, represents the most sensitive human health effect for a given exposure route and duration. ATSDR cannot make this judgement or derive an MRL unless information (quantitative or qualitative) is available for all potential systemic, neurological, and developmental effects. If this information and reliable quantitative data on the chosen endpoint are available, ATSDR derives an MRL using the most sensitive species (when information from multiple species is available) with the highest no-observed-adverse-effect level (NOAEL) that does not exceed any adverse effect levels. When a NOAEL is not available, a lowest-observed-adverse-effect level (LOAEL) can be used to derive an MRL, and an uncertainty factor of 10 must be employed. Additional uncertainty factors of 10 must be used both for human variability to protect sensitive subpopulations (people who are most susceptible to the health effects caused by the substance) and for interspecies variability (extrapolation from animals to humans). In deriving an MRL, these individual uncertainty factors are multiplied together. The product is then divided into the inhalation concentration or oral dosage selected from the study. Uncertainty factors used in developing a

substance-specific MRL are provided in the footnotes of the levels of significant exposure (LSE) tables that are provided in Chapter 2. Detailed discussions of the MRLs are presented in Appendix A.

Chapter 2. Health Effects

Tables and Figures for Levels of Significant Exposure (LSE)

Tables and figures are used to summarize health effects and illustrate graphically levels of exposure associated with those effects. These levels cover health effects observed at increasing dose concentrations and durations, differences in response by species and MRLs to humans for noncancer endpoints. The LSE tables and figures can be used for a quick review of the health effects and to locate data for a specific exposure scenario. The LSE tables and figures should always be used in conjunction with the text. All entries in these tables and figures represent studies that provide reliable, quantitative estimates of NOAELs, LOAELs, or Cancer Effect Levels (CELs).

The legends presented below demonstrate the application of these tables and figures. Representative examples of LSE tables and figures follow. The numbers in the left column of the legends correspond to the numbers in the example table and figure.

TABLE LEGEND

See Sample LSE Table (page C-5)

(1) Route of exposure. One of the first considerations when reviewing the toxicity of a substance using these tables and figures should be the relevant and appropriate route of exposure. Typically, when sufficient data exist, three LSE tables and two LSE figures are presented in the document. The three LSE tables present data on the three principal routes of exposure (i.e., inhalation, oral, and dermal). LSE figures are limited to the inhalation and oral routes. Not all substances will have data on each route of exposure and will not, therefore, have all five of the tables and figures. Profiles with more than one chemical may have more LSE tables and figures.

(2) Exposure period. Three exposure periods—acute (<15 days), intermediate (15–364 days), and chronic (≥365 days)—are presented within each relevant route of exposure. In this example, two oral studies of chronic-duration exposure are reported. For quick reference to health effects occurring from a known length of exposure, locate the applicable exposure period within the LSE table and figure.

(3) Figure key. Each key number in the LSE table links study information to one or more data points using the same key number in the corresponding LSE figure. In this example, the study represented by key number 51 identified NOAELs and less serious LOAELs (also see the three "51R" data points in sample LSE Figure 2-X).

(4) Species (strain) No./group. The test species (and strain), whether animal or human, are identified in this column. The column also contains information on the number of subjects and sex per group. Chapter 1, Relevance to Public Health, covers the relevance of animal data to human toxicity and Section 3.1, Toxicokinetics, contains any available information on comparative toxicokinetics. Although NOAELs and LOAELs are species specific, the levels are extrapolated to equivalent human doses to derive an MRL.

(5) Exposure parameters/doses. The duration of the study and exposure regimens are provided in these columns. This permits comparison of NOAELs and LOAELs from different studies. In this case (key number 51), rats were orally exposed to “Chemical X” via feed for 2 years. For a

more complete review of the dosing regimen, refer to the appropriate sections of the text or the original reference paper (i.e., Aida et al. 1992).

(6) Parameters monitored. This column lists the parameters used to assess health effects. Parameters monitored could include serum (blood) chemistry (BC), behavioral (BH), biochemical changes (BI), body weight (BW), clinical signs (CS), developmental toxicity (DX), enzyme activity (EA), food intake (FI), fetal toxicity (FX), gross necropsy (GN), hematology (HE), histopathology (HP), lethality (LE), maternal toxicity (MX), organ function (OF), ophthalmology (OP), organ weight (OW), teratogenicity (TG), urinalysis (UR), and water intake (WI).

(7) Endpoint. This column lists the endpoint examined. The major categories of health endpoints included in LSE tables and figures are death, body weight, respiratory, cardiovascular, gastrointestinal, hematological, musculoskeletal, hepatic, renal, dermal, ocular, endocrine, immunological, neurological, reproductive, developmental, other noncancer, and cancer. "Other noncancer" refers to any effect (e.g., alterations in blood glucose levels) not covered in these systems. In the example of key number 51, three endpoints (body weight, hematological, and hepatic) were investigated.

(8) NOAEL. A NOAEL is the highest exposure level at which no adverse effects were seen in the organ system studied. The body weight effect reported in key number 51 is a NOAEL at 25.5 mg/kg/day. NOAELs are not reported for cancer and death; with the exception of these two endpoints, this field is left blank if no NOAEL was identified in the study.

(9) LOAEL. A LOAEL is the lowest dose used in the study that caused an adverse health effect. LOAELs have been classified into "Less Serious" and "Serious" effects. These distinctions help readers identify the levels of exposure at which adverse health effects first appear and the gradation of effects with increasing dose. A brief description of the specific endpoint used to quantify the adverse effect accompanies the LOAEL. Key number 51 reports a less serious LOAEL of 6.1 mg/kg/day for the hepatic system, which was used to derive a chronic exposure, oral MRL of 0.008 mg/kg/day (see footnote "c"). MRLs are not derived from serious LOAELs. A cancer effect level (CEL) is the lowest exposure level associated with the onset of carcinogenesis in experimental or epidemiologic studies. CELs are always considered serious effects. The LSE tables and figures do not contain NOAELs for cancer, but the text may report doses not causing measurable cancer increases. If no LOAEL/CEL values were identified in the study, this field is left blank.

(10) Reference. The complete reference citation is provided in Chapter 8 of the profile.

(11) Footnotes. Explanations of abbreviations or reference notes for data in the LSE tables are found in the footnotes. For example, footnote "c" indicates that the LOAEL of 6.1 mg/kg/day in key number 51 was used to derive an oral MRL of 0.008 mg/kg/day.

FIGURE LEGEND

See Sample LSE Figure (page C-6)

LSE figures graphically illustrate the data presented in the corresponding LSE tables. Figures help the reader quickly compare health effects according to exposure concentrations for particular exposure periods.

(13) Exposure period. The same exposure periods appear as in the LSE table. In this example, health effects observed within the chronic exposure period are illustrated.

(14) Endpoint. These are the categories of health effects for which reliable quantitative data exist. The same health effect endpoints appear in the LSE table.

(15) Levels of exposure. Concentrations or doses for each health effect in the LSE tables are graphically displayed in the LSE figures. Exposure concentration or dose is measured on the log scale "y" axis. Inhalation exposure is reported in mg/m^3 or ppm and oral exposure is reported in mg/kg/day.

(16) LOAEL. In this example, the half-shaded circle that is designated 51R identifies a LOAEL critical endpoint in the rat upon which a chronic oral exposure MRL is based. The key number 51 corresponds to the entry in the LSE table. The dashed descending arrow indicates the extrapolation from the exposure level of 6.1 mg/kg/day (see entry 51 in the sample LSE table) to the MRL of 0.008 mg/kg/day (see footnote "c" in the sample LSE table).

(17) CEL. Key number 59R is one of studies for which CELs were derived. The diamond symbol refers to a CEL for the test species (rat). The number 59 corresponds to the entry in the LSE table.

(18) Key to LSE figure. The key provides the abbreviations and symbols used in the figure.

APPENDIX B

Table 2-X. Levels of Significant Exposure to [Chemical X] – Oral (1)

Figure key[a] (2)	Species (strain) No./group (4)	Exposure parameters (5)	Doses (mg/kg/day) (5)	Parameters monitored (6)	Endpoint (7)	NOAEL (mg/kg/day) (8)	Less serious LOAEL (mg/kg/day) (9)	Serious LOAEL (mg/kg/day) (9)	Effect
CHRONIC EXPOSURE									
51 (3)	Rat (Wistar) 40 M, 40 F	2 years (F)	M: 0, 6.1, 25.5, 138.0 F: 0, 8.0, 31.7, 168.4	CS, WI, BW, OW, HE, BC, HP	Bd wt	25.5	138.0		Decreased body weight gain in males (23–25%) and females (31–39%)
					Hemato	138.0			
					Hepatic		6.1[c]		Increases in absolute and relative weights at ≥6.1/8.0 mg/kg/day after 12 months of exposure; fatty generation at ≥6.1 mg/kg/day in males and at ≥31.7 mg/kg/day in females, and granulomas in females at 31.7 and 168.4 mg/kg/day after 12, 18, or 24 months of exposure and in males at ≥6.1 mg/kg/day only after 24 months of exposure
Aida et al. 1992 (10)									
52	Rat (F344) 78 M	104 weeks (W)	0, 3.9, 20.6, 36.3	CS, BW, FI, BC, OW, HP	Hepatic	36.3			
					Renal	20.6	36.3		Increased incidence of renal tubular cell hyperplasia
					Endocr	36.3			
George et al. 2002									
59	Rat (Wistar) 58M, 58F	Lifetime (W)	M: 0, 90 F: 0, 190	BW, HP	Cancer		190 F		Increased incidence of hepatic neoplastic nodules in females only; no additional description of the tumors was provided
Tumasonis et al. 1985									

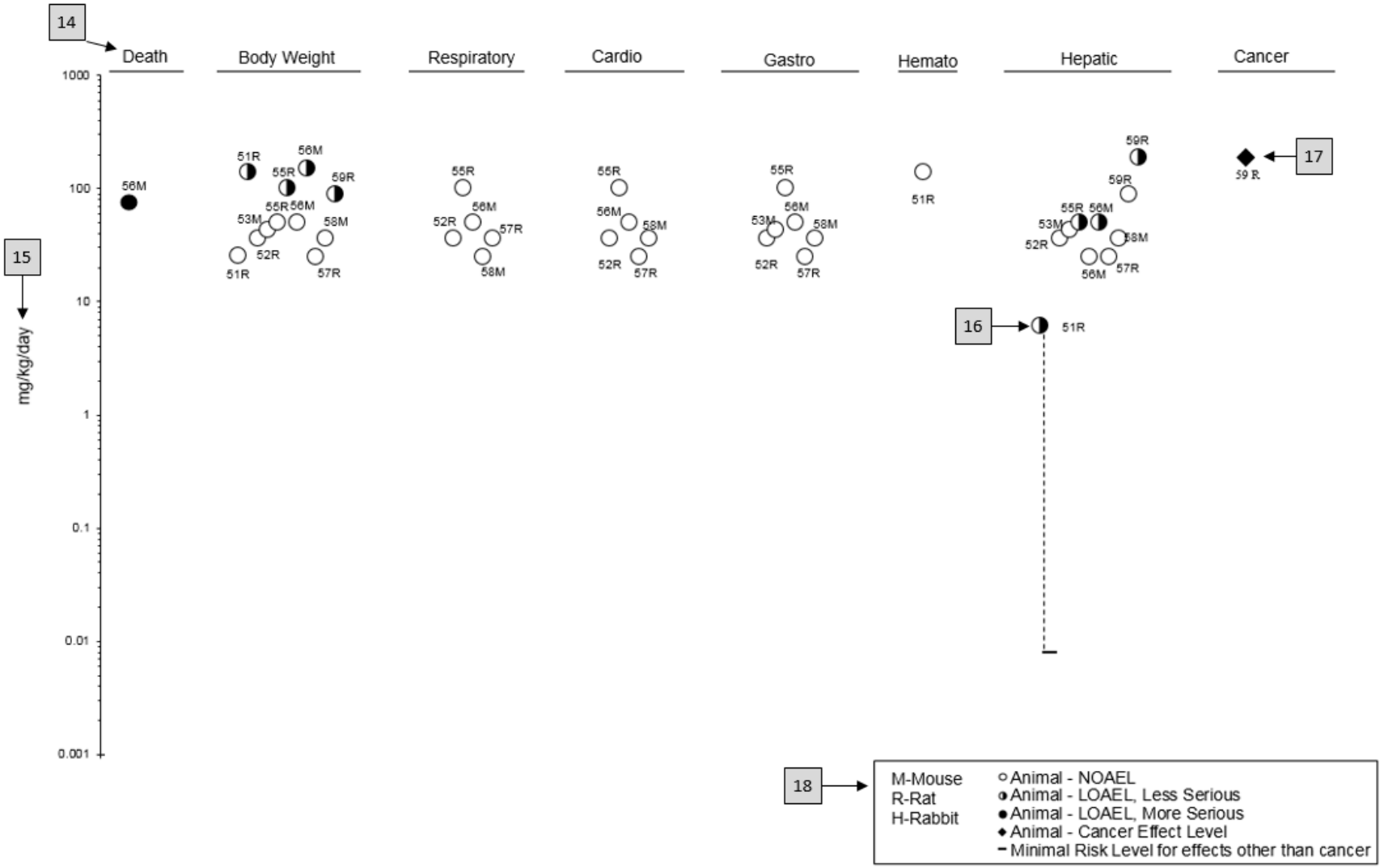
[a]The number corresponds to entries in Figure 2-x.

(11) [b]Used to derive an acute-duration oral minimal risk level (MRL) of 0.1 mg/kg/day based on the $BMDL_{05}$ of 10 mg/kg/day and an uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability).

[c]Used to derive a chronic-duration oral MRL of 0.008 mg/kg/day based on the $BMDL_{10}$ of 0.78 mg/kg/day and an uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability).

Figure 2-X. Levels of Significant Exposure to [Chemical X] - Oral

13 → Chronic (≥365 days)



APPENDIX D. QUICK REFERENCE FOR HEALTH CARE PROVIDERS

Toxicological Profiles are a unique compilation of toxicological information on a given hazardous substance. Each profile reflects a comprehensive and extensive evaluation, summary, and interpretation of available toxicologic and epidemiologic information on a substance. Health care providers treating patients potentially exposed to hazardous substances may find the following information helpful for fast answers to often-asked questions.

Primary Chapters/Sections of Interest

Chapter 1: Relevance to Public Health: The Relevance to Public Health Section provides an overview of exposure and health effects and evaluates, interprets, and assesses the significance of toxicity data to human health. A table listing minimal risk levels (MRLs) is also included in this chapter.

Chapter 2: Health Effects: Specific health effects identified in both human and animal studies are reported by type of health effect (e.g., death, hepatic, renal, immune, reproductive), route of exposure (e.g., inhalation, oral, dermal), and length of exposure (e.g., acute, intermediate, and chronic).
NOTE: Not all health effects reported in this section are necessarily observed in the clinical setting.

Pediatrics:

Section 3.2 Children and Other Populations that are Unusually Susceptible
Section 3.3 Biomarkers of Exposure and Effect

ATSDR Information Center

Phone: 1-800-CDC-INFO (800-232-4636) or 1-888-232-6348 (TTY)
Internet: http://www.atsdr.cdc.gov

The following additional materials are available online:

Case Studies in Environmental Medicine are self-instructional publications designed to increase primary health care providers' knowledge of a hazardous substance in the environment and to aid in the evaluation of potentially exposed patients (see https://www.atsdr.cdc.gov/csem/csem.html).

Managing Hazardous Materials Incidents is a three-volume set of recommendations for on-scene (prehospital) and hospital medical management of patients exposed during a hazardous materials incident (see https://www.atsdr.cdc.gov/MHMI/index.asp). Volumes I and II are planning guides to assist first responders and hospital emergency department personnel in planning for incidents that involve hazardous materials. Volume III—*Medical Management Guidelines for Acute Chemical Exposures*—is a guide for health care professionals treating patients exposed to hazardous materials.

Fact Sheets (ToxFAQs™) provide answers to frequently asked questions about toxic substances (see https://www.atsdr.cdc.gov/toxfaqs/Index.asp).

Other Agencies and Organizations

The National Center for Environmental Health (NCEH) focuses on preventing or controlling disease, injury, and disability related to the interactions between people and their environment outside the workplace. Contact: NCEH, Mailstop F-29, 4770 Buford Highway, NE, Atlanta, GA 30341-3724 • Phone: 770-488-7000 • FAX: 770-488-7015 • Web Page: https://www.cdc.gov/nceh/.

The National Institute for Occupational Safety and Health (NIOSH) conducts research on occupational diseases and injuries, responds to requests for assistance by investigating problems of health and safety in the workplace, recommends standards to the Occupational Safety and Health Administration (OSHA) and the Mine Safety and Health Administration (MSHA), and trains professionals in occupational safety and health. Contact: NIOSH, 395 E Street, S.W., Suite 9200, Patriots Plaza Building, Washington, DC 20201 • Phone: 202-245-0625 or 1-800-CDC-INFO (800-232-4636) • Web Page: https://www.cdc.gov/niosh/.

The National Institute of Environmental Health Sciences (NIEHS) is the principal federal agency for biomedical research on the effects of chemical, physical, and biologic environmental agents on human health and well-being. Contact: NIEHS, PO Box 12233, 104 T.W. Alexander Drive, Research Triangle Park, NC 27709 • Phone: 919-541-3212 • Web Page: https://www.niehs.nih.gov/.

Clinical Resources (Publicly Available Information)

The Association of Occupational and Environmental Clinics (AOEC) has developed a network of clinics in the United States to provide expertise in occupational and environmental issues. Contact: AOEC, 1010 Vermont Avenue, NW, #513, Washington, DC 20005 • Phone: 202-347-4976 • FAX: 202-347-4950 • e-mail: AOEC@AOEC.ORG • Web Page: http://www.aoec.org/.

The American College of Occupational and Environmental Medicine (ACOEM) is an association of physicians and other health care providers specializing in the field of occupational and environmental medicine. Contact: ACOEM, 25 Northwest Point Boulevard, Suite 700, Elk Grove Village, IL 60007-1030 • Phone: 847-818-1800 • FAX: 847-818-9266 • Web Page: http://www.acoem.org/.

The American College of Medical Toxicology (ACMT) is a nonprofit association of physicians with recognized expertise in medical toxicology. Contact: ACMT, 10645 North Tatum Boulevard, Suite 200-111, Phoenix AZ 85028 • Phone: 844-226-8333 • FAX: 844-226-8333 • Web Page: http://www.acmt.net.

The Pediatric Environmental Health Specialty Units (PEHSUs) is an interconnected system of specialists who respond to questions from public health professionals, clinicians, policy makers, and the public about the impact of environmental factors on the health of children and reproductive-aged adults. Contact information for regional centers can be found at http://pehsu.net/findhelp.html.

The American Association of Poison Control Centers (AAPCC) provide support on the prevention and treatment of poison exposures. Contact: AAPCC, 515 King Street, Suite 510, Alexandria VA 22314 • Phone: 701-894-1858 • Poison Help Line: 1-800-222-1222 • Web Page: http://www.aapcc.org/.

APPENDIX E. GLOSSARY

Absorption—The process by which a substance crosses biological membranes and enters systemic circulation. Absorption can also refer to the taking up of liquids by solids, or of gases by solids or liquids.

Acute Exposure—Exposure to a chemical for a duration of ≤14 days, as specified in the Toxicological Profiles.

Adsorption—The adhesion in an extremely thin layer of molecules (as of gases, solutes, or liquids) to the surfaces of solid bodies or liquids with which they are in contact.

Adsorption Coefficient (K_{oc})—The ratio of the amount of a chemical adsorbed per unit weight of organic carbon in the soil or sediment to the concentration of the chemical in solution at equilibrium.

Adsorption Ratio (Kd)—The amount of a chemical adsorbed by sediment or soil (i.e., the solid phase) divided by the amount of chemical in the solution phase, which is in equilibrium with the solid phase, at a fixed solid/solution ratio. It is generally expressed in micrograms of chemical sorbed per gram of soil or sediment.

Benchmark Dose (BMD) or Benchmark Concentration (BMC)—is the dose/concentration corresponding to a specific response level estimate using a statistical dose-response model applied to either experimental toxicology or epidemiology data. For example, a BMD_{10} would be the dose corresponding to a 10% benchmark response (BMR). The BMD is determined by modeling the dose-response curve in the region of the dose-response relationship where biologically observable data are feasible. The BMDL or BMCL is the 95% lower confidence limit on the BMD or BMC.

Bioconcentration Factor (BCF)—The quotient of the concentration of a chemical in aquatic organisms at a specific time or during a discrete time period of exposure divided by the concentration in the surrounding water at the same time or during the same period.

Biomarkers—Indicators signaling events in biologic systems or samples, typically classified as markers of exposure, effect, and susceptibility.

Cancer Effect Level (CEL)—The lowest dose of a chemical in a study, or group of studies, that produces significant increases in the incidence of cancer (or tumors) between the exposed population and its appropriate control.

Carcinogen—A chemical capable of inducing cancer.

Case-Control Study—A type of epidemiological study that examines the relationship between a particular outcome (disease or condition) and a variety of potential causative agents (such as toxic chemicals). In a case-control study, a group of people with a specified and well-defined outcome is identified and compared to a similar group of people without the outcome.

Case Report—A report that describes a single individual with a particular disease or exposure. These reports may suggest some potential topics for scientific research, but are not actual research studies.

Case Series—Reports that describe the experience of a small number of individuals with the same disease or exposure. These reports may suggest potential topics for scientific research, but are not actual research studies.

Ceiling Value—A concentration that must not be exceeded.

Chronic Exposure—Exposure to a chemical for ≥365 days, as specified in the Toxicological Profiles.

Clastogen—A substance that causes breaks in chromosomes resulting in addition, deletion, or rearrangement of parts of the chromosome.

Cohort Study—A type of epidemiological study of a specific group or groups of people who have had a common insult (e.g., exposure to an agent suspected of causing disease or a common disease) and are followed forward from exposure to outcome, and who are disease-free at start of follow-up. Often, at least one exposed group is compared to one unexposed group, while in other cohorts, exposure is a continuous variable and analyses are directed towards analyzing an exposure-response coefficient.

Cross-sectional Study—A type of epidemiological study of a group or groups of people that examines the relationship between exposure and outcome to a chemical or to chemicals at a specific point in time.

Data Needs—Substance-specific informational needs that, if met, would reduce the uncertainties of human health risk assessment.

Developmental Toxicity—The occurrence of adverse effects on the developing organism that may result from exposure to a chemical prior to conception (either parent), during prenatal development, or postnatally to the time of sexual maturation. Adverse developmental effects may be detected at any point in the life span of the organism.

Dose-Response Relationship—The quantitative relationship between the amount of exposure to a toxicant and the incidence of the response or amount of the response.

Embryotoxicity and Fetotoxicity—Any toxic effect on the conceptus as a result of prenatal exposure to a chemical; the distinguishing feature between the two terms is the stage of development during which the effect occurs. Effects include malformations and variations, altered growth, and *in utero* death.

Epidemiology—The investigation of factors that determine the frequency and distribution of disease or other health-related conditions within a defined human population during a specified period.

Excretion—The process by which metabolic waste products are removed from the body.

Genotoxicity—A specific adverse effect on the genome of living cells that, upon the duplication of affected cells, can be expressed as a mutagenic, clastogenic, or carcinogenic event because of specific alteration of the molecular structure of the genome.

Half-life—A measure of rate for the time required to eliminate one-half of a quantity of a chemical from the body or environmental media.

Health Advisory—An estimate of acceptable drinking water levels for a chemical substance derived by EPA and based on health effects information. A health advisory is not a legally enforceable federal standard, but serves as technical guidance to assist federal, state, and local officials.

Immediately Dangerous to Life or Health (IDLH)—A condition that poses a threat of life or health, or conditions that pose an immediate threat of severe exposure to contaminants that are likely to have adverse cumulative or delayed effects on health.

Immunotoxicity—Adverse effect on the functioning of the immune system that may result from exposure to chemical substances.

Incidence—The ratio of new cases of individuals in a population who develop a specified condition to the total number of individuals in that population who could have developed that condition in a specified time period.

Intermediate Exposure—Exposure to a chemical for a duration of 15–364 days, as specified in the Toxicological Profiles.

***In Vitro*—**Isolated from the living organism and artificially maintained, as in a test tube.

***In Vivo*—**Occurring within the living organism.

Lethal Concentration$_{(LO)}$ (LC_{LO})—The lowest concentration of a chemical in air that has been reported to have caused death in humans or animals.

Lethal Concentration$_{(50)}$ (LC_{50})—A calculated concentration of a chemical in air to which exposure for a specific length of time is expected to cause death in 50% of a defined experimental animal population.

Lethal Dose$_{(LO)}$ (LD_{Lo})—The lowest dose of a chemical introduced by a route other than inhalation that has been reported to have caused death in humans or animals.

Lethal Dose$_{(50)}$ (LD_{50})—The dose of a chemical that has been calculated to cause death in 50% of a defined experimental animal population.

Lethal Time$_{(50)}$ (LT_{50})—A calculated period of time within which a specific concentration of a chemical is expected to cause death in 50% of a defined experimental animal population.

Lowest-Observed-Adverse-Effect Level (LOAEL)—The lowest exposure level of chemical in a study, or group of studies, that produces statistically or biologically significant increases in frequency or severity of adverse effects between the exposed population and its appropriate control.

Lymphoreticular Effects—Represent morphological effects involving lymphatic tissues such as the lymph nodes, spleen, and thymus.

Malformations—Permanent structural changes that may adversely affect survival, development, or function.

Metabolism—Process in which chemical substances are biotransformed in the body that could result in less toxic and/or readily excreted compounds or produce a biologically active intermediate.

Minimal Risk Level (MRL)—An estimate of daily human exposure to a hazardous substance that is likely to be without an appreciable risk of adverse noncancer health effects over a specified route and duration of exposure.

Modifying Factor (MF)—A value (greater than zero) that is applied to the derivation of a Minimal Risk Level (MRL) to reflect additional concerns about the database that are not covered by the uncertainty factors. The default value for a MF is 1.

Morbidity—The state of being diseased; the morbidity rate is the incidence or prevalence of a disease in a specific population.

Mortality—Death; the mortality rate is a measure of the number of deaths in a population during a specified interval of time.

Mutagen—A substance that causes mutations, which are changes in the DNA sequence of a cell's DNA. Mutations can lead to birth defects, miscarriages, or cancer.

Necropsy—The gross examination of the organs and tissues of a dead body to determine the cause of death or pathological conditions.

Neurotoxicity—The occurrence of adverse effects on the nervous system following exposure to a hazardous substance.

No-Observed-Adverse-Effect Level (NOAEL)—The dose of a chemical at which there were no statistically or biologically significant increases in frequency or severity of adverse effects seen between the exposed population and its appropriate control. Although effects may be produced at this dose, they are not considered to be adverse.

Octanol-Water Partition Coefficient (K_{ow})—The equilibrium ratio of the concentrations of a chemical in *n*-octanol and water, in dilute solution.

Odds Ratio (OR)—A means of measuring the association between an exposure (such as toxic substances and a disease or condition) that represents the best estimate of relative risk (risk as a ratio of the incidence among subjects exposed to a particular risk factor divided by the incidence among subjects who were not exposed to the risk factor). An odds ratio that is greater than 1 is considered to indicate greater risk of disease in the exposed group compared to the unexposed group.

Permissible Exposure Limit (PEL)—An Occupational Safety and Health Administration (OSHA) regulatory limit on the amount or concentration of a substance not to be exceeded in workplace air averaged over any 8-hour work shift of a 40-hour workweek.

Pesticide—General classification of chemicals specifically developed and produced for use in the control of agricultural and public health pests (insects or other organisms harmful to cultivated plants or animals).

Pharmacokinetics—The dynamic behavior of a material in the body, used to predict the fate (disposition) of an exogenous substance in an organism. Utilizing computational techniques, it provides the means of studying the absorption, distribution, metabolism, and excretion of chemicals by the body.

Pharmacokinetic Model—A set of equations that can be used to describe the time course of a parent chemical or metabolite in an animal system. There are two types of pharmacokinetic models: data-based and physiologically-based. A data-based model divides the animal system into a series of compartments, which, in general, do not represent real, identifiable anatomic regions of the body, whereas the physiologically-based model compartments represent real anatomic regions of the body.

Physiologically Based Pharmacodynamic (PBPD) Model—A type of physiologically based dose-response model that quantitatively describes the relationship between target tissue dose and toxic endpoints. These models advance the importance of physiologically based models in that they clearly describe the biological effect (response) produced by the system following exposure to an exogenous substance.

Physiologically Based Pharmacokinetic (PBPK) Model—A type of physiologically based dose-response model that is comprised of a series of compartments representing organs or tissue groups with realistic weights and blood flows. These models require a variety of physiological information, including tissue volumes, blood flow rates to tissues, cardiac output, alveolar ventilation rates, and possibly membrane permeabilities. The models also utilize biochemical information, such as blood:air partition coefficients, and metabolic parameters. PBPK models are also called biologically based tissue dosimetry models.

Prevalence—The number of cases of a disease or condition in a population at one point in time.

Prospective Study—A type of cohort study in which a group is followed over time and the pertinent observations are made on events occurring after the start of the study.

Recommended Exposure Limit (REL)—A National Institute for Occupational Safety and Health (NIOSH) time-weighted average (TWA) concentration for up to a 10-hour workday during a 40-hour workweek.

Reference Concentration (RfC)—An estimate (with uncertainty spanning perhaps an order of magnitude) of a continuous inhalation exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious noncancer health effects during a lifetime. The inhalation RfC is expressed in units of mg/m^3 or ppm.

Reference Dose (RfD)—An estimate (with uncertainty spanning perhaps an order of magnitude) of the daily oral exposure of the human population to a potential hazard that is likely to be without risk of deleterious noncancer health effects during a lifetime. The oral RfD is expressed in units of mg/kg/day.

Reportable Quantity (RQ)—The quantity of a hazardous substance that is considered reportable under the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA). RQs are (1) ≥1 pound or (2) for selected substances, an amount established by regulation either under CERCLA or under Section 311 of the Clean Water Act. Quantities are measured over a 24-hour period.

Reproductive Toxicity—The occurrence of adverse effects on the reproductive system that may result from exposure to a hazardous substance. The toxicity may be directed to the reproductive organs and/or the related endocrine system. The manifestation of such toxicity may be noted as alterations in sexual behavior, fertility, pregnancy outcomes, or modifications in other functions that are dependent on the integrity of this system.

Retrospective Study—A type of cohort study based on a group of persons known to have been exposed at some time in the past. Data are collected from routinely recorded events, up to the time the study is undertaken. Retrospective studies are limited to causal factors that can be ascertained from existing records and/or examining survivors of the cohort.

Reverse Causation—Describes an association where the outcome results in a change in the biomarker of exposure.

Risk—The possibility or chance that some adverse effect will result from a given exposure to a hazardous substance.

Risk Factor—An aspect of personal behavior or lifestyle, an environmental exposure, existing health condition, or an inborn or inherited characteristic that is associated with an increased occurrence of disease or other health-related event or condition.

Risk Ratio/Relative Risk—The ratio of the risk among persons with specific risk factors compared to the risk among persons without risk factors. A risk ratio that is greater than 1 indicates greater risk of disease in the exposed group compared to the unexposed group.

Short-Term Exposure Limit (STEL)—A STEL is a 15-minute TWA exposure that should not be exceeded at any time during a workday.

Standardized Mortality Ratio (SMR)—A ratio of the observed number of deaths and the expected number of deaths in a specific standard population.

Target Organ Toxicity—This term covers a broad range of adverse effects on target organs or physiological systems (e.g., renal, cardiovascular) extending from those arising through a single limited exposure to those assumed over a lifetime of exposure to a chemical.

Teratogen—A chemical that causes structural defects that affect the development of an organism.

Threshold Limit Value (TLV)—An American Conference of Governmental Industrial Hygienists (ACGIH) concentration of a substance to which it is believed that nearly all workers may be repeatedly exposed, day after day, for a working lifetime without adverse effect. The TLV may be expressed as a Time-Weighted Average (TLV-TWA), as a Short-Term Exposure Limit (TLV-STEL), or as a ceiling limit (TLV-C).

Time-Weighted Average (TWA)—An average exposure within a given time period.

Toxicokinetic—The absorption, distribution, metabolism, and elimination of toxic compounds in the living organism.

Toxics Release Inventory (TRI)—The TRI is an EPA program that tracks toxic chemical releases and pollution prevention activities reported by industrial and federal facilities.

Uncertainty Factor (UF)—A factor used in operationally deriving the Minimal Risk Level (MRL), Reference Dose (RfD), or Reference Concentration (RfC) from experimental data. UFs are intended to account for (1) the variation in sensitivity among the members of the human population, (2) the uncertainty in extrapolating animal data to the case of human, (3) the uncertainty in extrapolating from data obtained in a study that is of less than lifetime exposure, and (4) the uncertainty in using lowest-observed-adverse-effect level (LOAEL) data rather than no-observed-adverse-effect level (NOAEL) data. A default for each individual UF is 10; if complete certainty in data exists, a value of 1 can be used; however, a reduced UF of 3 may be used on a case-by-case basis (3 being the approximate logarithmic average of 10 and 1).

Xenobiotic—Any substance that is foreign to the biological system.

APPENDIX F. ACRONYMS, ABBREVIATIONS, AND SYMBOLS

AAPCC	American Association of Poison Control Centers
ACGIH	American Conference of Governmental Industrial Hygienists
ACOEM	American College of Occupational and Environmental Medicine
ACMT	American College of Medical Toxicology
ADI	acceptable daily intake
ADME	absorption, distribution, metabolism, and excretion
AEGL	Acute Exposure Guideline Level
AIC	Akaike's information criterion
AIHA	American Industrial Hygiene Association
ALT	alanine aminotransferase
AOEC	Association of Occupational and Environmental Clinics
AP	alkaline phosphatase
APFO	ammonium perfluorooctanoate
AST	aspartate aminotransferase
atm	atmosphere
ATSDR	Agency for Toxic Substances and Disease Registry
AWQC	Ambient Water Quality Criteria
BCF	bioconcentration factor
BMD/C	benchmark dose or benchmark concentration
BMD_X	dose that produces a X% change in response rate of an adverse effect
$BMDL_X$	95% lower confidence limit on the BMD_X
BMDS	Benchmark Dose Software
BMR	benchmark response
BUN	blood urea nitrogen
C	centigrade
CAA	Clean Air Act
CAS	Chemical Abstract Services
CDC	Centers for Disease Control and Prevention
CEL	cancer effect level
CERCLA	Comprehensive Environmental Response, Compensation, and Liability Act
CFR	Code of Federal Regulations
Ci	curie
CI	confidence interval
cm	centimeter
CPSC	Consumer Products Safety Commission
CWA	Clean Water Act
DNA	deoxyribonucleic acid
DOD	Department of Defense
DOE	Department of Energy
DWEL	drinking water exposure level
EAFUS	Everything Added to Food in the United States
ECG/EKG	electrocardiogram
EEG	electroencephalogram
EPA	Environmental Protection Agency
ERPG	emergency response planning guidelines
F	Fahrenheit
F1	first-filial generation
FDA	Food and Drug Administration
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act

FR	Federal Register
FSH	follicle stimulating hormone
g	gram
GC	gas chromatography
gd	gestational day
GGT	γ-glutamyl transferase
GRAS	generally recognized as safe
HEC	human equivalent concentration
HED	human equivalent dose
HHS	Department of Health and Human Services
HPLC	high-performance liquid chromatography
HSDB	Hazardous Substance Data Bank
IARC	International Agency for Research on Cancer
IDLH	immediately dangerous to life and health
IRIS	Integrated Risk Information System
Kd	adsorption ratio
kg	kilogram
kkg	kilokilogram; 1 kilokilogram is equivalent to 1,000 kilograms and 1 metric ton
K_{oc}	organic carbon partition coefficient
K_{ow}	octanol-water partition coefficient
L	liter
LC	liquid chromatography
LC_{50}	lethal concentration, 50% kill
LC_{Lo}	lethal concentration, low
LD_{50}	lethal dose, 50% kill
LD_{Lo}	lethal dose, low
LDH	lactic dehydrogenase
LH	luteinizing hormone
LOAEL	lowest-observed-adverse-effect level
LSE	Level of Significant Exposure
LT_{50}	lethal time, 50% kill
m	meter
mCi	millicurie
MCL	maximum contaminant level
MCLG	maximum contaminant level goal
MF	modifying factor
mg	milligram
mL	milliliter
mm	millimeter
mmHg	millimeters of mercury
mmol	millimole
MRL	Minimal Risk Level
MS	mass spectrometry
MSHA	Mine Safety and Health Administration
Mt	metric ton
NAAQS	National Ambient Air Quality Standard
NAS	National Academy of Science
NCEH	National Center for Environmental Health
ND	not detected
ng	nanogram
NHANES	National Health and Nutrition Examination Survey

NIEHS	National Institute of Environmental Health Sciences
NIOSH	National Institute for Occupational Safety and Health
NLM	National Library of Medicine
nm	nanometer
nmol	nanomole
NOAEL	no-observed-adverse-effect level
NPL	National Priorities List
NR	not reported
NRC	National Research Council
NS	not specified
NTP	National Toxicology Program
OR	odds ratio
OSHA	Occupational Safety and Health Administration
PAC	Protective Action Criteria
PAH	polycyclic aromatic hydrocarbon
PBPD	physiologically based pharmacodynamic
PBPK	physiologically based pharmacokinetic
PEHSU	Pediatric Environmental Health Specialty Unit
PEL	permissible exposure limit
PEL-C	permissible exposure limit-ceiling value
PFBA	perfluorobutanoic acid
PFBS	Perfluorobutane sulfonic acid
PFDA	perfluorodecanoic acid
PFDoDA	perfluorododecanoic acid
PFHpA	perfluoroheptanoic acid
PFHxA	perfluorohexanoic acid
PFHxS	perfluorohexane sulfonic acid
PFNA	perfluorononanoic acid
PFOA	perfluorooctanoic acid
FOSA	perfluorooctane sulfonamide
PFOS	perfluorooctane sulfonic acid
PFUnA	perfluoroundecanoic acid
pg	picogram
PND	postnatal day
POD	point of departure
ppb	parts per billion
ppbv	parts per billion by volume
ppm	parts per million
ppt	parts per trillion
REL	recommended exposure level/limit
REL-C	recommended exposure level-ceiling value
RfC	reference concentration
RfD	reference dose
RNA	ribonucleic acid
SARA	Superfund Amendments and Reauthorization Act
SCE	sister chromatid exchange
SD	standard deviation
SE	standard error
SGOT	serum glutamic oxaloacetic transaminase (same as aspartate aminotransferase or AST)
SGPT	serum glutamic pyruvic transaminase (same as alanine aminotransferase or ALT)
SIC	standard industrial classification

SMR	standardized mortality ratio
sRBC	sheep red blood cell
STEL	short term exposure limit
$T_{1/2}$	Half-life
TLV	threshold limit value
TLV-C	threshold limit value-ceiling value
TRI	Toxics Release Inventory
TSCA	Toxic Substances Control Act
TWA	time-weighted average
UF	uncertainty factor
U.S.	United States
USDA	United States Department of Agriculture
USGS	United States Geological Survey
USNRC	U.S. Nuclear Regulatory Commission
VOC	volatile organic compound
WBC	white blood cell
WHO	World Health Organization

>	greater than
≥	greater than or equal to
=	equal to
<	less than
≤	less than or equal to
%	percent
α	alpha
β	beta
γ	gamma
δ	delta
μm	micrometer
μg	microgram
q_1^*	cancer slope factor
–	negative
+	positive
(+)	weakly positive result
(–)	weakly negative result



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REVIEW PAPER

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Developing innovative treatment technologies for PFAS-containing wastes

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ABSTRACT

The release of persistent per- and polyfluoroalkyl substances (PFAS) into the environment is a major concern for the United States Environmental Protection Agency (U.S. EPA). To complement its ongoing research efforts addressing PFAS contamination, the U.S. EPA's Office of Research and Development (ORD) commissioned the PFAS Innovative Treatment Team (PITT) to provide new perspectives on treatment and disposal of high priority PFAS-containing wastes. During its six-month tenure, the team was charged with identifying and developing promising solutions to destroy PFAS. The PITT examined emerging technologies for PFAS waste treatment and selected four technologies for further investigation. These technologies included mechanochemical treatment, electrochemical oxidation, gasification and pyrolysis, and supercritical water oxidation. This paper highlights these four technologies and discusses their prospects and the development needed before potentially becoming available solutions to address PFAS-contaminated waste.

Implications: This paper examines four novel, non-combustion technologies or applications for the treatment of persistent per- and polyfluoroalkyl substances (PFAS) wastes. These technologies are introduced to the reader along with their current state of development and areas for further development. This information will be useful for developers, policy makers, and facility managers that are facing increasing issues with disposal of PFAS wastes.

PAPER HISTORY
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Innovative team approach

The presence of persistent per- and polyfluoroalkyl substances (PFAS) in the environment is one of the most pressing environmental issues of the 21st century (Lim 2019; Lindstrom, Strynar, and Libelo 2011; Pan et al. 2017; Sunderland et al. 2019). There is high interest in understanding and addressing PFAS contamination among industry, state and federal government, and internationally. In April of 2020, the United States Environmental Protection Agency (U.S. EPA) formed the PFAS Innovative Treatment Team (PITT) to explore innovative tools and methods for destroying all the carbon fluorine (C-F) bonds in PFAS-containing waste. This interdisciplinary team consisted of eleven scientists and engineers from the Office of Research and Development (ORD) and operated under the framework of a "Tiger Team" as used by The National Aeronautics and Space Administration (NASA) Dempsey et al. (1964) or "Skunk Works" teams as used by corporations Lockheed (2021). During the team's six-month lifetime, it investigated numerous technologies to address PFAS contamination in various waste streams, with a goal of exploring several promising and understudied technologies. The team was provided with financial resources to initiate the study of different treatment technologies at the laboratory, pilot, or field scales. The work resulted in improved understanding and advancement of four innovative technologies to supplement ongoing EPA research into PFAS treatment technologies. These technologies are electrochemical oxidation (EO), supercritical water oxidation (SCWO), mechanochemical degradation (MCD), and gasification and pyrolysis. Efforts were also initiated to quantify PFAS destruction in traditional waste treatment processes such as conventional thermal incineration. Results from laboratory and field work initiated by or supported by the PITT are ongoing and will be presented in future publications.

In this paper, we provide an overview of PFAS waste disposal and destruction issues, a summary of the five PFAS-containing waste streams that were considered, a discussion of the four innovative technologies studied, and preliminary thoughts about the applicability of technologies to different waste streams. Technical results from laboratory and field tests of PFAS treatment by individual technologies will follow in future publications.

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The PFAS challenge

The nature of the C-F bond makes PFAS difficult to destroy (Lemal 2004; O'Hagan 2008; Tsang, Burgess, and Babushok 1998). The small size and high electronegativity of fluorine creates the strongest bond in organic chemistry when bonded to carbon, which results in fairly inert molecules with very low surface energies (Lemal 2004; O'Hagan 2008). Molecules and polymers containing many C-F bonds are generally highly stable and unreactive. C-F bonds are very rare in nature Goldman (1969), but are abundant in several classes of industrially-produced chemicals, including PFAS. The PFAS class of compounds includes thousands of different substances (Lindstrom, Strynar, and Libelo 2011) which differ primarily by the number of C atoms in the alkyl chain and the functional groups typically attached to the end of the chain.

The first reported synthesis of a C-F bond was in 1862 Borodin, (1862). In 1890, Moissan described perfluorinated alkanes (Moissan 1890a, 1890b), a new class of fluorinated molecules that had been synthesized and isolated. He noted that the fluorinated compounds were particularly inert and stable; tetrafluoromethane could only be destroyed completely by heating it over sodium (Moissan 1890a, 1890b). Tetrafluoromethane and fluorinated alkyl molecules were first isolated in 1926[14], mass produced as precursors and refrigerants by the mid-1930's (Daudt 1935; Okazoe 2009), and incorporated in greases by 1940 Gaylor (1940).

Research, development, and application of PFAS accelerated at the start of World War II, with strong links to the Manhattan Project. Liquid perfluorinated molecules and inert and formable fluoropolymers played a vital role in uranium enrichment, protective coatings, and other military applications Okazoe (2009). Many of these compounds were first developed in the late 1930s (IG Farbenindustrie, 1937, Plunkett 1941). Following World War II, companies involved in the war effort patented and developed commercial uses for the growing number of PFAS. Over the last 70 years, PFAS have been used in a variety of products. Products making extensive use of PFAS molecules include the following: protective non-stick and stain-resistant coatings Dinglasan-Panlilio and Mabury (2006), surfactants, fire-fighting agents (DeYoung 1994; Roth et al. 2020), temperature resistant products, food-contact materials (Schaider et al. 2017; Trier, Granby, and Christensen 2011), cosmetics Schultes et al. (2018), and more (Lemal 2004; Prevedouros et al. 2006). These synthetic compounds are used by industry, consumers, governments, and militaries around the world.

The characteristics that make PFAS useful in a wide variety of products also make them extremely difficult to destroy. The C-F bond is resistant to breakdown from ultraviolet light, biological processes, and heat. As a result, PFAS have become widespread persistent pollutants found around the world (Cicerone 1979; Cordner et al. 2019; Giesy and Kannan 2001; Lindstrom, Strynar, and Libelo 2011; Martin et al. 2002; McCord and Strynar 2019; Ravishankara et al. 1993; Stock et al. 2004). Some PFAS are detrimental to the environment (Ghisi, Vamerali, and Manzetti 2019; Ivy et al. 2012; Pan et al. 2017) and human health (Sunderland et al. 2019; Szilagyi, Avula, and Fry 2020). People can be exposed to PFAS from contaminated drinking water, fish, and food packaging (Sunderland et al. 2019; Trier, Granby, and Christensen 2011). Dermal, organ, and inhalation exposure to PFAS are all possible, especially for workers involved in making or processing PFAS and PFAS-containing materials where inhalation is the most likely route (Heydebreck et al. 2016). The best studied perfluoroalkyl acids are perfluorooctanesulfonic acid (PFOS) and perfluorooctanoic acid (PFOA). These long-chain PFAS can bioaccumulate over time to reach harmful levels and are linked to liver, immunological, developmental, reproductive, endocrine, cardiovascular, and cancer effects (Council 2020). With PFAS being in the blood of 98% of Americans, (Calafat et al. 2007) and PFAS' associated adverse health effects (Eriksen et al. 2009; Grandjean et al. 2012; Lewis, Johns, and Meeker 2015; Lindstrom, Strynar, and Libelo 2011; Sunderland et al. 2019), it is important to find effective methods to reduce the amount of PFAS in the sources of our exposure.

PFAS are present in several high-volume waste streams. These include municipal solid waste, landfill leachate, wastewaters treated at wastewater treatment plants, sewage sludge and biosolids from sewage treatment, and both concentrated and diluted Aqueous Film Forming Foam (AFFF). Treating the PFAS in these wastes requires methods that ensure PFAS and other harmful chemicals do not continue to accumulate in people or the environment. It is relatively easy to remove the functional group from a PFAS molecule, and thus destroy the initial compound; however, this often results in the formation of a different PFAS or organofluorine compound (Krusic, Marchione, and Roe 2005; Krusic and Roe 2004). This can create misleading information about the ultimate destruction of PFAS. The destruction and removal efficiency (DRE) for a treatment method can be very high, but the C-F bonds may all still be intact Watanabe et al. (2018). C-F bonds can require temperatures over 1400°C to be

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destroyed thermally (Tsang, Burgess, and Babushok 1998) or highly reducing or oxidizing conditions for destruction at lower temperatures (Costello and McCarthy 1984; Lebeau and Damiens 1926; Moissan 1890a), so PFAS and organofluorine byproducts can be released into the environment from most treatment methods. Many of these partially destroyed PFAS molecules can react and be oxidized in the atmosphere or groundwater to form more harmful species, such as carboxylic acid functionalized PFAS (Ellis et al. 2004; Martin et al. 2006; Prevedouros et al. 2006; Stock et al. 2004). Ideal destruction methods would mineralize all the fluorine in the PFAS, breaking all the C-F bonds, to ensure that no organofluorine byproducts are produced.

The disposal and destruction of PFAS in contaminated sources is a complex area of research. PFAS are found throughout the world in most every matrix, making one universal destruction technique nearly impossible. Each of the main sources of PFAS needs to be carefully examined to determine the best approach for remediation or destruction.

PFAS waste streams

To properly address the PFAS waste management issues, it was first necessary to identify significant PFAS-containing waste streams and to understand both the nature of those wastes and the magnitude of the PFAS issue regarding those wastes. The five waste streams the PITT focused on were (1) landfill leachate, (2) biosolids, (3) contaminated soils, (4) granular activated carbon (GAC) or ion exchange resins used to remove PFAS from other media, and (5) AFFF, both concentrated and diluted. Each of these waste streams will be summarized in this section to provide a context for the PFAS destruction techniques explored in the following section.

Landfill leachate

In 2018 the U.S. disposed over 310 million metric tons of waste in nonhazardous waste landfills (U.S. EPA 2020), of which at least half was municipal solid waste (MSW). MSW contains many consumer products known to contain PFAS, including food contact paper (lined to-go containers), textiles (carpets and clothing), cleaning agents, and non-stick cookware, among others. Since PFAS are not considered hazardous wastes and are not accounted for accordingly, other commercial or industrial wastes deposited in landfills may also contain these compounds.

Modern sanitary landfills are lined with a plastic membrane overlying low-permeability soil, such as clay, which limits groundwater contamination but requires treating the collected leachate. Because of the direct leaching of PFAS-containing wastes, landfill leachate tends to have elevated PFAS levels compared to municipal wastewater alone (Huset et al. 2011; Masoner et al. 2020). Lang et al. (2017) estimated 61.1 million m^3 of leachate were generated across the U.S. in 2013, comprising between 563 and 638 kg of PFAS.

Landfill leachate also tends to have higher suspended solids, chemical oxygen demand (COD), ammonia, salinity, and alkalinity than municipal wastewaters (Kjeldsen et al. 2002). The high organic matter loading makes leachate difficult to treat on its own, and any treatment of landfill leachate specifically for PFAS may require pre-treatment for co-contaminants (Wiszniowski et al. 2006). Many wastewater treatment plants do not contain the necessary treatment technologies (specially designed granular activated carbon, ion-exchange resins, reverse osmosis, and nanofiltration systems) to fully remove PFAS from treated water (Aro et al. 2021; Pan, Liu, and Ying 2016). As a result, the PFAS compounds have the potential to remain in the treated effluent.

Biosolids

In addition to the treated wastewater, wastewater treatment plants must also safely manage the residual sludges produced during treatment processes. Influent wastewater streams generally contain PFAS in various concentrations, which are then detected in both the treated effluent water and in the biosolids and sludges (Aro et al. 2021; Chiavola et al. 2020; Dauchy et al. 2017; Gallen et al. 2018a; Loganathan et al. 2007a). In the U.S., solids and sludge are largely either incinerated (~16%), landfilled (~22%), or land applied (~52%) U.S. EPA (2019). For land application, time and temperature treatment of WWTP solids to form Class A or B biosolids is regulated by the EPA to reduce pathogens, vector attraction, and heavy metals before use as nutrients and soil conditioners (U.S. EPA 1994). Since 2001, a growing number of studies (Alder and van der Voet 2015; Chen et al. 2012; Gallen et al. 2018b; Loganathan et al. 2007b; Michigan Department of Environment, 2020; Navarro et al. 2016; Schultz et al. 2006; Sepulvado et al. 2011; Sinclair and Kannan 2006; Venkatesan and Halden 2013; Yoo et al. 2009; Yu et al. 2009) have shown PFAS in biosolids in the U.S. and internationally with typical results showing PFOA levels from <20 µg/kg to as much as 240 µg/kg dry wt. These surveys show a larger range in

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PFOS levels generally from <30 μg/kg to over 1000 μg/kg. The concentrations of individual PFAS in biosolids show a high spatial and temporal variability. As a result of several rare cases of land-applied biosolids containing high PFAS levels, some states in the U.S. (such as the state of Maine) limit the PFAS concentration of biosolids that are to be land applied. Addressing PFAS in biosolids in a way that allows beneficial reuse or resource recovery is a priority.

Contaminated soil

The heterogenous nature of soils creates complex interactions with PFAS. The soil's organic content and surface charges are the major contributors to PFAS retention. Soils with high organic content can interact with and retain PFAS through hydrophobic interactions, while ions in the soil can interact with PFAS electrostatically. The length of the PFAS chain is the main factor in the PFAS partitioning into soil. As the tail gets longer, the PFAS is retained more due to the increasing size and greater hydrophobicity. A charged functional group can also increase retention (Mejia-Avendaño et al. 2020; Xiao et al. 2019).

The strong chemical structure of PFAS and their bonding with soil makes them challenging to eliminate from soil environments. Washington et al. calculated global soil loadings for eight PFAS compounds (Washington et al. 2019). The combined estimated load for the eight PFAS compounds ranged from 1500 to 9000 metric tons, with mean estimates of approximately 1000 metric tons for both PFOA and PFOS. These results indicate that soil has the potential to be a reservoir for PFAS. Soil concentrations reported for PFAS-contaminated sites are generally orders-of-magnitude greater than background levels (Brusseau, Anderson, and Guo 2020). Maximum reported PFOS concentrations for primary contaminated sites ranged upwards of several hundred mg/kg. For example, a maximum PFOS concentration of 373 mg/kg was measured at an AFFF source zone on a U.S. military installation (Brusseau, Anderson, and Guo 2020). PFAS soil concentrations for secondary-source contaminated sites (e.g., land application of municipal biosolids and use of contaminated ground water for irrigation) were about two orders of magnitude lower (Brusseau, Anderson, and Guo 2020).

Methods for PFAS removal from contaminated soils have been reviewed by Ross et al. (2018), Mahinroosta and Senevirathna (2020), and Bolan et al. (2021). Mature treatment technologies for soils include incineration, soil stabilization, excavation, and *ex-situ* thermal treatment Ross et al. (2018). Recent field tests focusing on sampling methods development have examined soil incineration of AFFF-contaminated soil as a secondary goal, cumulating in a proposed test method, OTM-45 (Merrill and Ryan 2021), and the Department of Defense's (DoD's) associated Strategic and Environmental Research and Development Program (SERDP) final report for Project ER19-1408 to be released on SERDP's web site, www.SERDP.org. More experimental methods include soil washing, advanced oxidation or reduction processes, and ball milling (Bolan et al. 2021). Since the goal of the PITT was to identify technologies that would destroy PFAS completely, we focused on destruction methods versus stabilization or removal (e.g., washing or thermal desorption) methods.

Granular activated carbon/anion exchange resins

While ingestion of contaminated food has been identified as the primary PFOA exposure pathway for the general population, drinking water can become the primary exposure pathway in communities with contaminated water (Vestergren and Cousins 2009). To provide protection from long term exposure to PFOS and PFOA in drinking water, the U.S. EPA established drinking water health advisory concentration limits for PFOS and PFOA of 70 ng/L in 2016 (U.S. EPA 2016a, 2016b). To meet these limits, granular activated carbon (GAC) and anion exchange resins (AEX) have been utilized as treatment methods for separating PFAS from liquid streams, often at a fraction of the cost of other separation technologies such as nanofiltration and reverse osmosis (Dickenson and Higgins 2016). GAC and AEX are commonly used to separate PFAS from liquid streams during groundwater site remediation and manufacturing as well as drinking water treatment. GAC removes PFAS compounds from liquid by surface adsorption whereas AEX involves ion exchange onto a positively-charged surface. The surfaces of both materials become saturated over time and no longer able to remove PFAS. GAC can be regenerated but eventually loses its effectiveness and must be disposed. AEX resins used for treatment of PFAS may or may not be amenable to regeneration. Spent GAC and AEX are currently either landfilled or incinerated. The former may lead to PFAS ending up in the landfill leachate.

Aqueous film-forming foams

AFFF are water-based foams that are used to extinguish fires, generally those involving flammable liquids, and are commonly used with aircraft operations. They generally contain a mixture of hydrocarbon and fluorocarbon surfactants, such as fluorotelomers with zwitterionic, cationic,

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and anionic properties along with PFOA or PFOS (D'Agostino and Mabury 2014; Place and Field 2012). Fluorinated surfactants' unique characteristics help the foams spread and isolate the fuel from the air, smothering the fire (Moody and Field 2000). The presence of fluorine on the molecules also makes PFAS effective free radical scavengers, which reduce the availability of hydroxyl radicals, the primary chemical component that serves to maintain combustion in conventional fires (Tsang, Burgess, and Babushok 1998).

There is a stockpile of existing AFFF materials, estimated at 9.9 million gallons (United Nations Environment Programme 2004), that now requires disposal to prevent environmental contamination. Most of these older formulations contain C_8 PFAS foams and have been phased out in favor of C_6 PFAS foams (Lim 2019), but still require disposal of the legacy stockpile. Alternate formulations that do not contain PFAS are being developed (Vergun 2019), but disposal of the legacy AFFF is problematic for both the military and civilian stockpiles.

According to a United Nations Environmental Program (UNEP) report United Nations Environment Programme (2004), quantities of AFFF are generally expressed as volume percentages of concentrate. In the foam industry, concentrates are typically referred to as "3%" or "6%" concentrate, depending on the mixture rate with water. For example, if the container of foam concentrate is labelled as 3%, then 97 volumes of water are mixed with 3 volumes of PFAS to make 100 volumes of foam solution for fire application. The high water concentration of AFFF, whether the concentrate or the diluted form, is problematic for multiple types of disposal methods.

On-going ORD research efforts, including projects initiated by the PITT, are underway to identify the temperature and time requirements for effective combustive destruction of AFFF. In addition to these combustion/incineration technologies, less developed, non-thermal approaches were considered by the PITT.

Potential, non-combustion treatment technologies

The PITT considered an array of different treatment technologies to deal with the five PFAS-containing waste streams discussed previously. The PITT specifically focused on promising technologies that were underfunded or understudied and that showed potential to advance with limited time and resources. The team considered various factors in its decision process, including those listed in Figure 1. The selection or dismissal of technologies was not an indication of the potential of those technologies, as the PITT passed over many promising technologies for a variety of different reasons unrelated to technology potential.

The PITT elected to focus on four emerging technologies, including:

- Mechanicochemical destruction (ball milling)
- Electrochemical oxidation
- Supercritical water oxidation (SCWO)
- Pyrolysis/gasification

Each of these technologies has characteristics that generally align them with specific PFAS-contaminated materials. Research Briefs are available on the PITT's web site, https://www.epa.gov/chemical-research/pfas-innovative-treatment-team-pitt, to explain each of the technologies, their benefits, and areas where more research is needed.

The factors related to treatment selection and process management are shown in Figure 2 for each of the four focus technologies. Selection of an applicable technology must consider how the waste characteristics and processing requirements match up with the capabilities of the technology. Factors related to engineering design considerations are indicated in Figure 2 for each of the four treatment technologies. The size or volume of the system is a function of the treatability goals and effectiveness, and whether the system is meant to be mobile or stationary. Consideration of energy consumption, capital costs, technology complexity, and lifecycle also are required considerations. In some cases, additional pretreatment unit operations such as de-watering or addition of co-mingling agents will either be required or aid in the effectiveness of the technology. Post-treatment unit operations may also be required such as when a by-product of the process is expected. For example, PFAS breakdown may result in smaller C-number molecules that are volatile, requiring gas treatment to prevent emissions.

- Effectiveness
- Readiness (e.g.: timeline, maturity, capacity)
- Applicability
- Cost
- Specificity (phase and characteristics of the contaminated matrix)
- Safety of outputs (potential for formation of harmful by-products)
- Required development remaining
- Constraints
- Opportunity level for PITT to influence development

Figure 1. Decision factors discussed about all technologies. Four technologies were selected based upon these factors.

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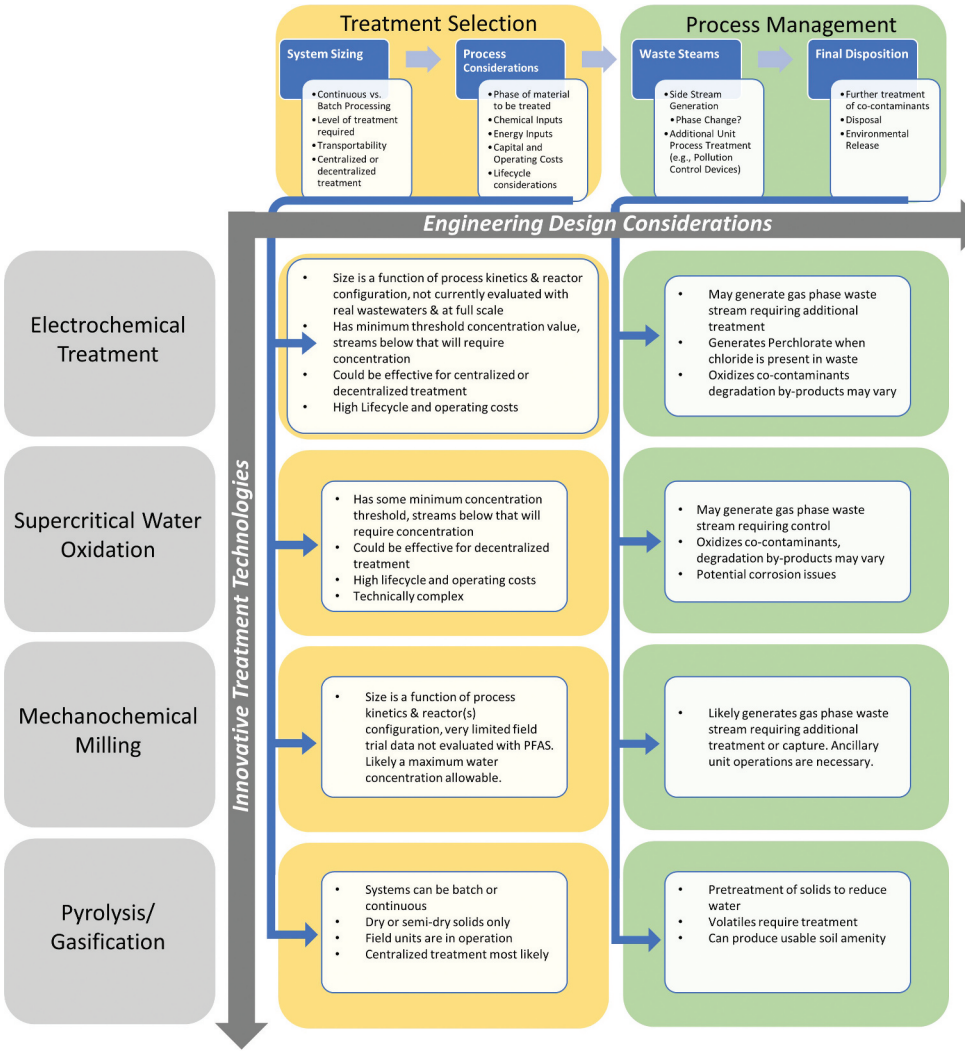


Figure 2. Innovative treatment technology considerations.

Waste-specific characteristics, such as the physical form of the PFAS waste source, are a major determinant of technology suitability. Figure 3 defines the technology selection in terms of the waste factors, handling characteristics, volume, co-contaminants, site locations, and concentrations. The PFAS phase, for example liquid leachate or soil contaminant, is a major factor in technology selection.

Mechanochemical milling

Treatment of solid or semi-solid matrices in high energy ball mills has been proposed as a means of treating PFAS-containing soils and dewatered sludges. Mechanochemical degradation (MCD) has been undertaken on organic-contaminated media (Cagnetta et al. 2016) often with silica or alkaline earth elements to react with fluorine and encourage reactivity. Reactive radicals, electrons, heat, and even plasma in localized areas of the crystalline structures are created by the impact of stainless steel milling balls in the rotating vessel (Nakayama 2010). Effective destruction (> 99%) of PFAS molecules, including PFOS and PFOA, have been demonstrated in laboratory scale ball mills using pure PFAS compounds and different co-milling reagents (including calcium oxide, potassium hydroxide, lanthanum oxide, sodium persulfate, and sand) (Bolan et al. 2021; Cagnetta et al. 2016, 2017; Lu et al. 2017; Wang et al. 2019; Zhang et al. 2013). Mechanochemical treatments have been effective in soil remediation for chlorinated chemicals (Bolan et al. 2021); however, the treatment of PFAS-containing wastes at a commercially relevant scale (~3 tons per hour) using mechanochemical methods requires additional study. In particular, the effectiveness of MCD at destroying PFAS in soil matrixes (with or without co-milling reagents) remains to be proven.

MCD could be developed in a portable (trailer-based) system or set up on-site for extensive remediation projects, as some commercial companies (e.g., Environmental

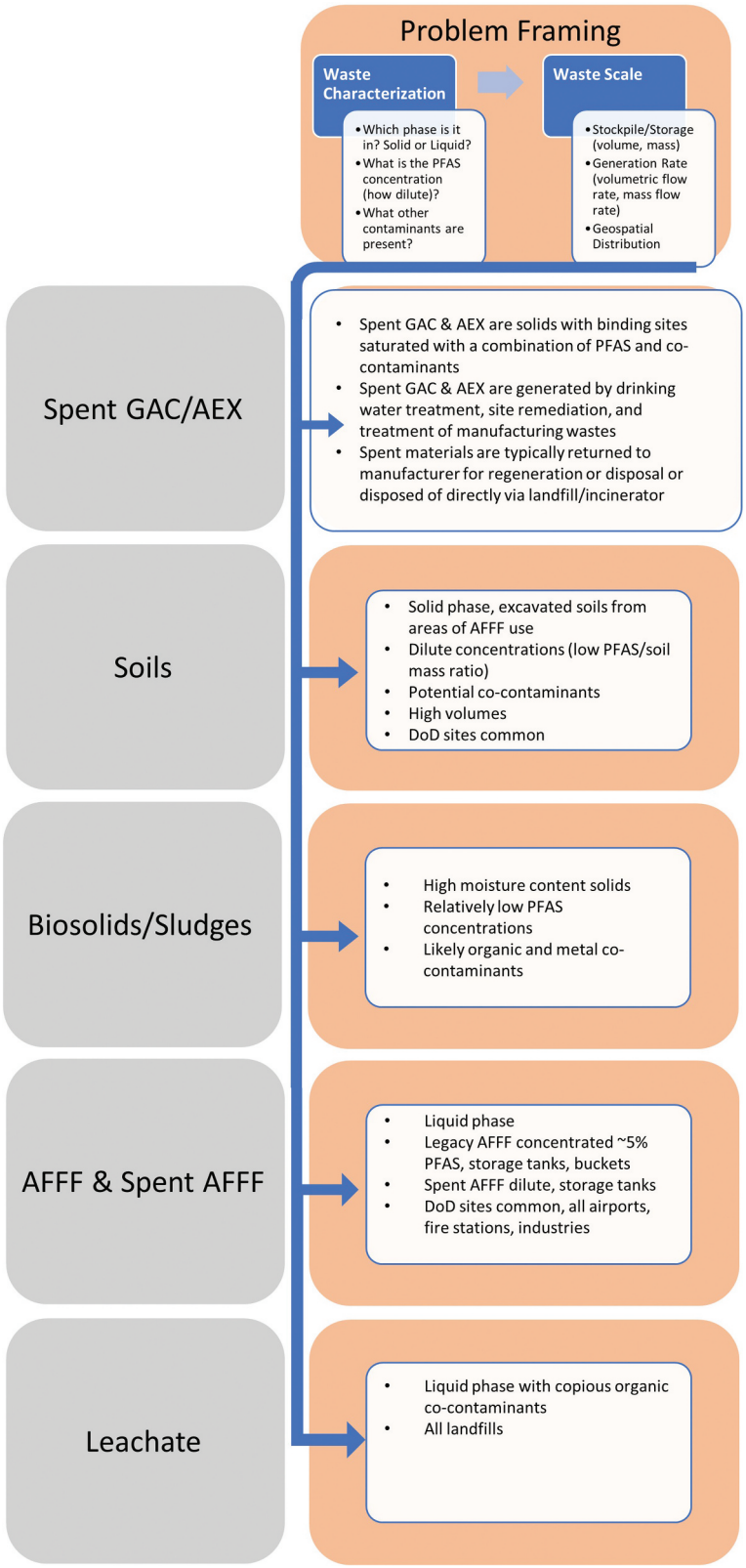


Figure 3. Characteristics of PFAS contaminated matrices.

Decontamination Limited) have done successfully in the past (Bulley 2020). In addition, MCD is unique in that it treats materials in the solid phase without combustion, as compared with most other non-combustion techniques that remove the PFAS from the solid phase into the liquid (e.g., soil washing) or gas (e.g., thermal desorption) phases for treatment. Despite this, it is possible that gaseous PFAS compounds could be released during the MCD treatment, especially in open systems, so a full MCD treatment system must address potential release of volatile PFAS during treatment.

MCD is most amenable to highly contaminated soils, requiring a pre-treatment drying step to remove moisture from the soils, followed by addition of one or more co-milling reagents to react with the PFAS molecules. The resulting soils would be much finer than the initial soils and would lose much of their microstructure. This may affect the fertility of the treated soils. However, mixing treated soils with untreated soils (such as clean soil from off-site) could allow partial recycling of site soils without the potential mechanical and biological issues associated with the milled soils. Another potential issue is the impact of any co-milling reagents on the resulting treated soils. For this reason, non-reactive reagents (such as silicon dioxide or other metal oxides) are preferable to highly reactive (e.g., oxidizing, such as persulfate, or reducing, such as metal hydrides) or pH-modifying (such as acids or hydroxides) reagents. PFAS impacted soils have recently been remediated with and without co-milling reagents in a benchtop ball-mill and showed over 99% destruction of the PFAS with limited byproducts that the authors could analytical resolve (Turner et al. 2021). Further studies are needed to evaluate the gas phase emissions and to better determine any PFAS byproducts.

It is possible that MCD could be used to treat other solid waste streams containing high PFAS concentrations, such as dried GAC. This is especially true with the addition of co-milling reagents to add reactivity and change the mechanical properties of the milled solid. Experiments are required to determine the feasibility of such approaches. More information on MCD can be found in the U.S. EPA's Mechanochemical Degradation Research Brief (Shields and Whitehill 2021).

Electrochemical treatment

Electrochemical oxidation (EO) has been used to oxidize pollutants by means of passing an electrical current through a solution. The electronegativity and electron affinity of fluorine allows the C-F bond to be broken and the fluorine atoms reduced when a high overpotential, > 3 V, is applied to a solution (Niu et al. 2016). The general destruction mechanism involves a step-wise removal of CF_2 groups and the synthesis of shorter carboxylic acid PFAS until all the carbons have been defluorinated (Le et al. 2019; Niu et al. 2016). PFAS, including PFOA and PFOS, have been destroyed using several different types of electrodes in a search to improve efficiencies and allow for process scale up. Materials such as boron-doped diamond (Liao and Farrell 2009; Soriano, Gorri, and Urtiaga 2017), titanium suboxides (Le et al. 2019; Wang et al. 2020), and tin and lead oxides (Niu et al. 2016) have been used to successfully degrade PFAS in aqueous solutions. The process has been demonstrated on PFAS at the bench and pilot scale (Liang et al. 2018; Nzeribe et al. 2019). The technology has advantages of being able to operate at ambient temperature and pressure. EO is diffusion limited and the remediation tends to slow as the concentration of PFAS decreases, so innovative electrode and reactor designs are needed to allow for scale-up. Other issues involve the buildup of minerals on the anode, possible formation of perchlorate and other inorganic by-products, and the generation of volatile by-products which need to be treated. Investigations into the use of an electric field to transport and destroy PFAS in soils have been started and show promise (Skinn 2019) as have tests on landfill leachate (Pierpaoli et al. 2021) and AFFF contaminated groundwater (Schaefer et al. 2019). Further information on this relatively low-energy technology is available in the U.S. EPA's Electrochemical Oxidation Research Brief (Krause, Magnuson, and Crone 2021).

Supercritical Water Oxidation (SCWO)

Above 374°C and 22.11 MPa, water reaches its supercritical state in which it behaves as a solvent for organics and enhances chemical oxidation reactions in the presence of an oxidizing agent such as O_2, air, or hydrogen peroxide. SCWO has been demonstrated to treat halogenated waste materials including polychlorinated biphenyls (Abeln et al., 2001; Kim et al. 2010b). SCWO is able to quickly treat not only water based waste streams, but also sludges (Svanström et al. 2004) and slurries (Kim et al. 2010a); providing a possible way to treat most any pumpable matrix. Although SCWO requires initial energy input to reach the required temperature and pressure, the resulting reactions often produce a significant amount of heat themselves, which can be harvested to keep driving the process. The concentration of oxidizable organic molecules in the material being processed must be controlled and moderated, either by diluting the influent stream or adding additional fuels (such as alcohols). During the oxidation process, SCWO produces many acidic species, such as

sulfuric acid (from sulfur-containing species) and hydrochloric acid (from chlorine-containing species). This results in a drop in pH during the process, which can cause significant corrosion of the reactor if not addressed. This can be addressed to some extent by adding alkalinity to the influent solution. The increased alkalinity of the modified influent solution may aid in the destruction of PFAS, as alkaline solutions show greater destruction and mineralization of PFOS at subcritical conditions than acidic or neutral solutions (Wu et al. 2019). An additional challenge of SCWO is the precipitation of salts. The three major product streams from a SCWO reactor are the effluent, any evolved gases, and the salts that precipitate out from the reaction. A thorough evaluation of PFAS destruction using SCWO must address all of these product streams. Secondary air treatment technology might be necessary if gaseous PFAS compounds are evolved as a result of the SCWO process. There are different manifestations of SCWO based on different process designs. The PITT explored four different manifestations of SCWO technology to evaluate the potential for PFAS destruction.

Technical challenges being addressed when creating commercial products (374water 2020; Battelle Memorial Institute 2019; General Atomics, industrial Supercritical Water Oxidation (iSCWO) 2021; Jama et al. 2020) for the widespread implementation of SCWO include the buildup of corrosive gases during the oxidation reaction, the precipitation of salts, and the high energy requirements. More information is available in the U.S. EPA's Research Brief on SCWO (Sahle-Damesessie and Krause 2021).

Pyrolysis/gasification

Pyrolysis decomposes solid or semi-solid materials at temperatures typically in the 300°C to 1000°C range in an oxygen-free environment. Gasification is similar to pyrolysis but operates at temperatures typically in the 800°C to 1650°C range with substoichiometric quantities of oxygen in a partial combustion process to provide additional energy to the process (Fytili and Zabaniotou 2008; Patel et al. 2020; Winchell et al. 2020). Pyrolysis can form a useful char (Boni et al. 2021) product whereas gasification typically forms low carbon ash. Both processes can generate a hydrogen-rich synthetic gas (syngas) depending on operating conditions. The high temperatures and residence times achieved by the combination of pyrolysis or gasification, followed directly by combustion of the hydrogen-rich syngas stream in a thermal oxidizer could potentially destroy PFAS by breaking apart the chemicals into inert or less recalcitrant constituents, although this remains a subject for further research. Compared to traditional incineration, pyrolysis and gasification require much lower air flows than incineration, which reduces the size and capital expense of air pollution control equipment. More information is available in the U.S. EPA's Research Brief on Pyrolysis and Gasification of Biosolids (Acheson et al. 2021).

Waste source and treatment technology applicability

Some of the basic factors and characteristics involved in matching PFAS waste sources with these four innovative technologies are illustrated in Figure 2 for the technology focus and Figure 3 for the waste focus. These illustrations are meant to serve as a general guide to technology selection, management of the processes, and issues to consider PFAS waste characteristics.

A crosswalk between the technology factors for these four treatment methods and the PFAS waste characteristics, coupled with a literature review on the level of technology development, is presented in Figure 4. These factors are synthesized into an assessment of Technology Readiness Level (TRL, see Figure 4 Key in Figure 5) by the PITT to understand both the waste type applicability and state of development of each technology. These assessments were made by the PITT based on available information and the team's best judgement and should be considered a starting point for discussion and technology development prioritization.

Spent **GAC/AEX** are solids that may be applicable to treatment in MCD or pyrolysis/gasification systems but are not applicable to liquid-based waste treatment technologies such as SCWO or EO. To our knowledge, no PFAS-laden GAC/AEX tests with MCD and pyrolysis/gasification have been undertaken. The crushing and grinding of the MCD systems seem an appropriate test for the solid GAC media. The elevated temperatures in pyrolysis/gasification systems may be an issue for the polymer-based AEX resins, causing melting and fouling of internal systems.

Like GAC/AEX, PFAS-contaminated **soils** could be treated in an MCD or pyrolysis/gasification unit. Both systems are ideally suited for treatment of solid materials. MCD has been used to treat soils contaminated with polychlorinated biphenyls, achieving over 99% mass reduction of soil contamination (Bulley 2020). We could not find literature references to pyrolysis/gasification treatment of PFAS-laden soils. However, the material handling characteristics and treatment operations are similar to those used in thermal decontamination of PFAS-contaminated soils (Bolan et al. 2021; Crownover et al. 2019; Sörengård et al. 2020). Therefore, although the

	Electrochemical Oxidation	SCWO	Mechanochemical degradation	Pyrolysis
Spent GAC / AEX	TRL 4[a] - AEX still bottom	N/A[j]	TRL 2[i,j]	TRL 1[j]
Soils	TRL 1[b]	N/A[j]	TRL 5/6[i]	TRL 1[j]
Biosolids / Sludges	N/A[j]	TRL 6[e]	TRL 1[j]	TRL 7[k]
Spent and unused AFFF	TRL 4/6[a, c]	TRL 7[e-h]	TRL 3/4[f]	N/A[j]
Landfill Leachate	TRL 4[d]	TRL 4[g]	N/A[j]	N/A[j]

a. AECOM (Liang 2018); b. Faraday (Skinn 2019); c. (Schaefe, 2019); d. (Pierpaoli 2021); e. (374Water 2020); f. (General Atomics 2021); g. Aquarden (Jama 2020); h. (Battelle 2019); i. EDL (Bolan 2021); j. PITT assessment; k. (BioForceTech 2019)

Figure 4. Crosswalk between PFAS waste and innovative Technology Readiness Level (TRL). Colors correspond to TRL categories described in Figure 5. No color indicates the non-applicability (N/A) of the technology to the waste stream.

Phase	TRL	Description
Research	1	Basic Principles observed
	2	Technology concept formulated
	3	Experimental proof of concept
Development	4	Technology validated in lab
	5	Technology validated in relevant environment
	6	Technology demonstrated in relevant environment
Deployment	7	System prototype demonstration in operational environment
	8	System complete and qualified
	9	Actual system proven in operational environment

Figure 5. Key for Figure 4 technology readiness levels. Note that technologies were rated for PFAS destruction and may not capture their full capacity or overall readiness-level for other treatment purposes. Source: https://www.twi-global.com/technical-knowledge/faqs/technology-readiness-levels.

release and treatment of volatiles requires additional study, the treatment of PFAS-contaminated soils using pyrolysis or gasification is technically feasible.

Biosolids/sludges are likely candidates for treatment in MCD, pyrolysis/gasification, and SCWO systems. Sufficiently dewatered sludge, or wet sludge dried by addition of co-milling agents, would be applicable to treatment in an MCD system. No such tests with PFAS have been identified in the literature. Preliminary studies on sludge treatment with SCWO have shown strong reductions in PFOS and PFOA levels in the processed effluent (374water 2020). PFAS testing on a biosolids pyrolysis system (Bioforcetech Corporation 2019) was repeated in a test commissioned by the PITT confirming high levels of degradation of the target PFAS compounds analyzed in the feed. Results on the latter are expected to be published in 2021.

A common source of stockpiled, legacy **AFFF** concentrate has been designated for upcoming SCWO, EO, and laboratory-scale MCD testing by the PITT with four SCWO providers. This legacy AFFF is comprised of 8-carbon PFAS, acids, and zwitterions as well as nonfluorinated surfactants and stabilizing chemicals (Material Safety Data Sheet FC-203CF 1996). Preliminary results from these tests show up to 99% degradation of the initial ("target") PFAS compounds in the feed. However, in all cases, the potential non-target byproducts have yet to be analyzed.

PITT efforts

A variety of mechanisms have been put into place to test AFFF destruction using SCWO with four commercial companies. Where feasible, a common AFFF source has been used for these tests. These studies have achieved preliminary results; complete studies on emissions and byproducts are underway. Hydrothermal oxidation, the lower pressure, lower temperature variant of SCWO, is undergoing both laboratory scale tests at EPA and pilot-scale commercial tests (as a SCWO pretreatment step). EO of dilute AFFF is undergoing testing in a laboratory scale reactor, accompanied by collection and analysis of the off-gases for volatile fluorinated organics. Laboratory scale studies of MCD of AFFF-laden soil are being conducted at EPA and a commercial laboratory. Scale-up plans and emission sampling tests have been proposed to extend this effort. Sludge gasification studies have been conducted at a field unit. The destruction of inlet PFAS is being assessed as well as the potential for fluorinated compound emissions. Preliminary results from all four technology/waste tests are expected to be available by 2022.

Summary

Four non-combustion technologies were highlighted for their potential to treat PFAS-laden waste streams: electrochemical oxidation, mechanochemical degradation, supercritical water oxidation, and pyrolysis/gasification. Considerations regarding technology factors and waste characteristics were cross-walked to aid in selection of treatment options. Finally, the technology applicability and readiness level were presented based upon existing, published information. Refinement of technology selection will be dependent on additional data; currently, only extremely limited testing results are available. Programs put into place by the PITT to examine these technologies are expected to yield published results by the end of 2022.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

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DEPARTMENT OF HEALTH AND HUMAN SERVICES

NATIONAL INSTITUTES OF HEALTH

NATIONAL INSTITUTE OF ENVIRONMENTAL HEALTH SCIENCES

Hearing on "Examining the Federal response to the risks associated with per- and polyfluoroalkyl substances (PFAS)"

Testimony before the
Senate Committee on Environment and Public Works

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Chairman Barrasso, Ranking Member Carper, Distinguished Members of the Senate Committee on Environment and Public Works, thank you for inviting me to testify at this hearing on a topic of increasing interest to the scientific community and to the greater public. I am Linda Birnbaum, the Director of the National Institute of Environmental Health Sciences (NIEHS) within the National Institutes of Health (NIH). I am also the Director of the National Toxicology Program (NTP), which serves to develop and coordinate toxicological testing across the Department of Health and Human Services, to conduct hazard assessments of toxic substances, and to manage the Interagency Coordinating Committee on the Validation of Alternative Methods. For the past 40 years I have conducted primary research in toxicology, and I am here today in my role as Director of NIEHS to provide a scientific perspective about the large, complex, and ever-expanding class of chemicals known as per and polyfluoroalkyl substances (PFAS).

The National Institute of Environmental Health Sciences (NIEHS)

The NIEHS is one of several Federal agencies actively working to address various aspects related to PFAS. The NIEHS mission, as set forth under the Public Health Service Act, is to conduct and support research, training, and health information dissemination with respect to environmental factors that may affect human health, directly or indirectly.[1] With this mandate, NIEHS researchers use state-of-the-art science and technology to investigate the interplay between environmental exposures, human biology, genetics, and human disease to help prevent illness, morbidity, and mortality, and improve human health. No age group or disease is beyond the NIEHS mission. Considering this fact, NIEHS researchers collaborate with their peers at the other NIH Institutes and Centers focused on specific life stages, organ systems, or diseases.

NIEHS also has responsibilities under the Superfund Amendments and Reauthorization Act of 1986 (SARA) which created the Worker Training Program (WTP) and the Superfund Research Program (SRP) within NIEHS.[2] The SRP is a broad university-based research program capable of addressing the wide array of scientific uncertainties facing the national Superfund program. Within this purview is the development of methods and technologies to detect hazardous substances in the environment; advanced techniques for the detection, assessment, and evaluation of the effects on human health of hazardous substances; methods to assess the risks to human health presented by hazardous substances; and basic biological, chemical, and physical methods to reduce the amount and toxicity of hazardous substances.

For nearly three decades,[3] NIEHS has been the leading Federal agency sponsoring basic research investigating health effects associated with human exposures to PFAS. NIEHS-

[1] Section 463 of the Public Health Service Act. (42 USC 285l).
[2] Sections 126(g) and 209(b) of the Superfund Amendments and Reauthorization Act of 1986. Public Law 99-499. October 17, 1986. (42 USC 9660a and 42 USC 9660, respectively).
[3] Harris MW, Birnbaum LS. Developmental Toxicity of Perfluorodecanoic Acid in C57BL/6N Mice. *Fundam. Appl. Toxicol*. 1989; 12(3):442-448. DOI: 10.1093/toxsci/12.3.442.

supported research uses human observational studies, animal models, *in vitro* tissue and cell culture systems, *in silico* approaches, and high throughput screening to study the effects of environmental exposure.

The most conclusive research focuses on a single chemical to understand the cause and effect on human health. While studying potentially toxic chemicals, we are largely limited to natural history and population-based studies that attempt to find connections between populations exposed and health effects in the real world. For that reason, you will hear me talk about "associations" – certain health effects happened to more people than normal in populations that are exposed.

The research conducted to date reveals associations between PFAS exposures and a variety of specific adverse human health outcomes. These include the potential for effects on children's cognitive and neurobehavioral development, immune system dysfunction, endocrine disruption, obesity, diabetes, lipid metabolism, and cancer. While knowledge about these epidemiologic associations has steadily expanded in recent years, many questions remain unanswered. The NIEHS and NTP, in coordination with other Federal agencies and State and local governments, continue to conduct research to enhance our understanding of the potential mechanisms and biological processes through which PFAS may be affecting human health. NIEHS coordinates and participates in governmental health research to assure applicability, disseminate findings, and prevent duplication of effort. To this end, NIEHS continues to co-host and participate in numerous symposia and collaborative working groups.

Per and Polyfluoroalkyl Substances (PFAS)

Before detailing the health effects associated with PFAS exposures, it is necessary to describe this class of chemicals. First created in the 1930s and 1940s, PFAS include some 4,700 man-made chemicals that contain fluorine atoms bonded to a carbon chain.[4] The carbon-fluorine bond is one of the strongest ever created by man and is rarely seen in nature. The unique chemical composition of PFAS imparts desirable physical and chemical properties for consumer and industrial products, such as oil and water repellency, high and low temperature stability, and friction reduction. These properties have led to PFAS incorporation in a wide range of consumer products, including textiles, paper products, semiconductors, automotive and aerospace components, cookware, food packaging, and stain repellant clothing. In addition, PFAS play an important role in industrial processes and aqueous film-forming foams (AFFF) that are used as a firefighting tool.

Our scientific understanding of PFAS compounds stems almost entirely from studies on a select few. Perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS) have been manufactured the longest, are the most widespread in the environment, and are the most well-studied PFAS to date. PFOA was used in the production of fluoropolymers such as Teflon®, and PFOS in the production of the original line of Scotchgard® water repellant products. PFOA and PFOS are considered "long-chain" PFAS due to the length of their carbon chain

[4] While approximately 4,700 fluorine-containing, man-made compounds have been created, not all of these compounds have entered into commerce or been actively used.

backbones and have been studied for several decades. A wide range of "short-chain" PFAS have been introduced recently as alternatives to the linear, "long-chain" compounds. All PFAS have garnered increased attention by both the scientific community and the public. Current efforts within the NIEHS and NTP to greatly enhance our understanding of additional long-chain as well as short-chain PFAS are detailed later in this testimony.

The chemical composition of PFAS impart high stability for product design, and this characteristic makes PFAS extremely stable in the environment. In fact, PFAS and complex PFAS degradation products remain in the environment for so long that scientists are unable to accurately estimate an environmental half-life. As PFAS are incorporated into more diverse processes and products, they have greater potential for release into the environment. Manufacturing and processing facilities, airports, and military installations that use firefighting foams are contributors to PFAS releases into the air, soil, and water, including both surface and groundwater sources of drinking water.[5] Because PFAS are resistant to environmental degradation processes, they are subject to long-range atmospheric and oceanic current transport. PFAS have been identified in both environmental and biological samples collected in some of the most remote areas on earth.

As new knowledge is acquired about the breadth of exposures in many communities and the potential hazards to human health, questions arise about whether continued use of PFAS in specific applications is necessary, or if alternatives exist that may be less harmful but still provide sufficient performance. As part of our portfolios, NIEHS and NTP contribute substantively to the field of alternatives assessment to ensure harmful chemicals are not replaced by similarly harmful but less well-studied related compounds.

Human Exposures

Humans are exposed to PFAS through myriad pathways, practices, and products. Ingestion, particularly through drinking water, is the predominant human exposure pathway for many individuals or communities,[6] but recent studies suggest that other exposure pathways, including inhalation and dermal absorption, are significant for human exposure.[7,8,9,10] Some PFAS

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bioaccumulate, leading to concentrations in animals and humans that are significantly higher than the surrounding environment, and they enter the human food chain.[11,12,13]

Human exposures to PFAS are extremely widespread. The Centers for Disease Control and Prevention's (CDC) National Center for Health Statistics' 2011–2012 U.S. National Health and Nutrition Examination Survey (NHANES) reported detectable PFAS blood serum concentrations in virtually all individuals (97 percent).[14] The most recent NHANES data indicate a reduction in serum concentrations of PFOS and PFOA since they were voluntarily phased out of production in the United States beginning in 2002 and 2006, respectively. Replacement PFAS have subsequently been rapidly introduced into the market and exposure is more difficult to assess accurately due to a lack of analytical standards.

Health Effects Research

Our understanding of the health effects associated with PFAS and our ability to draw conclusions regarding the contribution of any specific PFAS to human disease is based on combined data from multiple studies investigating epidemiologic associations in human cohort studies, biological plausibility and pathways in animal studies, mechanistic effects in human tissue and cell culture systems, and rapid high-throughput screening. It is important to note that epidemiology studies alone cannot definitively prove causation, and while animal studies are an important marker of scientific discovery, they may not be perfect predictors of effects in humans. By combining and carefully considering data across multiple types of studies, we can begin to build an understanding of how PFAS impact human health and recommend steps to mitigate deleterious impacts.

When investigating possible human health effects of chemical compounds distributed in the environment, it is also important to recognize that effects from exposure to mixtures pose unique challenges. While studies indicate adverse health effects due to exposures from certain PFAS, such as PFOA and PFOS, we have only limited or no data on which to base conclusions for the majority of PFAS. Our current scientific method involves using our understanding of the biological and chemical processes being influenced by the few well-studied chemicals to extrapolate potential conclusions about structurally similar compounds which we can reasonably expect to act through the same pathways and have similar effects. More research is needed to identify causal relationships between exposure to PFAS and adverse health effects in

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humans.

Decreased Immune System Function

As early as 1978, scientists observed immunotoxicity in non-human primates exposed to PFAS.[15] In 2016, NTP conducted a systematic literature review which concluded that PFOA and PFOS are presumed to be a hazard to healthy immune system function in humans.[16] This conclusion is based on a high level of evidence that PFOA and PFOS suppressed the antibody response in animal studies, and a moderate level of evidence that these chemicals affect multiple aspects of the immune system in humans. Adult PFAS exposure has also been associated with decreases in antibody production.[17] NTP is building on this 2016 systematic review to evaluate immunotoxicity of six related PFAS: PFDA, PFNA, PFHxA, PFBA, PFBS and PFHxS.[18]

Cancer

The epidemiological data on associations between PFAS and cancer risk are limited. Those published studies were recently summarized by the Agency for Toxic Substances and Disease Registry (ATSDR) in their Draft Toxicological Profile for Perfluoroalkyls.[19] According to the Toxicological Profile, "Occupational and community exposure studies have found increases in the risk of testicular and kidney cancer associated with PFOA. No consistent epidemiologic evidence for other cancer types were found for PFOA.[20,21] For PFOS, one occupational exposure study reported an increase in bladder cancer,[22] but this was not supported by subsequent occupational studies. General population studies have not consistently reported increases in malignant tumors for PFOS. Epidemiologic studies examining other perfluoroalkyl compounds consisted of two case-control studies. No increases in breast cancer risk were observed for PFHxS or PFNA; an increased breast cancer risk was observed for PFOSA.[23]

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[18] The six PFAS for which the National Toxicology Program is building on its 2016 systematic review to evaluate immunotoxicity are: perfluorodecanoic acid (PFDA); perfluorononanoic acid (PFNA); perfluorohexanoic acid (PFHxA); perfluorobutanesulfonic acid (PFBS); perfluorobutanesulfonic acid (PFBS); and perfluorohexanesulfonic acid (PFHxS).

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Another case- control study did not find increases in prostate cancer for PFOA, PFOS, PFHxS, PFNA, PFDeA, or PFUA.[24] However, among men with a first-degree relative with prostate cancer, associations were found for PFOA, PFOS, PFHxS, PFDeA, and PFUA, but not for PFNA."[25]

Child Development

PFOA and PFOS cause developmental toxicity in animals.[26,27,28] Human epidemiology studies also show associations between some PFAS and developmental effects.[29] One human study found that PFAS exposure during pregnancy was associated with decreased birth weight and head circumference only in males.[30] Similar decreases in birth weight have been reported in rodents for over a decade.[31] Recent findings from NIEHS-supported epidemiological studies of a cohort of mothers and babies showed that prenatal exposure to PFOS is associated with cognitive effects and decreased ability to regulate behavior in school-age children. However, no similar association was observed in this study for PFOA exposure.[32]

A review of the epidemiological literature by an NIEHS-funded scientist summarized findings from several prospective cohorts on the relationship between prenatal exposure to certain PFAS and neurodevelopmental and neurobehavioral outcomes – for example, cognitive abilities, psychomotor development, attention-deficit hyperactivity disorder, and cerebral palsy. So far, the available body of evidence is inconsistent with respect to these associations, both with respect to which compounds may have adverse effects and timing of potential windows of vulnerability. Additional studies are needed to resolve these questions.[33] Animal

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Perfluorinated Compounds in Danish Women: A Case-Control Study Nested in The Danish National Birth Cohort. *Cancer Causes Control*. 2014; 25(11):1439-1448. DOI: 10.1007/s10552-014-0446-7.

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studies are consistent with and provide additional biological plausibility for the developmental effects observed in the human studies.[34,35]

Endocrine Disruption

Studies suggest that some PFAS may interfere with healthy hormonal function in the body. Our endocrine system controls our basic physiology, including metabolism, growth, fertility, and development. Human studies suggest a concern that early-life exposures to some PFAS may contribute to altered insulin resistance.[36,37] Although further confirmation is required, the findings from one study suggest that exposures to some PFAS during pregnancy may influence lipid metabolism and glucose tolerance.[38] A study of pregnant women in Cincinnati found that those with higher prenatal PFAS levels had children with higher body fat levels at age eight[39]—a finding reinforced by other epidemiological studies[40,41] and similar effects on excessive body weight gain reported for experimental animals.[42] It appears that some PFAS may also affect body weight later in life. Scientists at the Harvard School of Public Health have found that adults with higher blood levels of some PFAS have lower resting metabolic rates, meaning they burn fewer calories while resting, which makes it difficult for them to maintain weight loss.[43]

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[42] Hines EP, White SS, Stanko JP, Gibbs-Flournoy EA, Lau C, Fenton SE. Phenotypic Dichotomy Following Developmental Exposure to Perfluorooctanoic Acid (PFOA) in Female CD-1 Mice: Low Doses Induce Elevated Serum Leptin and Insulin, and Overweight in Mid-Life. *Mol. Cell. Endocrinol.* 2009 May 25; 304(1-2):97-105. DOI: 10.1016/j.mce.2009.02.021.

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Effects on weight gain have been seen in numerous animal studies,[44,45,46] supporting this association in humans. It is particularly concerning that some PFAS alter thyroid hormone homeostasis that regulates metabolism and growth.[47,48,49]

Fertility is another outcome related to endocrine effects. A literature review of recent human epidemiologic evidence on the association between exposure to some PFAS and measures of human fertility show effects on the probability of conception.[50,51] In addition, several recent studies have shown that the duration of breastfeeding decreases with increasing blood concentrations of certain PFAS.[52,53] This is similar to 2006 findings in animals reporting impaired mammary gland development and lactation during and after pregnancy in mice.[54]

NIEHS Extramural PFAS Research Portfolio

NIEHS currently funds over 40 academic-based research projects that explore the health consequences of PFAS exposures. These projects include fundamental and human-based research projects that are funded through competitive awards using various NIH grant mechanisms. Concomitant with the recent emergence of public concerns about PFAS exposures, NIEHS has received a large increase in the number of grant applications and awarded more grants in this research area over the past year. For example, since September 2018, NIEHS has

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[49] Preston EV, Webster TF, Oken E, Claus Henn B, McClean MD, Rifas-Shiman SL, Pearce EN, Braverman LE, Calafat AM, Ye X, Sagiv SK. Maternal Plasma per- and Polyfluoroalkyl Substance Concentrations in Early Pregnancy and Maternal and Neonatal Thyroid Function in a Prospective Birth Cohort: Project Viva (USA). *Environ. Health. Perspect.* 2018 Feb 27; 126(2):027013. DOI: 10.1289/EHP2534.

[50] Bach CC, Vested A, Jørgensen K, Bonde JP, Henriksen TB, Toft G. Perfluoroalkyl and Polyfluoroalkyl Substances and Measures of Human Fertility: A Systematic Review. *Crit. Rev. Toxicol.* 2016 Oct; 46(9):735-55. DOI: 10.1080/10408444.2016.1182117.

[51] Jørgensen KT, Specht IO, Lenters V, Bach CC, Rylander L, Jönsson BAG, Lindh CH, Giwercman A, Heederik D, Toft G, Bonde JP. Perfluoroalkyl substances and time to pregnancy in couples from Greenland, Poland and Ukraine. *Environmental Health*. 2014; 13:116. DOI: 10.1186/1476-069X-13-116.

[52] Timmermann CA, Budtz-Jørgensen E, Petersen MS, Weihe P, Steuerwald U, Nielsen F, Jensen TK, Grandjean P. Shorter Duration of Breastfeeding at Elevated Exposures to Perfluoroalkyl Substances. *Reprod. Toxicol.* 2017 Mar; 68:164-170. DOI: 10.1016/j.reprotox.2016.07.010.

[53] Romano ME, Xu Y, Calafat AM, Yolton K, Chen A, Webster GM, Eliot MN, Howard CR, Lanphear BP, Braun JM. Maternal Serum Perfluoroalkyl Substances During Pregnancy and Duration of Breastfeeding. *Environ. Res.* 2016 Aug; 149:239-246. DOI: 10.1016/j.envres.2016.04.034.

[54] White SS, Calafat AM, Kuklenyik Z, Villanueva L, Zehr RD, Helfant L, Strynar MJ, Lindstrom AB, Thibodeaux JR, Wood C, Fenton SE. Gestational PFOA Exposure of Mice is Associated with Altered Mammary Gland Development in Dams and Female Offspring. *Toxicol. Sci.* 2007 Mar; 96(1):133-44. DOI: 10.1093/toxsci/kfl177.

awarded 10 new research project grants—representing a more than 30% increase in its extramural PFAS portfolio—focused on PFAS, many of which are investigating early life exposures (*in utero* and early childhood) and long-term health effects. Moreover, over the past seven months (September 2018-March 2019), NIEHS grantees have published 28 manuscripts detailing the health impacts of PFAS exposures. This list of manuscripts is attached to my testimony.

NIEHS Superfund Research Program (SRP)

Recently, NIEHS competitively awarded a five-year grant to the University of Rhode Island to fund its "Sources, Transport, Exposure and Effects of PFASs (STEEP) Superfund Research Program Center" (Fiscal Years 2017-2022).[55] The Center is assessing the impact of PFAS exposures on immune dysfunction and metabolic abnormalities by examining the health of nine-year-old children from birth cohorts in the Faroe Islands (Denmark).[56] Recent results from a prospective study of over 1,000 children show that weakened immune response is correlated with PFAS exposure.[57] The Center is also tracing unique PFAS chemical fingerprints at a contaminated groundwater site on Cape Cod, Massachusetts, leading to exposure through drinking water, as a function of PFAS chemistry, geochemistry, and distance from the source. Additionally, the Center is developing and validating novel passive sampling tools for PFAS to measure time weighted average concentrations for some PFAS and their volatile precursors. These tools can be deployed to aid site managers in their risk characterization.[58] Promising results to date indicate that these sampling tools can be effective monitors for airborne PFAS, a route that may contribute significantly to PFAS fate, transport, and human exposure. Finally, the Center is engaging communities and advising stakeholders on ways to effectively reduce human exposure to PFAS. Other NIEHS Superfund Research Program Centers are providing technical assistance regarding PFAS to State and local governments, water authorities, and private well users. The Brown University Superfund Research Center has developed Geographical Information Systems (GIS)-based databases for identifying municipalities at risk for PFAS exposure based on past land use data.[59,60] Other research at the University of Arizona is also developing groundwater modeling tools to predict how PFAS move in the subsurface, helping to

[55] NIH Grant No. P42ES027706. Sources, Transport, Exposure and Effects of PFASs (STEEP). McCann, Alyson. University of Rhode Island. Awarded August 30, 2017. NIH RePORTER Link.
[56] Dassuncao C, Pickard H, Pfohl M, Tokranov AK, Li M, Mikkelsen B, Slitt A, Sunderland EM. Phospholipid Levels Predict the Tissue Distribution of Poly- and Perfluoroalkyl Substances in a Marine Mammal. *Environ. Sci Technol. Lett.* 2019; 6(3):119-125. DOI: 10.1021/acs.estlett.9b00031.
[57] Budtz-Jørgensen E, Grandjean P. Application of Benchmark Analysis for Mixed Contaminant Exposures: Mutual Adjustment of Perfluoroalkylate Substances Associated with Immunotoxicity. *PLoS One.* 2018; 13(10):e0205388. DOI: 10.1371/journal.pone.0205388.
[58] Dixon-Anderson E, Lohmann R. Field-Testing Polyethylene Passive Samplers for the Detection of Neutral Polyfluorinated Alkyl Substances in Air and Water. *Environ. Toxicol. Chem.* 2018; 37:3002-3010. DOI: 10.1002/etc.4264.
[59] Guelfo J, Adamson DT. Evaluation of a National Data Set for Insights into Sources, Composition, and Concentrations of Per- and Polyfluoroalkyl Substances (PFASs) in U.S. Drinking Water. *Environ. Pollut.* 2018; 236:505-513. DOI: 10.1016/j.envpol.2018.01.066.
[60] Guelfo J, Marlow T, Klein D, Savitz D, Frickel S, Crimi M, Suuberg EM. Evaluation and Management Strategies for Per- and Polyfluoroalkyl Substances (PFASs) in Drinking Water Aquifers: Perspectives from Impacted U.S. Northeast Communities. *Environ. Health Perspect.* 2018; 126:13. DOI: 10.1289/ehp2727.

understand where to target remediation approaches.[61,62,63,64] SRP grantees have continued to work closely with Federal and State officials to translate scientifically defensible findings to guide best practices for PFAS monitoring and management—including several outreach efforts within regions impacted by PFAS—such as the New England States (Northeast Waste Management Officials' Association), as well as Michigan, North Carolina and New York. These outreach efforts also extend to communities grappling with the complexities of PFAS exposure and the uncertainties of risk.

The Superfund Research Program has been a key player in developing new solutions to PFAS contamination. Through Small Business Innovation Research (SBIR) grants, the Program provides support to scientists and engineers developing novel technologies for mitigation and remediation of PFAS in the environment. NIEHS SBIR grantee CycloPure, Inc., is developing novel, high-affinity cyclodextrin polymers for the cost-effective remediation of PFAS from water.[65] In another NIEHS SBIR project, EnChem Engineering, Inc. is developing and demonstrating an innovative combined *in-situ* / *ex-situ* technology to cost-effectively expedite treatment of PFAS at Superfund sites. The technology includes a mobile unit that combines a wash cycle using a non-toxic sugar, followed by an intense extraction and destruction process. Their results show more than 99% removal.[66] Yet another *in-situ / ex-situ* process is being developed by Lynntech, Inc. and utilizes plasma-based technology to decompose PFAS in water.[67] Additionally, the Michigan State University and Texas A&M University Superfund Research Centers are developing strategies to remediate PFAS via energy efficient nanoreactors capable of breaking the carbon-fluorine bond, as well as hydrogel sorbents to extract PFAS, respectively.[68,69,70] Also of note, the University of California, Berkeley Superfund Research

[61] NIH Grant No. P42ES004940. Sequestration Processes for Attenuation and Treatment of Arsenic and Other Toxic Elements in Mine Waters. Brusseau, Mark. University of Arizona. Awarded August 1, 2017. NIH RePORTER Link.
[62] Brusseau ML. The Influence of Molecular Structure on the Adsorption of PFAS to Fluid-Fluid Interfaces: Using QSPR to Predict Interfacial Adsorption Coefficients. *Water Res.* 2019; 152:148-158. DOI: 10.1016/j.watres.2018.12.057.
[63] Brusseau ML. Assessing the Potential Contributions of Additional Retention Processes to PFAS Retardation in the Subsurface. *Sci. Total Environ.* 2018; 613:176-185. DOI: 10.1016/j.scitotenv.2017.09.065.
[64] Brusseau M, Yan N, Van Glubt S, Wang Y, Chen W, Lyu Y, Dungan B, Carroll K, Holguin FO. Comprehensive Retention Model for PFAS Transport in Subsurface Systems. *Water Res.* 2019; 148:41-50. DOI: 10.1016/j.watres.2018.10.035.
[65] NIH Grant No. R43ES029401. Remediation of Perfluorinated Chemicals in Water Using Novel High-Affinity Polymer Adsorbents. Barin, Gokhan. CycloPure, Inc. Awarded March 22, 2018. NIH RePORTER Link.
[66] NIH Grant No. R43ES028649. Bench Scale Studies of Novel In-situ Aquifer Remediation of Recalcitrant Fluorinated Organic Compounds at Superfund Sites. Ball, Raymond. EnChem Engineering, Inc. Awarded August 28, 2017. NIH RePORTER Link.
[67] NIH Grant No. R43ES030250. Continuous Removal/Disposal System for the Concurrent Sorption and Breakdown of Contaminants into Harmless Precipitates. Miller, Joseph. Lynntech, Inc. Awarded September 18, 2018. NIH RePORTER Link.
[68] NIH Grant No. P42ES027704. Mitigation of Chemical and Mixture Effects Through Broad-Acting Sorbents. Phillips, Timothy. Texas A&M University. Awarded August 31, 2017. NIH RePORTER Link.
[69] Huang PJ, Hwangbo M, Chen ZY, Liu YN, Kameoka J, Chu KH. Reusable Functionalized Hydrogel Sorbents for Removing Long- and Short-Chain Perfluoroalkyl Acids (PFAAs) and GenX from Aqueous Solution. *ACS Omega*. 2018; 3(12):17447–17455. DOI: 10.1021/acsomega.8b02279.
[70] Tian H, Gao J, Li H, Boyd SA, Gu C. Complete Defluorination of Perfluorinated Compounds by Hydrated Electrons Generated from 3-Indole-acetic-acid in Organomodified Montmorillonite. *Sci. Rep.* 2016; 6:32949. DOI:

Center is combining biological and chemical treatment options to degrade and destroy PFAS and AFFF.[71,72,73]

NIEHS Time-Sensitive Research Awards

In addition to its regular funding programs, NIEHS has used a mechanism to support time-sensitive research opportunities related to PFAS. Time-sensitive grants are a rapid mechanism used to support research that characterizes initial exposures, collects human biological samples, and collects human health and exposure data.[74] Researchers at the Colorado School of Public Health, the University of Colorado Anschutz Medical Campus, and the Colorado School of Mines are studying PFAS exposures in residents near Colorado Springs whose wells and public water systems were contaminated with a wide range of PFAS, including high levels of perfluorohexane sulfonate (PFHxS).[75,76] This time-sensitive study started near the peak of exposure after contamination was discovered and will explore ways to measure how exposure levels to PFAS in the residents change over time.

In 2016, elevated levels of GenX, a short-chain PFAS containing an ether link generated in the production of non-stick coatings, were detected in North Carolina's Cape Fear River. The Cape Fear River provides drinking water for approximately 300,000 people and a production facility had been releasing GenX upstream. NIEHS funded a study at North Carolina State University to address community questions about GenX exposure and health effects, including GenX's potential toxicity, how it is stored in the body, and how long it remains in the environment.[77,78] Sampling results to date indicate elevation of GenX above the North Carolina Department of Health and Human Services health goal—140 parts per trillion—in treated water from at least

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10.1038/srep32949.

[71] Bruton TA, Sedlak DL. Treatment of Aqueous Film-Forming Foam by Heat-Activated Persulfate Under Conditions Representative of In Situ Chemical Oxidation. *Environ. Sci. Technol.* 2017; 51:13878-13885. DOI: 10.1021/acs.est.7b03969.

[72] Bruton TA, Sedlak DL. Treatment of Perfluoroalkyl Acids by Heat-Activated Persulfate Under Conditions Representative of In Situ Chemical Oxidation. *Chemosphere*. 2018; 206:457-464. DOI: 10.1016/j.chemosphere.2018.04.128.

[73] Yi S, Harding-Marjanovic KC, Houtz EF, Gao Y, Lawrence JE, Nichiporuk RV, Iavarone A, Zhuang W, Field JA, Sedlak DL, Alvarez-Cohen L. Biotransformation of AFFF Component 6:2 Fluorotelomer Thioether Amido Sulfonate Generates 6:2 Fluorotelomer Thioether Carboxylate Under Sulfate-Reducing Conditions. *Environ. Sci. Technol. Lett.* 2018; 5:283-288. DOI: 10.1021/acs.estlett.8b00148.

[74] National Institute of Environmental Health Sciences. Time-Sensitive Research Opportunities in Environmental Health. Internet: https://www.niehs.nih.gov/research/supported/timesensitve/index.cfm.

[75] NIH Grant No. R21ES029394. Exposure and Health Effects from Poly- and Perfluoroalkyl Substances in Colorado Water. Adgate, John L. University of Colorado Denver. Awarded December 13, 2017. NIH RePORTER Link.

[76] Gill N. Exposure Study to Assess People and Water Near Colorado Springs; Toxic Chemicals Have Contaminated Water Supplies for 65,000. *CU Anschutz Today*. December 21, 2017. Internet: https://www.cuanschutztoday.org/exposure-study-assess-people-water-near-colorado-springs.

[77] NIH Grant No. R21ES029353. Assessing Impact of Drinking Water Exposure to GenX (Hexafluoropropylene Oxide Dimer Acid) in the Cape Fear River Basin, North Carolina. Hoppin, Jane. North Carolina State University Raleigh. Awarded on October 31, 2017. NIH RePORTER Link.

[78] Peake T. Researchers Receive Grant to Study GenX Exposure in New Hanover County Residents. *NC State News*. November 1, 2017. Internet: https://news.ncsu.edu/2017/11/genx-study/.

one water treatment plant,[79] and groundwater-fed drinking water wells without granular activated carbon filtration.[80] Many other PFAS were also measured in treated Cape Fear River tap water. GenX was not detected in the tap water of homes whose groundwater was treated with granular activated carbon filtration. Blood and urine levels reported to date as part of this ongoing analysis reveal that PFOA, PFOS, and additional known and unknown PFAS have been detected in the study population. In rodent models, NTP is studying how GenX moves through the body and whether it affects function of the placenta, immune system, liver, and other tissues.

NTP REACT Program

The NTP Responsive Evaluation and Assessment of Chemical Toxicity, or REACT, Program is broadening our understanding of PFAS by studying over a hundred compounds that fall into different subclasses based on similarities in chemical properties. Scientists will be able to compare one PFAS to another, determine the relationship between chain length and other structural features and toxicity, and inform on whether there are common or overlapping patterns of toxicity.

REACT uses a combination of approaches. One project analyzes the chemical structure of PFAS compounds to see what information is available in databases for that compound or others with similar structure. Chemical structure plays a major role in how chemicals interact and chemicals with similar structure often have similar toxicity. This computer-based step is known as *in silico* screening. Based on *in silico* results, chemicals can be selected for further targeted laboratory testing with cells, known as *in vitro* testing. Examples include testing whether PFAS cause cells to die or substantially alter the function of human liver, placenta, or mammary gland derived cells. Some of these tests are similar to, or a refinement of, those used in the automated Toxicology in the 21st Century (Tox21) Program, a Federal collaboration among the NIH, the U.S. Environmental Protection Agency (EPA), and the U.S. Food and Drug Administration (FDA).[81] The *in vitro* data are then examined to prioritize select chemicals for toxicity testing in animals, known as *in vivo* studies, so the data can be considered all together. REACT is a collaborative program with EPA. Both NTP and EPA are contributing complementary resources to coordinate and share what is learned about individual chemicals.

Current Challenges

Real-world human exposures to PFAS involve complex mixtures, not individual chemicals. This fact complicates both the science of exposure and the assessment of health risks.[82] Currently, analytical techniques are limited for determining which specific PFAS are contained in a given

[79] North Carolina Department of Environmental Quality. GenX Results. Internet: https://www.ncwater.org/?page=690&Action=doGraphs.
[80] Leonard L. North Carolina Department of Environmental Quality. Latest test results show elevated levels of GenX in 30 more private wells. December 13, 2017. Internet: https://deq.nc.gov/news/press-releases/2017/12/13/latest-test-results-show-elevated-levels-genx-30-more-private-wells.
[81] U.S. Environmental Protection Agency. Toxicology Testing in the 21st Century (Tox21). Internet: https://www.epa.gov/chemical-research/toxicology-testing-21st-century-tox21.
[82] Kotthoff M, Bücking M. Four Chemical Trends Will Shape the Next Decade's Directions in Perfluoroalkyl and Polyfluoroalkyl Substances Research. *Front. Chem.* 2018 Apr 5; 6:103. DOI: 10.3389/fchem.2018.00103.

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complex mixture. Further, toxicological information on these combined PFAS mixtures remains incomplete. Additional research is needed to assess environmental exposures to mixtures and determine their combined effects.

Apart from the challenge of characterizing PFAS in environmental samples is the challenge of studying PFAS in the human body. Our present understanding is that the time required for elimination of PFAS from the human body can vary. While some longer chain molecules may remain in the blood for years, shorter chain PFAS may be more quickly eliminated. Differences in elimination rates of longer and shorter chain PFAS complicates biomonitoring as well as toxicological studies. However, lack of biological persistence does NOT mean lack of toxicity, particularly for chemicals like PFAS that may have consistent daily exposures.

Traditional methods for measuring the body burden of PFAS—namely analyzing serum—are not as effective for shorter chain PFAS as for longer chain PFAS. Scientists are beginning to measure PFAS in urine,[83] in plasma, and in whole blood, as well as in serum.[84] These expanded biomonitoring techniques for sampling and analyses will further inform our understanding of exposures and risks. Using these techniques, many scientists are rightly focusing on measuring the total exposure to all PFAS as opposed to the past focus on one substance in isolation. This is important as it allows for understanding cumulative effects of PFAS mixtures as a class. Examining the person in the context of the measure of all the exposures they have experienced in their lifetime and how they relate to their health is in step with the latest science.

Approaching PFAS as a class for assessing exposure and biological impact is the most prudent approach to protect public health. Based upon their persistent nature, widespread exposure, and known toxicity, it begs the question: does the net value of PFAS production and use for modern-day convenience outweigh the likely risks to public health and associated healthcare costs? Thus, scientific and technology innovation is critical to enable a shift to safer alternatives, as appropriate.

Manufacturers have begun recently to produce and market AFFF devoid of any PFAS. Such fluorine-free AFFF is now being used at Heathrow Airport in London, United Kingdom and at major airports in Sweden. It will be important to evaluate these alternatives for potential health effects as well.

Federal Collaboration

NIEHS and the NTP will continue to provide scientific leadership with respect to PFAS research. Communication and collaboration both within the Department of Health and Human Services, and across the Federal Government, about PFAS is intensifying. In February 2018, a Federal information exchange meeting about PFAS was held on the NIH campus in Bethesda, Maryland.[85] NIEHS was among other Federal agencies that participated at the PFAS National

[83] Hartmann C, Raffesberg W, Scharf S, Uhl M. Perfluoroalkylated Substances in Human Urine: Results of a Biomonitoring Pilot Study. *Biomonitoring* 2017; 4:1-10. DOI: 10.1515/bimo-2017-0001.
[84] Poothong S, Thomsen C, Padilla-Sanchez JA, Papadopoulou E, Haug LS. Distribution of Novel and Well-Known Poly- and Perfluoroalkyl Substances (PFASs) in Human Serum, Plasma, and Whole Blood. *Environ. Sci. Technol*. 2017 Nov 21; 51(22):13388-13396. DOI: 10.1021/acs.est.7b03299.
[85] Lenox K. Federal Agencies Exchange PFAS Updates. *NIEHS Environmental Factor*. 2018, Mar. Internet:

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Leadership Summit hosted by EPA in May 2018.[86] Within the Department of Health and Human Services and primarily through NTP, NIEHS works closely with the FDA and the CDC on PFAS matters. Additionally, NIEHS is specifically being consulted by ATSDR on the design and conduct of the exposure assessments and health studies authorized by the National Defense Authorization Act for Fiscal Year 2018, as amended.[87]

Conclusion

Thank you again for allowing me to share a scientific perspective on this important topic. In closing, I note that NIEHS is well-positioned to continue contributing essential scientific knowledge about this complex and large class of chemicals. This knowledge can help regulators make sound, science-based decisions and informs the medical and public health communities about the potential health effects associated with exposure to PFAS. I welcome your questions.

https://factor.niehs.nih.gov/2018/3/science-highlights/pfas/index.htm.

[86] U.S. Environmental Protection Agency. EPA PFAS National Leadership Summit and Engagement. May 22-23, 2018. Internet: https://www.epa.gov/pfas/pfas-national-leadership-summit-and-engagement.

[87] Sec. 316 of the National Defense Authorization Act for Fiscal Year 2018. Public Law 115-91. December 12, 2017.

Appendix to Testimony of Linda S. Birnbaum, Ph.D., D.A.B.T., A.T.S.
Director, National Institute of Environmental Health Sciences (NIEHS) and
National Toxicology Program (NTP), National Institutes of Health (NIH)
Senate Committee on Environment and Public Works Hearing – March 28, 2019

LIST OF CITATIONS TO PUBLICATIONS ABOUT PER- AND POLYFLUOROALKYL SUBSTANCES (PFAS) AUTHORED BY NIEHS SCIENTISTS AND GRANTEES (January 1, 2018—March 13, 2019)

NIEHS OFFICE OF THE DIRECTOR (OD) –

Ritscher A, Z Wang, M Scheringer, JM Boucher, L Ahrens, U Berger, S Bintein, SK Bopp, D Borg, AM Buser, I Cousins, J DeWitt, T Fletcher, C Green, D Herzke, C Higgins, J Huang, H Hung, T Knepper, CS Lau, E Leinala, AB Lindstrom, J Liu, M Miller, K Ohno, N Perkola, Y Shi, L Smastuen Haug, X Trier, S Valsecchi, K van der Jagt and L Vierke
Zurich Statement on Future Actions on Per- and Polyfluoroalkyl Substances (PFASs) [Journal Article]
Environmental Health Perspectives (2018) v. 126 (8): pp. 84502
Full-Text at: http://dx.doi.org/10.1289/ehp4158

NIEHS DIVISION OF INTRAMURAL RESEARCH (DIR) –

Impinen A, MP Longnecker, UC Nygaard, SJ London, KK Ferguson, LS Haug and B Granum
Maternal levels of perfluoroalkyl substances (PFASs) during pregnancy and childhood allergy and asthma related outcomes and infections in the Norwegian Mother and Child (MoBa) cohort [Journal Article]
Environment International (2019) v. 124 pp. 462-472
Full-Text at: http://dx.doi.org/10.1016/j.envint.2018.12.041

Iszatt N, S Janssen, V Lenters, C Dahl, H Stigum, R Knight, S Mandal, S Peddada, A Gonzalez, T Midtvedt and M Eggesbo
Environmental toxicants in breast milk of Norwegian mothers and gut bacteria composition and metabolites in their infants at 1 month [Journal Article]
Microbiome (2019) v. 7 (1): pp. 34
Full-Text at: http://dx.doi.org/10.1186/s40168-019-0645-2

Rosen EM, AL Brantsaeter, R Carroll, L Haug, AB Singer, S Zhao and KK Ferguson
Maternal Plasma Concentrations of Per- and polyfluoroalkyl Substances and Breastfeeding Duration in the Norwegian Mother and Child Cohort [Journal Article]
Environmental Epidemiology (2018) v. 2 (3): e027
Full-Text at: http://dx.doi.org/10.1097/ee9.0000000000000027

Rush EL, AB Singer, MP Longnecker, LS Haug, A Sabaredzovic, E Symanski and KW Whitworth
Oral contraceptive use as a determinant of plasma concentrations of perfluoroalkyl substances among women in the Norwegian Mother and Child Cohort (MoBa) study [Journal Article]
Environment International (2018) v. 112 pp. 156-164
Full-Text at: http://dx.doi.org/10.1016/j.envint.2017.12.015

Singer AB, KW Whitworth, LS Haug, A Sabaredzovic, A Impinen, E Papadopoulou and MP Longnecker
Menstrual cycle characteristics as determinants of plasma concentrations of perfluoroalkyl substances (PFASs) in the Norwegian Mother and Child Cohort (MoBa study) [Journal Article]
Environmental Research (2018) v. 166 pp. 78-85
Full-Text at: http://dx.doi.org/10.1016/j.envres.2018.05.019

NIEHS NATIONAL TOXICOLOGY PROGRAM (NTP) DIVISION –

Patlewicz G, AM Richard, AJ Williams, CM Grulke, R Sams, J Lambert, PD Noyes, MJ DeVito, RN Hines, M Strynar, A Guiseppi-Elie and RS Thomas
A Chemical Category-Based Prioritization Approach for Selecting 75 Per- and Polyfluoroalkyl Substances (PFAS) for Tiered Toxicity and Toxicokinetic Testing [Journal Article]
Environmental Health Perspectives (2019) v. 127 (1): pp. 14501
Full-Text at: https://doi.org/10.1289/ehp4555

Behl M, K Ryan, JH Hsieh, F Parham, AJ Shapiro, BJ Collins, NS Sipes, LS Birnbaum, JR Bucher, PMD Foster, NJ Walker, RS Paules and RR Tice
Screening for Developmental Neurotoxicity at the National Toxicology Program: The Future is Here [Journal Article]
Toxicological Sciences: an official journal of the Society of Toxicology (2018) v. 167 (1): pp.6-14
Full-Text at: http://dx.doi.org/10.1093/toxsci/kfy278

Blake BE, H Cope and SE Fenton
An In Vitro Screen of a Panel of Perfluoroalkyl Substances and an In Vivo Assessment of Effects on Placental and Fetal Growth [Meeting Abstract]
Birth Defects Res. (2018) v. 110 (9): pp. 770-770
Full-Text at: http://dx.doi.org/10.1002/bdr2.1355

Blake BE, SM Pinney, EP Hines, SE Fenton and KK Ferguson
Associations between longitudinal serum perfluoroalkyl substance (PFAS) levels and measures of thyroid hormone, kidney function, and body mass index in the Fernald Community Cohort [Journal Article]
Environ. Pollut. (2018) v. 242 pp. 894-904
Full-Text at: http://dx.doi.org/10.1016/j.envpol.2018.07.042

Frawley RP, M Smith, MF Cesta, S Hayes-Bouknight, C Blystone, GE Kissling, S Harris and D Germolec
Immunotoxic and hepatotoxic effects of perfluoro-n-decanoic acid (PFDA) on female Harlan Sprague-Dawley rats and B6C3F1/N mice when administered by oral gavage for 28 days [Journal Article]
Journal of Immunotoxicology (2018) v. 15 (1): pp. 41-52
Full-Text at: http://dx.doi.org/10.1080/1547691x.2018.1445145

Patisaul HB, SE Fenton and D Aylor
Animal models of endocrine disruption [Review]
Best Practice and Research: Clinical Endocrinology and Metabolism (2018) v. 32 (3): pp. 283-297
Full-Text at: http://dx.doi.org/10.1016/j.beem.2018.03.011

NIEHS GRANTEES –

Agier L, X Basagaña, L Maitre, B Granum, PK Bird, M Casas, B Oftedal, J Wright, S Andrusaityte, M de Castro, E Cequier, L Chatzi, D Donaire-Gonzalez, R Grazuleviciene, LS Haug, AK Sakhi, V Leventakou, R McEachan, M Nieuwenhuijsen, I Petraviciene, O Robinson, T Roumeliotaki, J Sunyer, I Tamayo-Uria, C Thomsen, J Urquiza, A Valentin, R Slama, M Vrijheid and V Siroux
Early-life exposome and lung function in children in Europe: an analysis of data from the longitudinal, population-based HELIX cohort [Journal Article]
Lancet Planet. Health (2019) v. 3 (2): pp. e81-e92
Full-Text at: https://doi.org/10.1016/S2542-5196(19)30010-5

Ammitzbøll C, L Börnsen, ER Petersen, AB Oturai, HB Søndergaard, P Grandjean and F Sellebjerg
Perfluorinated substances, risk factors for multiple sclerosis and cellular immune activation [Journal Article]
Journal of Neuroimmunology (2019) v. 330 pp. 90-95
Full-Text at: https://doi.org/10.1016/j.jneuroim.2019.03.002

Annunziato KM, CE Jantzen, MC Gronske and KR Cooper
Subtle morphometric, behavioral and gene expression effects in larval zebrafish exposed to PFHxA, PFHxS and 6:2 FTOH [Journal Article]
Aquatic Toxicology (2019) v. 208 pp. 126-137
Full-Text at: https://doi.org/10.1016/j.aquatox.2019.01.009

Bassler J, A Ducatman, M Elliott, S Wen, B Wahlang, J Barnett and MC Cave
Environmental perfluoroalkyl acid exposures are associated with liver disease characterized by apoptosis and altered serum adipocytokines [Journal Article]
Environ. Pollut. (2019) v. 247 pp. 1055-1063
Full-Text at: http://dx.doi.org/10.1016/j.envpol.2019.01.064

Brusseau ML
The influence of molecular structure on the adsorption of PFAS to fluid-fluid interfaces: Using QSPR to predict interfacial adsorption coefficients [Journal Article]
Water Research (2019) v. 152 pp. 148-158
Full-Text at: http://dx.doi.org/10.1016/j.watres.2018.12.057

Brusseau ML, N Yan, S Van Glubt, Y Wang, W Chen, Y Lyu, B Dungan, KC Carroll and FO Holguin
Comprehensive retention model for PFAS transport in subsurface systems [Journal Article]
Water Research (2019) v. 148 pp. 41-50
Full-Text at: http://dx.doi.org/10.1016/j.watres.2018.10.035

Cordner A, VY De la Rosa, LA Schaider, RA Rudel, L Richter and P Brown
Guideline levels for PFOA and PFOS in drinking water: the role of scientific uncertainty, risk assessment decisions, and social factors [Journal Article]
Journal of Exposure Science and Environmental Epidemiology (2019) v. 29 (2): pp. 157-171
Full-Text at: http://dx.doi.org/10.1038/s41370-018-0099-9

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Environmental Health

REVIEW Open Access

A pathway level analysis of PFAS exposure and risk of gestational diabetes mellitus

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Abstract

Per- and polyfluoroalkyl substances (PFAS) have been found to be associated with gestational diabetes mellitus (GDM) development, a maternal health disorder in pregnancy with negative effects that can extend beyond pregnancy. Studies that report on this association are difficult to summarize due to weak associations and wide confidence intervals. One way to advance this field is to sharpen the biologic theory on a causal pathway behind this association, and to measure it directly by way of molecular biomarkers. The aim of this review is to summarize the literature that supports a novel pathway between PFAS exposure and GDM development. Epidemiological studies demonstrate a clear association of biomarkers of thyroid hormones and glucose metabolism with GDM development. We report biologic plausibility and epidemiologic evidence that PFAS dysregulation of maternal thyroid hormones and thyrotropin (TSH) may disrupt glucose homeostasis, increasing the risk of GDM. Overall, epidemiological studies demonstrate that PFAS were positively associated with TSH and negatively with triiodothyronine (T3) and thyroxine (T4). PFAS were generally positively associated with glucose and insulin levels in pregnancy. We propose dysregulation of thyroid function and glucose metabolism may be a critical and missing component in the accurate estimation of PFAS on the risk of GDM.

Keywords: PFAS, Gestational diabetes mellitus, Thyroid hormones, Glucose metabolism

Introduction

Per- and polyfluoroalkyl substances (PFAS) are synthetic organic chemicals used ubiquitously in numerous industries that contaminate food, water, and air, resulting in human exposure. Some PFAS can be bioaccumulative and toxic. Elevated prenatal PFAS levels have been associated with maternal health disorders, including gestational diabetes mellitus (GDM). GDM is an endocrine disorder that develops during pregnancy and can have lasting impacts on maternal health, partly through conversion to type II diabetes (T2D). The mechanism of GDM development is poorly understood and generally not addressed in epidemiologic investigations on PFAS exposures and their impacts. We review the literature for candidate biomarkers of GDM, specifically highlighting those measuring maternal thyroid and maternal glucose homeostasis. We then review the epidemiologic literature for evidence of PFAS as a putative cause of GDM by way of molecular biomarkers in a pathway that connects PFAS toxicity to GDM. We propose that a systematic approach to examining this pathway through validated biomarker measurement can improve the understanding of the pathophysiology of GDM. Additionally, we suspect that this approach will reveal at which points PFAS have their strongest effects.

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Introduction to perfluoroalkyl and polyfluoroalkyl substances (PFAS)

PFAS are a family of synthetic chemicals diverse in size and structure that contain at least one perfluoroalkyl moiety (C_nF_{2n+1}-). Frequently studied PFAS include perfluorooctane sulfonate (PFOS), perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUnDA), and perfluorohexane sulfonic acid (PFHxS). PFAS have both hydrophobic and oleophobic characteristics that are used in commercial products to increase stain repellency and non-stick qualities, food processing equipment and packaging to reduce oil transfer, firefighting activities as a component of aqueous film forming foams, and industrial and manufacturing practices for purposes such as liquid cooling [1, 2]. Due to their strong carbon-fluorine bonds, they are resistant to degradation [1]. Regulations have been enacted in many countries to eliminate the most common PFAS, mainly PFOS and PFOA; yet they continue to circulate after production stops due to their ability to persist and accumulate in the environment [1]. Furthermore, new short-chain perfluoroalkyl substances and other structurally diverse PFAS developed by industries as replacements to PFOA and PFOS are increasingly being found to be equally toxic [1]. Therefore, understanding the biological effects of this class of chemicals continues to be a priority.

In a National Health and Nutrition Examination Survey (NHANES) study of 7876 US participants ≥12 years old, four of the 12 PFAS measured (PFOS, PFOA, PFNA, and PFHxS) were detected in 95% of serum samples [3]. Although concentrations of some PFAS, like PFOS, generally declined over the period of 1999 to 2008, others remained constant or increased, including PFOA and PFNA [3]. PFAS levels are highly prevalent globally and measured consistently in US and non-US cohorts [4]. The average half-life of PFAS in the human body varies, with long-chain compounds having increased half-lives and ability to bioaccumulate [1, 5]. Serum half-life measurements have mainly been reported for a selection of long-chain PFAS (PFOS, PFOA, and PFHxS), with a range from 3 to 5 years [1, 6]. Xu et al. found that these legacy PFAS had half-lives of approximately 2 to 3 years in a Swedish cohort and accounted for 90% of the total serum PFAS compared to short-chain PFAS (i.e. perfluorobutane sulfonic acid, perfluoroheptanoic acid), which had half-lives of less than 1.5 years and lower bioavailability [7]. All PFAS, however, have relatively long environmental half-lives due to minimal degradation and can remain bioavailable to humans over long periods of time through exposures such as drinking water [1].

PFAS impacts on maternal health and pregnancy

Acute hormonal and immune changes underway during pregnancy render women and their fetuses particularly vulnerable to PFAS exposure. As women drink more during pregnancy, water may be a significant exposure source. PFAS plasma levels in pregnant women in Shanghai, a particularly contaminated region, were higher in those drinking tap water compared to purified or bottled water, independent of diet [8]. There is epidemiological evidence that PFAS affects maternal health during pregnancy, including contributing to GDM development. Clinically, GDM is diagnosed as type I diabetes (T1D) or T2D presenting in the second or third trimester of pregnancy that is not preexisting [9]. GDM prevalence in 2015 was 8.8% of pregnancies resulting in live births worldwide [10]. GDM increases the risk of low or high birth weight, preterm birth, and preeclampsia [9]. GDM can also have a long-lasting negative impact on maternal health through its conversion into T2D in approximately 50% or more of women [11] and by initiating vascular changes that increase cardiovascular disease risk [9].

Even though associations of PFAS with diabetes and GDM risk have been reported, there has been minimal work in outlining a detailed and plausible biological mechanism that starts with PFAS and ends with glucose dysfunction that is a proximal cause of GDM [1]. Without a theory on the endocrine mechanism that links this exposure and outcome, associations are difficult to interpret. We narrow the pathway described here to include thyroid hormones (THs) in the maintenance of glucose homeostasis in pregnant and non-pregnant populations. We hypothesize that this is a primary, but not exclusive, pathway relevant to this exposure and outcome. PFAS can disrupt the function of the maternal thyroid, which is a master regulator of glucose homeostasis. Proper thyroid functioning is a known determinant of successful pregnancy outcomes and fetal development, therefore pregnant women are particularly at risk of PFAS thyrotoxicity. This review examines epidemiologic research on circulating biomarkers of thyroid function (hormones, thyroid autoantibodies), glucose metabolism (blood glucose, glucose tolerance), and insulin secretion (insulin resistance) to offer mechanistic insight into subclinical and clinical GDM (Fig. 1). We propose a biologic pathway where PFAS disruption of maternal thyroid function alters circulating TH concentrations and downstream glucose homeostasis, resulting in an increased risk for GDM development.

PFAS encompass thousands of compounds that may elicit different biological responses, particularly new and emerging compounds whose toxicities have yet to be examined. The majority of these compounds, however, have not been studied in relation to the proposed

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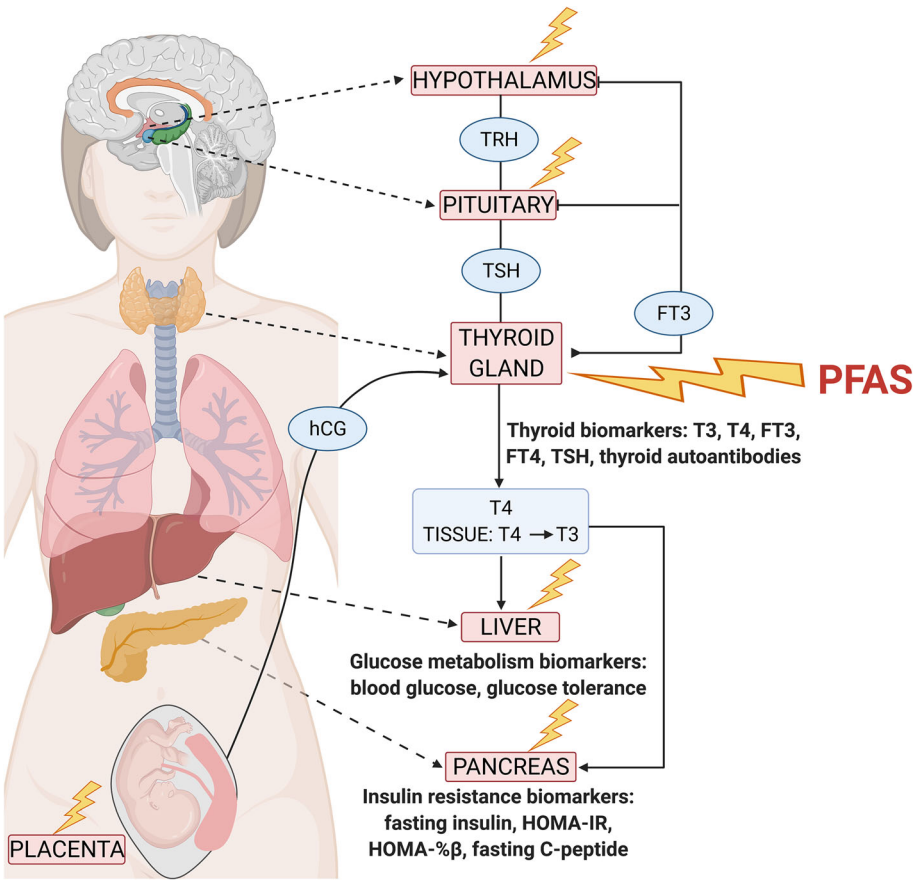


Fig. 1 Novel biomarkers in the pathway between PFAS exposure and gestational diabetes mellitus (GDM) development. PFAS targets the hypothalamic–pituitary–thyroid axis, disrupting thyroid hormone homeostasis, which may contribute to GDM development. While the thyroid may be the main target for hormone disruption, PFAS can also exert toxicity to other tissues involved in this regulatory pathway. Abbreviations: FT3, free triiodothyronine; FT4, free thyroxine; hCG, human chorionic gonadotropin; HOMA-%β, homeostatic model assessments of β-cell function; HOMA-IR, homeostatic model of assessment of insulin resistance; T3, triiodothyronine; T4, thyroxine; TRH, thyrotropin-releasing hormone; TSH, thyrotropin

pathway. Therefore, this review is limited to PFAS with the highest circulating concentrations ubiquitously measured in the population and most commonly studied in the literature (Supplemental Table 2), mainly PFOA, PFOS, PFNA, PFDA, PFUnDA, and PFHxS, though there are many PFAS which are not toxic or biologically active in the same way.

Thyroid hormones regulate normal maternal glucose homeostasis in non-pregnant and pregnant women

The thyroid gland, by way of its hormones, regulates multiple developmental and metabolic pathways involved in energy storage and expenditure, cardiovascular function, and glucose metabolism [12]. TH secretion is controlled by the hypothalamic-pituitary-thyroid axis (Fig. 1) [12, 13]. Briefly, thyrotropin-releasing hormone (TRH) is released by the hypothalamus, which stimulates the release of thyrotropin (TSH) by the pituitary gland [12, 13]. TSH binds to its receptor on the thyroid gland, which results in the production and release of the THs triiodothyronine (T3) and thyroxine (T4) [12]. Circulating THs can be in their biologically active unbound forms free T3 (FT3) or free T4 (FT4) but are primarily bound to transport proteins (over 99%) that facilitate their movement and binding to their receptors across the body [12, 13]. Circulating THs inhibit TRH and TSH release, completing the normal negative feedback loop [12]. Types 1, 2, and 3 iodothyronine deiodinases (D1, D2, and D3, respectively) catalyze the conversion of THs into their biologically active or inactive forms [13]. While circulating TH levels are typically held constant at the 'global level' by the hypothalamic-pituitary-thyroid axis, tissue-specific expression and activity of TH receptors, transporters, and deiodinases can modulate TH concentrations to allow for local regulation of TH-controlled processes [14, 15].

Glucose production and storage by the liver is one example of tissue-specific TH action. THs regulate glucose

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levels by increasing glucose production in the liver. On a transcriptional level, THs regulate several liver enzymes in the gluconeogenesis and glycogenolysis pathways to increase glucose levels, including phosphoenolpyruvate carboxykinase and glucose-6-phosphatase [12, 16, 17]. Additionally, THs regulate glucose transporters. THs increase expression of the hepatic glucose transporter GLUT2, increasing glucose output [17, 18]. T3 increases *GLUT4* gene expression in all cells, which encodes the transporter responsible for the insulin-mediated uptake of glucose [12, 17].

THs also modulate glucose homeostasis by regulating the differentiation and function of the islet cells of the maternal pancreas. Pancreatic islet cells, which produce insulin, express TH receptors [12, 15]. Insulin signals for all cell types in the body to take up glucose and store it as glycogen to maintain physiologically appropriate levels of glucose in the blood. The binding of T3 to the islet transcription factor MAFA results in the maturation of islet cells to become glucose-responsive and insulin-secreting [12, 15].

Disorders in the adult thyroid can disrupt blood glucose control. Hyperthyroidism is defined as low concentrations of TSH and elevated FT3 and/or FT4 concentrations [19]. The thyroid produces excess THs, increasing gluconeogenesis and resulting in elevated blood glucose levels [13, 15] and hepatic insulin resistance [17]. High doses of THs can also impair islet function in the pancreas, resulting in reduced insulin secretion and subsequent glucose intolerance [15]. Hence, hyperthyroidism is a cause of diabetes mellitus both through glucose overproduction and by inhibition of glucose uptake by the cells [13, 15]. Hypothyroidism is clinically defined as elevated TSH and low FT4 concentrations [19] and has also been associated with diabetes mellitus. In this disorder, liver gluconeogenesis is inhibited [17]. The effects of hypothyroidism include altered phosphorylation of insulin signaling proteins, dysregulation of leptin in the hypothalamus, and impaired adipose and skeletal muscle function (such as diminished blood flow and muscle oxidative capacity) [17]. The net result of these changes is increased insulin resistance in the peripheral tissues, resulting in a reduction in glucose utilization and altered glucose homeostasis [17, 18].

Autoimmune thyroid diseases (AITDs) are a main cause of toxicity to the thyroid system and therefore may also indirectly contribute to glucose homeostasis disruption. AITDs are the most common causes of hyperthyroidism and hypothyroidism [20]. These include Graves' disease (GD), associated with hyperthyroidism, and Hashimoto's thyroiditis (HT), associated with hypothyroidism. Therefore, markers of AITDs can also be considered as biomarkers for thyroid function. The presence of autoantibodies, namely thyroid peroxidase antibody (TPOAb) and thyroglobulin antibody (TgAb), have been measured in individuals who develop autoimmunity [21]. TPOAb targets the thyroid peroxidase (TPO) enzyme, which is responsible for adding iodine to thyroglobulin (Tg), the precursor of T3 and T4 [13, 22] and TgAb targets Tg [22]. Hence, elevation of either or both TPOAb and TgAb are indicators of potential toxicity to the system. While genetic factors account for approximately 70% of AITDs, environmental toxicant exposures, including PFAS, are also associated with elevated thyroid autoimmunity biomarker levels [23].

Thyroid and glucose regulation by the fetal placenta during pregnancy

The fetal placenta produces a variety of hormones that enter maternal circulation and stimulate maternal hormone production, including THs [24]. In the first 10 weeks of pregnancy, there is a steady increase in human chorionic gonadotropin (hCG) produced by placental trophoblasts [24]. In the period in which hCG reaches its peak physiological concentrations (3–10 weeks), hCG can bind to the maternal thyroid TSH receptor. Due to its similar structure to TSH, hCG can stimulate TH release, particularly FT4 [24, 25]. The transport protein TBG also rises during this time alongside hCG, stimulated by estrogen, increasing total T4 levels [25]. TSH release by the pituitary gland is suppressed by rising THs and hCG due to the negative feedback relationship [25, 26]. Placental factors other than hCG, such as placental growth factor (PLGF), may also regulate the normal function of maternal thyroid [27, 28].

The fetal thyroid gland does not begins its own hormone production until 16 weeks, yet the fetus requires THs to regulate multiple processes before this point, including successful embryonic implantation and fetal neurodevelopment [29, 30]. The fetus is fully dependent on maternal TH production in the first trimester [31, 32]. Insufficient first trimester maternal TH levels are associated with pregnancy loss and impaired neuropsychomotor development of the child [30, 33, 34]. Transplacental transfer of maternal THs into the fetal compartment is facilitated by TH binding proteins and transporters in the placenta [31, 35]. Deiodinase enzymes in the placenta (D2, D3) regulate fetal exposure to maternal THs, either by metabolizing T4 into the bioactive T3 or inactivating the hormones [35].

In a study of 18,683 pregnant women, women at the 85th–95th percentile of the first trimester hCG multiple of the median (MoM, a measure of hCG that is normalized for gestational age) had 6.4–11.8% lower risk of GDM incidence, respectively [36]. In this study, FT4 mediated 21.4% of the hCG-GDM association [36]. The placenta may be an important mediator in the regulation of glucose homeostasis by the maternal thyroid and subsequent risk of GDM. Higher first trimester hCG was

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associated with a reduced risk of GDM development and negatively associated with blood glucose levels [36, 37]. As gestational age advances, there is a net effect of changing maternal and placental physiology whereby maternal insulin resistance and blood glucose and free fatty acid (FFA) levels increase [9]. Glucose is transported through the placenta to the fetus to fuel growth [9]. Pancreatic β cell hyperplasia and hypertrophy occur to stimulate more insulin production and to counteract the increasing insulin resistance [9]. After the delivery of the child and the placenta, maternal insulin sensitivity returns to pre-pregnancy levels. The placenta plays an active role in this phenomenon [9] and is under-utilized as a source of biomarkers to study maternal glucose homeostasis.

Gestational diabetes mellitus (GDM): definition and candidate biomarkers

When glucose homeostasis is highly disrupted in pregnancy due to a dysregulation of the mechanisms described above, women are at risk of developing GDM. GDM is defined as clinical hyperglycemia detected during pregnancy, secondary to insulin resistance [9]. Insulin resistance indicates that the cells are not able to properly utilize glucose leading to glucose levels in the blood that have reached a level high enough to become toxic to the cells. Risk factors such as obesity, advanced age, and family history of diabetes can lead to pancreatic β cell dysfunction, resulting in insufficient insulin production to overcome the insulin resistance associated with pregnancy [9, 38]. This causes blood sugar and FFA levels to increase [9, 38].

Connecting the dots between thyroid function and gestational diabetes mellitus (GDM)

A causal relationship between THs and the risk of GDM is also supported by epidemiologic findings. Leng et al. reported that higher first trimester TSH levels were weakly associated with increased risk of GDM in a Chinese population of 7258 women (adjusted odds ratio (OR) 1.13, 95%CI 1.00, 1.27) [39]. In a longitudinal study of 321 pregnant women, FT3 and the FT3/FT4 ratio were positively associated with risk of GDM [40]. The adjusted OR of the highest versus lowest quartile of FT3 was 4.25 (95%CI 1.67, 10.80) in the first trimester and 3.89 (95%CI 1.50, 10.10) in the second trimester. For the FT3/FT4 ratio, the adjusted OR was 8.63 (95%CI 2.87, 26.00) in the first trimester and 13.60 (95%CI 3.97, 46.30) in the second trimester. Disruption of T3 and T4 expression as a result of hypothyroidism and hyperthyroidism is also associated with the risk of GDM [41, 42]. Some of these estimates are highly imprecise. Statistical power can be improved by increased sample size and by greater precision in the measurement of the physiologic process, which we aim to promote in this review.

Thyroid autoantibodies have also been studied as causes of GDM. In a meta-analysis, Yang et al. evaluated epidemiological studies published between 1997 and 2014 that assessed the association of thyroid autoantibodies with GDM. Among 21 studies, there was a weak association between thyroid autoantibodies and the risk of GDM (pooled relative risk (RR) 1.12, 95%CI 1.03, 1.22), and it was comparable when women with thyroid dysfunction were included (RR 1.18, 95%CI 1.06, 1.31) [43]. A more recent meta-analysis of thyroid dysfunction and thyroid autoimmunity by Jia et al. of 11 studies assessing thyroid function across the three trimesters of pregnancy showed a strong association between subclinical hypothyroidism, with TPOAb positivity, and GDM (OR 3.22, 95%CI 1.72, 6.03) [44]. Similarly, evidence of an association between TPOAb and GDM was reported in a prospective study of 1683 women by Huang et al., where TPOAb positive women in early pregnancy had an increased risk of GDM (RR 2.54, 95%CI 1.04, 6.23) [45]. These women also had significantly higher TSH and lower FT4 levels compared to euthyroid women. The presence of serum TPOAb and TgAb are indicators of higher GDM risk, making them candidate biomarkers of GDM either alone or in the context of clinically diagnosed thyroid disorders.

Candidate biomarkers to examine thyroid hormone and glucose disruption in gestational diabetes mellitus (GDM) development

GDM is diagnosed by increased values in blood glucose measurements [38, 46]. Testing strategies in pregnancy vary and include a one-step 2-h 75 g oral glucose tolerance test (OGTT) or a two-step process which starts with a 1-h 50 g oral glucose challenge test (OGCT) followed by a diagnostic 3-h 100 g OGTT in women who screen positive for GDM [47]. These tests are routinely performed starting at 24–28 weeks and can serve as a source of biomarkers for use in epidemiologic studies (Fig. 1). Fasting, post-load (post consumption of a glucose-rich drink used in challenge described above), or specific glucose values can be used to assess a woman's glucose tolerance during pregnancy.

In research studies, concurrent insulin measurement can be performed to assess insulin resistance and β cell function. A widely validated clinical and epidemiological tool is the homeostasis model assessment (HOMA), which is derived from a mathematical assessment of the balance between hepatic glucose output and insulin secretion based on fasting levels of both glucose and insulin [46, 48]. The Matsuda index is an another method to determine insulin sensitivity from the OGTT [49]. Fasting levels of C-peptide are indicators of pancreatic β cell

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function. C-peptide is released by these cells during insulin secretion but it degrades slower than insulin, making it a stable biomarker of insulin secretion [50]. Based on the OGTT, a pregnant woman may be diagnosed with isolated hyperglycemia (IH) or impaired glucose tolerance (IGT, a less mild form of glucose intolerance). The researcher can make use of her clinical diagnoses (binomial) or the continuously measured analytes such as fasting glucose or insulin levels to assess associations of THs and subclinical and clinical risk of GDM.

Candidate thyroid biomarkers to examine the causal pathway in maternal circulation include the hormones T3, T4, FT3, FT4, and TSH and thyroid autoantibodies (TPOAb and TgAb) (Fig. 1). These measures are ordered by the obstetrician commonly in the first trimester, but only in women presenting with symptoms of thyroid disease or who are at high risk (those who have an autoimmune disease such as T1D or family history of thyroid disease) [51, 52]. Small changes in FT3 and FT4 can result in a proportionally larger change in TSH due to their negative feedback relationship; therefore, TSH is the more reliable screening analyte for clinical diagnosis [51]. FT4, an accurate marker of thyroid function, is measured concurrently with TSH or in a follow-up screening to confirm a thyroid disorder diagnosis [51, 53]. Thyroid autoantibodies are also measured clinically in a small subset of women to determine women at risk of developing thyroid disorders in pregnancy, particularly those who have an autoimmune disease or have a family history of thyroid disease [51]. Measuring a full panel of THs in the research setting would allow for the most accurate understanding of thyroid function and also allow for assessment for intra- versus inter-individual variability [51, 52].

In the last 10 years, new methods for the measurement of THs have been developed using liquid chromatography-tandem mass spectrometry (LC-MS/MS) that have improved the sensitivity and accuracy of detection [54]. Due to the presence of TH carrier proteins in serum, free TH dissociation and reassociation to binding proteins can occur during sample processing, therefore altering the TH output measurements [54, 55]. This is a particular problem in biospecimens derived from pregnant women, due to the spike in TBG during pregnancy [25, 54]. Equilibrium dialysis or ultrafiltration prior to LC-MS/MS removes TH binding proteins prior to measurement of FT3 and FT4, reducing their interference [54]. LC-MS/MS allows for greater specificity compared to immunoassays by identifying analytes of interest by size rather than by antibodies. Antibodies have varied specificity to analytes and may cross-react with other metabolites in biospecimens, risking false positive results [55]. Sensitivity is enhanced because non-specific binding of antibodies and other types of interference that can mask the antibody-antigen reaction in immunoassays is avoided. This also allows for a reduced lower limit of detection of the analyte of interest [55]. LC-MS/MS methods have recently been developed for thyroid autoantibodies, but so far have comparable accuracy to the immunoassays [53].

Pregnant women are diagnosed with thyroid disease (i.e. hypothyroidism and hyperthyroidism) by comparing TH and antibody levels to pregnancy-specific reference ranges [52, 53]. These trimester-specific ranges account for changes in thyroid biomarker levels during pregnancy, such as the rise in FT4 and drop in TSH during the first trimester, that would otherwise appear as subclinical or clinical thyroid disease when compared to reference ranges for nonpregnant adults [52]. Universal trimester-specific ranges have been difficult to set due to differences in measurement protocols and population differences, such as iodine intake and ethnicity [52]. However, clinical laboratories can create their own reference ranges [52]. Outside of cut-offs and clinically significant TH levels, continuously measured TH biomarkers representing expression across the range of normal to abnormal variation are valuable in epidemiologic investigations to understand exposure effects and to link exposures to outcomes.

PFAS exposure and risk of gestational diabetes mellitus (GDM) development and candidate biomarkers: evidence in the literature

Epidemiologic literature was reviewed and summarized according to specific criteria (Supplement: Methods). Data were extracted and compiled in the Supplemental Tables 1–8 and summarized in Table 1. Forest plots were prepared for representative PFAS that displayed the most significant associations with selected outcomes (Figs. 2, 3). Null coefficients are not interpretable but collectively may offer insight on relationships that were underpowered but worthy of considering in future studies. Therefore, we created criteria to assess the direction of association for all outcomes moving beyond a p value of less than 0.05, as described in the Methods (Supplement).

PFAS and GDM

Out of the 11 epidemiological studies that reported on the association of PFAS and GDM [56–66], four (44%) demonstrated a positive association, differing by PFAS compound (Table 1, Supplemental Table 3) [56, 59, 62, 63]. Overall, higher PFAS levels were associated with higher risk of GDM development. Effect sizes were small and confidence intervals were wide, indicating lack of precision and/or potential bias. The strongest associations were found with PFOA, PFOS, and PFHxS, which are presented in a forest plot comparison (Fig. 2).

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Table 1 Summary of the epidemiologic studies reviewed, according to the direction of association of PFAS exposure on outcomes in the proposed pathway

Outcome	Positive	Negative	Null, Positive Trend[b]	Null, Negative Trend[b]
GDM	36% (4/11 studies)	0% (0/11 studies)	91% (10/11 studies)	64% (7/11 studies)
Maternal Blood				
Glucose homeostasis biomarkers[a]	73% (8/11 studies)	18% (2/11 studies)	45% (5/11 studies)	45% (5/11 studies)
T3	50% (2/4 studies)	50% (2/4 studies)	25% (1/4 studies)	25% (1/4 studies)
FT3	33% (2/6 studies)	33% (2/6 studies)	17% (1/6 studies)	17% (1/6 studies)
T4	20% (1/5 studies)	40% (2/5 studies)	20% (1/5 studies)	20% (1/5 studies)
FT4	20% (2/10 studies)	40% (4/10 studies)	20% (2/10 studies)	30% (3/10 studies)
TSH	45% (5/11 studies)	18% (2/11 studies)	18% (2/11 studies)	9% (1/11 studies)

Abbreviations: *GDM* gestational diabetes mellitus, *FT3* free triiodothyronine, *FT4* free thyroxine, *T3* triiodothyronine, *T4* thyroxine, *TSH* thyrotropin
[a]Glucose homeostasis biomarkers: isolated hyperglycemia, impaired glucose tolerance, blood glucose and insulin levels, insulin resistance
[b]Criteria defined in detail in the Methods (Supplement)

PFAS exposure and biomarkers of glucose homeostasis

We next examined the association of PFAS with biomarkers of glucose homeostasis that could putatively mediate the risk of GDM. These biomarkers do not indicate frank disease but are meaningful in understanding mechanism and chronic disease risk. A two-fold increase in serum PFHxS in Danish pregnant women was positively associated with fasting glucose and insulin levels (percentage change 1.7% (95%CI 0.2, 3.2) and 7.7% (95%CI 0.1, 15.9), respectively) [67]. Additionally, higher PFNA was associated with increased fasting insulin and β cell function (percentage change 12.1% (95%CI 0.7, 24.8) and 12.4% (95%CI 2.2, 23.7), respectively) [67]. Eleven epidemiological studies reported on the association of PFAS with glucose and insulin biomarkers in pregnant women [56–58, 60, 61, 64, 65, 67–70], with eight (73%) finding a positive association, depending on the PFAS [56–58, 60, 64, 65, 67, 69] (Table 1, Supplemental Table 4). Only two studies (18%) reported negative associations [68, 70], though levels of PFAS were lower in their study cohort compared to others examining this association (Supplemental Table 2). Additionally, the Mehta et al. study only included overweight and obese pregnant women enrolled in a stress-reduction intervention as a means to control weight gain and therefore this cohort may not reflect the general population [70]. There was evidence of nonmonotonic associations in the case of PFAS and glucose biomarkers, though these were ultimately null associations after adjustment for confounders (Supplemental Table 4). The general positive association of PFAS with glucose homeostasis biomarkers in these studies is in agreement with epidemiological studies examining PFAS exposure in non-pregnant individuals with both normal and elevated baseline glucose levels. In these populations, PFAS was found to be associated with an increased risk of diabetes and disruption of glycemic indicators, including increased blood glucose, glycated hemoglobin (HbA1c), and insulin secretion [71, 72].

PFAS exposure and biomarkers of thyroid function

An NHANES study of three independent study periods between 1999 and 2006 reported that among US non-pregnant adults ($n = 3974$), participants with thyroid disease had greater serum PFAS levels than those without [73]. A recent meta-analysis of 12 studies examining the effects of PFAS exposure on TH levels in adults found that PFAS are positively associated with FT4 and TSH but negatively associated with total T3 and T4 [74]. In a subgroup analysis of four studies with pregnant women within this meta-analysis ($N = 1735$), PFAS were not associated with THs [74]. Information on gestational age at the time of blood sampling for PFAS and TH measurements was not collected or controlled for; yet gestational age is a source of variability in both measures. This could be one reason for a null association.

There were 13 individual studies reviewed that measured the associations of PFAS and THs in maternal circulation [75–87], with ten (77%) finding a positive or negative association [75, 76, 79–85, 87] (Table 1, Supplemental Tables 5–7). Berg and colleagues published two studies examining associations of PFAS with maternal TH levels using the same cohort. Results were extracted from their 2015 study only [76, 88]. Generally, PFAS were negatively associated with THs and positively associated with TSH (Table 1). This is consistent with the idea that PFAS disrupts TH homeostasis, resulting in a reduction in TH levels that can stimulate TSH secretion due to the negative feedback relationship. In a systematic review examining the relationship between PFAS and THs, Ballesteros et al. also concluded that PFHxS and PFOS are generally found to be positively associated with maternal TSH in pregnancy [89]. Of the studies

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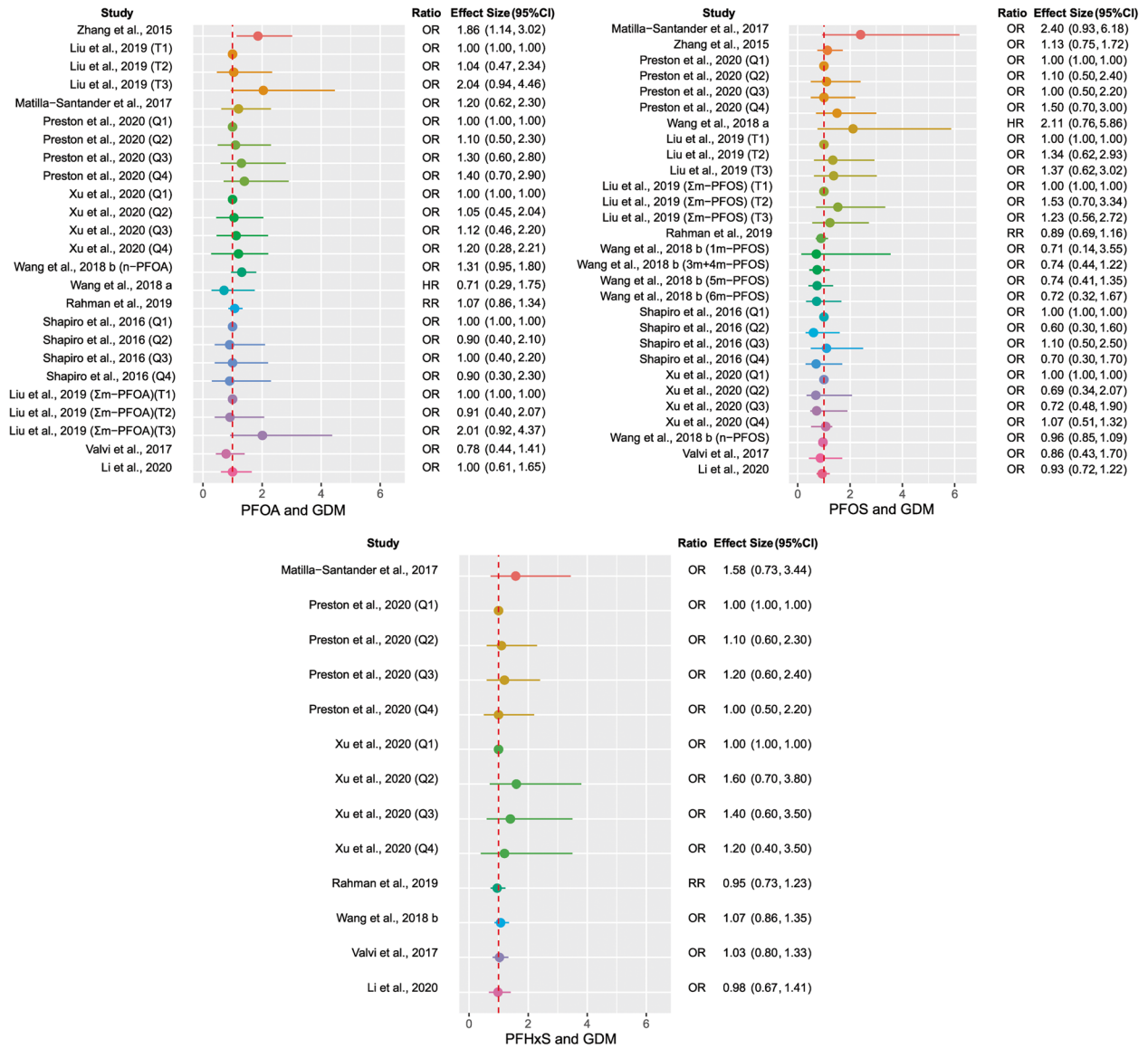


Fig. 2 Summary statistics of selected PFAS on gestational diabetes mellitus (GDM) development in the epidemiologic literature. Abbreviations: HR, hazard ratio; OR, odds ratio; PFOA, perfluorooctanoic acid; PFOS, perfluorooctane sulfonate; PFHxS, perfluorohexane sulfonic acid; RR, risk ratio

included in our review, the strongest PFAS and thyroid biomarker relationship was with TSH (Fig. 3).

Of the six studies that performed a subanalysis stratifying women by thyroid antibody status, all six reported that maternal thyroid autoimmunity altered PFAS disruption of the thyroid based on effect measure modification (Supplemental Table 8) [75, 79–82, 85]. In these studies, it is not presumed that PFAS increased autoimmunity but that PFAS may exacerbate the autoimmune damage to further dysregulate TH production [79, 82, 85]. Itoh et al. found that maternal PFAS levels were associated with FT3 in both maternal thyroid autoantibody positive and negative groups [79]. Consistent with studies that did not measure thyroid autoantibody status, Webster et al. found that PFAS increased TSH and lowered FT4 levels in TPOAb positive pregnant women. Effects were milder in pregnant women with normal TPOAb levels [85]. However, in a recent US study, TPOAb positive women had a negative association between PFAS exposure and TSH levels [81], inconsistent with other studies reporting a positive association [75, 76, 82, 83, 85]. The authors noted that this discrepancy could be a result of study design differences, particularly iodide status of their study population, which affects TH synthesis [81, 85]. Overall, the results indicate that thyroid autoimmunity is a key variable to consider.

Beyond disrupting maternal TH homeostasis, PFAS can be transferred from maternal circulation to the fetus to alter fetal TH levels. Several studies included in this

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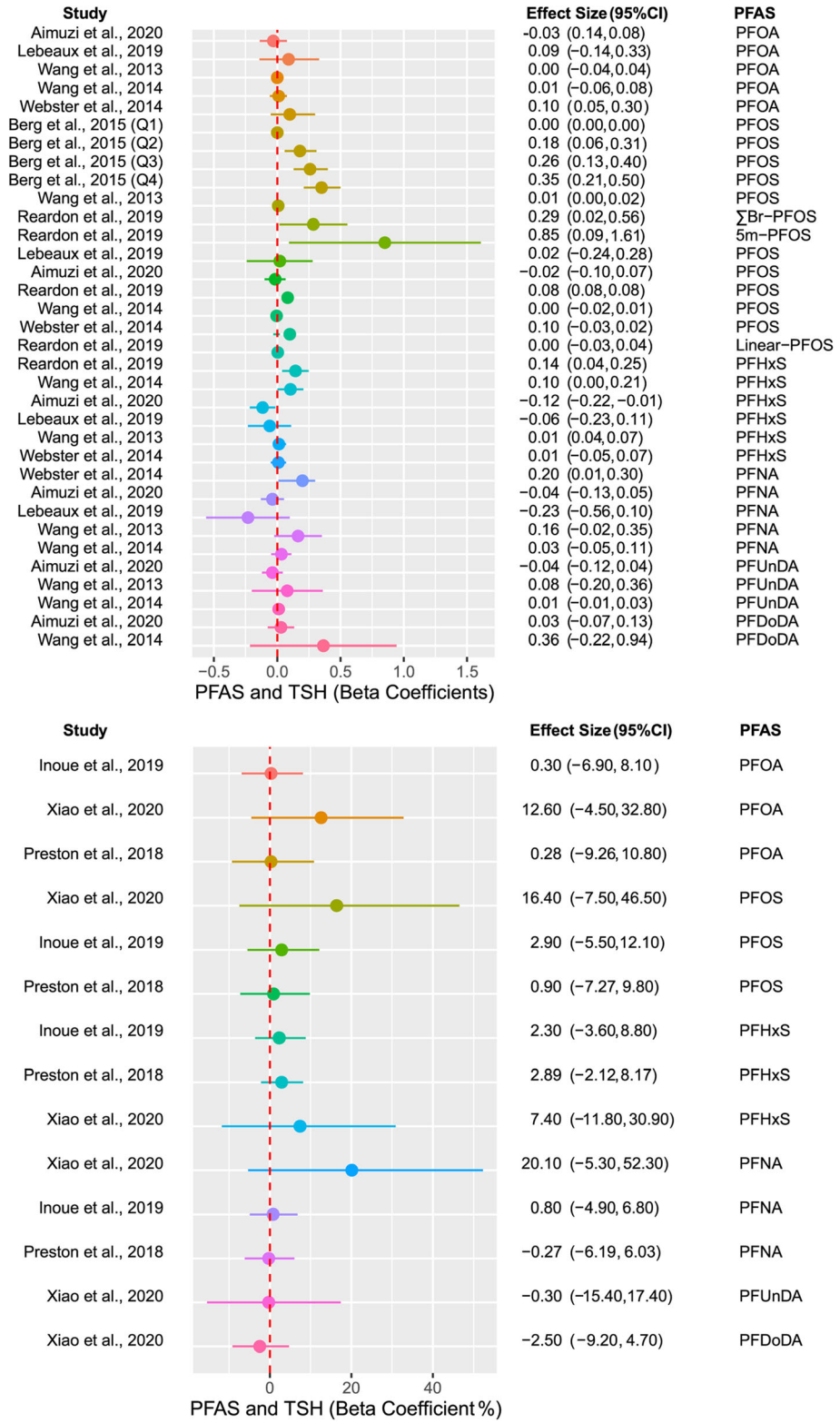


Fig. 3 (See legend on next page.)

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Fig. 3 Summary statistics of selected PFAS on thyrotropin (TSH) in the epidemiologic literature. Displayed is a representative forest plot of the PFAS and TSH relationship for selected PFAS where not all units are the same across all studies. The aim is to assess the overall direction and strength of association. Abbreviations: PFOA, perfluorooctanoic acid; PFOS, perfluorooctane sulfonate; PFHxS, perfluorohexane sulfonic acid; PFNA, perfluorononanoic acid; PFUnDA, perfluoroundecanoate; PFDoDA, perfluorododecanoate; TSH, thyrotropin

review examined the impact of PFAS on fetal THs by measuring the association of PFAS levels with cord blood and neonatal heel stick TH levels [80, 81, 83, 86, 87]. While results varied, maternal PFAS levels were generally positively associated with fetal and neonatal TSH and negatively with T3 and T4. THs regulate metabolic and physiological pathways that determine pregnancy outcomes (i.e. birth size) and development (i.e. neurodevelopment) [35]. Therefore, in addition to affecting maternal health, PFAS thyrotoxicity in pregnancy may alter fetal growth and development, with long-lasting impacts on child health.

Future directions: epidemiological modeling of PFAS and gestational diabetes mellitus (GDM)

To accurately estimate the association of PFAS and GDM risk, study design plays an important role. Longitudinal, unlike cross-sectional, studies allow for the identification of temporality in the relationship. For example, Reardon et al. found that PFHxS was positively associated with TSH and negatively with FT4 [82]. The association was time-dependent for TSH, with the effect size decreasing as gestation progressed [82]. Measuring biomarkers at standardized points throughout gestation can yield less biased conclusions on the relationships proposed here and improve generalizability and transportability to other populations.

This pathway approach primarily strengthens the potential for causal inference by way of a more accurate and precise measurement of the phenomenon of interest. It reduces bias due to measurement error by capturing information on physiologically relevant processes and by including multiple measures within an individual. If biomarkers are measured longitudinally in pregnancy, that likewise strengthens the potential for measuring a causal effect.

The subdiscipline in epidemiology called 'causal inference' lays out specific techniques for estimation, inference, and quantitative analysis of bias [90, 91]. The basis of this approach is the potential outcomes framework. This is a hypothetical framework by which all people who are unexposed in a study and have a set of outcomes can essentially be taken back in time, and then be given the exposure in order to measure their set of outcomes under that condition. The probability of outcome under the two scenarios can be compared to get a causal effect estimate. This is not feasible in real life. The objective is to approximate this scenario by applying analytical techniques. Most pregnant women are exposed to PFAS so the idea of a no exposure group is also unrealistic. For this reason, we might instead estimate the difference in risk if all pregnant women had PFAS levels at the 75th percentile versus all women who had levels at the 25th percentile.

A first step in this approach is to generate a directed acyclic graph (DAG) that can structure the relationship of the exposure and the outcome in relationship to confounders, intermediate variables, and colliders [92]. This is a way to identify sources of structural bias that can be minimized by collecting information, adjustment, or inverse probability weighting. Confounders are those variables which are common causes of the X (exposure), the M (intermediate), and Y (outcome) variables in three categories: 1) confounders of PFAS (X) and GDM (Y); 2) confounders of PFAS (X) and intermediate biomarkers (M, maternal thyroid and pancreas, fetal placenta); and 3) confounders of intermediate biomarkers (M) and GDM (Y). Effect modifiers are variables such as thyroid autoimmune biomarkers which might be operating in tandem with PFAS exposure in some fashion but may not necessarily be intermediates in a causal pathway. Effect modifiers can be identified and evaluated based on knowledge of mechanism and previously reported findings, but they cannot be represented in a DAG [93]. Analytical strategies for constructing DAGs are described elsewhere [92, 94, 95]. Statistical approaches to analyze these associations are outside of the scope of this review, but also an important area in developing this pathway-level approach. G methods have been proposed for the analysis of complex, longitudinal data with time-varying confounders [96]. Methods that allow for single or multiple mediators may also be useful in estimation of PFAS effects in the presence of measures of placental, thyroid, liver, and/or pancreatic function [97].

Adjustment for variables that are not confounders can also lead to bias in the estimation of the exposure and outcome association [98]. Variables that were adjusted for but may not be confounders in the studies reviewed are noted in Supplemental Table 1. To qualify as a confounder, the variable must precede the exposure in time and plausibly be a common cause of the exposure and the outcome.

Discussion

The evidence of PFAS exposure as a direct cause of GDM in the epidemiological literature is weak. This

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conclusion could change in future studies that consider the indirect pathway and measure the biomarkers proposed here. Based on the results of this review, we theorize that PFAS can alter TH homeostasis in pregnancy by causing higher TSH levels and lower T3 and T4 levels, disrupting downstream glucose metabolism. TH disruption may be happening at the level of the thyroid gland, the hypothalamus, the pituitary, or the fetal placenta (Fig. 1).

There is experimental evidence of the toxicity of PFAS to the thyroid. PFOS and PFHxS were found to inhibit iodide uptake in the FRTL-5 rat thyroid follicular cell line [99, 100]. These effects were not seen with PFOA, indicating compound specific effects on the thyroid [99]. In zebrafish, PFUnDA and PFOA induced changes in the expression of genes involved in TH metabolism and excretion [101, 102] while perfluorotridecanoic acid (PFTrDA) upregulated genes related to TH activation and synthesis [101]. Furthermore, PFAS may competitively bind to TH binding proteins to displace T4 [103] and to increase transcript expression of D1, an enzyme that converts T4 to T3, in the thyroids of rats [104]. All together, PFAS may therefore be altering TH homeostasis by direct interaction with the thyroid gland and through mechanisms that control TH availability. Based on the results of our literature review, PFAS may also exacerbate underlying thyroid autoimmunity. Approximately 30% of pregnant women during early pregnancy were found to be TBOAb/TgAb positive [105], confirming that this is a variable that cannot be ignored.

From our findings, we infer that PFAS exposure also causes glucose and insulin levels to increase in pregnancy. We propose that changes in glucose and insulin levels here may be a secondary consequence of thyroid dysregulation. This may also reflect direct toxicity of PFAS to the maternal liver and/or pancreas that could disrupt maternal and fetal glucose homeostasis during pregnancy. Molecular docking analysis found that PFOS, PFOA, and PFHxS can directly bind multiple peroxisome proliferator-activated receptor (PPAR) isoforms in the liver that regulate glucose metabolism [106]. Glycogen depletion and mitochondrial dysfunction are PPAR-independent mechanisms by which PFAS exerts toxicity in the liver [107]. In carp, a closely related species to zebrafish, PFOS exposure inhibited liver glucokinase gene expression, which regulates glucose uptake into the liver, and depleted glycogen stores in the liver, therefore altering glucose homeostasis [108]. Glycogen depletion was also observed in the livers of pregnant mice exposed to PFAS, along with hepatocellular alterations, such as hypertrophy and necrosis [109]. Additionally, Sant et al. determined that zebrafish embryonic exposure to PFAS induced morphological changes to the pancreas of zebrafish larvae, including reduced β cell area for insulin production, as well as reduced gene expression of hormones related to glucoregulation [110].

The majority of PFAS examined demonstrated null associations with GDM and the biomarkers proposed in this causal pathway. This may be a result of limitations in study design when examining PFAS cross-sectionally or sampling cases and controls versus a prospective cohort design. Findings could also be null due to measurement error or incorrect model specification. Incorrect model specification here includes the omission of interactions or intermediate variables. These are all issues that the proposed pathway approach can address. Another issue is that due to this being a large and diverse class of chemicals, the toxicological profiles of different PFAS vary dramatically. Therefore, there may be only particular compounds that impact the specific biomarkers proposed in this pathway. Biomarker-based studies are needed to narrow these lists and identify these specific relationships. Additionally, human exposure to PFAS is typically through complex mixtures and multiple routes of exposure, rather than to individual compounds [1]. In mixtures, the compounds have been shown to exert both additive and synergistic toxicities; therefore, examining the overall mixture effect may be more accurate in depicting the human exposure [111]. Few studies included in our review examined the combined effects of the PFAS on the outcomes. Chan et al. examined the association of the molar sum of PFHxS, PFOA, and PFOS with hypothyroxinemia in pregnant women but found no additive or synergistic effect, as compared to analyzing each compound alone [77]. Three studies [58, 70, 80] used Bayesian Kernel Machine Regression (BKMR) as a statistical approach to determine the PFAS mixture effect while also providing associations for each compound in the mixture to understand which are the main drivers of the overall association [112]. Correlations between mixture components are also taken into account [112]. With this approach, a PFAS mixture (six compounds) was associated with plasma glucose levels [58]. The single compounds PFOS and 2-(N-methyl-perfluorooctane sulfonamide) acetate (N-MeFOSAA) were the main contributors to this overall association [58]. Mixture modeling can be useful in identifying the 'bad actors.' There is no perfect model to account for these complex relationships, which is one reason it is impossible to conclude that PFAS associations with GDM are truly null.

Impairments in placental function can affect maternal health during pregnancy (i.e. preeclampsia). It follows that PFAS-induced effects on the placenta may also adversely affect the mother, as well as the fetus. The placenta is exposed to PFAS from maternal circulation [113]. In animal models, PFAS exposure causes both increases and decreases in placental weight, inhibits

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activity and gene expression of placental hormones, and induces necrosis and histopathological alterations, with compound specific effects [109, 114, 115]. Rodents do not produce hCG, so this model does not translate directly. Placental biomarkers altered by PFAS exposure during pregnancy, including but not exclusive to hCG, should be prioritized when studying this causal pathway.

Reverse causation, whereby thyroid dysfunction precedes and causes physiological differences that affect maternal PFAS concentrations, cannot be eliminated. For example, THs regulate multiple processes in the kidney, including the glomerular filtration rate (GFR), which is the rate of fluid filtered by the kidneys. Renal clearance is the primary mode of excretion of PFAS and a lower GFR is associated with higher serum PFAS levels [116]. Any alteration in thyroid function could in theory change the GFR and rate of excretion of PFAS, therefore affecting serum levels of PFAS. Watkins et al. measured the direction of association between PFOA and kidney function by comparing measured versus model-predicted (based on environmental exposure estimates) serum PFOA levels, which are dependently and independently influenced by GFR, respectively [116]. Only the measured PFOA was associated with kidney dysfunction, which may indicate that PFAS is a result of rather than the cause of the kidney function. Other physiological processes have been proposed that may demonstrate reverse causality in the causal association of PFAS with health outcomes [117]. Adding confounders related to circulating PFAS levels, such as GFR, into the model of PFAS exposure and GDM development could address this issue of reverse causation, but it cannot be completely eliminated when measures are cross-sectional in pregnancy.

The pathway proposed here may be influenced by a broad range of environmental exposures beyond PFAS. For example, organochlorine pesticides (OCPs) used for crop protection, such as dichlorodiphenyl trichloroethane and hexachlorobenzene, are also potential candidates for disruption of thyroid function [118, 119]. Several OCPs were associated with TSH and FT4 levels in cord plasma [120] and with the risk of developing GDM [121]. Likewise, exposure to brominated flame retardants, such as polybrominated diphenyl ethers (PBDEs), have been positively associated with GDM risk [122]. PBDEs are negatively associated with total T3 and T4 in cord serum and with altered birth outcomes, including lower IQ [123–125]. Furthermore, endocrine disruptors in plastics and personal care items, including phthalates and bisphenol A, have been shown to affect TH regulation including impairment of iodine uptake and inhibition of TH homeostasis [126] and phthalates have been found to be associated with hCG, glucose intolerance, and GDM [127–129].

GDM is a common pregnancy complication that may progress to adverse maternal and child health outcomes and can be better appreciated as a consequence of environmental exposure preconception and during pregnancy. Here, we present the evidence to date on PFAS as a relevant exposure. On the clinical side, this knowledge can be used to distinguish patients at different levels of risk for GDM and conversion of GDM to T2D, and also to formulate stronger individual-level interventions related to PFAS exposures through diet and drinking water in pregnancy. On the level of public health, these types of studies can be useful in generating causal knowledge on population-level exposures that increase the risk of GDM to motivate interventions when supported by the evidence. If consensus is reached regarding specific and harmful effects of PFAS, these types of studies could motivate innovation in the design of chemicals used in food packaging and commercial products. The biomarkers proposed in this pathway can be monitored in large cohorts to evaluate efficacy of PFAS regulation and/or more targeted types of interventions.

Conclusions

Epidemiological evidence strongly supports that problems with thyroid function can disrupt glucose homeostasis, which we posit may be a driving force for the development of GDM and may be a mechanism through which PFAS exposures exert toxicity in pregnant women. This mechanism may offer an opportunity to more precisely and accurately quantify the associations of PFAS (and other thyroid-disrupting chemicals) with GDM. The relationship between TH biomarkers and GDM as well as glucose biomarkers and GDM are well established, however the combination of these two associations into one common pathway is relatively unexplored. PFAS exposure may be an upstream environmental lever that interferes with thyroid function and disrupts downstream glucose homeostasis.

Abbreviations

AITDs: Autoimmune thyroid diseases; D1: Type 1 iodothyronine deiodinases; D2: Type 2 iodothyronine deiodinases; D3: Type 3 iodothyronine deiodinases; FFA: Free fatty acid; FT3: Free triiodothyronine; FT4: Free thyroxine; GDM: Gestational diabetes mellitus; hCG: Human chorionic gonadotropin; HOMA: Homeostasis model assessment; PLGF: Placental growth factor; PFAS: Per- and polyfluoroalkyl substances; T1D: Type I diabetes; T2D: Type II diabetes; T3: Triiodothyronine; T4: Thyroxine; TBG: Thyroxine-binding globulin; Tg: Thyroglobulin; TgAb: Thyroglobulin antibody; TH: Thyroid hormone; TPO: Thyroidperoxidase; TPOAb: Thyroid peroxidase antibody; TRH: Thyrotropin-releasing hormone; TSH: Thyrotropin

Supplementary Information

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Additional file 1: Methods. Supplemental Table 1. Summary of studies examining PFAS and gestational diabetes (GDM), glucose, or

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thyroid biomarkers in pregnancy. **Supplemental Table 2.** Summary of PFAS and biomarker levels in cohorts. **Supplemental Table 3.** Association of PFAS with gestational diabetes mellitus (GDM). **Supplemental Table 4.** Association of PFAS with glucose and insulin biomarkers. **Supplemental Table 5**: Evidence of PFAS association with free and total triiodothyronine (T3) in pregnancy. **Supplemental Table 6:** Evidence of PFAS association with free and total thyroxine (T4) in pregnancy. **Supplemental Table 7:** Evidence of PFAS association with thyrotropin (TSH) in pregnancy. **Supplemental Table 8:** Evidence of PFAS association with thyroid hormones by thyroid autoantibody status in pregnancy.

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Authors' contributions
JA conceptualized the study and oversaw the analysis. RLB, HW, FF, MB conducted the literature review, completed the analysis, and drafted the manuscript. RLB and JJA led the writing and editing of the manuscript. MF, CAN, CLH, DDM, and JC provided critical feedback. All authors read and approved the final manuscript.

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Availability of data and materials
All data generated or analyzed during this study are included in this published article [and its supplementary information files].

Declarations

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Not applicable.

Consent for publication
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Competing interests
The authors declare that they have no competing interests.

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The Madrid Statement on Poly- and Perfluoroalkyl Substances (PFASs)

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As scientists and other professionals from a variety of disciplines, we are concerned about the production and release into the environment of an increasing number of poly- and perfluoroalkyl substances (PFASs) for the following reasons:

1. PFASs are man-made and found everywhere. PFASs are highly persistent, as they contain perfluorinated chains that only degrade very slowly, if at all, under environmental conditions. It is documented that some polyfluorinated chemicals break down to form perfluorinated ones (D'Eon and Mabury 2007).
2. PFASs are found in the indoor and outdoor environments, wildlife, and human tissue and bodily fluids all over the globe. They are emitted via industrial processes and military and firefighting operations (Darwin 2011; Fire Fighting Foam Coalition 2014), and they migrate out of consumer products into air (Shoeib et al. 2011), household dust (Björklund et al. 2009), food (Begley et al. 2008; Tittlemier et al. 2007; Trier et al. 2011), soil (Sepulvado et al. 2011; Strynar et al. 2012), ground and surface water, and make their way into drinking water (Eschauzier et al. 2012; Rahman et al. 2014).
3. In animal studies, some long-chain PFASs have been found to cause liver toxicity, disruption of lipid metabolism and the immune and endocrine systems, adverse neurobehavioral effects, neonatal toxicity and death, and tumors in multiple organ systems (Lau et al. 2007; Post et al. 2012). In the growing body of epidemiological evidence, some of these effects are supported by significant or suggestive associations between specific long-chain PFASs and adverse outcomes, including associations with testicular and kidney cancers (Barry et al. 2013; Benbrahim-Tallaa et al. 2014), liver malfunction (Gallo et al. 2012), hypothyroidism (Lopez-Espinosa et al. 2012), high cholesterol (Fitz-Simon et al. 2013; Nelson et al. 2009), ulcerative colitis (Steenland et al. 2013), lower birth weight and size (Fei et al. 2007), obesity (Halldorsson et al. 2012), decreased immune response to vaccines (Grandjean et al. 2012), and reduced hormone levels and delayed puberty (Lopez-Espinosa et al. 2011).
4. Due to their high persistence, global distribution, bioaccumulation potential, and toxicity, some PFASs have been listed under the Stockholm Convention (United Nations Environment Programme 2009) as persistent organic pollutants (POPs).
5. As documented in the Helsingør Statement (Scheringer et al. 2014),
   a. Although some of the long-chain PFASs are being regulated or phased out, the most common replacements are short-chain PFASs with similar structures, or compounds with fluorinated segments joined by ether linkages.
   b. While some shorter-chain fluorinated alternatives seem to be less bioaccumulative, they are still as environmentally persistent as long-chain substances or have persistent degradation products. Thus, a switch to short-chain and other fluorinated alternatives may not reduce the amounts of PFASs in the environment. In addition, because some of the shorter-chain PFASs are less effective, larger quantities may be needed to provide the same performance.
   c. While many fluorinated alternatives are being marketed, little information is publicly available on their chemical structures, properties, uses, and toxicological profiles.
   d. Increasing use of fluorinated alternatives will lead to increasing levels of stable perfluorinated degradation products in the environment, and possibly also in biota and humans. This would increase the risks of adverse effects on human health and the environment.
6. Initial efforts to estimate overall emissions of PFASs into the environment have been limited due to uncertainties related to product formulations, quantities of production, production locations, efficiency of emission controls, and long-term trends in production history (Wang et al. 2014).
7. The technical capacity to destroy PFASs is currently insufficient in many parts of the world.

Global action through the Montreal Protocol (United Nations Environment Programme 2012) successfully reduced the use of the highly persistent ozone-depleting chlorofluorocarbons (CFCs), thus allowing for the recovery of the ozone layer. However, many of the organofluorine replacements for CFCs are still of concern due to their high global warming potential. It is essential to learn from such past efforts and take measures at the international level to reduce the use of PFASs in products and prevent their replacement with fluorinated alternatives in order to avoid long-term harm to human health and the environment.

For these reasons, we call on the international community to cooperate in limiting the production and use of PFASs and in developing safer nonfluorinated alternatives. We therefore urge scientists, governments, chemical and product manufacturers, purchasing organizations, retailers, and consumers to take the following actions:

Scientists:

1. Assemble, in collaboration with industry and governments, a global inventory of all PFASs in use or in the environment, including precursors and degradation products, and their functionality, properties, and toxicology.
2. Develop analytical methods for the identification and quantification of additional families of PFASs, including fluorinated alternatives.
3. Continue monitoring for legacy PFASs in different matrices and for environmental reservoirs of PFASs.
4. Continue investigating the mechanisms of toxicity and exposure (e.g., sources, fate, transport, and bioaccumulation of PFASs), and improve methods for testing the safety of alternatives.
5. Bring research results to the attention of policy makers, industry, the media, and the public.

Governments:

1. Enact legislation to require only essential uses of PFASs, and enforce labeling to indicate uses.
2. Require manufacturers of PFASs to
   a. conduct more extensive toxicological testing,
   b. make chemical structures public,
   c. provide validated analytical methods for detection of PFASs, and
   d. assume extended producer responsibility and implement safe disposal of products and stockpiles containing PFASs.
3. Work with industry to develop public registries of products containing PFASs.
4. Make public annual statistical data on production, imports, and exports of PFASs.

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5. Whenever possible, avoid products containing, or manufactured using, PFASs in government procurement.
6. In collaboration with industry, ensure that an infrastructure is in place to safely transport, dispose of, and destroy PFASs and PFAS-containing products, and enforce these measures.

Chemical manufacturers:

1. Make data on PFASs publicly available, including chemical structures, properties, and toxicology.
2. Provide scientists with standard samples of PFASs, including precursors and degradation products, to enable environmental monitoring of PFASs.
3. Work with scientists and governments to develop safe disposal methods for PFASs.
4. Provide the supply chain with documentation on PFAS content and safe disposal guidelines.
5. Develop nonfluorinated alternatives that are neither persistent nor toxic.

Product manufacturers:

1. Stop using PFASs where they are not essential or when safer alternatives exist.
2. Develop inexpensive and sensitive PFAS quantification methods for compliance testing.
3. Label products containing PFASs, including chemical identity and safe disposal guidelines.
4. Invest in the development and use of nonfluorinated alternatives.

Purchasing organizations, retailers, and individual consumers:

1. Whenever possible, avoid products containing, or manufactured using, PFASs. These include many products that are stain-resistant, waterproof, or nonstick.
2. Question the use of such fluorinated "performance" chemicals added to consumer products.

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OFFICE OF ENVIRONMENTAL HEALTH HAZARD ASSESSMENT

Public Health Goals

FIRST PUBLIC REVIEW DRAFT

Perfluorooctanoic Acid and Perfluorooctane Sulfonic Acid in Drinking Water

July 2021

Pesticide and Environmental Toxicology Branch
Office of Environmental Health Hazard Assessment
California Environmental Protection Agency

KD1061

Proposed Public Health Goals for Perfluorooctanoic Acid and Perfluorooctane Sulfonic Acid in Drinking Water

July 2021

Prepared by

Pesticide and Environmental Toxicology Branch

Office of Environmental Health Hazard Assessment

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PREFACE

Public Health Goal (PHG) technical support documents provide information on health effects from contaminants in California drinking water. PHGs are developed for chemical contaminants based on the best available data in the scientific literature and using the most current principles, practices, and methods used by public health professionals. These documents and the analyses contained therein provide estimates of the levels of contaminants in drinking water that would pose no significant health risk to individuals consuming the water on a daily basis over a lifetime.

Under the California Safe Drinking Water Act of 1996 (Health and Safety Code, Section 116365), the Office of Environmental Health Hazard Assessment (OEHHA) develops PHGs for drinking water contaminants in California based exclusively on public health considerations. OEHHA periodically reviews PHGs and revises them as necessary based on the occurrence of the respective chemical in California drinking water supplies and the availability of new scientific data. This document presents proposed PHGs for perfluorooctanoic acid and perfluorooctane sulfonic acid.

OEHHA is releasing this draft assessment for public comment and independent, external peer review. OEHHA will address the comments in a second draft and will release it for further public comment before publishing the final assessment.

PHGs published by OEHHA are for use by the State Water Resources Control Board (SWRCB) in establishing primary drinking water standards (California Maximum Contaminant Levels, or CA MCLs). Whereas PHGs are to be based solely on scientific and public health considerations without regard to economic cost considerations, MCLs adopted by SWRCB consider economic factors and technological feasibility. State law requires that MCLs be set at a level that is as close as feasible to the corresponding PHG, placing emphasis on the protection of public health. PHGs established by OEHHA are not regulatory and represent only non-mandatory goals. Under federal law, CA MCLs established by SWRCB must be at least as stringent as the federal MCL if one exists.

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List of Commonly Used Abbreviations

ACTH – adrenocorticotropic hormone
ADD – acceptable daily dose
ALP – alkaline phosphatase
ALT – alanine aminotransferase
ASF – age sensitivity factor
AST – aspartate aminotransferase
ATP – adenosine triphosphate
ATSDR – Agency for Toxic Substances and Disease Registry
AUC – area under the curve
BMD – benchmark dose
BMDL – lower 95% confidence limit of the benchmark dose
BMDS – benchmark dose software
BMI – body mass index
BMR – benchmark response
BUN – blood urea nitrogen
BW – body weight
cAMP – cyclic adenosine monophosphate
CAR – constitutive androstane receptor
CAT – catalase
CBG – corticosteroid binding globulin
CCK – cholecystokinin
CI – confidence interval
CL – clearance
CL_F – fecal clearance
CL_R – renal clearance
CORT – corticosterone
CSF – cancer slope factor
DART – developmental and reproductive toxicity
DEN – diethylnitrosamine
DNA – deoxyribonucleic acid
DWI – daily water intake
E2 – estradiol
EDS – ethane dimethyl sulfonate
ER – endoplasmic reticulum
ERβ – estrogen receptor beta
FOSE – perfluorooctane sulfonamide ethanol
fT4 – free thyroxine
FTOH – fluorotelomer alcohol
GA – gestational age
GD – gestation day
GFR – glomerular filtration rate
GGT – gamma-glutamyl transpeptidase

GluR2 – glutamate receptor 2
GnRH – gonadotropin-releasing hormone
GSH – glutathione
GSH-Px – glutathione peroxidase
HDL – high-density lipoprotein
HPC – health-protective concentration
HR – hazard ratio
IARC – International Agency for Research on Cancer
IgE – immunoglobulin E
IgG – immunoglobulin G
IL – interleukin
IQR – interquartile range
IVIVE – in vitro to in vivo extrapolation
i.v. – intravenous
KKS – kallikrein-kinin system
KO – knockout
L/kg-day – liters per kilogram body weight per day
LCMRL – lowest concentration minimum reporting level
LDH – lactate dehydrogenase
LDL – low-density lipoprotein
LH – luteinizing hormone
LMS – linearized multistage
LOAEC – lowest-observed-adverse-effect concentration
LOAEL – lowest-observed-adverse-effect level
LXR – liver X receptor
MCL – maximum contaminant level
MCMC – Markov chain Monte Carlo
MDA – malondialdehyde
µg/L – micrograms per liter
µg/ml – micrograms per milliliter
mg/kg-day – milligrams per kilogram body weight per day
MOA – mode of action
ng/L – nanograms per liter
ng/ml – nanograms per milliliter
NHANES – National Health and Nutrition Examination Survey
NIS – sodium-iodide symporter
NK – natural killer
NL – notification level
NMDA – n-methyl-d-aspartate
NO – nitric oxide
NOAEC – no-observed-adverse-effect concentration
NOAEL – no-observed-adverse-effect level
NTP – National Toxicology Program

OAT – organic anion transporter
OEHHA – Office of Environmental Health Hazard Assessment
8-OHdG – 8-hydroxy-2'-deoxyguanosine
OR – odds ratio
OVA – ovalbumin
P4 – progesterone
PBDE – polybrominated diphenyl ether
PBPK – physiologically-based pharmacokinetic
PCB – polychlorinated biphenyl
PCNA – proliferating cell nuclear antigen
PFAS – per- and polyfluoroalkyl substances
PFCA – polyfluorinated carboxylic acid
PFOA – perfluorooctanoic acid
PFOS – perfluorooctane sufonic acid
PHG – public health goal
PK – pharmacokinetic
PND – postnatal day
POD – point of departure
PPAR – peroxisome proliferator-activated receptor
ppb – parts per billion
ppm – parts per million
ppt – parts per trillion
PTFE – polytetrafluoroethylene
PXR – pregnane X receptor
RL – reference level
RNA – ribonucleic acid
ROS – reactive oxygen species
RR – relative risk
RSC – relative source contribution
RXR – retinoic X receptor
SDH – sorbitol dehydrogenase
SGA – small for gestational age
SIR – standardized incidence ratio
SMR – standardized mortality ratio
SOD – superoxide dismutase
SRBC – sheep red blood cells
SWRCB – State Water Resources Control Board
$T_{1/2}$ – half-life
T3 – triiodothyronine
T4 – thyroxine
TBG – thyroxine-binding globulin
TC – total cholesterol
TFE – tetrafluoroethylene

TK – toxicokinetic
TNF-α – tumor necrosis factor alpha
TSH – thyroid stimulating hormone
tT4 – total thyroxine
TTR – transthyretin
UCMR3 – US EPA's Third Unregulated Contaminant Monitoring Rule
UF – uncertainty factor
V_d – volume of distribution
WT – wild-type

SUMMARY

For more than a half-century, perfluorooctanoic acid (PFOA) and perfluorooctane sulfonic acid (PFOS) were widely used in industrial applications and consumer products, notably, PFOA in nonstick cookware and PFOS in stain and water-repellant fabrics and in fire-fighting foams. The manufacture of these chemicals was phased out in the US following concerns about their extreme persistence in the environment and their detection in virtually all human blood serum samples. Although levels in the environment have declined from their peak around the year 2000, PFOA and PFOS continue to be present in the environment and are found in California drinking water. Because exposure to these chemicals is so prevalent and elimination times are so long, it is critical to understand the toxicity associated with these compounds, and their impacts on human health.

Public Health Goals for PFOA and PFOS

Scientific studies show adverse health effects of PFOA and PFOS in people exposed at environmental levels, and similar effects in laboratory animals. There is evidence from epidemiologic studies that exposure to PFOA increases the risk of kidney cancer. Human exposure to PFOA is also associated with liver and immune system toxicity and increased total cholesterol, and there is suggestive evidence of an association with risk of preeclampsia and pregnancy-related hypertension. PFOS is associated with immune system toxicity and increased total cholesterol in humans, with suggestive evidence of an association with risk of preeclampsia and pregnancy-related hypertension. The effects seen in humans are supported by studies in laboratory animals, which show that PFOA and PFOS can cause liver toxicity, immunotoxicity, thyroid toxicity, developmental/reproductive toxicity, and cancer.

This draft document presents proposed public health goals (PHGs) for PFOA and PFOS in drinking water, based on the most sensitive health effects. A PHG is the concentration of a contaminant in drinking water that is estimated to pose no significant health risk to individuals consuming the water on a daily basis over a lifetime. The proposed PHG for PFOA is based on kidney cancer in humans, while the proposed PHG for PFOS is based on liver and pancreatic tumor data from rat studies. This draft document also identifies health-protective concentrations (HPCs) for noncancer effects of PFOA and PFOS. The dose-response data from human studies were sufficient for derivation of the HPCs for these compounds, with the most sensitive noncancer endpoints being liver damage for PFOA and clinically relevant increased total cholesterol for PFOS.

Table S1 shows the proposed PHGs and HPCs for PFOA and PFOS.

Table S1. Proposed Public Health Goals and Health-Protective Concentrations

Chemical Name	PHG (ppt)	PHG Effect(s)	HPC (ppt)	HPC Effect
Perfluorooctanoic acid	0.007	Kidney cancer (human data)	3	Increased risk of liver damage (human data)
Perfluorooctane sulfonic acid	1	Cancer (animal data)	2	Increased total cholesterol (human data)

HPC, health-protective concentration; PHG, public health goal; ppt, parts per trillion (equivalent to nanograms per liter or ng/L)

PHGs are not regulatory requirements, and are based solely on protection of public health without regard to cost impacts or other factors. PHGs form the basis of California's Maximum Contaminant Levels (MCLs) for drinking water, which are established by the State Water Resources Control Board (SWRCB), and each MCL must be set as close to the corresponding PHG as is economically and technologically feasible. PHGs are developed for chemical contaminants based on the best available data in the scientific literature and using the most current principles, practices, and methods used by public health professionals, and on comprehensive analyses of information on the toxicology of each compound.

Derivation of the PHGs and HPCs

As noted above, a number of adverse health effects have been observed in studies in humans and laboratory animals exposed to PFOA and PFOS. The proposed PHGs are based on the cancer endpoint because it is the most sensitive effect. PHGs are set at a level where the cancer risk is estimated to be one per one million persons exposed over a lifetime. The PHGs, as well as the noncancer HPCs, also include considerations of sensitive and highly exposed populations.

The proposed PHGs and noncancer HPCs are different from the reference levels supporting the notification levels OEHHA recommended to SWRCB in 2019.[1] This is due to a number of factors, including the availability of new studies, the use of human data when possible, and improved toxicokinetic analyses.

Systematic Literature Search

The proposed PHGs and noncancer HPCs are based on epidemiologic and laboratory animal studies OEHHA identified through systematic literature searches described in appendices to this document. In addition, OEHHA identified studies published before 2016 from the reference lists of assessments published by the US Environmental Protection Agency (US EPA, 2016b; US EPA, 2016d), the National Toxicology Program (NTP, 2016), the International Agency for Research on Cancer (IARC, 2017a), and the Agency for Toxic Substances and Disease Registry (ATSDR, 2018a). Finally, in 2019, OEHHA issued a data call-in to request information from the public. For each pertinent study identified in this manner, OEHHA evaluated the methods and quality in order to base the PHGs and noncancer HPCs on studies of sufficient quality.

Toxicokinetic Analyses to Address Persistence in Humans

PFOA and PFOS strongly bioaccumulate in humans and to a much lesser degree in animals. To address the issue of the persistence of PFOA and PFOS in the human body in its dose-response analyses, OEHHA used serum concentrations as a measure of the internal exposure. Fortunately, all the available human toxicity studies OEHHA selected to use in the dose-response analyses report serum concentrations for PFOA or PFOS. OEHHA converted serum

[1] In 2019, OEHHA developed reference levels of 0.1 ppt for PFOA based on pancreatic and liver tumors in rats and 0.4 ppt for PFOS based on liver tumors in rats. However, the cancer-based reference levels were lower than could be reliably detected in drinking water using currently available technologies. Thus, OEHHA recommended that SWRCB set the notification levels for PFOA and PFOS at the lowest levels that could be reliably detected in drinking water (5.1 ppt for PFOA and 6.5 ppt for PFOS). OEHHA also developed noncancer reference levels of 2 ppt for PFOA based on liver toxicity in mice and 7 ppt for PFOS based on immunotoxicity in mice (OEHHA, 2019).

levels to chronic intake doses (amount of chemical consumed per unit body weight) using toxicokinetic (TK) approaches. In establishing human equivalent doses associated with health effects observed in animal studies, OEHHA also used a TK approach to address the large differential in chemical half-life between animals and humans.

OEHHA conducted an extensive review of the literature and analyzed different TK approaches in order to select the best method to address this matter. The TK approaches OEHHA selected for use rely on the application of a clearance factor. OEHHA conducted a comprehensive review of the literature before it selected the most appropriate clearance value to use in its analyses. A human clearance factor of 2.8×10^{-4} L/kg-day was developed for PFOA and 3.9×10^{-4} L/kg-day for PFOS. This improves the approach used previously (OEHHA, 2019).

PFOA Health Effects Basis for PHG and HPC

Kidney cancer in humans as the sensitive endpoint for the PHG: A large case-control study of PFOA and kidney cancer in 10 study centers across the US was recently published by the National Cancer Institute (Shearer et al., 2021).[2] This prospective population-based study, along with previous epidemiologic studies in an area of high environmental exposure (Barry et al., 2013; Vieira et al., 2013) and in an occupational setting (Steenland and Woskie, 2012), provide strong evidence that PFOA causes kidney cancer in humans. The occupational study by Raleigh et al. (2014) did not report an association between PFOA and kidney cancer, but used an inhalation exposure model that was not validated. Lack of information on potential confounding, a potentially inappropriate comparison group, and limited statistical power are other possible reasons why this study may have missed a true association. Multiple analyses performed by the researchers of all four studies show that the positive results for kidney cancer and PFOA are very unlikely to be due to chance. Also, evidence of dose-response patterns, a criterion commonly used for evaluating causality, is seen in each of the four studies, and the prospective nature of these studies assures that PFOA exposure came before kidney cancer development, which helps rule out the possibility of reverse causality.

OEHHA performed a number of evaluations and detailed analyses on whether the biases or problems frequently seen in epidemiologic studies could have caused the positive associations reported. This includes evaluating potential problems related to participant recruitment and selection, in categorizing participants' PFOA exposure, and in classifying people with and without kidney cancer. Overall, these analyses show that each of these issues was either very minor or likely to have led to underestimates, rather than overestimates, of kidney cancer risk from PFOA exposure.

A number of other factors are known to cause or are suspected to cause kidney cancer, and these could potentially confound the relationship between PFOA and kidney cancer. The studies used in OEHHA's dose-response analyses accounted for the most important of these (e.g., sex, age, tobacco smoking, and being overweight or obese) with statistical adjustments or stratified analyses. For all other factors, including the presence of other PFAS, all available evidence shows that they were either not prevalent enough or their associations with PFOA or kidney cancer were not strong enough to cause major confounding.

[2] Paper became available online in September, 2020 at: https://academic.oup.com/jnci/article/113/5/580/5906528

Overall, the body of evidence on PFOA and kidney cancer from human studies meets the modified Hill (1965) criteria that are commonly used to evaluate causality. The carcinogenicity of PFOA in humans is supported by the results of animal cancer bioassays. A recent NTP study in rats concluded there is "clear evidence of carcinogenic activity of PFOA" in male rats and "some evidence of carcinogenic activity of PFOA" in female rats (NTP, 2020). It is also supported by in vivo and in vitro mechanistic studies.

Dose-response analysis of the human kidney cancer data for the PFOA PHG: The epidemiologic studies by Shearer et al. (2021) and Vieira et al. (2013) include data sufficient for quantifying cancer risks. Both studies involved large sample sizes and therefore had good statistical power to identify true effects. Both studies had a good range of PFOA exposures, included large numbers of participants with exposure levels close to those seen in the general US population, and adjusted or stratified for other major kidney cancer risk factors. In these two studies, PFOA exposure was assessed using either directly measured serum PFOA levels in each individual (Shearer et al., 2021), which are good indicators of long-term PFOA exposure, or individual PFOA serum levels estimated using a validated exposure model (Vieira et al., 2013). Evaluations show that the impacts of exposure misclassification from either of these methods are likely to be mostly minor and unlikely to have caused the elevated risks reported in either study.

The cancer slope factors derived from these studies are within about six-fold of each other. To make maximum use of both these strong studies, the geometric mean of the cancer slope factors, 0.0026 per ng/kg-day, is used to derive the proposed PHG of 0.007 parts per trillion (ppt) for PFOA (Table S1).

Human liver toxicity as the basis for the noncancer HPC for PFOA: Several epidemiologic studies provide sufficient data to derive the noncancer dose-response benchmarks for immunotoxicity, liver toxicity and increased cholesterol health effects. The proposed noncancer HPC for PFOA is based on an increased risk of liver toxicity, as indicated by elevated alanine aminotransferase (ALT) levels (Gallo et al., 2012) exceeding clinically based reference levels used by the International Federation of Clinical Chemistry and Laboratory Medicine. The study involved residents of the Mid-Ohio Valley who lived near a chemical plant known to have emitted PFOA into the surrounding environment, and whose ALT increased with increasing serum levels of PFOA. This is the basis of an acceptable daily dose of 0.87 ng/kg-day and a proposed noncancer HPC of 3 ppt for PFOA (Table S1).

PFOS Health Effects Basis for PHG and HPC

PFOS PHG based on cancer endpoint: Although there are a few epidemiologic studies that show some association of PFOS with breast, liver, and bladder cancer, the results are mixed or the sample sizes are small. OEHHA did not identify any epidemiologic studies of PFOS that could be used for quantifying cancer risk in humans. Thus, the proposed PHG for PFOS is based on cancer data in laboratory animals. The cancer slope factor of 15.6 per mg/kg-day, derived from liver and pancreatic tumors in male rats exposed through the diet for two years (Butenhoff et al., 2012b), is used to derive the proposed PHG of 1 ppt for PFOS (Table S1).

Increased cholesterol in humans as the basis for the noncancer HPC for PFOS: Sensitive noncancer endpoints for PFOS are immunotoxicity and alterations in lipid metabolism. Total cholesterol appeared to be a somewhat more sensitive endpoint, with the large study by

Steenland et al. (2009) of residents near the West Virginia facility who drank contaminated water and had elevated blood serum levels of PFOS. Based on the relationship in this study between PFOS and serum lipids (Steenland et al., 2009), a point of departure for PFOS was derived for an increased risk of elevated total cholesterol above the clinical reference level published by the American Heart Association. This is the basis of an acceptable daily dose of 0.64 ng/kg-day and a proposed noncancer HPC of 2 ppt for PFOS (Table S1).

Evaluation of Exposure Parameters

Calculation of the PHGs entails making assumptions about the amount of water people consume and, in the calculation of the HPCs for noncancer effects, an additional assumption regarding the relative source contribution, that is, the proportion of exposures to a chemical attributed to tap water as part of total exposure from all sources, including food.

Drinking water intake: OEHHA used age-specific water ingestion estimates derived from a nationwide survey of food and beverage intake from approximately 20,000 individuals (see OEHHA (2012)). These age-specific intake rates are normalized to body weight. This enables addressing the higher rate of ingested water (per unit body weight) of the young. In considering levels of water intake to assume for the PFOA and PFOS assessment, OEHHA reviewed the literature that could inform drinking water trends and intake in California and performed a detailed evaluation of US EPA's recently updated chapter on water consumption in its *Exposure Factors Handbook* (US EPA, 2019) that used nationwide survey data from 2005-2010.

OEHHA found that US EPA's updated drinking water intake rates may not be representative of California's residents due to a number of factors such as greater numbers of jobs performed in outdoor settings (e.g., farm work and construction) for longer periods throughout the year, and lifestyle and recreation, which all require proper hydration, particularly in California's hot summer climate. Thus, OEHHA retained the peer reviewed drinking water intake rates that it developed in 2012. A lifetime-weighted average drinking water intake rate of 0.053 L/kg-day was used in deriving the PHGs and HPCs.

Relative source contribution for PFOA and PFOS: OEHHA applied US EPA's Exposure Decision Tree Approach (US EPA, 2000) and found a lack of information about sources of exposure to support a value other than the default value of 20%. This determination is consistent with other regulatory bodies, including US EPA, Health Canada, and the State of New Jersey.

Conclusion

PFOA and PFOS are extremely persistent in the environment and are present in virtually all human blood serum samples. New analyses in this assessment have improved the estimates of clearance rates for PFOA and PFOS. Clearance in humans is much slower than in laboratory animals, with half-lives of several years in humans versus several days in animals. There is mounting evidence that environmental levels of PFOA and PFOS can adversely affect human health, and studies in laboratory animals support the effects observed in humans exposed to these chemicals through the environment. Large, well-conducted epidemiologic studies form the basis for the proposed PHG for PFOA and HPCs for PFOA and PFOS, while a well-conducted study in laboratory animals is the basis for the proposed PHG for PFOS.

1. INTRODUCTION

Perfluorooctanoic acid (PFOA) and perfluorooctane sulfonic acid (or perfluorooctane sulfonate, PFOS) are organic acids that consist of a chain of eight carbons, where all the hydrogens on the carbon chain have been replaced with fluorines. The two chemicals differ in that PFOA has a carboxylic acid at one end of the carbon chain, whereas PFOS has a sulfonic acid group. Both chemicals belong to a large class of chemicals called per- and polyfluoroalkyl substances (PFAS), of which PFOA and PFOS are the best studied.

The strong carbon-fluorine bonds of these compounds make them resistant to biological and environmental degradation. As such, PFAS confer stain, grease, heat, and water resistant properties to consumer products when they are applied, which has made the commercial use of these chemicals desirable. Historically, PFAS have been used in myriad products, including fabrics, carpets, cookware, cleaning products, and aqueous fire-fighting foam.

Due to their resistance to biological and environmental degradation, most PFAS have long half-lives in humans and the environment. Coupled with many industrial uses, the environmental persistence of PFAS has resulted in widespread exposure. Even though PFOA and PFOS were voluntarily phased out in the United States (PFOS in 2002 and PFOA by 2015), exposure to these chemicals still occurs due to their environmental persistence, their use in imported goods, and the use of products manufactured before the phase-out. Additionally, PFOA and PFOS can result from the breakdown of other PFAS. PFOA and PFOS have been detected in California drinking water, despite the absence of manufacturing of these compounds in California. Furthermore, Biomonitoring California reports that PFOA and PFOS were found in the serum of >99% and >97%, respectively, of participants across all study cohorts. This indicates that exposure to these chemicals is widespread in California.

Numerous adverse health effects in humans and laboratory animals have been associated with exposure to PFOA and PFOS, including liver toxicity, immunotoxicity, thyroid toxicity, reproductive/developmental toxicity, effects on lipids and cholesterol, and cancer. In 2019, OEHHA derived cancer reference levels (RLs) for PFOA and PFOS in drinking water (OEHHA, 2019).

- 0.1 ng/L (nanograms/liter) or parts per trillion (ppt) for PFOA, based on pancreatic and liver tumors in male rats;
- 0.4 ng/L (or ppt) for PFOS, based on liver tumors in male rats.

OEHHA also developed RLs for noncancer effects as follows:

- 2 ng/L (or ppt) for PFOA, based on liver toxicity in female mice;
- 7 ng/L (or ppt) for PFOS, based on immunotoxicity in male mice.

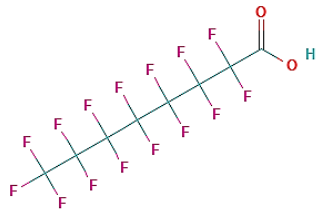
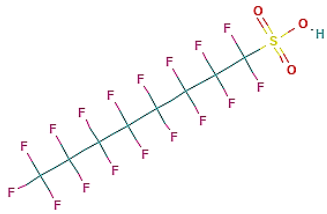
OEHHA reviewed previous evaluations by US EPA (2016a, 2016b, 2016c, 2016d), New Jersey Drinking Water Quality Institute (2017, 2018), and ATSDR (2018a) and concluded those evaluations comprehensively and effectively identified the published literature prior to their respective dates of publication. Thus, in deriving Public Health Goals (PHGs) for PFOA and PFOS, OEHHA performed a systematic literature search and thoroughly evaluated human, animal, and mechanistic toxicity studies published from 2016 onward. Additionally, OEHHA conducted rigorous evaluations of toxicokinetics, drinking water intake rates, and the relative

source contribution of drinking water, and used its own health risk assessment methodology when deriving health-protective concentrations in this document.

1.1. Physical and Chemical Properties

The physical and chemical properties for PFOA and PFOS can be found in Table 1.1.1. The structures of PFOA and PFOS can be linear or branched and formation of these structures is dependent on the manufacturing process. Both chemicals are solid at room temperature and have low vapor pressure. PFOA and PFOS are stable and are resistant to environmental degradation. In solution, PFOA exists as the perfluorooctanoate anion and is a strong acid. PFOS also exists as an anion in solution; however, pKa values can only be estimated.

Table 1.1.1. Physical and Chemical Properties of PFOA and PFOS

	Perfluorooctanoic acid (PFOA)	**Perfluorooctane sulfonic acid (PFOS)**
Formula	$C_8HF_{15}O_2$	$C_8HF_{17}O_3S$
CAS No.	335-67-1	1763-23-1
Structure		
Molecular weight (g/mole)	414.09	500.13
Physical state at ambient temperature	White powder	White powder
Melting point (°C)	54.3 (HSDB, 2020a) 55 (US EPA, 2020)	≥400 (potassium salt) (EFSA, 2008)
Boiling point (°C)	192 (HSDB, 2020a) 189 (US EPA, 2020)	249 (HSDB, 2020b) 258–260 (US EPA, 2016b; US EPA, 2016d)
Density (g/cm^3)	1.792 (20 °C) (HSDB, 2020a)	No data
Solubility in water (mg/L)	3,300 (25 °C) (HSDB, 2020a)	680 (OECD, 2002) 570 (potassium salt in pure water) (ATSDR, 2021) 519 (20 ± 0,5°C) (EFSA, 2008) 680 (24 - 25°C) (EFSA, 2008)
Vapor pressure (mm Hg)	0.525 at 25 °C (measured) (Hekster et al., 2003) 0.962 at 59.25 °C (measured) (Kaiser et al., 2005)	2.0×10^{-3} at 25 °C estimated US EPA EPI Suite) (HSDB, 2020b)

	Perfluorooctanoic acid (PFOA)	Perfluorooctane sulfonic acid (PFOS)
Henry's Law constant (atm. m^3/mol)	9.08×10^{-2} (25 °C, estimated US EPA EPI Suite) (HSDB, 2020a)	4.1×10^{-4} (25 °C, estimated US EPA EPI Suite) (HSDB, 2020b) 3.05×10^{-9} atm. m^3/mol pure water (potassium salt) (EFSA, 2008)
Log K_{ow}	4.81 (estimated US EPA EPI Suite) (HSDB, 2020a) Ammonium perfluorooctanoate (APFO): 0.7 (EFSA, 2008)	4.49 (estimated US EPA EPI Suite) (HSDB, 2020b)
Acidity, pKa	-0.5-4.2 (HSDB, 2020a) 2.8 (US EPA, 2016b) 2.5, 2 to 3 (Prevedouros et al., 2006)	<1.0 (estimated) (HSDB, 2020b) -3.3 (calculated value for acid) (Brooke et al., 2004)

2. METHODOLOGY

Development of a PHG for a chemical in drinking water entails a four-part process.

2.1. Systematic Literature Search and Toxicological Evaluation

The toxicological evaluation of a chemical starts with a review of the available literature. A systematic literature search is conducted, using a comprehensive search string and multiple scientific databases (Pubmed, Embase, Scopus, etc.). Details on the literature search are provided in Appendix 1. Briefly, a PECO (population, exposure, comparator, outcome) statement that outlines inclusion/exclusion criteria is developed for initial reference screening. The title and abstract of each reference are screened by a minimum of two reviewers, and each reference is included or excluded based on criteria outlined in the PECO statement. A separate search was conducted specifically for human epidemiology studies as outlined in Appendix 7. Subsequently, a full-text review of included references is conducted to ensure that the studies are relevant for PHG development.

2.2. Study Evaluation

2.2.1. Animal Studies

The findings from studies that meet the PECO criteria are critically evaluated and the quality of each study is assessed. OEHHA's criteria for study quality evaluation include the following:

- appropriate number of animals per dose group
- an untreated control group plus a minimum of two dose groups
- appropriate exposure duration
- relevant route of exposure
- appropriate test species
- appropriate statistical analysis
- biological significance of endpoints
- adequate reporting.

2.2.2. Human Studies

For epidemiology studies, several factors were evaluated when assessing study quality, including study design, factors related to bias, exposure and outcome methods, and confounding. Specific details for OEHHA's study quality evaluation for human epidemiology studies are provided in Chapters 5 and 6, and in Appendix 7.

2.3. Evaluation of Health Hazards

For each major toxicity endpoint (e.g. hepatotoxicity, immunotoxicity, reproductive toxicity, cancer, etc.), relevant studies are reviewed to identify the health hazards induced by chemical exposure. This review considers factors such as consistency across multiple studies, exposure-reponse relationships, biological plausibility, and relevance to human health. Furthermore, consideration is given to the potential molecular and cellular mechanisms by which toxicity is induced (modes of action).

2.4. PHG Derivation

After a review of the toxicity studies of suitable quality and identification of relevant hazards, the most sensitive endpoints from studies determined to be relevant to human health are selected, and analyses of the dose-response relationships are performed. The adverse effect or a physiological change that leads to an adverse effect that occurs at the lowest dose is selected as the critical effect from which a PHG is derived.

If a chemical has been identified as a human or animal carcinogen, health-protective water concentrations are determined for both cancer and noncancer effects and the lowest value is selected as the PHG.

2.4.1. Deriving Health-Protective Concentrations for Noncancer Effects

Calculation of a health-protective concentration for noncancer effects involves a four-step approach: determination of the point of departure (POD), estimation of an acceptable daily dose (ADD), determination of the relative source contribution (RSC) and calculation of a health-protective drinking water concentration (C).

Point of Departure (POD)

The POD is the dose of a chemical (in units of milligrams per kilogram of body weight per day, mg/kg-day) from a study in animals or humans that is used as a starting point for calculation of the ADD. The POD is typically determined by fitting a mathemathical model to the dose-response data. OEHHA generally uses the publicly available Benchmark Dose Software (BMDS) program developed and maintained by the US Environmental Protection Agency (US EPA; https://www.epa.gov/bmds). BMDS fits mathematical models to the data and determines the dose (benchmark dose or BMD) that corresponds to a pre-determined level of response (benchmark response or BMR). The BMR is typically set at 5% above the background or the response of the control group for dichotomous data. For continuous data, a BMR of one standard deviation from the control mean is typically used when there are no data to indicate what level of response is biologically significant (US EPA, 2012). In order to account for the uncertainty of the data, the model also calculates the 95% lower confidence limit of the BMD, called the BMDL (L stands for the lower confidence limit). For PHG development, OEHHA uses the BMDL as the POD for the calculation of a health-protective drinking water concentration when the data are amenable to BMD modeling. When data are not amenable to BMD modeling, OEHHA uses the no-observed-adverse-effect level or concentration (NOAEL or NOAEC), or lowest-observed-adverse-effect level or concentration (LOAEL or LOAEC) approach in identifying the POD.

Application of BMD modeling for noncancer effects mitigates some of the limitations of the NOAEL/LOAEL approach, including:

- dependence on dose selection and sample size;
- inability to account for uncertainty and variability of experimental results due to the characteristics of the study design;
- the need to use an uncertainty factor when a NOAEL cannot be determined in a study; and
- inability to account for the shape of the dose-response curve.

Acceptable Daily Dose (ADD)

The ADD is the estimated maximum average daily dose of a chemical (in mg/kg-day) that can be consumed by a human for an entire lifetime without adverse effects. This is similar to the term "reference dose" used by US EPA. To determine the ADD, the POD is adjusted by factors that account for uncertainties and variabilities in the risk assessment, such as differences between animals and humans, and differences among humans in response to a chemical exposure. These factors are combined into a composite uncertainty factor (UF).

Uncertainty and Variability Factors (UF)

When developing health-protective levels for noncancer effects based on animal toxicity studies, OEHHA generally applies a combined UF of 300 (OEHHA, 2008).

These UFs are:

- 10 for interspecies extrapolation, accounting for possible differences in the way laboratory animals and humans respond to the chemical, consisting of
  - $\sqrt{10}$ for toxicokinetics
  - $\sqrt{10}$ for toxicodynamics
- 30 for intraspecies variability, which accounts for some human subpopulations, such as children and the elderly, possibly being more sensitive to the chemical than the general population, consisting of
  - $\sqrt{10}$ for toxicodynamics
  - 10 for toxicokinetics.

These default factors are applied unless data support an alternative value. A table of default UFs for ADD derivation is presented in Appendix 2. Additional adjustments may be included depending on the limitations of available data.

The ADD is calculated using the following equation:

$$\mathrm{ADD} = \mathrm{POD} \div \mathrm{UF}.$$

Relative Source Contribution (RSC)

The RSC is the proportion of exposures to a chemical attributed to tap water, as part of total exposure from all sources (including food and air). The RSC values typically range from 20% to 80% (expressed as 0.20 to 0.80), and are determined based on available environmental monitoring data. For certain PHGs, the RSC can be as high as 1.0 (tap water is the only source of the chemical) when it is deemed appropriate. OEHHA uses this approach to ensure that the PHG identifies a level of a drinking water contaminant that would pose no significant health risk after taking into account exposures to all other sources (see Appendix 4 for details).

Daily Water Intake Equivalent (DWI)

To calculate a PHG for a chemical, the ADD is converted to a concentration in drinking water that accounts for the total exposure to the chemical that people receive from using tap water. It includes intake from ingestion as well as inhalation and dermal contact with the chemical in tap water from household uses (e.g., drinking, cooking, bathing, and showering). Inhalation exposure can take place when the chemical volatilizes out of the water during cooking or showering. Dermal absorption of the chemical can occur during bathing and other household uses of tap water.

DWI is expressed in units of liters or liter equivalents per kilogram of body weight per day (L/kg-day or L_{eq}/kg-day, respectively). Liter equivalents represent the equivalent of the amount of tap water one would have to drink to account for the exposure to a chemical in tap water through oral, inhalation, and dermal routes. However, due to the physicochemical properties of PFOA and PFOS, inhalation and dermal exposure through household uses of tap water are expected to be negligible.

For oral intake rates, the PHG program uses age-specific water ingestion estimates (OEHHA, 2012) derived from a nationwide survey of food and beverage intake from approximately 20,000 people (US Department of Agriculture's Continuing Survey of Food Intake of Individuals 1994-1996, 1998 dataset). These age-specific intake rates, normalized to body weight and expressed as L/kg-day, indicate that drinking water ingestion per unit body weight is higher in infants than in adults. Previous PHGs using ingestion rates of 2 L/day for adults and 1 L/day for a 10 kg child are being updated with these more refined estimates. While US EPA has recently updated drinking water ingestion rates in their Exposure Factors Handbook (US EPA, 2019), OEHHA has not adopted these new ingestion rates for reasons described in Appendix 3.

Derivation of the Health-Protective Concentration (C)

Following the determination of the ADD, the health-protective concentration (C, in milligrams per liter, mg/L or in micrograms per liter, µg/L) in drinking water can be derived by incorporating the DWI and RSC of the chemical:

$$\mathrm{C} = (\mathrm{ADD} \times \mathrm{RSC}) \div \mathrm{DWI}.$$

2.4.2. Deriving Health-Protective Concentrations for Cancer Effects

Calculation of a health-protective concentration for cancer effects involves a three-step approach: determination of a POD from which a cancer potency can be determined, estimation of an average daily dose, and calculation of a health-protective drinking water concentration (C).

Cancer Dose-Response Analyses and Cancer Potency Derivation

Standard methods for estimation of lifetime theoretical cancer risks are employed in the development of cancer potencies based on animal studies (US EPA, 2005; OEHHA, 2009; US EPA, 2012). The estimated cancer potency, also referred to as the cancer slope factor (CSF), is a measure of the carcinogenic potential of a compound. It is often reported in units of 1/(mg/kg-day) or $(\mathrm{mg/kg\text{-}day})^{-1}$ and is derived by fitting a linear low-dose extrapolation using US EPA's BMDS Multistage-Cancer model (US EPA, 2012) to the tumor incidence data from an animal carcinogenicity bioassay.

Method for Calculating Cancer Potency

Development of cancer potency estimates from animal bioassays includes consideration of:

- the quality, suitability, and sensitivity of the available animal bioassay studies; for example, the thoroughness of experimental protocol, the temporal exposure pattern, the degree to which dosing resembles the expected manner of human exposure, the duration of the study, the purity of test material, the number and size of exposed groups, and the extent of tumor occurrence
- the cancer sites and types from the selected experiments most appropriate for characterizing the cancer potency; where there are multiple sites with significant tumor findings in a selected experiment, a multisite analysis is performed to describe the overall carcinogenic potential
- whether a dose-response model that assumes the absence of a carcinogenic threshold dose should be used or whether there are compelling mechanistic data to support an alternative approach
- interspecies scaling of animal cancer potency to human cancer potency
- physiologic, TK and metabolic information for possible use in extrapolating from test animals to humans, from high to low dose, and from one exposure route to another.

Calculating Average Daily Dose

A mathematical model is fit to dose-response data from animal studies. For studies that do not involve daily administration of a fixed mg/kg body weight amount, an average daily dose (in units of mg/kg-day) is calculated. This is done by adjusting the administered or nominal dose, accounting for days of dosing during the week and total dosing weeks during the experimental period. For studies using variable doses, the weighted mean dose is calculated considering the dosing frequency and duration of the various administered doses.

Dose-Response Model

Information on the mode of action (MOA) involved in the carcinogenesis of a chemical is evaluated to determine whether human cancer risk should be estimated using the default assumption of low dose linearity or otherwise. Unless there is sufficiently compelling evidence, OEHHA uses a non-threshold approach and a linearized multistage (LMS) cancer model to calculate the chemical's cancer potency, expressed as the CSF. This is accomplished by using the BMDS Multistage-Cancer model developed by US EPA (BMDS version 2.7). The model calculates the lifetime probability of developing a tumor (p) induced by an average daily dose (d) using the following equation:

$$p(d) = \beta + (1- \beta) \times \exp[-(q_1 d + q_2 d^2 + ... + q_i d^i)].$$

The q_i are parameters of the model, which are taken to be constants and are estimated from the animal cancer bioassay data. As recommended by US EPA (2012), $q_i \geq 0$ for all i. For example, with four dose groups, the Multistage-Cancer model can have a maximum of four parameters, β, q_1, q_2, and q_3. When dose is expressed in units of mg/kg-day, q_1 is given in units of $(\text{mg/kg-day})^{-1}$. The q_1 parameter is, for small doses, the ratio of excess lifetime cancer risk to the average daily dose received. The parameter β provides the basis for estimating the

background lifetime probability of the tumor (i.e., when dose d is zero, the probability of cancer, p, is equal to β).

The Multistage-Cancer model defines the probability of developing a tumor at a single site. For carcinogens that induce tumors at multiple sites and/or in different cell types at the same site in a particular species and sex, US EPA's BMDS can be used to derive maximum likelihood estimates (MLEs) for the parameters of the multisite carcinogenicity model by summing the MLEs for the individual multistage models from the different sites and/or cell types. This multisite model provides a basis for estimating the cancer potency of a chemical that causes tumors at multiple sites.

Adjusting for Human-Animal Differences

In the absence of reliable TK information, the human cancer slope factor (CSF_{human}) is estimated by assuming the chemical dose per body weight scaled to the three-quarters power produces the same degree of effect in different species. Under this assumption, the CSF_{animal} is multiplied by the ratio of human to animal body weights raised to the one-fourth power when animal cancer potency is expressed in units of $(mg/kg\text{-}day)^{-1}$:

$$CSF_{(human)} = CSF_{(animal)} \times (\text{body weight}_{(human)} \div \text{body weight}_{(animal)})^{1/4}$$

When data are available, separate oral and inhalation cancer potencies may be calculated and they are applied to each specific exposure route. Since it is unusual to have a cancer bioassay through dermal exposure, OEHHA generally uses the oral cancer potency for estimating cancer risk through the dermal route. Similarly, when an inhalation cancer potency is not available, the oral cancer potency is used to estimate cancer risk through the inhalation route. If only an inhalation cancer potency is available, then it will be applied to all routes when determining the PHG.

Accounting for Increased Susceptibility During Early-in-Life Exposures

When determining cancer risk, OEHHA applies age sensitivity factors (ASFs, unitless) to account for the increased susceptibility of infants and children to carcinogens (OEHHA, 2009). A weighting factor of 10 is applied for exposures that occur from the 3rd trimester to <2 years of age, and a factor of 3 is applied for exposures that occur from 2 through 15 years of age. However, analysis of recent PFOA bioassay data from the National Toxicology Program (NTP, 2020) indicates that perinatal exposure (from gestation day (GD) 6 through postnatal day (PND) 21) does not increase cancer risk, compared to later-in-life exposures, in rats (see Section 5.7.2 for details). Thus, ASFs are not applied in the calculation of cancer based health-protective concentrations for PFOA and, due to structural and toxicological profile similarities, for PFOS as well.

Derivation of the Health-Protective Concentration (C)

The health-protective water concentration (C) for carcinogenic effects can be calculated using the following equation:

$$C = R \div (CSF \times DWI)$$

where,

R = default risk level of one in one million, or 10^{-6}

CSF = cancer slope factor, in $(mg/kg\text{-}day)^{-1}$

DWI = lifetime average daily water intake rate.

Water consumption rates are described in the noncancer methodology section, and the underlying principles do not change when examining cancer endpoints.

3. PRODUCTION, USE, AND ENVIRONMENTAL OCCURRENCE

3.1. Production and Use

PFOA and PFOS are produced by two main methods: by electrochemical fluorination (ECF) or by telomerization (US EPA, 2016b; US EPA, 2016d). Historically, the ECF method has been the main source for PFOA and PFOS production (Prevedouros et al., 2006; Paul et al., 2009). In addition to the linear form of PFOA or PFOS, 20-30% of the PFOA or PFOS produced by the ECF method are branched isomers (Paul et al., 2009; Beesoon et al., 2011). In contrast, primarily linear isomers of PFOA and PFOS are produced in the telomerization process, and the relative fraction of the linear isomer in the analyzed sample can be used to trace the manufacturing origin of the compound mix in the environment or human serum (US EPA, 2016b; US EPA, 2016d).

The main use of PFOA has been as a processing aid in the manufacturing of fluoropolymers such as polytetrafluoroethylene (PTFE) and polyvinylidene fluoride (Prevedouros et al., 2006). PTFE was widely used as the functional component of non-stick pans, but partly as a result of the US EPA PFOA Stewardship Program[3] and Canada's PFAS Environmental Performance Agreement,[4] this and other uses of PFOA have been steadily declining in North America (Vierke et al., 2012). As the processing aid, PFOA acts to solubilize fluoromonomers to facilitate polymerization (Prevedouros et al., 2006). PFOA was also added to aqueous polymer dispersions used for paints, photographic film additives and in the textile finishing industry (OECD, 2006; Vierke et al., 2012). Additionally, PFOA and other polyfluorinated carboxylic acids (PFCAs) are indicated in patents of various consumer products, including floor polishes, cleaning formulations, hair care products, medical inhalers, fuel additives, air fresheners and textile treatments (references summarized in Prevedouros et al. (2006)). In one case study, high levels of PFOA and PFOS in the serum samples of one Canadian family were attributed to repeated carpet treatments in their house (Beesoon et al., 2012). PFOA was used as a component of aqueous fire-fighting foams from approximately 1965-1975, and this use has contributed to environmental contamination near related facilities such as airports and military bases (Prevedouros et al., 2006; Ahrens et al., 2015; Darlington et al., 2018).

PFOS has been used in the production of firefighting foams, semiconductors, hydraulic fluids and photolithography (D'Eon and Mabury, 2011b). The major consumer product-related uses for PFOS include water-repellant treatment for clothes, stain and dirt-resistant treatment for carpets, and oil and grease-repellant treatments for paper and packaging (Paul et al., 2009). PFOS-containing fire-fighting foams continue to be held in stock (OECD, 2006). Specific industrial uses of PFOS and its salts included as a mist-suppressing agent (i.e., fume suppressant) in the mining industry, as an antireflective agent in the photographic industry, as photo-resistant and antireflective coatings, as an anti-erosion additive in the aviation industry, and as a surfactant (fume suppressant) in the metal plating industry (OECD, 2006). PFOS is found as an impurity in food packaging (Bagley et al., 2017). Both PFOA and PFOS appear to be used in certain pesticide products (OECD, 2006; Liu et al., 2017c).

[3] https://www.epa.gov/assessing-and-managing-chemicals-under-tsca/fact-sheet-20102015-pfoa-stewardship-program
[4] https://www.canada.ca/en/environment-climate-change/services/environmental-performance-agreements/list/perfluorocarboxylic-acids-overview.html

PFOA and PFOS can accumulate in the environment as impurities or degradation products of other PFAS (Prevedouros et al., 2006; Paul et al., 2009).

3.2. Environmental Occurrence and Human Exposure

PFOA and PFOS are manmade compounds and are not known to occur naturally. The historical worldwide production of PFOA was estimated at a total of 3,600-5,700 tons from 1951-2004, and the estimated direct global environmental emissions were 400-700 tons over the same period (Prevedouros et al., 2006). One of the major PFOA production sites in the US was the DuPont Washington Works plant, located in West Virginia near the Ohio border. The historical worldwide production of PFOS was estimated at 96,000 tons from 1970-2002, and estimated total global emissions were 6,800-45,250 tons during that time period (Paul et al., 2009). Other reports estimated lower PFOS production and emissions (Armitage et al., 2009; Wang et al., 2017b). The main PFOS manufacturing site in the US was at the 3M Company in Decatur, Alabama (Wang et al., 2017b). PFOA and PFOS are resistant to degradation in the environment (Parsons et al., 2008; Sáez et al., 2008). While manufacturing and direct use of PFOA and PFOS have declined in Western countries, these compounds have continuing presence in the environment. China continues production of PFOA and PFOS, and yearly environmental release from all sources was estimated at 40 tons of PFOA and 70 tons of PFOS (Liu et al., 2017c).

The presence of PFOA and PFOS in groundwater and surface water is of major concern. These chemicals can reach groundwater and surface waters via direct emissions of liquid waste and leaching of contaminated sites, such as landfills and airfields. In addition, due to their unique physicochemical properties, PFOA and PFOS can also reach groundwater from air emissions followed by deposition from air to soil with precipitation and migration through soil to groundwater (Davis et al., 2007). Both DuPont Washington Works and 3M Decatur plants caused PFOA and PFOS contamination of the drinking water in the nearby communities.

Air

Due to their ionic nature, PFOA and PFOS have low volatility (US EPA, 2014). Ionic PFAS such as PFOA and PFOS have low vapor pressures and predominantly bind to particles (Abbott et al., 2007; Haug et al., 2011b; Vierke et al., 2011). When directly introduced into the atmosphere, particle-bound PFAS settle to the ground following rain events (Barton et al., 2007; Davis et al., 2007). When dissolved in water, PFOA and PFOS tend to concentrate at the air-water interface, and for this reason, aerosols formed with the action of ocean waves have much higher PFOA levels than in the water (McMurdo et al., 2008). Aerosol transport by air currents likely contributes to the long-range transport of PFAS, which may explain the presence of these chemicals in the organisms in the Arctic (Martin et al., 2004a; Smithwick et al., 2006). PFOS levels remained constant in the particle phase of the air in the Arctic in 2006-2012 (Hung et al., 2016).

Commonly, PFAS air studies focused on precursors of PFOA and PFOS (Dreyer et al., 2009; Cai et al., 2012a; Del Vento et al., 2012; Wang et al., 2015b). Precursor compounds FTOHs (fluorotelomer alcohols) and FOSEs (perfluorooctane sulfonamide ethanols) play an important role in the mechanisms of air transport of PFOA and PFOS. These substances are used as grease-proofing agents in various consumer products such as carpets and food packaging and, due to their volatility, are often present at higher concentrations in the air than PFOA and PFOS. Once in an organism, FTOHs and FOSEs can break down to PFOA and PFOS, and FTOHs in

indoor office air significantly predicted PFOA serum concentrations in office workers (Fraser et al., 2012). Precursors can also degrade to PFOA and PFOS in the environment due to microbial action (Lee et al., 2010; Liu and Mejia Avendaño, 2013). Due to their neutral nature, these precursor compounds are much more efficiently transported into the air from wastewater and landfills (Ahrens et al., 2011; Vierke et al., 2011), and could also contribute to long-range transport of PFOA and PFOS (Jahnke et al., 2007a; Schenker et al., 2008).

PFAS air concentrations are generally higher in urban areas compared to rural areas. PFOA and PFOS concentrations in the gas fractions of outside air in an urban area (Albany, New York) averaged 2.86 (range, 1.89-6.53) picograms per cubic meter (pg/m^3) and 1.42 (0.94-3) pg/m^3, respectively (Kim and Kannan, 2007). For the particle fraction of the outside air, mean PFOA and PFOS concentrations were reported at 1.57 (range, 0.76-4.19) pg/m^3 and 0.66 (range, 0.35-1.16) pg/m^3, respectively, in samples collected in 2006 (Kim and Kannan, 2007). In two urban areas in Japan during 2000-2001, PFOS outdoor air concentrations were 0.6-5.3 pg/m^3 (Sasaki et al., 2003). In a different study of urban areas in Japan, with samples collected from 2001-2003, PFOA air concentrations were 2.0-263 pg/m^3 and PFOS concentrations were 0.7-5.2 pg/m^3 (Harada et al., 2005b). The exposure due to the high PFOA air levels (geometric mean of 263 pg/m^3) observed in the Kyoto area were comparable with the estimated exposure from food intake in the area. In rural areas of northwestern Europe, PFOA and PFOS concentrations in the outdoor air were 6.4 and 0.34 pg/mg^3, respectively (combined means from available data), and for urban areas, these values were 232 and 33 pg/m^3, respectively (Barber et al., 2007; Egeghy and Lorber, 2011). In an urban area in Germany (Hamburg), PFOA and PFOS in the outdoor air were present at <0.2-2.6 pg/m^3 and 0.4-1.6 pg/m^3, respectively (Jahnke et al., 2007b).

Reports on PFOA and PFOS in indoor air are scarce. Based on the observation of twenty-fold higher indoor levels of EtFOSA (ethylperfluorooctanesulfonamide) and MeFOSEA (methylperfluorooctanesulfoamidethylacrylate) compared to outdoor levels (Shoeib et al., 2005), some exposure models assumed that the indoor concentrations of PFOA and PFOS would also be twenty-fold higher than their respective outdoor levels, pegging the estimates at about 30-50 ng/m^3 (Egeghy and Lorber, 2011; Lorber and Egeghy, 2011). Mean PFOA levels in the particulate phase of indoor air in Tromso, Norway was 4.4 ng/m^3 (Barber et al., 2007). In a case study of a family with very high PFAS exposure due to repeated carpet treatments, PFOA and PFOS indoor air concentrations in the dust samples in a residence were as high as 150 and 1,100 ng/g respectively (Beesoon et al., 2012).

Soil

PFOA and PFOS can bind effectively to different types of soil and sediment, and the organic content in the solid phase appears to be the main determinant of binding (Higgins and Luthy, 2006). High organic content in sewage sludge results in absorption of PFOA and PFOS from wastewater (Zareitalabad et al., 2013), with negative implications for downstream sludge uses, such as for fertilizer in agricultural settings. Based on a review of available studies for PFOA and PFOS, Zareitalabad et al. (2013) reported average soil-adsorption coefficients ($\log K_{OC}$) of 2.1 log(l/kg) and 3.0 log(l/kg), respectively. In the FAO (Food and Agriculture Organization of the United Nations) soil mobility classification scheme, these correspond to the moderately mobile class (US EPA, 2009).

Strynar et al. (2012) analyzed sixty 'background' soil samples (i.e., without known PFAS contamination sources) from around the world and noted that the US soils had generally higher

PFAS background levels than some of the other countries. The authors hypothesized, that this would correlate with the relatively higher PFAS serum levels in US residents (Strynar et al., 2012). Among the reported results, PFOA was present at 1.56-31.7 ng/g in soil samples from North Carolina, Kentucky, Texas and Indiana, and PFOS was present at 0.606-2.55 ng/g. The levels of PFOA and PFOS in the agricultural soil in the Chicago area were greatly increased with the addition of bio-solids, with a maximum detected PFOS level of 483 ng/g (Sepulvado et al., 2011). The concentration of certain precursors, such as MeFOSAA (2-N-methylperfluorooctane sulfonamidoacetic acid) was high in the applied sludge but not in the augmented soil, indicating likely conversion to PFOS upon application (Sepulvado et al., 2011).

Global soil analysis of samples from each continent revealed PFAS accumulation in the Northern hemisphere (Rankin et al., 2016). The highest PFOS concentrations were detected in Europe, and the highest PFOA level occurred in one sample in Asia. Among the North American soil samples, three were from California, which contained 11-101 pg/g PFOA and 3.2-14.2 pg/g PFOS (Rankin et al., 2016). The soil level data of Rankin et al. (2016) were modeled to predict the global soil load of PFOA and PFOS (Washington et al., 2019), which was about 1,000 metric tons for either compound.

Indoor dust contained very high levels of PFOA and PFOS, at 100-500 ng/g for samples from the UK, Canada, France, Germany, Japan and US, while homes from Sweden, Norway, Thailand and Kazakhstan demonstrated much lower levels (Kubwabo et al., 2005; Kato et al., 2009; Goosey and Harrad, 2011; Haug et al., 2011b). Levels were also high in the dust from cars, classrooms and offices in the UK (Goosey and Harrad, 2011). High levels were found in the dust from the daycare centers in the US (mean values, 142 and 201 ng/g for PFOA and PFOS, respectively) and Sweden (~40 ng/g for either) (Strynar and Lindstrom, 2008; Björklund et al., 2009). Harrad et al. (2010) reviewed available data and suggested that carpeting and use of floor wax could be contributing to the presence of PFAS in the house dust.

Water

PFOA and PFOS occur in groundwater and surface waters, and in public drinking water in the US and around the world. PFOA and PFOS were included in US EPA's Third Unregulated Contaminant Monitoring Rule (UCMR3) which extensively analyzed sources of drinking water across the US from 2013-2015, with method reporting limits (detection limit) for PFOA and PFOS at 20 and 40 ng/L, respectively (US EPA, 2017). Out of 4,920 public water systems (PWS) analyzed, PFOA was detected in 117 (2.4%) and PFOS in 95 (1.9%). PFOA and PFOS were more frequently detected in the groundwater than surface water and were strongly correlated with the presence of major industrial sites (Hu et al., 2016). Although having tested widely across the US, the low detection rates under UCMR3 could be due to the relatively high detection limits of 20 ng/L for PFOA and 40 ng/L for PFOS. Several UCMR3-tested areas in California had 20-70 ng/L PFOA and/or 40-200 ng/L PFOS in drinking water (Hu et al., 2016).

In the subset of UCMR3 results for California,[5] 0.83% of water samples contained PFOA or PFOS at the level of or greater than the detection limit. The average PFOA concentration among these samples was 28 ng/L, and the average PFOS concentration was 57 ng/L.

[5] https://www.waterboards.ca.gov/drinking_water/certlic/drinkingwater/PFOA_PFOS, accessed September 2020

The results of the more recent drinking water monitoring program carried out by SWRCB are summarized in Table 3.2.1.[6] This program comprised four rounds of testing in 2019-2020. Due to lower detection limits, the detection rate was higher compared to the UCMR3 dataset, and the average values are lower. The program utilized US EPA Method 537.1, with lowest concentration minimum reporting levels (LCMRLs) of 0.82 ng/L for PFOA and 2.7 ng/L for PFOS (Shoemaker and Tettenhorst, 2018).

Table 3.2.1. PFOA/PFOS public water system testing results in California (2019-2020)

Dates	PFAS	Samples (N)	% detected	Arithmetic mean including non-detects (ng/L)	Arithmetic mean excluding non-detects (ng/L)	Geometric mean excluding non-detects (ng/L)
04/01/19-06/30/19	PFOA	570	40	5.71	14.4	8.71
07/01/19-09/30/19	PFOA	653	43	5.31	12.4	8.16
10/01/19-12/31/19	PFOA	920	33	4.79	14.5	9.01
01/01/20-06/30/20	PFOA	771	38	5.27	13.9	7.89
04/01/19-06/30/19	PFOS	570	45	11.5	25.5	14.6
07/01/19-09/30/19	PFOS	653	47	11.5	24.5	14.0
10/01/19-12/31/19	PFOS	920	40	10.4	26.1	13.3
01/01/20-06/30/20	PFOS	772	42	9.7	22.8	11.8

In a different study, the median of 57 reported PFOA concentrations in US surface waters was 41 ng/L, and for PFOS (N=56), 42 ng/L (Zareitalabad et al., 2013). These values were highest among ten nations analyzed, and for comparison, PFOA and PFOS median values for China (N=86, 83) were 1.8 ng/L and 0.89 ng/L, respectively, while for Canada (N=74, 70) PFOA and PFOS median values were 0.1 ng/L and 2.2 ng/L, respectively (Zareitalabad et al., 2013). These surface water data were based on several reports (Hansen et al., 2002; Boulanger et al., 2005; Giesy et al., 2006; Nakayama et al., 2007).

In a summary of published studies, PFOA and PFOS levels in river water (including rivers from Europe and China) were generally within 1-10 ng/L and 0.1-10 ng/L, respectively (Zhao et al., 2015b). Higher PFOA levels (22-260 ng/L) were observed in the Yangtze river (So et al., 2007).

Another contributor to PFOA and PFOS in surface waters is stormwater runoff. PFOA and PFOS were detected in 100% of stormwater runoff samples in the Minneapolis, MN, area at concentrations up to 30.6 and 155.8 ng/L, respectively (Xiao et al., 2012). These levels were significantly higher than rainfall concentrations (8.4 and 6.7 ng/L for PFOA and PFOS, respectively), indicating leaching of PFOA and PFOS from urban surfaces during the storm events. In a different study conducted in New York state, PFOA and PFOS concentrations in runoff reached 29.3 and 14.6 ng/L, respectively, and were also higher than concentrations in rain or snow (Kim and Kannan, 2007).

The discharge from municipal wastewater treatment plants (WWTPs) appears to be a major contributor to increased PFOA and PFOS concentrations in surface water (Becker et al., 2008). PFOA and PFOS in wastewater discharges are persistent despite wastewater treatment

[6] https://www.waterboards.ca.gov/pfas/drinking_water.html, accessed September 2020, accessed September 2020

(Sinclair and Kannan, 2006; Schultz et al., 2006a; Becker et al., 2008). Certain wastewater primary treatments, such as activated sludge treatment, can result in increased levels, presumably as a result of degradation of precursor compounds such as fluorotelomer alcohols (Sinclair and Kannan, 2006; Schultz et al., 2006b; Eriksson et al., 2017a). Oxidation and microbial processes can also contribute to transformation of precursor compounds to PFOA and PFOS (Houtz and Sedlak, 2012).

PFOA and PFOS are ubiquitously present throughout the world's oceans, and their concentrations in surface ocean water can reach several hundred pg/L (Ahrens et al., 2009; Ahrens et al., 2010; Benskin et al., 2012; Cai et al., 2012b; Zhao et al., 2012; González-Gaya et al., 2014; Zhao et al., 2015b). As the result of bioaccumulation, PFOA and PFOS are present in marine organisms, and consumption of seafood can be a major source of PFAS in humans.

Biota and Food

PFOA and PFOS have been described as proteinophilic compounds (Kelly et al., 2009; Xia et al., 2013), and as a result they readily distribute into living organisms. PFAS did not significantly correlate with several classes of lipophilic compounds such as PCBs (polychlorinated biphenyls), DDTs (dichlorodiphenyltrichloroethanes) and PBDEs (polybrominated diphenyl ethers) in a bioaccumulation study in fish and birds in the Barents Sea food web, consistent with the distinct physicochemical properties of PFAS (Haukås et al., 2007).

The marine environments in the industrialized parts of the world, e.g., the Baltic Sea, are of particular concern for PFOA and PFOS pollution, as high levels were found in fish, birds and marine mammals (Kratzer et al., 2011; Gebbink et al., 2016; de Wit et al., 2020). Gebbink et al. (2016) reported dramatic trophic magnification of PFOS in the food chain, but PFOA levels were fairly similar in the living organisms in the ecosystem. This study also reported enrichment of the linear PFOS isomer up the food chain, with approximately 90% of total PFOS in the top organisms (Gebbink et al., 2016). Accordingly, fish and seafood are among the top contributors to PFOA and PFOS in the diet (Haug et al., 2010; Noorlander et al., 2011).

Trophic magnification of PFOA and PFOS and selective enrichment of linear PFOS (n-PFOS) were also found in the food web of Taihu Lake, China (Fang et al., 2014). In this study, n-PFOS concentrations in fish were 10-90 ng/g. High biomagnification of PFOS was also observed in the eastern Arctic food webs (Tomy et al., 2004; Haukås et al., 2007). In this ecosystem, PFOA did not appear to be subject to trophic magnification (Tomy et al., 2004). Martin et al. (2004b) observed trophic magnification of PFOS in the Lake Ontario food chain but suggested that conversion of PFOS precursors to PFOS could contribute more to trophic magnification than PFOS bioaccumulation, at least in this particular ecosystem.

Metabolic conversion from precursor compounds can be a major source of PFAS exposure in marine organisms (Gebbink et al., 2016). Pilot whales, as well as crustaceans, lack the enzyme necessary for conversion of PFOSA (perfulorooctane sulfonamide) to PFOS, and contained much higher levels of PFOSA than PFOS in a study in the North Atlantic (Dassuncao et al., 2017). Moreover, PFOSA levels in whale muscle demonstrated a sharp decline from 2001 onward, whereas PFOS levels in whale muscle were increasing. Based on these observations and exposure models, this study concluded that high PFOS levels in most marine organisms, as well as recent declines of PFOS in marine organisms other than whales could be primarily driven by exposure to PFOS precursor(s) (Dassuncao et al., 2017). Due to the efficient and reversible retention by soil, PFOA and PFOS are readily taken up by plants and are subject to

further bioaccumulation in livestock (Ghisi et al., 2019). Stahl et al. (2009) observed a statistically significant concentration-dependent carryover of PFOA and PFOS from spiked soil (0.25-50 mg/kg) to maize, oats, wheat and potatoes. In this study, PFOA levels in foodstuffs exceeded the background levels at 0.25 mg/kg spiked concentration in soil (lowest studied), while PFOS in plants exceeded background levels at 1 mg/kg. PFOA and PFOS were also detected in lettuce, tomatoes, carrots, peas, celery and radishes (Blaine et al., 2013; Blaine et al., 2014; Bizkarguenaga et al., 2016). Cereals and cereal products were the main source of PFOA in the Norwegian diet, followed by meat and dairy, and with much smaller contribution from fruits and vegetables among other food groups (Haug et al., 2010). In a different study, PFOA contribution from vegetables (18 pg/kg-day) was comparable to that of meat products (22 pg/kg-day) (Gebbink et al., 2015b).

Animals appear to bioaccumulate PFOA and PFOS, as discussed in more detail in Chapter 4 of this document. As a result of feed and drinking water contamination, meat, eggs and dairy contain high levels of PFAS. Generally, animal products (meat, eggs, fish and dairy) tend to dominate dietary intake of PFOS, while for PFOA, cereals and vegetables have a comparable contribution (Tittlemier et al., 2007; Haug et al., 2010; Noorlander et al., 2011; Vestergren et al., 2012; Gebbink et al., 2015b). Although food packaging materials and non-stick cookware contain PFAS, it is unclear whether they significantly contribute to PFOA and PFOS intake with food (Jogsten et al., 2009).

The dietary studies discussed in this section were not specific to California or US diet, and the contribution of diet to overall exposure for California residents is further discussed in Appendix 4 of this document.

Human Exposure

Humans are exposed to PFOA and PFOS through diet, drinking water, ingestion of dust, inhalation of indoor and outdoor air, and through metabolic conversion of PFAS precursors, such as FOSEs and FTOHs. Appendix 5, Table A5.1 summarizes exposure studies that analyzed different routes of exposure (Egeghy and Lorber, 2011; Haug et al., 2011a; Lorber and Egeghy, 2011). The major exposure contribution in adults is food (71-87%), followed by drinking water (7.5-23%). The contribution of PFOA in ingested dust is also significant in adults (14.7-19.8%), whereas that of PFOS is smaller (1.6-5.5%). The biggest difference in infants and young children compared to adults is larger contribution from ingested dust, attributed to increased floor level playing activity and hand to mouth transfer. These exposure assessments did not consider indirect exposure resulting from exposure to PFOA and PFOS precursors in the environment, and the subsequent conversion to PFOA and PFOS in the organism. The contribution from precursor compounds to the overall PFOA and PFOS exposure is generally thought to be low (<10%) based on exposure assessments that estimate the 50th percentiles of exposures from different routes (Vestergren et al., 2008; Gebbink et al., 2015a; Gebbink et al., 2015b). However, in the high-exposure scenario, based on the 95th percentile of exposure via all routes, conversion from precursors was estimated to contribute up to 55% and 80% of overall exposure to PFOA and PFOS, respectively (Vestergren et al., 2008). Based on the study of temporal trends of PFOA, PFOS and precursors in diet, consumer products and human serum, it was suggested that precursor compounds may significantly contribute to the overall PFOA exposure in the Norwegian population (D'Eon and Mabury, 2011b).

Additional exposure studies found comparable values to those presented in Appendix 5, Table A5.1 (Noorlander et al., 2011; Vestergren et al., 2012; Gebbink et al., 2015a; Balk et al., 2019).

However, some studies predicted higher background exposure to PFOA and PFOS (Trudel et al., 2008; Fromme et al., 2009; Karrman et al., 2009). For example, Trudel et al. (2008) estimated PFOA exposure levels for an intermediate exposure scenario for infants, children and adults at 9.8, 7.6 and 2.5 ng/kg-day, respectively. For PFOS, exposure levels for an intermediate exposure scenario for infants, children and adults were at 54.6, 22.1 and 15.3 ng/kg-day, respectively. These values, particularly for PFOS, are higher compared to the studies in Table A5.1 in Appendix 5. While different exposure routes were analyzed, individual contributions to overall exposure were not specified in Trudel et al. (2008).

Occupational PFOA exposure can be much higher, e.g., 158 ng/kg-day for male adults in one estimate, and driven by inhalation (Vestergren and Cousins, 2009). In the general population, air pollution can provide an exposure contribution equal to that of diet (Harada et al., 2005b).

Contaminated drinking water can also become the main source of exposure. Vestergren and Cousins (2009) estimated that at 519 ng/L or higher, the contribution of drinking water to the overall PFOA exposure would be greater than 75%. The cutoff of 519 ng/L was from reported PFOA drinking water concentrations in Arnsberg, Germany (Hölzer et al., 2008). These exposure conditions were also met in exposure studies in Little Hocking and Lubeck (US), where the PFOA concentration in drinking water was 3,550 and 500 ng/L, respectively, and with PFOS exposure studies in Ronneby, Sweden, where the PFOS concentration in drinking water was 8,000 ng/L (Thompson et al., 2010; Li et al., 2018c). The PFOA exposure from drinking water in the Veneto region of Italy was close, with a median of 319 ng/L (Pitter et al., 2020).

Biomonitoring

Due to their ubiquitous presence in the environment, PFOA and PFOS have been detected in close to 100% of human serum samples. The available publications reporting PFOA and PFOS serum levels are too numerous to provide a comprehensive review. In the US, the National Health and Nutrition Examination Survey (NHANES) compiles the data for PFAS levels, including PFOA and PFOS, in serum for the general population. Data from this survey are available independently and in published reports (Calafat et al., 2007a; Calafat et al., 2007b; Kato et al., 2011; Jain, 2018). In 2013-2014, the weighted average PFOA serum concentration was 2.0 ng/ml, and for PFOS the weighted average serum concentration was 4.1 ng/ml (Jain, 2018). Mirroring PFOA and PFOS trends in manufacturing and uses serum concentrations increased through the 90s and then declined. The published studies on the time trends of PFOA and PFOS serum levels are summarized in Appendix 5, Table A5.2. While decreases in serum concentrations were observed in all studies from the US, Western Europe and Japan over 2000-2015, one study from South Korea (Seo et al., 2018) did not find a clear trend within the 2006-2015 period. Time trend studies from other countries and parts of the world are lacking, and it is possible that the decrease in PFOA and PFOS serum concentration observed over the last decade would be specific to industrial countries, where use and environmental pollution has been decreasing.

Several studies analyzed PFOA and PFOS serum levels in California residents, they are summarized in Table 3.2.2. These include studies conducted by the Biomonitoring California[7] program as well as other published studies of Californians. Generally, the PFOA and PFOS serum levels are consistent with NHANES data. Studies reporting time trends in the PFOA and

[7] https://dev.biomonitoring.ca.gov/

PFOS serum concentrations in California also found decreases from earlier peaks (Wang et al., 2011; Olsen et al., 2012; Kim et al., 2020).

Table 3.2.2. PFOA and PFOS biomonitoring studies in California

Study	Area	Participants	PFAS	Year-C_{serum}(ng/ml)[a]	Reference
CHDS[b]	Northern California	Pregnant women, archived samples (N=105)	PFOA	Arithmetic means 1960s[c]: 0.30 1980s: 3.17 2009: 2.21	Wang et al. (2011)
			PFOS	Arithmetic means 1960s[c]: 45.90 1980s: 30.60 2009: 9.44	
CHARGE[b]	Northern California	Women (mothers) (N=450)	PFOA[d]	2009: 1.66; 2010: 1.37 2011: 1.26; 2012: 1.14 2013: 1.02; 2014: 0.94 2015: 0.80; 2016: 0.68	Kim et al. (2020)
			PFOS[d]	2009: 4.86; 2010: 4.1 2011: 3.78; 2012: 3.42 2013: 3.02; 2014: 2.8 2015: 2.42; 2016: 2.12	
Red Cross plasma samples[b]	Los Angeles	Adult plasma donors (N=100)	PFOA[d]	2000/01: 4.0 2006: 2.7 2010: 1.9	Olsen et al. (2012)
			PFOS[d]	2000/01: 35.0 2006: 14.3 2010: 7.7	
MIEEP	San Francisco	Pregnant women (N=49-77)	PFOA	2010-2011: 0.47 (95th pctl: 2.14)	Website,[e] Morello-Frosch et al. (2016)
			PFOS	2010-2011: 2.55 (95th pctl: 7.25)	
FOX	California	Firefighters (N=101)	PFOA	2010-2011: 3.75 (95th pctl: 9.54)	Website,[e] Dobraca et al. (2015)
			PFOS	2010-2011: 12.5 (95th pctl: 24.7)	
CTS	California	Female teachers (N=1,257-1,759)	PFOA	2011-ongoing: 2.46 (95th pctl: 6.22) 2011-2015: 2.996 (mean)	Website,[e] Hurley et al. (2018)
			PFOS	2011-ongoing: 6.80 (95th pctl: 19.5) 2011-2015: 8.539 (mean)	Website,[e] Hurley et al. (2018)
		Subset of CTS subjects with identifiable source of drinking water (N=1,333)	PFOA	2011-2013: 2.45 (1,263 subjects with non-detectable PFOA[c] in drinking water); 3.47 (70 subjects with detectable levels of PFOA in drinking water)	Hurley et al. (2016)
			PFOS	2011-2013: 6.76 (1,240 subjects with non-detectable PFOS[c] in drinking water); 8.51 (93 subjects with detectable PFOS in drinking water)	
BEST-1	Central Valley	Adults (N=110)	PFOA	2011-2012: 1.97 (95th pctl: 4.7)	Website[e]
			PFOS	2011-2012: 7.00 (95th pctl: 25.8)	

Study	Area	Participants	PFAS	Year-C_{serum}(ng/ml)[a]	Reference
BEST-2	Central Valley	Adults (N=337)	PFOA	2013: 1.49 (95th pctl: 4.57)	Website[e]
			PFOS	2013: 5.21 (95th pctl: 17.6)	
MAMAS	California	Pregnant women (N=200)	PFOA	2012-2015: 1.24 (95th pctl: 2.81)	Website[e]
			PFOS	2012-2015: 4.2 (95th pctl: 12.3)	
ACE 1	San Francisco Bay Area	Chinese adults (N=96)	PFOA	2016: 1.41 (90th pctl: 2.85)	Website[e]
			PFOS	2016: 6.51 (90th pctl: 19.3)	
ACE 2	San Francisco Bay Area	Vietnamese adults (N=99)	PFOA	2017: 1.69 (90th pctl: 3.06)	Website[e]
			PFOS	2017: 7.47 (90th pctl: 22.9)	
CARE-LA	Los Angeles county	Adults (N=425)	PFOA	2018: 1.04 (95th pctl: 3.06)	Website[e]
			PFOS	2018: 2.13 (95th pctl: 8.33)	
CARE-2	Riverside, San Bernardino, Imperial, Mono, and Inyo counties	Adults (N=358)	PFOA	2019: 0.98 (95th pctl: 2.70)	Website[e]
		Adults (N=357)	PFOS	2019: 2.40 (95th pctl: 8.72)	

ACE, Asian/Pacific Islander community exposures; BEST, biomonitoring exposures study; CARE-LA, California regional exposure study, Los Angeles county; CHARGE, childhood autism risk from genetics and environment study; CARE-2, California Regional Exposure Study, Region 2; CHDS, child health and development studies; CTS, California teachers study; FOX, firefighter occupational exposures project; MAMAS, measuring analytes in maternal archived samples; MIEEP, maternal and infant environmental exposure project

[a] Geometric mean if not indicated otherwise; pctl, percentile

[b] CHDS (except 2009 data), CHARGE and Olsen et al. (2012) studies were not part of the Biomonitoring California program

[c] Non-detects were as assigned a value of LOD (limit of detection)/$\sqrt{2}$ for each analyte

[d] Graphical data were quantified using GetData Graph Digitizer software (version 2.26)

[e] https://biomonitoring.ca.gov/, last accessed May 2021

4. TOXICOKINETICS

PFOA and PFOS strongly bioaccumulate in humans and, to a much lesser degree, in animals. For such compounds, dose-response analysis is usually based on an internal dose metric such as serum concentrations, and the results are converted to applied doses using toxicokinetic (TK) approaches. All available human toxicity studies report serum concentrations for PFOA or PFOS, necessitating an adequate TK approach to convert human dose-response data to an oral dose, which serves as the basis for the PHG. Similarly, animal studies that report applied doses would require conversion to serum concentrations prior to dose-response analysis, and those that report serum levels would require conversion to human equivalent doses in deriving an acceptable daily dose. This chapter summarizes available TK information for these compounds in humans and animals (Sections 4.1-4.5), considers available physiologically-based pharmacokinetic models (Section 4.6) and analyzes in more detail the estimation of serum clearance for these compounds (Sections 4.7-4.9). As will be explained in the text, applying a clearance factor is a relatively simple method to convert serum concentrations to applied doses, and has been used in this capacity by US EPA for its PFOA and PFOS assessments (US EPA, 2016b; US EPA, 2016d). After reviewing this approach in detail, OEHHA provides updated clearance estimates for PFOA and PFOS (Section 4.9).

4.1. Species Differences: PFOA and PFOS Serum Half-lives

PFAS, including PFOA and PFOS, demonstrate dramatic differences in TK[8] properties among species, and in some cases, between sexes, as demonstrated for half-life ($T_{1/2}$) estimates in Table 4.1.1. Except for human $T_{1/2}$ values, data are from a recent review that summarized available studies in rat, mouse and monkey (Pizzurro et al., 2019). For human $T_{1/2}$, values in the table were chosen by US EPA for their PFOA and PFOS assessments. Human $T_{1/2}$ values are discussed in detail in Section 4.7. Additionally, PFOA $T_{1/2}$ was 5.5-7 hours in the rabbit, 10.6-20.1 days in the dog and 2.7-5.6 days in the Japanese macaque (Hanhijärvi et al., 1988; Kudo and Kawashima, 2003; Harada et al., 2005a).

Table 4.1.1. PFOA and PFOS serum half-lives in species (Pizzurro et al., 2019)

	PFOA		**PFOS**	
	female	**male**	**female**	**male**
Rat	1.9-4.6 h (<25mg/kg) 16.2 h (25 mg/kg) 24 h (50 mg/kg)	1.5-15 d (<25 mg/kg) 6.5 d (25 mg/kg) 4.4 d (50 mg/kg)	24-83 d	26-82 d
Mouse	1.2 d (20 mg/kg-d[a]) 15.6 d (1 or 10 mg/kg)	21.7 d (1 or 10 mg/kg)	38 d (1 mg/kg-d) 30 d (20 mg/kg-d)	43 d (1 mg/kg-d) 36 d (20 mg/kg-d)
Cynomolgus monkey	32.6 d	20 d	110-200 d	132-200 d
Human	2.3 years (US EPA, 2016b)		5.4 years (US EPA, 2016d)	

d, days; h, hours

[a] 17 days (multi-dose treatment)

[8] The terms “toxicokinetic” and “pharmacokinetic” have been used interchangeably in past PHG documents. In this document, OEHHA is using the term toxicokinetic, unless specifically referring to physiologically based pharmacokinetic models, for consistency.

Human PFOA $T_{1/2}$ is ~ 200x that of the rat (male) and ~40x that of the mouse. Human PFOS $T_{1/2}$ is ~ 40x that of the rat or mouse. Such dramatic differences in half-lives indicate that interspecies dose extrapolations have to be done using TK considerations, such as clearance rates or PBPK (physiologically based pharmacokinetic) models. Another interesting aspect of interspecies TK differences is the sex difference for the rat PFOA $T_{1/2}$ values, with much faster elimination in the female rat (Table 4.1.1). Other species, including human, do not demonstrate sex differences in PFOA $T_{1/2}$ values. The observation of sex differences in PFOA elimination in the rat allowed characterization of the underlying mechanisms of renal reabsorption involving specific membrane transporters. While absorption, distribution and metabolism of PFOA and PFOS demonstrate interspecies similarity, differences in excretion appear to underlie interspecies differences in PFAS kinetics.

4.2. Absorption

PFOA and PFOS are well absorbed with oral administration in animal studies. In male CD rats, 93% of the oral gavage dose (11 mg/kg) was absorbed after 24 hours (unpublished report cited in US EPA (2016b)). Similarly, oral absorption efficiency was at least 92-93% in male Sprague Dawley rats exposed to 5 or 20 mg/kg of PFOA via gavage (Cui et al., 2010). In the same study, absorption of PFOS in male rats was approximately 98% for either 5 or 20 mg/kg oral gavage doses (Cui et al., 2010). The PFOA absorption efficiency in male C57BL/6 mice was 98% over 48 hours following a gavage dose of approximately 7.5 mg (Jandacek et al., 2010). In a PFOA mouse study, the area under the concentration-time curve (AUC) was measured over 24 hours for serum concentrations following a single intravenous (i.v.) or oral gavage dose, and the resulting i.v. to oral AUC ratio was close to 1, indicating 100% absorption efficiency (Fujii et al., 2015). In male Sprague Dawley rats, the absorption of a single oral dose at 4.2 mg/kg PFOS was estimated to be >95% (Chang et al., 2012). PFOS was fully absorbed in female white New Zealand rabbits following an oral gavage dose of 0.2 µg/kg (Tarazona et al., 2016). When PFOS was alternatively introduced by i.v. or oral gavage to male or female Sprague Dawley rats at 2 mg/kg, the plasma concentration curves overlapped at ≥2 hours in either sex, indicating 100% absorption (Huang et al., 2019a).

In humans, PFOA was rapidly absorbed with oral doses of 50-1,200 mg in a clinical trial of cancer patients, as indicated by peak plasma concentrations at 2-4 hours in most study participants (Elcombe et al., 2013; IARC, 2017a; Convertino et al., 2018). However, in a sizable fraction of the 43 subjects in this study, peak plasma concentrations were steadily increasing over the initial 24 hours of measurement, indicating a slower absorption/distribution process and significant kinetic variability in this population (Elcombe et al., 2013). No studies of human PFOS oral absorption were identified. PFOA and PFOS human exposure models demonstrated good correlation of predicted and observed serum concentrations, with the assumption of high oral absorption efficiency (Haug et al., 2011a; Noorlander et al., 2011; Vestergren et al., 2012).

Several PFOA and PFOS exposure models, originating with Trudel et al. (2008) used PFOA/PFOS oral absorption efficiency values of 0.66, 0.8 and 0.91 to represent low, intermediate and high exposure scenarios, respectively (Trudel et al., 2008; Gebbink et al., 2015a; Balk et al., 2019). This scale was derived from the distribution of recovery of [^{14}C]PFOA (carbon-14 radioisotope labeled PFOA) doses (as percentage of total administered) in urine and tissues among both sexes of rat, mouse, hamster and rabbit (Hundley et al., 2006). This study did not consider elimination via bile excretion/fecal route, and produced much lower PFOA absorption estimates, e.g., 77% and 57% in male and female mice, respectively, than most other reports. Therefore, the resulting assumption of lower human absorption efficiency, such

as reported in Trudel et al. (2008) is not supported by most available data. Based on available human and animal studies, oral absorption of PFOA and PFOS appears to be equal to or greater than 90%.

PFOA was dermally absorbed in rabbits and rats (Kennedy Jr, 1985; US EPA, 2016b). In an unpublished study, O'Malley and Ebbens (1981) observed increased mortality in female and male New Zealand white rabbits at 1,000 and 2,000 mg/kg PFOA applied dermally for 14 days, while no animals died at 100 mg/kg (US EPA, 2016b).

Dermal permeability coefficients (K_p) for PFOA were $9.49 \pm 2.86 \times 10^{-7}$ cm/h and $3.25 \pm 1.51 \times 10^{-5}$ cm/h, for human and rat skin respectively, as determined in vitro (Fasano et al., 2005).

In an unpublished study, Hinderliter (2003) demonstrated effective absorption of PFOA in rats through inhalation exposure to a PFOA aerosol (US EPA, 2016b). Plasma PFOA concentrations increased proportionally to the applied dose, and reached higher levels in male rats, presumably due to sex-specific excretion differences.

4.3. Distribution

PFOA and PFOS are widely distributed in the body with preferential accumulation in the liver, plasma and kidney (Appendix 6, Table A6.1). Liver appears to have the highest levels of PFOA in rats and male mice, but in female mice plasma concentrations are higher than those in the liver. In two monkey and two human studies, liver had lower PFOA concentrations than serum (Griffith and Long, 1980; Butenhoff et al., 2004a; Maestri et al., 2006; Mamsen et al., 2019). For PFOS, all species but humans had strong liver accumulation, and in human studies, evidence was mixed, with highest levels of PFOS in the liver in two studies (Olsen et al., 2003c; Maestri et al., 2006). Taken together, these data suggest toxicokinetic differences between species.

PFOA and PFOS can cross the placenta and accumulate in the fetus, with liver as one of the fetal target organs (Mamsen et al., 2019). PFOA and PFOS can transfer via lactation in humans (reviewed in Pizzurro et al. (2019)), resulting in decreased body burden in the mother and increased blood concentrations in the infant. This may be of concern, particularly for PFOA, since concentrations in the infant plasma can become much higher relative to maternal plasma. In a German study of 53 mother-infant pairs randomly sampled from the general population and of which 37 infants were exclusively breast-fed, the median concentration of PFOS was nearly the same in the maternal and infant serum at 6 months of age, while PFOA concentrations were 4.6-fold higher in the infants, on average (Fromme et al., 2010). In infants from a Faroe Islands study, PFOS levels in plasma increased on average at a rate of 29.2% per month over the first 18 months of life, while PFOA levels increased on average at a rate of 27.8% per month (Mogensen et al., 2015a). Overall the values increased approximately 4-fold for either compound, the majority of increase was during the first 11 months and correlated with breast-feeding. In the same study, at the age of 5 years, the PFOA and PFOS values decreased relative to the peak at 11-18 months.

The effects of breastfeeding on increasing PFOA and PFOS concentrations at 1 year of age were also reported in a study of 101 German infants (Abraham et al., 2020). However, in a cross-sectional study of 300 children in Texas, plasma concentrations of PFOA or PFOS steadily increased for 0-3, 3-6, 6-9 and 9-13 years of age groups, indicating that a possible early life spike in plasma concentrations would have dissipated by 3 years of age (Schecter et al., 2012). This could be partly due to growth dilution. Several recent kinetic and PBPK models

addressed infant kinetics of PFAS (Verner et al., 2016; Brochot et al., 2019; Goeden et al., 2019).

In general, PFAS distribution in the body has been thought to be driven by protein binding (Kennedy et al., 2004; Cheng and Ng, 2017). High levels of PFOA and PFOS accumulation in plasma and liver are likely driven by binding to specific proteins, such as serum albumin and liver fatty acid-binding protein (L-FABP). Published reports on PFOA and PFOS binding to proteins are summarized in Appendix 6, Table A6.2. PFOA and PFOS bind to albumin with particularly high affinity, often with the dissociation constant at ~10^{-6} M. Detailed analysis of PFOA-albumin binding energies indicates non-covalent binding with likely involvement of Van-der-Waals forces and hydrogen bonds (Qin et al., 2010).

Binding of PFOA and PFOS to certain targets, such as plasma albumin, L-FABP and transthyretin appears to be of the same magnitude as binding constants reported for their endogenous ligands (Luebker et al., 2002; D'Eon et al., 2010; MacManus-Spencer et al., 2010). Displacement of endogenous ligands from carrier or transporter proteins has been hypothesized as one of the possible mechanisms of action in PFOA/PFOS toxicity.

While binding to blood proteins is important, PFOA and PFOS do not appear to distribute to red blood cells or lipoproteins to a significant extent in either humans or rats (Johnson et al., 1984; Kärrman et al., 2006; Ehresman et al., 2007; Kudo et al., 2007; Butenhoff et al., 2012c; Hanssen et al., 2013; Jin et al., 2016). In contrast, PFOA and PFOS appear to be efficiently distributed to the liver, likely due to the demonstrated L-FABP binding (Luebker et al., 2002).

At physiological pH, PFOA and PFOS are charged and therefore, would not be able to cross membranes via passive transport. Several transporter proteins are likely to be involved in PFOA and PFOS transport (Appendix 6, Table A6.3). Transporter studies primarily involved cell-cultures (in vitro system), in which kinetics of PFAS absorption into cells or across a cell barrier were compared to that of a negative control, such as cells lacking the transporter of interest. In some studies, such as Kummu et al. (2015), the efficiency of in vitro transport was correlated with transporter expression levels for human organ samples.

Important physiological roles for PFAS-active transporters have been proposed and include:

- in the kidney, secretion and/or reabsorption of PFAS, which drives overall PFAS kinetics; transporters are suggested to be responsible for species/sex kinetic differences;
- in the liver, bile acid transporters (Zhao et al., 2015a) appear to mediate re-uptake of PFAS following secretion in bile, likely contributing to enterohepatic circulation of these compounds;
- in the placenta, organic anion transporter (OAT) 4 may be involved in transport of PFAS into the fetus (Kummu et al., 2015).

There are limited animal studies on distribution of PFOA and PFOS isomers. When administered orally as a mixture of isomers to rats, both PFOA and PFOS demonstrated organ-specific and sex-specific differences among isomers after 38 days following a single dose or after 38 days of daily treatments (Benskin et al., 2009; De Silva et al., 2009). While some isomers demonstrated higher levels of accumulation in some organs, the underlying mechanisms or similarity with human systems remain unclear. Branched PFOS isomers were eliminated faster than n-PFOS (linear form) in rats given PFAS-spiked food for 77 days (Ross et al., 2012).

In humans, n-PFAS isomers appear to be enriched (relative to other isomers and the source of exposure) and with longer half-lives compared to other isomers (Zhang et al., 2013b; Zhou et al., 2014). Longer retention of linear isomers could be due to tighter binding to blood proteins (Beesoon and Martin, 2015; Gao et al., 2015b).

4.4. Metabolism

PFOA and PFOS are not known to be metabolized (EFSA, 2018). Based on the distribution and excretion of ionic and non-ionic fluorine in female rats administered an unspecified dose of PFOA (mix of linear and branched isomers) and followed for 96 hours, Ophaug and Singer (1980) hypothesized that PFOA did not undergo Phase I metabolism and was excreted intact. Single dose i.v. studies in rats of both sexes and in vitro microsomal incubations with radioactively labelled PFOA did not detect covalently modified or Phase II conjugated metabolites of PFOA (Vanden Heuvel et al., 1991; Goecke et al., 1992; Kuslikis et al., 1992). For example, only parent [^{14}C]PFOA was excreted in urine and bile of male and female rats treated with a single i.v. dose of 9.4 µmol/kg PFOA and followed for 6 hours (bile) or up to 28 days (male rats) (Vanden Heuvel et al., 1991). Consistent with the general lack of metabolism, only 1.3-5.2% of ^{14}C activity was recovered in the expired air from mice, rats and hamsters over 120 hours after a single oral gavage dose of [^{14}C]PFOA (10 mg/kg) (Hundley et al., 2006). Based on elution properties of PFOA excreted in rats exposed to a single intraperitoneal (i.p.) dose (50 mg/kg) and followed for 96 hours, Ylinen et al. (1989) also concluded that the compound would not be conjugated with glucuronic or amino acid groups, indicating no Phase II metabolism. While PFOA metabolism studies in humans and studies with PFOS are lacking, it is generally assumed that both compounds are inert to metabolism in humans and are excreted intact (US EPA, 2016b; US EPA, 2016d).

4.5. Excretion

Excretion pathways of PFOA and PFOS include:

1) Renal or urinary excretion, which occurs in all mammalian species and appears to be dominant in fast eliminators, e.g., in the case of PFOA elimination in the female rat.
2) Fecal or gastrointestinal excretion appears to play a more important role in slow eliminators, such as humans; likely subject to enterohepatic circulation.
3) Elimination pathways via pregnancy and lactation in human females (Wong et al., 2014).

Several studies in rats determined that renal excretion was higher than fecal excretion for PFOA and PFOS. With a single i.p. dose of PFOA (20 mg/kg), cumulative elimination in urine (50-75% of the dose) was more than 20-fold higher than excretion in feces, in both female and male rats (Kudo et al., 2001). During subchronic exposure in male rats, the daily amount of PFOA excreted in urine was ≥2-fold higher than the amount excreted in feces over 28 days at either 5 or 20 mg/kg-day doses (Cui et al., 2010). For PFOS, approximately similar amounts were excreted in urine and feces for the first 10 days at either 5 or 20 mg/kg-day doses, with progressively relatively higher amounts of PFOS excreted in urine at 10+ days (Cui et al., 2010). Ohmori et al. (2003) found that in male and female rats injected with a single dose of PFOA or other perfluorocarboxylic acids (48.65 mmol/kg), increasing PFAS chain length correlated with longer $T_{1/2}$ and lower renal clearance. Regression of renal clearance versus total clearance for all data points (PFAS/sex combinations) produced good fit (r^2 = 0.981) and a slope of 0.48, suggesting that renal clearance would account for approximately half of overall excretion. Additional rat studies are consistent with these general conclusions (Ophaug and Singer, 1980;

Kojo et al., 1986; Vanden Heuvel et al., 1991; Luebker et al., 2005a; Katakura et al., 2007; Benskin et al., 2009; Chang et al., 2012; Gao et al., 2015a).

In male and female mice administered a single dose of PFOA and followed for 24 hours, urinary clearance was 7.6x higher than fecal clearance with an i.v. dose (0.31 µmol/kg), and 2.5x higher with a gavage dose (3.13 µmol/kg) (Fujii et al., 2015). Similar to rats (Ohmori et al., 2003), increasing chain length of perfluoroalkyl carboxylate (C7-C14) resulted in progressively lower urinary clearance and progressively higher fecal clearance, with approximately similar amounts eliminated via urine or feces for PFDA (perfluorodecanoic acid, C10).

In humans, multiple reports directly measured PFOA and PFOS renal clearance in occupationally exposed subjects and in the general population (Table 4.5.1). All studies were in Asia (China, Japan). Despite a wide range of observed serum concentrations, renal clearance values for both PFOA and PFOS were generally narrowly distributed. The only exception was the PFOA clearance value from the Zhang et al. (2013b) study, which at 0.79 ml/kg-day was higher than the rest of the values, with the next highest value for PFOA clearance at 0.09 ml/kg-day. Considering it an outlier, the geometric mean of the remaining six studies for PFOA renal clearance was 0.059 ml/kg-day (using an average for two values in Harada et al. (2005a)). For PFOS, the geometric mean of six studies was 0.016 ml/kg-day.

Table 4.5.1. Human renal clearance (CL_R) studies for PFOA and PFOS

Reference	Chemical	Population	CL_R (ml/kg-day)[a]	C_{serum} (ng/ml)[a]
Fu et al. (2016)	PFOA	Occupational (N=302)	0.067 (9×10^{-5}-2.4)	1,052
	PFOS		0.01 (5×10^{-5}-0.54)	5,624
Gao et al. (2015b)	PFOA	Occupational (N=36)	0.09 (0.01-2.17)	2.66-10,515
	PFOS		0.01 (0.0002-0.07)	37.9-36,625
Zhang et al. (2015)	PFOA	General adult (N=54)	0.071 (adults)	2.47
	PFOS		0.026 (adults)	8.62
Fujii et al. (2015)	PFOA	General adult (N=10)	0.044	5.96
Zhou et al. (2014)	PFOA	Fishermen (N=16-39)	0.075 (0.02-0.263)	43.5 (34.7-52.4)
	n-PFOS		0.015 (0.001-0.092)	8,940 (7,280-10,600)
Zhang et al. (2013b)	n-PFOA	General adult (N=7-20)	0.79 (0.48-1.1)	3 (0.24-28)
	n-PFOS		0.031 (0.021-0.042)	15 (0.91-50)
Harada et al. (2005a)	PFOA	General adult (N=20)	0.033 (M) 0.027 (F)	7.9-12 (M) 7.6-14 (F)
	PFOS		0.012 (M) 0.019 (F)	12.6-26.3 (M) 11.2-23.5 (F)

[a] Range indicated in parenthesis when reported
C_{serum}, concentration in serum; n-PFOA, n-PFOS, linear isomers

Han et al. (2012) compared available PFOA renal clearances (CL_R) for different species and sexes to their corresponding glomerular filtration rates (GFR). The adapted table for this comparison is presented in Appendix 6 (Table A6.4), with added values for male and female rat, and an OEHHA-derived human clearance value. Assuming the unbound fraction in blood (f_u) as 0.02, comparison of the $GFR \times f_u$ (amount passively accessible to tubular secretion) and CL_R (amount eventually excreted) values provides insight into whether the compound is actively secreted or reabsorbed during the renal filtration process. This comparison demonstrated dramatic interspecies differences, with net renal tubular secretion in some systems (rabbit, female rat) to account for very high clearance values, and a wide range of reabsorption efficiencies with other species (Appendix 6,Table A6.4). According to this calculation, human

kidney reabsorbed 99.8% of PFOA. This mechanism would explain the exceptionally long human $T_{1/2}$ relative to animals.

Renal reabsorption was found to be hormone-dependent in rats (Ylinen et al., 1989; Vanden Heuvel et al., 1992; Kudo et al., 2002). Multiple receptors at the basolateral and apical membranes of tubular cells have been implicated in this process (Kato et al., 2002; Kudo et al., 2002; Katakura et al., 2007; Yang et al., 2009a; Weaver et al., 2010; Han et al., 2012). Renal reabsorption has been consistently incorporated into PBPK models of PFAS.

While more PFOA and PFOS would be expected to be eliminated in feces than in urine in humans, measurements in feces were below the detection limit in the three available studies (Beesoon et al., 2012; Genuis et al., 2013; Fujii et al., 2015). PFAS are more readily detected in the urine compared to feces due to the significantly lower detection limits. However, in one study both PFOA and PFOS could be detected in stool samples following treatment with cholestyramine in eight individuals with high PFAS body burdens (Genuis et al., 2013), providing evidence for the presence of enterohepatic circulation of these compounds. Cholestyramine is a resin used to immobilize certain lipophilic compounds in the gastrointestinal (GI) tract, preventing their reabsorption and therefore, interrupting their enterohepatic cycle.

Further evidence for enterohepatic circulation of PFOA and PFOS came from the measurements of their biliary clearance in humans. Harada et al. (2007b) measured PFOA and PFOS in the bile samples from four elderly patients, and estimated mean biliary clearance as 1.06 and 2.98 ml/kg-day, respectively. These values are dramatically higher than the urinary clearances, and would also greatly exceed the overall plasma clearances given the kinetic assumptions used in that study. Therefore, the authors concluded that a large fraction of PFOA and PFOS secreted with bile would be reabsorbed via an enterohepatic circulation mechanism, and estimated that the reabsorbed fractions were 0.89 and 0.97 for PFOA and PFOS, respectively. Using similar methodology, Fujii et al. (2015) reported biliary clearance for PFOA as 0.044±0.01 ml/kg-day (mean ± standard deviation, N=5). Both studies reported very close ratios of PFOA concentration in bile to its concentration in serum, 0.21 (Harada et al., 2007b) and 0.25 (Fujii et al., 2015). Harada et al. (2007) estimated the bile to serum ratio for PFOA was 0.60 (N=4).

Enterohepatic circulation for PFAS appears to be observed in other species. Vanden Heuvel et al. (1991) reported a fairly high level of excretion in bile for [^{14}C]PFOA in bile duct-cannulated rats exposed to a single i.v. dose (9.4 µmol/kg) and followed for 6 hours. There was no difference between male and female rats, and in male rats, bile excretion likely contributed to elimination through feces, which after 28 days added up to approximately 35% of the total dose. These results were consistent with another rat study (Kudo et al., 2001).

In mice treated with increasing doses of PFOA (12.5, 25, 50 µmol/kg-day) via oral gavage for 4 weeks, Minata et al. (2010) observed a high degree of PFOA concentration in bile. At the low dose, the concentration in bile was 2.8-fold higher than in blood, and at mid and high doses, 16.7-fold and 33.9-fold higher, respectively. Such high excretion rates in bile combined with the relatively long $T_{1/2}$ estimates in mouse (Table 4.1.1) would also imply a high level of re-absorption of PFOA although the report did not address this question.

Additional PFAS elimination routes in humans include pregnancy, lactation, birth, and menstrual loss of blood in females. Several studies modeling human biomonitoring data observed better concordance of predicted and observed serum concentrations for females when terms for loss

through menstrual blood and lactation were included in the overall elimination constant (Wong et al., 2014; Gomis et al., 2017). Table 4.5.2 lists individual terms of the elimination constant used in Gomis et al. (2017), calculated using published parameters, with the resulting values in the second column and overall percent contribution to total elimination in the third column. This study estimated lower $T_{1/2}$ values for both PFOA and PFOS compared to most other studies, which would result in higher plasma elimination.

Table 4.5.2. Elimination rate (Gomis et al., 2017) by term (year^{-1})[a]

$$k_{elim}(t_{age}) = \frac{B'_w(t_{age})}{B_w(t_{age})} + \frac{ln2}{T_{1/2}} + \frac{M_e}{V_d \times B_w} + \frac{BM_t \times I_m \times U}{V_d \times B_w}$$

Formula term	Corresponding elimination rate (y^{-1})		% of total
Growth dilution $\frac{B'_w(t_{age})}{B_w(t_{age})}$	0 in adults		
Plasma elimination $\frac{ln2}{T_{1/2}}$	PFOA	0.33	37.3
	PFOS	0.21	54.8
Loss to menstrual blood $\frac{M_e}{V_d \times B_w}$	PFOA	0.057	6.57
	PFOS	0.049	12.9
Loss to lactation $\frac{BM_t \times I_m \times U}{V_d \times B_w}$	PFOA	0.50	56.1
	PFOS	0.12	32.3

[a] for an average adult woman 30-40 years old; body weight (B_w) = 74.8 kg (US EPA, 2011a); half-life ($tT_{1/2}$) 2.1 years (PFOA, American women), 3.3 years (PFOS, American women); volume of distribution (V_d) 200 ml/kg (PFOA), 235 ml/kg (PFOS); menstrual blood loss (M_e) 868 ml/year; serum:milk transfer ratio (BM_t), 0.029 (PFOA), 0.0085 (PFOS); average volume of milk (I_m) 700 ml/day; unit correction factor (*U*) 365 days/year.

The overall comparison for different terms in the elimination rate for an adult woman indicates that contribution of elimination via menstrual blood may not be significant, while PFAS loss through lactation would be sizable. The model assumes only one pregnancy and birth per woman-lifetime, and a lactation period of 6 months, therefore the overall effect of lactation on the life-averaged elimination rate would be higher for a woman who has more than one child. One limitation of this approach was using fixed previously reported V_d estimates, which may not be optimal, as described in Section 4.8 of this document.

4.6. Physiologically-Based Pharmacokinetic Models

A large number of physiologically based pharmacokinetic (PBPK) models have been developed for PFOA and PFOS, and they are summarized in Table A6.5 in Appendix 6. The models were developed for different species, including humans and differ in complexity. The majority of models incorporate the renal reabsorption loop, which was first hypothesized as underlying the sex differences in PFOA kinetics between fast-eliminating female rats and slow eliminating male rats. It is assumed that renal transporters (Appendix 6, Table A6.3) mediate transfer of PFAS, including PFOA and PFOS, at renal interfaces, and that the net effect of secretion and reabsorption could explain the species differences in PFAS elimination (Appendix 6, Table A6.4). The majority of PFAS PBPK models describe renal reabsorption with Michaelis-Menten kinetics, with observed data-optimized K_m and V_{max} parameters.

Andersen et al. (2006) developed the first model with renal reabsorption, in the monkey. The model contains only three compartments: central compartment (~plasma), tissue compartment and filtrate compartment for renal elimination. The reabsorption loop was modeled as the back-flow from the filtrate to the central compartment. Six parameters in the model were optimized based on observed data, including reabsorption parameters, compartmental transfer constants, V_d and the proportion of free compound in blood. The resulting model described well the PFOA kinetics of a single dose (10 mg/kg), as well as kinetics of the approach to steady state during repeat dosing and following the discontinuation of daily treatment (20 mg/kg-day). A similar approach was used for PFOS, and while a single dose (2 mg/kg) demonstrated good convergence with the observed data, predictions for repeated treatment experiments (0.03, 0.15, 0.74 mg/kg-day) were much higher than the observed values in an independent validation study.

There were two important directions of PFAS PBPK modelling that started with the Andersen et al. (2006) model. In the first direction, Tan and coworkers and later, Loccisano and coworkers, continued to add compartments and expand to other species, successively developing a five-compartment model for rat and monkey (Tan et al., 2008), an eight-compartment model for rat (Loccisano et al., 2012a), a nine-compartment model for monkey and human (Loccisano et al., 2011), and culminating in the addition of gestational and lactational compartments for rat (Loccisano et al., 2012a) and human (Loccisano et al., 2013). The Loccisano et al. (2011) human model was further optimized at the organ level (Fabrega et al., 2014) using contemporaneously published human cadaver data. These sequentially developed models re-used certain parameters developed in previous iterations. One of the limitations of the rat models is the use of limited TK studies for optimization and validation and reliance on unpublished data. Another limitation of several models is using a time-dependent function for some parameters, such as V_d and free fraction in blood. While time-dependence was employed for a better fit of the observed kinetic data (particularly for longer treatments), the biological basis underlying such an adaptation of a physiological parameter is unclear and lacks experimental justification.

The second important derivation of the Andersen et al. (2006) model was the development of the complex probabilistic optimization of the same basic structure for two compounds (PFOA, PFOS) and three species (rat, mouse, monkey). This approach (Wambaugh et al., 2013) was used by US EPA in the risk assessment of PFOA and PFOS (US EPA, 2016b; US EPA, 2016d). Some of the optimized parameter values have very wide confidence intervals that span orders of magnitude, indicating a high level of uncertainty. The model optimization for rat and mouse relied on a limited number of kinetic studies, even though multiple studies are available in the literature. Certain parameter values, such as the filtrate compartment volume had biologically implausible values, highlighting the fact that the model was not physiologically based. It appears that, at least for some species, the model may have limited predictive power outside the range of concentrations used in the optimization process. For example, OEHHA ran the Wambaugh et al. (2013) model with kinetic data from a 28-day oral study in mice (Li et al., 2017b) that has doses below the range of doses used for optimization of the model and found that at the lowest PFOA dose (0.05 mg/kg-day), the model predicted approximately 10 times higher serum concentration than was reported (Appendix 6, Table A6.6). The code for the model was obtained from the authors and was adapted for Berkeley Madonna, a mathematical modeling software package.

Rodriguez et al. (2009) developed simplified 2- to 3-compartment models for PFOA in mice. The model for non-pregnant mice included a more complex renal recirculation circuit with an

additional compartment for renal plasma. The models that include gestation and lactation in mice did not include renal reabsorption and the dam was modeled as a single compartment. The model relied on data from only three published studies for optimization and validation. The model had a tendency to overestimate plasma levels. However, in OEHHA's comparison of mouse models (Appendix 6, Table A6.6), this model performed well at predicting plasma concentration at 0.05 mg/kg-day in the 28-day oral study by (Li et al., 2017b). The model code was adapted for Berkeley Madonna.

Unlike traditional PBPK methods that optimize a certain number of parameters for a better fit of observed data, Cheng and Ng (2017) developed a complex 19-compartment PFOA model for rat that utilized 72 independent parameters without fitting any parameters to data. Instead, parameter values and ranges were developed through expert knowledge and used for Monte-Carlo analysis of uncertainty ranges for predicted serum concentrations. The fit to the observed data was excellent and the uncertainty ranges were narrow. However, the study only modeled a single dose scenario and was validated with limited data. The model was re-iterative and not differential equation-based, which made it computationally challenging. While this appears to be the most biologically-informed approach of all published models to date, its utility for risk assessment is limited because it cannot handle repeated dose exposures.

Worley and Fisher (2015) developed a novel PFOA rat model with detailed mechanism of active transport in the renal reabsorption loop. This included active transport from filtrate to proximal tubule cells via apical transporters, active transport from kidney to proximal tubule cells via basolateral transporters and active flux from proximal tubule cells to blood. The transporter kinetics were described using Michaelis-Menten kinetics or first-order transfer, and in some cases was informed by in vitro parameters adjusted to the in vivo system (in vitro to in vivo extrapolation or IVIVE). The model was calibrated for single dose oral gavage and i.v. experiments and demonstrated good agreement with observed serum, liver and urine concentrations. However, the model relied on a limited number of studies for development and validation, including unpublished data. This model was further developed into a PFOA human model (Worley et al., 2017a) and a PFOS rat, mouse, monkey and human model (Chou and Lin, 2019). The human PFOA model only considered human serum data from a single study for optimization and a single study for validation, despite including many non-serum compartments (Worley et al., 2017a). The model has not been validated with additional human kinetic data that are available.

The multi-species PFOS model of Chou and Lin (2019) and the related read-across report (Chou and Lin, 2020) is an attempt to develop a comprehensive PBPK model framework for use in risk assessment. The main contribution of this research was to develop uncertainty distributions for different parameters and species, which were used in a somewhat traditionally defined PFOS model with more detailed GI and kidney compartments and simplified compartment for the rest of the body. Many starting parameters in the model were values optimized to data in the related Loccisano models, and since Bayesian-Markov chain Monte Carlo (MCMC) optimization did not appear to change these original values much (within 20% of the prior values), the overall parameter space was not very different compared to the Loccisano models. However, unlike the Loccisano suite of models for PFOS, free fraction in blood was modeled as a constant. The models demonstrated generally good agreement of predicted and observed values for the rat, mouse and monkey. For humans, the model failed to accurately predict organ levels (liver, kidney). The organ predictions for rats were generally accurate for one study used for validation. Similar to other PFAS PBPK models, this model relied on the same handful of animal studies and did not evaluate the vast majority of published data.

Although this model was further used for human risk assessment (Chou and Lin, 2020), its utility would be limited given poor predictive power at the organ level.

Two simplified human models have been reported for predictions of gestational and lactational transfer (Verner et al., 2016; Goeden et al., 2019). Verner et al. (2016) modeled mother and child as two compartments, with placental transfer (gestational) and lactational transfer (post-birth) parameters, and values for V_d and $T_{1/2}$, lactation, and growth characteristics summarized from available literature. Evaluating the Verner model with available plasma data in infants and children (Fromme et al., 2010; Granum et al., 2013; Mogensen et al., 2015a) provided acceptable fit (R^2 = 0.5-0.6 for regression of individual predictions). The model predicted a peak in infant plasma levels at cessation of breastfeeding, e.g., at 6 months for the Fromme et al. (2010) study. For PFOA, the child/mother plasma ratio reached 4.5 at the 50th percentile, 7.8 at the 95th percentile and a maximum 15.3. For PFOS these values were <1, 3 and 7, respectively. Much lower ratios for PFOS were attributed by the authors to less efficient transfer to milk.

The Goeden et al. (2019) model for PFOA was similar the the Verner model overall. Apart from slightly different parameter values, the main differences were: consideration for bottle-fed infants, adjusting V_d for age, and a detailed break-down of age-dependent water consumption rates. The model also considered central tendency as well as upper percentiles for its parameter values. This model demonstrated good fit of predicted to observed plasma data (e.g., R^2 = 0.7 for PFOA based on Fromme et al. (2010) data). The model also predicted a peak in plasma concentration in breast-fed infants at the age of termination of breast-feeding. At their peak, PFOA concentrations in infants were 6-fold higher than maternal serum at delivery.

Both human lactational models predicted an increase in plasma concentration, particularly for PFOA during the first year of life attributed to breast-feeding. This could be important for relevant developmental endpoints. However, it is important to emphasize that the exact level in infants depends on the mother's exposure and the context of exposure.

In addition to more complex models, multiple PFOA and PFOS exposure studies utilized a one-compartment model, typically to back-calculate exposure from serum concentrations (Washburn et al., 2005; Fromme et al., 2007; Trudel et al., 2008; Karrman et al., 2009; Vestergren and Cousins, 2009; Haug et al., 2010; Niisoe et al., 2010; Thompson et al., 2010; Egeghy and Lorber, 2011; Lorber and Egeghy, 2011; Shin et al., 2011; Lorber et al., 2015; Zhang et al., 2015; Gomis et al., 2017; Balk et al., 2019; Zhang et al., 2019b). These models had different assumptions for absorption efficiency, V_d and $T_{1/2}$ values; some of these issues are discussed later in this document.

There are several common conclusions regarding PFOA and PFOS PBPK models. Most models have been developed in series, with the rat PFOA model as the first. The rat PFOA models relied on the same limited number of studies, some unpublished, for optimization and validation, and at the expense of multiple other published kinetic studies. None of the published animal models have been extensively tested against published studies with kinetic data to determine overall prediction efficiency and dose range applicability, i.e., whether the models can predict serum concentrations outside the range used for their optimization. US EPA used the Wambaugh et al. (2013) model in their PFOA and PFOS risk assessment, but as it happens the studies used for the optimization of the model were also the critical studies chosen for dose-response in the toxicity assessment (US EPA, 2016b; US EPA, 2016d). It remains unclear, whether this or another model can be effectively applied to a study not used in the model

development, especially if the predicted doses or plasma concentrations are outside the range used for model optimization.

Despite well-established species differences in PFOA and PFOS toxicokinetics, physiological parameters optimized in a PBPK model or measured in vitro for one species are often used for a PBPK model in another species without adjustment. This is usually motivated by the lack of data in the species of interest but can also mask possible interspecies differences in physiological parameters. In order to better fit observed data, some models incorporated biologically implausible mechanisms, such as making certain physiological parameters time-dependent. Finally, although several human PBPK models were developed, only one was optimized at the organ level, with limited success (Fabrega et al., 2014), underscoring the need for more detailed human data.

Use of PFOA and PFOS PBPK models in risk assessment

The fact that there are dramatic differences in PFOA/PFOS half-lives between species necessitates incorporation of kinetic considerations in extrapolating dose from animal studies to humans. Even when evaluating human studies, which are often based on serum concentrations, kinetic considerations are important in conversion of the point of departure to an exposure concentration. Due to well understood challenges in this area, most animal and human PFOA and PFOS toxicity studies include plasma or serum level measurements in the reports, as detailed in Chapters 5 and 6 of this document.

The available PFOA and PFOS animal PBPK models have not been validated with the majority of published kinetic data and their use for the purposes of risk assessment would require an extensive review. OEHHA's work with two mouse models (Appendix 6, Table A6.6) suggested that models can err dramatically outside their optimized range and specifically at lower doses, which are of particular interest for POD determinations. However, since most animal studies of interest have reported serum concentrations, use of this metric appears to be least uncertain for the PFOA/PFOS assessment, and still fairly precise compared to modeled average concentrations when half-lives are much longer than the treatment interval.

In most animal species with half-lives at approximately 1 month (Table 4.1.1), day-to-day fluctuations in serum concentrations would be small compared to the steady state concentration, and using reported serum concentrations would add the least uncertainty to the dose-response analysis. Female and male mice at lower PFOA dose (1 mg/kg) demonstrate fairly long half-lives of 15-20 days (Table 4.1.1), and a similar argument applies, i.e., using reported serum concentrations would add the least uncertainty. While male rats demonstrate shorter PFOA $T_{1/2}$ estimates of 1.5-15 days (Table 4.1.1), fluctuations in serum concentrations and the resulting difference of the modeled average and reported serum concentration are expected to be small relative to other uncertainties in this risk assessment. In this case, using the reported serum concentration would provide a slightly more conservative dose metric of adverse effect, assuming levels were measured prior to daily dosing in a repeated dose experiment.

Due to the long half-lives of PFOA and PFOS in humans, a simple one-compartment model with first order elimination appears to be most effective in predicting serum concentration in humans (US EPA, 2016b; US EPA, 2016d). The use of this model ultimately requires determination of the clearance rate (CL), and three methods for CL calculation are considered in the following sections of this document. The first method for CL determination, previously employed by US

EPA for PFOS (US EPA, 2016d), involves independent estimates for $T_{1/2}$ and V_d, which are then used to calculate CL. The second method analyzes epidemiologic data in specific exposure situations, which allows direct CL calculation. This method was effectively used by US EPA for PFOA CL determination (US EPA, 2016b). The third method considers enterohepatic circulation of PFOA and PFOS as the limiting CL factor, and provides upper-bound CL estimates based on limited experimental data. The following sections of this document provide detailed review of published parameter estimates ($T_{1/2}$ and V_d) and application of these three methods to CL calculation.

4.7. Half-life ($T_{1/2}$) Estimate for PFOA and PFOS in Humans

Humans demonstrated longer half-lives than animals, on the order of years versus days or weeks. The available data are summarized in Table 4.7.1 for PFOA and in Table 4.7.2 for PFOS. For the calculation of the CLs, US EPA used $T_{1/2}$ estimates of 2.3 years for PFOA (Bartell et al., 2010) and 5.4 years for PFOS (Olsen et al., 2007). The Bartell et al. (2010) PFOA study was based on tracing the drop in serum concentrations in a subset of the C8 Panel following discontinued exposure from drinking water. Importantly, it was established that C8 Panel exposures were likely long enough to reach steady-state levels in exposed subjects (an important assumption in TK modeling). In this exposure scenario, very high initial exposures resulted in high initial serum concentrations and the ongoing exposures from other sources, such as diet, would have little effect on the time-dependent decrease of plasma concentrations. In a reanalysis of the C8 Panel data, with more subjects and longer follow-up time, the Li et al. (2017e) study produced a slightly higher $T_{1/2}$ value of 2.7 years, which appears to be the best available estimate for PFOA $T_{1/2}$ to date. The same robust analytical method applied to PFOA TK data from Ronneby in Sweden, also produced a PFOA $T_{1/2}$ estimate of 2.7 years (Li et al., 2017e; Li et al., 2018c). Thus, the estimated PFOA $T_{1/2}$ of **2.7 years** reported by Li et al. (2017e) and Li et al. (2018c) is deemed the most appropriate value for use by OEHHA.

Other comparable studies of drinking water exposure to PFOA, such as for the Arnsberg and Decatur sites (Brede et al., 2010; Worley et al., 2017b), produced PFOA $T_{1/2}$ estimates of 2.3-3.9 years, which are close to 2.7 years. Brede et al. (2010) investigated paired plasma concentrations following detection of elevated PFOA levels in drinking water in Ansberg, Germany. This study had shorter follow-up (2 years), mixed composition of subjects, including childen (who demonstrated TK properties distinct from adult participants), unclear exposure history and importantly, significantly lower water consumption in exposed individuals compared to controls. These factors would increase the uncertainty of the final estimated $T_{1/2}$ compared to those derived from the Li et al. (2017e, 2018c) studies.

Worley et al. (2017b) analyzed decreased PFOA levels in environmentally exposed subjects in Decatur, Alabama. While the follow-up was 6 years, the study involved relatively few participants (45, vs. 455 in the reanalysis of C8 Panel data by Li et al. (2017e)), exposure history was not clear, and most importantly, PFOA $T_{1/2}$ was estimated using a one-comaprtment TK model that required an assumption of a specific PFOA V_d value. As described in the following section, identifying a correct PFOA V_d value may pose its own problems, and therefore, this indirect $T_{1/2}$ estimation method would introduce additional levels of uncertainty.

While most studies estimate PFOA $T_{1/2}$ within 2.4-4.8 years, the encompassed values are mostly derived in situations of relatively high PFOA exposure, such as those occurring in Little Hocking, Ohio, in the C8 Panel studies. However, high starting PFOA exposures may not correctly predict kinetic behavior at lower environmental concentrations, and specifically with the

overall approximation of a first-order elimination model, high and low exposure scenarios may provide different $T_{1/2}$ estimates. Consistent with this hypothesis, Seals et al. (2011) found a longer $T_{1/2}$ value (8.5-10.1 years) in the C8 Panel participants with lower PFOA exposure.

Other types of PFOA $T_{1/2}$ studies, such as occupational studies or analysis of disappearance trend in the general population (Table 4.7.1) produce somewhat higher estimates, likely due to underestimating or ignoring ongoing exposures. Studies that include considerations of urinary clearance (Zhang et al., 2013b; Fu et al., 2016) are based on the premise of the predominance of renal elimination, and moreover, depend on a chosen V_d value. Similarly, the $T_{1/2}$ determination based on dynamic population modeling by Gomis et al. (2017) relied on an a priori chosen V_d value. As discussed in Section 4.8, estimation and subsequent use of a V_d value could bring an additional level of uncertainty to toxicokinetic analysis. Finally, Dourson et al. (2019), based on previously available kinetic data from controlled dosing of human cancer patients at relatively high levels, assumed that the steady state for PFOA exposure would be achieved by approximately 36 weeks and estimated plasma concentration at that point. Then, using the steady state assumptions, they determined the resulting $T_{1/2}$. In addition to a number of other issues, this evaluation assumed and did not experimentally determine the time to steady state. While the underlying kinetic data could be useful for further analysis, OEHHA does not consider the PFOA $T_{1/2}$ reported in the Dourson et al. (2019) study to be of sufficient quality or representative of environmental exposure.

Table 4.7.1. Human half-life estimates for PFOA

Reference	Population	N	$T_{1/2}$ (years)	Method
Burris (2002) as cited in Harada et al. (2005a)	Occupational	9	4.4	3M study, unpublished
Olsen et al. (2007)	Occupational	26	3.5^{GM}	Retired workers followed for 5 years
Spliethoff et al. (2008)	General (infants)	2,640	4.1	Disappearance $T_{1/2}$ due to declining levels
Costa et al. (2009)	Occupational	16	4.8	Former workers followed for 2-29 years
Brede et al. (2010)	Arnsberg, 2y	138	3.3	Drinking water pollution, decline
Bartell et al. (2010)	C8 Panel, 1y	200	2.3	Drinking water pollution, decline
Seals et al. (2011)	C8 Panel	1,573	2.9	Cross-sectional study, higher exposure
			8.5-10.1	Cross-sectional study, lower exposure
Zhang et al. (2013b)	General	66	2.8	Calculated from urinary clearance
Gomis et al. (2016)	Occupational	4	2.4	Accounted for ongoing exposure
Fu et al. (2016)	Occupational	207	11.7^{GM}	Calculated from urinary clearance
Gomis et al. (2017)	General	120	1.8-2.4	Population-based cross-sectional model
Li et al. (2017e)	Ronneby; C8 Panel, 4y	455	2.7	Drinking water pollution, decline
Worley et al. (2017b)	Decatur	45	3.9	Drinking water decline, one-compartment model with V_d assumption
Li et al. (2018c)	Ronneby	106	2.7	Drinking water pollution, decline
Dourson et al. (2019)	Clinical trial	NA	<0.7	Assumed steady state at given dose
Xu et al. (2020a)	Occupational	17	1.5-1.8	Airport workers followed for 5 months

^{GM} geometric mean; NA, not applicable.

The PFOS $T_{1/2}$ of 5.4 years used by US EPA was determined based on elimination kinetics in retired workers (Olsen et al., 2007). Several disappearance studies in the general population, which analyzed declining levels of PFOS in industrialized countries, produced lower values of 3.3-4.8 years (Yeung et al., 2013b; Gomis et al., 2017). Using rigorous analysis and high and discontinued environmental exposure data, Li et al. (2018c) estimated PFOS $T_{1/2}$ at **3.4 years**. This value is consistent with most recent reports (Table 4.7.2), and is chosen by OEHHA as the best estimate based on available evidence.

Table 4.7.2. Human half-life estimates for PFOS

Reference	Population	N	$T_{1/2}$ (years)	Method
Burris (2002) as cited in Harada et al. (2005a)	Occupational	9	8.7	3M study, unpublished
Olsen et al. (2007)	Occupational	26	5.4	Retired workers followed up for 5 years
Spliethoff et al. (2008)	General (infants)	2,640	4.4	Disappearance $T_{1/2}$ due to declining levels
D'Eon and Mabury (2011b)	Review of studies	NA	5.4	Disappearance $T_{1/2}$ due to declining levels
Glynn et al. (2012)	General	413	8.1	Disappearance $T_{1/2}$ due to declining levels
Olsen et al. (2012)	General	600	4.3	Cross-sectional, population based
Yeung et al. (2013b)	General	420	4.3-4.8	Disappearance $T_{1/2}$ due to declining levels
Zhang et al. (2013b)	General	66	22^{GM}	Calculated from urinary clearance
Fu et al. (2016)	Occupational	207	4.1^{GM}	Calculated from urinary clearance
Gomis et al. (2017)	General	120	3.3-5	Population-based cross-sectional model
Li et al. (2017e)	C8 Panel, 4y	455	3.7	Drinking water pollution, decline
Worley et al. (2017b)	Decatur	45	3.3	Probabilistic model of C8 Panel data
Li et al. (2018c)	**Ronneby**	**106**	**3.4**	**Drinking water pollution, decline**
Xu et al. (2020a)	Occupational	17	1.7-2.9	Airport workers followed for 5 months

^{GM} geometric mean; NA, not applicable.

Data are available on PFOA and PFOS plasma levels in California residents in several Biomonitoring California studies, with samples collected as early as 2011-2012 and as recent as 2018 (https://biomonitoring.ca.gov; these data have been reported in several publications (Hurley et al., 2016; Hurley et al., 2018; Kim et al., 2020). These studies demonstrated decreases in serum PFOA and PFOS levels in California residents over the 2009-2016 period. Kim et al. (2020) reported that in California mothers of young children (N=450), average PFOA serum concentration decreased from 1.65 ng/ml in 2009 to 0.7 ng/ml in 2016 and for PFOS, from 4.8 ng/ml in 2009 to 2 ng/ml in 2016. Assuming no additional input (disappearance method), these rates of decrease would correspond to $T_{1/2}$ estimates of about 6 years for either compound, higher than the chosen $T_{1/2}$ values derived by Li et al. (2017e) and Li et al. (2018c). Similar to the conclusion of the Seals et al. (2011) study, higher values could be due to lower exposure levels and/or continuous background exposures.

4.8. PFOA and PFOS Volume of Distribution (V_d) and Clearance Rate (CL)

Published estimates for PFOA and PFOS V_d values are presented in Table 4.8.1. These mainly comprise two types of studies, the first being studies with original TK experimental data and assessments, and the second being applied studies, such as those modeling human exposure, which derived novel V_d values based on previously published data and suited to the specific assumptions of the study.

Table 4.8.1. Estimates of V_d of PFOA and PFOS

Reference	Data Source	PFAS	Species	V_d (ml/kg)	Method
Harada et al. (2003)	Seacat et al. (2002)	PFOS	monkey (cynomolgus) 26 weeks	300 (corrected to 541 by OEHHA)[a]	Subchronic oral study, 1-compartment, ss assumption, although calculation appears to be incorrect[a]
Kemper et al. (2003) unpublished	same study	PFOA	rat	211 - 264	Single i.v. or oral dose, non-compartmental; reported in Vestergren and Cousins (2009)
Ohmori et al. (2003)	same study	PFOA	rat (m)	196	Single i.v. dose, serum concentrations fitted to 2-compartment model
			rat (f)	201	
Butenhoff et al. (2004a)	same study	PFOA	monkey (m)	181	Single i.v. dose, non-compartmental analysis; $V_d = \frac{Dose \times AUMC}{AUC^2}$
			monkey (f)	198	
Washburn et al. (2005)	Noker (unpublished)	PFOA	monkey (m)	1,810-5,210	Subchronic oral study, 1-compartment, steady state[b]; $V_d = \frac{Dose}{k_e \times C_{ss}}$
			monkey (f)	2,460-6,340	
	Butenhoff et al. (2002)		monkey (m)	1,260-3,730	
	Butenhoff et al. (2004b)		rat (m)	270	
			rat (f)	430	
	Palazzolo (1993), unpublished		rat (m)	100-550	
Vestergren and Cousins (2009)	Griffith and Long (1980)	PFOA	monkey (rhesus) 90 days	1,480-4,470	Subchronic oral studies; steady state with 1-compartment elimination ($T_{1/2}$=25 days); $V_d = \frac{Dose}{k_e \times C_{ss}}$
	Butenhoff et al. (2004a)	PFOA	monkey (cynomolgus) 6 months	1,300-4,470	
Niisoe et al. (2010)	Harada et al. (2007b)	PFOA	human	300	Mass balance for excretion through bile, assuming 0.88 efficiency for reabsorption and 3.5-year $T_{1/2}$
	Vestergren and Cousins (2009)	PFOA	human	464-780	95% confidence of the regression line for human intake-serum concentration; steady state assumption
Thompson et al. (2010)	Emmett et al. (2006), Dupont	PFOA	human	170	Steady state with 1-compartment elimination ($T_{1/2}$=2.3 years); $V_d = \frac{Dose}{k_e \times C_{ss}}$
Chang et al. (2012)	same study	PFOS	rat (m)	649-765	Oral or i.v. single dose in jugular-cannulated rat
			rat (f)	521-586	
			rat (m)	666-1,228	Single oral dose with long follow-up; data fit to non-compartmental model;
			rat (f)	468-484	
			mouse (m)	263-290	
			mouse (f)	258-261	

Reference	Data Source	PFAS	Species	V_d (ml/kg)	Method
			monkey (m) (cynomologus)	202	$V_d = \frac{Dose \times AUMC}{AUC^2}$
			monkey (f) (cynomologus)	274	
Fujii et al. (2015)	same study	PFOA	mouse (m)	180	Single i.v. dose, fitted to 2-compartment model; $V_d = Dose/C(0)$
			mouse (f)	150	
Kim et al. (2016b)	same study	PFOA	rat (m)	112	Single i.v. dose; $V_d = \frac{Dose \times AUMC}{AUC^2}$
			rat (f)	171	
		PFOS	rat (m)	383	
			rat (f)	351	
		PFOA	rat (m)	106	Single oral dose; $V_d = \frac{Dose \times AUMC}{AUC^2}$
			rat (f)	154	
		PFOS	rat (m)	280	
			rat (f)	289	
Iwabuchi et al. (2017)	same study	PFOA	rat (m)	150	Single oral dose, V_d calculated as ratio of dose to AUC and k_e; k_e from first order elimination model
		PFOS	rat (m)	960	
Huang et al. (2019a)	same study	PFOS	rat (m)	681[c]	Single i.v. dose, serum concentrations fitted to 2-compartment model
			rat (f)	421[c]	
			rat (m)	78.5-524[c]	Single oral dose, serum concentrations fit to 2-compartment model
			rat (f)	55.4-315[c]	
			rat (m)	299[c]	5-day gavage study, serum concentrations fit to 2-compartment model
			rat (f)	222[c]	
Dzierlenga et al. (2020)	same study	PFOA	rat(m)	153[c]	Single i.v. dose, serum concentrations fit to 2-compartment model
			rat(f)	207[c]	
			rat(m)	154-202	Single oral dose, serum concentrations fit to 1-compartment model
			rat(f)	79.2-342[c]	Single oral dose, serum concentrations fit to 2-compartment model

AUC, area under the curve; AUMC, area under the first moment curve; k_e, elimination constant; ss, steady state.

[a] The calculation is based on two C_{ss} points (Seacat et al., 2002): at 0.03 mg/kg-day, C_{ss}=16 mg/L (ppm) and at 0.15 mg/kg-day, C_{ss} = 80 mg/L (ppm). At steady state, intake = elimination, i.e., $Dose = V_d \times C_{ss} \times k_e$. Assuming that $k_e = ln2/T_{1/2}$, the equation can be rewritten as $V_d = Dose \times T_{1/2}/(C_{ss} \times ln2)$. With $T_{1/2}$ of 200 days, at 0.03 mg/kg-day: $V_d = 0.03 \times \frac{200}{16 \times 0.693} = 541\ ml/kg$, and at 0.15 mg/kg-day: $V_d = 0.15 \times \frac{200}{80 \times 0.693} = 541\ ml/kg$ as well, different from the reported 300 ml/kg. Furthermore, the assumption of steady state may not be quite correct in this case, since $T_{1/2}$ is estimated as 200 days, about equal to the duration of the study. In a 1-compartment model described as $C_{ss} = \alpha \times (1 - e^{-k_e \times t})$, serum concentration would equal ½ of the C_{ss} at $t = T_{1/2}$. Therefore, V_d could be further increased two-fold.

[b] For use in the human 1-compartment model (Washburn et al., 2005), V_d values derived from subchronic studies were much higher than 0.2 L/kg, based on a single dose rat study; however, "Discussion with Dr. Joseph Rodricks and Dr. John Butenhoff, the corresponding author of the cynomolgus monkey

toxicokinetic paper (Butenhoff et al., 2004a), confirmed that the fractional volumes of distribution based on the subchronic monkey study may be preferable."
[c] Sum of the central and peripheral compounds in a 2-compartment model.

A third type of study analyzed the fit of kinetic models to animal data and identified V_d or a comparable property through an optimization algorithm (Andersen et al., 2006; Wambaugh et al., 2013). These computational models describe the body as a single compartment or a series of compartments, and optimize the model parameters, which describe PFAS distribution and excretion, to fit TK datasets, such as plasma concentration profiles. Andersen et al. (2006) developed a simplified model for PFOA and PFOS in the monkey using kinetic data from a single i.v. dose study and from 6-month oral studies (Butenhoff et al., 2002; Seacat et al., 2002; Butenhoff et al., 2004a). In this model, PFAS was distributed between the central and peripheral compartments, and was excreted through a separate renal compartment with a renal reabsorption loop. The central compartment was described by its volume of distribution (V_{dc}). The peripheral (tissue) compartment was described in terms of compartmental transfer rate from central to tissues (k_{12}) and compartmental transfer rate from tissue back to central (k_{21}). Although the V_d for the tissue compartment was not formally defined, it can be calculated using the following formula (Wambaugh et al., 2013):

$$V_{dt} = \frac{k_{12} \times V_{dc}}{k_{21}}$$

The overall V_d can then be estimated as the sum of V_{dc} and V_{dt}. Table 4.8.2 presents parameter values estimated for chronic oral exposure in monkeys, and the resulting V_{dt} and V_d values.

Table 4.8.2. OEHHA-derived estimates of V_d from the computational model of Andersen et al. (2006)

PFAS	V_{dc} (ml/kg)*	k_{12} (1/h)*	k_{21} (1/h)*	V_{dt} (ml/kg), calculated	V_d (ml/kg), calculated
PFOA	140	3.3	0.1	4,620	4,800
PFOS	220	3.3	0.1	7,260	7,500

*Values reported in Andersen et al. (2006)

The resulting V_d values are much higher than the estimates for the central compartment due to the high predicted distribution of PFOA and PFOS to tissues. Similar to other V_d estimates obtained as optimized parameters in kinetic models of PFAS, these V_d values are subject to many uncertainties, and are less reliable than V_d values directly estimated from experimental data.

Previously used PFOA and PFOS CL and V_d estimates

To calculate PFOA and PFOS clearance rates (CL), US EPA utilized a two-step method:

1) identify V_d and $T_{1/2}$ from animal or human studies;
2) calculate CL using the formula $CL = V_d \times (\frac{ln2}{T_{1/2}})$, based on steady state assumption.

The calculated values for PFOA and PFOS CLs were 1.4×10^{-4} L/kg-day and 8.1×10^{-5} L/kg-day, respectively (US EPA, 2016b; US EPA, 2016d). Half-lives of 2.3 and 5.4 years were used, respectively, as described above. However, selection of V_d values poses new questions in light of the recently available data.

US EPA estimated a human PFOA V_d value of 170 ml/kg. It was derived from an epidemiologic study (C8 cohort) assuming steady state and $T_{1/2}$ of 2.3 years (Thompson et al., 2010). US EPA noted that this value is similar to estimates of V_d in monkey, obtained from a single dose experiment (Butenhoff et al., 2004a). A V_d of this magnitude would indicate primarily extracellular distribution of PFOA, and it has been criticized as likely too low when compared to higher V_d values obtained from subchronic monkey experiments with repeated dosing (Washburn et al., 2005; Vestergren and Cousins, 2009). It has been suggested that V_d estimates based on subchronic monkey studies would be preferable for chronic exposures in humans (Washburn et al., 2005).

Citing the lack of credible studies for a PFOS V_d, US EPA adopted the following strategy. Starting with the PFOA V_d of 170 ml/kg, a factor of 1.35 was applied based on the observation in the modeling paper of Andersen et al. (2006) that the optimized V_{dc} value for PFOS was 20-50% higher than the PFOA value. Thus, US EPA estimated a human PFOS V_d value of 230 ml/kg. Relying on a modeling study would not be optimal since compartment volumes are only some of the optimized parameters and the accuracy of their values can be compromised in order to obtain the best data fit. Moreover, as explained in the previous section, V_{dc} values determined in this study were not representative of the V_d, but rather of volume of the central compartment, which would have a much smaller contribution to the overall V_d in this model Finally, multiple published PFOS V_d studies are now available, obviating the need for indirect considerations.

Consideration of subchronic monkey studies for V_d estimates

Several subchronic monkey studies reported measurements of PFOA and PFOS steady state levels (Butenhoff et al., 2002; Seacat et al., 2002; Butenhoff et al., 2004a) that may be more appropriate for estimating V_d values than those obtained from single dose animal experiments (Table 4.8.1).

For example, in the Butenhoff et al. (2004a) study, daily oral doses of 3, 10 and 20 mg/kg administered to 4-6 monkeys/group over 6 months resulted in steady state serum concentrations of 81±40, 99±50 and 156±103 µg/ml, respectively. Based on these values and the steady state assumption, Vestergren and Cousins (2009) estimated V_d as 1,300-4,470 ml/kg, which is substantially higher than 181-198 ml/kg reported in the original study derived from a single-dose experiment (10 mg/kg) in male and female monkeys (Butenhoff et al., 2004a). Although serum concentration results for subchronic experiments were reported, the authors did not calculate the corresponding steady state V_d. However, they noted that the plasma levels at steady state were lower than expected, which would drive the corresponding V_d values higher. The authors suggested that this could be due to incomplete absorption (due to the fact that fecal PFOA dropped dramatically when the dietary exposure stopped), and secondly, due to the possibility that some PFOA retained in the body could be trapped in the enterohepatic loop and therefore be absent from the plasma pool. Both of these reasons appear plausible and none of the available subchronic monkey studies included controls to account for less than complete absorption and enterohepatic circulation. The uncertainty in the outcome of this approach also sheds light on the limitations of the steady state assumption. Similar to the monkey, enterohepatic circulation plays an important role in PFOA and PFOS PK in humans, necessitating an alternative enterohepatic circulation-based approach to estimating V_d and clearance rate.

Updated PFOA V_d estimate

Exposure to PFOA from the PFAS manufacturing plant at the border of Ohio and West Virginia (C8 Panel site) appears to have occurred at a constant level during 1985-2005 (Shin et al., 2011), after which the main source of exposure ceased and plasma levels started declining. Assuming PFOA $T_{1/2}$ at 2.3-2.7 years, the exposure duration was sufficient to reach steady state (about eight half-lives). Therefore, at the end of this period, V_d can be calculated using the following formula, where C_{ss} is plasma concentration at steady state:

$$V_d = Dose \times T_{1/2} \div (C_{ss} \times ln2).$$

Thompson et al. (2010) used this approach and calculated the applied dose, as daily amount of PFOA in consumed drinking water, averaged at two locations in the C8 region: in Lubeck, where PFOA in drinking water was 500 ng/L (500 ppt), and in Little Hocking, where PFOA in drinking water was 3,550 ng/L (3,500 ppt). These values are consistent with an independent report from the C8 Panel studies (Shin et al., 2011). However, predating the majority of C8 Panel studies, Emmett et al. (2006) was used as the source of serum concentration value for Little Hocking: specifically, 448 ng/ml in subjects using 'Little Hocking system water only' as their drinking water source (N=291). Besides this specified category, Emmett et al. (2006) reported mean serum concentrations for Little Hocking in general (N=478) and Belpre (N=14), another nearby location, as 478 and 321 ng/ml, respectively. For Lubeck, the average plasma concentration for non-occupationally exposed individuals (N=12) was 68 ng/ml (Emmett et al., 2006).

Reported serum values from the C8 Science Panel studies, which were exposure and health studies conducted in the same area one year later and on a large scale, are different from those reported by Emmett et al. (2006). Frisbee et al. (2009) reported 227.58, 42.96 and 92.36 ng/ml for Little Hocking, Belpre and Lubeck, respectively, with 82-87% population coverage (percent that participated in the study). While the Frisbee et al. (2009) C8 Panel samples were taken a year later than Emmett et al. (2006), such a large difference in values is unexpected given the long $T_{1/2}$ of PFOA. The more likely cause is the difference in sample selection, which in the Emmett et al. (2006) study included preliminary selection of households based on a certain expected level of air and water exposure supplemented with some number of volunteered samples. This selection may have introduced unaccounted for bias toward higher than average levels in plasma. In contrast, the C8 Panel studies, including Frisbee et al. (2009) aimed for full population coverage and randomization, and as such, have reported better average values for the population.

Applying the Thompson et al. (2010) approach to the calculation of V_d, using serum concentrations reported in Frisbee et al. (2009), and assuming a $T_{1/2}$ of 2.3 years, a reported average water consumption 1.4 L, absorption efficiency (f_a) 0.91 and a reported average body weight (BW) 71.8 kg, the resulting V_d, averaged between Little Hocking and Lubeck, equals **225 ml/kg**, as follows:

$$V_d = \frac{C_w \times 1.4\ L/d \times f_a \times T_{1/2}}{C_{serum} \times ln2 \times BW}$$

Little Hocking:

$$V_d = \frac{3{,}550 \times 1.4 \times 0.91 \times 839.5}{227.58 \times ln2 \times 71.8} = 335\ ml/kg$$

Lubeck:

$$V_d = \frac{500 \times 1.4 \times 0.91 \times 839.5}{92.36 \times ln2 \times 71.8} = 116\ ml/kg$$

Average: 225 ml/kg or 0.225 L/kg.

Similar to the original analysis, the calculation assumes 0.91 absorption efficiency. The resulting value for V_d is not much higher than the previously used 170 ml/kg (US EPA, 2016b) and appears much lower than the values derived from the subchronic monkey studies. The reasons for this discrepancy remain unclear, although one possible reason could be the choice of the PFOA $T_{1/2}$ value.

Updated PFOS V_d and CL estimates

Most published animal studies (Table 4.8.1) produced higher directly measured V_d estimates for PFOS than the value of 220 ml/kg derived by EPA using a kinetic model-based approximation. The only analysis relying on subchronic data (Harada et al., 2003) produced a V_d of 541 ml/kg when the calculation was corrected, as described in the footnote in Table 4.8.1. This calculation was performed with the assumption of steady state, even though the duration of exposure was only about one $T_{1/2}$. In a one-compartment model, plasma concentration at one $T_{1/2}$ would only reach 50% of the steady state level. Applying this adjustment results in a V_d estimate of 1,080 ml/kg. Substituting this V_d value and the PFOS $T_{1/2}$ value of 3.4 years into the clearance formula, the updated CL estimate is obtained:

$$CL = V_d \times (\frac{ln2}{T_{1/2}}) = 6.0\times10^{-4}\ \text{L/kg/day}.$$

Li et al. (2018c) described a PFOS exposure case from drinking water that was somewhat similar to the PFOA C8 Panel exposure scenario (Little Hocking and Lubeck). In 2013, the PFOS concentration in outgoing water from the two water works in Ronneby, Sweden was 8,000 ng/L, while median serum concentration in Ronneby residents exposed for at least 10 years was 372 ng/ml (Li et al., 2018c; Silva et al., 2020). While exposure was occurring from the mid-1980s, exact time-dependent levels are unknown. However, assuming that steady state was reached in 2013, V_d can be calculated as follows:

$$V_d = \frac{C_w \times 1.4\ L/d \times f_a \times T_{1/2}}{C_{serum} \times ln2 \times BW} = \frac{8{,}000 \times 1.4 \times 0.9 \times 1{,}241}{372 \times ln2 \times 70} = 693\ \text{ml/kg}.$$

In this calculation, daily water consumption was 1.4 L/day, absorption efficiency 0.9, $T_{1/2}$ was 3.4 years, and the default average body weight of 70 kg was applied.

The PFOS V_d estimate derived from human epidemiologic data produced a higher value (693 ml/kg) than that of PFOA (225 ml/kg). This can be due to experimental uncertainty or true chemical differences.

4.9. Alternative Approaches and Calculation of Clearance (CL)

Given the wide range for PFOA V_d estimates, additional approaches to data analysis should be considered. Moreover, direct determination of clearance may help to decrease uncertainty due to separate determinations of $T_{1/2}$ and V_d.

PFOA CL and V_d estimates based on biliary clearance

This approach has been previously described (Fujii et al., 2015). It is assumed that the PFOA overall CL (which can be expressed as the function of $T_{1/2}$ and V_d), is the sum of renal (CL_R) and fecal (CL_F) clearances. While CL_F cannot be measured directly due to experimental limitations, it can be expressed as PFOA biliary clearance adjusted for biliary reabsorption (f_b). Biliary reabsorption for PFOA would likely be similar to the overall absorption efficiency, which based on animal studies, is $f_b \geq 0.9$, as described in Section 4.2. The resulting formulas for the plasma clearance (CL) and V_d are:

$$CL = CL_R + (1 - f_b) \times \frac{C_{bile}}{C_s} \times Bile_flux$$

$$V_d = \frac{T_{1/2}}{ln2}(CL_R + (1 - f_b) \times \frac{C_{bile}}{C_s} \times Bile_flux).$$

Bile flux was estimated as 5 ml/kg-day (Davies and Morris, 1993). In two available studies with human patients, the ratio of bile to serum concentrations ($\frac{C_{bile}}{C_s}$) for PFOA was 0.21 and 0.25, with an average 0.23 (Harada et al., 2007b; Fujii et al., 2015). As described Section 4.5, the geometric mean of human CL_R for 6 studies was 0.060 ml/kg-day. Inputting these values in the formulas results in **CL ≤1.75x10^{-4} L/kg-day**, and V_d ≤248 ml/kg. Similar to other approaches, this method predicts a somewhat lower V_d for PFOA. The main limitations include the reliability of human CL_R and biliary clearance studies.

PFOA CL determination from exposure studies

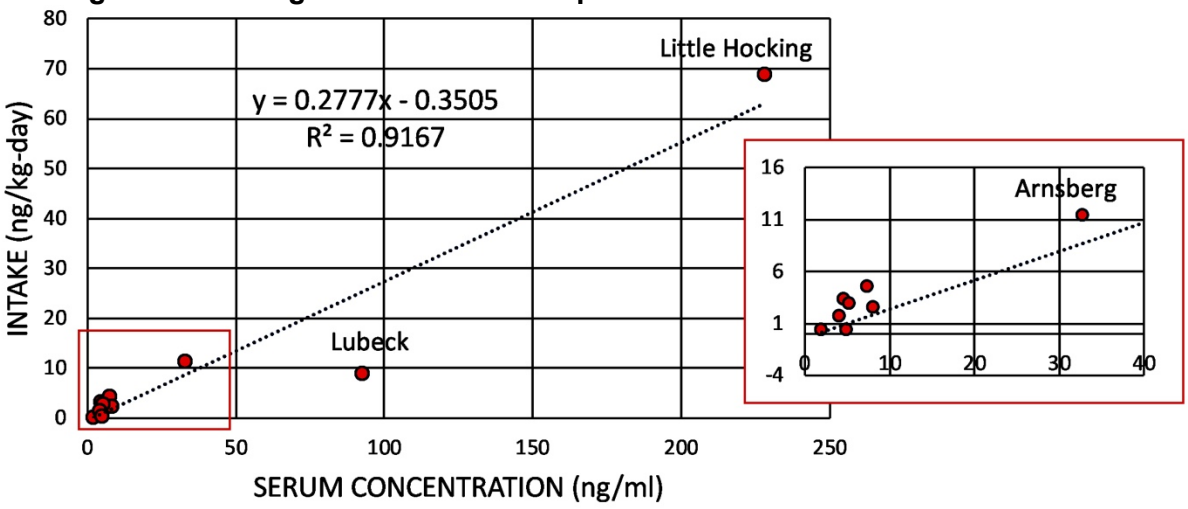
As an extension of the steady state-based approach used to estimate V_d based on Little Hocking and Lubeck data, as described above, other exposure studies that reported matching serum concentrations can be added to the dataset, and all data points can be fitted to a linear regression. In addition to the studies of exposure through polluted drinking water (Little Hocking, Lubeck, Arnsberg), several studies approximated exposure through all routes and some dietary studies reported diet as the primary exposure route. All these exposure assessments are assumed to occur at steady state levels. Details of the studies included in the dataset are provided in Table 4.9.1. The serum data for the Thompson et al. (2010) entry was updated as described in Section 4.8.

Table 4.9.1. PFOA Exposure studies that accounted for primary route(s) of exposure and reported plasma or serum concentrations

Reference	Population	Exposure	Intake (ng/kg-day)	Serum (ng/ml)
Fromme et al. (2007)	Bavaria, 2005	Diet	3.3 (females)	4.6
			4.4 (males)	7.4
Trudel et al. (2008)	North America, Europe, 1999-2007	All routes	2.5 (North America)	8.1
			2.9 (Europe)	5.3
Brede et al. (2010)	Arnsberg, Germany (men)	Drinking water	11.4	32.8
Thompson et al. (2010), Frisbee et al. (2009)	C8, Lubeck	Drinking water	9	92.4
	C8, Little Hocking		69	228
Haug et al. (2011a)	Norway, 2008	All routes	0.27	2
Lorber and Egeghy (2011)	USA, 2003-2004	All routes	1.6	4.1
Vestergren et al. (2012)	Sweden, 1999	Diet	0.35	5

The result of the linear regression for this data set is presented in Figure 4.9.1. The slope of the regression line in this graph is in fact clearance, which equals 0.28 ml/kg-day or **2.8×10^{-4} L/kg-day**.

Figure 4.9.1. Regression of PFOA exposure studies



PFOS CL determination based on the exposure through drinking water in Ronneby, Sweden

For PFOS, OEHHA considered a similar regression approach to that described in the previous section for PFOA, using all exposure studies. However, other than the scenario of increased intake from drinking water in Ronneby, Sweden (Li et al., 2018c), the rest of the available exposure assessments were at the lower range, rendering the regression approach non-informative. Therefore, analysis of the Ronneby data sets was more straightforward.

Three studies have been published that characterized exposure of Ronneby residents to PFOS in drinking water (Li et al., 2018c; Andersson et al., 2019; Silva et al., 2020). During approximately 1985-2013, about one third of households in Ronneby, Sweden received drinking water contaminated with PFAS from a nearby military airport. While the exact levels of

exposure throughout this period are not known, PFOS concentration in drinking water was 8,000 ng/L at the end of the exposure period (Li et al., 2018c). Extensive biomonitoring started approximately 6 months after the end of the exposure, and during the 2014-2016 time frame, 3,418 residents of Ronneby participated. The categorization of PFOS exposure within this population is presented in Table 4.9.2. Overall, this group comprised 7% of the Ronneby population in 2013, with a slightly higher percentage of females, 55% of study participants vs. 47% in the overall population of Ronneby (Andersson et al., 2019).

Table 4.9.2. Exposure[#] to PFOS in residents of Ronneby, Sweden

Reference	N	Exposure characterization	Descriptive statistic	C_{serum} ng/ml
Li et al. (2018c)	3,418	Original group: general population of Ronneby	mean±st.dev. median	245±234 176
Andersson et al. (2019)	2,347	Exposed for at least one year during 1985-2013	median[a]	222
	2,003	Exposed for at least one year during 2005-2013	median[a]	261
Silva et al. (2020)	1,845	Exposed for at least one year ending in 2013, <2 year olds excluded	median[b]	279
	1,176	Exposed for at least 10 years ending in 2013	median[b]	372
	506	Exposed for at least 29 years ending in 2013	median[b]	485

st.dev., standard deviation.
[#] Exposure is defined as residence at an address serviced by the affected water system.
[a] Separate values reported for men and women, averaged values presented here were calculated based on the reported numbers for each category.
[b] Separate values reported for men and women, averaged values presented here were calculated based on the reported percentages for each category.

According to the data presented in Table 4.9.2, the PFOS median serum concentrations increased with longer presumed exposure, which was determined as residence at an address serviced by the affected water system. The exposed individuals are more likely to attain the steady state (assumption for CL calculation) when exposed for longer periods of time, in this case for ≥10 years. This subgroup still contains a large number of subjects (N=1,176), increasing confidence in the choice of median as a reporting metric. In age composition, 64.6% of this group were 19-65 years, which is the population subgroup likely to be described by the steady state model. However, 24.3% were 66-94 years (Silva et al., 2020), the population subgroup with further increasing PFOS serum levels (Li et al., 2017e) that cannot be described by the steady state model. Such increases in older subjects have been described in other population studies of PFAS, and could be possibly attributed to declining kidney function with age, and as a result, decreased PFAS elimination. The fraction of these subjects in the '≥10 years' group is relatively low, and the resulting effect on serum concentration would be low.

In contrast, the fraction of 66-94 years comprises 45.7% of the '≥ 29 years' exposure group (Silva et al., 2020), and would violate the steady state assumption, were this group selected for analysis. Although longer confirmed exposure would be generally considered better for this type of analysis, the high fraction of the older age group and the overall lower number of participants (N=506) were among the reasons not to consider this subpopulation for analysis. Using these data (66 to94-year-olds) for clearance estimate would require a different kinetic

model and better understanding of the age-dependent changes in PFOA and PFOS excretion that currently exists.

The PFOS serum level reported for residents exposed for at least 10 years (ending in 2013) was 372 ng/ml (median, N=1,176). The method was the same as described in Section 4.8, except CL and not V_d was estimated, obviating the need for a $T_{1/2}$ estimate:

$$CL = \frac{C_w \times 1.4\ L/d \times f_a}{C_{serum} \times BW} = \frac{8{,}000 \times 1.4 \times 0.9}{372 \times 70} = 0.39 \text{ ml/kg-day} = \mathbf{3.9x10^{-4}\ L/kg\text{-}day}.$$

In this calculation, daily water consumption was 1.4 L/day, absorption efficiency 0.9, and average body weight 70 kg; C_w is the concentration of 8,000 ng/L in water.

PFOS CL and V_d estimates based on biliary clearance

Formulas for CL and V_d based on bile clearance developed above for PFOA in this document can also be applied to PFOS:

$$CL = CL_R + (1 - f_b) \times \frac{C_{bile}}{C_s} \times Bile_flux$$

$$V_d = \frac{T_{1/2}}{ln2}\left(CL_R + (1 - f_b) \times \frac{C_{bile}}{C_s} \times Bile_flux\right).$$

Bile flux was estimated as 5 ml/kg-day (Davies and Morris, 1993). The bile to serum ratio was 0.6 based on a study with 4 patients (Harada et al., 2007b). As described Section 4.5, the geometric mean of human CL_R for 6 studies was 0.016 ml/kg-day. Inputting these values in the formulas results in CL ≤ $3.16x10^{-4}$ L/kg-day, or V_d≤616 ml/kg. In conclusion, similar to other approaches, this method predicts a somewhat lower V_d for PFOS. The main limitations include the reliability of human CL_R and biliary clearance studies.

CL conclusions

Table 4.9.3 presents the summary of CL considerations developed in this document, labeled as possible options for this assessment. The corresponding V_d values are also included for comparison purposes. The CLs developed by US EPA (options A-1 and S-1 for PFOA and PFOS, respectively) were calculated using separately defined V_d and $T_{1/2}$ values. For PFOA, US EPA used the exposure data at the site of drinking water contamination in Ohio and West Virginia (C8 Panel) to calculate both V_d and $T_{1/2}$. While OEHHA concurs that these exposure conditions provided the best available system for estimating human V_d at the time, an updated calculation using a better quality report on serum concentrations (Frisbee et al., 2009) resulted in a higher PFOA V_d value (225 ml/kg, option A-2).

An alternative quantitation based on directly measured human CL_R and with consideration of PFOA biliary clearance suggested that PFOA V_d≤250 ml/kg (option A-4). In contrast, PFOA V_d estimates from chronic monkey studies (3,300 ml/kg) are much higher and may not be accurately applied to humans because of experimental limitations or species differences, such as decreased absorption efficiency in chronic gavage experiments in monkeys and inability to account for effects of biliary secretion/enterohepatic circulation of PFOA and extrapolate across species. Utilizing V_d from animal studies would introduce additional uncertainty in the assessment by relying on animal-to-human extrapolation of PK parameters. In this case,

OEHHA believes that using human data would be preferable and result in the least uncertainty; in addition, use of human data allows direct calculation of clearance, bypassing the separate steps of V_d and $T_{1/2}$ determination. In order to increase coverage of available human epidemiologic data, OEHHA performed a regression on human PFOA exposure data from multiple sites and exposure scenarios. The result of this analysis was a PFOA clearance rate of **$2.8x10^{-4}$ L/kg-day** (option A-3), which will be applied to conversion of serum levels to applied dose, as detailed elsewhere in this document. The alternative method considering biliary clearance of PFOA (option A-4) resulted in clearance rate of ≤$1.75x10^{-4}$ L/kg-day, which is fairly consistent with the preferred option A-3, given the underlying uncertainties of the method.

For PFOS, the previously used clearance rate value (option S-1) relied on several assumptions, and this approach should be updated given multiple newly available studies. This document outlines three distinct approaches to the updated PFOS clearance rate. Exposure to high PFOS levels in drinking water that occurred in Ronneby, Sweden, appears to be the best available data set for human exposures, and analysis of this data produced a reported $T_{1/2}$ of 3.4 years and clearance rate of $3.9x10^{-4}$ L/kg-day (option S-2). Using this $T_{1/2}$ together with the V_d from a chronic monkey study (1,080 ml/kg) would result in a CL of $6.0x10^{-4}$ L/kg-day. The alternative method considering biliary clearance of PFOS (option S-3) resulted in a CL ≤$3.2x10^{-4}$ L/kg-day, which is fairly consistent with option S-2. In summary, OEHHA considers option S-2 as the best available estimate for PFOS CL in humans, and will apply the PFOS CL of **$3.9x10^{-4}$ L/kg-day** to conversion of serum levels to applied dose, as detailed elsewhere in this document.

Table 4.9.3. Summary of V_d and CL considerations for PFOA and PFOS

Option	Method	$T_{1/2}$ (years)	V_d (ml/kg)	CL (10^{-4} L/kg-day)	References
PFOA					
A-1	Used by US EPA (2016a), based on human epidemiologic data; steady state	2.3	170	1.4	Emmett et al. (2006); Bartell et al. (2010); Thompson et al. (2010)
A-2	Updated Option A-1; human epidemiologic data; different reference for serum levels	2.3[a]	225	1.9	Frisbee et al. (2009); Bartell et al. (2010); Thompson et al. (2010)
A-3	Regression on exposure-serum data set; human epidemiologic data; steady state	-	-	**2.8**	Fromme et al. (2007); Trudel et al. (2008); Frisbee et al. (2009); Brede et al. (2010); Thompson et al. (2010); Haug et al. (2011a); Lorber and Egeghy (2011); Vestergren et al. (2012)
A-4	Upper-limit estimate based on human renal clearance and biliary clearance/reabsorption	2.7[a]	≤250	≤1.75	Harada et al. (2007b); Fujii et al. (2015); Li et al. (2017e)
PFOS					
S-1	Used by US EPA (2016b); steady state assumption; V_d derived as $1.35 \times V_d^{PFOA}$; human $T_{1/2}$	5.4	230	0.81	Andersen et al. (2006); Olsen et al. (2007); Thompson et al. (2010)

Option	Method	$T_{1/2}$ (years)	V_d (ml/kg)	CL (10^{-4} L/kg-day)	References
S-2	Human epidemiologic data (exposure-serum); steady state assumption	3.4[a]	1,160	**3.9**	Li et al. (2018c); Silva et al. (2020)
S-3	Upper-limit estimate based on human renal clearance and biliary clearance/reabsorption	3.4[a]	≤620	≤3.2	Harada et al. (2007b); (Li et al., 2018c)

[a]Used for V_d calculation only

5. EVIDENCE OF TOXICOLOGICAL EFFECTS

5.1. Immunotoxicity

NTP has previously reviewed the scientific literature on PFOS and PFOA and their relation to immunotoxicity (NTP, 2016). The NTP review included scientific information published up to May 18th, 2016. Based on this evidence, NTP reached the following conclusions for PFOA:

> "The NTP concludes that PFOA is presumed to be an immune hazard to humans based on a high level of evidence that PFOA suppressed the antibody response from animal studies and a moderate level of evidence from studies in humans. Although the strongest evidence for an effect of PFOA on the immune system is for suppression of the antibody response, there is additional, although weaker, evidence that is primarily from epidemiological studies that PFOA reduced infectious disease resistance, increased hypersensitivity-related outcomes, and increased autoimmune disease incidence. The evidence indicating that PFOA affects multiple aspects of the immune system supports the overall conclusion that PFOA alters immune function in humans."

With regards to PFOS, NTP reached the following conclusions (NTP, 2016):

> "The NTP concludes that PFOS is presumed to be an immune hazard to humans based on a high level of evidence that PFOS suppressed the antibody response from animal studies and a moderate level of evidence from studies in humans. Although the strongest evidence for an effect of PFOS on the immune system is for suppression of the antibody response, there is additional, although weaker, evidence that is primarily from studies in experimental animals that PFOS suppresses disease resistance and natural killer (NK) cell activity. The evidence indicating that PFOS suppresses multiple aspects of the immune system supports the overall conclusion that PFOS alters immune function in humans."

NTP's findings are consistent with assessments by US EPA (2016b), New Jersey DWQI (2017) and ATSDR (2018a), which evaluated much of the same immunotoxicity database and described the immunotoxic effects of PFOA and PFOS in animal studies and in humans. These assessments reported effects on the spleen and thymus of rodents (including changes in organ weight and lymphocyte populations), as well as a decreased ability of the immune system to respond to a challenge.

5.1.1. Recent Human Evidence

OEHHA's search strategy and study summary tables for the immunotoxicity of PFOA and PFOS in humans are in Appendix 7. All but two of the immunotoxicity studies OEHHA identified provided results for both PFOA and PFOS. Two studies reported information only for PFOS. This included the study by Xu et al. (2020b), which involved a community where the local water supply was contaminated with PFOS, and the study by Ammitzboll et al. (2019), which appeared to have measured both PFOA and PFOS but only presented immune results for PFOS. Six results were available for antibody responses, with the most common being response to tetanus and diphtheria vaccine. Thirty-five results were available for hypersensitivity-related outcomes including eleven for asthma, nine for eczema, seven for rhinitis, three for immunoglobulin E (IgE) levels, and five for allergy. Thirteen results were available for an infectious disease outcome or related symptom such as gastroenteritis or fever. Three studies provided results for C-reactive protein (CRP) or cytokine levels. Twelve studies

were prospective cohort studies, five were cross-sectional only, and six presented both prospective and cross-sectional results. One study was based on a case-control design but with a cross-sectional assessment of exposure and outcome. Studies were done in a variety of locations including the US, Faroe Islands, Norway, China, and Japan.

Antibody response: Most studies of PFOA and PFOS and antibody response, including those identified by OEHHA and those reviewed by (NTP, 2016), investigated anti-diphtheria and anti-tetanus immunoglobulin G (IgG) levels. The large majority of results on these outcomes are based on two cohorts of pregnant women and their offspring from the Faroe Islands. One of these cohorts involved children born in 1997-2000 (the "1997-2000 birth cohort") and the other involved children born in 2007-2009 (the "2007-2009 birth cohort"). In both studies, concentrations of PFOA and PFOS were measured in maternal serum during pregnancy and in the serum of offspring at various ages after birth. Children from the 1997-2000 birth cohort have been followed up to 13 years of age, and the children from the 2007-2009 cohort have been followed up to 5 years of age. The study by Kielsen et al. (2016) (reviewed in US EPA (2016b)) is the only other study besides the Faroe Islands cohorts to investigate associations between PFOA or PFOS and antibody response to diphtheria or tetanus vaccine. This study was done in twelve adults from Copenhagen, Denmark.

A summary of the results from all studies of PFOA or PFOS and antibody response to tetanus or diphtheria vaccine, either before or after the (NTP, 2016) review, are shown in Table 5.1.1. Most of these results are from various follow-up periods for the 1997-2000 Faroe Islands cohort. All studies presented results in terms of the percentage decrease in antibody levels associated with a two-fold increase in serum PFOA or PFOS levels. As seen in Table 5.1.1, the results vary greatly depending on the timing of the exposure and outcome assessment. However, the large majority of results are consistent with a decline in antibody levels with increasing PFOA or PFOS levels (i.e., the percent change in antibody levels with increasing PFOA or PFOS levels was negative). Many of these results, although not all, show a greater than 10-20% decrease in antibody levels for each two-fold increase in PFOA or PFOS, and several of these results are statistically significant. Overall, 69-85% of all studies' results show at least some decrease in antibody levels with increasing PFOA or PFOS exposure (Table 5.1.2), and in 10-45% of these, the decreases are statistically significant. These associations were seen in both cross-sectional and prospective analyses. The most consistent findings are for diphtheria vaccine response and PFOA, where 85% of results show a decrease in antibody levels with increasing PFOA levels and 45% of these results are statistically significant. In all three cohorts that evaluated diphtheria vaccine response (the Faroe Islands 1997-2000 cohort, the Faroe Islands 2007-09 cohort, and Kielsen et al. (2016)) at least some evidence of an inverse relationship between PFOA and PFOS and antibody response was seen (Table 5.1.1).

NTP (2016) identified several studies that evaluated associations between PFOA and PFOS and antibody response to vaccines other than those for diphtheria and tetanus, including mumps, measles, rubella (MMR), and influenza. Most of these studies found some evidence that IgG levels decreased with increasing PFOA or PFOS serum concentrations, although not all results were statistically significant and non-linear dose-response patterns were seen in some studies (Table 5.1.3). In the two studies published since the NTP (2016) review, the results were mixed. Using data from the US National Health and Nutrition Examination Survey (NHANES), Pilkerton et al. (2018) identified statistically significant inverse associations between both PFOA and PFOS and rubella IgG titers in adults but not in older children (Table 5.1.3). In contrast, in a study of 75 US adults, odds for influenza H1N1 seroconversion were higher in

those with higher PFOA or PFOS serum concentrations, although findings were borderline or not statistically significant (Stein et al., 2016a).

Table 5.1.1. Results of epidemiologic studies of PFOA or PFOS and anti-tetanus or anti-diphtheria vaccine response at different ages. Values are the percent change in IgG levels for each 2-fold increase in PFOA or PFOS concentration (please read the footnotes of this table, they are important for its understanding and interpretation).

PFOA	Outcome									
	Diphtheria					Tetanus				
	5 years pre	5 years post	7 years	13 years	Adult	5 years pre	5 years post	7 years	13 years	Adult
0	-16.2 **-18.9**[a*]	-6.2	**-22.8**			-10.5 **-22.2**[a*]	14.5	7.4		
1.5 years	4.2*					**-16.3***				
5 years	-6.8 18.3[a*]	-6.1	**-25.2**			-13.3 **-25.3**[a*]	-9.7	**-35.8**		
7 years			**-25.4**	-9.2*				-20.5	2.9*	
13 years				**-25.3***					-5.6*	
Adult					-8.2[b]					0.23[b]

PFOS	5 years pre	5 years post	7 years	13 years	Adult	5 years pre	5 years post	7 years	13 years	Adult
0	**-38.6** -14.0[a]	-20.6	-10.0			-10.1 -10.8[a]	-2.3	35.3		
1.5 years	17.5					-7.0				
5 years	-16.0 17.1[a]	-15.5	**-27.6**			-11.9 -9.1[a]	**-28.5**	-23.8		
7 years			**-30.3**	-25.6				-9.1	**45.4**	
13 years				-10.5					23.4	
Adult					**-11.9**[b]					-3.6[b]

* Studies published since the NTP (2016) review
"Pre" and "post" refer to pre- and post-vaccination antibody levels
All results are from the 1997-2000 Faroe Islands cohort except as noted below:
[a] 2007-09 Faroe Islands cohort
[b] Kielsen et al. (2016)
Bolded numbers in the column and row headings are the ages when the PFOA or PFOS (left most column) or IgG levels (third row) were measured. "0" represents maternal serum PFOA or PFOS levels measured during gestation; "pre" and "post" are pre- and post-immunization values at age 5.
Bolded results are statistically significant.
Red boxes represent cross-sectional evaluations; all others are prospective.
Example: in the box marked in blue, the upper value of -16.2 is the percent change in pre-vaccination diphtheria IG levels at 5 years of age for each 2-fold increase in PFOA at birth seen in the 1997-2000 Faroe Islands cohort. The lower value is the corresponding results from the 2007-09 Faroe Islands cohort (hence it is marked with an "a"). This lower number is statistically significant so it is bolded.
This table does not include the results of Abraham et al. (2020) (reviewed below).

Table 5.1.2. The numbers and percentages of studies of PFOA or PFOS and tetanus or diphtheria antibody levels showing inverse and/or statistically significant associations

			Total results			Inverse association			Statistically significant inverse association			Statistically significant positive association		
Exposure	**Outcome**	**N/%**	**Total**	**Cross**	**Pros**	**Total**	**Cross**	**Pros**	**Total**	**Cross**	**Pros**	**Total**	**Cross**	**Pros**
PFOA	Diphtheria	N	13	6	7	11	5	6	5	2	3	0	0	0
		%[a]							45%	40%	50%	0%	0%	0%
		%[b]				85%	83%	86%	38%	33%	43%	0%	0%	0%
	Tetanus	N	13	6	7	9	5	4	4	1	3	0	0	0
		%[a]							44%	20%	75%	0%	0%	0%
		%[b]				69%	83%	57%	31%	17%	43%	0%	0%	0%
PFOS	Diphtheria	N	13	6	7	11	5	6	4	2	2	0	0	0
		%[a]							36%	40%	33%	0%	0%	0%
		%[b]				85%	83%	86%	31%	33%	29%	0%	0%	0%
	Tetanus	N	13	6	7	10	5	5	1	1	0	1	0	1
		%[a]							10%	20%	0%	10%	0%	20%
		%[b]				77%	83%	71%	8%	17%	0%	8%	0%	14%

"Inverse association" refers to results showing decreasing IgG levels with increasing PFOA or PFOS concentrations; "Positive association" refers to increasing IgG levels with increasing PFOA or PFOS concentrations
Abbreviations: Cross, cross-sectional study; N, number of studies; Pros, prospective study
[a] Percentage of all results indicating an inverse association
[b] Percentage of all results
This table does not include the results of Abraham et al., 2020 (reviewed below)

Table 5.1.3. Epidemiologic studies on associations between PFOA or PFOS and antibody response to other vaccines

Vaccine	Exposure timing	Change in antibodies with PFOA[1]	Change in antibodies with PFOS[1]	Possible sources of heterogeneity	Reference
Rubella	Maternal 0-3 day post delivery	**-0.4 (-0.64 to -0.17)**	**-0.08 (-0.14 to -0.02)**	• developmental exposure metric	Granum et al. (2013)
	Children: current	**-8.9 (-14.6 to -2.9)**	**-13.3 (-19.9 to -6.2)**	• childhood exposure metric booster vaccination	Stein et al. (2016b)
	Adults	**p=0.002**	**p=0.03**	• outcome in all adults combined only F values for decline in IgG reported	Pilkerton et al. (2018)[2]
	Children 12-18 years old: current	p=0.80	p=0.25	• childhood exposure metric only F values for decline in IgG reported	Pilkerton et al. (2018)[2]
Mumps	Children: current	**-6.6 (-11.7 to -1.5)**	**-5.9 (-9.9 to -1.6)**	• childhood exposure metric booster vaccination	Stein et al. (2016b)
Measles	Maternal 0-3 day post delivery	-0.13 (-0.35 to 0.09)	-0.05 (-0.1 to 0.01)	• developmental exposure metric	Granum et al. (2013)
	Children: current	-3.4 (-16.7 to 11.9)	-2.9 (-17.3 to 13.9)	• childhood exposure metric booster vaccination	Stein et al. (2016b)
Influenza	Adult at vaccination	Antibody titer ratio 2nd -0.10 (-0.3 to 0.1) 3rd -0.07 (-0.28 to 0.14) **4th -0.22 (-0.43 to -0.01)** Antibody titer rise **2nd -0.28 (-0.51 to -0.06)** **3rd -0.37 (-0.60 to -0.13)** 4th -0.12 (-0.36 to 0.13)	Antibody titer ratio 2nd -0.06 (-0.26 to 0.14) 3rd -0.02 (-0.18 to 0.23) 4th -0.03 (-0.24 to 0.19) Antibody titer rise 2nd 0.03 (-0.19 to 0.26) 3rd 0.18 (-0.00 to 0.41) 4th -0.04 (-0.28 to 0.21)	• influenza H3N2 outcome is antibody rise or ratio by quartile of PFOA or PFOS compared to the first quartile; negative values indicate a decrease in antibody levels outcome in adults	Looker et al. (2014)

Vaccine	Exposure timing	Change in antibodies with PFOA[1]	Change in antibodies with PFOS[1]	Possible sources of heterogeneity	Reference
	Adult at vaccination	OR = 6.8 (1.0-48.1) p-trend = 0.07 (hemagglutinin inhibition) OR = 1.8 (0.7-4.3) p-trend = 0.27 (immunohistochemistry)	OR = 1.3 (0.2-7.3) p-trend = 0.81 (hemagglutinin inhibition) OR = 2.4 (0.9-6.6) p-trend = 0.12 (immunohistochemistry)	• influenza H1N1 ORs for seroconversion response to FluMist fourth vs. first quartile of PFOA or PFOS outcome in adults	Stein et al. (2016a)

Abbreviations: OR, odds ratio
Results in parentheses are 95% confidence intervals. Bolded results are statistically significant
Results published since the NTP (2016) review are highlighted in red
Data are presented in the format used by the NTP NTP (2016)
[1] Percent change in antibody concentration per 2-fold increase in PFOA or PFOS unless otherwise noted
[2] For Pilkerton et al. (2018), only F values are given
This table does not include the results of Abraham et al. (2020) (reviewed below)

Budtz-Jorgensen and Grandjean (2018) have published a benchmark dose (BMD) analysis of PFOA and PFOS and altered antibody response to tetanus and diphtheria vaccine using data from the 1997-2000 and 2007-2009 Faroe Islands cohorts. Individual data from these cohorts are not publically available. In one set of analyses, prenatal serum PFAS concentrations and IgG levels at age 5 years were assessed using combined data from the 1997-2000 and 2007-2009 cohorts. In another set of analyses, serum PFAS concentrations at age 5 years and IgG levels at age 7 years were assessed. Since data at age 7 years were not available from the 2007-2009 cohort, this later set of analyses only involved data from the 1997-2000 cohort. Analyses were done for each PFOA and PFOS separately, both adjusted and unadjusted for each other. A benchmark response of 5% was used. The benchmark doses were thus the PFOA or PFOS serum concentrations associated with a 5% decrease in anti-tetanus or anti-diphtheria IgG levels after vaccination, and the BMDLs were the lower one-sided 95% confidence intervals of these BMDs. Several dose-response models were evaluated, with the lowest BMD values and the best model fit occurring with the piece-wise linear model. The major characteristic of this model compared to a linear model is that the dose-response slope is allowed to change at the median exposure. Other variables in the models were age, sex, cohort (1997-2000 or 2007-2009), and booster type at age 5 (for analyses of IgG at age 7).

The results of the Budtz-Jorgensen and Grandjean (2018) BMD analyses are shown in Table 5.1.4. The lowest BMDs are for the analyses of PFOA at age 5 years and IgG levels at age 7 years. Adjustment of the PFOA BMD calculations for PFOS led to somewhat higher BMDs in most but not all cases. However, these adjustments overall had only small impacts on BMDLs, likely due to the increased variance that occurred from having two correlated variables (PFOA and PFOS) in the same model. Some of the BMDLs are lower in the analyses involving age 7 IgG levels than in the analyses of age 5 IgG levels. This may be partially due to the fact that the latter involves only the 1997-2000 cohort and therefore a smaller sample size. Overall, the lowest BMDLs were 0.10 and 0.12 ng/ml, which were seen in the unadjusted and adjusted analyses, respectively, of prenatal PFOA exposure and anti-diphtheria IgG levels at age 5 years. Importantly, these BMDLs appear to be well outside the range of PFOA and PFOS values measured in this cohort. That is, although the actual values for the measured PFOA and PFOS concentrations at age 5 were not specified numerically, they were displayed graphically and the lowest measured values appear to be about 6-7 ng/ml for PFOS and 1.2-1.3 ng/ml for PFOA (Grandjean et al., 2012). As such, these BMDLs are approximately 6- to 13-fold lower than the lowest measured value.

Table 5.1.4. Benchmark dose (BMD) modeling results using the piece-wise linear model from the Faroe Islands cohorts (Budtz-Jorgensen and Grandjean, 2018)

	Prenatal PFAS exposure and age 5 IgG (1997-2000 and 2007-2009 cohorts)						Age 5 PFAS exposure and age 7 IgG (1997-2000 cohort only)				
	Unadjusted			Adjusted for other PFAS			Unadjusted			Adjusted for other PFAS	
Tetanus	BMD	BMDL		BMD	BMDL		BMD	BMDL		BMD	BMDL
PFOS	2.59	1.05			1.64		1.45	0.56		3.57	0.72
PFOA	0.25	0.13		0.25	0.13		0.52	0.16		0.67	0.17
Diphtheria											
PFOS	1.62	0.83		2.51	0.95		0.98	0.49		1.21	0.54
PFOA	0.15	0.10		0.21	0.12		0.48	0.17		1.06	0.20

All numbers are in ng/ml serum
Some BMDs were not calculated or not provided by the authors. Explanation not provided.

Infectious diseases: In its review, NTP concluded that there was "low confidence" that PFOA was associated with infectious disease outcomes (NTP, 2016). This rating was based on the small number of studies on any specific outcome and the overall inconsistency of the findings that were available at the time. For most outcomes, OEHHA identified similar weaknesses in the literature published since the NTP review (NTP, 2016). For example, while a statistically significant association between increasing PFOA serum concentrations and increasing risk for fever has been reported (Dalsager et al., 2016), this outcome has not been examined in any other study (either before or after the NTP review). For other outcomes such as colds or gastroenteritis, studies published since the NTP review did not identify clear associations (Appendix 7, Table A7.3). Statistically significant increases with increasing PFOA were seen in three of the four studies of lower respiratory tract infections in children published since the NTP review (Impinen et al., 2018; Impinen et al., 2019; Kvalem et al., 2020), although clear increases were not seen in the large prospective Spanish study by Manzano-Salgado et al. (2019).

NTP also concluded that there was "low confidence" that PFOS was associated with infectious disease outcomes (NTP, 2016). Most of the studies NTP reviewed found no association with outcomes such as gastroenteritis, colds, or flu. One study found an association between PFOS and hospitalizations for any infectious disease, but only in females (Fei et al., 2010). Studies published since the NTP (2016) review on PFOS and gastroenteritis, colds, and cough have not identified clear associations (Table A7.3). However, statistically significant associations have been seen in studies in children for "any infection" (not necessarily hospitalizations) (Goudarzi et al., 2017), fever (Dalsager et al., 2016), gastroenteritis (Impinen et al., 2018), and lower respiratory tract infections (Impinen et al., 2018; Impinen et al., 2019; Kvalem et al., 2020) (Table A7.3). Links between PFOS and fever or lower respiratory tract infections were not examined in any of the studies reviewed by NTP (NTP, 2016).

Hypersensitivity: In their review, NTP (2016) found that that increasing serum concentrations of PFOA in children were consistently associated with asthma, increased total IgE, and several other indicators of immune hypersensitivity. However, NTP rated this evidence as "low confidence" primarily due to the cross-sectional nature of these studies.

Since the NTP (2016) review, at least three more cross-sectional studies in children have found associations between PFOA and asthma (Qin et al., 2017; Timmermann et al., 2017; Averina et

al., 2019). An association was also recently reported between serum PFOA concentrations at age 5 years and whether a child had ever had asthma up to age 13 years but only in children who had not received an MMR vaccination before age 5 (Timmermann et al., 2017). Three of the six studies evaluating possible associations between maternal or cord blood PFOA levels and childhood asthma reported elevated ORs in those with higher PFOA levels although these findings were not statistically significant (Goudarzi et al., 2016; Impinen et al., 2018; Beck et al., 2019) (Table A7.3). The three others reported mixed or negative results.

Most studies on PFOA and eczema published since the NTP (2016) review have not found clear or consistent associations. In a study of cord blood PFOA levels and childhood eczema up to age 24 months, an association was seen in females (OR = 2.52; 95% CI, 1.12-5.68) but not in males (OR not provided) (Chen et al., 2018b). Wen et al. (2019b) reported an elevated OR for eczema (OR = 1.89; 95% CI, 1.10-3.16), with the highest risks in those with the GST-T1null phenotype. None of the other studies on PFOA and eczema published since the NTP review found similar associations. Several of the recent studies assessing rhinitis found ORs greater than 1.2, but none were statistically significant. Recent studies on PFOA and IgE levels or allergy outcomes did not find clear or consistent associations.

For PFOS, NTP concluded that there was low confidence that exposure during childhood is associated with increased hypersensitivity responses based on the human studies they reviewed (NTP, 2016). While noting that several studies did identify associations between PFOS and asthma or serum IgE levels, the cross-sectional nature of these findings led to NTP's rating of low confidence. Most of the studies of PFOS and asthma published since the NTP review have not found clear associations. This includes two prospective studies of maternal serum or cord blood PFOS levels and asthma in young children (Goudarzi et al., 2016; Impinen et al., 2018). Three of the four studies of PFOS and lower respiratory tract infections published since the NTP review reported statistically significant associations between increasing PFOS exposures and this outcome in children (Impinen et al., 2018; Impinen et al., 2019; Kvalem et al., 2020). All of these were prospective studies. Clear or consistent associations were not seen in any of the more recent studies OEHHA reviewed of PFOS and eczema, IgE, or rhinitis.

Other outcomes: With regards to autoimmunity outcomes, NTP (2016) identified two studies that reported associations between PFOA exposures and ulcerative colitis. However, NTP rated this body of evidence as "low confidence" since both studies involved participants from the same study area. In the only study to examine a similar outcome since the NTP review, a clear association was not seen between PFOA and inflammatory bowel disease (Hammer et al., 2019), although the sample size was small (N=37 cases). NTP only identified one study of PFOS and an autoimmunity outcome. This study reported that prenatal concentrations of PFOS were associated with decreases in anti-actin IgG in a test for antibodies to several neural or non-neural antigens in 7-year-old children from the Faroe Islands (Osuna et al., 2014). This finding has not been replicated in another population. Two studies of PFOS and inflammatory bowel disease published since the NTP review have reported mixed results (Hammer et al., 2019; Xu et al., 2020b)(Hammer et al., 2019; Xu et al., 2020).

Abraham et al., 2020: This study was published after OEHHA's main literature search so is not included in the summary tables in Appendix 7. It is reviewed here because it provided information on diminished vaccine response, one of the outcomes OEHHA considered a potential critical effect. Briefly, this study used a cross-sectional design to investigate associations between diminished vaccine response and serum levels of PFOA and PFOS in one-year-old children living near Berlin, Germany. It included 101 children (51 boys and 50

girls) whose parents were recruited using announcements in newspapers and through pediatricians. Serum samples were collected in 1997-98 and stored frozen at -80 °C. The study was originally designed to investigate the effects of dioxins, PCBs, pesticides, heavy metals, and other environmental contaminants on the immune system and other biological parameters. Although not clearly described, it appeared that the researchers attempted to recruit children from industrial areas near Berlin, where exposure to these agents was likely to be high. In fact, levels of lead and several of the other agents were elevated, and serum levels of some of these (specifically PCBs and dioxins) were highly correlated with serum levels of PFOA and PFOS. The researchers reported statistically significant associations between increasing levels of PFOA and decreasing concentrations of diphtheria, influenza, and tetanus antibody. Results were adjusted for time since vaccination and the number of vaccinations. Similar associations were not seen for PFOS. A multivariate analysis using the function 'stepAIC' in the statistical program R with inclusion of dibenzo-p-dioxins (PCDDs), dibenzofurans (PCDFs), ndl-PCBs in addition to PFOA and PFOS revealed a highly significant influence of PFOA only. This study is discussed further in Chapter 6.

Other significant studies identified between January 2, 2020 (i.e., the end date of OEHHAs initial full literature review) and December 31, 2020 are listed in Appendix 7, Table A7.29.

5.1.2. Recent Animal Evidence

In addition to NTP (2016), US EPA (2016b) and New Jersey DWQI (2017) also evaluated the immunotoxicity of PFOA and PFOS. OEHHA evaluated animal studies from 2016 onward, and identified many toxicity endpoints related to immunotoxicity. Observed toxicity included changes in spleen and thymus weights, changes in lymphocyte populations, changes in cytokine levels, and immunosuppression. Studies for PFOA are summarized in Table 5.1.5.

Table 5.1.5. Summary of recent animal toxicity studies of PFOA reporting immune toxicity

Sex/Species	Exposure	Endpoints	NOAEL/LOAEL	Reference
Female C57BL/6N PPARα KO and WT mice (6/dose/group)	0, 7.5 or 30 mg/kg-day in drinking water for 15 days	**WT**: ↓ relative spleen and relative thymus weights; **WT and KO**: ↓SRBC-specific IgM antibody responses	LOAEL: 7.5 mg/kg-day for ↓ relative thymus weight in WT mice	DeWitt et al. (2016)
Female C57BL/6N mice (8/dose)	0, 0.94, 1.88, 3.75, or 7.5 mg/kg-day in drinking water for 15 days	↓ dinitrophenyl-ficoll (DNP)-specific IgM antibody response; ↓ relative spleen and thymus weight	NOAEL: 0.94 mg/kg-day for ↓ antibody response	DeWitt et al. (2016)
Female C57BL/6N mice (4/dose/group)	0, 3.75 or 7.5 mg/kg-day in drinking water for 10, 13 or 15 days	changes in splenic lymphocyte subpopulations	LOAEL: 3.75 mg/kg-day	DeWitt et al. (2016)

Sex/Species	Exposure	Endpoints	NOAEL/LOAEL	Reference
Male ICR mice (5/dose)	Treated mice were sensitized with OVA to induce active systemic anaphylaxis on days 0 and 7. OVA + 100 or 150 mg/kg 3 times on days 9, 11, and 13 orally[b]. Control mice had 150 mg/kg PFOA only or OVA only.	↓ rectal temperature; ↑ serum histamine, TNF-α, IgG1 and IgE levels	LOAEL: 100 mg/kg for ↑ TNF- α and IgE levels in sensitized mice	Lee et al. (2017)
Male Sprague Dawley rats (10/dose)	0, 150, or 300 ppm in feed (0, 14.7, or 29.5 mg/kg-day) for 16 weeks	↓ absolute and relative spleen weight; lymphoid follicle atrophy	LOAEL: 14.7 mg/kg-day for ↓ absolute and relative spleen weight	NTP (2020)
Female Sprague Dawley rats (10/dose)	0, 300, or 1,000 ppm in feed (0, 27.7, or 92.7 mg/kg-day) for 16 weeks	pigment in spleen	LOAEL: 27.7 mg/kg-day	NTP (2020)

[a] LOAEL/NOAEL not applicable for single dose studies.
[b] The specific manner of oral administration was not stated in study.
Abbreviations: GD, gestation day; IgE, immunoglobulin E; IgM, immunoglobulin M; IL-22, interleukin-22; KO, knockout; LOAEL, lowest-observed-adverse-effect level; NOAEL, no-observed-adverse-effect level; OVA, ovalbumin; PPARα, peroxisome proliferator-activated receptor alpha; SRBC, sheep red blood cells; TNF-α, tumor necrosis factor alpha; WT, wild-type

Immunotoxicity studies for PFOS published from 2016 onward are summarized in Table 5.1.6. Similar immunotoxic effects to PFOA were observed in these recent studies, including changes in spleen and thymus weight, changes in lymphocyte populations, and changes in cytokine levels.

Table 5.1.6. Summary of recent animal toxicity studies of PFOS reporting immune toxicity

Sex/Species	Exposure	Endpoints	NOAEL/LOAEL	Reference
Pregnant C57BL/6 mice (12/dose)	0, 0.1, 1, or 5 mg/kg-day via gavage from GD 1 - 17; pups examined at 4 and 8 weeks of age	**Male pups:** ↓ absolute spleen and thymus weight; ↓ thymic cellularity; changes in thymic lymphocyte population; **Pups of both sexes:** ↑ absolute liver weight; ↓ splenic cellularity; changes in splenic lymphocyte populations; ↓ splenic lymphocyte proliferation; ↓ splenic NK cell activity; ↓ splenic plaque forming cells; changes in IL-2 and IL-4 levels	NOAEL: 0.1 mg/kg-day for reduction of splenic NK cell activity in males	Zhong et al. (2016)
Male C57BL/6 mice (10/dose)	0, 2.5, 5, or 10 mg/kg-day via gavage for 30 days	↓ absolute spleen weight	NOAEL: 5 mg/kg-day	Xing et al. (2016)
C57BL/6 mice (sex not specified) (4/dose)	0 or 2 mg/kg via gavage for 25 days; mice were infected with *Citrobacter* at day 7	↓ in pathogen clearance at late stage infection; induction of IL-22 from ILC3 and Th17 cells; ↓ mucin	NA[a]	Suo et al. (2017)
Male Sprague Dawley rats (6/dose)	0, 1, or 10 mg/kg-day via gavage for 4 weeks	↑ serum TNF-α and IL-6 levels	LOAEL: 1 mg/kg-day	Han et al. (2018b)
Male ICR mice (5/dose)	Treated mice were sensitized with OVA to induce active systemic anaphylaxis on days 0 and 7. OVA + 50, 100 or 150 mg/kg, 3 times on days 9, 11 and 13 orally.[a] Control mice had 150 mg/kg PFOS only or OVA only.	↓ rectal temperature; ↑ histamine, TNF-α, IgG and IgE levels in sensitized mice	LOAEL: 50 mg/kg for ↑TNF-α and IgE levels	Lee et al. (2018)

Sex/Species	Exposure	Endpoints	NOAEL/LOAEL	Reference
Male and female Sprague Dawley rats (10/sex/dose)	0, 0.312, 0.625, 1.25, 2.5, or 5 mg/kg-day for 28 days via gavage	**Males**: ↓white blood cells; ↓neutrophils; ↓ eosinophils; ↓ relative thymus weight **Females**: ↓ relative thymus weight at 1.25 mg/kg-day (not statistically significant at higher doses)	NOAEL: 2.5 mg/kg-day for all endpoints in males	NTP (2019b)

[a] The specific manner of oral administration was not stated in study.
Abbreviations: GD, gestation day; IgE, immunoglobulin E; IgG, immunoglobulin G; IL-2, interleukin-2; IL-4, interleukin-4; IL-6, interleukin-6; LOAEL, lowest-observed-adverse-effect level; NK cell, natural killer cell; NOAEL, no-observed-adverse-effect level; OVA, ovalbumin; TNF-α, tumor necrosis factor alpha

5.1.3. Recent Mechanistic Evidence

OEHHA's recent literature search identified only one mechanistic study of PFOA immunotoxicity. Results from an in vitro study investigating the effects of PFOA on allergic inflammation showed that PFOA enhanced histamine release and changes in pro-inflammatory cytokines in RBL-2H3 rat cells through the NF-κB pathway (Lee et al., 2017).

For PFOS, OEHHA identified only a few studies that investigated the mechanisms for immunotoxicity in human cell lines. Yang et al. (2016) reported that PFOS inhibited 11β-hydroxysteroid dehydrogenase 1, an enzyme responsible for converting cortisone to cortisol, in human decidual stromal cells. Furthermore, exposure to PFOS attenuated the cortisone-induced reduction of the pro-inflammatory cytokines, IL-6 and IL-1β (Yang et al., 2016). In human lymphocytes, PFOS caused cytotoxicity by inducing oxidative stress, as evidenced by increased ROS formation, lipid peroxidation, glutathione depletion and activation of caspase-3 (Zarei et al., 2018).

Two recent mechanistic immunotoxicity studies of PFOS in animal cells or tissues were identified. In rat RBL-2H3 cells sensitized with anti-dinitrophenol (DNP), PFOS increased histamine and pro-inflammatory cytokine levels, and activated the NF-κB pathway (Lee et al., 2018). Additionally, a study in dolphins showed that PFOS caused an increase in CD4+ and CD8+ T cell proliferation and induced pro-inflammatory interferon-gamma (IFN-γ) in T cells (Soloff et al., 2017).

5.1.4. Conclusions

OEHHA found mostly consistent evidence that PFOA and PFOS are associated with suppressed antibody response in humans, in particular, suppressed response to diphtheria, tetanus, and rubella vaccines. OEHHA also identified evidence that PFOA or PFOS can affect other immune outcomes such as asthma or certain infectious diseases or related symptoms, although this effect was not seen in all studies.

OEHHA evaluated the possibility that some of the results reported in the epidemiologic studies reviewed here may have been caused by confounding, outcome or exposure misclassification, selection bias, or some other error. Most of the epidemiologic studies reviewed here provided only a limited assessment of confounding. However, there was no convincing evidence

indicating that the associations reported for PFOA or PFOS and diminished antibody responses were caused by confounding. For example, age is a potential confounder since there is good evidence that it is associated with both PFAS levels and a number of different aspects of immune function. Importantly though, almost all of the studies OEHHA reviewed either limited their participants to a narrow range of ages or they adjusted for age in their statistical analyses. Other factors like body mass index (BMI), socioeconomic indicators, smoking, and breast-feeding may also be related to both PFAS exposure and immune function but most of the evidence OEHHA reviewed suggests these associations are not strong enough to cause many of the PFAS-immunotoxicity associations that were reported. For example, based on data from US NHANES, Calafat et al. (2007b) reported geometric mean serum PFOA concentrations of 5.0 (95% CI, 4.5-5.5), 5.4 (95% CI, 4.9-5.9), and 5.6 (95% CI, 5.1-6.1) ng/ml for people with less than a high school education, a high school education, and greater than a high school education (an indicator of socioeconomic status), respectively. They also reported PFOA levels in non-smokers, passive smokers, and smokers of 4.9 (95% CI, 4.5-5.4), 5.5 (95% CI, 4.9-6.1), and 5.4 (95% CI, 4.9-5.9) ng/ml, respectively. Overall, the small differences in PFOA levels across these different categories of education or smoking do not appear great enough to cause the 10-20% decreases in antibody response reported in the studies OEHHA reviewed (Axelson, 1978). For breast-feeding, while this factor was associated with higher PFAS exposures in the Faroe Islands cohorts, it was not strongly associated with antibody concentrations (Mogensen et al., 2015b; Budtz-Jorgensen and Grandjean, 2018) and thus unlikely to have caused major confounding. Finally, with regards to BMI, the Faroe Islands researchers wrote that statistical adjustment for this factor had "virtually no impact on the results" (Mogensen et al., 2015a).

Chemical exposures that are highly correlated with PFOA or PFOS and have significant immune effects could also cause confounding. In the 1997-2000 Faroe Islands cohort, correlations between serum levels of PFOA and PFOS with polychlorinated biphenyls (PCBs) in 5-year-old children were 0.00 and 0.08, respectively, which are likely too low to cause significant confounding (Grandjean et al., 2012). In fact, statistical adjustments for PCB levels had little effect on PFOA or PFOS results (Grandjean et al., 2017a; Grandjean et al., 2017b). Methylmercury may have some effects on the immune system, however the authors of the Faroe Islands studies wrote, "…exposures to methylmercury and polychlorinated biphenyls were only weakly correlated with serum concentrations of the PFAS, and confounding by these other exposures could therefore be ignored" (Budtz-Jorgensen and Grandjean, 2018). Correlations between PFOS and PFOA with most other organochlorine chemicals also appeared to be fairly low (data presented only in figure form) (Oulhote et al., 2017).

Many of the individual PFAS are correlated with each other, and these correlations can potentially make it difficult to determine the individual effects of any single compound. In some studies, these correlations are fairly strong. For example, in 1,562 children and adults ages 12 years and older in the 1999-2000 NHANES, Calafat et al. (2007b) reported a Pearson correlation coefficient of 0.64 ($p < 0.01$) between PFOA and PFOS serum concentrations. In 652 Danish men, the Spearman correlation coefficient between PFOA and PFOS serum concentrations was 0.71 ($p < 0.0001$) (Eriksen et al., 2011). Despite these high correlations, several pieces of evidence suggest the effects of PFOA and PFOS on antibody levels may be independent of each other. First, the correlations between PFOA and PFOS reported in the Faroe Islands cohorts are less strong than those reported in the Calafat et al. (2007b) and Eriksen et al. (2011) studies. For example, Pearson correlation coefficients between PFOA and PFOS serum concentrations measured at age 7 years in the 1997-2000 Faroe Islands cohort ranged from 0.29 to 0.50 (Table 5.1.7). Second, adjustment of PFOA- or PFOS-antibody response associations for other PFAS in the 1997-2000 Faroe Islands cohort generally resulted

in only small changes in effect sizes (Table 5.1.4) (Mogensen et al., 2015a; Budtz-Jorgensen and Grandjean, 2018). Third, animal studies provide clear evidence of an association between exposure to PFOA and PFOS and suppression of antibody response (NTP, 2016).

Table 5.1.7. Pearson correlation coefficients between PFOA, PFOS, and PFHxS at ages 5 and 7 years in the 1997-2000 Faroe Islands cohort (Mogensen et al., 2015a)

	Levels at age 7 years		
	PFOS	**PFOA**	**PFHxS**
Age 5 years			
PFOS	0.77	0.06	0.43
PFOA	0.34	0.61	0.39
PFHxS	0.40	0.27	0.85
Age 7 years			
PFOS	--		
PFOA	0.29	--	
PFHxS	0.50	0.34	--

OEHHA evaluated several other potential biases but did not find clear evidence that these were likely to be responsible for the PFAS-antibody response associations identified. For example, selection bias is a possibility, and a number of studies did not report accurate or thorough data on refusal or participation rates. Although not explicitly stated, most studies appear to have involved convenience samples. In some instances, the use of convenience sampling might lead to concerns about selection bias. However, in the studies OEHHA reviewed, participants did not appear to have been selected in a manner that would have been associated with both their PFAS levels and their immune function. As such, there is no obvious reason why convenience sampling would have introduced major bias.

Inaccuracies in the methods used to assess exposure and outcome can also introduce bias. Almost all of the studies OEHHA reviewed assessed exposure using serum levels of PFOA or PFOS, which is generally a valid and widely accepted method for assessing PFAS exposures (NTP, 2016). Very few of the studies mentioned whether or not research personnel were blinded when assessing exposure or outcome. However, although not explicitly stated, there is some indication that the laboratory personnel measuring the PFAS levels in the Faroe Islands studies were blinded to the outcome status of the participants (e.g., mention is made of “coded” laboratory samples, and PFAS and antibody levels were measured in different labs) (Grandjean et al., 2012; Grandjean et al., 2017b). In general, all of the studies OEHHA reviewed appeared to have assessed immune outcomes using the same methods in all participants, regardless of their PFAS exposure levels. This suggests that most bias that may have resulted from inaccuracies in outcome assessment was likely non-differential and therefore likely to have biased results towards the null, not towards the associations seen.

A number of the studies OEHHA reviewed used cross-sectional designs. Evidence for reverse causality has been seen in studies of PFOA or PFOS and outcomes such as renal function or age of menopause (Dhingra et al., 2017). Reverse causality might also be a source of error in cross-sectional studies of immune responses if the presence of an adverse immune outcome may lead people to use certain products or have other lifestyle changes that increase their PFAS exposure. While this may be possible, OEHHA was unable to find evidence to support it, at least to an extent that it would have caused major bias. Importantly, associations between PFOA or PFOS and decreased vaccine response have also been seen in several prospective analyses (Table 5.1.1), which are likely to be less susceptible to this bias.

Many of the results OEHHA reviewed involved only a single serum measurement of PFOA or PFOS. This could potentially lead to bias from exposure misclassification if the latency between exposure and the outcome is long or if the serum levels of these agents fluctuate significantly over time. However, the half-lives of PFOS and PFOA in humans appears to be long. In a study in retired fluorochemical production workers followed for up to 5 years, the half-lives of serum PFOA and PFOS were 3.5 and 4.8 years, respectively (Olsen et al., 2007). In addition, although serum PFOA and PFOS concentrations appear to be declining over time in most people, *relative* levels seem to remain fairly stable. For example, in the 1997-2000 Faroe Islands cohort, correlation coefficients between levels measured at age 5 and levels measured at age 7 were 0.61 and 0.77 for PFOA and PFOS, respectively (Table 5.1.7). Overall, the long half-lives of PFOA and PFOS and the fact that relative levels seem to remain stable over time suggests that a single measurement of PFOA or PFOS can be a good long-term marker of exposure in many people.

In addition to the human evidence, OEHHA also found consistent evidence that PFOA and PFOS are associated with suppressed antibody response in experimental animals. NTP (2016) identified six animal studies (including the DeWitt et al. (2016) study described in Table 5.1.5) reporting immunosuppression in mice following exposure to PFOA, determined by decreases in antigen-specific IgM and/or IgG antibody production in response to a challenge with T-cell specific antigens. Similarly, NTP identified seven studies in mice reporting suppressed antibody responses following exposure to PFOS. In OEHHA's review of animal immunotoxicity studies published from 2016 onward, one study reported decreased sheep red blood cell (SRBC)-specific and dinitrophenyl-ficoll (DNP)-specific IgM antibody responses in mice exposed to PFOA (DeWitt et al., 2016). OEHHA did not identify any experimental animal studies of PFOS and effects on antibody responses published from 2016 onward. Additionally, OEHHA identified one mouse study of PFOA and one mouse study of PFOS, which reported increases in IgE levels following multiple exposures to 150 mg/kg of the respective chemical (Lee et al., 2017; Lee et al., 2018). In these studies, IgE levels were increased in animals sensitized with ovalbumin, and in non-sensitized animals compared to their respective controls.

NTP (2016) noted that there was evidence from experimental animal studies indicating PFOA and PFOS affect multiple aspects of the immune system in addition to the suppression of antibody response. The recent animal immunotoxicity studies of PFOA identified by OEHHA primarily reported changes in spleen and thymus weight. NTP (2020) also reported lymphoid follicle atrophy in male rats, and pigment in the spleen in female rats. Similar to PFOA, the recent animal immunotoxicity studies of PFOS reported changes in spleen and thymus weight, in addition to changes in thymus lymphocyte populations and cytokine levels. NTP (2016) considered these endpoints as secondary outcomes, because these endpoints are "less indicative of overall immunotoxicity." Nonetheless, NTP (2016) reported that reductions in murine spleen and/or thymus weight at moderate to high doses (>10 mg/kg-day), and reductions in murine thymus cellularity at high doses (>20 mg/kg-day) were consistently observed across multiple studies for both PFOA and PFOS. For changes in cytokine levels, NTP noted the inherent difficulty in determining whether clear and consistent patterns exist, due to differences in study design and cells/tissues examined. Additionally, Zhong et al. (2016) reported that gestational exposure to PFOS induced decreased NK cell activity in pups, supporting previous findings reported in NTP (2016).

In summary, based on this review of the recent literature and the past evidence reviewed by NTP (2016), OEHHA identified consistent evidence in the human epidemiologic literature and from experimental animal studies that PFOA and PFOS are associated with decreased antibody

responses (Table 5.1.8). OEHHA's evaluations suggest that the associations observed in the human epidemiologic studies are not due to confounding, exposure or outcome misclassification, selection bias, or reverse causation. Positive findings were also seen in a number of studies involving other immune outcomes such as asthma, rhinitis, or lower respiratory tract infections, especially for PFOA, and these, along with the experimental animal data, provide further support that PFOA and PFOS are immunotoxic. Importantly, several of these associations have involved exposure levels that are close to those seen in the general US population.

Table 5.1.8. Summary of OEHHA's conclusions regarding the human and experimental animal data on PFOA and PFOS and immunotoxicity

Outcome	PFOA	PFOS
Vaccine response in humans	Mostly consistent evidence for decreased vaccine response	Mostly consistent evidence for decreased vaccine response
Other immune-related outcomes in humans	-Some evidence for increased **asthma** and related outcomes but based mostly on cross-sectional data and not consistent across all studies -Some evidence for increases in **respiratory infections** in children but not consistent across all studies -Weak, mostly inconsistent, or no evidence for links to other outcomes	-Some evidence for increased **asthma** and related outcomes but based mostly on cross-sectional data and not consistent across all studies -Some evidence for increases in **respiratory infections** in children but not consistent across all studies -No associations or only weak evidence identified for other outcomes
Immunosuppression in animals	Consistent evidence for decreased antibody response	Consistent evidence for decreased antibody response
Effects on the spleen and/or thymus in animals	Consistent evidence for decreased spleen and/or thymus weight in mice	Consistent evidence for decreased spleen and/or thymus weight in mice
Other immune-related effects in animals	-Consistent evidence for decreased thymic cellularity -Few studies, consistent evidence for changes in NK cell activity	-Consistent evidence for decreased thymic cellularity -Few studies, consistent evidence for changes in NK cell activity

5.2. Liver Toxicity

US EPA has reviewed the human epidemiologic literature, published up to December 2015, on PFOA or PFOS and liver toxicity (US EPA, 2016b; US EPA, 2016d). In their reviews, US EPA identified a number of epidemiologic studies that investigated associations between PFOA and serum levels of liver enzymes such as alanine transaminase (ALT), aspartate aminotransferase (AST), and gamma-glutamyl transpeptidase (GGT). All of these studies were primarily in adults. Although findings were not consistent across all studies, several reported statistically significant associations between increasing serum levels of PFOA and increasing blood levels of these enzymes. These findings were seen in both high exposure settings (e.g., occupational cohorts) and general population studies, and were seen both in studies using cross-sectional and prospective designs. Several of these studies controlled for factors that could potentially confound results, including BMI, alcohol intake, smoking, medication use, socioeconomic status,

and medical history. In addition, several studies involved scenarios where PFOA was the predominant PFAS to which participants were exposed (US EPA, 2016b; US EPA, 2016d). Based on this evidence, US EPA concluded, "Overall, an association of serum PFOA concentration with elevations in serum levels of ALT and GGT has been consistently observed in occupational, highly exposed residential communities, and the U.S. general population. The associations are not large in magnitude, but indicate the potential to affect liver function" (US EPA, 2016b).

US EPA (2016b) identified one epidemiologic study of PFOA and liver disease (e.g., hepatitis, cirrhosis, fatty liver disease). In this study, an OR of 2.02 (95% CI, 0.50-8.10) was reported for non-hepatitis liver disease among the most highly exposed workers at a chemical plant that used PFOA, although the number of cases was small (35 total cases) (Steenland et al., 2015). Findings for hepatitis were not reported.

For animal toxicity, a thorough examination of the literature of PFOA was previously conducted by other agencies (US EPA, 2016a; US EPA, 2016b; New Jersey DWQI, 2017; ATSDR, 2018a). US EPA (2016b) identified several hepatotoxic endpoints in animal studies that were commonly reported in the scientific literature, namely increases in serum levels of liver enzymes (e.g., ALT and/or AST), increased relative liver weight, hepatocellular hypertrophy, and hepatocellular necrosis.

For PFOS, US EPA identified two epidemiologic studies that investigated associations with liver toxicity. The first was a cross-sectional study of serum PFOS and liver enzymes in 2,216 adults over age 20 from the 1999–2000 and 2003–2004 US NHANES (Lin et al., 2010). In linear regression models adjusted for age, gender, race/ethnicity, smoking, drinking status, education, BMI, metabolic syndrome, iron saturation status, and insulin resistance, a borderline statistically significant positive association was found between serum PFOS concentrations and serum ALT (linear regression coefficient (β) = 1.01; p=0.066). Clear associations with GGT and total bilirubin were not seen. The second study was a cross-sectional analysis of serum PFOS and liver enzymes from the C8 Health Study. This study involved 47,092 residents of the Mid-Ohio Valley, West Virginia, who lived near a chemical plant known to have emitted PFOA into the surrounding environment (Gallo et al., 2012). In linear regression models adjusted for age, physical activity, BMI, average household income, education, race, alcohol consumption, and smoking, increasing serum concentrations of PFOS were associated with increasing serum levels of ALT (regression coefficient between lognormal (ln) PFOS and ln ALT = 0.020 (95% CI, 0.014-0.026). A positive association was also seen with direct bilirubin (β = 0.029 (95% CI, 0.024-0.034)). Clear associations with GGT were not seen. US EPA did not identify any studies of PFOS and liver diseases like fatty liver disease, cirrhosis or hepatitis. Overall, US EPA concluded that, "The epidemiological data supporting liver damage based on serum ALT and GGT as reported by Gallo et al. (2012) are not strong enough to support an association of serum PFOS alone with liver damage in humans, because in most of the epidemiology studies the serum contains a mixture of PFAS and possibly other exogenous chemicals" (US EPA, 2016d).

PFOS exposure has been consistently shown to induce liver toxicity in experimental animals. A thorough examination of this literature was previously conducted by other agencies (US EPA, 2016c; US EPA, 2016d; New Jersey DWQI, 2018). In general, increases in absolute and/or relative liver weight, increased liver histopathological effects (e.g., hypertrophy, necrosis, etc.), and increased biomarkers of liver damage were reported.

5.2.1. Recent Human Evidence

OEHHA identified nine studies of PFOA or PFOS and liver toxicity published since December 2015 or that were otherwise not included in the most recent US EPA (2016b) and US EPA (2016d) reviews (summarized in Appendix 7, Tables A7.5 and A7.6). In general, for PFOA, the more recent studies OEHHA identified support the conclusions of US EPA (2016b) and US EPA (2016d). All of the studies that examined PFOA and liver enzymes in adults published since the US EPA review reported statistically significant associations between increasing serum PFOA concentrations and increasing blood levels of ALT, AST, and/or GGT. This includes two prospective studies (Darrow et al., 2016; Salihovic et al., 2018). Both of these studies adjusted or otherwise controlled for a number of potential confounding factors including cholesterol levels, BMI, medication use, socioeconomic status, alcohol, or smoking.

Three of the more recent studies OEHHA identified were done in children, with only one reporting statistically significant associations between PFOA and increased liver enzymes. The positive study (Attanasio, 2019) differed from the two others in that it involved somewhat older children (ages 12-19 versus ages 6-11 (Mora et al., 2018) and ages 8-12 (Khalil et al., 2018)). In this study, associations were seen between increasing serum PFOA concentrations and ALT, AST, and GGT in girls but not in boys, and fairly large unexplained changes in results were seen after statistical adjustments. Overall, the reason why fairly consistent findings have been seen in adults while results in children are mixed is not known.

With regards to PFOS, the prospective cohort study by Salihovic et al. (2018) in 1,002 elderly adults in Sweden reported an association between increasing serum levels of PFOS and increasing ALT ($p < 0.001$). The other three recent studies OEHHA identified that examined PFOS and liver toxicity presented mixed results (Gleason et al., 2015; Jain and Ducatman, 2018b; Nian et al., 2019). Overall, two of the three negative studies were done in children. The other was based on 3,573 adults in the 2011-14 NHANES (Jain and Ducatman, 2018b). OEHHA could not identify major differences in study quality that might explain these inconsistent results.

5.2.2. Recent Animal Evidence

OEHHA's review of recent animal studies, published from 2016 onward, is summarized in Table 5.2.1. PFOA exposure has consistently been shown to induce liver toxicity in experimental animals. In general, increases in absolute and/or relative liver weight, increased liver histopathology, and increased biomarkers of liver damage were observed.

Table 5.2.1. Summary of recent animal toxicity studies of PFOA reporting effects on the liver

Sex/Species/ Reference	Exposure	Serum/Plasma Concentrations (µg/ml)	Endpoints	NOAEL/LOAEL
Male CD-1 mice (3-5/dose) Beggs et al. (2016)	0 or 3 mg/kg-day via gavage for 7 days	NA	↑ relative liver weight	NA[a]
Male Kunming mice (4/dose) Liu et al. (2016)	0 or 10 mg/kg-day via gavage for 14 days	NA	↑ serum ALT, AST, ALP, and LDH; vacuolar degeneration; necrosis; inflammatory cell infiltration; ↑ oxidative stress (↑ TNF-α and IL-6, ↑ MDA and H_2O_2, ↓ SOD and CAT activity); ↑ caspase-3 activity (apoptosis)	NA[a]
Male and female C57BL/6 mice (6/sex/dose) Rebholz et al. (2016)	0 or 3.5 mg/kg of feed (fat- and cholesterol-containing diet) (~0.55 mg/kg BW-day, according to authors) for six weeks	**Males:** 0.002 or 26.9 **Females:** 0.028 or 44.3 at six weeks	**Both sexes:** ↑ relative liver weight	NA[a]
Male and female BALB/c mice (6/sex/dose) Rebholz et al. (2016)	0 or 3.5 mg/kg of feed (fat- and cholesterol-containing diet) (~0.55 mg/kg BW-day, according to authors) for six weeks	**Males:** 0.005 or 28.2 **Females:** 0.086 or 35.6 at six weeks	**Both sexes:** ↑ relative liver weight	NA[a]

Sex/Species/ Reference	Exposure	Serum/Plasma Concentrations (µg/ml)	Endpoints	NOAEL/LOAEL
Pregnant C57BL/6J mice (mated with FVB male mice) to produce hybrid offspring (6 for dams, 6-10/sex/dose for pups) van Esterik et al. (2016)	Dietary exposure to 0, 0.003, 0.01, 0.03, 0.1, 0.3, 1, and 3 mg/kg-day (targeted dose). Exposure started 2 weeks before mating and continued during mating (1 week), gestation (3 weeks), and lactation (3 weeks)	NA	**Both sexes:** nuclear dysmorphology (p=0.06) **Male pups:** ↑ absolute and relative liver weight; ↑ eosinophilic liver foci (p=0.07)	LOAEL: 0.003 mg/kg-day
Male BALB/c mice (5/dose) Yu et al. (2016)	0, 0.5, or 2.5 mg/kg-day via "oral infusion" for 28 days	0.011, 29.34, or 114.3 at 28 days	↑ absolute and relative liver weight; altered glucose metabolism	NOAEL: 0.5 mg/kg-day for ↑ liver weight
Male Sprague Dawley rats (5/dose) Cavallini et al. (2017)	Single dose of 0 or 150 mg/kg intragastrically, sacrifice after 48 or 96 hours	NA	↑ relative liver weight	NA[a]
Male SV129 WT and PPARα KO mice (4/dose) Das et al. (2017)	0 or 10 mg/kg-day via gavage for 7 days	NA	**WT and KO:** ↑ absolute and relative liver weight; hepatocellular hypertrophy; decreased DNA content	NA[a]
Male BALB/c mice (minimum of 8/dose)	0, 1, or 5 mg/kg-day orally[b] for 7 days	NA	↑ absolute liver weight; hepatocyte cytoplasmic vacuolization; ↑ serum ALT	LOAEL: 1 mg/kg-day for ↑ serum ALT levels

Sex/Species/ Reference	Exposure	Serum/Plasma Concentrations (µg/ml)	Endpoints	NOAEL/LOAEL
Hui et al. (2017)				
Male Wistar rats (5-12/dose) Kawabata et al. (2017)	Single oral dose of 0 or 50 mg/kg via gavage, sacrifice after 9 days	33.3 at 9 days after exposure. Control level not determined	↑ absolute and relative liver weight	NA[a]
Male and female BALB/c mice (30/sex/dose) Li et al. (2017b)	0, 0.05, 0.5, or 2.5 mg/kg-day via gavage for 28 days	**Males:** 0, 1.2, 5.9 or 13.4 **Females:** 0, 0.97, 2.7 or 9.5 at 28 days	↑ absolute liver weight; hepatocellular hypertrophy and apoptosis; mitochondrial morphology changes; changes in mitochondrial membrane potential; oxidative DNA damage (ROS generation)	LOAEL: 0.05 mg/kg-day for hepatic mito-chondrial membrane potential changes, apoptosis, oxidative DNA damage
Male Kunming mice (number not specified) Wu et al. (2017)	Single oral dose of 0 or 5 mg/kg via gavage	NA	↑ hepatic cytoplasmic vesicles; ↑ inflammatory cells around the hepatic portal area	NA[a]
Male BALB/c mice (3/dose) Yan et al. (2017)	0, 0.08, 0.31, 1.25, 5, or 20 mg/kg-day via gavage for 28 days	NA	hepatocyte swelling	Not provided[c]
Male BALB/c mice (20/dose) Zheng et al. (2017)	0 or 1.25 mg/kg-day orally[b] (for 28 days	0.04 or 55.5 at 28 days	↑ relative liver weight; altered glucose metabolism	NA[a]
Pregnant Kunming mice (8/dose) Qin et al. (2018)	0 or 5 mg/kg-day intragastricall y throughout gestation	NA	↑ ALT and AST in F1 pup serum on PND 21 (although the changes were not statistically significant)	NA[a]

Sex/Species/ Reference	Exposure	Serum/Plasma Concentrations (µg/ml)	Endpoints	NOAEL/LOAEL
Male Kunming mice (8/dose) Wu et al. (2018)	0, 1, or 5 mg/kg-day intragastrically for 21 days	NA	↑ absolute and relative liver weight; ↑ serum ALT and AST; ↑ hepatic vacuoles	NOAEL: 1 mg/kg-day for ↑ liver enzymes
Male C57BL/6 mice (6-8/dose) Crebelli et al. (2019)	0, 0.55, 5.5 and 28 mg/L in drinking water for 5 weeks (corresponding to 0, 0.1, 1, and 5 mg/kg-day)	0.0014, 3.094, 23.971, or 83.703 at 28 days	↑ absolute liver weight; cytoplasmic vacuolization; hepatocyte hypertrophy; irregular architecture of parenchyma; necrosis; ↑ serum ALT and AST	NOAEL: 1 mg/kg-day for liver histo-pathology, ↑ ALT and AST, and necrosis
Male miR-34A -/- KO and WT C57BL/6J mice[d] (15-18/dose) Cui et al. (2019)	0 or 5 mg/kg-day via gavage for 28 days	NA	**WT and KO:** ↑ relative liver weight; ↑ serum ALT, AST, and cholinesterase; ↓ total bile acid; swollen hepatocytes	NA[a]
Male BALB/c mice (12/dose) Guo et al. (2019)	0, 0.4, 2, or 10 mg/kg-day via gavage for 28 days	0.02, 13, 64, or 88	↑ absolute and relative liver weight; ↑ serum ALT and AST; hepatocellular hypertrophy; karyolysis; ↑ albumin; ↑ serum ammonia; ↓ serum BUN	LOAEL: 0.4 mg/kg-day based on ↑ liver weight, hepatocellular hypertrophy, and karyolysis
Pregnant Kunming mice (10/dose), female pups (5-10/dose) Li et al. (2019c)	0, 1, 2.5, 5, or 10 mg/kg-day via gavage from GD 1-17	NA	**Pups:** ↑ absolute and relative liver weight; swollen hepatocytes; vacuolar degeneration; dissolved nuclei; blurred liver architecture; ↑ serum ALT and AST; ↑ CAT, SOD, and 8-OHdG; ↓ histone acetylation	LOAEL: 1 mg/kg-day for ↑ serum ALT and AST

Sex/Species/ Reference	Exposure	Serum/Plasma Concentrations (µg/ml)	Endpoints	NOAEL/LOAEL
Male C57BL/6 mice (5/diet/treat-ment/time point) Li et al. (2019d)	0 or 1 mg/kg-day (in distilled water, presumably gavage) for 2, 8, or 16 weeks. Half of the animals were on low fat diet, other half on high fat diet	NA	↑ absolute and relative liver weight; hepatocellular hypertrophy and hyperplasia; hepatic lobular inflammation; ↓ in ALT; fibrosis induced by the high-fat diet	NA[a]
Male and female Sprague Dawley rats (10/sex/dose) NTP (2019a)	0, 0.625, 1.25, 2.5, 5, or 10 mg/kg-day (for males) and 0, 6.25, 12.5, 25, 50, or 100 mg/g-day (for females) via gavage for 28 days	**Males:** BD, 0.378, 0.503, 1.297, 3.34, or 10.9 **Females:** BD, 0.129, 0.292, 0.475, 1.67, or 6.71 at 28 days	hepatocyte hypertrophy; hepatocyte cytoplasmic alteration; ↑ absolute and relative liver weight; ↑ serum ALT and ALP **Males only:** ↑ serum AST and bilirubin	LOAEL: 0.625 mg/kg-day for hepatocyte cytoplasmic alteration and ↑ liver weight in males
Male APOE*3-Leiden.CETP transgenic mice[e] (8/dose) Pouwer et al. (2019)	0, 0.01, 0.291, or 30.2 mg/kg-day in diet for 6 weeks	0.005, 0.065, 1.524 or 144 at 6 weeks	↑ absolute and relative liver weight; ↑ ALT	NOAEL: 0.291 mg/kg-day for ↑ liver weight and ↑ ALT
Male APOE*3-Leiden.CETP transgenic mice (8/dose) Pouwer et al. (2019)	0, 0.01, 0.298, or 29.5 mg/kg-day in diet for 4 weeks	0, 0.051, 1.395, or 93.713 at 4 weeks	↑ absolute and relative liver weight; ↑ serum ALT; hepatic hypertrophy	NOAEL: 0.298 mg/kg-day for ↑ liver weight, ↑ ALT, hypertrophy and steatosis

Sex/Species/ Reference	Exposure	Serum/Plasma Concentrations (µg/ml)	Endpoints	NOAEL/LOAEL
Pregnant Kunming mice (10/dose) Song et al. (2019)	0 or 20 mg/kg-day via gavage from GD 1-7	NA	**Dams:** ↑ relative liver weight; increased oxidative stress (↑ MDA, ↓ SOD and GSH-Px)	NA[a]
Male C57BL/6NCrl mice (4-6/dose) Wen et al. (2019c)	0, 1, or 3 mg/kg-day via gavage for 7 days	NA	↑ absolute liver weight; ↑ carboxylesterase protein levels and activity	LOAEL: 1 mg/kg-day for ↑ liver weight
Male C57BL/6NTac WT and PPARα KO mice (4-6/dose) Wen et al. (2019c)	0 or 3 mg/kg-day via gavage for 7 days	NA	**WT and KO:** ↑ absolute liver weight (effect more pronounced in WT animals); ↑ carboxylesterase protein levels and activity	NA[a]
Pregnant CD-1 mice (11-13 dams/dose) Blake et al. (2020)	0, 1, or 5 mg/kg-day via gavage from ED 1.5 to ED 11.5 or ED 17.5	ED11.5: BD, 25.4 or 117.3 ED17.5: 0.211, 18.7 or 95.1	**Dams:** ↑ absolute and relative liver weight; hepatic effects (cytoplasmic alteration, hypertrophy, ↓ glycogen, eosinophilic granular cytoplasm, ↓ hepatic mitosis, ↑ apoptosis, necrosis, abnormal ultrastructure, ↓ rough ER); ↓ albumin and total protein levels; ↑ serum AST and SDH **Pups:** no reported liver effects	LOAEL: 1 mg/kg-day for ↑ liver weight and liver effects
Female Sprague Dawley rats (10/dose) NTP (2020)	0, 300, or 1,000 ppm in feed (0, 29.6, or 98.6 mg/kg-day) for 16 weeks	BD, 20.4, or 72.3 at 16 weeks	↑ absolute and relative liver weight; hepatocyte cytoplasmic alteration; hepatocyte hypertrophy; ↑ serum ALT and ALP	NOAEL: 300 ppm (29.6 mg/kg-day) for all liver endpoints
Male Sprague	0, 150, or 300 ppm in feed	BD, 193, or 243 at 16 weeks	↑ relative liver weight; liver necrosis; liver	LOAEL: 150 ppm

Sex/Species/ Reference	Exposure	Serum/Plasma Concentrations (µg/ml)	Endpoints	NOAEL/LOAEL
Dawley rats (10/dose) NTP (2020)	(0, 15.6, or 31.7 mg/kg-day) for 16 weeks		pigment; hepatocyte hypertrophy; hepatocyte cytoplasmic alteration; hepatocyte single cell death; ↑ serum ALT and ALP; ↑ bile salts	(15.6 mg/kg-day) for all liver endpoints
Male Sprague Dawley rats (10/dose) NTP (2020)	0, 20, 40, or 80 ppm in feed (0, 1.9, 4.0, or 7.9 mg/kg-day) for 16 weeks	BD, 81.4, 130.8, or 159.6 at 16 weeks	↑ absolute and relative liver weight; liver necrosis; liver pigment; hepatocyte cytoplasmic alteration; hepatocyte hypertrophy; hepatocyte single cell death; ↑ serum ALT and ALP	LOAEL: 20 ppm (1.9 mg/kg-day) for multiple liver endpoints
Female Sprague Dawley rats (50/dose) NTP (2020)	0, 300, or 1,000 ppm in feed (0, 18, or 63 mg/kg-day) for 107 weeks	BD, 20.4, or 72.3 at 16 weeks	liver necrosis; liver pigment; bile duct hyperplasia; hepatocyte cytoplasmic alteration; hepatocyte hypertrophy; hepatocyte single cell death; hepatocyte ↑ mitoses	LOAEL: 300 ppm (18 mg/kg-day) for hepatocyte cytoplasmic alteration and hepatocyte hypertrophy
Male Sprague Dawley rats (50/dose) NTP (2020)	0, 20, 40, or 80 ppm in feed (0, 1, 2.2, or 4.5 mg/kg-day) for 107 weeks	BD, 81.4, 130.8, or 159.6 at 16 weeks	liver cystic degeneration; liver eosinophilic and mixed cell focus; liver focal inflammation; liver necrosis; liver pigment; hepatocyte hypertrophy; hepatocyte cytoplasmic alteration; hepatocyte single cell death	LOAEL: 20 ppm (1 mg/kg-day) for liver necrosis, hepatocyte hypertrophy, hepatocyte cytoplasmic alteration

[a] LOAEL/NOAEL not applicable for single dose studies.
[b] The specific manner of oral administration was not stated in study.
[c] Histology data are presented in the supplementary materials, but specific doses at which hepatocyte swelling and lipid deposits become significant are not provided.
[d] miR-34A -/- KO mice do not produce the microRNA miR-34A. miR-34A functions as a tumor suppressor.
[e] APOE*3-Leiden.CETP transgenic mice are reported to have human-like lipoprotein metabolism.
Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BD, below limit of detection; BUN, blood urea nitrogen; CAT, catalase; ED, embryonic day; ER, endoplasmic reticulum; GD, gestation day; GSH-Px, glutathione peroxidase; HDL, high density lipoprotein; H_2O_2, hydrogen peroxide; IL-6, interleukin 6; KO, knockout; LDH, lactate dehydrogenase; LDL, low density lipoprotein; LOAEL, lowest-observed-adverse-effect level; MDA, malondialdehyde; NOAEL, no-observed-adverse-effect level; 8-OHdG, 8-hydroxy-2'-deoxyguanosine; PND, postnatal day; PPARα, peroxisome proliferator-activated receptor alpha; SDH, sorbitol dehydrogenase; SOD, superoxide dismutase; TNF-α, tumor necrosis factor alpha; WT, wild-type

Well-conducted studies demonstrating the lowest NOAELs/LOAELs are described here. Recently, NTP released toxicity data from subacute and chronic bioassays conducted in male and female Sprague Dawley rats. An additional cohort of animals was exposed to PFOA during gestation and lactation (perinatal exposure; 150 or 300 ppm PFOA in feed given to dams). An initial chronic study in male rats with concentrations of 0, 150, or 300 ppm in feed (0, 14.7, or 29.5 mg/kg-day) was ended at 21 weeks due to overt toxicity; however, it appears a subset of animals receiving these doses were examined at 16 weeks. The study was subsequently repeated with lower doses. Liver toxicity was observed in all of the studies, regardless of sex or duration. Common liver effects included increased relative liver weight, increased serum ALT and alkaline phosphatase (ALP), necrosis, increased liver pigment, hepatocyte cytoplasmic alteration and hypertrophy, and hepatocyte single cell death (NTP, 2019a; NTP, 2020). OEHHA identified lowest-observed-adverse-effect levels (LOAELs) of 0.625 mg/kg-day and 1 mg/kg-day for the 28-day and 107-week studies in male rats, respectively, for liver toxicity. This corresponds to plasma concentrations of 0.0507 and 0.0814 milligrams per milliliter (mg/ml), respectively (NTP, 2020). Plasma/serum concentration is the most appropriate dose metric for extrapolating toxicity data from rodent studies to humans because of the large difference in the chemical's biological half-life between rodents (1-3 weeks) and humans (2-3 years). This accounts for the accumulation of PFOA in humans due to the chemical's long half-life. Plasma concentration in the chronic male rat study was determined at 16 weeks, but because the plasma/serum half-life of PFOA is estimated to be 4-6 days in male rats (Lau et al., 2006; New Jersey DWQI, 2017), it is anticipated that by 16 weeks, a steady-state concentration would have been reached. Thus, the plasma concentration would remain relatively stable over the 107-week period of continuous dosing.

Li et al. (2017b) reported decreased body weight, increased absolute and relative liver weight, hepatocellular hypertrophy and apoptosis, lipid accumulation in hepatocyte cytoplasm, and other biomarkers of hepatotoxicity. Quantitative data for several endpoints and PFOA serum concentrations were presented as graphs. These graphical data were quantified using GetData Graph Digitizer software (version 2.26), and are presented in Table 5.2.2. Female mice were more sensitive to apoptosis than male mice. The administered dose of 0.05 mg/kg-day corresponds to a serum concentration of 0.97 microgram per milliliter (µg/ml) for females and 1.2 µg/ml for males, which was measured at the end of the exposure period. OEHHA identified a LOAEL of 0.05 mg/kg-day (serum concentration of 0.97 µg/ml) for changes in mitochondrial membrane potential (indicative of mitochondrial dysfunction), increases in biomarkers of apoptosis (caspase-9 and p53), and increased oxidative DNA damage (Li et al., 2017b).

Table 5.2.2. Dose metrics and endpoints in female BALB/c mice from Li et al. (2017b)

Administered dose (mg/kg-day)	Reported serum concentration (µg/ml)	Cells with mitochondrial membrane potential changes[a] (%)	Caspase-9 levels[a] (iU/g)	p53 levels[a] (iU/g)	8-OHdG[a] (ng/g)
0	0	1.2 ± 0.5	71.3 ± 4.2	28.9 ± 3.5	22.9 ± 7.3
0.05	0.97	12.3 ± 1.2**	130.2 ± 9.0**	46.8 ± 5.1**	68.6 ± 6.2**
0.5	2.7	17.6 ± 1.1**	157.9 ± 3.5**	58.3 ± 4.5**	87.9 ± 9.3**
2.5	9.5	39.3 ± 14.6**	220.9 ± 1.1**	69.0 ± 3.2**	96.8 ± 2.6**

[a] Mean ± standard deviation
8-OHdG, 8-hydroxydeoxyguanosine; iU/g, international units/gram

**p <0.01, statistical analysis (t-test) by OEHHA

van Esterik et al. (2016) reported non-statistically significant increases in liver histopathology (eosinophilic liver foci, nuclear dysmorphology, and lipid accumulation) in C57BL/6JxFVB mice exposed in utero and during lactation to PFOA. The authors also reported an increase in absolute liver weight in male F1 mice at 26 weeks of age. OEHHA obtained the individual animal liver weight data from the authors, and evaluated the absolute and relative liver weights for statistical and biological significance.

A thorough statistical analysis showed a significant increase in absolute and relative liver weight in male F1 mice at 26 weeks of age at all doses relative to control. Relative liver weights were obtained for F1 mice (sex unspecified), comprising 8 dose groups with approximately 10 animals from 2-5 litters in each group. Anova type II test with unbalanced design demonstrated statistical differences among groups (p=0.03) and lack of effect from specific litters. Levene's test for homogeneity of variance was not significant. However, normality of residuals was violated, as visually demonstrated by the lack of linearity in the normal Q-Q plot and significance in the Shapiro-Wilk normality test (p=0.0057). Thereafter, OEHHA applied a Kruskal-Wallis test (non-parametric analogue of Anova), which was significant (p=0.0047), indicating difference(s) among dose groups. Parametric and non-parametric t-tests demonstrated significant increases in all dose groups relative to the control group. Finally, rank-based trend tests (Spearman's rank correlation, Kendall's rank correlation) either for the full dataset or with exclusion of the control group were significant, indicating a positive trend in the dose-response data either in the presence ($p=2\times10^{-5}$ for both the Spearman's and Kendall's tests) or absence (p=0.007 and p=0.008, respectively) of the control group. Overall, the statistical analysis supports a dose-dependent increase for the relative liver weights in this experiment.

The relative liver weight values in the control group appear to be low in comparison to historical controls from the same research group and other sources, which may explain the observed increases in all treatment groups. OEHHA calculated a mean relative liver weight of 3.04% for control F1 animals, whereas mean relative liver weights following treatment ranged from 3.23-3.49% at 26 weeks (van Esterik et al., 2016). In a separate study from the same laboratory (van Esterik et al., 2014), the mean relative liver weight from control male mice was approximately 4.69% at 23 weeks. Additionally, reports from Jackson Laboratories state that the relative liver weight of the parent strain (male C57BL/6J mice) at 26 weeks is 4.30 ± 0.47%. This suggests that the control animals in (van Esterik et al., 2016) had unusually low liver weights that are not comparable to available historical values. However, Spearman's rank correlation and Kendall's rank correlation tests without the control group still showed a statistically significant trend (p <0.01 for both tests), indicating that the low control values were not solely responsible for the observed statistical significance.

Increased F1 liver weight appears to be a significant effect of PFOA exposure. However, there are several concerns that lower the overall confidence in this study. First, no details about the liver weight data are included in the publication. Second, the control liver weight values are below historical controls. Third, the authors reported that animals were fasted for 18 hours prior to sacrifice, which can lead to reductions in liver weight (Jensen et al., 2013).

Much like for PFOA, several animal studies published from 2016 onward reported various hepatotoxic endpoints following oral exposure to PFOS. Study details are summarized in Table 5.2.3.

Table 5.2.3. Summary of recent animal toxicity studies of PFOS reporting liver effects

Sex/Species	Exposure	Serum/Plasma Concentration (µg/ml)	Endpoints	NOAEL/LOAEL
Male CD-1 mice (3-5/dose) Beggs et al. (2016)	0 or 10 mg/kg-day via gavage for 7 days	NA	↑ relative liver weight	NA[a]
Pregnant mice (strain not specified) (3-5/dose) Mehri et al. (2016)	0, 1, 10, or 20 mg/kg-day orally[b] from GD 1-14	NA	fetal liver enlargement	doses that caused effect were not specified
Male Sprague Dawley rats (7/dose) Wan et al. (2016)	0, 1, or 10 mg/kg-day orally[b] for 4 weeks	NA	↑ absolute and relative liver weight; hepatocellular hypertrophy; cytoplasmic vacuolization; ↑ serum ALT and AST; inflammatory cellular infiltration; ↑ biomarkers of apoptosis	LOAEL: 1 mg/kg-day for ↑ serum ALT
Male C57BL/6 mice (10/dose) Xing et al. (2016)	0, 2.5, 5, or 10 mg/kg-day via gavage for 30 days	0.02, 70.2, 130.6, and 201.2 at 30 days	↑ relative liver weight; ↑ serum ALT, AST, ALP, and GGT; hepatocyte vacuolization and necrosis; hepatocyte hypertrophy; ↑ oxidative stress (↓ SOD, CAT, and GSH-Px); ↑ apoptosis	LOAEL: 2.5 mg/kg-day for ↑ relative liver weight and liver enzymes, oxidative stress, and apoptosis
Male C57BL/6 mice (5-6/dose) Zhang et al. (2016c)	0, 30, 60, or 120 mg/kg of feed (equivalent to 0, 3.48, 6.96, or 13.92 mg/kg BW-day)[c] for 21 or 23 days	0, 94.6, 176, and 392 at 21 days	↑ relative liver weight; ↑ ALT and bile acids; hepatocyte vacuolization and necrosis; ↑ MDA	LOAEL: 3.48 mg/kg-day for ↑ liver weight

Sex/Species	Exposure	Serum/Plasma Concentration (µg/ml)	Endpoints	NOAEL/LOAEL
Pregnant C57BL/6 mice (12/dose) Zhong et al. (2016)	0, 0.1, 1, or 5 via gavage from GD 1-17	NA	**Pups:** ↑ absolute liver weight at 4 weeks (but not significant at 8 weeks)	NOAEL: 1 mg/kg-day for ↑ liver weight
Domestic cats (72 in clinics and shelters) Bost et al. (2016)	Case control study	Range: BD to 0.121 Geometric mean: 0.0089	Significant association with PFOS and liver disease in the highest quartile	NA[a]
Male and female Cynomolgus monkeys (6/sex/dose) Chang et al. (2017)	0 or 14 mg/kg via gavage on three separate occasions over 422 days; maximum PFOS serum concentrations of 165 µg/ml for females and 160.8 µg/ml for males on day 365	NA	no toxicologically significant effects reported	NOAEL: 165 µg/ml serum PFOS
Male and female Sprague Dawley rats (12/sex/dose) Bagley et al. (2017)	0 or 100 ppm in feed (equivalent to 6 mg/kg-day for males and 6.6 mg/kg-day for females)	NA	**Both sexes:** ↑ absolute and relative liver weight; mild necrosis; hepatocellular hypertrophy **Males:** cytoplasmic vacuolization	NA[a]
Male WT and ERβ KO mice (8/dose/group) Xu et al. (2017)	0 or 5 mg/kg-day via gavage for 28 days	NA	**WT:** hepatocyte degeneration and vacuolization; ↓ bile acids **KO:** effects reported in WT animals not observed	NA[a]

Sex/Species	Exposure	Serum/Plasma Concentration (µg/ml)	Endpoints	NOAEL/LOAEL
Male Sprague Dawley rats (6/dose) Han et al. (2018a)	0, 1, or 10 mg/kg-day via gavage for 4 weeks	NA	hepatocellular hypertrophy; cytoplasmic vacuolization; ↑ serum ALT, AST; ↑ oxidative stress and apoptosis	LOAEL: 1 mg/kg-day for ↑ liver enzymes, oxidative stress, and apoptosis
Male Sprague Dawley rats (6/dose) Han et al. (2018b)	0, 1, or 10 mg/kg-day via gavage for 4 weeks	NA	↑ absolute liver weight; hepatocyte degeneration; cytoplasmic vacuolization; ↑ serum ALT, AST	NOAEL: 1 mg/kg-day for ↑ liver enzymes
Male C57BL/6 mice (5/dose) Huck et al. (2018)	normal diet: 0 or 0.089 mg/kg-day high fat diet: 0 or 0.087 mg/kg-day for 28 days	NA	**Normal diet:** ↑ relative liver weight	NA[a]
Female CD-1 mice (≥4/dose) Lai et al. (2018)	0, 0.3, or 3 mg/kg-day via gavage for 7 weeks	0.024, 33.8, and 109.6 at 7 weeks	↑ absolute and relative liver weight; yellowish liver; altered glucose metabolism	NOAEL: 0.3 mg/kg-day for liver effects
Pregnant Kunming mice (5/dose) Liang et al. (2019)	0, 0.5 or 5 mg/kg-day intragastrically throughout gestation (20.5 days)	NA	**Dams:** ↑ absolute liver weight; cytoplasmic vacuolization; inflammatory cell infiltration and cellular deformation	NOAEL: 0.5 mg/kg-day for ↑ liver weight and histopathology in dams

Sex/Species	Exposure	Serum/Plasma Concentration (µg/ml)	Endpoints	NOAEL/LOAEL
Male mice (strain not specified) (4/dose) Lv et al. (2018)	0 or 10 mg/kg-day via gavage for 3 weeks	NA	↑ relative liver weight; ↑ ALT, AST and LDH; inflammatory cell infiltration; edema; cytoplasmic vacuolization; necrosis; architectural disorganization; ↑ oxidative stress (↑ MDA and H_2O_2, ↓ GSH); ↑ biomarkers of apoptosis	NA[a]
Male and female Sprague Dawley rats (10/sex/dose) NTP (2019b)	0, 0.312, 0.625, 1.25, 2.5, or 5 mg/kg-day via gavage for 28 days	**Males:** BD, 23.73, 51.56, 94.26, 173.7, or 318.2 **Females:** 0.0543, 30.53, 66.97, 135.1, 237.5, or 413.6 at 28 days	**Both sexes:** hepatocyte hypertrophy; ↑ absolute and relative liver weight; ↑ ALT, ALP, bile salt/acid, albumin, and direct bilirubin; **Males:** ↑ AST; ↓ globulin; hepatocyte cytoplasmic vacuolization; **Females:** hepatocyte cytoplasmic alteration; ↑ total bilirubin	LOAEL: 0.312 mg/kg-day for ↑ relative liver weight in males and females
Male ICR mice (10/dose) Su et al. (2019)	0 or 10 mg/kg-day for 21 days; administration method not explicitly stated, but presumably via gavage	NA	↑ absolute liver weight; disordered liver lobule; hepatocyte vacuolization; hydropic degeneration of hepatocytes; inflammatory cell infiltration; ↑ serum ALT and AST; ↑ hepatic TNF-α and IL-6	NA[a]

[a] LOAEL/NOAEL not applicable for single dose studies.
[b] The specific manner of oral administration was not stated in study.
[c] Calculated by OEHHA using a BW of 0.267 kg and a consumption rate of 0.023 kg/day (from US EPA, 1988).

Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BD, below limit of detection; BW, body weight; CAT, catalase; ERβ, estrogen receptor beta; GD, gestation day; GGT, gamma glutamyl transferase; GSH, glutathione; GSH-Px, glutathione peroxidase; HDL, high density lipoprotein; H_2O_2, hydrogen peroxide; IL-6, interleukin-6; KO, knockout; LDH, lactate dehydrogenase; LDL, low density lipoprotein; LOAEL, lowest-observed-adverse-effect level; MDA, malondialdehyde; NOAEL, no-observed-adverse-effect level; SOD, superoxide dismutase; TNF-α , tumor necrosis factor alpha; WT, wild-type

Zhang et al. (2016c) administered PFOS in the diet of male C57BL/6 mice. Where data were presented as graphs, values were determined using GetData Graph Digitizer, version 2.26. The authors reported increases in relative liver weight, increased ALT, liver histopathology (vacuolization and necrosis), and oxidative stress. In a parallel study, PFOS-induced hepatotoxicity was exacerbated in animals receiving a marginal methionine/choline deficient diet compared to animals on a control diet (Zhang et al., 2016c). OEHHA identified a LOAEL of 3.48 mg/kg-day (serum concentration of 94.6 µg/ml) for increased liver weight.

5.2.3. Recent Mechanistic Evidence

For PFOA, results from mechanistic studies are consistent with effects observed in the liver in animal studies, such as oxidative stress and liver injury. Increased reactive oxygen species (ROS) production resulting in cytotoxicity has been observed in mouse hepatocytes exposed to PFOA (Sun et al., 2019b; Xu et al., 2019b). Binding studies have shown that PFOA binds to the antioxidant enzymes, superoxide dismutase (SOD) and catalase (CAT) (Xu et al., 2018; Xu et al., 2019b). These studies also reported PFOA increased SOD activity; however, it had no effect on CAT activity. The PFOA-induced increase in SOD activity resulted in increased apoptosis in hepatocytes.

A study by Zhang et al. (2016a) showed evidence of cell proliferation of human hepatocytes at low concentrations (50-200 µM) and cytotoxicity at high concentrations (>400 µM) of PFOA. Orbach et al. (2018) reported that apoptotic cell death occurred in multi-cellular hepatic organotypic culture models (OCMs) of either primary human or rat cells exposed to PFOA. Decreased glutathione (GSH), interleukin-10 (IL-10), and mitochondrial activity were observed in both cell types. Increased apoptosis was also observed in mouse liver AML12 cells (Wu et al., 2017). Furthermore, PFOA induced autophagosome formation in HepG2 cells (Yan et al., 2017).

Gene expression studies with PFOA (both in vivo and in vitro) have found changes associated with a myriad of physiological functions, including liver necrosis, carcinogenesis, peroxisome proliferator-activated receptor (PPAR) signaling, fatty acid metabolism, regulation of cell proliferation, apoptosis regulation and immune response (Beggs et al., 2016; Liu et al., 2016; Rebholz et al., 2016; Song et al., 2016; Das et al., 2017; Hui et al., 2017; Li et al., 2017b; Zheng et al., 2017; Cui et al., 2019; Li et al., 2019c; Li et al., 2019d; Song et al., 2019; Wen et al., 2019c). Metabolomic analyses also revealed that PFOA affects multiple metabolic pathways, including amino acid, carbohydrate, and lipid metabolism (Yu et al., 2016).

PFOA can bind to nuclear receptors found in the liver, such as PPARs (α, γ and β/δ) (Dong et al., 2016a; Rose et al., 2016; Rosenmai et al., 2018; Li et al., 2019a). In HepG2 cells, PFOA can activate human pregnane X receptor (hPXR), a nuclear receptor involved in xenobiotic metabolism in the liver and intestine (Zhang et al., 2017). Furthermore, Dong et al. (2016a)

reported PPARα, constitutive androstane receptor (CAR), and PXR effects in the liver of rats exposed to PFOA orally for 28 days.

Guo et al. (2019) reported that PFOA causes changes in urea cycle protein levels in mice, which caused an increase in serum ammonia levels, leading to liver effects such as hypertrophy and karyolysis.

For PFOS, oxidative stress has been implicated in liver injury. Increases in ROS production and lipid oxidation have been measured in hepatocytes exposed to PFOS (Wan et al., 2016; Khansari et al., 2017; Selano et al., 2019). PFOS can bind to SOD and CAT, and alter their activities (Han et al., 2018a; Xu et al., 2018; Xu et al., 2019b). Rats exposed to PFOS showed increases in ROS and nitric oxide (NO), and Kupffer cell activation induced hepatocyte proliferation via the nuclear factor- κB/tumor necrosis factor-α (NF-κB/TNF-α) pathway (Han et al., 2018a; Han et al., 2018b). Choline supplementation reduced PFOS-induced hepatic oxidative stress and changes in lipid metabolism in male C57BL/6 mice (Zhang et al., 2016c), but had no impact on steatosis in Sprague Dawley rats (Bagley et al., 2017).

Liver gene expression studies with PFOS (both in vivo and in vitro) have found changes associated with a myriad of physiological functions, including liver necrosis, carcinogenesis, PPAR signaling, fatty acid metabolism, regulation of cell proliferation, apoptosis, and immune response (Beggs et al., 2016; Dong et al., 2016a; Song et al., 2016; Bagley et al., 2017; Xu et al., 2017; Han et al., 2018a; Han et al., 2018b; Huck et al., 2018; Lv et al., 2018; Liang et al., 2019). A study by Yao et al. (2016) demonstrated that PFOS induces apoptosis in HepG2 cells via mitophagy (removal of damaged mitochondria via autophagy), leading to hepatotoxicity.

PFOS has been linked to increases in blood glucose and insulin resistance in animal studies. A study by Qiu et al. (2016b) aimed to investigate the mechanism leading to insulin resistance. They found that in HepG2 cells, PFOS interfered with the phosphatidylinositol 3-kinase-serine/threonine protein kinase, specifically protein kinase B (PKB, also known as AKT), signaling pathway and increased the expression of genes related to gluconeogenesis, such as phosphoenolpyruvate carboxykinase (PEPCK). The authors concluded that the inactivation of AKT by PFOS increased gluconeogenesis and insulin resistance in the liver.

PFOS can bind to transporters such as fatty acid binding protein and the T4 serum carrier protein, transthyretin (TTR) (Cheng and Ng, 2018; Sheng et al., 2018; Selano et al., 2019), displacing endogenous ligands and disrupting essential processes in the liver. PFOS can also induce ERβ in mice (Xu et al., 2017). ERβ knockout mice did not display signs of hepatotoxicity observed in wild-type animals exposed to PFOS, such as hepatocyte degeneration and decreases in hepatic cholesterol and bile acids, indicating that ERβ may be a possible molecular target of PFOS induced liver toxicity.

PFOS can activate hPXR, a nuclear receptor involved in xenobiotic metabolism in the liver and intestine (Zhang et al., 2017). Activation of PXR has been associated with altered lipid metabolism, endocrine disruption and carcinogenesis. The authors postulate that the activation of hPXR may be one mechanism leading to toxicity observed in animals and humans exposed to PFOS, and provide a PPARα independent mechanism for liver toxicity. PPARs α, γ and β/δ are other nuclear receptors that PFOS can bind to in the liver (Rosenmai et al., 2018; Li et al., 2019a). Binding of PFOS to these receptors has been associated with effects seen in the liver, such as cell proliferation and differentiation and lipid metabolism.

Adding to the information about possible mechanisms, Xu et al. (2017) demonstrated that, ERβ (estrogen receptor beta) knockout mice did not show the hepatotoxic effects (hydropic degeneration and vacuolization of hepatocytes, decreased hepatic cholesterol and bile acid levels) that were present in wild-type (WT) mice (Xu et al., 2017).

5.2.4. Conclusions

Overall, the recent epidemiologic evidence OEHHA identified supports the conclusions reached by US EPA: the majority of studies available to date support an association between PFOA and increases in liver enzymes in adults. While similar evidence has been seen in some studies of PFOS, this evidence overall is not as clear or consistent. Associations between PFOA and increased liver enzymes were reported in a number of different study populations, across several different exposure scenarios (occupational, high environmental, and general population exposures), in studies using both cross-sectional and prospective designs, and in studies that adjusted for the common causes of liver disease such as alcohol use, BMI, and medication use. In several studies that provided both adjusted and unadjusted results, findings changed very little after adjusting for the major determinants of liver toxicity (Gallo et al., 2012; Salihovic et al., 2018). Almost all of these studies used commonly accepted methods for assessing both exposure (serum PFOA) and outcome (blood levels of liver enzymes). Although blinding of researchers was not mentioned in most publications, descriptions of the methods that were provided suggest that participants' exposure and outcome were evaluated independently in most, if not all, of these studies.

Most of the studies OEHHA reviewed assessed liver enzyme levels using only a single serum measurement. Reliance on a single measurement like this could lead to some misclassification since liver enzyme levels may fluctuate over time in some people. However, since exposure and outcome seem to have been evaluated independently of each other in these studies, this misclassification would most likely bias results to the null, not towards the positive associations reported in most studies.

None of the recent studies of PFOA and liver toxicity identified by OEHHA controlled for other PFAS. However, several of the studies identified by US EPA (2016b) that reported associations between PFOA and liver toxicity involved occupational cohorts where PFOA exposures seemed to have far outweighed those of PFOS and other PFAS. This, combined with the fact that the results of human studies of PFOS and liver toxicity have been less consistent than those of PFOA, suggests that the associations identified for PFOA were actually related to PFOA and not entirely due to correlations between PFOA and PFOS. Further information on the potential of PFOS and other PFAS to have confounded the relationships seen between PFOA and liver toxicity, specifically increases in ALT, is presented in Section 6.1.1.

As noted by US EPA, the magnitude of the associations between PFOA and increased liver enzymes appears to be fairly small. For example, in the analysis of PFOA and ALT by Gallo et al. (2012), the partial coefficient of determination (R^2) value for PFOA was only 0.2%. Relatively small effect sizes were also seen in the more recent studies OEHHA identified in this search. For example in Jain and Ducatman (2018b), a 10% increase in PFOA was associated with only a 0.68% increase in ALT, a 0.71% increase in GGT, and a 0.49% increase in AST. Based on a linear extrapolation, this represents about a 6.8% increase in ALT and a 4.9% increase in AST for a doubling of PFOA levels. These fairly small effect sizes raise concerns that they may be due to relatively small amounts of residual confounding or bias. Importantly though, the fact that associations were seen across a number of different study populations and the fact that results

changed only slightly after adjustments for potential confounders in several studies are evidence that this is not the case.

The relatively small effect sizes reported in most of the studies of PFOA and liver enzymes might also raise concerns about their clinical relevance. These effect sizes are generally lower than what might be seen with overt hepatitis or other severe liver disease. Regardless, as shown for lead and other prevalent exposures, small effect sizes like this, which might have only minor or unnoticeable effects in an otherwise healthy individual, can have very important impacts on a population basis (Miller et al., 2009), especially for very common exposures like PFOA.

In summary, the current epidemiologic literature provides consistent evidence that PFOA can cause hepatotoxicity in humans, and in particular, increases in liver enzyme levels (Table 5.2.4). Consistent evidence for overt liver diseases has not been seen. However most of these studies involve relatively small sample sizes or small numbers of cases, and as such may not have sufficient statistical power or sensitivity to detect these types of effects. Findings for PFOS and liver toxicity in human studies are overall less consistent than those seen for PFOA but a number of human studies do suggest that PFOS could be hepatotoxic in humans.

In addition to the human evidence, exposure to PFOA and PFOS has consistently been shown to induce liver toxicity in experimental animals. Toxicity responses in the liver of rats and mice exposed to PFOA were remarkably consistent across studies (Table 5.2.1). Increased absolute and/or relative liver weight were observed in nearly all of the toxicity studies that included an assessment of the liver. Increased serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST), biomarkers of liver damage, were reported in several studies. Additionally, histopathological observations of the liver, including hypertrophy, cytoplasmic vacuolization, and oxidative stress were reported in multiple studies. Much like with PFOA, rats and mice exposed to PFOS have consistently displayed liver toxicities across studies, including increased absolute and/or relative liver weight, increased serum ALT and AST levels, histopathology including hypertrophy and vacuolization. Furthermore, the liver toxicity observed in rodent studies of PFOS is remarkably similar to toxicity observed in rodent studies of PFOA, suggesting that both chemicals are similar in their biological activities. The hepatotoxicity endpoints reported in recent animal studies (from 2016 onward) were quite consistent with data from earlier studies (prior to 2016).

Table 5.2.4. Summary of OEHHA's conclusions regarding the human and experimental animal data on PFOA and PFOS and liver toxicity

Outcome	PFOA	PFOS
ALT in humans	Mostly consistent evidence for increases in ALT	A few findings of increases in ALT
Other liver enzymes and related biomarkers in humans	Some evidence for increases in AST, GGT and bilirubin	A few findings of increases in AST, GGT, and bilirubin but findings are mixed
Liver disease in humans	A few positive associations but small sample sizes	No data
Increased liver weight in animals	Consistent evidence	Consistent evidence
Liver histopathology in animals (hypertrophy, vacuolization, etc.)	Consistent evidence	Consistent evidence

Outcome	PFOA	PFOS
Biomarkers of liver toxicity in animals (ALT, AST, etc.)	Consistent evidence	Consistent evidence

In general, perturbations of lipid homeostasis induced by PFOA and PFOS in animals differ considerably from responses in humans. This may be due to activation of PPARα, which is known to impact lipid metabolic processes more prominently in rodents than in humans. An in-depth evaluation of lipid homeostasis perturbation by PFOA and PFOS in humans and laboratory animals is presented below.

5.3. Perturbation of Lipid Homeostasis

US EPA (2016b) concluded that the human epidemiology studies on PFOA and serum lipid levels "have generally found positive associations between serum PFOA concentration and total cholesterol (TC) in the PFOA-exposed workers and the high-exposure community (i.e., increasing lipid level with increasing PFOA); similar patterns are seen with low-density lipoproteins (LDLs) but not with high-density lipoproteins (HDLs)."

In its review, US EPA identified a number of studies showing associations between PFOA and increasing total cholesterol levels (US EPA, 2016b). This included seven studies performed in three separate occupational cohorts (3M, DuPont-Washington Works, and Italy) and five studies performed in the highly exposed community near the DuPont facility in West Virginia (e.g., the C8 Health Project studies). Evidence of a positive association between serum PFOA concentrations and increasing serum levels of total cholesterol were reported in both cross-sectional and longitudinal analyses and in analyses that adjusted or controlled for factors such as age, gender, BMI, alcohol, smoking, time of hire and the use of lipid lowering medications. Steenland et al. (2015) did not find an association between high occupational PFOA exposures and self-reported use of prescription medications for high cholesterol, but did identify associations between increasing serum concentrations of PFOA and increasing total cholesterol, LDL cholesterol, and triglyceride levels. No association was seen with HDL. Associations were also seen between serum PFOA and total cholesterol in two of the three large population based cross-sectional studies identified by US EPA. US EPA also identified a number of studies that reported associations between PFOA and LDL, HDL, and triglyceride levels, although these associations were much less consistent than those seen for total cholesterol.

The ATSDR (ATSDR, 2018a) identified several additional studies published prior to 2016 that were not included in the 2016 US EPA review. This included two population based studies in either pregnant women (N=854) (Skuladottir et al., 2015) or children (N=225) (Zeng et al., 2015) that reported associations between PFOA and total cholesterol. It also included a cross-sectional study of highly exposed workers (N=55) and nearby community residents (N=132) in China, which found an inverse association between PFOA and HDL cholesterol (Wang et al., 2012), and a lower exposure community study in the UK which reported an association between prenatal PFOA levels and increased total cholesterol and LDL in the offspring at ages 7 and 15 years, but only in analyses confined to the lowest tertile of PFOA exposure (Maisonet et al., 2015). Clear associations were not seen in the upper two tertiles.

In its 2016 review of PFOS, US EPA (2016d) concluded that, "Multiple epidemiologic studies have evaluated serum lipid status in association with PFOS concentration. These studies

provide support for an association between PFOS and small increases in total cholesterol." They also noted that, "Evidence for associations between other serum lipids and PFOS is mixed, with some studies showing an association with measurements of concurrent HDL and/or LDL and others failing to measure the serum lipoprotein complexes." In its review, US EPA identified only two higher exposure studies, both done in the 3M occupational cohort. In the first, a cross-sectional study involving approximately 500 workers, associations were identified between increasing serum PFOS concentrations and increasing cholesterol and triglycerides (Olsen et al., 2003a). In the second (published in the same paper), a longitudinal analysis involving 175 workers in the same facilities, clear associations were not seen (Olsen et al., 2003a). Serum levels of both PFOS and PFOA were high in these workers (geometric means of 0.44-0.91 µg/ml for PFOS and 0.33-1.13 µg/ml for PFOA). No community studies with high PFOS exposures were identified. US EPA noted a number of population-based studies that examined associations between PFOS and lipid levels, with the large majority finding at least some evidence of an association with increased total cholesterol, some finding associations with increased LDL, and mostly mixed or null results for HDL and triglycerides.

US EPA (2016b) also identified multiple studies in animals that report decreases in serum cholesterol and triglyceride levels in rodents following exposure to PFOA. However, US EPA noted that "decreases in triglycerides, cholesterol, and lipoprotein complexes are an expected consequence of PPARα activation in rodents," thus these changes would be expected considering that PFOA is a known PPARα activator.

US EPA (2016d) identified several studies reporting decreased cholesterol levels following PFOS administration in experimental animals (rats, mice, and monkeys). Additionally, PFOS induced differential gene expression in genes involved with lipid and cholesterol metabolism and transport.

5.3.1. Recent Human Evidence

OEHHA identified 28 human epidemiologic studies of PFOA and PFOS and lipid levels published since (or otherwise not included in) the 2016 US EPA reviews. These studies are summarized in Appendix 7, Tables A7.8 and A7.9. Overall, the findings from the studies OEHHA reviewed are mostly consistent with US EPA's 2016 review and conclusions. That is, a number of studies reported associations between increasing levels of PFOA and increasing total cholesterol and increasing LDL in adults. This included several studies with overall high quality ratings. Most of this evidence involved cross-sectional assessments, although positive associations between PFOA and total cholesterol, LDL, and triglycerides were also seen in the cohort study by (Lin et al., 2019). Positive associations were seen in several studies that OEHHA rated as having high quality. Several studies also identified associations between increasing PFOA and increasing triglyceride concentrations, but this was not as consistent as seen for total cholesterol or LDL. The findings for HDL in adults and findings in children appear to be less consistent than seen for total cholesterol and LDL in adults.

Findings for PFOS were mostly similar to those of PFOA. That is, several studies identified associations with PFOS and increasing total cholesterol and LDL, including several with reasonably high quality score ratings. Some studies did not identify these associations, although no study reported a clear association between increasing PFOS and decreases in total cholesterol or LDL concentrations.

5.3.2. Recent Animal Evidence

OEHHA's review of recent PFOA animal studies examining changes in lipid homeostasis, published from 2016 onward, is presented in Table 5.3.1.

Table 5.3.1. Summary of recent animal toxicity studies of PFOA reporting effects on lipid homeostasis

Sex/Species/ Reference	Exposure	Serum/Plasma Concentrations (µg/ml)	Endpoints	NOAEL/LOAEL
Male and female C57BL/6 mice (6/sex/dose) Rebholz et al. (2016)	0 or 3.5 mg/kg of feed (fat- and cholesterol-containing diet) (~0.55 mg/kg BW-day, according to authors) for six weeks	**Males:** 0.002 or 26.9 **Females:** 0.028 or 44.3 at six weeks	**Both sexes:** ↑ plasma cholesterol	NA[a]
Male and female BALB/c mice (6/sex/dose) Rebholz et al. (2016)	0 or 3.5 mg/kg of feed (fat- and cholesterol-containing diet) (~0.55 mg/kg BW-day, according to authors) for six weeks	**Males:** 0.005 or 28.2 **Females:** 0.086 or 35.6 at six weeks	**Both sexes:** ↓ hepatic cholesterol **Males:** ↑ plasma cholesterol	NA[a]
Male BALB/c mice (5/dose) Yu et al. (2016)	0, 0.5, or 2.5 mg/kg-day via "oral infusion" for 28 days	0.011, 29.34, or 114.3 at 28 days	changes in lipid metabolism; changes in fatty acid biosynthesis	LOAEL: 0.5 mg/kg-day
Male SV129 WT and PPARα KO mice (4/dose) Das et al. (2017)	0 or 10 mg/kg-day via gavage for 7 days	NA	**WT:** increased liver triglycerides (mild steatosis)	NA[a]
Male BALB/c mice (minimum of 8/dose) Hui et al. (2017)	0, 1, or 5 mg/kg-day orally[b] for 7 days	NA	↓ free fatty acids and serum triglycerides; ↑ liver triglycerides	LOAEL: 1 mg/kg-day for ↓ serum triglycerides and ↑ liver triglycerides

Sex/Species/ Reference	Exposure	Serum/Plasma Concentrations (µg/ml)	Endpoints	NOAEL/LOAEL
Male and female BALB/c mice (30/sex/dose) Li et al. (2017b)	0, 0.05, 0.5, or 2.5 mg/kg-day via gavage for 28 days	**Males:** 0, 1.2, 5.9 or 13.4 **Females:** 0, 0.97, 2.7 or 9.5 at 28 days	lipid accumulation in cytoplasm of hepatocytes	NOAEL: 0.05 mg/kg-day
Male Kunming mice (number not specified) Wu et al. (2017)	Single oral dose of 0 or 5 mg/kg via gavage	NA	↑ hepatic LDL cholesterol; ↓ hepatic HDL cholesterol	NA[a]
Male BALB/c mice (3/dose) Yan et al. (2017)	0, 0.08, 0.31, 1.25, 5, or 20 mg/kg-day via gavage for 28 days	NA	lipid deposits	Not provided[b]
Pregnant Kunming mice (8/dose) Qin et al. (2018)	0 or 5 mg/kg-day intragastrically throughout gestation	NA	↑ triglycerides, and cholesterol in F1 pup serum on PND 21 (although the changes were not statistically significant)	NA[a]
Male Kunming mice (8/dose) Wu et al. (2018)	0, 1, or 5 mg/kg-day intragastrically for 21 days	NA	↓ serum triglycerides and HDL; ↑ triglycerides in liver; ↑ LDL in serum	NOAEL: 1 mg/kg-day for ↑ liver triglycerides
Male miR-34A -/- KO and WT C57BL/6J mice[c] (15-18/dose) Cui et al. (2019)	0 or 5 mg/kg-day via gavage for 28 days	NA	**WT and KO:** ↓ liver triglycerides	NA[a]

Sex/Species/ Reference	Exposure	Serum/Plasma Concentrations (µg/ml)	Endpoints	NOAEL/LOAEL
Male BALB/c mice (12/dose) Guo et al. (2019)	0, 0.4, 2, or 10 mg/kg-day via gavage for 28 days	0.02, 13, 64, or 88	steatosis	NOAEL: 2 mg/kg-day
Male C57BL/6 mice (5/diet/treat-ment/time point) Li et al. (2019d)	0 or 1 mg/kg-day (in distilled water, presumably gavage) for 2, 8, or 16 weeks. Half of the animals were on low fat diet, other half on high fat diet	NA	↓ steatosis induced by the high-fat diet	NA[a]
Male and female Sprague Dawley rats (10/sex/dose) NTP (2019a)	0, 0.625, 1.25, 2.5, 5, or 10 mg/kg-day (for males) and 0, 6.25, 12.5, 25, 50, or 100 mg/g-day (for females) via gavage for 28 days	**Males:** BD, 0.378, 0.503, 1.297, 3.34, or 10.9 **Females:** BD, 0.129, 0.292, 0.475, 1.67, or 6.71 at 28 days	↓ in cholesterol and triglyceride levels in males, but ↑ in females	LOAEL: 0.625 mg/kg-day for ↓ cholesterol and triglycerides in males
Male APOE*3-Leiden.CETP transgenic mice[d] (8/dose) Pouwer et al. (2019)	0, 0.01, 0.291, or 30.2 mg/kg-day in diet for 6 weeks	0.005, 0.065, 1.524 or 144 at 6 weeks	↓ plasma triglycerides, total cholesterol, and non-HDL cholesterol; ↑ plasma HDL cholesterol	NOAEL: 0.291 mg/kg-day for changes in plasma lipid contents
Male APOE*3-Leiden.CETP transgenic mice[d] (8/dose) Pouwer et al. (2019)	0, 0.01, 0.298, or 29.5 mg/kg-day in diet for 4 weeks	0, 0.051, 1.395, or 93.713 at 4 weeks	microvesicular steatosis; ↓ plasma triglycerides and non-HDL cholesterol; ↑ plasma HDL cholesterol	NOAEL: 0.298 mg/kg-day for steatosis

Sex/Species/ Reference	Exposure	Serum/Plasma Concentrations (µg/ml)	Endpoints	NOAEL/LOAEL
Pregnant CD-1 mice (11-13 dams/dose) Blake et al. (2020)	0, 1, or 5 mg/kg-day via gavage from ED1.5 to ED11.5 or ED17.5	ED11.5: BD, 25.4 or 117.3 ED17.5: 0.211, 18.7 or 95.1	**Dams:** ↓ serum triglycerides	LOAEL: 1 mg/kg-day

[a] LOAEL/NOAEL not applicable for single dose studies.
[b] Histology data are presented in the supplementary materials, but specific doses at which hepatocyte swelling and lipid deposits become significant are not provided.
[c] miR-34A -/- KO mice do not produce the microRNA miR-34A. miR-34A functions as a tumor suppressor.
[d] APOE*3-Leiden.CETP transgenic mice are reported to have human-like lipoprotein metabolism.
Abbreviations: BD, below limit of detection; BW, body weight; ED, embryonic day; HDL, high density lipoprotein; KO, knockout; LDL, low density lipoprotein; LOAEL, lowest-observed-adverse-effect level; NOAEL, no-observed-adverse-effect level; PND, postnatal day; PPARα, peroxisome proliferator-activated receptor alpha; WT, wild-type

Pouwer et al. (2019) examined the hepatic effects of PFOA using a transgenic mouse model that possesses human-like lipid metabolism. In addition to microvesicular steatosis and hepatotoxicity, PFOA induced changes in plasma lipids, including decreased plasma triglycerides, decreased plasma non-HDL cholesterol, and increased HDL cholesterol. The authors suggested that this mouse model presents a suitable representation of lipid metabolism in humans, as these data supported a phase I clinical trial of PFOA in humans that showed an association between higher PFOA levels and lowered cholesterol. OEHHA identified a NOAEL of 0.29 mg/kg-day for steatosis and changes in plasma lipid contents.

Much like for PFOA, several animal studies published from 2016 onward reported changes in lipid homeostasis following oral exposure to PFOS. Study details are summarized in Table 5.3.2.

Table 5.3.2. Summary of recent animal toxicity studies of PFOS reporting lipid effects

Sex/Species	Exposure	Serum/Plasma Concentration (µg/ml)	Endpoints	NOAEL/LOAEL
Male C57BL/6 mice (5-6/dose) Zhang et al. (2016c)	0, 30, 60, or 120 mg/kg of feed (equivalent to 0, 3.48, 6.96, or 13.92 mg/kg BW-day)[a] for 21 or 23 days	0, 94.6, 176, and 392 at 21 days	↑ hepatic triglycerides and lipid content; altered lipid metabolism	LOAEL: 3.48 mg/kg-day
Male and female Sprague Dawley rats (12/sex/dose) Bagley et al. (2017)	0 or 100 ppm in feed (equivalent to 6 mg/kg-day for males and 6.6 mg/kg-day for females)	NA	**Males:** ↓ serum cholesterol and triglyceride levels; ↑ liver free fatty acids and triglycerides **Females:** ↓ liver free fatty acids and triglycerides	NA[b]
Male WT and ERβ KO mice (8/dose/group) Xu et al. (2017)	0 or 5 mg/kg-day via gavage for 28 days	NA	**WT:** ↓ hepatic cholesterol **KO:** effect reported in WT animals not observed	NA[b]
Male C57BL/6 mice (5/dose) Huck et al. (2018)	normal diet: 0 or 0.089 mg/kg-day high fat diet: 0 or 0.087 mg/kg-day for 28 days	NA	**Normal diet:** ↑ relative liver weight; steatosis **High fat diet:** ↓ steatosis	NA[b]
Female CD-1 mice (≥4/dose) Lai et al. (2018)	0, 0.3, or 3 mg/kg-day via gavage for 7 weeks	0.024, 33.8, and 109.6 at 7 weeks	↑ liver triglycerides; ↓ serum triglycerides	LOAEL: 0.3 mg/kg-day for ↑ liver triglycerides
Pregnant Kunming mice (5/dose) Liang et al. (2019)	0, 0.5 or 5 mg/kg-day intragastrically throughout gestation (20.5 days)	NA	**Dams:** ↑ liver triglycerides; ↑ liver lipid droplets; **Pups:** ↑ liver triglycerides, total cholesterol, and LDL; ↓ HDL	NOAEL: **Dams**: 0.5 mg/kg-day for ↑ liver triglycerides **Pups**: 0.5 mg/kg-day for changes in liver lipid contents

Sex/Species	Exposure	Serum/Plasma Concentration (µg/ml)	Endpoints	NOAEL/LOAEL
Male and female Sprague Dawley rats (10/sex/dose) NTP (2019b)	0, 0.312, 0.625, 1.25, 2.5, or 5 mg/kg-day via gavage for 28 days	**Males:** BD, 23.73, 51.56, 94.26, 173.7, or 318.2 **Females:** 0.0543, 30.53, 66.97, 135.1, 237.5, or 413.6 at 28 days	**Both sexes:** ↓ cholesterol and triglycerides	LOAEL: 0.312 mg/kg-day for ↓ cholesterol in males
Male ICR mice (10/dose) Su et al. (2019)	0 or 10 mg/kg-day for 21 days; administration method not explicitly stated, but presumably via gavage	NA	↑ triglycerides and total cholesterol	NA[b]

[a] Calculated by OEHHA using a BW of 0.267 kg and a consumption rate of 0.023 kg/day (from US EPA, 1988).
[b] LOAEL/NOAEL not applicable for single dose studies.
Abbreviations: BD, below limit of detection; BW, body weight; HDL, high density lipoprotein; KO, knockout; LDL, low density lipoprotein; LOAEL, lowest-observed-adverse-effect level; NOAEL, no-observed-adverse-effect level; WT, wild-type

5.3.3. Recent Mechanistic Evidence

PFOA has been shown to disrupt lipid metabolism in the liver. One way PFOA does this is by changing the expression and activity of enzymes involved in fatty acid metabolism. Changes in fatty acid metabolism have been linked to liver disease. PFOA increases acyl-CoA oxidase activity in rat liver (Cavallini et al., 2017), and carboxylesterase mRNA and protein levels in male mice (Wen et al., 2019c). Carboxylesterases play a role in lipid metabolism and homeostasis (Lian et al., 2018). A proteomic study by Shao et al. (2018) showed that PFOA can bind to acetyl-CoA carboxylase a and b (Acaca and Acacb), enzymes involved in fatty acid metabolism. PFOA has also been shown to bind to human liver fatty acid-binding protein, which would displace uptake of fatty acids and thus potentially disrupt lipid regulation (Sheng et al., 2016; Sheng et al., 2018; Cheng et al., 2019). PFOA is also associated with changes in hepatic and serum cholesterol levels (Rebholz et al., 2016). Pouwer et al. (2019) showed that PFOA altered levels of cholesterol ester transfer protein, which subsequently changed cholesterol metabolism and homeostasis in male APOE*3 Leiden transgenic mice, which were made to have human-like lipoprotein metabolism.

For PFOS, liver gene expression studies (both in vivo and in vitro) have found changes associated with fatty acid metabolism (Beggs et al., 2016; Dong et al., 2016a; Song et al., 2016; Bagley et al., 2017; Xu et al., 2017; Han et al., 2018a; Han et al., 2018b; Huck et al., 2018; Lv et al., 2018; Liang et al., 2019).

Inflammation and endoplasmic reticulum stress have been associated with hepatic steatosis in mice exposed to PFOS (Su et al., 2019). Levels of TNF-α, a cell signaling protein associated with inflammatory stress, and endoplasmic reticulum stress proteins (activating transcription factor 6 [ATF6], Eukaryotic translation initiation factor 2 subunit 1 [eIF2α], 78kDa glucose-regulated protein [GRP78], X-box binding protein 1 [XBP1]) were increased in the liver in mice exposed to PFOS. ER stress pathways have been linked to hepatic steatosis (Zhang et al., 2014), thus perturbation to the normal processes may lead to liver toxicity.

5.3.4. Conclusions

As shown in Table A7.11 (Appendix 7), nine of the 16 studies in adults that reported on PFOA and total cholesterol (TC) or LDL identified some evidence of a positive association (marked as "+" or "(+)" in the table), two found no association (marked as "0"), one reported a negative association (increasing PFOA associated with a decrease in TC; marked as "-" or "(-)" in the table), and the others presented results that were difficult to interpret (marked as "U"). Of the seven studies of PFOA and TC or LDL that did not find positive associations, three reported positive associations with triglyceride levels. The average quality score in the studies that did and did not find some evidence of a positive association are 8.0 and 5.1, respectively. Only two of the nine of the studies of PFOA and TC or LDL in children reported some evidence of a positive association.

Eight of the 15 studies in adults that reported on PFOS and total cholesterol (TC) or LDL identified some evidence of a positive association, four found no association including the most recent NHANES study by (Liu et al., 2018b), one reported a negative association, and the others presented results that were difficult to interpret (marked as "U") (Appendix 7, Table A7.12). Only one of the PFOS studies that did not find evidence for a positive association with either TC or LDL reported an association for triglyceride levels. This was the relatively small cross-sectional study by (Yang et al., 2018) (N=145) and the major findings were not statistically significant. The average quality scores in the studies that did and did not report evidence of an association between PFOS and TC or LDL were 7.4 and 5.4, respectively. As with PFOA, the majority of results for PFOS in the recent studies in children were negative.

Several sources of possible bias in the studies reviewed were evaluated. First, OEHHA examined the possibility that confounding by diet or diet-related factors might have caused the associations between PFOA or PFOS and lipid levels reported in many of these studies. For example, if an increased consumption of certain foods (or greater exposure to food packaging) is associated with both increased PFOA exposure and with higher lipid levels, this could potentially confound any relationships between PFOA or PFOS and lipid levels. Since PFOA and PFOS have been found in a number of different foods (Appendix 7, Table A7.1), it is possible that increased dietary consumption overall (i.e., overall calorie intake) could be an important confounder. However, many of the studies OEHHA reviewed adjusted for BMI, waist circumference, or total calorie intake. Other studies adjusted for specific foods or dietary patterns (e.g., fat intake, Mediterranean diet) that might be related to total cholesterol levels. In a cross-sectional study of 965 Danish pregnant women, both mid-gestational serum PFOS and PFOA concentrations were found to be associated with increased meat or meat product intake and decreased vegetable intake (Skuladottir et al., 2015). However, the associations reported between PFOS and PFOA and total cholesterol changed very little with adjustments for fat, total calorie, or meat and vegetable intake (Table 5.3.3).

Table 5.3.3. Differences in mean total cholesterol levels (mmol/dl) between the fifth and first quintiles of serum PFOA or PFOS concentrations and p-trends across quintiles in Danish pregnant women (Skuladottir et al., 2015).

Adjustment	PFOA	PFOS
Age, parity, education, smoking and BMI	0.40 (p=0.008)	0.41 (p=0.007)
Plus fat intake and total calorie intake[1]	0.39 (p=0.01)	0.39 (p=0.01)
Plus meat, vegetable, and total calorie intake[1]	0.45 (p=0.003)	0.44 (p=0.004)

[1] Adjusted for these factors in addition to age, parity, education, smoking and BMI

Another factor suggesting that diet is not a major confounder in all of the studies reporting PFOA- or PFOS-lipid associations is that some of these associations have been seen in several high exposure studies. In occupational studies and community studies done in areas with known high drinking water contamination, most PFOA or PFOS intake is likely related to the environmental contamination or occupational exposure, and less is likely related to the dietary sources usually seen in the general population. As such, some of the usual diet-related sources of PFOA or PFOS are less likely to confound PFOS- or PFOA-lipid associations in these studies. For example, associations between PFOA and total cholesterol have been reported in several high exposure occupational studies where serum PFOA concentrations in the higher and lower PFOA exposure groups differ by 1,000 ng/ml or more (US EPA, 2016b). In contrast, most dietary studies suggest that dietary factors are usually only responsible for differences in serum PFOA of about 1-2 ng/ml or less (Jain, 2014; Skuladottir et al., 2015; Harris et al., 2017). While this might not apply to locally grown or produced foods, it would apply to other foods or food related items. Overall, the small contribution of many of the usual diet or diet-related sources to the overall exposure levels in the high exposure occupational or community studies suggest that confounding by these typical diet-related exposure sources is unlikely to be the cause of the associations identified in these studies.

Many of the studies identifying associations between PFOS or PFOA and lipid levels are cross-sectional studies. As mentioned above, studies of this design are frequently criticized based on their potential for reverse causation, or for the fact that they cannot assure that the most appropriate exposure window has been assessed (e.g., latency effects). With regards to the latter, the serum half-lives of both PFOA and PFOS are several years (Olsen et al., 2007), and studies have shown that serum concentrations measured in the same people over several years are generally very well correlated (Liu et al., 2018a). For example, Nøst et al. (2014), reported correlation coefficients between 0.60 and 0.84 for PFOA and PFOS in 53 Norwegian men in samples collected 6-8 years apart (Table 5.3.4). Overall, a single cross-sectional measurement of PFOA and PFOS concentrations in serum is likely to provide a fairly accurate picture of a person's exposure over a period of several years. Since OEHHA could not find convincing evidence that the latency between PFOA or PFOS exposure and adverse effects on lipid levels is longer than this, it seems that widespread major errors due to missing or inadequate exposure data is unlikely to have caused the associations reported in many of the studies reviewed.

Table 5.3.4. Spearman correlation coefficients for serum PFOA and PFOS concentrations measured in the same individuals over time (Nøst et al., 2014) (all p-values <0.05)

	Beginning and ending year of measurement			
Compound	**1979-1986**	**1986-1994**	**1994-2001**	**2001-2007**
PFOA	0.65	0.66	0.60	0.75
PFOS	0.84	0.65	0.62	0.81

Reverse casualty might be possible in some cross-sectional studies if having altered lipid levels or being diagnosed with a lipid related disease could lead to the use of certain medications or lead to changes in certain lifestyle factors that might affect one's PFOA or PFOS exposure. Again, this would seem less likely in the high exposure occupational or community studies, where these factors might not be the major determinants of exposure. In addition, several studies reported associations after excluding people taking lipid-lowering medications or after adjusting for use of these medications (Sakr et al., 2007; Steenland et al., 2009; Liu et al., 2018b; Jain and Ducatman, 2019; Lin et al., 2019). In addition, PFOA-lipid associations have been seen in a number of prospective studies (Olsen et al., 2003a; Sakr et al., 2007; Fitz-Simon et al., 2013), which might be less susceptible to reverse causality than some cross-sectional analyses.

Finally, few of the studies provided detailed information on participant selection or response/participation/refusal rates, and most appeared to involve convenience samples. Importantly though, none of the studies OEHHA reviewed appeared to have selected participants based on both PFOA and lipid levels, and thus widespread and important selection bias seems unlikely.

The evidence for PFOS also supports an association with increased total cholesterol and LDL levels in adults. Evidence based on high exposure scenarios appears to be limited to studies from a single occupational facility, where results have been mixed (Olsen et al., 2003a). Despite this, a number of large population based studies in adults with seemingly high quality have reported associations between PFOS and increases in total cholesterol (US EPA, 2016b; He et al., 2018; Dong et al., 2019). In most, if not all, of these studies serum PFOS levels are highly correlated with serum levels of PFOA or other PFAS. This raises the concern that some of the associations reported in these studies might be due to other PFAS. Because of these high correlations, appropriately adjusting for these other PFAS can be difficult in epidemiologic studies due to issues related to co-variance, and none of the PFOS-lipid studies OEHHA reviewed that identified positive associations with total cholesterol and LDL reported results with these adjustments. Some animal studies have identified PFOS related changes in lipid homeostasis in the liver, and this provides some biologic plausibility that PFOS can affect lipid metabolism in humans.

Using data from the 2003-14 NHANES on 8,948 adults, Dong et al. (2019) calculated benchmark doses (BMDs) using a hybrid approach. In Dong et al. (2019) this involved setting a cut-off point for elevated TC based on the distribution of TC values in the unexposed or lower exposed reference group. Here, this cut-off point was the upper 5th percentile of TC values in the lowest PFOA exposure group. The benchmark response was then defined as a 10% increase in values above this level. Reference doses (RfD) were then calculated using the following equation, where Vd is the volume of distribution (0.17 L/kg for PFOA and 0.23 L/kg for PFOS); $T_{1/2}$ is the half-life in serum (5.4 years for

PFOS and 2.3 years for PFOA); and BMDL is the lower 95% confidence interval of the BMD:

$$RfD = BMDL \times Vd \times Ln(2) \times 1{,}000/T_{1/2}$$

The results of these calculations are shown in Table 5.3.5. Based on data from the 2013-14 NHANES, the authors estimated that approximately 3.8% and 3.4% of the US population had PFOA or PFOS serum concentrations, respectively, above the BMDLs.

Table 5.3.5. Benchmark and reference doses for a 10% increase in serum total cholesterol based on 8,948 adults in NHANES 2003-14 (Dong et al., 2019)

Chemical	BMD (ng/ml)	BMDL (ng/ml)	RfD (ng/kg/day)
PFOA	10.5	5.6	0.8
PFOS	44.2	24.1	2.0

Abbreviations: BMD, benchmark dose; BMDL, lower 95% confidence interval of the BMD; NHANES, US National Health and Nutrition Examination Survey; RfD, reference dose

Although the hybrid approach has a number of advantages (see Crump (1995)), few details were provided in this paper on several important aspects of this approach or on other key issues, including the definition of the unexposed reference group, the distribution of PFOA or TC values in this group, model fit (e.g., the fit of linear versus non-linear models), the impact of potential confounders, or the role of reverse causality

For effects on lipid homeostasis in animals, several recent studies reported that PFOA decreased serum triglycerides and cholesterol in mice (Hui et al., 2017; Wu et al., 2018; Pouwer et al., 2019), which support the conclusions reached in other assessments. A study using APOE*3-Leiden.CETP transgenic mice, which are reported to have human-like lipoprotein metabolism, reported decreased plasma triglycerides and total cholesterol (Pouwer et al., 2019), which the authors suggest is predictive of human physiological responses to PFOA exposure. Conversely, one study reported that PFOA administered with a high-fat diet increased plasma cholesterol in mice (Rebholz et al., 2016). In rats, PFOA induced differential changes in triglyceride and cholesterol levels based on sex, where decreased levels were observed in males, and increased levels were observed in females (NTP, 2019a). Interestingly, several studies reported that levels of HDL and LDL cholesterol were differently impacted by PFOA exposure, although the results were inconsistent between studies (Wu et al., 2018; Pouwer et al., 2019).

Accumulation of triglycerides in the liver (steatosis) was also reported in several rodent studies (Das et al., 2017; Hui et al., 2017; Li et al., 2017b; Wu et al., 2018; Guo et al., 2019; Pouwer et al., 2019). Interestingly, this effect was not observed in PPARα knockout mice (Das et al., 2017), indicating that PPARα activation significantly contributes to the induction of hepatic steatosis. However, a few studies observed contradictory results in mice, where PFOA reduced liver triglycerides (Cui et al., 2019), and even reversed steatosis induced by a high-fat diet (Li et al., 2019d).

For PFOS, two recent studies in rats reported decreased serum cholesterol and triglycerides (Bagley et al., 2017; NTP, 2019b), although statistical significance was not reached for females in one study (Bagley et al., 2017). Conversely, one recent study in mice reported increased serum triglycerides and cholesterol (Su et al., 2019).

Much like PFOA, several studies reported increases in liver triglycerides in mice exposed to PFOS (Zhang et al., 2016c; Bagley et al., 2017; Lai et al., 2018; Liang et al., 2019), including in pups exposed gestationally (Liang et al., 2019). Gestational exposure also increased hepatic LDL levels, and decreased HDL levels (Liang et al., 2019). Huck et al. (2018) reported that PFOS induced steatosis in mice fed on a regular diet, but decreased steatosis in animals on a high-fat diet. In rats, levels of liver free fatty acids and triglycerides differed based on sex (increased in males, decreased in females) (Bagley et al., 2017). One study reported decreases in hepatic cholesterol in mice following PFOS exposure, but these effects were not observed in ERβ knockout animals (Xu et al., 2017).

In summary, the current epidemiologic literature provides evidence that PFOA and PFOS can cause increased total cholesterol in humans (Table 5.3.6). In contrast, some animal studies have shown decreased cholesterol with PFOA and PFOS exposure (Table 5.3.6). Different results in animals and humans may be explained by the stronger activity of PPARα in animals, which is involved in the metabolism of cholesterol and fatty acids.

Table 5.3.6. Summary of OEHHA's conclusions regarding the human and experimental animal data on PFOA and PFOS and lipids

Outcome	PFOA	PFOS
Total cholesterol in humans	-Mostly consistent associations with increased total cholesterol in adults -Findings are less consistent in children	-Mostly consistent associations with increased total cholesterol in adults -Findings are less consistent in children
Other lipids in humans	Some associations identified for increased LDL and TGs, and for decreased HDL but fewer studies	Data on LDL, TGs, and HDL are inconclusive
Lipid effects in animals (TGs and cholesterol, steatosis)	Evidence of decreased serum TGs and cholesterol in several studies, and mixed results for increased steatosis	Inconsistent evidence for decreased serum and hepatic cholesterol and TGs, and steatosis

HDL, high density lipoprotein; LDL, low density lipoprotein; TGs, triglycerides

5.4. Thyroid Toxicity

The US EPA has reviewed the scientific literature on PFOA and PFOS and thyroid toxicity (US EPA, 2016b; US EPA, 2016d). This review included literature published up to December 2015. In their reviews, US EPA identified a number of epidemiologic studies that investigated associations between PFOA or PFOS and thyroid hormone levels or more overt thyroid diseases.

For PFOA, US EPA (2016b) identified three epidemiologic studies linking this agent to thyroid disease, primarily hypothyroidism. The first was a cross-sectional study involving US NHANES adult participants (Melzer et al., 2010). Here, the adjusted ORs for self-reported current thyroid disease were 2.24 (95% CI, 1.38–3.65) in women and 2.12 (95% CI, 0.93–4.82) in men, comparing those in the upper quartile to those in the lower two quartiles of serum PFOA. The second study investigated parent-reported thyroid disease in 10,725 children 1-17 years of age living near a Teflon™ manufacturing facility in the Mid-Ohio Valley (USA) (Lopez-Espinosa et al., 2012). In analyses adjusted for age and sex, the OR for hypothyroidism for an interquartile

contrast of 13 to 68 ng/ml in serum PFOA was 1.54 (95% CI, 1.00-2.37). Clear associations with thyroxine (T4) or thyroid stimulating hormone (TSH) were not seen. The third study was a large investigation that included adults in the same community (Winquist and Steenland, 2014). Here, an environmental fate and transport model was used to estimate yearly air and drinking water PFOA concentrations. These were then combined with information on residential history, drinking water consumption rates, and water sources to estimate each participant's yearly PFOA intake rate. These intake rates were then used in an absorption/distribution/metabolism/ excretion model to generate yearly estimates of serum PFOA concentrations for each participant. For participants who worked in the local chemical plant, where PFOA exposures were especially high, an occupational exposure model using work history and modeled job- and department-specific serum concentrations was used to generate yearly serum PFOA concentration estimates for years when the participant worked at the plant. Information on thyroid disease was initially based on self-reports then, confirmed using medical records. Hazard ratios (HRs) controlling for sex, race, education, smoking, and alcohol use for all thyroid diseases combined across cumulative PFOA exposure quintiles were 1.00, 1.24, 1.27, 1.36, and 1.37 in women (p-trend = 0.03) and 1.00, 1.12, 0.83, 1.01, and 1.05 among men (p-trend = 0.85).

With regards to PFOA and thyroid hormone levels, US EPA concluded, "Association between PFOA and TSH also was seen in pregnant females with anti-TPO antibodies (Webster et al., 2014). In contrast, generally null associations were found between PFOA and TSH or thyroid hormones (triiodothyronine (T3) or T4) in people who have not been diagnosed with thyroid disease" (Table 5.4.1).

For PFOS, US EPA (2016d) concluded that the epidemiologic studies they reviewed provided "limited support" for an association between PFOS and thyroid disease. This conclusion was primarily based on two large cross-sectional studies using participants of the US NHANES. The first involved data from NHANES 1999-2000, 2003-2004, and 2005-2006 and included 1,900 males and 2,066 females ages 20 and older (Melzer et al., 2010). In men, the OR adjusted for age, ethnicity, education, BMI, smoking status, and alcohol consumption for self-reported currently treated thyroid disease in those with serum PFOS concentrations ≥36.8 ng/ml (fourth quartile) compared to those with serum PFOS ≤25.5 ng/ml (first and second quartiles) was 2.68 (95% CI, 1.03–6.98). In women, this OR was 1.27 (95% CI, 0.82–1.97). The second study included 1,181 subjects >20 years of age from the 2007-2008 and 2009-2010 NHANES (Wen et al., 2013). In analyses adjusted for age, race, drinking, smoking, and urinary iodine levels, a 1-unit increase in ln-PFOS serum concentration was associated with subclinical hypothyroidism in both men (OR = 1.98; 95% CI, 1.19–3.28) and women (OR = 3.03; 95% CI, 1.14–8.07).

US EPA (2016d) also identified a number of epidemiologic studies that investigated associations between PFOS and levels of thyroid hormones, including T4, free thyroxine (fT4), T3, and TSH. Based on these studies, US EPA concluded that in most groups, including children, the evidence linking PFOS to these hormones was inconsistent. An exception was seen for pregnant women where the three studies in this group all identified associations between increasing serum concentrations of PFOS and increasing serum concentrations of TSH (Wang et al., 2013; Webster et al., 2014; Berg et al., 2015). In one of these, the association was only seen in women with elevated levels of anti-thyroid peroxidase (anti-TPO) antibodies (Webster et al., 2014).

Table 5.4.1. Summary of US EPA's conclusions on epidemiologic studies of PFOA or PFOS and thyroid toxicity (US EPA, 2016b; US EPA, 2016d)

Outcome	PFOA	PFOS
Thyroid hormone levels	No clear associations overall ↑TSH in anti-TPO positive (one study)	Inconsistent overall ↑TSH in pregnancy (three studies)
Thyroid disease	Two positive, one negative study	"Limited evidence" overall (two NHANES studies)

US EPA (2016b) identified two studies examining thyroid effects in experimental animals exposed to PFOA (Butenhoff et al., 2002; Martin et al., 2007, as cited by US EPA (2016b)) These studies reported decreases in total T3 and free T3 in monkeys, and decreases in total T4 and free T4 in male rats, both without significant changes in TSH. No thyroid hormone changes were observed in female rats, presumably due to the higher clearance rate of PFOA compared to male rats and other species.

Compared to PFOA, the thyroid toxicity database for PFOS in animals is more extensive. US EPA (2016d) identified several studies reporting thyroid effects in animals exposed to PFOS (Seacat et al., 2002; Lau et al., 2003; Thibodeaux et al., 2003; Luebker et al., 2005; Chang et al., 2007; Martin et al., 2007; Chang et al., 2008; Yu et al., 2011, as reported in US EPA (2016d)). In general, a reduction in fT4 and total T4 (tT4) levels, without significant changes in TSH, was consistently observed in rats exposed to PFOS. The evaluated studies included studies in pregnant rats, where PFOS reduced T4 and T3 levels in dams (Thibodeaux et al, 2003, as reported in US EPA (2016d)), and T4 in pups (Lau et al., 2003, as reported in US EPA (2016d)). In monkeys, PFOS induced a significant decrease in total T3 and tT4 levels in females only, with no changes in TSH (Seacat et al., 2002, as reported in US EPA (2016d)).

5.4.1. Recent Human Evidence

Overall, OEHHA found 25 publications, published since the 2016 US EPA review, that examined associations between PFOA or PFOS and thyroid hormone levels in humans (Appendix 7, Table A7.7).

PFOA and thyroid hormone levels: In non-pregnant adults, the findings for TSH overall are mixed although the majority of results suggest a positive association (10 of 19 results), with three studies reporting statistically significant positive associations and none reporting statistically significant negative associations (Table A7.7). Two of the three studies reporting statistically significant positive associations were in females (Heffernan et al., 2018; Zhang et al., 2018) while the other involved females and males combined (Blake et al., 2018). The results were mixed in the other four studies in non-pregnant females. Two studies in non-pregnant adults reported statistically significant inverse associations between PFOA and fT4 (Olsen et al., 2007; Zhang et al., 2018), although results from the other 13 studies on fT4, and the results for T4, were mixed. OEHHA did not identify clear differences in study quality between those studies that did and did not identify statistically significant associations.

In pregnant females, the majority of results (7 of 11) also showed positive associations between PFOA and TSH although none were statistically significant. A statistically significant inverse

association with fT4 was reported in one study (Preston et al., 2018), but clear trends between PFOA and fT4 or T4 in pregnant women were not seen across the different studies.

In newborns (samples typically collected in cord blood), consistent associations were not seen between PFOA and TSH, T4, or fT4. Serum PFOA levels can vary by age, with higher levels in children under 10 months of age (Mogensen et al., 2015a). A statistically significant positive association was seen between maternal PFOA concentrations and cord blood TSH in one study (Kim et al., 2011b), and statistically significant positive associations were seen between cord blood PFOA and infant T4 or fT4 in two others (de Cock et al., 2014; Aimuzi et al., 2019). However, two of these studies involved small sample sizes (N=29 and N=31 in Kim et al., 2011b and De Cock et al., 2014, respectively), these findings were not consistent with those reported in the other studies in newborns, and none of these studies appeared to be of markedly higher quality than the other newborn studies. Major differences in results were not seen between males and females amongst newborns.

In the 17 results OEHHA identified in children older than newborns, two cross-sectional studies reported statistically significant inverse associations between PFOA and TSH in female participants (Lopez-Espinosa et al., 2012; Lewis et al., 2015), and one reported a statistically significant positive association between modeled maternal PFOA during pregnancy and blood T4 concentrations in offspring 1-5 years of age (males and females combined) (Lopez-Espinosa et al., 2012). Findings from the other studies were mixed. Clear differences in study quality were not seen between those studies reporting statistically significant associations and those that did not. Overall, consistent trends were not seen in subgroups of either male or female children.

PFOS and thyroid hormone levels: In studies in adults, clear trends across the different studies were not seen between PFOS and TSH or T4. For fT4, the majority of results (10 of 18) were consistent with a positive association between PFOS and this hormone. However, among the studies reporting results that were statistically significant, four were consistent with a positive association and two were consistent with an inverse association. Among the latter two, one did not adjust for any potential confounders (Li et al., 2017d) and the other involved participants in a case-control study of premature ovarian insufficiency (Zhang et al., 2018). No other major differences in study quality were identified between those studies reporting positive and inverse associations.

In their review, US EPA (2016b) identified three studies in pregnant women that reported positive associations between PFOS and TSH (Wang et al., 2013; Webster et al., 2014; Berg et al., 2015). Another study published before 2016 that was not included in the US EPA review reported an inverse association between PFOS and TSH in pregnant women, although the result was not statistically significant (Wang et al., 2014). Since the 2016 US EPA review, six cross-sectional studies and one prospective study have been published and most have reported inverse associations between PFOS and TSH in pregnant women, with two of the results being statistically significant. Clear differences in study quality between those studies reporting positive associations and those reporting inverse associations were not seen. Overall, findings on TSH and PFOS to date are inconsistent.

In male newborns, two of the eight studies reported statistically significant inverse associations between PFOS concentrations and TSH in either cord blood (Tsai et al., 2017) or maternal blood during pregnancy (Preston et al., 2018). In contrast, two of the studies in newborn

females showed the opposite effect. Results in the other groups of children, and for T4 and FT4, have also been mixed.

Thyroid diseases: OEHHA identified four studies of PFOA or PFOS and thyroid disease published since the 2016 US EPA reviews (US EPA, 2016b; US EPA, 2016d). In the first, in analyses adjusted for age, BMI, and alcohol intake, ORs for an association between serum concentrations of PFOA and PFOS and self-reported thyroid disease were 1.60 (95% CI, 0.96-2.82) and 0.94 (95% CI, 0.84-1.01), respectively, in 154 adult fishermen (women were not included) from Wisconsin (Christensen et al., 2016). The thyroid outcomes in this study included benign thyroid tumors, Hashimoto's disease, Grave's disease, hypothyroidism, hyperthyroidism, goiter/enlarged thyroid gland, and "other thyroid or endocrine problem". The median serum concentration of PFOA in the men in this study was 2.5 ng/ml (IQR, 1.8-3.3 ng/ml).

In the second study, a case-control study of congenital hypothyroidism in South Korean infants, unadjusted mean serum PFOA concentrations were lower in controls (N=13) than cases (N=27) (means of 2.12 and 5.40 ng/ml, respectively; $p < 0.01$) (Kim et al., 2016a). Major differences between cases and controls were not seen for PFOS. Few details were provided on subject selection procedures or on the methods used to control for or evaluate potential confounding.

In the third study, Dufour et al. (2018) examined associations between PFOA and PFOS concentrations in cord blood from 221 mother-infant pairs and maternal hypothyroidism. Mothers were considered to be hypothyroid if levothyroxine use was recorded in their medical records. Mean PFOA and PFOS concentrations were 0.88 ng/ml (standard deviation (SD), 0.79) and 0.80 ng/ml (SD, 0.52), respectively. Odds ratios for the first through the fourth quartiles of PFOA concentrations were 1.00 (reference), 4.42 (95% CI, 1.23-21.14), 3.22 (95% CI, 0.88-15.38), and 5.62 (95% CI, 1.64-26.11). Odds ratios for the first through the fourth quartiles of PFOS concentrations were 1.00 (reference), 1.76 (95% CI, 1.23-21.14), 3.22 (95% CI, 1.08-10.92), and 2.95 (95% CI, 0.98-10.07). Odds ratios were adjusted for maternal age and tobacco use. There were 37 cases of hypothyroidism overall but the numbers in each quartile of PFOA or PFOS were not provided.

The fourth study involved an area in Ronneby, Sweden in which the local water supply was contaminated with PFAS from firefighting foams used at a nearby military air field practice site (Andersson et al., 2019). Serum levels of 257, 280 and 15 ng/ml were reported for PFHxS, PFOS and PFOA, respectively, in a biomonitoring study of local residents. Hyper- and hypothyroidism in the population was assessed by linking population registry information to registries on deaths, hospitalizations, clinic visits, and prescription medications. Overall, living at an address supplied by municipal water from the contaminated waterworks was not associated with clear effects on either outcome, in either men or women.

5.4.2. Recent Animal Evidence

Thyroid effects have been reported in animals environmentally exposed to PFAS. Levels of T3 were negatively associated with PFAS in polar bears and hooded seals (Bourgeon et al., 2017; Grønnestad et al., 2018) and PFOA was associated with hyperthyroidism in domestic cats (Bost et al., 2016).

NTP recently released subacute (28 days) and chronic (16 or 107 weeks) bioassays for PFOA conducted in male and female Sprague Dawley rats. Animals were given PFOA in feed (doses

are provided in Table 5.4.2). For the chronic studies, an additional group of animals was exposed to PFOA during gestation and lactation (perinatal exposure). Although the initial chronic study in male rats with concentrations of 0, 150, or 300 ppm (0, 14.7, or 29.5 mg/kg-day) in feed was ended at 21 weeks due to overt toxicity, it appears a subset of animals receiving those doses were examined at 16 weeks, and the study was repeated with lower doses. Results are summarized in Table 5.4.2. Thyroid follicular cell hypertrophy was observed in male and female rats in the 28-day studies, and in female rats in the 107-week studies. Thyroid toxicity was not observed in female rats in the 16-week studies and male rats in the 107-week studies (NTP, 2020). It should be noted, however, that male rats exposed perinatally in the 107-week studies had higher incidences of thyroid follicular cell hypertrophy, although statistical significance was not reached (p=0.087, Fisher's exact test, performed by NTP). OEHHA identified a LOAEL of 0.625 mg/kg-day for males and 6.25 mg/kg-day for females (corresponding to a plasma concentrations of 50.7 and 0.49 µg/ml, respectively) for changes in thyroid hormone levels in the 28-day studies, and a NOAEL of 14.7 mg/kg-day (plasma concentration of 193 µg/ml) for thyroid follicular cell hypertrophy and changes in thyroid weight in male rats in the chronic studies.

Table 5.4.2. Thyroid toxicity from subacute and chronic studies of PFOA in Sprague Dawley rats (NTP, 2019a; NTP, 2020)

Sex	Exposure	Endpoints	NOAEL/LOAEL
Male (10/dose)	0, 0.625, 1.25, 2.5, 5, or 10 mg/kg-day via gavage for 28 days	Thyroid follicular cell hypertrophy (trend); ↑ relative thyroid weight; ↓ TSH, T3, fT4 and tT4	LOAEL: 0.625 mg/kg-day for changes in thyroid hormones
Female (10/dose)	0, 6.25, 12.5, 25, 50, or 100 mg/kg-day via gavage for 28 days	Thyroid follicular cell hypertrophy; ↑ TSH; ↓ fT4 and tT4	LOAEL: 6.25 mg/kg-day for increased TSH
Male (10/dose)	0, 150, or 300 ppm in feed (0, 14.7, or 29.5 mg/kg-day) for 16 weeks	↓ relative and ↑ absolute thyroid weight; thyroid follicular cell hypertrophy	NOAEL: 14.7 mg/kg-day for all thyroid endpoints
Male (10/dose)	0, 20, 40, or 80 ppm in feed (0, 1.8, 3.7, or 7.5 mg/kg-day) for 16 weeks	↓ absolute thyroid weight (not significant at the highest dose)	NOAEL: 1.8 mg/kg-day for
Female (50/dose)	0, 300, or 1,000 ppm in feed (0, 18, or 63 mg/kg-day) for 107 weeks	Thyroid follicular cell hypertrophy	NOAEL: 18 mg/kg-day

LOAEL, lowest-observed-adverse-effect level; NOAEL, no-observed-adverse-effect level; TSH, thyroid stimulating hormone; T3, triiodothyronine; fT4, free thyroxine; tT4, total thyroxine

For PFOS, NTP (2019b) conducted subacute studies in male and female Sprague Dawley rats. Animals were given 0, 0.312, 0.625, 1.25, 2.5, or 5 mg/kg-day PFOS via oral gavage for 28 days. Decreases in T3, fT4, and tT4 were observed in both sexes, while decreased absolute thyroid weight was reported in males only (NTP, 2019b). OEHHA identified a LOAEL of 0.312 mg/kg-day (corresponding to plasma concentrations of 23.7 and 30.5 µg/ml for males and females, respectively) based on decreases in fT4 and tT4 in both sexes.

A study in male and female cynomolgus monkeys given 14 mg/kg PFOS via oral gavage on three separate occasions over an observation period of 422 days showed a slight reduction in serum tT4 in both sexes (Chang et al., 2017). There were no significant changes in TSH or fT4. The authors did not consider the reduction in tT4 to be toxicologically relevant because a sufficient reservoir of inactive (bound to protein) T4 remained available to maintain thyroid hormone homeostasis.

5.4.3. Recent Mechanistic Evidence

Proper thyroid function is critical for appropriate metabolism and development. Evidence suggests that PFOA can impact thyroid gland function and perturb thyroid hormone homeostasis. Several recent mechanistic studies showed that PFOA can bind to TTR, a transport protein that carries T4 (Ren et al., 2016; Zhang et al., 2016b; Kar et al., 2017). PFOA effectively displaced T4 binding to TTR, which led to an increased amount of free T4 and an increased T4 uptake rate into rat hepatocytes (Selano et al., 2019). However, PFOA did not bind to thyroxine-binding globulin (TBG), another thyroid hormone transport protein (Ren et al., 2016).

PFOA did not inhibit cyclic adenosine monophosphate (cAMP) production induced by TSH in rat thyroid FRTL-5 cells (Croce et al., 2019), suggesting that PFOA did not interfere with TSH endocrine signaling. Furthermore, (Buckalew et al., 2020) report that PFOA inhibits iodide uptake in rat thyroid cells (FRTL-5), and in hNIS-HEK293T-EPA cells that express the human sodium-iodide symporter (hNIS) . NIS-mediated transport of iodide into the thyroid gland is critical for thyroid hormone synthesis, and disruption of this pathway can lead to thyroid dysfunction. In contrast, Wang et al. (2019a) did not observe any iodide uptake inhibition by PFOA in hNIS-HEK293T-EPA cells.

Like PFOA, PFOS can bind to TTR (Ren et al., 2016; Zhang et al., 2016b; Kar et al., 2017; Xin et al., 2018a), but not to TBG (Ren et al., 2016). However, PFOS inhibited iodide uptake more potently than PFOA in rat thyroid FRTL-5 cells and in hNIS-HEK293T-EPA cells (Wang et al., 2019a; Buckalew et al., 2020). PFOS also interacts with thyroid hormone receptors, and can stimulate cell proliferation in rat pituitary cancer (GH3) cells, a process which is regulated by thyroid hormone receptors (Xin et al., 2018a).

Similar to PFOA, PFOS did not inhibit cAMP production induced by TSH in rat thyroid FRTL-5 cells (Croce et al., 2019).

Dong et al. (2016a) reported expression changes in genes related to thyroid hormone homeostasis, including genes affected by constitutive androstane receptor/pregnane X receptor (CAR/PXR) activation.

5.4.4. Conclusions

Overall, for PFOA and thyroid hormone levels in humans, OEHHA did not find clear and consistent trends across the different epidemiologic studies reviewed. The majority of findings identified suggested that PFOA in adults (pregnant or not pregnant) might be associated with an increase in TSH. However, a number of studies reported the opposite effect and no obvious study design factor or other reason was found that explained this variability in results. OEHHA also did not see consistent associations between PFOS and thyroid hormone levels in humans. US EPA (2016d) identified three epidemiologic studies that reported positive associations

between PFOS and TSH in pregnant women. However, the four studies published since US EPA's review (US EPA, 2016b) (or otherwise not included in that review) reported essentially opposite findings.

The large majority of epidemiologic studies OEHHA reviewed on PFOA or PFOS and thyroid hormone levels were cross-sectional studies, where the temporal relationship between the exposure and the outcome can sometimes be difficult to discern. It is possible that people with altered thyroid hormone status may use certain products, change their diets, or otherwise have certain lifestyle changes that lead to increased PFOA or PFOS exposure ("reverse causation"). However, OEHHA found no evidence in the current literature to suggest that this is likely to be the case. In addition, the very large majority of people in the studies OEHHA reviewed had thyroid hormone levels that were within normal reference ranges, despite their PFOS or PFOA exposure. This suggests that the differences in thyroid hormone levels that were seen in people with different PFOA or PFOS levels in some studies were likely to have been too small to have caused major changes in lifestyle, diet, or product use that markedly affected their PFOA or PFOS intake. Overall, OEHHA found little evidence and little reason to believe the associations identified in some studies, or that the inconsistency overall, were a result of reverse causation.

Participation rates appeared to vary across studies although not all studies provided sufficient information to fully assess these rates or to assess any impacts of possible selection bias. However, OEHHA found little indication that selection bias was a major concern in these studies since most selected their participants prior to measuring PFAS or thyroid hormone levels. As such, participants' selection was most likely independent of their exposure or outcome status and therefore unlikely to have introduced major bias in most studies.

As seen in Table A7.7 (Appendix 7), the studies OEHHA reviewed on thyroid hormone levels were adjusted for a number of the more prevalent determinants of thyroid hormone levels or risk factors for thyroid disease including age, sex, smoking, race/ethnicity, and BMI. No major differences were found in the results from those studies that did or did not adjust for these factors. Only a few studies adjusted for other PFAS or for other chemical exposures (besides smoking) that might influence thyroid hormone levels (Shrestha et al., 2015; Byrne et al., 2018). Shrestha et al. (2015) assessed correlations between PFAS and PCBs and polybrominated diphenyl ethers (PBDEs), and between thyroid hormone levels and PCBs and polybrominated diphenyl ethers (PBDEs). Their results are shown in Table 5.4.3 and overall the correlations between PFOA or PFOS and these other chemical agents appear too low to have caused major confounding. Overall, OEHHA did not find clear evidence that confounding caused the inconsistency in the findings reviewed here.

Table 5.4.3 Pearson correlation coefficients between serum PCBs, PBDEs, PFOA, PFOS, and thyroid hormones (Shrestha et al., 2015)

	PFOS	PFOA	TSH	fT4	T4
Serum ∑ PCBs (ng/g serum total lipids)	0.30 (0.01)	0.16 (0.15)	0.04 (0.71)	0.08 (0.46)	0.09 (0.40)
Serum ∑ PBDEs (ng/g serum total lipids)	0.02 (0.86)	0.04 (0.68)	-0.02 (0.85)	-0.13 (0.23)	-0.10 (0.35)

The numbers in parentheses are p-values
Abbreviations: fT4, free thyroxine; ΣPBDEs, total polybrominated diphenyl ethers; ΣPCBs, total polychlorinated biphenyls; T4, total thyroxine; TSH, thyroid stimulating hormone

OEHHA identified only three epidemiologic studies of more overt thyroid conditions and PFOA or PFOS published since the 2016 US EPA reviews. Although these studies presented some suggestive evidence of an association between PFOS or PFOA and certain thyroid conditions,

small sample sizes, unusual dose-response patterns, lack of information on subject selection or potential confounders, or lack of replication limited the ability to make firm conclusions regarding these studies (Table 5.4.4).

The recent NTP studies in rats demonstrated that PFOA can adversely impact the thyroid. Decreases in T3 and T4 were observed in both male and female rats in the 28-day studies. This is somewhat consistent with previous results in rats, where T4 was significantly reduced, but only in male rats. The NTP studies also reported thyroid follicular cell atrophy in both male and female rats, regardless of duration (28 days to 107 weeks). These data suggest that PFOA can affect thyroid tissue directly, but more research is needed to determine the full impact of PFOA on thyroid function (Table 5.4.4).

The most recent PFOS studies from (NTP, 2019b) support previous reports in the literature that PFOS reduces T3 and T4 levels in rats. The overall body of evidence from the animal literature suggests that PFOS negatively impacts thyroid hormone levels (Table 5.4.4). Recent mechanistic studies suggest that PFOS may interact with thyroid hormone transporters and receptors in animals, which is similar to results reported in mechanistic studies with human thyroid hormone transporters and receptors (US EPA, 2016d).

Table 5.4.4. Summary of OEHHA's conclusions regarding the human and experimental animal data on PFOA and PFOS and thyroid toxicity

Outcome	PFOA	PFOS
Thyroid hormone levels in humans	Many studies but highly inconsistent results	Many studies but highly inconsistent results
Thyroid disease in humans	Limited number of studies, no convincing evidence	Limited number of studies, no convincing evidence
Thyroid hormone levels in animals	Limited number of studies, but positive trends were reported	Positive evidence

5.5. Developmental and Reproductive Toxicity

In its finalized *Toxicological Profile for Perfluoroalkyls*, ATSDR noted there is some epidemiologic evidence that PFOA and PFOS are associated with impaired fertility (longer time to pregnancy and infertility) but the results are not consistent across studies and some studies have found associations between maternal PFOA or PFOS exposure and decreased birth weight, which was attenuated by about 50% after accounting for maternal glomerular filtration rates (ATSDR, 2021). ATSDR also noted inconsistent findings related to several other reproductive outcomes associated with exposure to PFOA and PFOS. NTP has not published reviews of the epidemiologic literature on developmental and reproductive toxicity (DART) of PFOA or PFOS.

In the Health Effects Support Documents for PFOA and PFOS, US EPA's review of the epidemiologic literature on DART effects of each of these chemicals included literature published from 2009 through 2015 (US EPA, 2016b; US EPA, 2016d). US EPA's review focused on the outcomes of pregnancy-related hypertension, preeclampsia, measures of fetal growth, and pubertal development. Related to these outcomes, US EPA reviewed epidemiologic studies examining gestational age, measures of fetal growth, miscarriage,

preterm birth, birth defects, postnatal growth and maturation (including neurodevelopment and pubertal development), risk of adult obesity following prenatal exposure, pregnancy-related hypertension, preeclampsia, fecundity, sperm count, and semen quality (US EPA, 2016b).

For PFOA, US EPA found that the data on DART were suggestive of an association with risk of pregnancy-induced hypertension or preeclampsia and the possibility of an effect of reduced birth weight. US EPA also noted there was conflicting evidence from two studies for altered puberty onset in females and there were limited data suggesting reduced fertility and fecundity in females, and an association with attention deficit hyperactivity disorder in children in studies of both a highly exposed community and the general population.

For PFOS, US EPA reviewed epidemiologic studies examining measures of developmental outcomes such as fetal growth restriction, puberty, as well as pregnancy-related hypertension, preeclampsia, gestational diabetes, and fertility (US EPA 2016d). In summarizing, US EPA noted that despite study limitations, including uncertainty concerning the possible effect of low glomerular filtration rate (GFR), "…the association between PFOS exposure and birth weight for the general population cannot be ruled out."

GFR is the flow rate of blood being filtered by the kidneys. It increases in the first half of pregnancy and declines slightly in the second half of pregnancy. Women whose GFR fails to increase sufficiently in pregnancy have been shown to have smaller babies (Verner et al., 2016). GFR may also influence urinary excretion of PFAS; people with lower GFR have higher blood levels of PFAS (Verner et al., 2016). Thus, GFR may confound the association between PFAS and some DART outcomes, such as lower birth weight, because changes in both may be due in some part to effects of low GFR (Savitz, 2014; Verner et al., 2016). In addition, maternal plasma volume expands in early pregnancy, diluting PFAS concentrations. Lower plasma volume expansion is also associated with reduced fetal growth and possibly also reduced GFR (Vesterinen et al., 2015) and may confound any association between PFAS exposure and fetal growth. However, the potential for confounding by GFR and plasma volume expansion appears greater when PFAS exposure is based on measurements in blood obtained in late pregnancy. Pregnancy hemodynamics appear less likely to substantially confound studies of effects of PFAS on fetal growth when serum or plasma is sampled early in pregnancy (Verner et al., 2016; Sagiv et al., 2018; Steenland et al., 2018).

US EPA also noted that a small set of studies reported associations with gestational diabetes, preeclampsia, and pregnancy-induced hypertension in populations with serum PFOS concentrations of 12-17 ng/ml (US EPA, 2016d).

Furthermore, US EPA found that the overall data suggest a consistent association between PFOS exposure and fertility and fecundity measures, despite concern over GFR and reverse causation. Studies examining semen quality were largely null (US EPA, 2016d).

Given US EPA's findings regarding reproductive and developmental effects of PFOA and PFOS, OEHHA evaluated the most recent literature, published since US EPA's reviews, for the following outcomes: pregnancy-related hypertension and preeclampsia, measures of fetal growth, pubertal development, and fertility and fecundity.

Developmental and reproductive effects of PFOA in animals were described in assessments by US EPA (2016b), New Jersey DWQI (2017) and ATSDR (2021). US EPA (2016b) reported numerous reproductive effects in animals, including reduced fertility in male mice, decreased

litter size, increased resorptions and stillbirths, and increased time to parturition. US EPA (2016b) also reported multiple developmental effects in rodents, including reduced postnatal growth, delays in developmental landmarks, reduced ossification, and delayed mammary gland development. Based on this evidence, PFOA was listed under Proposition 65 as a chemical known to the state of California to cause reproductive toxicity in 2017.

Developmental and reproductive effects of PFOS in animals were also described in assessments by US EPA (2016d), New Jersey DWQI (2018) and ATSDR (2021). US EPA (2016d) identified no studies reporting reduced fertility, but reported histopathological lesions in male rat testes, and increased pup mortality. It was hypothesized that neonatal mortality is caused by the interaction of PFOS with lung surfactant components, which alters lung morphology and pulmonary surfactant function in developing rodents. Adverse developmental effects include reduced pup body weight, developmental delays, and altered hormone and glucose regulation. As such, PFOS was listed under Proposition 65 as a chemical known to the state of California to cause reproductive toxicity in 2017.

5.5.1. Recent Human Evidence

Pregnancy-related hypertension and preeclampsia: OEHHA identified two studies of the association between PFOA or PFOS exposure and preeclampsia published since January 2016 (Huang et al., 2019b; Wikström et al., 2019), one of which also examined pregnancy-induced hypertension (Huang et al., 2019b). Two additional studies re-examined previously published data to determine whether uncertainties in exposure or geocoding of reconstructed PFOA exposures biased the epidemiologic findings regarding associations between PFOA and preeclampsia. The authors found that these sources of uncertainty did not have significant impacts on those findings (Avanasi et al., 2016a; Avanasi et al., 2016b); these studies will not be discussed further.

The preeclampsia studies are summarized in Appendix 7, Tables A7.13 and A7.14. Preeclampsia is a condition in which the pregnant woman is hypertensive because of reduced renal excretion associated with a decrease in GFR (US EPA, 2016b). Wikström et al. (2019) conducted a prospective cohort study in Sweden using early pregnancy (median 10 weeks) serum PFAS measurements and preeclampsia in the same pregnancy. The median serum PFOA concentration was 1.61 ng/ml and the median PFOS concentration was 5.39 ng/ml. A doubling in early pregnancy serum PFOS was associated with increased risk of preeclampsia for all women (OR = 1.53; 95% CI, 1.07-2.20) and nulliparous women OR = 2.02; 95% CI, 1.26-3.29). When the comparison was highest vs. lowest quartile of serum PFOS, the increase in risk was greater: OR = 2.68; 95% CI, 1.17-6.12). ORs were adjusted for parity (except when stratified by parity), age, weight, and smoking (based on serum cotinine). PFOA was also associated with increased risk of preeclampsia, but the ORs were somewhat smaller and not statistically significant. Wikström et al. (2019) did not adjust for the presence of other PFAS. A strength of this study is the use of early pregnancy PFAS measurements, which would avoid potential confounding by GFR. The correlations among some PFAS were relatively high: for PFOA and PFOS, r = 0.60; for PFOA and PFNA, r = 0.66; and for PFOS and PFNA, r = 0.55. PFNA was also associated with preeclampsia, with ORs that were somewhat higher than those for PFOA and statistically significant.

In the Huang et al. (2019b) cross-sectional study, cord blood samples were collected from 686 women shortly after birth and tested for PFAS. The median serum PFOA concentration was 6.98 ng/ml and the median PFOS concentration was 2.38 ng/ml. Risk of gestational

hypertension was 3.3% and risk of preeclampsia was 2.8%, and 91.5% of the women were nulliparous. PFOA was associated with non-statistically significant increases in risk of preeclampsia, while PFOS was associated with non-statistically significant reductions in risk. Although the authors note that PFOS levels in cord blood are highly correlated with PFOS in maternal blood, they also acknowledge that previous studies have reported that PFOA and PFOS decreased across pregnancy due to increased GFR and subsequent increased rate of elimination in urine. It is therefore possible that the measured PFAS levels do not represent levels at other points in the pregnancy that may have been more relevant to risks of hypertensive disorders of pregnancy (Huang et al., 2019b).

Huang et al. (2019b) also examined gestational hypertension. While ORs for the second tertile of cord serum PFOA and PFOS, compared to the first tertile, suggested protective effects, the associations were not statistically significant. The ORs for continuous exposure also suggested no associations (Huang et al., 2019b).

Measures of fetal growth – birth weight: The large number of studies examining PFOA and birth weight are summarized in Appendix 7, Table A7.15. Two recent cross-sectional studies reported statistically significant lower birth weight among children with higher PFOA concentrations in maternal or cord serum around the time of birth. Kwon et al. (2016) reported a change of −77.93 g (95% CI, −153.56 - −2.30) per unit change in log PFOA, and Li et al. (2017c) reported that each ln-unit change in total PFOA (i.e., linear and branched) was associated with a change of –112.7 g (95% CI, –171.9 - –53.5) and each ln-unit change in linear PFOA (n-PFOA) was associated with a change of –85.0 g (95% CI, –133.6 - –36.5) in birth weight. The findings from both of these studies are not adjusted for GFR or plasma volume expansion, which could confound the associations with samples in late pregnancy or around the time of birth (Verner et al., 2016; Govarts et al., 2018; Sagiv et al., 2018).

Six prospective cohort studies and a case-cohort study also reported statistically significant lower birth weight in association with serum or plasma PFOA concentrations during pregnancy. Two of these studies included samples with relatively high risk for lower birth weight. The largest change in birth weight was reported by Lauritzen et al. (2018) for the Swedish sub-sample (N=159, median 2nd trimester maternal serum PFOA concentration = 2.33 ng/ml): for male infants, β = –526 g (95% CI, –828 - –222) per ln-unit increase in PFOA. This case-cohort study included SGA cases, from Sweden and Norway, selected predominantly (82%) from parous participants who were considered high-risk for SGA; however, results for the Norwegian sub-sample were null (Lauritzen et al., 2018). Hjermitslev et al. (2020) studied PFOA exposure in Greenlandic Inuit women with high smoking rates and possibly high exposure to persistent organic pollutants through traditional diet, although the serum PFOA concentrations (sampled between 7 and 40 weeks gestation for one group and before the end of gestation week 13 for another group) were not high: median (range) = 1.06 (0.10-7.26) ng/ml. A 1 ng/ml increase in maternal serum PFOA was associated with a change in birth weight of –119 g (95% CI, –202 - –36.6) (adjusted for GA and other potential confounders) (Hjermitslev et al., 2020).

Meng et al. (2018) reported a more modest change in birth weight of –35.6 g (95% CI, –66.3 - –5.0) per doubling of PFOA measured in maternal plasma in the first two trimesters (92% in first trimester). Wikström et al. (2019) reported a ln-unit increase in maternal serum PFOA sampled at 10 weeks (median) was associated with a change in birth weight of –68 g (95% CI, –112 - –24) for all children, with a slightly stronger association in girls of –86 g (95% CI, –145 - –26). For both of these studies, the serum samples were generally taken before changes associated with GFR and increased blood volume would be expected to become important confounders.

For the remaining cohort studies that reported associations with lower birth weight, coefficients were β = –51.4 g (95% CI, –97.2 - –5.7) per ln-unit PFOA increase (Starling et al., 2019), β = –63.77 g (–122.83 - –4.71) per 2-SD increase in ln PFOA (Lenters et al., 2016), and β = –197 g (95% CI, –391 - –3) per log-unit PFOA increase (Minatoya et al., 2017). Blood samples were taken at various times or in late pregnancy in these studies, and no consideration of potential confounding by GFR was reported. Starling et al. (2019) adjusted for gestational weight gain and both Starling et al. (2019) and Minatoya et al. (2017) adjusted for gestational age at blood draw.

Twenty-three of the recent studies, including 11 prospective cohort, one retrospective cohort, and 11 cross-sectional studies, reported no statistically significant associations between prenatal PFOA and birth weight or birth weight *z*-score. Four large cohort studies with sample sizes ranging from N=1,202 to N=1,705 assessed PFOA exposure mainly in the first trimester of pregnancy, thereby minimizing concerns over confounding or reverse causation associated with GFR or weight gain, and reported no statistically significant associations between prenatal PFOA exposure and birth weight or birth weight *z*-score (Bach et al., 2016; Ashley-Martin et al., 2017; Manzano-Salgado et al., 2017; Sagiv et al., 2018). The median PFOA concentrations in these studies were between 1.7 ng/ml and 5.8 ng/ml in maternal plasma (Ashley-Martin et al., 2017; Manzano-Salgado et al., 2017; Sagiv et al., 2018) and 2.0 ng/ml in maternal serum (Bach et al., 2016).

Some investigators examined possible sex differences in effects of prenatal PFOA exposure on birth weight. Of the six studies that reported possible stronger associations between PFOA and lower birth weight in girls, two reported statistically significant associations in girls but not boys: Hjermitslev et al. (2020) reported β = –161 g (95% CI, –283 - –40.1) for female infants, and β = –81.2 g (95% CI, –194 - 31.2) for male infants. Wikström et al. (2019) reported β = –86 g (95% CI, –145 - –26) for girls and β = –49 g (–113 - 15) for boys. Of four studies reporting a possible stronger association with lower birth weight in boys, Lauritzen et al. (2017) reported a statistically significant association, β = –526 g (95% CI, –828 - –222) in boys and β = –156 g (95% CI, –541 - 228) in girls.

Studies examining PFOS and birth weight are summarized in Appendix 7, Table A7.16. Three recent studies reported large, statistically significant associations between prenatal PFOS exposure and lower birth weight in small to modest samples: one cross-sectional study (N=98) reported that each log-unit increase in umbilical cord serum PFOS was associated with a change in birth weight of –417.3 g (95% CI, –742.1 - –92.4). The mean PFOS concentration was 4.07 ng/ml (Xu et al., 2019a). Another cross-sectional study (N=317) with a median cord serum PFOS concentration of 3.0 ng/ml reported that each ln-unit increase in PFOS was associated with a change in birth weight of –150.6 g (95% CI, –225.4 - –75.7) in male infants and –26.6 g (95% CI, –125.1 - 71.8) in females (Li et al., 2017c). Although the authors of these studies adjusted for some important potential confounders, the possibility of confounding or reverse causation associated with GFR due to exposure assessment at time of birth is of some concern, as are the substantial changes with adjustment for confounding. Lauritzen et al. (2017) conducted a case-cohort study with SGA cases selected predominantly from parous participants who were at high-risk for SGA in Sweden and Norway. In the Swedish sub-sample (N=159, median 2nd trimester maternal serum PFOS concentration = 16.4 ng/ml), the change in mean birth weight was –292 g (95% CI,–500 - –84) per ln-unit increase in PFOS. Results for the Norwegian sub-sample (N=265), with a median serum PFOS concentration of 9.74 ng/ml, were null (Lauritzen et al., 2017).

Three large prospective cohort studies, each of which included more than 1,500 participants, assessed PFOS exposure in early pregnancy and reported statistically significant associations with lower birth weight. Bach et al. (2016), with a median maternal serum PFOS concentration of 8.3 ng/ml, observed associations only for 2nd quartile compared to 1st quartile exposure, β = –86 g (95% CI, –159 - –13) for all births and β = –93 g (95% CI, –157 - –29) for term births. The association per IQR (4.8 ng/ml) of PFOS was null for all births, with contrasting but non-statistically significant associations in girls (β = –32 g (95% CI, –71 - 7)) and boys (β = 26 g (95% CI, –13 - 65)) (Bach et al., 2016). Wikstrom et al. (2019), with a median maternal serum PFOS concentration of 5.38 ng/ml, reported a change in birth weight of –46 g (95% CI, –88 - –3) per ln-unit increase in PFOS for all children, –85 g (95% CI, –145 - –25) for girls, and –13 g (95% CI, –73 - 47) for boys (Wikström et al., 2019). Meng et al. (2018) reported a change in birth weight of –45.2 g (95% CI, –76.8 - –13.6) per doubling of PFOS exposure in a population with a median maternal plasma PFOS concentration of 30.1 ng/ml (Meng et al., 2018). By assessing PFOS exposure early in pregnancy, these prospective studies reduce concerns about confounding and reverse causation associated with GFR and increased blood volume.

Two further prospective cohort studies with later pregnancy exposure assessment reported associations with reduced birth weight. In the study by Marks et al. (2019), each unit increase in PFOS concentration (median 13.8 ng/ml) was associated with a change in birth weight of −8.50 g (95% CI, −15.93 - −1.07). In a sensitivity analysis including only 1st trimester samples (N=115), associations were consistent with the entire study sample. Valvi et al. (2017) reported a change in birth weight of approximately −150 g (p <0.05; presented graphically) for boys of the Faroe Islands with median maternal serum PFOS of 27.2 ng/ml.

One study (N=62) reported that prenatal PFOS exposure was associated with an increase in birth weight of 596 g (95% CI, 89 - 1,103) in girls. The median umbilical cord plasma PFOS concentration was 1.600 ng/ml and there was no association in boys (de Cock et al., 2016). No other studies reported statistically significant increases in birth weight associated with PFOS.

Twenty-two studies, including eight cross-sectional, one retrospective cohort, and 14 prospective cohort studies, reported no statistically significant associations between prenatal PFOS exposure and birth weight, term birth weight, birth weight *z*-score, or birth weight for GA *z*-score. Some of these studies included large samples, and PFOS concentrations ranged from less than 1 ng/ml in cord serum to more than 25 ng/ml in maternal plasma or maternal serum. For example, in a prospective cohort study (N=1,202), Manzano-Salgado et al. (2017) assessed PFOS in maternal plasma (median 6.05 ng/ml) in the first half of pregnancy and reported no association with birth weight. Sagiv et al. (2018) also assessed PFOS in early pregnancy and evaluated many potential confounders, including GFR and plasma albumin, and reported that maternal plasma PFOS (median 25.7 ng/ml) was not statistically significantly associated with term birth weight (β = −17.9 g (95% CI, −40.9 - 5.1)) in this cohort of 1,645 mother-infant pairs.

Measures of fetal growth – small for gestational age: Six studies, summarized in Appendix 7, Table A7.15, examined associations between exposure to PFOA and risk of SGA infants (Wang et al., 2016; Lauritzen et al., 2017; Manzano-Salgado et al., 2017; Govarts et al., 2018; Wikström et al., 2019; Xu et al., 2019a). Two of the smaller studies, one of which was a cross-sectional study in Hangzhou, China and had low exposure concentrations (median umbilical cord serum concentration=1.05 ng/ml) (Xu et al., 2019a), and one of which was a prospective cohort study in Taiwan with median 3rd trimester maternal serum PFOA concentrations of 2.34 ng/ml and 2.37 ng/ml for female and male infants, respectively (Wang et al., 2016), reported no associations with SGA.

The remaining four studies were conducted in Europe and had conflicting results within and between studies. The case-cohort study by Lauritzen et al. (2017) included parous participants from Sweden and Norway and sampled for women at high risk of SGA. In the sample of women from Norway (N=265), whose median PFOA concentration in second trimester maternal serum was 1.62 ng/ml, PFOA was associated, though not statistically significantly, with lower odds of SGA, OR = 0.66 (95% CI, 0.33-1.33). In Swedish women (N=159), with a median second trimester maternal serum PFOA concentration of 2.33 ng/ml, prenatal PFOA exposure was associated with a large and statistically significant increase in risk of SGA for all children (OR = 5.25; 95% CI, 1.68-16.4), and for boys (OR = 6.55; 95% CI, 1.14-37.5); but the increase was not statistically significant for girls (OR = 4.73; 95% CI, 0.79-28.3) (Lauritzen et al., 2017).

Govarts et al. (2018) analyzed pooled cross-sectional data for cohorts from Belgium, Norway, The Netherlands, and Slovakia (N=693), and reported evidence of an increase in risk for SGA overall, OR = 1.64 (95% CI, 0.97-2.76) per IQR cord serum PFOA. The association was statistically significant among mothers who smoked during pregnancy, OR = 2.18 (95% CI, 1.02-4.64), but not among nonsmokers, OR = 1.51 (0.87-2.63). Some cord serum PFOA levels were measured, but for two cohorts and part of a third cohort, cord serum PFOA levels were estimated using PFOA measured in breast milk. The median (IQR) cord serum PFOA concentration was 0.550 (0.299-1.200) ng/ml but varied by cohort. The magnitude, but not the direction, of the effect varied by cohort (data not reported). The authors did not address potential confounding by GFR (Govarts et al., 2018).

In a prospective cohort study with 1,202 participants in Spain, Manzano-Salgado et al. (2017) reported that first trimester maternal PFOA (median concentration 2.35 ng/ml) was associated with increased risk of SGA in male infants, OR = 1.18 (95% CI, 0.82-1.69) and decreased risk (protective effect) in female infants, OR = 0.72 (95% CI, 0.50-1.04), though neither was statistically significant. In another large prospective cohort study in Sweden (N=1,533), Wikström et al. (2019) reported increased risk of SGA birth associated with first trimester PFOA exposure (median serum concentration 1.61 ng/ml) for all children, OR = 1.43 (95% CI, 1.03-1.99), driven mainly by increased risk among girls, OR = 1.96 (95% CI, 1.18-3.28). The association was weaker and not statistically significant for boys, OR = 1.16 (95% CI, 0.75-1.78) (Wikström et al., 2019).

Five recent epidemiologic studies, summarized in Appendix 7, Table A7.16, examined the association between maternal PFOS exposure and SGA birth. Xu et al. (2019a) conducted a small (N=98) cross-sectional study using cord serum PFOS (median concentration of 4.07 ng/ml). The analysis was adjusted for important confounders and tap water consumption, but not GFR. (Xu et al., 2019a) reported a large association, with adjusted OR = 4.138 (95% CI, 1.07-15.98) per log-unit increase in PFOS; the unadjusted OR = 1.64. There was a large change in the OR after adjustment (Xu et al., 2019a).

Govarts et al. (2018) analyzed pooled cross-sectional data from four cohorts (N=693), and reported cord serum PFOS was associated with increased risk of SGA among mothers who smoked during pregnancy, OR = 1.63 (95% CI, 1.02-2.59). Among nonsmokers, PFOS was associated with decreased risk of SGA, OR = 0.66 (95% CI, 0.61-0.72). The median (IQR) cord serum PFOS concentration was 1.98 ng/ml but varied by cohort. Cord serum PFOS levels were either measured directly, or for most participants, PFOS levels were estimated based on breast milk PFOS levels. The magnitude, but not the direction, of the effect varied by cohort (data not reported). The authors did not address potential confounding by GFR (Govarts et al., 2018).

The case-cohort study by Lauritzen et al. (2017) included parous participants from Sweden and Norway and sampled for women at high risk of SGA. In the sample of women from Norway (N=265), with median second trimester PFOS concentration of 9.74 ng/ml, maternal PFOS exposure was not associated with SGA. In the Swedish cohort (N=159, median PFOS concentration = 16.4 ng/ml), a ln-unit increase in maternal PFOS was associated, though not statistically significantly, with SGA, OR = 2.51 (95% CI, 0.93-6.77) (Lauritzen et al., 2017).

In a prospective cohort study with 1,202 participants, Manzano-Salgado et al. (2017) reported that first trimester maternal PFOS (median concentration 6.05 ng/ml) was not associated with increased risk of SGA. In another large prospective cohort study (N=1,533), (Wikström et al., 2019) reported some non-statistically significant associations with first trimester PFOS exposure (median serum concentration 5.38 ng/ml). For all children, OR = 1.19 (95% CI, 0.87-1.64); girls, OR = 1.40 (95% CI, 0.83-2.35); and boys, OR = 1.08 (95% CI, 0.72-1.63) per ln-unit increase in prenatal serum PFOS (Wikström et al., 2019).

Pubertal development: OEHHA identified two recent retrospective cohort studies of PFOA and pubertal development (Appendix 7, Table A7.17), one of which also investigated the relationship between PFOS and pubertal development. Ernst et al. (2019) collected multiple puberty-related indicators at multiple times from most of their study participants beginning at age 11 years, and had PFOA measurements from the first trimester of the children's gestation. Plasma PFOA medians were reported separately for boys and girls in the two groups (samples) of study participants identified from within the cohort as a whole, and ranged between 4.1 and 5.1 ng/ml. No consistent patterns of associations between PFOA and pubertal indicators were evident in this cohort (Ernst et al., 2019).

Di Nisio et al. (2020), in contrast, assessed onset of menarche when participants were approximately 18 years old, and used lifelong residence in the area as a proxy for exposure. In a subset of participants with actual PFOA measurements, the median was 28.7 ng/ml in serum of exposed participants, and 2.6 ng/ml in "control" participants. The mean age at menarche was 164 days ($p < 0.001$) later in those who lived in the high PFOA area. PFOS concentrations were similar in the two groups (3.25 and 3.15 ng/ml) (Di Nisio et al., 2020).

Ernst et al. (2019) also examined PFOS in association with pubertal development and reported that in girls, early gestation PFOS exposure in the middle tertile (28.1-38.4 ng/ml and 23.3-31.5 ng/ml in the two study participant 'samples') was associated with earlier indicators of pubertal development compared to the lowest tertile (Appendix 7, Table A7.18). The highest exposure tertile was not consistently associated with pubertal development in girls. In boys, PFOS exposure above the lowest tertile appeared to be associated with earlier genital development and voice break, although most comparisons were not statistically significant. PFOS was not associated with other indicators of puberty in boys (Ernst et al., 2019).

Fertility and fecundity: Four recent studies examined possible associations between a woman's exposure to PFOA and fertility or fecundity (Appendix 7, Table A7.19). A cross-sectional study of 1,251 pregnant women examined early pregnancy plasma PFOA levels (median 5.03 ng/ml in nulliparous women and 3.43 ng/ml in parous women) and reported no association with time to pregnancy (TTP; an indicator of fecundability, which is defined as the probability of pregnancy per cycle) in nulliparous women. Although a statistically significant association with reduced fecundability in parous women was observed, the authors hypothesized that "the association in parous women was due to residual confounding" (Bach et al., 2018). A small (N=99) prospective cohort study also found no association between pre-

conception serum PFOA (geometric mean 2.79 ng/ml) and fecundability or ovarian reserve in women who had no history of fertility or fertility-related conditions (Crawford et al., 2017).

A very small prospective cohort study in a fertility clinic examined 34 women with a mean plasma PFOA concentration of 2.44 ng/g before in vitro fertilization (IVF). There were no statistically significant correlations between PFOA and blastocyst conversion, fertilization, ovarian function, or response to gonadotropin stimulation (McCoy et al., 2017). Another fertility clinic study was a case-control study with 157 cases of endometriosis-related infertility and 178 controls who had no reproductive endocrine disorders but were seeking infertility treatment for male reproductive dysfunction. Plasma PFOA (median 14.67 ng/ml among cases and 12.09 ng/ml among controls) was not associated with endometriosis-related infertility (Wang et al., 2017a).

The four studies that examined the relationship between PFOA and fertility or fecundity also examined PFOS (Appendix 7, Table A7.20). In a cross-sectional study of 1,251 pregnant women, Bach et al. (2018) found no association between early pregnancy plasma PFOS (median concentration 30.2 ng/ml in nulliparous women and 26.0 ng/ml in parous women) and TTP in nulliparous women, but reduced fecundability in parous women. However, the authors attributed the association in parous women to confounding (Bach et al., 2018). A small (N=99) prospective cohort study of women who had no history of infertility or fertility-related conditions and were attempting to conceive found no association between pre-conception serum PFOS (geometric mean 9.29 ng/ml) and fecundability or ovarian reserve (Crawford et al., 2017).

McCoy et al. (2017) examined associations between plasma PFOS before IVF treatment (mean concentration 6.52 ng/g) and ovarian function, ovarian response, and fertilization among women undergoing IVF (N=34). Higher plasma PFOS levels were associated with lower plasma estradiol, r = –0.47 pg/ml ($p < 0.05$), but no other outcomes (McCoy et al., 2017).

A case-control study in a fertility clinic compared 157 cases of endometriosis-related infertility with 178 controls who had no reproductive endocrine disorders but were seeking infertility treatment for their male partners' reproductive dysfunction. The median plasma PFOS concentration was 6.40 ng/ml among cases and 6.60 ng/ml among controls. Women in the highest PFOS exposure tertile were less likely to have endometriosis-related infertility than women in the lowest tertile, OR = 0.66 (95% CI, 0.36 - 1.21), though the association was not statistically significant. The association was similar in an analysis that was restricted to women who had never been pregnant. In a sensitivity analysis, plasma PFOS was statistically significantly associated with lower odds of endometriosis-related infertility, OR = 0.47 (95% CI, 0.22 - 0.99), when restricted to women who had no other gynecologic pathology. This study also reported inverse or null associations between endometriosis-related infertility and other PFAS, with the exception of PFBS, which was associated with increased risk of endometriosis-related infertility (Wang et al., 2017a).

5.5.2. Recent Animal Evidence

Studies of PFOA exposure reporting developmental and reproductive toxicity effects published from 2016 onward are summarized in Tables 5.5.1, 5.5.2, and 5.5.3.

Table 5.5.1. Summary of recent animal toxicity studies of PFOA reporting developmental toxicity

Sex/Species	Exposure	Endpoints	NOAEL/LOAEL	Reference
Pregnant C57BL/6/Bkl mice (6/dose)	0 or 0.3 mg/kg-day in diet from GD 1-21	**Pups:** ↑ body weight; changes in femur and tibia bone morphometric properties; ↓ bone tissue mineral density	NA[a]	Koskela et al. (2016)
Pregnant C57BL/6J mice (6/dose for dams; 6-10/dose for pups)	Dietary exposure to 0, 0.003, 0.01, 0.03, 0.1, 0.3, 1, or 3 mg/kg-day (targeted dose to dams); exposure started 2 weeks before mating and continued during mating (1 week), gestation (3 weeks), and lactation (3 weeks)	**Dams:** ↓ litter size at two highest doses **Pups (both sexes):** ↓ body weight at PND 4; nuclear dysmorphology (p=0.06) **Male pups:** ↑ liver weight; ↑ eosinophilic liver foci (p=0.07)	**Dams:** NOAEL: 0.3 mg/kg-day for ↓ litter size **Pups:** NOAEL: 0.003 mg/kg-day for ↓ body weight in females on PND 4	van Esterik et al. (2016)
Pregnant CD-1 mice, male pups used for behavioral tests, 1 per litter per test. (4-17/dose, varied by behavioral test)	0, 0.1, 0.3, or 1 mg/kg-day via gavage from GD 1-17	**Pups:** ↑ ambulatory activity on PND 18; ↓ activity in animals injected with methamphetamine	NOAEL: 0.3 mg/kg-day based on ↑ ambulatory activity	Goulding et al. (2017)
Pregnant Kunming mice (10/dose), male offspring (7-10/dose) evaluated for effects on PND 21 and PND 70	0,1 ,2.5, or 5 mg/kg-day via gavage from GD 1-17	**Pups:** ↓ number of surviving mice at weaning; changes in absolute testis weight; ↓ serum testosterone levels (except in low dose animals on PND 70); ↓ Leydig cells; vacuolization of Sertoli cells; ↓ spermatozoa	LOAEL: 1 mg/kg-day for ↓ serum testosterone	Song et al. (2018)

Sex/Species	Exposure	Endpoints	NOAEL/LOAEL	Reference
Pregnant Kunming mice (10/dose), female pups (5-10/dose)	0, 1, 2.5, 5, or 10 mg/kg-day via gavage from GD 1-17	**Pups:** ↓ body weight; ↓ survival; ↑ absolute and relative liver weight; swollen hepatocytes; liver cell vacuolar degeneration and dissolved nuclei; blurred liver architecture; ↑ serum ALT and AST; ↑ CAT, SOD, and 8-OHdG; ↓ histone acetylation	LOAEL: 1 mg/kg-day for ↑ serum ALT and AST	Li et al. (2019c)

[a] LOAEL/NOAEL not applicable for single dose studies.
Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; CAT, catalase; GD, gestation day; LOAEL, lowest-observed-adverse-effect level; NOAEL, no-observed-adverse-effect level; PND, postnatal day; SOD, superoxide dismutase; 8-OHdG, 8-hydroxydeoxyguanosine

van Esterik et al. (2016) evaluated the developmental toxicity of PFOA in C57BL/6J mice. Toxicity in the F1 generation was monitored in 6-10 pups from 2-5 litters in each dose group. Decreased litter sizes were reported at the two highest doses. Additionally, several developmental effects were reported in pups, including the following: increased liver weight, and eosinophilic liver foci (p=0.07) in males; decreased femur length and femur weight, decreased quadriceps femoris muscle weight, decreased adipocyte cell size, and decreased serum triglycerides and cholesterol in females; and decreased body weight at PND 4, decreased tibia length, and hepatocellular anisokaryosis and karyomegaly in pups of both sexes (p=0.06). The decrease in body weight persisted until adulthood on a standard diet, but reverted to control levels once animals were placed on a high fat diet. Body weight data on PND 4 were digitized using GetData Graph Digitizer software (version 2.26), and evaluated for statistical significance ($p < 0.001$; student's T-test determined by OEHHA). OEHHA determined a NOAEL of 0.003 mg/kg-day based on decreased body weight in female pups on PND 4.

Table 5.5.2. Summary of recent animal toxicity studies of PFOA reporting reproductive toxicity in male animals

Sex/Species	Exposure	Endpoints	NOAEL/LOAEL	Reference
Male BALB/c mice (20/dose)	0, 1.25, 5, or 20 mg/kg-day via gavage for 28 days	↓ fertility; ↓ litter weight after mating; disruption of blood-testis barrier; ↑ testicular IgG; ↑ TNF-α in testis	LOAEL: 1.25 mg/kg-day for disruption of blood-testis barrier	Lu et al. (2016a)
Male BALB/c mice (11/dose)	0, 1.25, 5, or 20 mg/kg-day via gavage for 28 days	**Epididymis:** ↓ triglycerides and cholesterol; ↓ relative weight; ↑ MDA levels; ↓ GSH-Px levels	LOAEL: 1.25 mg/kg-day for ↓ in relative epididymis weight	Lu et al. (2016b)

Sex/Species	Exposure	Endpoints	NOAEL/LOAEL	Reference
Male BALB/c mice (6/dose)	0, 1.25, 5, or 20 mg/kg-day via gavage for 28 days	changes in levels of proteins involved with endocytosis and blood-testis barrier	NOAEL: 1.25 mg/kg-day	Lu et al. (2017)
Male Kunming mice (number not specified)	0 or 10 mg/kg-day intragastrically for 21 days	atrophy of seminiferous tubules; disorganization of seminiferous epithelium; absence of spermatozoa; depletion of spermatogonial cells; detachment of germ cells; ↓ absolute testis weight; ↓ epididymal sperm count; ↑ MDA; ↓ SOD and CAT activity	NA[a]	Yuan et al. (2017)
Male BALB/c mice (15/dose)	0, 1.25, 5, or 20 mg/kg-day via gavage for 28 days	changes in CBG protein levels in testes; ↑ CBG and corticosterone levels in serum; ↓ adrenocorticotropic hormone levels in serum	LOAEL: 1.25 mg/kg-day for ↑ CBG levels in testis and serum	Sun et al. (2018b)
Male C57BL/6 mice (6-8/dose)	0, 0.55, 5.5, or 28 mg/L in drinking water for 5 weeks (0, 0.1, 1, and 5 mg/kg-day)	no observed DNA damage in testis; no change in testicular weight	NOAEL: 5 mg/kg-day	Crebelli et al. (2019)
Male Sprague Dawley rats (12/dose)	0, 25, or 50 mg/kg-day via gavage for 9 days; PFOA administration started 7 days after EDS treatment to kill Leydig cells	slowed recovery of serum testosterone; ↓ PCNA+ cells	LOAEL: 25 mg/kg-day for slowed recovery of serum testosterone	Lu et al. (2019)
Male and female Sprague Dawley rats (10/sex/dose)	0, 0.625, 1.25, 2.5, 5, or 10 mg/kg-day for 28 days via gavage	**Males:** ↑ relative testis weight; ↓ absolute epididymis weight; ↓ cauda epididymis weight; ↓ cauda epididymis sperm count	NOAEL: 2.5 mg/kg-day for ↑ relative testis weight and ↓ cauda epididymis weight	NTP (2019a)
Male Sprague Dawley rats (10/dose)	0, 150, or 300 ppm in feed (0, 14.7, or 29.5 mg/kg-day) for 16 weeks	↓ absolute testis weight	NOAEL: 14.7 mg/kg-day	NTP (2020)

[a] LOAEL/NOAEL not applicable for single dose studies.
Abbreviations: CAT, catalase; CBG, corticosteroid binding globulin; EDS, ethane dimethyl sulfonate; GSH-Px, glutathione peroxidase; IgG, immunoglobulin G; LOAEL, lowest-observed-adverse-effect level; MDA, malondialdehyde; NOAEL, no-observed-adverse-effect level; PCNA, proliferating cell nuclear antigen; PFOA, perfluorooctanoic acid; SOD, superoxide dismutase; TNF-α, tumor necrosis factor alpha

Table 5.5.3. Summary of recent animal toxicity studies of PFOA reporting reproductive toxicity in female animals

Sex/Species	Exposure	Endpoints	NOAEL/LOAEL	Reference
Male and female C57BL/6 mice (6/sex/dose)	0 or 3.5 mg/kg of feed (~0.55 mg/kg-day, according to authors)	↑ cholesterol in the ovary (p=0.069) and mammary glands (p <0.05)	NA[a]	Rebholz et al. (2016)
Pregnant Kunming mice (12/dose)	0, 2.5, 5, or 10 mg/kg-day via gavage from GD 1-7 or GD 1-13	**Dams:** ↑ number of resorbed embryos; ↑ serum estradiol; ↓ serum progesterone; ↓ number of corpora lutea; ↓ ratio of corpora lutea to ovarian areas; ↑ CAT and SOD activity, H_2O_2, and MDA levels in ovary; ↑ apoptosis protein markers (p53 and Bax) in ovary	LOAEL: 2.5 mg/kg-day for ↑ oxidative stress, ↑ apoptosis markers and ↓ in number of corpora lutea	Chen et al. (2017b)
Pregnant Kunming mice (10/dose)	0 or 20 mg/kg-day via gavage from GD 1-7	**Dams:** ↓ absolute and relative uterus weight; ↑ markers of uterine apoptosis	NA[a]	Song et al. (2019)
Female Sprague Dawley rats (10/dose)	0, 300, or 1,000 ppm in feed (0, 27.7, or 92.7 mg/kg-day) for 16 weeks	ovarian cysts	NOAEL: 27.7 mg/kg-day	NTP (2020)
Female Sprague Dawley rats (50/dose)	0, 300, or 1,000 ppm in feed (0, 18, or 63 mg/kg-day) for 107 weeks	squamous metaplasia in the endometrium	LOAEL: 18 mg/kg-day	NTP (2020)

Sex/Species	Exposure	Endpoints	NOAEL/LOAEL	Reference
Pregnant CD-1 mice (11-13 dams/dose)	0, 1, or 5 mg/kg-day via gavage from embryonic day 1.5 to embryonic day 11.5 or embryonic day 17.5	**Dams:** ↑ relative gestational weight gain; ↑ absolute, but ↓ relative placenta weight; placental lesions (labyrinth congestion, atrophy, fibrin clots, necrosis, and nodules) **Embryos:** ↓ viable weight	**Dams:** NOAEL: 1 mg/kg-day for placenta effects **Embryos:** NOAEL: 1 mg/kg-day	Blake et al. (2020)

[a] LOAEL/NOAEL not applicable for single dose studies.
Abbreviations: CAT, catalase; GD, gestation day; H_2O_2, hydrogen peroxide; LOAEL, lowest-observed-adverse-effect level; MDA, malondialdehyde; NOAEL, no-observed-adverse-effect level; SOD, superoxide dismutase

For PFOS, studies reporting developmental and reproductive toxicity effects published from 2016 onward are summarized in Tables 5.5.4, 5.5.5, and 5.5.6.

Table 5.5.4. Summary of recent animal toxicity studies of PFOS reporting developmental toxicity

Sex/Species	Exposure	Endpoints	NOAEL/LOAEL	Reference
Pregnant Sprague Dawley rats (10/dose)	0, 5, or 20 mg/kg via gavage from GD 12-18	**Dams:** ↓ body weight; ↓ placenta weight; **Pups:** ↓ fetal body weight in males; ↑ serum corticosterone	LOAEL: 5 mg/kg-day for ↑ serum corticosterone in pups	Li et al. (2016a)
Pregnant mice (strain not specified, 3-5/dose)	0, 1, 10, or 20 mg/kg-day orally[a] from GD 1-14	**Fetus:** ↑ body weight; ↑ crown-rump length; brain necrosis; umbilical hernia; liver enlargement; brain anomaly	NOAEL: 1 mg/kg-day for ↑ crown-rump length	Mehri et al. (2016)
Pregnant C57BL/6 mice (10-12/dose), pups (12/dose)	0, 0.1, 1, or 5 mg/kg-day via gavage from GD 1-17	**Male pups:** ↓ serum testosterone; ↑ estradiol	NOAEL: 0.1 mg/kg-day for ↓ serum testosterone[b]	Zhong et al. (2016)
Pregnant CD-1 mice (6/dose)	0 or 0.3 mg/kg-day via gavage from GD 1-18.5	No apical toxicity endpoints observed	NA[c]	Lai et al. (2017a)
Pregnant CD-1 mice (6-8/dose), male pups (4/dose)	0, 0.3, or 3 mg/kg-day via gavage throughout gestation	**Male pups:** ↓ serum testosterone; ↓ epididymal sperm count; changes in polyunsaturated fatty acid levels	NOAEL: 0.3 mg/kg-day for ↓ serum testosterone and epididymal sperm count	Lai et al. (2017b)

Sex/Species	Exposure	Endpoints	NOAEL/LOAEL	Reference
Pregnant C57BL/6 mice (6/dose), pups (12/dose)	0 or 0.3 mg/kg-day via gavage throughout gestation; animals injected with 5 mg/kg DEN on PND 15	↑ ALT and AST in pups	NA[c]	Lai et al. (2017b)
Pregnant CD-1 mice (8/dose)	0, 0.5, 2.5, or 12.5 mg/kg-day via gavage from GD 1-17	↓ fetal weight; ↓ crown-rump length; ↓ placental weight and diameter; placental histopathology (reduced blood vessel branching, vascular collapse, atresia, basement membrane breakage)	LOAEL: 0.5 mg/kg-day for reduced placental weight on GD 18	Chen et al. (2018a)
Pregnant Kunming mice (5/dose)	0, 0.5, or 5 intragastrically throughout gestation (20.5 days)	**Pups:** ↑ liver triglycerides, total cholesterol, and LDL; ↓ HDL	NOAEL: 0.5 mg/kg-day	Liang et al. (2019)
Pregnant Sprague Dawley rats (4-5/dose for dams, 8 pups per litter tested)	0 or 1 mg/kg-day in gelatin to dams from GD 1 to PND 21	**Dams:** no observed adverse effects **Pups:** ↑ activity in open-field behavioral tests	NA[c]	Reardon et al. (2019)

[a] The specific manner of oral administration was not stated in study.
[b] Statistically significant effect at the mid-dose, but not at the high dose.
[c] LOAEL/NOAEL not applicable for single dose studies.
Abbreviations: DEN, diethylnitrosamine; GD, gestational day; HDL, high density lipoprotein; LDL, low density lipoprotein; LOAEL, lowest-observed-adverse-effect level; NOAEL, no-observed-adverse-effect level; PND, postnatal day

Table 5.5.5. Summary of recent animal toxicity studies of PFOS reporting reproductive toxicity in male animals

Sex/Species	Exposure	Endpoints	NOAEL/LOAEL	Reference
Male ICR mice (10/dose)	0, 0.5, 5, or 10 mg/kg-day via gavage for 4 weeks	↓ sperm count; ↓ blood-testis barrier integrity	NOAEL: 0.5 mg/kg-day	Qiu et al. (2016a)
Male C57 mice (12/dose)	0, 0.5, or 10 mg/kg-day via gavage for 5 weeks	↓ absolute and relative testis weight, sperm count, serum testosterone levels; vacuolization in spermatogonia, spermatocytes and Leydig cells; ↑ apoptotic cells in testes, apoptosis related proteins; ↑ ERα and ERβ protein expression; ↓ PCNA+ cells	LOAEL: 0.5 mg/kg-day for ↓ testis weight, ↑ testicular lesions, and ↑ apoptosis	Qu et al. (2016)
Male Sprague Dawley rats (5/dose)	0, 5, or 10 mg/kg-day via gavage for 21 days	↓ absolute testis and seminal vesicle weight; ↓ serum testosterone; ↓ sperm count; delayed Leydig cell differentiation	LOAEL: 5 mg/kg-day for ↓ sperm count and ↓ seminal vesicle weight	Li et al. (2018b)

Abbreviations: ERα,β, estrogen receptor alpha, beta; LOAEL, lowest-observed-adverse-effect level; NOAEL, no-observed-adverse-effect level; PCNA, proliferating cell nuclear antigen

Table 5.5.6. Summary of recent animal toxicity studies of PFOS reporting reproductive toxicity in female animals

Sex/Species	Exposure	Endpoints	NOAEL/LOAEL	Reference
Female ICR mice (20/dose)	0 or 10 mg/kg-day orally for 30 days	Prolongation of duration of diestrus; ↓ number of corpora lutea; ↓ serum levels of P4, LH and GnRH on day 7; ↓ serum levels of GnRH, E2, T4 and T3 on day 14; ↑ serum levels of CORT on day 14	NA[a]	Wang et al. (2018)
Male and female Sprague Dawley Rats (10/sex/dose)	0, 0.312, 0.625, 1.25, 2.5, or 5 mg/kg-day for 28 days via gavage	**Females:** ↑ testosterone	NOAEL: 0.625 mg/kg-day for ↑ testosterone	NTP (2019b)

[a] LOAEL/NOAEL not applicable for single dose studies.
Abbreviations: CORT, corticosterone; E2, estradiol; GnRH, gonadotropin-releasing hormone; LH, luteinizing hormone; LOAEL, lowest-observed-adverse-effect level; NOAEL, no-observed-adverse-effect level; P4, progesterone; T3, triiodothyronine; T4, thyroxine

5.5.3. Recent Mechanistic Evidence

Developmental toxicity

Animal studies have reported altered organ differentiation in offspring exposed to PFOA during gestation. To investigate mechanisms by which PFOA could affect organ development, a number of in vitro studies have looked at expression of key genes during the developmental stage. In a study comparing the effects of endocrine disrupting chemicals on rhesus monkey embryonic stem cells, PFOA was found to alter the expression of genes related to cellular infiltration by leukocytes, lung injury, liver necrosis, hypertrophy, and stress response (Midic et al., 2016). PFOA altered mRNA and protein expression in the testicular Dlk1-Dio3 imprinted gene cluster (important for the control of growth and development) in F1 rats exposed in utero (Song et al., 2018). Zhou et al. (2017) reported that PFOA induced changes in myocardial differentiation (measured by expression of the myosin heavy chain 6 (myh6)) gene in mouse embryonic R1 stem cells.

Stress response from PFOA exposure can alter organ development. PFOA induced an increase in ROS in human mesenchymal stem cells, and also altered expression of genes that encode surface marker proteins involved in cell-cell interactions, potentially affecting the cells' ability to both differentiate and self-renew (Liu et al., 2019a).

Changes in adiposity have been seen in animals and in humans from prenatal exposure to PFOA. These changes have been linked to activation of PPARγ. Liu et al. (2019a) observed upregulation of adipogenic markers, including PPARγ and fatty acid synthase, in mesenchymal stem cells. Gestational exposure to PFOA decreased expression of acetyl-histone H3, acetyl-histone H4 and increased expression of acyl-CoA thioesterase 1, long-chain acyl-CoA synthetase and palmitoyl-CoA oxidase 1 in the liver of F1 female mouse pups (Li et al., 2019b). Changes in expression of these genes can alter lipid metabolism and can lead to liver damage.

Similar to PFOA, PFOS can alter expression of genes involved in development. (Chen et al., 2018a) reported expression changes in genes related to angiogenesis in mouse placenta following exposure to PFOS. In J1 mouse embryonic stem cells, PFOS induced changes in expression of neural markers during global differentiation, suggesting the potential for developmental neurotoxicity (Yin et al., 2018). PFOS also inhibited placental 11-β hydroxysteroid dehydrogenase 2 in isolated rat microsomes, and altered expression of placental genes (Li et al., 2016b).

Changes in adiposity have been seen in animals and in humans because of prenatal exposure to PFOS. These changes have been linked to activation of PPARγ. Upregulation of adipogenic markers, which include PPARγ and fatty acid synthase, as well as promotion of adipogenic differentiation in mesenchymal stem cells were observed (Liu et al., 2019a; Liu et al., 2019b).

Cardiovascular effects from PFOS exposure have been seen in animals. To investigate the effect of PFOS on cardiac development, Zhang et al. (2016f) exposed D3 embryonic stem cells to PFOS and reported a significant decrease in embryoid body diameter, and a decrease in mRNA and protein levels of cardiac-specific transcription factors and mesodermal markers. As with PFOA, Zhou et al. (2017) reported that PFOS induced changes in myocardial differentiation in the mouse R1 embryonic stem cell line.

Changes in behavior were observed in pups exposed to PFOS from gestation through lactation, as noted in Table 5.5.4 (Reardon et al., 2019). The authors postulate that the changes in behavior are the result of altered metabolite profiles in the brain of F1 pups from PFOS exposure.

Reproductive toxicity

Effects on the male reproductive system have been observed in animal toxicity studies. Molecular studies show that PFOA can alter cell structure in male reproductive organs. In BALB/c mice, PFOA altered epididymal gene expression related to lipid metabolism, altered epididymal fatty acid composition, activated the protein kinase B/adenosine monophosphate protein kinase (AKT/AMPK) signaling pathway, and increased oxidative stress in sperm (Lu et al., 2016b). PFOA disrupted junctions between isolated mouse Sertoli cells, and altered the levels of proteins associated with endocytosis and the blood-testis barrier (Lu et al., 2016a; Lu et al., 2017).

PFOA has been shown to affect male hormone production. PFOA reduced testosterone levels in animals that were treated with ethane dimethyl sulfonate (EDS) to eliminate Leydig cells, and in isolated seminiferous tubules from EDS-injected animals (Lu et al., 2019). PFOA also altered gene and protein expression related to steroidogenesis in testis and seminiferous tubules in rats (Lu et al., 2019), and affected genes related to apoptosis in mouse testis (Yuan et al., 2017). In mouse Leydig tumor cells, PFOA reduced mitochondrial membrane potential, resulting in inhibition of steroidogenesis and reduced progesterone concentration in cells (Zhao et al., 2017). However, in another study, corticosteroid binding globulin (CBG) expression was increased and in turn, increased progesterone levels and steroidogenesis (Sun et al., 2018b).

Tian et al. (2019) reported that mouse Leydig tumor cells exposed to PFOA showed a reduction in androgen receptor (AR) gene expression and upregulation of pregnane X receptor (PXR). PFOA also decreased protein levels of Nrf2, and reduced antioxidant capacity in mouse testis (Yuan et al., 2017). In an in vitro model of human spermatogenesis using male human embryonic stems cells, PFOA decreased markers for primary spermatocytes and spermatogonia (Steves et al., 2018). However, in an in vitro study in human sperm cells, PFOA did not cause genotoxicity (Emerce and Cetin, 2018).

Female reproductive effects have also been observed in animal studies. Decreased oocyte viability and increases in ROS production in mouse ovaries were observed following exposure to PFOA (Lopez-Arellano et al., 2019). Studies have shown that disruption of gap junction intercellular communication because of PFOA exposure may result in a decrease in oocyte viability (Lopez-Arellano et al., 2019). Additionally, exposure to PFOA during gestation induced increased biomarkers of apoptosis in the uterus of pregnant mice (Song et al., 2019). Long-term exposure of trophoblast stem cells to PFOA affected genes related to cysteine metabolism and interleukin signaling, indicating suppression of viral response (Midic et al., 2016), thus potentially leaving the placenta and embryo more susceptible to viral infection.

PFOS administered to pregnant CD-1 mice altered testicular gene expression in F1 males (Lai et al., 2017a). PFOS also altered gene expression in the fetal liver of CD-1 mice, and activated the liver X receptor/ retinoid X receptor (LXR/RXR) pathway (Lai et al., 2017b). PFOS inhibited androgen production, increased apoptosis, and altered gene expression in rat Leydig cells (Li et al., 2018b). Qiu et al. (2016a) reported that PFOS altered expression of proteins related to the blood-testis barrier in mice in vivo and in isolated Sertoli cells.

Additionally, Qu et al. (2016) reported that PFOS altered expression levels of proteins related to apoptosis, and changed expression levels of estrogen receptors in the testis.

PFOS can interfere with actin microfilaments in Sertoli cells and can cause disruption in human Sertoli cell tight junction permeability (Li et al., 2016a; Chen et al., 2017a; Gao et al., 2017). Expression of HILI (piwi-like RNA-mediated gene silencing 2) in primary spermatocytes was decreased (Steves et al., 2018). Changes in expression of this gene can result in mutations in spermatids. In an in vitro study in human sperm cells, PFOA did not cause DNA damage (Emerce and Cetin, 2018).

5.5.4. Conclusions

Overall, the evidence for an association between prenatal PFOA exposure and lower birth weight remains mixed. A large association was seen in births to a relatively small group of women in Sweden, but not for the Norwegian group in the study with cases selected primarily from a high-risk population. Other associations reported from cross-sectional and other prospective cohort studies were more modest. The majority of studies reported no statistically significant associations with birth weight, despite some large samples and some PFOA concentrations >5 ng/ml (although most were much lower). A clear relationship between PFOA concentration and risk of decreased birth weight is not apparent.

Although a few studies reported large decreases in birth weight associated with prenatal PFOS exposure, larger prospective studies reported more modest associations, and the majority of recent studies, including large prospective studies with a range of PFOS concentrations, reported no statistically significant associations between prenatal exposure to PFOS and birth weight. The relationship between prenatal PFOS exposure and decreased birth weight remains unclear.

While half of the studies of SGA reported that prenatal PFOA exposure was statistically significantly associated with increased risk of SGA in one part of the sample, the results were inconsistent within and across studies. For women who were at elevated risk of delivering SGA infants, including those who smoked during pregnancy, PFOA exposure was associated with increased risk except among a Norwegian cohort (Lauritzen et al., 2017; Govarts et al., 2018). Other studies reported conflicting data on differences by sex, with one reporting a stronger association in female infants than in males (Wikström et al., 2019), while another reported a possible protective effect in females (Manzano-Salgado et al., 2017).

PFOS was associated with a statistically significant increased risk of SGA in two cross-sectional studies, with one study reporting increased risk among women who smoked during pregnancy, and decreased risk of SGA among non-smokers. The case-cohort and prospective cohort studies reported no associations and non-statistically significant associations between prenatal exposure to PFOS and risk of SGA birth.

OEHHA's review of the literature published since 2016 identified little new epidemiologic evidence supporting an association between higher PFOA levels in women and decreases in fertility or fecundity.

The few epidemiologic studies of PFOS exposure and fertility and fecundity published since 2016 provide little new epidemiologic evidence supporting an association between PFOS and

reduced fertility or fecundity, although a recent case-control study suggests a possible association between PFOS exposure and reduced risk of endometriosis-related infertility.

For PFOA, a number of studies in mice reported reproductive toxicity following exposure to PFOA for 1-4 weeks. In male mice, studies reported decreased testis and epididymis weights and sperm count, and effects on the blood-testis barrier. NTP (2019a) reported decreased absolute cauda epididymis weight and sperm count, and increased relative testis weight in male rats after 28 days of exposure via oral gavage. In a 16-week oral gavage study, decreased absolute testis weight was also observed in male rats (NTP, 2020). In female mice, studies reported decreases in litter size, changes in hormone levels, and effects on the ovary. Generally, these data support earlier findings reported by US EPA and others that PFOA is a developmental and reproductive toxicant (Table 5.5.7).

For PFOS, recent studies identified multiple adverse effects on reproductive health, such as decreases in testis and/or epididymis weights, decreases in sperm count, increases in apoptosis and apoptosis markers in the ovary or testis, changes in hormone levels, and changes in estrous cycle. Several studies also reported adverse effects in offspring exposed to PFOS during gestation, including reduced growth, changes in lipid and hormone homeostasis, and behavioral alterations. OEHHA did not identify any recent studies examining effects of PFOS on fetal lungs. Nonetheless, the recent data support earlier findings that PFOS adversely affects reproduction and development in animals (Table 5.5.7).

Table 5.5.7. Summary of OEHHA's conclusions regarding the human and experimental animal data on PFOA and PFOS and developmental and reproductive toxicity

Outcome	PFOA	PFOS
Pregnancy-related hypertension and preeclampsia in humans	Data are suggestive of an association with risk of preeclampsia and pregnancy-related hypertension	Data are suggestive of an association with risk of preeclampsia and pregnancy-related hypertension
Measures of fetal growth in humans	Inconsistent evidence for decreased birth weight and risk of SGA	Inconsistent evidence for decreased birth weight and risk of SGA
Pubertal development	-Sparse, inconsistent evidence for changes in onset of puberty in prenatally exposed girls and boys -Sparse evidence for delayed puberty in girls and boys associated with postnatal exposure to high concentrations	-Sparse, inconsistent evidence for earlier puberty in prenatally exposed girls and boys -Sparse evidence for delayed puberty in girls and boys associated with postnatal exposure to high concentrations
Fertility and Fecundity in humans	Inconsistent evidence for reduced fertility or fecundity in women	Inconsistent evidence for reduced fertility or fecundity in women
Testicular/epididymal effects in animals	Positive evidence for decreased testicular and epididymal weight, decreased sperm count, and disruption of the blood-testis barrier	Positive evidence for decreased testicular and epididymal weight, and decreased sperm count

Outcome	PFOA	PFOS
Fertility/litter size effects in animals	Positive evidence for decreased litter sizes and pup survival	-No evidence for effects on fertility -Positive evidence of increased neonatal mortality
Adverse effects in animal offspring	Positive evidence for reduced fetal/birth weight, and increased liver effects in pups exposed during gestation	Positive evidence for decreased body weight and changes in hormone homeostasis in pups exposed during gestation

5.6. Neurotoxicity

In their review, US EPA (2016b) did not identify clear or consistent evidence from human epidemiologic studies linking PFOA or PFOS to neurotoxicity outcomes.

US EPA (2016b) identified two studies that reported behavioral effects in mice following exposure to PFOA. In one study, a single exposure to PFOA on postnatal day 10 induced changes in habituation and activity patterns months later, alongside protein expression changes of neuroactive proteins in the brain (Johansson et al., 2009, as reported by US EPA (2016b)). Additionally, gestational exposure induced sex-specific changes in exploratory behavior in pups (Onishchenko et al., 2011, as reported by US EPA (2016b)).

US EPA's literature review of the neurotoxic effects of PFOS in laboratory animals found inconsistent results for PFOS-induced learning and memory impairment, as determined by water maze testing (Luebker et al., 2005; Butenhoff et al., 2009; Long et al., 2013; Wang et al., 2015, as reported by US EPA (2016d)). Mechanistic studies revealed potential neurotoxic effects of PFOS, including effects on excitatory amino acids (Yang et al., 2009, as reported by US EPA (2016d)), changes in gene expression of neuroactive compounds and inflammatory markers (Wang et al., 2010; Zeng et al., 2011, as reported by US EPA (2016d)), and decreased neurite growth in cultured hippocampal cells (Liao et al., 2009, as reported by US EPA (2016d)).

5.6.1. Recent Animal Evidence

Guo et al. (2019) exposed male BALB/c mice (12/dose) to 0, 0.4, 2 or 10 mg/kg-day PFOA via oral gavage for 28 days and observed decreased glutamic acid content and increased glutamate synthetase in the mouse brain at the high dose. Additionally, in utero exposure to 5 mg/kg-day PFOA induced an increase, compared to controls, in cortical nerve cells and effectors of cell proliferation (including nerve growth factor) in Kunming mouse pups (Qin et al., 2018). However, an acute exposure in male rats (single oral dose of 50 mg/kg) had no effect on memory function (Kawabata et al., 2017).

Several studies reported neurotoxic effects following oral PFOS exposure. Changes in dopamine levels and dopaminergic gene expression in the hippocampus and cortex of rodents have been observed. Following a single PFOS exposure of 11.3 mg/kg on PND 10, (Hallgren and Viberg, 2016) observed changes in gene expression involved in dopaminergic signaling in mice at 2 months of age. In adult male rats, an exposure of 0.5 mg/kg-day was associated with an increase in dopamine receptor subtype 2 (D2) gene expression in the prefrontal cortex; other dopaminergic system changes were noted at doses of 1 mg/kg-day and higher (Salgado et al.,

2016). No deficits in tyrosine hydroxylase or dopamine aminotransferase were observed in mice (Patel et al., 2016).

Increased hallmarks of Alzheimer's disease were observed in adult rats exposed both pre- and perinatally to PFOS (Zhang et al., 2016e). Increased Tau mRNA and elevated protein levels, increased phosphorylation of Tau, and elevated β-amyloid aggregation were observed in the hippocampus of 90-day-old rats exposed pre- or postnatally to PFOS in drinking water (1.7-15 mg/L). A reduction in nuclei in the dentate gyrus of the hippocampus, an abnormality which the authors argue is consistent with memory dysfunction, was reported in mice exposed during gestation (20 mg/kg-day, administered to dams) (Mehri et al., 2016). PFOS exposure of Wistar dams from GD 11-20 at 1 or 2 mg/kg-day resulted in a reduction in glutamate receptor 2 (GluR2) expression to 82% of control at 1 mg/kg-d and 44% of control ($p < 0.01$) at 2 mg/kg-day in the cortex of the pups measured on PND 4 (Ishida et al., 2017). An increase in kainic acid-induced excitotoxicity was also observed in the rat pups exposed during gestation, likely due to the decrease in cortical GluR2 expression (Ishida et al., 2017). Excitotoxicity due to excess calcium influx results in neuronal cell damage and death, and is implicated in neurodegenerative disease (Dong et al., 2016b). Recently, Zhang et al. (2019a) reported changes in synaptic plasticity (decreased long-term potentiation, lower input/output and paired pulse facilitation curves, and decreased facilitated excitatory post-synaptic potentials in the hippocampus) in rats exposed both pre- and postnatally to PFOS. These results are similar to observed results from an earlier study that reported repression of long term potentiation in the hippocampus of Sprague Dawley rats exposed to PFOS via intracerebroventricular injection (Zhang et al., 2016d). Reardon et al. (2019) reported that rat pups exposed to 1 mg/kg-day PFOS from GD 1 to PND 21 (given to dams during gestation and lactation) had increased activity in open-field behavior tests, suggesting neurobehavioral changes due to chemical exposure.

5.6.2. Recent Mechanistic Evidence

PFOA increased nerve growth factor (NGF) in mouse neurons ex vivo (Qin et al., 2018), and altered neurotransmitter levels in mouse brain (Yu et al., 2016).

Much like in the animal toxicity literature, several in vitro studies have reported neurotoxic effects of PFOS exposure. (Dong et al., 2016b) exposed C17.2 neural stem cells to PFOS, and observed impaired cell cycle proliferation, and changes in protein and mRNA expression of components in the Wnt signaling pathway.

PFOS altered expression of dopaminergic genes in the cortex and hippocampus of male mouse pups (Hallgren and Viberg, 2016), and dopamine receptors in the amygdala, prefrontal cortex, and hippocampus of male rats (Salgado et al., 2016). PFOS reduced the number of dopaminergic neurons in a culture of primary mesencephalic neurons, and inhibited vesicular uptake of dopamine in HEK293 cells expressing the human vesicular monoamine transporter 2 (Patel et al., 2016).

Guo et al. (2017) exposed SK-N-SH neuroblastoma cells to PFOS, and observed changes in cellular morphology (shrunken and round cells), inhibition of cell growth, and changes in protein and mRNA levels of brain-derived neurotrophic factor (BDNF). The authors also reported increased methylation of the BDNF promoter, and changes in expression of genes for DNA methyltransferases, suggesting an epigenetic basis for the inhibition of cell growth. In a different human neuroblastoma cell line (SK-SY5Y), PFOS induced ROS, increased apoptotic markers, and changes in cellular morphology (smaller unattached cells, retraction of

pseudopods) (Sun et al., 2018a). A similar study in SK-SY5Y neuroblastoma cells reported increased oxidative stress, and markers of apoptosis, possibly mediated by the JNK signaling pathway (Sun et al., 2019a). Additionally, increased ROS and markers of apoptosis were observed in HAPI rat microglia following exposure to PFOS (Ge et al., 2016). PFOS induced cytotoxicity in SK-SY5Y cells, as indicated by lactose dehydrogenase release (Patel et al., 2016).

Oh et al. (2017) reported that PFOS causes endoplasmic reticulum stress in rat embryo primary cortical neurons. In neonatal rat primary hippocampal neurons and astrocytes, PFOS induced oxidative stress, changes in cellular morphology (shrinkage, rounding, detachment), autophagy, increased apoptosis, changes in glutamate/glutamine levels, and stunted neurite outgrowth (Li et al., 2017f). Additionally, when primary hippocampal neurons were exposed to the cellular medium used during PFOS experiments in astrocytes (termed astrocyte conditional media), increases in apoptosis and n-methyl d-aspartate (NMDA) receptor gene and protein expression were observed (Wang et al., 2019b). The authors proposed that increases in D-serine levels in the extracellular medium (released by astrocytes in response to PFOS exposure), led to neuronal toxicity mediated by NMDA receptor activation.

In neuronal differentiated PC12 (pheochromocytoma) cells, PFOS altered cell morphology (shrunken cells), increased ROS production, increased caspase-3 activity (marker of apoptosis), and induced autophagy (Li et al., 2017a).

PFOS altered expression of Tau mRNA and protein in the brain of F1 rats exposed pre- and postnatally. PFOS also induced accumulation of β-amyloid in the hippocampus, and changed expression levels of amyloidogenesis related genes (Zhang et al., 2016e).

Berntsen et al. (2018) reported that PFOS-induced cytotoxicity in cerebellar granular neurons in vitro can be modulated by the presence of NMDA receptor antagonists and calcium chelators. The authors suggest that PFOS induces excitotoxicity, and subsequent cell death, through activation of glutamate receptors and subsequent influx of extracellular calcium. PFOS exposure reduced glutamate receptor GluR2 expression (AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid, receptor subunit) and increased excitotoxicity in primary cerebral cultures from rat fetuses on GD 18 (Ishida et al., 2017). Additionally, PFOS altered expression of AMPA-related genes in primary rat hippocampal neurons, and changed glutamate receptor levels in rats exposed both pre- and postnatally (Zhang et al., 2019a).

Taken together, there is a substantial body of in vivo and in vitro evidence that suggests that PFOS has neurotoxic potential. These in vitro studies support findings in animal toxicity studies (discussed above) that PFOS can have adverse effects on various parts of the central nervous system.

5.6.3. Conclusions

The evidence of PFOA-induced neurotoxicity in animals is very limited (Table 5.6.1). Changes in behavior, in addition to alterations in glutamatergic homeostasis and nerve cell proliferation suggest that PFOA may induce neurotoxic effects in animals, but more study is needed to draw any firm conclusions.

Recent animal and mechanistic studies of PFOS-induced neurotoxicity highlight effects that were not identified previously, including effects on dopaminergic signaling, excitotoxicity and

changes in glutamate signaling, and changes in synaptic plasticity. Additionally, hallmarks of Alzheimer's disease were observed in one study, which could potentially be associated with memory deficits observed in previous studies. In vitro studies in various cell lines also reported oxidative stress in response to PFOS exposure. As such, the evidence of PFOS-induced neurotoxicity is increasing, and the current body of evidence suggests PFOS can induce neurotoxicity via multiple modes of action (Table 5.6.1).

Table 5.6.1. Summary of OEHHA's conclusions regarding the experimental animal data on PFOA and PFOS and neurotoxicity

Outcome	PFOA	PFOS
Changes in glutamatergic signaling in animals	Positive evidence	Positive in vivo and in vitro mechanistic evidence
Behavioral alterations in animals	Positive evidence	Positive evidence
Learning and memory impairment in animals	No evidence	Inconsistent evidence, based on water maze results
Changes in dopaminergic signaling in animals	No evidence	Positive in vivo and in vitro mechanistic evidence

5.7. Cancer

US EPA reviewed the literature for PFOA and cancer published up to December 2015 and concluded that there was "suggestive evidence of carcinogenic potential" for PFOA and that, "Epidemiology studies demonstrate an association of serum PFOA with kidney and testicular tumors among highly exposed members of the general population" (US EPA, 2016b). In 2017 the International Agency for Research on Cancer (IARC) classified PFOA as possibly carcinogenic to humans (Group 2B) based on limited evidence in humans and in experimental animals (IARC, 2017a). With regards to the human evidence, IARC concluded that, "A positive association was observed for cancers of the testis and kidney."

With regards to PFOS and cancer, US EPA (2016b) concluded that, "A small number of epidemiology studies of PFOS exposure and cancer risk are available. While these studies do report elevated risk of bladder and prostate cancers, limitations in design and analysis preclude the ability to make definitive conclusions." In particular, US EPA noted the small numbers of cases in many of these studies and the fact that the results in several of the studies linking PFOS to increased risks of bladder or prostate cancer were not statistically significant. In addition, US EPA noted that, "…some [human epidemiologic studies of PFOS and cancer] are confounded by failure to adjust for smoking" but did not present clear evidence to support this assertion. IARC has not reviewed PFOS.

In addition to reviewing the results of previous reviews by the US EPA (US EPA, 2016b; US EPA, 2016d), ATSDR (2018a), and others, OEHHA reviewed the epidemiologic literature on PFOA and PFOS and cancer, with a focus on cancer of the kidney, testis, bladder, breast, prostate, and pancreas. Detailed summaries of PFOA and cancer are shown in Appendix 7, Tables A7.21-27.

For animal studies, OEHHA reviewed all the available animal carcinogenicity data, including the recent NTP (2020) two-year bioassay of PFOA in male and female Sprague Dawley rats. Tumor incidence data and descriptions of rare tumor types are presented below.

5.7.1. Human Evidence

PFOA

Bladder cancer: For bladder cancer, epidemiologic studies have not found clear associations. The high exposure occupational study by Raleigh et al. (2014) identified an elevated relative risk in the most highly exposed workers (hazard ratio (HR) = 1.66; 95% CI, 0.86-3.18) but this was based on a relatively small number of cancer cases and involved limited or no information on potential confounders like smoking. In the other occupational cohort (Steenland and Woskie, 2012; Steenland et al., 2015), relative risk estimates were below 1.0 in the most highly exposed workers. Studies in highly exposed communities were also negative (Barry et al., 2013; Vieira et al., 2013; Mastrontonio et al., 2017).

Breast cancer: Some intriguing results have been reported for breast cancer, but because of the somewhat limited evidence and various weaknesses in these studies, firm conclusions cannot be made based solely on these studies. For example, Mastrontonio et al. (2017) found a statistically significant increase in breast cancer mortality in an area with relatively high drinking water contamination (relative risk (RR) = 1.11; 95% CI, 1.02-1.20), although the increase was small and this study was based on an ecologic design with very limited information on potential confounders. Bonefeld-Jorgensen et al. (2014) reported elevated RRs in women over 40 years old, but with an unusual dose-response pattern (highest RRs in the 2nd and 4th quintiles, all other RRs near 1.0). In a population based study, Mancini et al. (2020) identified elevated PFOA-related risks for estrogen receptor negative (OR = 7.73; 95% CI, 1.46–41.08) and progesterone receptor negative cases (OR = 3.44; 95% CI, 1.30–9.10) in women in the 2nd vs. 1st quartiles of serum PFOA, but ORs were lower and not statistically significant for the higher quartiles. The quartile cutoff points in these analyses appear to be based on all breast cancer cases, regardless of estrogen or progesterone receptor status. The total numbers of cases in the estrogen receptor negative and progesterone receptor negative analyses were 26 and 57, respectively. There were 194 controls. The numbers of cases and controls in each PFOA exposure category for these analyses were not provided.

Most of the studies OEHHA identified had only limited information on known breast cancer risk factors, and several studies assessed exposure using only a single or a small number of blood samples. The serum elimination half-life of PFOA appears to be fairly long (approximately 3-4 years) (Olsen et al., 2007). However, the latency period between PFOA exposure and breast cancer (if an association exists) is unknown, and assessing exposure based on a single serum sample or samples collected only during a single short window of time could miss or underestimate the impacts of exposure fluctuations or relevant exposure periods. These issues would most likely bias study results towards an underestimate of the true cancer risks.

Kidney cancer: OEHHA identified seven human studies of PFOA and kidney cancer (Table A7.23). Two of these studies are not informative, either because of the ecologic nature of the exposure data (Mastrontonio et al., 2017), or because of the very small number of cases (Girardi and Merler, 2019). Four of the remaining five studies reported statistically significant associations between PFOA and kidney cancer or renal cell carcinoma (RCC) incidence (Barry et al., 2013; Vieira et al., 2013; Shearer et al., 2021) or mortality (Steenland and Woskie, 2012). Evaluations of bias, confounding, and all other major aspects of causal inference for these studies all suggest that the results of these positive studies represent real effects. These evaluations are discussed in Chapter 6. One study, the occupational study by (Raleigh et al., 2014), did not find an association between PFOA and kidney cancer. A detailed analysis of the

potential reasons the results of this study differ from most of the other human studies of PFOA and kidney cancer is presented in Chapter 6. Overall, the findings from the human epidemiologic studies, combined with data from animal and mechanistic research, provide strong evidence that PFOA is a cause of kidney cancer.

Liver cancer: Liver cancer is relatively rare and the small numbers of liver cancer cases included in the studies OEHHA reviewed limited the ability of most of these studies to adequately investigate the relationship between PFOA and this cancer type. In the study that appears to have involved the highest PFOA exposure levels, Girardi and Merler (2019) reported SMRs of 1.02 (95% CI, 0.12-7.21; N=1 case), 2.72 (95% CI, 0.69-11.0; N=2 cases), and 3.07 (95% CI, 1.15-8.18; N=4 cases) for liver cancer by tertiles of estimated cumulative PFOA exposure, respectively, compared to regional rates. Compared to railroad workers, mortality rate ratios were even higher. Information on alcohol consumption or other potential confounders was not available, although it seems somewhat unlikely that confounding would cause relative risks this high. The liver cancer SMR was also elevated in workers who reportedly did not work directly with PFOA (SMR = 2.71; 95% CI, 1.02-7.22), although serum sampling results in a subset of these workers showed that they also had significant PFOA exposure (mean = 977 ng/ml). In another high exposure occupational study, Steenland and Woskie (2012) reported SMRs for liver cancer above 2.0 in PFOA exposed workers in the 1st and 3rd quartiles of cumulative PFOA exposure compared to workers at a nearby facility who were not exposed to PFOA. The number of cases was small (N=10 exposed cases overall) and SMRs in the other quartiles were near or below 1.0. In a study of tetrafluoroethylene (TFE) workers co-exposed to PFOA, Consonni et al. (2013) reported an SMR of 2.00 (95% CI, 0.54-5.12) for those in the highest PFOA category of cumulative exposure (not shown in Tables). However, every worker was also exposed to TFE, which has been linked to liver cancer in rodents (IARC, 2017b). In the 3M occupational cohort, SMRs for PFOA exposed workers were mostly near or below 1.0, although the number of cases was small (N=8 cases in all quartiles). Overall, the results of several high exposure occupational studies provide some evidence that PFOA is associated with liver cancer, although the small sample sizes of these studies limit the usefulness of these studies for dose-response analyses.

The lower exposure non-occupational studies all reported relative risks near 1.0, although the numbers of cases were small (e.g., the C8 studies), exposure ranges were limited (i.e., the population based study by Eriksen et al., 2009), or exposure assessment was ecological (i.e., Mastrontonio et al. (2017)). Because of these issues, these studies by themselves also cannot be used to make definitive conclusions regarding PFOA and liver cancer or to adequately assess dose-response relationships.

Pancreatic cancer: Pancreatic cancer is relatively rare. Because of this, cohort studies need very large sample sizes in order to have enough cases and sufficient statistical power to detect true associations in a convincing manner. Most of the studies OEHHA reviewed, including the high exposure occupational cohort studies, had relatively small sample sizes overall and therefore small numbers of pancreatic cancer cases, and clear associations have not been seen in these studies. By far the largest non-ecologic study OEHHA identified was the nested case-control study by Eriksen et al. (2009), which was based on a cohort of 57,053 people and had 128 cases of pancreatic cancer. In this population based study involving low exposure levels, relative risk estimates adjusted for smoking and diet generally seemed to increase with increasing quartiles of serum PFOA: 1.00 (reference), 0.88 (95% CI, 0.49-1.57), 1.33 (95% CI, 0.74-2.38), 1.55 (95% CI, 0.85-2.80), although the trend was not statistically significant (RR = 1.03 (95% CI, 0.98-1.10) for each 1 ng/ml increase in PFOA). Overall, while some limited

supporting evidence is present, firm conclusions regarding pancreatic cancer cannot be made based solely on the human epidemiologic literature published to date.

Prostate cancer: Several studies of prostate cancer, including the two high exposure occupational studies, have reported relative risk estimates above 1.0. Relative risk estimates in the most recent reports from these occupational studies range from 1.32 to 1.88 although none are statistically significant. Mortality HRs of 3.0 (95% CI, 0.9–9.7, N=10 cases) and 6.6 (95% CI, 1.1–37.7, N=2 cases) were reported for the two highest exposure groups in an earlier report from the 3M occupational cohort (Lundin et al., 2009). However, in a later report with six additional years of follow-up, relative risk estimates were much lower (HR = 1.32; 95% CI, 0.61-2.84) (Raleigh et al., 2014). The authors of the later report, which included additional "non-exposed" workers from a nearby 3M facility and more complex exposure assessment methologies, wrote that the earlier analyses "were limited by the qualitative nature of the exposure assessment and that the lowest exposed members of the population were more likely to be research and development professionals with lower overall baseline risks." A rationale for why research and development professionals would have 3-6 times lower risks than the other workers in the earlier study was not given. And, it would generally be expected that the potentially less accurate exposure assessment in the earlier study would bias results to the null, not towards the large SMRs that were reported. Given the fairly wide confidence intervals reported in each study, it is possible the inconsistency between the earlier and later reports from the 3M cohort is due to chance. Currently however, the reasons for these inconsistent results are unknown.

One factor to consider when looking at the data on prostate cancer as a whole is that none of the prostate cancer studies adjusted for prostate cancer screening. This could have potentially confounded some of the results of these studies, although OEHHA could not find any evidence that PFOA exposure is strongly related to cancer screening. Overall, the human evidence linking PFOA to prostate cancer has a number of limitations that prevent firm conclusions being made regarding PFOA and prostate cancer based on this evidence alone.

Testicular cancer: Testicular cancer is fairly rare, and too few cases of this cancer were identified in the occupational cohorts for the findings from these studies to be informative by themselves. However, elevated risks of testicular cancer were seen in two studies involving people living in areas with high drinking water contamination. In the retrospective cohort study of residents living near the DuPont facility in West Virginia, HRs of 1.00, 1.04, 1.91, and 3.17 were reported by quartile of exposure (p-trend = 0.04) (Barry et al., 2013). And, mostly similar findings were seen in the study by Vieira et al. (2013), which involved the same study area but used different methods for assessing exposure, different methods for ascertaining cancer cases, and different control groups. These findings are further supported by the elevated mortality relative risk of 1.86 (95% CI, 0.81-4.27) reported for residents of a highly contaminated area in Italy (Mastrontonio et al., 2017). The former is an ecologic study, but there is no reason to suspect that ecologic fallacy or exposure misclassification caused this elevation, and no major confounders are obvious. Research has also shown that risks of testicuilar cancer are elevated in firefighters, an occupation where PFOA exposures can be high (Soteriades et al., 2019). Overall, the epidemiologic literature to date suggests that PFOA is associated with testicular cancer.

PFOS

Fewer human epidemiologic data are available for PFOS and cancer so these studies are reviewed narratively here and summary tables are not provided.

Bladder cancer: Studies of highly exposed occupational cohorts are limited to a single facility, a chemical manufacturing plant in Decatur, Alabama, with geometric mean PFOS serum levels of about 1-2 ppm (or µg/ml) in the most highly exposed workers. PFOA was also present, with a mean serum level of 0.899 ppm reported (Olsen et al., 2003d). In a study of all workers for the period 1961 to 1997, bladder cancer mortality was elevated in the most highly exposed workers compared to Alabama state rates (SMR = 12.77; 95% CI, 2.63-37.35) although this involved only three exposed cases (Alexander et al., 2003). All of the bladder cancer deaths in this cohort were in the most highly exposed group. Information on potential confounders such as smoking were not available. In a follow-up study, current and past employees were sent a questionnaire in an attempt to identify incident cases of bladder cancer (Alexander and Olsen, 2007). The response rate was 74 percent, and 11 cases of bladder cancer were identified. The standardized incidence ratio (SIR) for the cohort overall compared to the US population was 1.28 (95% CI, 0.64-2.29) for men and women combined. The SIR was elevated in women (SIR = 6.42; 95% CI, 0.78-23.18; N=2 cases) but neither of these two cases worked in jobs with obvious high PFOS exposure.

Breast cancer: Two deaths from breast cancer were reported in the only high exposure occupational cohort study (SMR = 1.57; 95% CI, 0.19-5.66) (Alexander et al., 2003). No information on common breast cancer risk factors was available other than age, and findings for breast cancer incidence were not reported. Mastrontonio et al. (2017) found a slight increase in breast cancer mortality for a community with PFOS and PFOA drinking water contamination (SMR = 1.11; 95% CI, 1.02-1.20) although this study used an ecologic design and there was no individual information on exposure or most relevant confounders. All other studies of PFOS and breast cancer have involved relatively lower general population exposures. In the Danish National Cohort, clear associations with breast cancer incidence were not found overall (Bonefeld-Jorgensen et al., 2014), although some indication of an interaction between PFOS and the aromatase (CYP19) CC genotype was seen (p-interaction = 0.055, 36 cases in the CC genotype-higher PFOS exposure category) (Ghisari et al., 2017). In a case-control study nested in a large cohort of French women, breast cancer ORs adjusted for smoking, exercise, diet, and several reproductive and development factors were above 1.0 and statistically significant but without clear dose-response trends overall (Mancini et al., 2020). Dose-response trends were more consistent for estrogen receptor positive (p-trend = 0.04, 132 cases overall) and progesterone receptor positive tumors (p-trend = 0.02, 98 cases overall) but these findings have yet to be replicated in another study population. Replication (i.e., the Hill criterion of "consistency") is an important element in evaluating causality (Bradford Hill, 1965). However, the lack of replication does not mean that this finding is not real, and replication is not a required element of causal inference. Another general population study did not find evidence of increased breast cancer incidence with increasing perinatal serum levels of PFOS but did not examine breast cancer subtypes or genetic variants (Cohn et al., 2020). Several studies in which PFOS levels were measured after or near the time of cancer diagnosis have reported associations between PFOS and breast cancer (e.g., Tsai et al., 2020). Given the long half-life of PFOS in human blood, the exposure levels measured in these studies could represent exposures that occurred prior to cancer development. However, this is currently difficult to evaluate since data on the latency of PFOS-related cancer is not available. Overall, while a number of intriguing findings for PFOS and breast cancer have been reported, small sample

sizes, narrow exposure ranges, lack of data on potential confounders, latency issues, and lack of replication in particular subgroups have all limited the ability to make firm conclusions based on these results.

Liver cancer: The only high exposure occupational cohort study of liver cancer was too small to provide useful information regarding this cancer (only two liver cancer deaths total and only one in a high exposure job) (Alexander et al., 2003). A population based study involving mostly low exposures found no clear association between a single PFOS serum measurement and liver cancer (Eriksen et al., 2009). Overall, because of small sample sizes or limited exposure ranges the human epidemiologic literature on its own cannot be used to make conclusions regarding PFOS and liver cancer.

Prostate cancer: The results of the two studies that reported on PFOS exposure and prostate cancer were inconsistent. No significant associations were observed between prostate cancer and PFOS-exposed jobs within a PFOS manufacturing facility in Decatur, Alabama. The OR for those with high PFOS exposure for more than 1 year was 1.08 (95%CI, 0.44–2.69); the OR for the combined category of those with low or high PFOS exposure for 1 year or more was 1.36 (95%CI, 0.61–3.02) (Grice et al., 2007). In a case-cohort analysis within the Danish general population, an increase in prostate cancer was observed for the three upper quartiles of PFOS serum levels compared with the lowest quartile. For the lowest vs. the highest quartile, the incidence rate ratio (IRR) was 1.38 (95% CI, 0.99-1.93) (Eriksen et al., 2009). When PFOS was analyzed as a continuous variable, the IRR was 1.05 (95% CI, 0.97-1.14).

The inconsistent results may be partially explained by the differences in the method of exposure assessment between the two studies. In the occupational cohort, cumulative PFOS exposure was estimated based on a job-exposure matrix up to the year of the diagnosis. In the Danish cohort, serum samples were collected prospectively at the time of enrollment.

Other cancer types: Associations between PFOS and other cancer types have been examined, but to date clear and consistent associations with these other types have not been identified (Alexander et al., 2003; Olsen et al., 2004; Grice et al., 2007; Eriksen et al., 2009; Mastrontonio et al., 2017)

5.7.2. Animal Evidence

PFOA

Cancer bioassays in laboratory animals published prior to 2016 have been thoroughly described previously (US EPA, 2016b; New Jersey DWQI, 2017; IARC, 2017a). The studies published prior to 2016 are briefly described below, and significant tumor findings observed in these studies are presented in Table 5.7.1.

Table 5.7.1. Significant tumor incidences and cases of hyperplasia following exposure to PFOA (studies published prior to 2016)

Sex/Species	Exposure	Tumor type	Dose (mg/kg-day)	Incidence[a]	Reference
Male Sprague Dawley rats (50/dose)	Dietary for 106 weeks	Leydig cell adenoma	0, 1.3, or 14.2	0/33, 2/36, 7/44*	Butenhoff et al. (2012a), data from (Sibinsky, 1987)
		Pancreatic acinar cell hyperplasia		3/46, 1/46, 10/47	Caverly Rae et al. (2014)
Female Sprague Dawley rats (50/dose)		Ovarian tubular hyperplasia	0, 1.6, or 16.1	0/48, 7/50, 15/47	Butenhoff et al. (2012a)
Male Sprague Dawley rats (76-79/dose)	Dietary for 104 weeks	Hepatocellular adenoma	0[b] or 13.6	1/79, 10/76*	Biegel et al. (2001)
		Pancreatic acinar cell adenoma or carcinoma	0[b] or 13.6	1/79, 8/76*	
		Leydig cell adenoma	0[b] or 13.6	2/78, 8/76*	
Pregnant CD-1 mice (6-14 dams/dose or 21-37 female pups/dose)	Drinking water from GD 1-17, pups followed for 18 months	Hepatocellular adenoma	0, 0.01, 0.1, 0.3, 1, or 5	0/29, 1/29, 1/37, 4/26,* 0/31, 1/21	Filgo et al. (2015)
		Hepatic hemangio-sarcoma	0, 0.01, 0.1, 0.3, 1, or 5	0/29[c], 0/29 0/37, 1/26 0/31, 2/21	

[a] Incidence is number of animals with tumors/effective number of animals (animals that died prior to the first appearance of the tumor were excluded)
[b] Pair-fed control, fed same amount of food as treated group
[c] Significant trend ($p < 0.01$, determined by Filgo et al. (2015))
*$p < 0.05$, pairwise comparison with Fisher's exact test, statistical analysis by OEHHA
GD, gestation day

Sibinsky (1987), as reported by Butenhoff et al. (2012a), administered 0, 30, or 300 ppm PFOA to Sprague Dawley rats (0, 1.3, or 14.2 mg/kg-day for males; 0, 1.6 or 16.1 mg/kg-day for females) in the diet for 105-106 weeks. In male animals, a significant increase in Leydig cell adenomas was observed in the high dose group, and a positive trend with increasing dose was identified ($p=0.005$). An increase in pancreatic acinar cell hyperplasia was also observed in the high dose males following a re-evaluation of the Butenhoff et al. (2012a) data by Caverly Rae et al. (2014). In females, a significant increase in ovarian tubular hyperplasia was observed at the mid and high dose. However, a re-evaluation performed by Mann and Frame (2004) for E. I. du

Pont de Nemours and Company reclassified the ovarian tubular hyperplasia as gonadal stromal hyperplasia and found no significant increases in ovarian hyperplasia. Furthermore, an increase in mammary gland fibroadenoma in the high-dose group was initially reported in females, but a follow-up examination by a Pathology Working Group that included participants from former PFOA manufacturers (DuPont and 3M Company) found no significant increase over controls (Table 5.7.2 (Hardisty et al., 2010)).

Table 5.7.2. Mammary gland fibroadenoma data (single and multiple tumors) in female Sprague Dawley rats from Butenhoff et al. (2012a) and (Hardisty et al., 2010)

Tumor Type	0 ppm	30 ppm	300 ppm
Fibroadenomas	10/46	19/45	21/44*
Fibroadenomas re-evaluated by PWG	18/50	22/50	23/50

PWG, Pathology Working Group (Hardisty et al., 2010)
*p <0.05, reported by (Butenhoff et al., 2012a)

Biegel et al. (2001) administered 0 or 300 ppm (0 or 13.6 mg/kg-day) to male Sprague Dawley rats in the diet for 24 months. Statistically significant increases in tumors were reported at multiple sites; specifically liver (hepatocellular adenomas), pancreas (acinar cell adenomas or carcinomas), and testis (Leydig cell adenomas). Significant increases in pancreatic acinar cell hyperplasia and Leydig cell hyperplasia were also observed.

Filgo et al. (2015) exposed three different strains of pregnant mice (CD-1, 129/SV WT, and 129-SV PPARα KO) to doses of PFOA in drinking water ranging from 0 to 5 mg/kg-day from GD 1-17. Female offspring were observed for 18 months. A significant increase in hepatocellular adenomas, and a significant trend for hepatic hemangiosarcomas, were observed in the CD-1 F1 generation. Liver tumors were not observed in 129/SV WT mice. In the 129/SV PPARα KO mice, hepatocellular adenomas were observed at incidences of 0/6, 1/10, 1/10, 1/9, and 2/9 in the control, 0.1, 0.3, 1, and 3 mg/kg-day groups, respectively. It should be noted that the liver was the only organ evaluated in these studies and that exposures occurred only during the prenatal life stage.

Recently, NTP published the technical report on chronic cancer bioassays of PFOA administered in feed to male and female Sprague Dawley rats (NTP, 2020) (see Section 5.2.2). In these studies, in addition to groups exposed post-weaning via the diet for 107 weeks, NTP included groups that had perinatal (gestational and lactational) exposures plus post-weaning dietary exposures to PFOA.

In the male rat study, significant increases in hepatocellular adenomas and pancreatic acinar cell adenomas/adenocarcinomas were observed (Table 5.7.3). The incidence of hepatocellular adenoma was significantly increased at both 40 and 80 ppm by pairwise comparison with controls, with a significant dose-related trend ($p < 0.001$). In addition, four hepatocellular carcinomas were observed in the 300/80 ppm (perinatal/postweaning) group, but not in any other groups (Table 5.7.4). Although this increase was not statistically significant, hepatocellular carcinoma is a rare tumor in male rats with a historical control incidence of 0/340. The incidence of pancreatic acinar cell adenoma or adenocarcinoma combined (Table 5.7.3) was significantly increased in all three PFOA-treated groups by pairwise comparison with control, with a significant dose-related trend ($p < 0.001$). Pancreatic acinar cell adenocarcinoma

is a rare tumor type in male rats (historical control incidence of adenocarcinoma is 2/340), and was observed in all post-weaning treated groups with and without perinatal exposure, but not in controls (0/0 ppm) or the perinatal only exposure group (300/0 ppm) (Tables 5.7.3 and 5.7.4).

Table 5.7.3. Hepatocellular and pancreatic tumor incidences in male Sprague Dawley rats exposed to PFOA in the diet for 107 weeks (NTP, 2020)

Concen-tration in feed (ppm)	Dose (mg/kg-day)	Plasma concentration (mg/L)	Hepatocellular adenoma	Pancreatic acinar cell adenocarcinoma	Pancreatic acinar cell adenoma or adenocarcinoma
0	0	BD	0/36***	0/36	3/43***
20	1.0	81.4	0/42	3/42	29/49***
40	2.3	130.8	7/35**	1/36	26/41***
80	4.8	159.6	11/37***	3/38	32/40***

BD: below the limit of detection; values were considered zero for dose-response analysis.
Tumor incidence is expressed as the number of tumor-bearing animals over the number of animals alive at the time of first occurrence of the tumor.
Treatment group tumor incidences with asterisks indicate significant results from Fisher pairwise comparison with controls (conducted by OEHHA): **, p <0.01; ***, p <0.001.
Control group tumor incidences with asterisks indicate significant results from exact trend test (conducted by OEHHA): ***, p <0.001.

Table 5.7.4. Hepatocellular and pancreatic tumor incidences in male Sprague Dawley rats exposed to PFOA perinatally and in the diet for 107 weeks (NTP, 2020)

Perinatal/post-weaning concentration in feed (ppm)	Hepatocellular carcinoma	Hepatocellular adenoma or carcinoma	Pancreatic acinar cell adenocarcinoma	Pancreatic acinar cell adenoma or adenocarcinoma
0/0	0/36	0/36	0/36	3/38
300/0	0/35	0/35	0/35	7/39
300/20	0/38	1/38	2/38	20/42***
300/40	0/38	5/38*	1/38	30/43***
300/80	4/39	12/39***	3/39	30/41***

Tumor incidence is expressed as the number of tumor-bearing animals over the number of animals alive at the time of first occurrence of the tumor.
Treatment group tumor incidences with asterisks indicate significant results from Fisher pairwise comparison with controls (conducted by OEHHA): *, p <0.05; ***, p <0.001.

In the female rat study, pancreatic acinar cell adenoma/adenocarcinoma and uterine adenoma/adenocarcinoma were observed (Tables 5.7.5 and 5.7.6). Pancreatic acinar cell adenomas and adenocarcinomas are both rare in female rats (historical control incidence of 0/340 for adenoma and 0/340 for adenocarcinoma) and were observed in the PFOA-treated female rats. Specifically, there was one acinar cell adenoma and one adenocarcinoma in the 1,000 ppm group (Table 5.7.5), three acinar cell adenomas, one adenocarcinoma, and one ductal adenocarcinoma in the 300/1,000 ppm group (Table 5.7.6), and none in controls or any

other treated groups. NTP concluded that, "[T]here was some evidence of carcinogenic activity in female rats based on the increased incidence of pancreatic acinar cell adenoma or adenocarcinoma combined." There was a statistically significant increase of uterine adenocarcinoma at 1,000 ppm with a dose-related trend ($p < 0.05$). Besides uterine tumors, two types of rare tumors were observed and noted by NTP. Hepatocellular carcinomas were observed in the 1,000 ppm group (three carcinomas) as well as the 300/1,000 ppm group (four carcinomas), compared to one carcinoma in controls and none in any other treated groups (Table 5.7.6). NTP (2020) noted that hepatocellular carcinoma is a rare tumor in female rats (historical control incidence, 1/340) and the observed increase may be treatment-related.

Table 5.7.5. Pancreatic acinar cell and uterine tumor incidences in female Sprague Dawley rats exposed to PFOA in the diet for 107 weeks (NTP, 2020)

Concen-tration in feed (ppm)	Dose (mg/kg-day)	Plasma concentration (mg/L)	Pancreatic acinar cell adenoma or adenocarcinoma	Uterine adeno-carcinoma	Uterine adenoma or adenocarcinoma
0	0	BD[a]	0/24[b]	1/32*	2/32
300	18	20.4	0/30	5/39	5/39
1,000	63	72.3	2/27	8/35*	8/35

BD: below the limit of detection; values were considered zero for dose-response analysis.
Tumor incidence is expressed as the number of tumor-bearing animals over the number of animals alive at the time of first occurrence of the tumor.
Treatment group tumor incidences with asterisks indicate significant results from Fisher pairwise comparison with controls (conducted by OEHHA): *, $p < 0.05$.
Control group tumor incidences with asterisks indicate significant results from exact trend test (conducted by OEHHA): *, $p < 0.05$.

Table 5.7.6. Tumor incidences in female Sprague Dawley rats exposed to PFOA perinatally and in the diet for 107 weeks (NTP, 2020)

Perinatal/ post-weaning concen-tration in feed (ppm)	Pancreatic acinar cell adenoma or adeno-carcinoma	Pancreatic acinar cell adeno-carcinoma	Pancreatic ductal adeno-carcinoma	Hepato-cellular carcinoma	Uterine adeno-carcinoma	Uterine adenoma or adeno-carcinoma
0/0	0/30[a]	0/24	0/40	1/23	1/40	2/40
150/300	0/38	0/36	0/42	0/32	3/43	3/43
300/1,000	4/33	1/24	1/41	4/23	5/41	5/41

Tumor incidence is expressed as the number of tumor-bearing animals over the number of animals alive at the time of first occurrence of the tumor.
[a] This denominator is different than that from Table 5.7.5 because it was calculated using the day of first occurrence from the 300/1,000 ppm group (day 673).

NTP (2020) noted that, "The additional effect of perinatal exposure in combination with postnatal exposure was uncertain and limited to the observation of hepatocellular carcinomas" for the male rat study, and, "The combined perinatal and postweaning exposure was not observed to

change the neoplastic or nonneoplastic response compared to the postweaning exposure alone" for the female rat study.

PFOS

Summaries of the sole report of carcinogenicity bioassays (Butenhoff et al., 2012b) for PFOS have been previously published (US EPA, 2016d; New Jersey DWQI, 2018). The study design and significant results are briefly described below.

Butenhoff et al. (2012b) published a report of carcinogenicity studies from 2002 by 3M (Thomford, 2002), a former PFOS manufacturer. In these studies, male and female Sprague Dawley rats were administered 0, 0.5, 2, 5, or 20 ppm potassium PFOS (K^+PFOS; 0, 0.024, 0.098, 0.242, or 0.984 mg/kg-day for males; 0, 0.029, 0.120, 0.299, or 1.251 mg/kg-day for females) in the diet for two years. Due to mortality issues, female rats in the 2 ppm group were administered K^+PFOS in the diet for 103 weeks, instead of 105 weeks. An additional group, referred to as the "recovery group" here, was administered 20 ppm PFOS for one year, and then control diet for the next year (data not shown).

A statistically significant increase in hepatocellular adenoma incidence was observed in both male and female animals at the highest dose. Positive trends for hepatocellular adenomas were reported in both sexes. Hepatocellular carcinoma is a rare tumor in female Sprague Dawley rats, with a historical control incidence of 0/765 (Baldrick, 2005) and 3/1,314 (Charles River, 2004)[9]. One hepatocellular carcinoma was observed in female rats; thus combined hepatocellular adenoma/carcinoma incidence was also increased in female rats. An increase in pancreatic islet cell carcinoma (by trend) was also observed in male rats. Tumor incidence data for male and female rats are summarized in Tables 5.7.7 and 5.7.8 respectively. Additionally, in female rats, increases in rare thyroid follicular cell adenoma and carcinoma combined were observed (although not statistically significant) and increased incidence of mammary fibroadenoma was observed in the low-dose female rat group (Table 5.7.9). It should be noted that the relatively low effective number of female rats was not due to high levels of premature mortality (mortality in treated groups was comparable to controls), but due to the fact that the first incidence of some tumors appeared quite late in the bioassay (day 653 for hepatocellular carcinoma, day 666 for hepatocellular adenoma, day 671 for thyroid follicular cell adenoma, and day 731 for thyroid follicular cell carcinoma).

While no increase of thyroid follicular cell adenoma was observed in the groups of male rats fed PFOS in the diet for two years, a statistically significantly increased incidence of thyroid follicular cell adenoma was observed in the group of male rats fed 20 ppm PFOS in the diet for one year followed by control diet for another year, in comparison to the control group (control, 3/31; 20 ppm in diet for one year, 9/29; $p < 0.05$). In female rats, one rare thyroid follicular cell adenoma was observed in the group fed 20 ppm PFOS in the diet for one year followed by control diet for another year, and none was seen in the controls. No increases of other tumors were observed

[9] Charles River (2004) includes studies that were initiated or published between 1989 and 2002. In general, the more relevant historical control data are provided by studies conducted within 2-3 years of the Thomford (2002) study. The Thomford (2002) study started in 1998 and last two years. Therefore, a subset of studies from Charles River (2004), initiated or published between 1995 and 2002, were used in OEHHA's analysis.

in these groups of male or female rats fed PFOS in the diet for one year followed by control diet for another year.

Table 5.7.7. Hepatocellular and pancreatic tumor incidences in male Sprague Dawley rats exposed to K^+PFOS in the diet for 2 years (Thomford, 2002; Butenhoff et al., 2012b)

Concentration in feed (ppm)	Dose (mg/kg-day)	Serum conc. (mg/L)[a]	Hepatocellular adenoma[b]	Pancreatic islet cell adenoma[c]	Pancreatic islet cell carcinoma[d]	Pancreatic islet cell adenoma or carcinoma[c]
0	0	0.014	0/41**	4/44	1/38*	5/44
0.5	0.024	2.64	3/42	3/44	2/41	5/44
2	0.098	12.1	3/47	4/48	2/44	6/48
5	0.242	32.3	1/44	4/46	5/44	8/46
20	0.984	121	7/43**	4/44	5/40	9/44

[a] Calculated by OEHHA
[b] First occurrence at day 512
[c] First occurrence at day 465
[d] First occurrence at day 542
Tumor incidence is expressed as the number of tumor-bearing animals over the number of animals alive at the time of first occurrence of the tumor.
Treatment group tumor incidences with asterisks indicate significant results from Fisher pairwise comparison with controls (calculated by OEHHA): **, p <0.01.
Control group tumor incidences with asterisks indicate significant results from exact trend test (conducted by OEHHA): *, p <0.05; **, p <0.01.

Table 5.7.8. Hepatocellular tumor incidences in female Sprague Dawley rats exposed to K^+PFOS in the diet for 2 years (Thomford, 2002; Butenhoff et al., 2012b)

Concentration in feed (ppm)	Dose (mg/kg-day)	Serum conc. (mg/L)[a]	Hepatocellular adenoma[b]	Hepatocellular carcinoma[c]	Hepatocellular adenoma or carcinoma[c]
0	0	0.841	0/28**	0/28	0/28**
0.5	0.029	5.49	1/26	0/29	1/29
2	0.120	23.0	1/15	0/16	1/16
5	0.299	66.4	1/28	0/31	1/31
20	1.251	215	5/31*	1/32	6/32*

[a] Calculated by OEHHA
[b] First occurrence at day 666
[c] First occurrence at day 653
Tumor incidence is expressed as the number of tumor-bearing animals over the number of animals alive at the time of first occurrence of the tumor.
Treatment group tumor incidences with asterisks indicate significant results from Fisher pairwise comparison with controls (reported by study authors): *, p <0.05.

Control group tumor incidences with asterisks indicate significant results from exact trend test (conducted by OEHHA): **, p <0.01.

Table 5.7.9. Thyroid and mammary gland tumor incidences in female Sprague Dawley rats exposed to PFOS in the diet for 2 years (Thomford, 2002; Butenhoff et al., 2012b)

Concen-tration in feed (ppm)	Dose (mg/kg-day)	Serum conc. (mg/L)[#]	Thyroid follicular cell adenoma[a,b]	Thyroid follicular cell carcinoma[a]	Thyroid follicular cell adenoma or carcinoma[a,b]	Mammary gland fibroadenoma[c]
0	0	0.841	0/26	0/24	0/26	20/60
0.5	0.029	5.49	0/25	0/15	0/25	27/50*
2	0.120	23.0	0/14	0/9	0/14	20/48
5	0.299	66.4	2/26	1/15	3/26	24/49
20	1.251	215	1/30	0/25	1/30	11/60

[#] Calculated by OEHHA
[a] Thyroid follicular cell adenomas and carcinomas are both rare in female Crl:CD (SD) BR rats (adenoma: 0.55% and carcinoma: 0 (Baldrick, 2005); adenoma: 0.86%, carcinoma: 0.50%, based on studies initiated or published between 1995-2002 in Crl:CD (SD) BR rats and reported by Charles River (2004)). There was only one thyroid follicular cell carcinoma that was observed during terminal sacrifice on day 731. The authors conducted terminal sacrifices on a series of dates, as early as day 719. All terminally sacrificed animals with thyroid gland examined were included in the denominators for thyroid follicular cell carcinoma.
[b] First occurrence at day 671
[c] The first occurrence of mammary gland fibroadenoma happened within the first year on day 229, therefore, incidence of mammary gland fibroadenoma for the control and 20 ppm groups includes 10 animals each from the one-year interim sacrifice group. For the 0.5, 2, and 5 ppm groups, there were no interim sacrifice groups at one year.
Tumor incidence is expressed as the number of tumor-bearing animals over the number of animals alive at the time of first occurrence of the tumor. Treatment group tumor incidences with asterisks indicate significant results from Fisher pairwise comparison with controls (reported by study authors): *, p <0.05.

5.7.3. Mode of Action and Mechanistic Considerations

PPARα

PFOA and PFOS are known activators of PPARα, as several studies have reported activation of rodent and human PPARα in controlled in vitro systems (Takacs and Abbott, 2007; Wolf et al., 2008a; Wolf et al., 2012). PPARα is a nuclear receptor that regulates genes that modulate the transport and metabolism of many biomolecules, including lipids, lipoproteins, glucose/glycerol, cholesterol, bile acid, and amino acids (Rakhshandehroo et al., 2010). Activation of PPARα leads to an increase in peroxisome number and size, and changes in peroxisomal and microsomal fatty acid β-oxidation levels.

Previous assessments have examined the role of PPARα in PFOA-induced toxicity (US EPA, 2016b; US EPA, 2016d; New Jersey DWQI, 2017; IARC, 2017a; New Jersey DWQI, 2018). These reviews included analyses of PPARα-related endpoints in rodents, effects of PFOA on PPARα knockout mice, and in vitro studies with rodent and human PPARα in various cell

systems. US EPA (2016b) indicated that mechanistic data for PFOA supports the PPARα MOA for liver tumors in rats (Butenhoff et al., 2012a), and may play indirect roles in testicular tumor induction. Conversely, (US EPA, 2016d) indicated that the available evidence does not adequately support a PPARα MOA for PFOS. IARC (2017a) reported that there is moderate evidence for many potential mechanisms for PFOA-induced toxicity (including PPARα). OEHHA reviewed the recent literature exploring the role of PPARα in PFOA toxicity.

Many studies have demonstrated that PFOA can induce liver toxicity independent of PPARα activation. Filgo et al. (2015) administered 0, 0.1, 0.3, 1, or 3 mg/kg-day PFOA to pregnant 129/Sv WT and PPARα KO 129/Sv mice via oral gavage from GD 1-17, and followed the female offspring for 18 months. PPARα KO F1 females had a significant increase in non-neoplastic liver lesions, including hepatocyte hypertrophy, bile duct hyperplasia, and hematopoetic cell proliferation, and an increase in hepatocellular adenomas was seen although it did not reach statistical significance (see Section 5.7.2).

Wen et al. (2019c) administered 0 or 3 mg/kg-day PFOA to C57BL/6NTac WT and PPARα KO mice via oral gavage for 7 days. Both WT and KO animals had increased liver weights, although the effect was more pronounced in WT animals. Additionally, PFOA induced changes in carboxylesterase protein expression in WT versus KO animals. Estrogen receptor alpha (ERα) mRNA expression was increased in KO animals, and there was also suggestive evidence that KO animals had greater CAR and PXR activation than WT animals.

Das et al. (2017) administered 0 or 10 mg/kg-day PFOA to male SV129 WT and PPARα KO mice via oral gavage for seven days. Both WT and KO animals showed liver toxicity, including increased relative liver weight, hepatocellular hypertrophy, decreased hepatic DNA content, and increased hepatic triglycerides (significant in WT animals only). Wild-type animals also displayed mild steatosis that was absent in KO animals. A gene expression analysis indicated that PFOA altered expression of genes related to lipid catabolism, fatty acid synthesis, and triglyceride synthesis in both WT and KO mice, although the effect was more pronounced in WT animals.

Wolf et al. (2008b) administered 0, 1, 3, or 10 mg/kg-day PFOA via oral gavage to 129S1/SvlmJ WT and PPARα KO mice for 7 days. Both WT and KO mice showed liver toxicity, including increased relative and absolute liver weight, hepatocyte hypertrophy, and increased labeling index (only at the high dose in KO mice). However, PPARα KO mice had an increase in vacuole accumulation. Hepatic ultrastructural changes in treated PPARα KO mice (compared to PPARα KO control mice) included decreased cytoplasmic glycogen granules, less rough endoplasmic reticulum, and cytoplasmic rarefaction.

Gene expression studies in the liver of WT and PPARα KO mice revealed PPARα-independent alterations in response to administration of PFOA and PFOS. PFOA (0, 1, or 3 mg/kg-day orally for 7 days) administered to PPARα-KO mice induced changes in the expression genes related to fatty acid metabolism, inflammation, xenobiotic metabolism, and cell cycle control (Rosen et al., 2008b). PFOS (0, 3 or 10 mg/kg-day orally for 7 days) administered to PPARα-KO mice induced changes in the expression of genes related to cholesterol biosynthesis, oxidative phosphorylation, ribosome biogenesis, and bile acid/cholesterol homeostasis (Rosen et al., 2010). These results suggest that PFOA and PFOS can induce biological activity in a PPARα-independent manner, and that all physiological effects of PFOA and PFOS should not be attributed to activation of PPARα.

Rebholz et al. (2016) reported that 3.5 mg/kg-day PFOA in the diet for five weeks activated PPARα in male BALB/c mice, but not in female BALB/c mice or C57BL/6 mice of both sexes. Nonetheless, C57BL/6 mice exposed to PFOA showed increased relative liver weight and changes in plasma and hepatic cholesterol levels. Furthermore, the authors reported that BALB/c mice have more PPARα than C57BL/6 mice, and that treatment with PFOA increased PPARα mRNA expression in male BALB/c mice, but not in C57BL/6 mice of either sex. Increased PPARα mRNA is an indicator of PPARα receptor activation (Yaacob et al., 2001; Blanquart et al., 2004; Rebholz et al., 2016), suggesting that PPARα is more readily activated in BALB/c mice compared to C57BL/6 mice. Li et al. (2017b) observed that PFOA (0.05 mg/kg-day via oral gavage for 28 days) induced liver toxicity (changes in mitochondrial membrane potential, apoptosis, and oxidative DNA damage) in female BALB/c mice without PPARα activation.

In summary, many studies have shown that PFOA and PFOS can induce biological activity and hepatotoxicity that is independent of PPARα activation. This indicates that the toxicity observed in rodent studies may not act entirely through the PPARα activation pathway. As such, OEHHA cannot conclude that all hepatotoxic endpoints of PFOA and PFOS in rodents are the result of PPARα activation. Many chemicals that increase peroxisome proliferation in rodents also induce liver tumors. However, there is substantial debate about whether hepatic effects of PPARα-activating compounds in rodents are relevant to humans due to interspecies differences in activation characteristics.

Klaunig et al. (2003) presents a list of key events in the hypothesized pathway leading from PPARα activator treatment to liver tumor development. The key events identified in the proposed tumor progression pathway are 1) activation of PPARα, 2) perturbation of cell proliferation and apoptosis, and 3) selective clonal expansion. Klaunig et al. (2003) noted that rats and mice are more responsive than humans to some effects of PPARα activators in the liver: peroxisome proliferation, induction of fatty acid β-oxidation metabolic pathways, minimum ligand concentration for receptor activation, maximum receptor activation. They suggested that the liver tumor induction observed from exposure to some PPARα activators in rats and mice is not relevant to human cancer risk assessment.

The PPARα rat/mouse liver tumor mode of action (MOA) hypothesis proposed by Klaunig et al. (2003) depended in part on studies by Peters et al. (1997) and Ward et al. (1998) using PPARα KO mice derived from WT SV129 mice. Peters et al. (1997) found that 11 months of treatment with the PPARα agonist Wy-14,643 did not induce liver tumors in nine PPARα KO mice but did induce multiple hepatocellular tumors in the corresponding WT mice. Diethyl hexyl phthalate (DEHP) also did not induce peroxisome proliferation in PPARα KO mice after 24 weeks of exposure (Ward et al., 1998). Klaunig et al. (2003) suggested that a long-term DEHP cancer bioassay in PPARα KO mice would also be negative.

A number of PPARα activator studies in rats and mice, which have generated data relevant to the proposed PPARα tumor MOA, have been performed since the publication of that MOA hypothesis. Much of those data have been reviewed by Guyton et al. (2009) and Rusyn and Corton (2012).

Recently, Corton et al. (2018) reviewed the evidence for a PPARα MOA for hepatic tumorigenesis in rodents. Similar to Klaunig et al. (2003), the authors identified key events associated with PPARα activators, including PPARα receptor activation, cell growth pathway

alteration, perturbations in hepatocyte growth and survival, and the selective clonal expansion of preneoplastic foci cells, and hypothesized additional MOAs for tumorigenesis when PPARα is not involved. For example, Ito et al. (2007) found that exposure of SV129-derived PPARα KO mice to 100 or 500 ppm DEHP in the diet for up to 22 months resulted in the induction of liver tumors in those mice, with a significant trend for dose-response. These data indicate that PPARα activators may induce liver tumors by a mechanism independent of PPARα activation. However, Corton et al. (2018) argue that the absence of significant increases in liver tumors in WT mice (of the same strain) at the same doses complicate the interpretation of these results. Furthermore, they proposed alternate MOAs for tumor induction in PPARα-null mice, including activation of CAR, and the involvement of steatosis and inflammation, none of which preclude PPARα activation in WT animals.

Yang et al. (2007) developed transgenic mice (from a WT SV129 mouse) that constitutively expressed activated PPARα in hepatocytes in a targeted manner. The authors then used these mice to study whether the activation of PPARα in hepatocytes only is sufficient to induce liver tumors. These transgenic mice demonstrated peroxisome proliferation (including increased PCoA activity) and hepatocyte proliferation at 8 to 10 weeks of age. However, no liver tumors were noted in more than 20 mice at 11 months of age. In contrast, WT mice fed 0.1% Wy-14,643 in the diet for 11 months developed hepatocellular carcinomas (exact number of tumor-bearing animals not provided by authors). These data indicate that PPARα activation by itself is not sufficient to induce hepatocarcinogenesis. However, Corton et al. (2018) argue that the VP16PPARα fusion protein does not function in the precise manner that endogenous PPARα does. The authors claim that differences in downstream protein-protein interactions and differences in global transcription profiles induced by VP16PPARα (compared to endogenous PPARα) explain why these transgenic mice do not develop liver tumors.

Recently, Filgo et al. (2015) reported liver tumors were observed in female offspring (at 18 months of age) of PPARα KO mice, but not WT mice following gestational PFOA exposure. This result supports the observations in the Ito et al. (2007) study, that a PPARα activator can induce tumors in PPARα KO animals, but not in WT animals. It is unclear why PPARα KO animals developed tumors, whereas their WT counterparts did not. However, if the PPARα carcinogenesis MOA proposed by Klaunig et al. (2003) and Corton et al. (2018) is correct, then one would expect the opposite result.

The development of tumors in PPARα KO mice indicates that there are additional MOAs that contribute to DEHP- and PFOA-induced tumorigenesis. Corton and colleagues suggested activation of CAR, or increased steatosis and inflammation as alternate MOAs for liver carcinogenesis in rodents. Recent studies have reported that PFOA interacts with CAR in PPARα KO mice (Wen et al., 2019c), and altered gene expression of transcriptional targets associated with CAR in wild type and PPARα KO mice (Rosen et al., 2008a; Rosen et al., 2008b; Rosen et al., 2017). Similarly, PFOS altered levels of CAR-related transcripts in rats (Dong et al., 2016a) and in mice (Rosen et al., 2017).Furthermore, oral administration of PFOA or PFOS in rats induced significant increases in *Cyp2b1* and *Cyp2b2* expression, a marker of increased CAR activity, compared to controls (Elcombe et al., 2010; Elcombe et al., 2012a; NTP, 2019a; NTP, 2019b), CAR activation by PFOA is greater in PPARα KO mice compared to WT mice (Wen et al., 2019c). Conversely, in vitro nuclear receptor assays with PFOS showed no activity for rat CAR (Bagley et al., 2017). Interaction with CAR may be one of many additional MOAs for PFOA and PFOS, and one should not exclude other mechanisms simply

because one MOA has been identified. Additional evidence is needed before CAR activation is accepted as an additional MOA for PFOA-induced carcinogenesis.

Alterations in lipid metabolism and hepatic inflammation are commonly observed following exposure to PFOA and PFOS. However, it is unclear if these effects directly contribute to carcinogenesis, as the majority of rodent studies evaluating lipid metabolism and liver inflammation are of acute or subacute durations, which are not suitable for determining carcinogenic potential. Additional data are needed to determine whether increased liver lipid content and inflammation are sufficient to induce carcinogenicity in rodents.

Corton et al. (2018) argue that the microenvironment following PPARα activation in transgenic mice with constitutive PPARα activity is different enough from WT mice to prevent the formation of liver tumors, despite major biomarkers of PPARα activation (peroxisome proliferation, hepatomegaly, and decreases in serum triglycerides and free fatty acids) being present in both WT (given a PPARα activator) and transgenic animals. This explanation suggests that the typical physiological responses following PPARα activation are inconsequential for hepatic tumorigenesis in rodents, and seems to undermine the importance of the hypothesized sequelae of events with regards to tumorigenesis.

In summary, these lines of evidence suggest that PFOA-induced hepatotoxicity, including carcinogenesis, in rodents is not solely the result of PPARα activation. Liver toxicity was observed in PPARα KO mice, and in studies where PPARα was not activated (Filgo et al., 2015; Rebholz et al., 2016; Das et al., 2017; Li et al., 2017b; Wen et al., 2019c). Furthermore, rodent carcinogenicity studies with other PPARα activators have demonstrated that PPARα activation is not required for liver tumor induction, and that constitutive activation of PPARα does not lead to tumorigenesis. Therefore, OEHHA considers rodent liver toxicity studies, including carcinogenicity studies, to be relevant to human health. This is consistent with the position outlined in (IARC, 2017a) for PFOA, where human relevance could not be excluded based on the available evidence.

Klaunig et al. (2003) proposed that Leydig cell tumors and pancreatic acinar cell tumors are also induced by activation of PPARα in rodents, and along with liver tumors comprise a PPARα mediated “tumor triad.” The proposed MOA for pancreatic acinar cell tumors in rats is that PPARα activation leads to altered bile acid composition, which causes an increase in cholecystokinin (CCK) levels, which is a growth factor for acinar cells. Binding to CCK1 receptors leads to acinar cell proliferation, and subsequently neoplasia (Caverly Rae et al., 2014). This MOA was proposed predominantly based on studies of the PPARα agonist Wy-14,643. The authors argue that since human acinar cells do not have functional CCK receptors, this MOA is of minimal relevance to humans, and chemicals that cause pancreatic acinar cell tumors in rats are unlikely to induce tumors in humans. The authors also posit that human pancreatic tumors are predominantly ductal, and typically do not originate from acinar cells, unlike rodents, where ductal tumors are rare and acinar cell tumors more common.

However, there is little experimental support for the PPARα MOA for these tumor types (Peraza et al., 2006). Biegel et al. (2001) reported that 300 ppm (13.6 mg/kg-day) PFOA in the diet for two years induced pancreatic acinar proliferation in male Sprague Dawley rats, but the PPARα model agonist Wy-14,643 did not, suggesting that PPARα is not involved in pancreatic tumor formation, and that a different MOA is responsible for tumor induction. Additionally, NTP (2020) reported a large increase in pancreatic acinar cell tumors in male rats, a modest increase in liver

tumors, and no significant increase in Leydig cell tumors. If the proposed PPARα MOA is dominant, then one would expect liver tumors to be the most prevalent tumor type.

Furthermore, Murphy et al. (2008) reported that CCK activates calcium signaling and enzyme secretion in human pancreatic acinar cells, indicating that this pathway does have direct human relevance. Additionally, site concordance is not necessary when determining human relevance of animal tumors, so the difference in pancreatic tumor types between humans and rodents is not considered. Without additional experimental evidence linking pancreatic and testicular tumors in rodents solely to PPARα activation, OEHHA concludes that there is no scientific basis to exclude these tumor types for evaluation of human cancer risk.

It is likely that carcinogenesis occurs through multiple modes of action. In the *Key Characteristics of Carcinogens* section below, possible carcinogenic mechanisms are organized by key characteristics and discussed in greater detail.

Key Characteristics of Carcinogens

A comprehensive review of the more than 100 agents known to cause cancer in humans identified 10 key characteristics (KCs) of carcinogens (Smith et al., 2016; IARC, 2020). As the name implies, KCs are characteristics of agents that cause cancer, in contrast to the hallmarks of cancer (Hanahan and Weinberg, 2000; Hanahan and Weinberg, 2011), which are properties of cancer cells and neoplasms, and also in contrast to modes of action, which are sequences of key events that transform normal cells into malignant tumors. Mode of action analysis depends on prior knowledge sufficient to hypothesize how an agent might cause cancer, knowledge that too often is incomplete. The KCs can encompass many types of mechanistic endpoints and are not constrained to previously formulated hypotheses, allowing a broader consideration of multiple mechanistic pathways and hypotheses. OEHHA uses this approach to systematically identify, organize, and summarize information on mechanisms of carcinogenesis.

For this assessment of PFOA and PFOS, OEHHA reviewed the evidence identified through literature searches on five of the KCs (Table 5.7.10).

Table 5.7.10. Evidence relevant to the key characteristics of carcinogens for PFOA and PFOS[1]

Key Characteristic	Example of Relevant Evidence	Relevant Evidence Reviewed for PFOA	Relevant Evidence Reviewed for PFOS
1. Is electrophilic or can be metabolically activated	Parent compound or metabolite with an electrophilic structure (e.g., epoxide, quinone), formation of DNA and protein adducts	NR	NR
2. Is genotoxic	DNA damage (DNA strand breaks, DNA–protein cross-links, UDS), intercalation, gene mutations, cytogenetic changes (e.g., CAs, MN)	✓	✓
3. Alters DNA repair or causes genomic instability	Alterations of DNA replication or repair (e.g., topoisomerase II, base-excision or double-strand break repair)	NR	NR

Key Characteristic	Example of Relevant Evidence	Relevant Evidence Reviewed for PFOA	Relevant Evidence Reviewed for PFOS
4. Induces epigenetic alterations	DNA methylation, histone modification, microRNA expression	NR	NR
5. Induces oxidative stress	Oxygen radicals, oxidative stress, oxidative damage to macromolecules (e.g., DNA, lipids)	✓	✓
6. Induces chronic inflammation	Elevated white blood cells, myeloperoxidase activity, altered cytokine and/or chemokine production	NR	NR
7. Is immunosuppressive	Decreased immunosurveillance, immune system dysfunction	✓	✓
8. Modulates receptor-mediated effects	Receptor inactivation/activation (e.g., ER, PPAR, AhR) or modulation of endogenous ligands (including hormones)	✓	✓
9. Causes immortalization	Inhibition of senescence, cell transformation	NR	NR
10. Alters cell proliferation, cell death, or nutrient supply	Increased proliferation, decreased apoptosis, changes in growth factors, energetics and signaling pathways related to cellular replication or cell cycle control, angiogenesis	✓	✓

[1] This review was conducted based on information identified through a focused literature search conducted in March 2020. A checkmark (✓) means that evidence for that particular KC was reviewed, and NR stands for "not reviewed." Source of the KCs and examples: Smith et al. (2016); IARC (2019). Any of the 10 characteristics in this table could interact with any other (e.g., oxidative stress, DNA damage, and chronic inflammation), which when combined provides stronger evidence for a cancer mechanism than would one KC alone.
Abbreviations: AhR, aryl hydrocarbon receptor; CA, chromosomal aberration; ER, estrogen receptor; MN, micronuclei; PPAR, peroxisome proliferator-activated receptor; UDS, unscheduled DNA synthesis.

The evidence relevant to these five KCs for PFOA and PFOS is briefly summarized below. More detailed discussions of the findings from studies of PFOA and PFOS relevant to these five KCs are provided in Appendix 8.

Overall, the evidence relevant to the KCs of carcinogens reviewed for PFOA is summarized as follows:

- KC2: PFOA has been tested in many genotoxicity test systems that have assessed numerous endpoints indicative of either mutagenicity, chromosomal effects, or DNA damage. Several studies provide evidence that PFOA causes DNA damage -- measured as increases in DNA strand breaks (in human cell lines and in non-mammalian species), γ-H2AX (in a human cell line), and 8-hydroxydeoxyguanosine (8-OHdG, a marker of oxidative DNA damage, in human cell lines and in rodent liver). Some studies provide evidence that PFOA may have chromosomal effects, while others do not, and several studies provide evidence that PFOA is not mutagenic.

- KC5: A number of studies indicate that PFOA may cause oxidative stress. Two studies in rodents and two studies in human cells have shown that PFOA led to increased 8-OHdG, while one study in human cells reported mixed results and two studies (one in exposed humans and one in a unicellular organism) found no effect. Several studies, including one in mice, four in human HepG2 cells, and one in a mouse cell line, have shown that PFOA increased intracellular production of reactive oxygen species (ROS), while a study in human-hamster hybrid cells showed increased intracellular production of both ROS and reactive nitrogen species (RNS). Increased lipid peroxidation was observed in mice, in human erythrocytes exposed in vitro, and in a rat cell line. PFOA also has been shown to alter total antioxidant capacity (TAC), antioxidant enzyme content or activity, and glutathione levels in mice, fish, and in in vitro studies of human erythrocytes and human HepG2 cells.
- KC7: Several animal studies have shown that PFOA suppresses IgM production as either a T cell dependent antibody response (TDAR) or T cell independent antibody response (TIAR), reduces cellularity and proliferation of T cells and B cells, and reduces the number of neutrophils. The preponderance of evidence shows that PFOA can suppress the immune system in ways that would allow neoplastic cells to escape immune surveillance, survive, and replicate to form tumors.
- KC8: Several animal studies have shown that PFOA alters gene expression in the liver, and that these effects are mediated through ERα, PPARα, PPARγ, PXR, and CAR. Evidence that PFOA can bind to or activate ERα, PPARα, and possibly PPARβ/δ comes from in silico modeling studies (human ERα), studies in human cells or cell lines (ERα, PPARα), and studies in animal tissue preparations or cell lines (ERα, PPARβ/δ). There is also evidence from studies in animals that PFOA can modulate levels of endogenous hormones, including estradiol, progesterone, testosterone and thyroid hormones, and possibly levels of growth factors in the testis and mammary gland.
- KC10: Several animal studies provide evidence that PFOA increases cell proliferation, based on respiratory tissue hyperplasia observed in rats, and stimulated mammary gland development in mice. In addition, PFOA increased levels of regulatory cell cycle proteins in a human breast epithelial cell line, and increased cell proliferation in multiple studies of human breast and ovarian cell lines.

Overall, the evidence relevant to the KCs of carcinogens reviewed for PFOS is summarized as follows:

- KC2: PFOS has been tested in many genotoxicity test systems that have assessed numerous endpoints indicative of either mutagenicity, chromosomal effects, or DNA damage. Some studies provide evidence that PFOS is mutagenic (in transgenic mice and fish and in transgenic mouse cells in vitro), several studies provide evidence of chromosomal effects (e.g., induction of micronuclei (MN) in rodents and zebrafish), and several studies provide evidence of DNA damage (e.g., induction of DNA strand breaks in rats, zebrafish, and other non-mammalian species).
- KC5: A number of studies indicate that PFOS may cause oxidative stress. Several studies, including one in rats, three in human HepG2 cells, two in rodent cells or cell lines, and one in *C. elegans*, have shown that PFOS increased intracellular production of ROS. Increased lipid peroxidation was observed in one study in rats and two studies in rat cells or cell lines. PFOS has also been shown to alter antioxidant enzyme activity and glutathione levels in one study in rats and two studies in human HepG2 cells.

- KC7: Several animal studies have shown that PFOS suppresses TDAR/TIAR IgM production and reduces cellularity of T cells and B cells. PFOS also suppresses NK cell activity, as shown in one study in cultured human blood cells and three studies in mice, although one other mouse study reported an increase in NK cell activity. The preponderance of evidence shows that PFOA and PFOS can suppress the immune system in ways that would allow neoplastic cells to escape immune surveillance, survive, and replicate to form tumors.
- KC8: Several animal studies have shown that PFOS alters the expression of genes that are regulated by ERα, PPARα, PPARγ, PXR, and CAR, and one reporter gene study shows PFOS activates murine PPARβ/δ in vitro. The evidence for the estrogenic effect of PFOS also comes from increased ER reporter activity in human cells in vitro, increased proliferation of estrogen-responsive human breast cancer cell lines in several studies, weak binding to ER in fish, and similar gene expression patterns between PFOS and E2 in fish. One reporter gene study indicates PFOS inhibited androgen receptor (AR) activation by dihydrotestosterone (DHT). There is also evidence from animal studies that PFOS can decrease thyroid hormone levels and increase estradiol levels.
- KC10: Two studies in rats provide evidence that PFOS increases cell proliferation and inhibits apoptosis in the liver, with the latter effect being long-lived. In multiple studies of human fetal liver, breast and ovarian cell lines, PFOS increased cell proliferation. In addition, PFOS altered the expression of proteins linked to cell proliferation, including increasing levels of regulatory cell cycle proteins and growth factors in a human fetal liver cell line, and increased cell proliferation in multiple studies of human breast, ovarian, and fetal liver cell lines.

5.7.4. Conclusions

Based on the evidence of cancer in human and animal studies (Table 5.7.11), OEHHA determined that PFOA and PFOS should be evaluated as carcinogens.

The association of PFOA and kidney cancer in humans provides strong evidence of carcinogenicity. Additionally, hepatic and pancreatic acinar tumors have been reported in animal carcinogenicity studies of PFOA, while hepatic and pancreatic islet tumors have been reported in animal carcinogenicity studies of PFOS. OEHHA determined that the cancer data derived from animal studies are relevant to human health, and support the positive cancer findings in the human epidemiology data. PFOA appears to act through multiple MOAs, and the PPARα MOA does not adequately explain the incidences of pancreatic and testicular tumors reported. Furthermore, there is considerable uncertainty whether hepatic tumors in rodents are solely induced by activation of PPARα, so the liver tumors in rodents induced by PFOA and PFOS should not be dismissed due to the assumption that it lacks human relevance.

Mechanistic studies of PFOA and PFOS have shown that these chemicals possess several of the key characteristics of carcinogens, including the ability to induce oxidative stress, inflammation, and modulate receptor-mediated effects. Additionally, there is suggestive evidence that PFOA and PFOS are genotoxic, thus a genotoxic MOA for cancer remains plausible. These data support the human and animal evidence that PFOA and PFOS present a carcinogenic hazard.

Table 5.7.11. Summary of OEHHA's conclusions regarding the human and experimental animal data on PFOA and PFOS and cancer

Outcome	PFOA	PFOS
Bladder cancer in humans	Mostly no association	Few studies, no clear or consistent associations
Breast cancer in humans	High relative risks in some subgroups but yet to be confirmed	High relative risks in some subgroups but yet to be confirmed
Kidney cancer in humans	Strong evidence, including a large multicentric study, mostly positive findings	Few studies, no clear or consistent associations
Liver cancer in humans	Some positive associations from occupational studies but small sample sizes	Few studies, small sample sizes
Pancreatic cancer in humans	Weak evidence, most studies with small sample sizes	Few studies, no clear or consistent associations
Prostate cancer in humans	Mostly no association	Few studies, no clear or consistent associations
Testicular cancer in humans	Some positive associations but small sample sizes	No studies
Liver tumors in animals	Positive evidence in both sexes of rats, identified by multiple laboratories, with one study showing evidence of transplacental carcinogenicity in mice	Positive evidence in both sexes of rats
Pancreatic tumors in animals	Positive evidence in male rats (multiple laboratories) and female rats	Positive evidence in male rats
Testicular tumors in animals	Positive evidence in multiple studies	Not reported
Uterine tumors in animals	Positive evidence in one study	Not reported

5.8. Other Toxic Effects

Effects of PFOA and PFOS on the immune system, liver, thyroid, reproduction and development, and the nervous system have been described in their respective sections above. Additional noncancer toxicities are reported in this section.

US EPA (2016b) reported that reduction in body weight was commonly observed in animal studies of PFOA. Additional toxicity endpoints in animal studies included increased absolute and/or relative kidney weight in rats (Butenhoff et al., 2004; Cui et al., 2009, as reported in US EPA, 2016b), effects on the lung, including pulmonary congestion, in rats (Cui et al., 2009, as reported in US EPA 2016b), effects on the adrenal cortex in male rats (Butenhoff et al., 2004, as reported in US EPA, 2016b), and increased serum leptin and insulin levels in mice (Hines et al., 2009, as reported in US EPA, 2016b).

Like PFOA, US EPA (2016d) reported that PFOS reduced body weight in rats and monkeys. In monkeys, oral administration of PFOS induced gastrointestinal toxicity (anorexia, emesis, black stool), lipid depletion in the adrenals, atrophy of pancreatic exocrine and serous alveolar cells, and pulmonary necrosis and inflammation (Goldenthal et al., 1978; Goldenthal et al., 1979; Seacat et al., 2002, as reported in US EPA, 2016d). Acute inhalation exposure in rats induced breathing disturbances and discoloration of the lung (Rusch et al., 1979, as reported in US EPA, 2016d).

5.8.1. Recent Animal Evidence

Animal studies of PFOA and PFOS, published from 2016 onward, reporting toxicity outcomes outside of the broad categories discussed in previous sections are described below.

Decreases in body weight gain were reported in a number of short-term studies of PFOA in rodents (Hui et al., 2017; Suo et al., 2017; Du et al., 2018; Crebelli et al., 2019; Cui et al., 2019; Song et al., 2019). However, one study did report an increase in body weight (Koskela et al., 2016).

Several PFOA studies reported adverse effects in adipose tissue in mice, including decreased adipose tissue mass (Li et al., 2019d; Pouwer et al., 2019) and increased adipocyte cell size (van Esterik et al., 2016). Du et al. (2018) reported adipose tissue histopathology (including atrophy, nucleolus deformation, cytoskeletal impairment, vacuolization, and reduced organelles), increased adipose cell death, and reduced leptin and adiponectin levels in the blood of mice. PFOA can activate uncoupling protein 1 (UCP1) in brown adipose tissue, which is normally activated by fatty acids (Shabalina et al., 2016). The result of activation of this protein leads to heat production and energy consumption in animals.

PFOA has been associated with adverse effects on bone health. Koskela et al. (2016) reported changes in bone density and morphometric properties in mice exposed to PFOA in utero. Additionally, in utero exposure to PFOA reduced tibia and femur length (along with a decrease in quadriceps femoris muscle weight) in mice (van Esterik et al., 2016).

Increased blood glucose levels (Zheng et al., 2017; Du et al., 2018) and increased insulin levels (Wu et al., 2017; Du et al., 2018; Wu et al., 2018) were reported in several mouse studies of PFOA. Furthermore, PFOA induced changes in pancreatic cytoarchitecture and serum trypsinase levels (Wu et al., 2017), and changes in glucose metabolism/homeostasis pathways (Wu et al., 2017; Zheng et al., 2017) in male mice.

Much like PFOA, PFOS has been reported to cause decreases in body weight gain in rodents (Li et al., 2016b; Qu et al., 2016; Wan et al., 2016; Xing et al., 2016; Bagley et al., 2017; Wang et al., 2018; Su et al., 2019). Other effects in rodents include decreased absolute kidney weight (Xing et al., 2016), sensitivity to light (Bagley et al., 2017), increased serum corticotropic releasing factor (CRF), adrenocorticotropic hormone (ACTH) and corticosterone (Salgado-Freiria et al., 2018), and changes in gut metabolism and gut bacteria (Xu et al., 2017; Lai et al., 2018; Salgado-Freiria et al., 2018). Acute exposure to PFOS increased mortality, rough hair, constipation, anorexia, asthenia, syncope, and reduced food consumption in mice (Xing et al., 2016).

PFOS has been associated with an early reduction in blood glucose following glucose or insulin administration in female mice (Lai et al., 2018), and increased insulin levels in the serum of male mice (Su et al., 2019).

5.8.2. Recent Mechanistic Evidence

Mechanistic studies of PFOA and PFOS, published from 2016 onward, that fall outside the broad toxicity categories above are described below.

In a metabolomics study using rat kidney mesangial cells, a model of diabetic kidney disease, PFOA altered amino acid biosynthesis and metabolism (Gong et al., 2019). Fibrosis and inflammation were also observed in PFOA exposed cells. In frog kidney cells, PFOA caused alterations in DNA/RNA (genotoxicity), secondary protein structure, lipids, and fatty acids in cells that formed monolayers and in cells differentiating into dome structures (Gorrochategui et al., 2016). Low doses of PFOA had a greater effect on cells forming monolayers while high doses had a greater effect on dome forming structures.

Animal studies show that PFOA can impact bone development, and this is supported by several in vitro studies. (Koskela et al., 2017) reported that PFOA induced an increase in osteoclasts derived from human peripheral blood, and an increase in osteoclast resorption area. In MC3T3-E1 osteoblast cells, PFOA increased levels of oxidative stress (increased ROS and mitochondrial superoxide), adversely impacted mitochrondrial function (increased membrane potential collapse, decreased adenosine triphosphate (ATP) production, increased cardiolipin peroxidation, and increased cytochrome c release), and osteoblast differentiation (decreased collagen synthesis, decreased alkaline phosphatase (ALP) activity, and decreased mineralization) (Choi et al., 2017). PFOA also altered mRNA and protein levels of transcription factors and the expression of other genes related to osteogenic differentiation in human mesenchymal stem cells (Liu et al., 2019a; Pan et al., 2019). In mouse osteoblast cells (MC3T3-E1), PFOA altered levels of osteoblast differentiation markers, increased the number of multinucleated osteoclasts, and altered bone resorption levels (Koskela et al., 2016).

Berntsen et al. (2017) exposed primary cerebellar granular neurons from rat pups to PFOA, and reported that PFOA accumulated diffusely in cell membranes. The authors also reported that vitamin E protected against cytotoxicity, suggesting that oxidative stress may be involved in PFOA-induced cytotoxicity, but there was no observed increase in ROS or lipid peroxidation.

PFOA induced markers of oxidative stress in human erythrocytes (Pan et al., 2018). PFOA also increased ROS levels and autophagy in the mitochondria and endoplasmic reticulum of A549 lung cancer cells via activation of the MAPK pathway and inhibition of the PI3K/AKT pathway (Xin et al., 2018b). Inhibition of autophagy increased PFOA toxicity, suggesting that autophagy has a protective function.

Studies have shown that PFOA can bind to steroid hormone receptors (Kang et al., 2016; Behr et al., 2018; Chaparro-Ortega et al., 2018; Qu et al., 2018; Cao et al., 2019; McComb et al., 2019; Xin et al., 2019). Interaction with steroid hormone receptors can alter normal hormone secretion. PFOA decreased adrenocorticotropic hormone, and increased corticosteroid binding globulin (CBG) and cortisone in mice (Sun et al., 2018b). Additionally, PFOA can bind to transporters such as fatty acid binding protein and T4 serum carrier-protein transthyretin (TTR) (Selano et al., 2019), displacing endogenous ligands and disrupting essential processes in the

liver. PFOA has also been shown to bind to pepsin, an enzyme in the stomach that breaks proteins down into polypeptides (Yue et al., 2016).

Associations between PFOA and changes in lipid metabolism and homeostasis have been reported in human epidemiology studies and animal toxicity studies, and these endpoints are supported by in vitro mechanistic studies. Adipocyte differentiation and lipid accumulation were observed in human and mouse adipocytes exposed to PFOA (Ma et al., 2018; Li et al., 2019b). In T3-L1 preadipocytes, PFOA caused a dose-dependent increase in adipocyte proliferation and increase in accumulation of lipids and triglycerides (Ma et al., 2018).

In a study comparing gene expression analyses of endocrine disrupting chemicals, PFOA altered genes related to cellular infiltration, necrosis and hypertrophy, and NRF2-mediated oxidative stress in rhesus monkey embryonic cells (Midic et al., 2016). In rhesus monkey trophoblast stem cells, PFOA induced gene expression changes in many different pathways, including cysteine metabolism, interleukin signaling, and PPAR, among others (Midic et al., 2018).

Recent in vitro studies indicate that PFOA can impact pancreatic cells. Liu et al. (2018d) showed that PFOA impaired specification of pancreatic progenitor cells from human embryonic cells. Additionally, PFOA decreased cell viability, increased apoptosis and oxidative stress and caused mitochondrial membrane potential collapse in rat pancreatic β-cell-derived RIN-m5F cells (Suh et al., 2017).

PFOA can activate the kallikrein-kinin system (KKS) in mice and in human retina endothelial cells. KKS is involved in regulating inflammation, blood pressure and vascular permeability (Liu et al., 2017a; Liu et al., 2018c).

PFOA induced decreased cell viability, cell morphology changes and increased intracellular calcium in chicken primary embryo cardiomyocytes (Lv et al., 2019). The observed toxicity was due to changes in protein expression of Wnt5a/Frizzled2, which regulates calcium homeostasis. Further, interaction of PFOA with PPARα was shown to play a role in regulating these proteins.

PFOA can partition into bacterial and model phospholipid bilayers, and affect lateral phospholipid interactions within the bilayer (Fitzgerald et al., 2018). While the significance of these findings to human health is unclear, alteration of the cellular membrane lipid bilayer could theoretically impair the function of many proteins embedded in the lipid bilayer.

In a metabolomics study of a diabetic kidney disease model of rat kidney mesangial cells, PFOS increased oxidative stress and changed amino acid biosynthesis and metabolism, which subsequently lead to increased fibrosis and inflammation (Gong et al., 2019). PFOS caused epithelial-mesenchymal transition (EMT)-associated renal fibrosis in rat tubular epithelial cells (Chou et al., 2017). Renal fibrosis was shown to be through Sirt1-mediated PPARγ deacetylation. PFOS induced apoptosis of rat renal tubular cells was also shown to be associated with Sirt1 and a PPARγ-dependent mechanism (Wen et al., 2016). Like PFOA, PFOS caused alteration in DNA/RNA, secondary protein structure, lipids, and fatty acids in frog kidney cells (Gorrochategui et al., 2016). The authors suggest that the nucleic acid alterations are indicative of genotoxicity.

PFOS suppressed osteogenic differentiation in human bone marrow derived mesenchymal stem cells via inhibition of calcium deposition and changes in mRNA levels of osteogenic markers

and transcription factors (Liu et al., 2019b). Liu et al. (2019b) also report changes in markers of bone turnover. Similarly, (Pan et al., 2019) reported changes in mRNA and protein levels of genes involved in osteogenic differentiation in human mesenchymal stem cells.

PFOS can activate UCP1 in brown adipose tissue, which is normally activated by fatty acids (Shabalina et al., 2016). Activation of this protein leads to heat production and energy consumption in animals. The authors suggest this may pose a risk of altered energy metabolism to newborns and infants, who normally have brown adipose tissue and can be exposed to PFOS through breastmilk.

Several in vitro and in silico studies demonstrate that PFOS affects adipogenesis and may be an environmental risk factor for obesity. PFOS induced adipogenesis in vitro in murine-derived 3T3-L1 preadipocytes and in vivo in mice through activation of Nrf2 signaling (Xu et al., 2016). In mice, PFOS also induced PPARγ and CCAAT-enhancer binding protein α expression, a transcription factor involved in adipogenesis and adipocyte differentiation. Molecular docking analyses show that PFOS can bind to PPARγ (as well as PPAR α and β), which are important regulatory pathways for adipogenesis (Li et al., 2018a). PFOS also increased lipid content in 3T3-L1 cells in a dose-dependent manner and enhanced expression of adipogenic related genes. However, PFOS decreased lipid accumulation in human differentiating adipocytes and affected DNA methylation of adipogenic genes, although gene expression was not affected, suggesting minimal impacts on adipogenic pathways (van den Dungen et al., 2017).

A binding study by Kumar et al. (2018) showed that PFOS binds to the alpha4-helical domain in proteins involved in cholesterol biotransformation and transport in mitochondria.

Chen et al. (2018c) and Chen et al. (2018d) reported increased markers of inflammation (glial fibrillary acidic protein and proliferating cell nuclear antigen (PCNA)), changes in cytokine expression (IL-1β, TNF-α), and modulation of signal transduction pathways (NF-κB, JAK2/STAT3) in C6 glioma cells (astrocytes) exposed to PFOS. Similarly, PFOS activated the JAK2/STAT3 pathway in astrocytes from newborn rat pups (Chen et al., 2018d). The authors propose that PFOS activates NF-κB and JAK2/STAT3 signaling, which subsequently activates astrocytes and leads to the secretion of pro-inflammatory cytokines. Chen et al. (2018d) also exposed SH-SY5Y human neuroblastoma cells to the cellular media used in the experiments with the C6 glioma cell line (containing cytokines secreted from astrocytes following exposure to PFOS). The neuroblastoma cells displayed elevated biomarkers of apoptosis (increased activation of caspase 3 and cleavage of poly (ADP-ribose) polymerase (PARP)), and the authors claim that TNF-α secreted from astrocytes in response to PFOS exposure is responsible.

PFOS altered gene and protein expression of neuroendocrine hormones (such as gonadotropin releasing hormone, luteinizing hormone, and follicle stimulating hormone) in the hypothalamic-pituitary-thyroid axis of rats (López-Doval et al., 2016). Additionally, PFOS induced changes in corticotropic releasing factor and glucocorticoid receptors in the brain of male rats (Salgado-Freiria et al., 2018), and reduced kisspeptin expression (which regulates gonadotropin releasing hormone in the hypothalamus) in female mice (Wang et al., 2018). PFOS can interact with steroid hormone receptors, which can result in subsequent changes to hormone secretion (Kang et al., 2016; Behr et al., 2018; Chaparro-Ortega et al., 2018; Qu et al., 2018; Cao et al., 2019; McComb et al., 2019). In mouse Leydig tumor cells, PFOS disrupted membrane potential, increased ROS production and disrupted progesterone production (Zhao et al., 2017).

Liu et al. (2018d) showed the PFOS impaired specification of pancreatic progenitor cells from human embryonic cells. Disrupted development of the pancreas can lead to metabolic diseases such as diabetes, which have been linked to PFOS exposure in people.

PFOS can activate the KKS in mice and in human retina endothelial cells. KKS is involved in regulating inflammation, blood pressure and vascular permeability (Liu et al., 2017a; Liu et al., 2018c).

A mechanism for bioaccumulative properties of PFOS may be through binding to phospholipids in lung cells and adipocytes (Sanchez Garcia et al., 2018). In a docking study, PFOS was shown to bind to human serum albumin (HSA) through electrostatic forces and hydrogen bonds (Liu et al., 2017b). This binding resulted in decreased esterase activity of HSA.

ToxCast High-Throughput Toxicity Screening

ToxCast™ (US EPA Toxicity Forecaster) is a chemical prioritization research program developed by the US EPA (Dix et al., 2007; Judson et al., 2010; Kavlock et al., 2012). ToxCast includes data generated by the Tox21 (Toxicity Testing in the 21st Century) program, which is a multi-agency collaboration between the National Institute of Environmental Health Sciences/National Toxicology Program, the National Center for Advancing Translational Sciences, the US Food and Drug Administration, and US EPA's National Center for Computational Toxicology. ToxCast utilizes various in vitro and zebrafish systems to identify chemical activity in a battery of high-throughput screening (HTS) assays. OEHHA explored ToxCast data on PFOA and PFOS using information that is publicly available on the Computational Toxicology (CompTox) Chemicals Dashboard.[10]

The ToxCast database on the CompTox Chemicals Dashboard reported that PFOA was active in 81 of the 1,073 assays in which it was tested, PFOA ammonium salt was active in 58 of the 860 assays in which it was tested, PFOS was active in 260 of the 1,165 assays in which it was tested, PFOS potassium salt was active in 179 of the 895 assays in which it was tested, and PFOS lithium salt was active in 26 of the 238 assays in which it was tested. ToxCast assays in which PFOA, PFOA ammonium salt, PFOS, PFOS potassium salt, and PFOS lithium salt were active are shown in Appendix 9, Tables A9.1-A9.5, respectively. Although there were several curve-fitting flags (flag details are not included in Appendix 9) associated with some ToxCast assays in which PFOA, PFOS, and their salts were active, OEHHA did not apply these flags as a cutoff for excluding assays, for the following reasons. First, these curve-fitting flags are subject to change as the ToxCast data analysis pipeline evolves (Thomas et al., 2019). Second, completely filtering out all active assay calls that have curve-fitting flags is not recommended because potential biological signals could be omitted (Judson et al., 2016).

There are several limitations that exist in the PFOA and PFOS ToxCast datasets. First, the purity grades of PFOA, PFOA ammonium salt, PFOS, PFOS potassium salt, and PFOS lithium used in the ToxCast assays were not reported, while the purity grades of the test substances used in the Tox21 assays are reported as "unknown/inconclusive," based on the Tox21 quality control analyses.[11] Also, PFOA and PFOS and their salts are surfactants, and the disposition of these chemicals in ToxCast in vitro assay systems (other than the zebra fish assays) remain uncertain. It has been acknowledged that chemical disposition and partitioning can greatly

[10] https://comptox.epa.gov/dashboard, accessed on May 3, 2021

[11] https://tripod.nih.gov/tox21/samples, accessed on May 3, 2021

affect the accuracy of predictions from in vitro test systems.[12] Additionally, the surfactant properties of PFOA and PFOS may cause cell lysis and cytotoxicity at high concentrations in cell-based assays, e.g., in human bronchial epithelial cells (Sorli et al., 2020). Due to these limitations, the ToxCast data on PFOA and PFOS and their salts are not considered to contribute much to the overall body of mechanistic evidence for these chemicals.

5.8.3. Conclusions

PFOA induces multiple toxicity endpoints in experimental animals, including reduction in body weight, adverse effects on adipose tissue, negative impacts on bone health, and perturbations of glucose homeostasis. In vitro mechanistic studies of PFOA on adipose tissue and osteoclasts/osteoblasts support the in vivo evidence. Other mechanistic studies reveal additional potential MOAs of PFOA, including inducing oxidative stress, interaction with steroid hormone receptors, perturbation of metabolism, disruption of normal macromolecule structure, and inducing fibrosis and inflammation in rat kidney cells.

Much like PFOA, PFOS commonly induces reductions in body weight in animals. Other endpoints have been reported in the literature, but consistent observations across studies are not observed. Mechanistically, PFOS behaves similarly to PFOA in many of the evaluated studies, including effects on bone and adipose tissue, generation of ROS, interactions with steroid hormone receptors, and induction of fibrosis and inflammation in rat kidney cells.

[12] Tox21 Cross-Partner Projects. 4. *In vitro* Chemical Disposition. Available: https://tox21.gov/projects/. Crizer D, Sipes N, Waidyananthaet S et al (2020): *In Vitro* Disposition of Tox21 Chemicals: Initial Results and Next Steps. Available: https://www.epa.gov/sites/production/files/2020-10/documents/7_david_crizer_epa_nams_conference_2020_508c.pdf

6. DOSE-RESPONSE ASSESSMENT

6.1. Noncancer Dose-Response Analyses and Acceptable Daily Dose Derivation

6.1.1. Perfluorooctanoic Acid

Human Studies

Based on its review of the currently available human studies, OEHHA has determined that the most sensitive noncancer endpoints for PFOA are immunotoxicity, liver toxicity, and alterations in lipid metabolism or production. A number of studies have also linked PFOA to adverse effects related to the thyroid gland and alterations of thyroid hormone levels; however, these findings were too inconsistent for OEHHA to make firm conclusions regarding these outcomes. Associations with several reproductive and developmental outcomes have also been reported. While the positive associations reported in some of these studies are cause for concern, overall, the findings from study to study were also somewhat inconsistent, and OEHHA was unable to identify studies that could be used to accurately evaluate the dose-response patterns for these outcomes.

In the following sections, OEHHA presents the human studies it considered as candidate studies for its POD determinations.

Immunotoxicity

A number of animal and human studies have provided evidence that PFOA can increase the risks of immune-related diseases or otherwise adversely affect the immune system. With regards to human studies, increased serum levels of PFOA have been linked to diminished antibody levels in response to vaccinations for diphtheria, tetanus, rubella, mumps, and influenza (Hib) (Granum et al., 2013; Looker et al., 2014; Stein et al., 2016b; Grandjean et al., 2017a; Grandjean et al., 2017b; Pilkerton et al., 2018; Abraham et al., 2020). These findings have been seen in both adults and children, although the greatest effect sizes have been reported in children. For example, decreases in tetanus and diphtheria antibody concentrations of 25% or more have been reported for each doubling of PFOA serum concentration in children from the Faroe Islands (Grandjean et al., 2017a) (Table 5.1.1). A number of other studies have identified associations between PFOA and immune-related diseases such as asthma or respiratory tract infections (Qin et al., 2017; Timmermann et al., 2017; Impinen et al., 2018; Averina et al., 2019; Kvalem et al., 2020), although the evidence on these outcomes is less consistent than that seen for diminished vaccine response.

The most sensitive endpoints for vaccine response appear to be response to childhood influenza, tetanus, and diphtheria vaccinations. The human studies that considered these endpoints include research from two Faroe Islands cohorts and the study in healthy one-year old children in Germany by Abraham et al. (2020). These studies, and the PODs that OEHHA derived from them, are discussed below.

Abraham et al. (2020):

NOAEC/LOAEC method: Details of the Abraham et al. (2020) study are provided in Chapter 5. The results from this study for influenza (Hib), tetanus, and diphtheria antibody levels are shown in Table 6.1.1. The NOAECs are highlighted in green in this table. These are the mean PFOA serum concentrations in the highest quintile of PFOA that did not show a statistically significant difference in antibody levels, which in all three cases is the 4th quintile, compared to the reference category (the lowest quintile of PFOA). The p-values in this table were calculated by the study authors using linear regression models adjusted for the number of vaccinations the child had received and for the time since the last vaccination. As shown, the NOAECs ranged from 18.9 to 19.4 ng/ml. The authors also presented data by PFOA deciles, which involved smaller sample sizes and less statistical power but showed similar NOAECs (20.5 to 22.4 ng/ml).

Table 6.1.1. Mean serum antibody concentrations by quintiles of serum PFOA (ng/ml) from Abraham et al. (2020). The NOAECs are highlighted in green.

Anti-Hib (mg/dl)					
Quintile	**PFOA[1]**	**N**	**Mean[2]**	**SD**	**p-value**
1	3.4	20	1.84	0.68	Ref
2	8.5	20	1.84	0.71	0.98
3	14.8	20	1.84	0.84	0.98
4	19.4	20	1.50	0.55	0.09
5	25.7	18	1.19	0.6	0.003

Anti-tetanus (mg/L)					
Quintile	**PFOA[1]**	**N**	**Mean[2]**	**SD**	**p-value**
1	3.4	20	1.07	0.31	Ref
2	8.5	20	1.04	0.44	0.77
3	14.5	20	1.02	0.39	0.67
4	18.9	20	1.02	0.35	0.61
5	25.3	20	0.74	0.36	0.003

Anti-diphtheria (IU/ml)					
Quintile	**PFOA[1]**	**N**	**Mean[2]**	**SD**	**p-value**
1	3.4	20	0.50	0.41	Ref
2	8.5	20	0.57	0.29	0.56
3	14.5	20	0.64	0.36	0.26
4	18.9	20	0.40	0.45	0.49
5	25.3	20	0.13	0.53	0.02

Abbreviations: Ref, reference category; SD, standard deviation
[1] Mean PFOA serum concentrations (ng/ml) in each quintile
[2] Log_{10} values of the adjusted antibody levels

Knee function method: The authors of Abraham et al. (2020) also estimated NOAECs using the knee function method. Here, they used the breakpoint in a piecewise linear regression model with two segments to estimate the 'knee' function, the point at which the association between increasing PFOA and decreasing antibody levels begins to occur. An informative visual display of these results can be seen in Figure 3 of the Abraham et al. (2020) publication. Based on this method, the study authors reported NOAECs of 12.2, 16.9, and 16.2 ng/ml for Hib, tetanus, and diphtheria, respectively.

BMD method: The means of the log10 transformed adjusted antibody concentrations at each PFOA quintile are shown in Table 6.1..1. OEHHA performed BMD modeling using a 10% decrease in vaccine response as the BMR. A BMR of 10% is within the lower range of the response levels seen in this study, which ranged from 0 to 86%. A one SD change from the control mean (quantile 1) was not used because it was generally outside of the observed range of responses. BMD modeling based on clinical reference levels for long- or short-term immunity were also not performed because mean antibody levels in all PFOA quintiles appeared to fall above clinical reference levels (Plotkin, 2010).

Because a 10% decrease in the mean of the log10 values is not the same as a 10% decrease on the absolute (non-logged) scale, the BMR was estimated after back-transforming the log10 values shown in Table 6.1.1 out of the log10 scale (see model run outputs in Appendix 10). This should be considered a somewhat conservative estimate (i.e., slightly less than 10%) since the mean of the log10 transformed antibody levels is typically lower than the log10 of the mean of the absolute (non-logged) levels. Using this BMR, the Hill model gave a BMD_{10} of 16.8 ng/ml and a $BMDL_{10}$ of 2.8 ng/ml (p-value for model fit=0.99) for Hib. This BMD:BMDL ratio is fairly large (e.g., >5) highlighting the uncertainty in this particular dataset. The corresponding BMD_{10} and $BMDL_{10}$ values for tetanus antibody levels were 20.0 and 6.5 ng/ml (p-value for model fit=0.68). For diphtheria antibody levels, the corresponding values were 17.0 and 11.4 ng/ml (p-value for model fit=0.35). Overall, the effect of a 10% decrease in antibody response is likely to be minor in most children. However, the impacts of these small decreases could be much more important in children who already have compromised or borderline-compromised immune systems for other reasons. As such, these small effects could have important implications for the population as a whole, especially given the very widespread nature of PFOA exposure.

Faroe Islands studies:

NOAEC/LOAEC method: Associations between serum PFOA concentrations and serum antibody levels in response to diphtheria and tetanus vaccinations in children were assessed in two Faroe Islands cohorts (Grandjean et al., 2017a). Details of these studies are provided in Appendix 7, Table A7.3. Categorical data, which can be used to develop NOAECs, were not available from the peer-reviewed publications but results for diphtheria have been published in a preliminary report from the Europen Food Safety Authority (EFSA, 2020). These are shown in Table 6.1.2. As seen, the NOAEC is a serum level of 4.75 ng/ml. Categorical data for tetanus were not available.

Table 6.1.2. Mean serum diphtheria antibody concentrations at age 7 by quintiles of serum PFOA (ng/ml) at age 5 from the Faroe Islands cohort (EFSA, 2020). The NOAEC is highlighted in green.

Quintile	PFOA[1]	N	Mean[2]	SD	p-value
1	2.75	86	0.15	1.60	Ref
2	3.45	86	-0.23	1.97	0.22
3	4.05	86	-0.28	1.89	0.11
4	4.75	86	0.13	2.09	0.94
5	6.10	86	-0.60	1.90	0.007

Abbreviations: Ref, reference category; SD, standard deviation
[1] Mean PFOA serum concentrations (ng/ml) in each quintile
[2] Log2 values of mean serum antibody concentrations

BMD method: As reviewed in Chapter 5 and shown in Table 5.1.4, BMD calculations have been performed by two of the Faroe Islands study investigators (Budtz-Jorgensen and Grandjean, 2018). Using a piecewise model with a 5% decrease in antibody levels as the BMR, the authors reported a BMD_{05} and $BMDL_{05}$ of 0.67 and 0.17 ng/ml, respectively, for tetanus antibody, and a BMD_{05} and $BMDL_{05}$ of 1.06 and 0.20 ng/ml, respectively, for diphtheria antibody. Both of these BMDLs are well below the lowest PFOA serum concentration observed in this study. OEHHA performed its own BMD modeling and obtained mostly similar results, with large BMD:BMDL ratios (e.g., >5) and BMDLs that were also well below the range of observed values. The most likely reason the BMD:BMDL ratios were so large was the high degree of variability (i.e., the very large standard deviations) in antibody levels seen in each PFOA exposure category.

Liver Toxicity

As reviewed above, animal and human studies have shown that PFOA can cause liver toxicity. The most consistent liver toxicity-related endpoint seen in the human epidemiologic data are increases in the liver enzyme, ALT. By far the two largest studies of PFOA and ALT in humans are those of Gallo et al. (2012) and Darrow et al. (2016), both performed in the C8 study area (mid-Ohio River Valley, West Virginia and Ohio). Both of these studies were cross-sectional and both involved over 30,000 adults. Details on the Darrow et al. (2016) study can be found in Appendix 7, Table A7.5. The Gallo et al. (2012) study was reviewed by US EPA (2016b). The Gallo et al. (2012) and Darrow et al. (2016) studies were similar in all major aspects of study design except that while Gallo et al. (2012) based PFOA exposure on measured PFOA serum concentrations, the Darrow et al. (2016) study based PFOA exposure on modeled PFOA serum levels. These modeled levels were developed using the participant's residences, PFOA drinking water concentrations, emissions data, environmental fate and transport models, and other factors. Further details on this exposure model can be found in Shin et al. (2011).

Both the Darrow et al. (2016) and Gallo et al. (2012) studies evaluated the relationship between PFOA and ALT using two general types of analyses. In the first, subjects were divided into quantiles of PFOA exposure (quintiles in Darrow et al. (2016) and deciles in Gallo et al. (2012)), and linear regression models were used to compare mean ALT levels in each non-reference quantile to the mean ALT level in the lowest (reference) quantile. In the second type of analysis, a cut-off point was used to define high or elevated ALT, and ORs for having an ALT level above this cutoff point were calculated for each non-reference quantile compared to the lowest (reference) quantile. The cutoff values used to define elevated ALT levels in both studies were 45 IU/L for men and 34 IU/L for women. These are clinically based reference levels used

by the International Federation of Clinical Chemistry and Laboratory Medicine (Schumann et al., 2002), and were approximately the 90th percentile of all ALT values in these studies.

ALT means:

NOAEC/LOAEC method: OEHHA selected the Darrow et al. (2016) study involving modeled PFOA exposure levels for its dose-response analysis of mean ALT concentrations. Gallo et al. (2012) presented similar data for measured serum PFOA concentrations in figure form and these also showed a dose-response relationship between increasing PFOA and increasing ALT. A validation study has shown a good correlation between measured and modeled PFOA serum levels in the C8 cohort (Shin et al., 2011). The results from Darrow et al. (2016) are shown in Table 6.1.3. Sample sizes in each PFOA quintile were not provided, so they were estimated by dividing the total sample size by five. The mean PFOA concentrations in each quintile were also not provided, so OEHHA used the category midpoints. An exception to this was quintile 5. Here a value of 507 ng/ml was used since another publication involving a subsample of the C8 cohort (Vieira et al., 2013) showed that this was likely to be the most common exposure level, or close to the most common exposure level, in participants with modeled exposures above 81 ng/ml, the lower cut-off point of the 5th quintile in Darrow et al. (2016). This value had no impact on the LOAEC or NOAEC derived from this study, and the effect of selecting this value on BMD calculations was evaluated in sensitivity analyses. The Darrow et al. (2016) study authors used a linear regression analysis to calculate the difference in adjusted mean ALT levels between PFOA quintiles 2-5 and quintile 1. Because ALT levels were lognormal transformed for these analyses, the regression coefficients (b) can be used to calculate the percent differences in ALT values between these quintiles (on an absolute, non-logged scale) using the equation: percent difference = $[\exp(b) - 1] \times 100\%$. As seen in Table 6.1.3, the NOAEC is 8.6 ng/ml.

Table 6.1.3. Regression coefficients for the mean differences in serum ALT by quintile of modeled serum PFOA (ng/ml) from Darrow et al. (2016). The NOAEC is highlighted in green.

Quintile	N	PFOA[1]	b	95% CI
1	6,145	4.2	0	Ref
2	6,145	8.6	0.001	-0.016-0.018
3	6,145	19.0	0.023	0.007-0.040
4	6,145	54.1	0.036	0.019-0.053
5	6,145	507	0.048	0.031-0.066

Abbreviations: b, regression coefficient; CI, confidence interval; Ref, reference category

[1] Category midpoints for PFOA serum concentrations (ng/ml) except highest quintile based on data presented in Vieira et al. (2013)

BMD method: OEHHA performed a BMD analysis of the Darrow et al. (2016) data shown in Table 6.1.3, but when data from all five quintiles were used, these calculations resulted in either non-convergence, wide BMD:BMDL ratios (e.g., >5), or BMDLs that were far below the range of observed PFOA values. As such, these results were not used to derive a POD. Similar results were obtained when other estimates of the mean or median PFOA concentration in quintile 5 were used (e.g., the estimated quintile 5 mean of 235 ng/ml based on Gallo et al. (2012)). When the highest PFOA quintile was excluded, the Hill model for a BMR_{01} (a 1% increase in

mean ALT from the reference category) gave a BMD_{01} and $BMDL_{01}$ of 14.1 and 8.0 respectively. The regression coefficient in quintile 5 corresponds to a difference of 4.9%, so a BMR_{05} was outside the range of observed responses. A BMR involving a one standard deviation change in ALT was also above the range of observed responses (i.e., >4.9% increase in mean ALT from the reference category).

ORs for high ALT:

NOAEC/LOAEC method: OEHHA selected the Gallo et al. (2012) study for its analyses of ORs for high ALT in the C8 cohort because the results of this study showed a more consistent and clear dose-response pattern than seen in Darrow et al. (2016). The ORs from the Gallo et al. (2012) study are shown in Table 6.1.4. Mean PFOA concentrations in each decile were not provided with the OR results, so they were based on digitized data from Figure 1 of the Gallo et al. (2012) publication. As seen in Table 6.1.4, the NOAEC is 9.8 ng/ml. The OR at the LOAEC (decile 3; OR = 1.19) represents about a 1.5% increase in the prevalence of having a high ALT compared to the prevalence in lowest decile (i.e., estimated prevalences of 10.4% vs. 8.9% in deciles 3 and 1, respectively).

BMD method: OEHHA used two general analytic approaches to calculate a BMD for the Gallo et al. (2012) ORs: the Generalized Least Squares for Trend Estimation (glst) method (Greenland and Longnecker, 1992) performed in Stata version 15, and analyses using the US EPA BMDS. Because a BMR of 5% was above the range of observed effect sizes in this study, a BMR of 1% (that is, a 1% increase in the prevalence of having elevated ALT compared to the lowest decile) was selected. This BMR is consistent with the guidance provided by US EPA (US EPA, 2012) in that it is in the lower range of the observed effect sizes, and it likely represents an important adverse effect (discussed in further detail below). BMD analyses using all ten deciles resulted in relatively poor model fit and BMDs that were not well correlated with the observed data. BMD analyses using only deciles 1-5 resulted in much improved model fit and, on visual inspection, resulted in BMDs that correlated well with the observed data. Using deciles 1-5, the BMD_{01} and $BMDL_{01}$ based on the glst method (p-value for model fit=0.96) were 12.4 and 10.6 ng/ml, respectively. The corresponding values using BMDS (Hill model; p-value for model fit=0.96) were 11.8 and 10.6 ng/ml, respectively.

Table 6.1.4. Odds ratios for elevated ALT by deciles of measured PFOA serum concentrations (ng/ml) from Gallo et al. (2012). The NOAEC is highlighted in green.

Decile	PFOA[1]	N	OR	95% CI
1	5.8	4,645	1.00	Ref
2	9.8	4,645	1.09	0.94-1.26
3	13.5	4,645	1.19	1.03-1.37
4	18.0	4,645	1.26	1.09-1.45
5	24.2	4,645	1.40	1.22-1.62
6	32.7	4,645	1.39	1.21-1.60
7	47.1	4,645	1.31	1.14-1.52
8	70.9	4,645	1.42	1.23-1.64
9	117.9	4,645	1.40	1.21-1.62
10	353.1	4,645	1.54	1.33-1.78

Abbreviations: CI, confidence interval; OR, odds ratio;
Ref, reference category
[1] Mean PFOA concentrations (ng/ml) in each decile based on digitized data from Figure 1 in Gallo et al. (2012)

Serum Lipid Concentrations

A number of human studies have identified associations between increasing serum concentrations of PFOA and increasing serum concentrations of total cholesterol (TC). OEHHA considered three epidemiologic studies for dose-response analyses of PFOA and TC: Dong et al. (2019), Lin et al. (2019), and Steenland et al. (2009). Associations between PFOA and TC were more consistent in studies of adults than in studies of children, and these three studies were the largest studies in adults that identified associations between PFOA and TC. In addition, each of these three studies presented results that were adjusted for several potentially important confounders (e.g., age, gender, and BMI); each used established methods to assess PFOA exposure and lipid levels; and each presented data in a format that could be used for dose-response analyses (e.g., mean differences or ORs).

Lin et al. (2019):

The Lin et al. (2019) study included subjects who were participants of a clinical trial of the effect of lifestyle modifications on pre-diabetes. This study included 888 pre-diabetic adults who were recruited from 27 medical centers in the US during the years 1996-1999. The study involved both cross-sectional and prospective components, with the results of both components providing evidence of an association between PFOA and increased TC. For its POD calculations, OEHHA used the results from the cross-sectional component because they were presented in a format that was more amendable to dose-response analysis and there was no convincing evidence that reverse causality was responsible for the effects identified (reverse causality is discussed in greater detail in the summary of PFOS PODs below).

NOAEC/LOAEC method: The results of Lin et al. (2019) are shown in Table 6.1.5. As shown, the NOAEC for TC was 4.2 ng/ml. Results for LDL and TG are shown for comparison purposes, and are similar to those for TC. The mean TC concentration in each PFOA quartile was not provided by the study authors; only mean differences were given. However, the authors did report that the mean TC level in all subjects combined was 204 (SD, ±35.4) mg/dl. Using this value, and the sample sizes and mean differences, the mean TC level in the lowest quartile can be estimated to be about 195 mg/dl. Given this, the mean difference of 10.13 mg/dl at the LOAEC represents about a 5% increase in TC compared to the lowest PFOA exposure group.

Table 6.1.5. Mean differences in serum TC, TG, and LDL by quartiles of serum PFOA (ng/ml) from Lin et al. (2019). The NOAEC is highlighted in green.

Quartile	N	PFOA[1]	TC[2]	TG[2]	LDL[2]
1	221	2.6 (2.0-3.0)	Ref	Ref	Ref
2	222	4.2 (3.8-4.5)	2.00 (-4.64-8.65)	15.92 (-2.15-34.00)	-0.30 (-6.38-5.77)
3	227	5.6 (5.3-6.2)	10.13 (3.56-16.70)	14.03 (-3.83-31.89)	7.88 (1.87-13.88)
4	228	8.4 (7.4-10.3)	13.36 (6.63-20.10)	36.80 (18.49-55.10)	6.70 (0.55-12.86)

Abbreviations: LDL, low-density lipoprotein; Ref, reference category; TC, total cholesterol; TG, triglycerides

[1] Median PFOA (IQR) in each quartile
[2] Mean differences in lipid levels compared to quartile 1; data in parentheses are 95% confidence intervals

BMD method: OEHHA performed a BMD analysis of the Lin et al. (2019) data for TC shown in Table 7.5 using the Hill model and a BMR of 5%. Standard deviations for the mean differences ($SD_{mean\ differences}$) were derived from the confidence intervals using the following equations:

> Standard error (SE) = (upper 95% confidence interval – lower 95% confidence interval) ÷ 3.92
>
> $SD_{mean\ differences} = SE \div \sqrt{([1 \div N_{ref}] + [1 \div N_{exp}])}$, where N_{ref} is the sample size in quartile 1 and N_{exp} is the sample size in quartiles 2-4 (Cochrane Collaboration, 2011). The SD in the reference group (quartile 1) was estimated by using the average of the SDs in quartiles 2-4.

The BMD_{05} and $BMDL_{05}$ were 5.5 and 4.6 ng/ml, respectively. The BMDS was unable to calculate model fit parameters but the visual display showed an excellent model fit.

Dong et al. (2019):

Using data from US NHANES for the years 2003-2014 on 8,948 adults, Dong et al. (2019) calculated a BMD for PFOA and TC using a hybrid approach (Crump, 1995). Here, the cut-off point for elevated TC was set at the upper 5th percentile of TC values in the lowest PFOA exposure group (the actual TC value at this cutoff point was not provided), and the BMR was defined as a 10% increase in the number of people with TC values above this level. Further details on this analysis are provided in Section 5.3.4. Using this method, Dong et al. (2019) reported a BMD_{10} and $BMDL_{10}$ of 10.5 and 5.6 ng/ml, respectively (Table 5.3.5). Key variables or other key results such as the cut-off point used to define elevated TC or model fit parameters were not provided.

Steenland et al. (2009):

Steenland et al. (2009) performed a cross-sectional investigation of the relationship between serum PFOA levels and serum lipid levels in 46,294 adults from the C8 cohort. Serum samples and data on potentially important confounders were collected in the years 2005-2006. In their statistical analyses, the authors analyzed serum TC levels as both a continuous and a categorical variable. In the latter, ORs for having an elevated TC concentration were calculated using a value of ≥240 mg/dl to define elevated TC. This value has been a commonly used guideline for defining high TC (US DHHS, 2005). Fifteen percent of the study population had TC concentrations above this level. Subjects taking cholesterol-lowering medications were excluded, and results were adjusted for age, gender, BMI, education, smoking, exercise, and current alcohol consumption. Results were similar in people who fasted prior to serum collection compared to those who did not. Associations with TC were also seen for PFOS but the correlation between PFOA and PFOS was only moderate (R=0.32), the mean serum concentrations of PFOA were markedly higher than those of PFOS (80.3 vs. 22.4 ng/ml, respectively), and adjustment of the PFOA results for PFOS had only relatively small effects.

TC means

NOAEC/LOAEC method: Regression coefficients for the mean differences in TC concentrations between deciles 2-10 and decile 1 are shown in Table 6.1.6. These coefficients were

developed by the study authors using TC values that were lognormal transformed. As such, the regression coefficients can be used to calculate the percent difference in TC values in deciles 2-10 compared to decile 1 using the equation: percent difference = [exp(b) − 1] × 100%. The SEs and p-values for the regression coefficients in Table 7.6 were provided by the study authors and were based on their linear regression analyses. The mean PFOA concentrations in each PFOA decile were not provided so they were estimated using deciles from another C8 study (Gallo et al., 2012). The sample sizes in each decile were also not provided so they were estimated using the total sample size divided by 10. SDs were estimated from the SEs using the equations provided above for mean differences. As shown in Table 6.1.6, the increase in TC in decile 2 compared to decile 1 was statistically significant. Thus, no NOAEC can be derived from these data, and the LOAEC is 9.8 ng/ml.

Table 6.1.6. Regression coefficients for the mean differences in serum TC and related results by deciles of serum PFOA (ng/ml) from Steenland et al. (2009). The LOAEC is highlighted in blue.

Decile	PFOA	N	Regression results for lnTC						Estimated percent change in TC		
			b	SE	p	CI_L	CI_U	SD	Change[1]	CI_L	CI_U
1	5.8	4,629	0	Ref				0.192	Ref		
2	9.8	4,629	0.01	0.004	0.0026	0.002	0.018	0.192	1.01%	0.22%	1.80%
3	13.5	4,629	0.02	0.004	<0.0001	0.012	0.028	0.192	2.02%	1.22%	2.82%
4	18.0	4,629	0.03	0.004	<0.0001	0.022	0.038	0.192	3.05%	2.24%	3.86%
5	24.2	4,629	0.04	0.004	<0.0001	0.032	0.048	0.192	4.08%	3.27%	4.90%
6	32.7	4,629	0.03	0.004	<0.0001	0.022	0.038	0.192	3.05%	2.24%	3.86%
7	47.1	4,629	0.04	0.004	<0.0001	0.032	0.048	0.192	4.08%	3.27%	4.90%
8	70.9	4,629	0.04	0.004	<0.0001	0.032	0.048	0.192	4.08%	3.27%	4.90%
9	117.9	4,629	0.04	0.004	<0.0001	0.032	0.048	0.192	4.08%	3.27%	4.90%
10	353.1	4,629	0.05	0.004	<0.0001	0.042	0.058	0.192	5.13%	4.31%	5.95%

Abbreviations: b, regression coefficient; CI_L, lower 95% confidence interval; CI_U, upper 95% confidence interval; ln, lognormal; Ref, reference; SD, standard deviation; SE, standard error; TC, total cholesterol
[1] The percent increase in TC values compared to decile 1

BMD method: Using data on all PFOA deciles and a BMR_{01} gave a poor model fit for all models using BMDS. Removing the highest dose group gives a BMD_{01} and $BMDL_{01}$ of 9.9 and 8.6 ng/ml, respectively (p-value for model fit=0.06). Removing the next three highest exposure groups did not improve model fit. However, if the highest five dose groups are removed, model fit improves substantially but the BMD_{01} and $BMDL_{01}$ change only slightly (9.8 and 8.5 ng/ml, respectively; p-value for model fit=0.97).

ORs for elevated TC

NOAEC/LOAEC method: The ORs from Steenland et al. (2009) for having an elevated TC level are shown in Table 6.1.7. Samples sizes in each quartile were not provided so they were estimated using the total sample size divided by four. The mean PFOA concentrations in each quartile were also not provided, so OEHHA used the category midpoints. An exception to this was quartile 4. Here a value of 507 ng/ml was used since another publication involving a subsample of the C8 cohort (Vieira et al., 2013) showed that this was likely to be the most

common exposure level, or close to the most common exposure level, in participants with modeled exposures above 67 ng/ml, the lower cut-off point of the 4th quartile in Steenland et al. (2009). This estimate had no effect on the selection of the NOAEC or LOAEC or on BMD calculations (see below). As seen in Table 6.1.7, the OR in the second quartile is statistically significant, and the LOAEC is 19.9 ng/ml (rounded from 19.85 ng/ml).

BMD method: BMD calculations were not performed using these data because both the glst and US EPA BMDS require information on the prevalence of the outcome in each exposure group, data that were not provided. In some instances, ORs can be used to estimate disease prevalence. In this study however, the prevalence of the outcome was fairly high (15%) and it is unclear whether, and by how much, ORs might lead to an overestimation of risk or prevalence.

Table 6.1.7. Odds ratios for elevated serum TC by quartiles of serum PFOA (ng/ml) from Steenland et al. (2009). The LOAEC is highlighted in blue.

Quartile	PFOA[1]	N	OR	95% CI
1	6.55	11,574	1.00	Ref
2	19.85	11,574	1.21	1.12-1.31
3	46.75	11,574	1.33	1.23-1.43
4	507	11,574	1.38	1.28-1.50

Abbreviations: CI, 95% confidence interval; OR, odds ratio; Ref, reference

[1] Category midpoints for PFOA serum concentrations (ng/ml) except highest quartile based on data presented in Vieira et al. (2013)

Summary: PFOA PODs

A summary of the PODs for PFOA derived from the candidate studies identified by OEHHA are shown in Table 6.1.8. These PODs ranged from 2.8 to 19.9 ng/ml (mean = 10.9 ng/ml). The lowest value of 2.8 ng/ml was the $BMDL_{10}$ for a decrease in antibody levels in response to influenza vaccine from the study by Abraham et al. (2020). Although this value was within the range of the PFOA levels observed in this study, it was associated with a large BMD:BMDL ratio (e.g., >5). The highest value was the LOAEC for ORs for elevated serum TC from the C8 cohort (Steenland et al., 2009). Aside from the Steenland et al. (2009) LOAEC for elevated TC, the highest PODs were for some of the NOAECs for vaccine response from Abraham et al. (2020). This is not surprising given this study's small sample size and low statistical power. After excluding the two LOAECs in Table 6.1.8, the PODs ranged from 2.8 to 19.4 ng/ml, with a mean of 10.4 ng/ml.

Table 6.1.8. Summary of potential PODs for PFOA

Candidate studies	Effect	Method	POD/notes
Immunotoxicity			
Abraham et al. (2020)	Diphtheria antibody levels	NOAEC/LOAEC	NOAEC = 18.9 ng/ml
	Hib antibody levels	NOAEC/LOAEC	NOAEC = 19.4 ng/ml
	Tetanus antibody levels	NOAEC/LOAEC	NOAEC = 18.9 ng/ml

Candidate studies	Effect	Method	POD/notes
	Diphtheria antibody levels	Knee function	NOAEC = 16.2 ng/ml
	Hib antibody levels	Knee function	NOAEC = 12.2 ng/ml
	Tetanus antibody levels	Knee function	NOAEC = 16.9 ng/ml
	Diphtheria antibody levels	BMDS: Hill model	BMD_{10} = 17.0 ng/ml $BMDL_{10}$ = 11.4 ng/ml
	Hib antibody levels	BMDS: Hill model	BMD_{10} = 16.8 ng/ml $BMDL_{10}$ = 2.8 ng/ml
	Tetanus antibody levels	BMDS: Hill model	BMD_{10} = 20.0 ng/ml $BMDL_{10}$ = 6.5 ng/ml
Faroe Islands (Grandjean et al., 2017a; Budtz-Jorgensen and Grandjean, 2018)	Diphtheria antibody levels	NOAEC/LOAEC	NOAEC = 4.75 ng/ml
	Tetanus antibody levels	NOAEC/LOAEC	Data not available
	Diphtheria antibody levels	BMDS: Hill and Piecewise models	$BMDL_{05}$ outside observed range; large BMD:BMDL ratio
	Tetanus antibody levels	BMDS: Hill and Piecewise models	$BMDL_{05}$ outside observed range; large BMD:BMDL ratio
Liver Toxicity			
Darrow et al. (2016)	Mean ALT levels	NOAEC/LOAEC	NOAEC = 8.6 ng/ml
	Mean ALT levels	BMDS: multiple models, all exposure levels	Large BMD:BMDL ratios; non-convergence
	Mean ALT levels	BMDS: Hill model, quintile 5 excluded	BMD_{01} = 14.1 ng/ml $BMDL_{01}$ = 8.0 ng/ml
	Elevated ALT ORs	NOAEC/LOAEC	Inconsistent dose-response curve – used Gallo et al. (2012)
	Elevated ALT ORs	glst	Inconsistent dose-response curve – used Gallo et al. (2012)
	Elevated ALT ORs	BMDS	Inconsistent dose-response curve – used Gallo et al. (2012)
Gallo et al. (2012)	Mean ALT levels	NOAEC/LOAEC	Data in figure form – used Darrow et al. (2016)
	Mean ALT levels	BMDS	Data in figure form – used Darrow et al. (2016)

Candidate studies	Effect	Method	POD/notes
	Elevated ALT ORs	NOAEC/LOAEC	**NOAEC = 9.8 ng/ml**
	Elevated ALT ORs	glst	BMD_{01} = 12.4 ng/ml $BMDL_{01}$ = 10.6 ng/ml
	Elevated ALT ORs	BMDS: Hill model	BMD_{01} = 11.8 ng/ml $BMDL_{01}$ = 10.6 ng/ml
Lipid Concentrations			
Lin et al. (2019)	Mean TC levels	NOAEC/LOAEC	NOAEC = 4.2 ng/ml
	Mean TC levels	BMDS: Hill model	BMD_{05} = 5.5 ng/ml $BMDL_{05}$ = 4.6 ng/ml
Dong et al. (2019)	Mean TC levels	NOAEC/LOAEC	Information not provided
	Mean TC levels	BMDS	Information not provided
	Mean TC levels	Hybrid model	BMD_{10} = 10.5 ng/ml $BMDL_{10}$ = 5.6 ng/ml
Steenland et al. (2009)	Mean TC levels	NOAEC/LOAEC	No NOAEC LOAEC = 9.8 ng/ml
	Mean TC levels	BMDS: Hill model, deciles 1-5	BMD_{01} = 9.8 ng/ml $BMDL_{01}$ = 8.5 ng/ml
	Elevated TC ORs	NOAEC/LOAEC	No NOAEC LOAEC = 19.9 ng/ml
	Elevated TC ORs	BMDS/glst	Prevalence data not provided

OEHHA selected the NOAEC of 9.8 ng/ml for elevated ALT from the Gallo et al. (2012) study as the POD for its PFOA ADD calculations. While this study does not provide the lowest POD, it does offer the following advantages for dose-response and risk assessment calculations.

Very large sample size (N=46,452). This is by far the largest of the candidate studies OEHHA reviewed for its POD calculations. The very large sample size helps reduce the probability that findings are due to chance, allows for the detection of relatively subtle effect sizes, and helps to increase the likelihood that study findings are broadly generalizable. In addition, because of the large sample size, effect estimates could be examined in a relatively large number of exposure categories (i.e., deciles in this case) with good statistical power. This allowed for a more precise determination of the NOAEC compared to some of the smaller studies OEHHA considered for its POD calculations.

Valid method for assessing exposure. Exposure was assessed using a single measured serum concentration of PFOA in each participant. Serum measurements are a commonly used and widely accepted method for assessing PFOA exposure. The long half-life of PFOA in serum suggests that a single serum measurement is likely to provide an accurate and precise

indication of a person's true long-term exposure. Based on the methodologic details provided in the study publication there is no indication that the serum samples were collected, or PFOA concentrations were measured, in a way that would have differed between those with higher or lower ALT levels. Because of this, the major effect of misclassification of PFOA exposure would most likely be non-differential and most likely bias results towards the null (i.e., in the direction of finding no effect) (Jurek et al., 2005). Non-differential misclassification of exposure could have biased some of the ORs in the middle exposure categories (i.e., in deciles 2-9) away from 1.0. However, OEHHA could find no evidence that this bias was substantial, and a number of other, lower-exposure studies (Gleason et al., 2015; Jain and Ducatman, 2018b; Salihovic et al., 2018; Attanasio, 2019; Nian et al., 2019) identified statistically significant PFOA-ALT associations at PFOA concentrations similar to the LOAEC in Gallo et al. (2012).

Clinically relevant outcome. The cutoff points used to define elevated ALT in Gallo et al. (2012) were based on clinical reference levels published by the International Federation of Clinical Chemistry and Laboratory Medicine (Schumann et al., 2002). ALT concentrations above these reference levels have been associated with increases in both liver-related mortality and all-cause mortality (Kwo et al., 2017). For example, based on participants from US NHANES 1999-2008, Ruhl and Everhart (2012) found that ALT levels >30 IU/L in men or >19 IU/L in women were associated with a >8-fold increase in mortality from liver disease (hazard ratio = 8.2; 95% CI, 2.1-31.9). In addition, a study by Lee et al. (2008) involving 47,182 residents of Olmsted County, MN, found ALT concentrations >45 IU/L for men and >29 IU/L for women were associated with a 34% increase in mortality from all causes (SMR = 1.34; 95% CI, 1.05-1.71). These findings do not necessarily mean that people with PFOA exposures above a certain level will have an 8.2-fold higher risk of death from liver disease or a 34% higher risk of death overall. However, elevated ALT is an indicator of liver toxicity. As such, these findings highlight the potential clinical importance of having high ALT and the importance of the pathological processes that cause these elevated ALT levels. The clinical relevance of these ALT levels is also highlighted by the fact that the American College of Gastroenterology recommends that people with ALT levels >33 IU/L in men and >25 IU/L in women undergo a clinical evaluation, including a physical exam, screening for alcohol and hepatotoxic medication use, testing for viral hepatitis, assessment for nonalcoholic fatty liver disease and alcoholic liver disease, and screening for hereditary liver diseases (Kwo et al., 2017).

Consistency of findings. The Gallo et al. (2012) findings linking PFOA to elevated levels of ALT are not only consistent with research done in other study areas and other study populations (see Appendix 7, Table A7.5), these findings are also internally consistent. In this and the Darrow et al. (2016) study (which used mostly the same study participants), PFOA-ALT associations were examined using a variety of different approaches and analyses, and all provided evidence that PFOA was associated with increased ALT. These approaches and analyses included using both modeled and measured PFOA exposures; evaluating PFOA and ALT as both continuous and categorical variables; assessing PFOA exposure as both cumulative lifetime and yearly annual average exposure; examining other biomarkers of liver toxicity such as GGT and bilirubin; and performing subgroup analyses based on age, sex, and occupational exposure. Overall, the internal and external consistency of the Gallo et al. (2012) findings provides very strong evidence that the associations identified in this study are real (Bradford Hill, 1965).

No major confounding identified. The findings in Gallo et al. (2012) were adjusted or otherwise controlled for a number of factors that could potentially affect ALT levels, including age, sex, alcohol intake, socioeconomic status, fasting, race, smoking, BMI, exercise, and

insulin resistance. These include some of the most prevalent and strongest risk factors for liver disease. A number of other factors can affect ALT levels including use of certain medications, viruses, other chemical exposures, or genetic disorders. However, OEHHA could not find convincing evidence that these are prevalent enough, or related strongly enough to both ALT and PFOA, to cause the elevated ORs seen in Gallo et al. (2012) (Axelson, 1978; Schlesselman, 1978).

Effects are unlikely to be solely due to PFOS. A particular concern for studies of PFOA is the potential for confounding by other PFAS. This is especially true for confounding by PFOS since in many populations, PFOA and PFOS exposures are highly correlated and PFOS is the predominant PFAS exposure. OEHHA evaluated the possibility that PFOS, and not PFOA, was responsible for the associations identified in Gallo et al. (2012) and found this to be unlikely for a number of reasons.

First and perhaps most importantly, animal data have shown that PFOA can cause liver toxicity independent of exposure to other PFAS, including PFOS. Second, the C8 studies involved an area that had high levels of PFOA contamination, and mean serum concentrations of PFOA were higher than those of PFOS (mean serum levels of 83.0 and 23.3 ng/ml, respectively). By itself, the fact that PFOA was the predominant PFAS exposure here does not ensure that it caused the effects reported in Gallo et al. (2012). However, the fact that PFOA is known to cause liver toxicity in laboratory animals, combined with the fact that PFOA exposures in this area were very high, is highly suggestive that PFOA was the causative agent.

The third piece of evidence that the PFOA findings of Gallo et al. (2012) were due to PFOA and not PFOS was that the correlation between these two agents in the C8 study area was only moderate, well below the correlations seen in other studies. For example, correlation coefficients for PFOA and PFOS as high as 0.64 have been reported in US NHANES (Calafat et al., 2007b). In the C8 cohort, the serum PFOA-PFOS correlation coefficient was 0.32 (Steenland et al., 2009). Another important finding is that the magnitude of the association between PFOS and ALT was not markedly higher than that seen with PFOA. In fact, it was a little lower (i.e., regression coefficients of 0.020 for lnPFOS (ng/ml) and lnALT (IU/L), and 0.022 for lnPFOA (ng/ml) and lnALT (IU/L)). These findings are important because the likelihood that a factor will cause major confounding is not simply related to whether or not that factor is associated with both the exposure and the outcome of interest, but more importantly, its related to the relative magnitude of these associations (Axelson, 1978). Overall, the fact that the association between PFOS and ALT was not markedly stronger than that between PFOA and ALT, combined with the fact that PFOA and PFOS were only moderately correlated, make it highly unlikely that PFOS was an important cause of the effects attributed to PFOA in this study.

One method to evaluate whether the associations between the potential confounding factor and the exposure and outcome of interest are strong enough to cause major confounding is to compare the main study results before and after statistically adjusting for that factor. Although the authors of the Gallo et al. (2012) paper did not present PFOA-ALT results adjusted for PFOS, an illustration of the likely impact that this adjustment would have had can be seen in another C8 study. In the C8 study examining PFAS and serum TC (Steenland et al., 2009), the effect size for PFOS was much larger than that seen for PFOA (e.g., regression coefficients of 0.01112 for lnPFOA (ng/ml) and 0.02660 for PFOS (ng/ml), respectively, with serum lnTC (mg/dl)). Here, because the magnitude of the association with TC is much stronger for PFOS than for PFOA, one might not be surprised if confounding by PFOS could have caused the association reported for PFOA. However, when the authors of this study adjusted the PFOA-TC

results for PFOS, the PFOA-TC effect size was only reduced by 20-30%, and the overall trend of increasing TC with increasing PFOA remained (more detailed results were not provided in the publication). The most likely reason the PFOA-TC effect did not decrease even further (e.g., to zero) after this adjustment is that the PFOA-PFOS correlation was only moderate, and not strong enough to cause major confounding. For ALT, because the effect of PFOS was not larger than that for PFOA, the impact of confounding by PFOS is likely to be even less than the 20-30% seen in the analysis of TC.

The results of Lin et al. (2010) provide another example of the likely impact of confounding by PFOS or other PFAS on the relationship between PFOA and ALT. This study involved a cross-sectional evaluation of several PFAS and serum liver enzymes in 2,216 adults (18 years of age or older) in the 1999-2000 and 2003-2004 US NHANES. An NOAEC, LOAEC, or BMD was not developed from this study because the main categorical results were not adjusted for potentially important confounders. However, the authors did report regression coefficients for associations between ALT and PFOA and PFOS in analyses that were adjusted for age, gender, race/ethnicity, smoking, alcohol drinking, education, BMI, insulin resistance, metabolic syndrome, and iron saturation status. Results were presented both in analyses where PFOA and PFOS were entered into the linear regression models on their own, and in analyses where these agents were entered together (as well as with PFHxS and PFNA). The results of these analyses are shown in Table 6.1.9. As seen, while the regression coefficient for PFOA increased after adjustment for PFOS, the regression coefficient for PFOS decreased after adjustment for PFOA. As a whole, these results provide further evidence that PFOA can affect ALT levels, and that this effect is not strongly confounded by PFOS.

Table 6.1.9. Linear regression coefficients (β) for association between serum log-PFOA or log-PFOS (ng/ml) and serum ALT (IU/L) in Lin et al. (2010)

Analysis	Log-PFOA	Log-PFOS
Each PFAS entered into the model on its own	β=1.86 p=0.005	β=1.01 p=0.066
Each PFAS entered into the model with other PFAS	β=2.19 p=0.009	β=-0.19 p=0.769

No obvious selection bias. The C8 study has been estimated to include >80% of all residents of the affected areas. This high participation rate suggests that major errors due to selection bias are highly unlikely. In other studies, participation rates were much lower or were unclear or not reported (e.g., Lin et al. (2019)).

NOAEC is available. In some of the studies and outcome metrics that OEHHA considered for POD calculations, LOAECs were available but NOAECs were not. While this is not a fatal flaw, using a LOEAC rather than an NOAEC would add additional uncertainty to the ADD calculations.

Appropriate study design. The Gallo et al. (2012) analyses were cross-sectional, and a common criticism of cross-sectional studies is the possibility of reverse causation. However, OEHHA could not find any convincing evidence or plausible reason to believe that the alterations in serum ALT levels, or the liver diseases that typically cause them, would cause widespread and major increases in serum PFOA concentrations. This is particularly clear when looking at the Gallo et al. (2012) analyses of mean ALT concentrations by increasing categories

of PFOA exposure. Here, while the ALT levels increased by approximately 11% from the first to the tenth decile of PFOA, the mean PFOA levels across these deciles increased by over 5,000%. OEHHA was not able to identify any evidence or any plausible mechanism by which such a relatively small increase in serum ALT (or the underlying factors that might cause these relatively small increases) could cause such a huge increase in serum PFOA.

Another criticism of cross-sectional studies is that because they measure exposure and outcome at the same time, the most relevant exposure period might be missed. This is especially important in scenarios where the latency period between the exposure and outcome is long. However, as discussed above, the half-life of PFOA in human serum is several years. As such, a measurement of PFOA at a single point in time likely represents PFOA exposure over a fairly extended period of time. In addition, the half-life of ALT in plasma is short: about 47 hours (Kim et al., 2008). ALT levels typically rise rapidly (within a few hours or days) after a toxic exposure begins (Kim et al., 2008), and fall rapidly after exposure cessation (Curtis and Sivilotti, 2015; McGovern et al., 2015). Overall, the long half-life of serum PFOA, combined with the acute nature of ALT as a marker of hepatic injury, suggest that the cross-sectional approach used in Gallo et al. (2012) is a valid method for assessing PFOA-ALT associations.

Acceptable Daily Dose Calculation

To calculate the acceptable daily dose (ADD), which is an estimated maximum daily dose of a chemical that can be consumed by humans for an entire lifetime without toxic effects, the POD is divided by the composite uncertainty factor (UF). The ADD for PFOA was calculated using the NOAEC of 9.8 ng/ml for elevated ALT from the Gallo et al. (2012) study and the clearance factor of 0.28 ml/kg-day discussed in Chapter 4, with a UF of $\sqrt{10}$ for intraspecies variation. The clearance factor was used to convert the POD, which was expressed as a serum concentration of PFOA (in units of ng/ml), into a daily intake rate in units of nanograms per kilogram of body weight per day (ng/kg-day). OEHHA typically applies an intraspecies UF of 30 when deriving an ADD from an animal study, and this can be reduced to 1 when using human data. Here, the intraspecies UF is reduced to $\sqrt{10}$ rather than 1 for the following reasons. The Gallo et al. (2012) study involved a very large population of adults and the participation rate was about 80%. This large sample size and high participation rate suggests the study participants were a good representation of the population of the study area as a whole, and likely included a diverse group of people in terms of ages, health status, smoking and other chemical exposures, nutrition, socioeconomic status, and other factors. The study did not include children. While a few studies have identified associations between PFOA and ALT concentrations in children, the most consistent evidence linking PFOA to ALT has been in adults, and OEHHA could not find other convincing evidence that the hepatotoxic effects of PFOA are likely to be substantially greater in children than adults. In addition, since PFOA is not metabolized readily, there are not likely large kinetic differences between children and adults. A UF of $\sqrt{10}$ for intraspecies variation rather than 1 was applied because the C8 study population was not diverse in terms of race or ethnicity. In addition, it did not examine other potential susceptibility factors such as obesity or genetics. Some data suggest that obesity or certain genetic polymorphisms might increase susceptibility to PFAS (Ghisari et al., 2017; Jain and Ducatman, 2018b; Jain and Ducatman, 2019; Wen et al., 2019a). However, these data were limited (for a variety of reasons) and OEHHA was unable to identify consistent or convincing evidence that this susceptibility might be greater than a factor of $\sqrt{10}$. Finally, there is strong evidence for immunotoxicity of PFOA. The NOAECs from the immunotoxicity studies ranged from 4.75 to 19.4 ng/ml for response to vaccination in children. In the Faroe Islands study, depressed response to childhood vaccinations was seen at mean serum levels of 6.1 ng/ml, lower than the

mean serum concentration identified as the NOAEC for elevated ALT in Gallo et al. (2012). OEHHA chose not to use PODs from these studies because of the limited sample sizes and associated uncertainty in the data. However, these studies point to the potential for immunotoxicity to occur below the NOAEC for elevated ALT in adults. This is another reason a UF for intraspecies variation larger than 1 is warranted.

ADD = (POD × CF) ÷ UF = (9.8 ng/ml × 0.28 ml/kg-day) ÷ √10 = **0.87 ng/kg-day**.

Animal Studies

Liver, immune system, thyroid, and developmental/reproductive toxicities are the major noncancer effects for PFOA in experimental animals. Of these effects, liver toxicity appears to be the most sensitive endpoint. OEHHA identified PODs from the animal studies for comparison with those derived using human studies. Among the animal studies that exhibited a dose-response, Table 6.1.10 lists candidate critical studies for PFOA that report serum/plasma levels of PFOA and that OEHHA considers are of sufficient quality. However, because there are sufficient human data available to calculate an ADD and further develop a health-protective concentration without the uncertainty of extrapolating from animals to humans, OEHHA did not derive ADDs from these animal studies.

Table 6.1.10. Candidate critical studies for noncancer effects of PFOA in animals

Reference	Sex/Species	Dose/Route of Exposure/Duration	Endpoint	NOAEL/LOAEL (mg/kg-day)	NOAEL/LOAEL/ BMDL (mg/L)
Liver Toxicity					
Yu et al. (2016)	Male BALB/c mice (5/dose)	0, 0.5, or 2.5 mg/kg-day via oral infusion for 28 days	↑ relative liver weight	NOAEL: 0.5	NOAEL: 29.3 $BMDL_{1SD}$: 22.6
Li et al. (2017b)	Male and female BALB/c mice (30/sex/ dose)	0, 0.05, 0.5, or 2.5 mg/kg-day via gavage for 28 days	hepatic mitochondrial membrane potential changes, apoptosis, oxidative DNA damage	LOAEL: 0.05	LOAEL: 0.97 $BMDL_{1SD}$: 0.11 (↑ p-53 levels)
Guo et al. (2019)	Male BALB/c mice (12/dose)	0, 0.4, 2, or 10 mg/kg-day via gavage for 28 days	↑ relative liver weight, hepatocellular hypertrophy, and karyolysis	LOAEL: 0.4	LOAEL: 13 $BMDL_{1SD}$: 1.71
Blake et al. (2020)	Pregnant CD-1 mice (11-13 dams/dose)	0, 1, or 5 mg/kg-day via gavage from ED1.5 to ED11.5 or ED17.5	liver effects (↑ cell death) in dams, ED11.5	LOAEL: 1	LOAEL: 25.4 $BMDL_{05}$: 3.0

BMD modeling (BMDS, version 2.7) was performed on the candidate critical study datasets in Table 6.1.10, using plasma/serum concentration as the dose metric, and the results are summarized in Table 6.1.11.

Table 6.1.11. BMD modeling of noncancer endpoints from candidate PFOA animal studies

Study	Endpoint	Model	Goodness of fit p-value	BMD_{1SD} (mg/L)	$BMDL_{1SD}$ (mg/L)
Yu et al. (2016)	↑ relative liver weight	Linear[a]	0.901	32.1	22.6
Li et al. (2017b)	↑ p-53 levels	Hill	0.8453	0.15	0.11
Li et al. (2017b)	↑ mitochondrial membrane potential changes	Poor model fit			
Li et al. (2017b)	↑ caspase-9 levels	Poor model fit			
Li et al. (2017b)	↑ 8-OHdG	Poor model fit			
Guo et al. (2019)	↑ relative liver weight	Exponential4	0.413	2.55	1.71
Blake et al. (2020)	↑ relative liver weight (ED11.5)	Linear[a]	0.204	16.4	12.9
Blake et al. (2020)	↑ relative liver weight (ED17.5)	Linear	0.708	7.0	5.1
Blake et al. (2020)	↑ liver cell death (ED11.5)	Gamma[a]	0.995	6.4[b]	3.0[b]

[a] Additional models produced the exact same results
[b] This dataset was modeled with a benchmark response of 5% above background, therefore the values represent BMD_{05} and $BMDL_{05}$
8-OHdG, 8-hydroxy-2-deoxyguanosine; ED, embryonic day

The LOAEL of 0.05 mg/kg-day from Li et al. (2017b) for hepatic mitochondrial membrane potential changes and increased apoptosis and oxidative DNA damage corresponds to a serum concentration of 0.97 mg/L. These endpoints were also frequently observed in in vitro studies (Wu et al., 2017; Orbach et al., 2018; Sun et al., 2019b; Xu et al., 2019b). While these endpoints are not frank effects, they may be considered upstream effects in the continuum of changes resulting in liver toxicity, which includes hepatocyte hypertrophy and increased liver weight as reported in the Li et al. (2017b) study. BMD modeling of increased p-53 levels, a biomarker of apoptosis, from the Li et al. (2017b) resulted in a $BMDL_{1SD}$ of 0.11 mg/L.

A NOAEL of 0.003 mg/kg-day was identified from the van Esterik et al. (2016) study (Table 5.5.1), based on reduced female pup body weight on PND 4 in animals exposed to PFOA during gestation and lactation. However, serum concentrations were not reported in this study, and due to the complexity of the dosing scheme (PFOA was administered to dams during pregnancy and lactation, making it difficult to determine whether the effect was due to in utero exposure, lactational exposure, or both), and lack of a reliable kinetic model, serum concentrations could not be determined. Therefore, this study is not considered as a candidate critical study.

6.1.2. Perfluorooctane Sulfonic Acid

Human Studies

Based on its review of the available human studies, OEHHA has determined that the sensitive noncancer endpoints for PFOS are immunotoxicity and alterations in lipid metabolism or production. Several studies have also linked PFOS to adverse effects related to the thyroid gland; however, these findings are too inconsistent from study to study to make firm conclusions regarding these outcomes. Research in laboratory animals has shown that PFOS can cause hepatoxicity. However, the human epidemiologic evidence linking PFOS to increases in liver enzyme levels or other hepatotoxic effects is not as robust or consistent as that seen for PFOA. Associations with several reproductive and developmental outcomes have also been reported. While the positive associations reported in some of these studies are cause for concern, overall, the findings from study to study were also somewhat inconsistent, and OEHHA was unable to identify studies that could be used to accurately evaluate the dose-response patterns for these outcomes.

In the following sections, OEHHA presents the human studies that were considered as candidate studies for POD determination.

Immunotoxicity

A number of animal and human studies have provided evidence that PFOS can increase the risks of immune-related diseases or otherwise adversely affect the immune system. With regards to human studies, increased serum levels of PFOS have been linked to diminished antibody levels in response to vaccinations for diphtheria, tetanus, rubella, and mumps (Granum et al., 2013; Stein et al., 2016b; Grandjean et al., 2017a; Grandjean et al., 2017b; Pilkerton et al., 2018). These findings have been seen in both adults and children, although the greatest effect sizes and most consistent results have been reported in children. For example, decreases in tetanus and diphtheria antibody concentrations of >30% have been reported for each doubling of PFOS serum concentration in children from the Faroe Islands (Grandjean et al., 2017a) (Table 5.1.1). A few studies have identified associations between PFOS and immune-related diseases such as asthma, eczema, and lower respiratory tract infections (Dalsager et al., 2016; Goudarzi et al., 2017; Qin et al., 2017; Averina et al., 2019; Manzano-Salgado et al., 2019; Kvalem et al., 2020) although the evidence for these outcomes is less consistent than that seen for diminished vaccine response.

For immunotoxicity, OEHHA selected the studies of PFOS and diminished response to diphtheria and tetanus vaccinations performed in the Faroe Islands cohorts as candidate critical studies. Clear associations between PFOS and diminished antibody response to diphtheria, influenza, and tetanus vaccines were not seen in the recently published study in one-year old children by Abraham et al. (2020). The exact reasons for the inconsistency between the Faroe Islands studies and the Abraham et al. (2020) study for PFOS are unknown although the latter involved a much more limited age range (Abraham et al. (2020) only included one-year-old children, while the Faroe Islands studies followed children from birth up to age 17 years old). In addition, Abraham et al. (2020) involved only cross-sectional analyses, while the Faroe Islands studies included both cross-sectional and prospective analyses. Finally, Abraham et al. (2020) had far fewer children than the Faroe Islands cohorts. The Faroe Islands studies, and the PODs that can be derived from them, are discussed below.

Faroe Islands studies:

NOAEC/LOAEC method: Associations between serum PFOS concentrations and antibody levels in response to diphtheria and tetanus vaccinations in children were assessed in two Faroe Islands cohorts (Grandjean et al., 2017a). Details of these studies are provided in Appendix 7, Table A7.4. Categorical data, which can be used to develop NOAECs, were not available from the peer-reviewed publications but results for diphtheria have been obtained from a draft report by the European Food Safety Authority (EFSA, 2020). These are shown in Table 6.1.12. As seen in this table, the LOAEC and NOAEC are 26.0 and 20.6 ng/ml, respectively. The LOAEC and NOAEC developed based on PFOS deciles were similar. Although the decrease in antibody response in quintile 3 was statistically significant, the analyses by exposure deciles showed a large degree of variability in responses around the quintile 3 exposure levels (e.g., for PFOS levels between 14 and 20 ng/ml). As such, OEHHA selected quintile 5 rather than quintile 3 as the LOAEC, and quintile 4 rather than quintile 2 as the NOAEC. Categorical data for tetanus were not available.

Table 6.1.12. Mean serum diphtheria antibody concentrations at age 7 by quintiles of serum PFOS (ng/ml) at age 5 from the Faroe Islands cohorts (EFSA, 2020). The NOAEC is highlighted in green.

Quintile	PFOS[1]	N	Mean[2]	SD	p-value
1	11.5	86	0.25	1.81	Ref
2	14.9	86	-0.07	1.91	0.32
3	17.4	86	-0.48	1.89	0.01
4	20.6	86	-0.16	1.90	0.15
5	26.0	86	-0.39	1.94	0.03

Abbreviations: Ref, reference category; SD, standard deviation
[1] Mean PFOS serum concentrations (ng/ml) in each quintile
[2] Log2 values of mean serum antibody concentrations

BMD method: As reviewed in Chapter 5 and shown in Table 5.1.4, BMD calculations have been performed by the Faroe Islands study investigators (Budtz-Jorgensen and Grandjean, 2018). Using a piecewise model with a 5% decrease in antibody levels as the BMR, the authors reported a BMD_{05} and $BMDL_{05}$ of 3.57 and 0.72 ng/ml, respectively, for tetanus antibody, and a BMD_{05} and $BMDL_{05}$ of 1.21 and 0.54 ng/ml, respectively, for diphtheria antibody. Both of these BMDLs are well below the lowest PFOS serum concentration observed in this study. OEHHA performed its own BMD modeling and obtained results with large BMD:BMDL ratios (e.g., >5) and BMDLs that were also well below the range of observed values. The most likely reason the BMD:BMDL ratios were so large was the high degree of variability (as indicated by the large SDs) in antibody levels seen in each PFOS exposure category.

Serum Lipid Concentrations

A number of human studies have identified associations between increasing serum concentrations of PFOS and increasing serum concentrations of total cholesterol (TC). OEHHA considered the following studies for dose-response analyses of PFOS and TC.

- Dong et al. (2019)
- Steenland et al. (2009)
- Frisbee et al. (2009)

- Starling et al. (2014)

These studies were selected because each provided some evidence of a statistically significant association between PFOS and TC; each involved a relatively large sample size (i.e., had sufficient statistical power); each adjusted or otherwise accounted for the strongest and most prevalent risk factors for elevated TC levels (e.g., age, gender, BMI, and socioeconomic status); each used validated and well accepted methods for assessing both exposure and outcome (i.e., serum concentrations of PFOS and TC); none showed evidence of selection bias; and each presented results in a format that could potentially be used for dose-response assessment (e.g., ORs or mean TC levels by categories of serum PFOS).

Dong et al. (2019):

Using data from US NHANES for the years 2003-2014 on 8,948 adults, Dong et al. (2019) calculated a BMD and BMDL for PFOS and TC using a hybrid approach (Crump, 1995). Here, the cut-off point for elevated TC was set at the upper 5th percentile of TC values in the lowest PFOS exposure group (the actual TC value at this cutoff point was not provided), and the BMR was defined as a 10% increase in the number of people with TC values above this level. Further details on this analysis are provided in Section 5.3.4. Using this method, Dong et al. (2019) reported a BMD_{10} and $BMDL_{10}$ of 44.2 and 24.1 ng/ml, respectively (Table 5.3.5). Key variables or other key results such as the cut-off point used to define elevated TC or the model fit parameters were not provided.

Steenland et al. (2009) and Frisbee et al. (2009):

Steenland et al. (2009) performed a cross-sectional investigation on the relationship between serum PFOS and serum lipid concentrations in 46,294 adults from the C8 cohort. Details of this study are provided in the PFOA dose-response assessment above. Results were presented for analyses using both PFOS and TC as categorical and continuous variables. Increases in mean serum TC levels were seen in increasing deciles of serum PFOS. The regression coefficients representing the difference in lognormal TC values between deciles 2-10 and decile 1 were 0.01, 0.01, 0.03, 0.03, 0.04, 0.04, 0.05, 0.06, and 0.06, respectively (these results are reported in the footnotes of Table 3 in Steenland et al. (2009)). Overall, these coefficients indicate a pattern of increasing TC levels with increasing PFOS exposure although standard errors or p-values were not provided. Adjusted mean TC concentrations by deciles of PFOS with 95% confidence intervals were presented in figure form, and this also shows a clear pattern of increasing TC concentrations with increasing PFOS exposure.

The authors also calculated ORs for having an elevated TC concentration for PFOS quartiles 2-4 compared to quartile 1. Here, a TC value of ≥240 mg/dl was used to define elevated TC. This value has historically been used for defining high TC (US DHHS, 2005), and 15% of the study population were above this level. The results of these analyses are shown in Table 6.1.13. Samples sizes in each quartile were not provided, so they were estimated using the total sample size divided by four. Here, the OR in quartile 2 is statistically significant, and the LOAEC is 16.4 ng/ml. BMD calculations were not performed using these data because both the glst and US EPA BMDS require an estimate of the disease prevalence in each exposure group, data that were not provided. In some instances, ORs can be used to estimate disease prevalence. In this study however, the prevalence of the outcome was fairly high (e.g., >10%) and it is unclear whether, and by how much, the ORs here might lead to an overestimation of risk or prevalence.

Table 6.1.13. Odds ratios for an elevated serum TC level by quartiles of serum PFOS concentrations (ng/ml) from Steenland et al. (2009). The LOAEC is highlighted in blue.

Quartile	PFOS[1]	N	OR	95% CI
1	6.6	11,574	1.00	Ref
2	16.4	11,574	1.14	1.05-1.23
3	23.8	11,574	1.28	1.19-1.39
4	34.0	11,574	1.51	1.40-1.64

Abbreviations: CI, 95% confidence interval; OR, odds ratio; Ref, reference

[1] Category midpoints for PFOS serum concentrations (ng/ml) except for the highest quartile which is the midpoint based on digitized data from Figure 3 of Steenland et al. (2009)

While the Steenland et al. (2009) study only included adults, another study in the C8 area performed the same analyses in children. Frisbee et al. (2009) was a cross-sectional study in the C8 area that measured serum PFOS and serum lipid concentrations in 12,476 children and adolescents ages 1-18 years old. Both studies enrolled participants and collected serum samples in the same years (2005-2006). Mean (±SD) serum PFOA and PFOS concentrations were 69.2 (±111.9) ng/ml and 22.7 (±12.6) ng/ml, respectively, which were similar to those reported in the C8 study of adults discussed above (Steenland et al., 2009). In linear regression models with adjustments for age, sex, BMI, fasting, and exercise, increasing PFOS serum concentrations were associated with increases in TC and LDL. The actual regression coefficients were not provided but the authors reported that the mean differences in TC and LDL levels in the 5th compared to the 1st quintile of PFOS were 8.5 mg/dl and 5.8 mg/dl, respectively. For PFOA, the corresponding values were lower: 4.6 mg/dl and 3.8 mg/dl, respectively. Figures showing clear increases in mean TC and LDL levels with increasing quintiles of PFOS were provided. Somewhat similar patterns were seen for PFOA, although the magnitude of these associations appear less strong than for PFOS. Mean HDL concentrations also seemed to generally increase with increasing quintiles of PFOS, although the dose-response pattern was more variable (i.e., the increases were not monotonic across all exposure levels).

ORs for having elevated TC by quintiles of PFOS were presented, and are shown in Table 6.1.14. Here, the cut-off for elevated TC was based on the American Heart Association guideline for children of >170 mg/dl (Benjamin et al., 2017). ORs were presented for PFOS quintiles 2-5, with quintile 1 as the reference group. Quintile cut-off points or medians were not provided, but the medians could be estimated based on digitization of the article's Figure 3. As seen, the LOAEC was 16 ng/ml, which is very close to the LOAEC of 16.4 ng/ml seen in the C8 adults.

Table 6.1.14. Odds ratios for elevated serum TC by quintiles of serum PFOS (ng/ml) from Frisbee et al. (2009). The LOAEC is highlighted in blue.

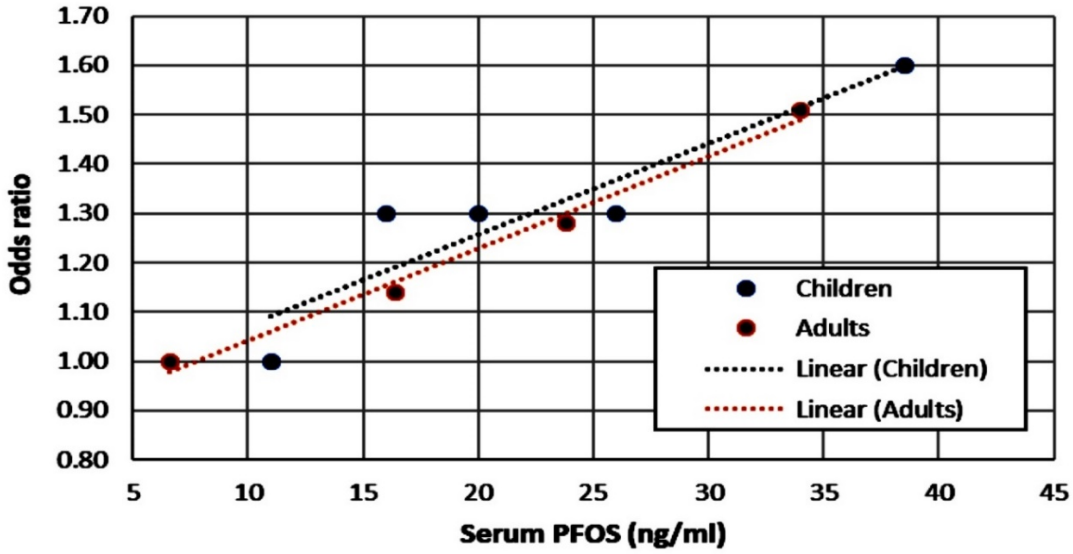
Quintile	PFOS[1]	N	OR	95% CI
1	11	2,495	1.0	Ref
2	16	2,495	1.3	1.1-1.4
3	20	2,495	1.3	1.2-1.5
4	26	2,495	1.3	1.2-1.6
5	38	2,495	1.6	1.4-1.9

Abbreviations: CI, 95% confidence interval; OR, odds ratio; Ref, reference

[1] Category medians for PFOS serum concentrations (ng/ml) based on digitized data from the paper's Figure 3

In order to help explore whether children might be more susceptible to the lipid-altering effects of PFOS than adults, OEHHA compared the PFOS-TC ORs reported in Steenland et al. (2009) to those in Frisbee et al. (2009). The ORs from both studies are shown in Figure 6.1.1. Unweighted linear regression slopes are also shown and fit both sets of data well (R^2s >0.80). As seen, the dose-response slopes for adults and children are very similar, suggesting that based on PFOS serum levels, children are not more susceptible to the effects of PFOS on TC than adults.

Figure 6.1.1. Odds ratios for elevated serum TC by quantiles of serum PFOS (ng/ml) in adults Steenland et al. (2009) and children Frisbee et al. (2009) from the C8 study area



Starling et al. (2014):

This was a cross-sectional analysis of 891 pregnant women enrolled in the Norwegian Mother and Child (MoBa) Cohort Study in 2003–2004. Non-fasting plasma samples were obtained at mid-pregnancy and analyzed for 19 different PFAS including PFOA and PFOS. Plasma concentrations of TC, LDL, HDL, and TGs were also measured. Median (IQR) PFOA and PFOS concentrations were 2.25 (1.66-3.03) ng/ml and 13.03 (10.31-16.60) ng/ml, respectively. The authors reported that each lognormal increase in PFOS was associated with an increase in TC of 8.96 mg/dl (95% CI, 1.70-16.22). These results were adjusted for age, pre-pregnancy BMI, nulliparous or inter-pregnancy interval, duration of breastfeeding, parity, education, smoking at mid-pregnancy, gestational weeks at blood draw, and oily fish consumption. The association between PFOA and TC was not statistically significant (each lognormal increase was associated with a 2.58 mg/dl (95% CI, -4.32-9.47) increase in TC.

The mean differences in TC for PFOS quartiles 2-5 compared to quartile 1 are shown in Table 6.1.15. As seen, none of these mean differences was statistically significant. As such, no NOAEC or LOAEC could be identified.

OEHHA entered the data in Table 6.1.15 into the US EPA BMDS, using various dose-response models. The SDs of the mean differences were estimated using the confidence intervals provided in the publication and the equations above. The absolute mean TC concentration (in mg/dl) in PFOS quartile 1 was provided in the publication. Using this, the absolute mean TC concentrations in the other PFOS quartiles could be estimated by adding the mean differences in TC reported for these other quartiles to the absolute mean TC concentration in quartile 1.

The mean difference in TC in PFOS quartile 4 compared to PFOS quartile 1 of 7.59 mg/dl represents about a 3.7% increase in mean TC across these quartiles. Because of this, OEHHA selected a 1% increase in TC, rather than a 5% or 10% increase, as the BMR. Using this BMR, the Hill model gave a BMD_{01} and $BMDL_{01}$ of 14.5 and 12.3 ng/ml, respectively (p-value for model fit=0.37). Fit was not improved with the other models.

A potential weakness of the Starling et al. (2014) study for dose-response assessment is that a statistically significant association was seen between PFOS and **increasing** concentrations of HDL (the “good cholesterol”). For example, the increases in TC, LDL, and HDL for each lognormal increase in PFOS were 8.96 mg/dl (95% CI, 1.70-16.22), 6.48 (95% CI, −0.07-13.03) and 4.39 (95% CI, 2.37-6.42), respectively. Since HDL is a component of TC, these results suggest that a fairly substantial portion of the effect seen for TC is due to HDL and therefore may not necessarily be adverse. Another potential weakness is that PFOS was highly correlated with several other PFAS (several Spearman correlation coefficients >0.60). In addition, the authors noted that the PFOS-TC association was attenuated after adjustment for these other PFAS. Further details of this particular analysis were not provided, making it difficult to evaluate the overall importance of this effect.

Table 6.1.15. Mean difference and absolute mean serum TC concentrations by quartiles of serum PFOS concentrations (ng/ml) from Starling et al. (2014)

			Mean differences (mg/dl)				Absolute means (mg/dl)		
Quartile	PFOS[1]	N	Mean difference	CI_L	CI_U	SD	Mean	CI_L	CI_U
1	5.2	223	0	Ref		41.26	207.59	Ref	
2	11.7	223	-3.35	-10.34	3.64	37.64	204.24	197.25	211.23
3	14.8	223	3.06	-4.93	11.05	43.02	210.65	202.66	218.64
4	20.5	223	7.59	-0.42	15.60	43.13	215.18	207.17	223.19

Abbreviations: CI_L, lower 95% confidence interval; CI_U, upper 95% confidence interval; Ref, reference; SD, standard deviation

[1] The midpoint of each PFOS category for quartiles 1-3. For quartile 4, the upper border was not provided so the midpoint between the 75th and 95th percentile was used.

Summary: PFOS PODs

A summary of the PODs derived from the candidate studies identified by OEHHA are shown in Table 6.1.16. These PODs ranged from 12.3 to 24.1 ng/ml in serum (mean = 17.9 ng/ml). After removing the two LOAECs (both from C8 studies), the range remained the same but the average changed slightly (19.0 ng/ml). The lowest POD was the $BMDL_{01}$ of 12.3 ng/ml calculated using the data in Norwegian pregnant women from Starling et al. (2014). The highest was the $BMDL_{10}$ of 24.1 ng/ml generated by Dong et al. (2019) from the data in adults in US NHANES using the hybrid approach.

Table 6.1.16. Summary of potential PODs for PFOS

Candidate studies	Effect	Method	POD/notes
Immunotoxicity			
Faroe Islands (Grandjean et al., 2017a; Budtz-Jorgensen and Grandjean, 2018)	Diphtheria antibody levels	NOAEC	NOAEC = 20.6 ng/ml
	Tetanus antibody levels	NOAEC	Data not available
	Diphtheria antibody levels	BMDS: Hill and Piecewise models	$BMDL_{05}$ outside observed range; large BMD:BMDL ratios
	Tetanus antibody levels	BMDS: Hill and Piecewise models	$BMDL_{05}$ outside observed range; large BMD:BMDL ratios
Lipid Concentrations			
Dong et al. (2019)	Mean TC levels	NOAEC/LOAEC	Information not provided
	Mean TC levels	BMDS	Information not provided

Candidate studies	Effect	Method	POD/notes
	Mean TC levels	Hybrid model	BMD_{10} = 44.2 ng/ml $BMDL_{10}$ = 24.1 ng/ml
Steenland et al. (2009)	Elevated TC ORs	NOAEC/LOAEC	No NOAEC **LOAEC = 16.4 ng/ml**
	Elevated TC ORs	BMDS/glst	Prevalence data not provided
Frisbee et al. (2009)	Elevated TC ORs	NOAEC/LOAEC	No NOAEC LOAEC = 16 ng/ml
	Elevated TC ORs	BMDS/glst	Prevalence data not provided
Starling et al. (2014)	Mean TC levels	NOAEC/LOAEC	Categorical results not statistically significant
	Mean TC levels	BMDS: Hill model	BMD_{01} = 14.5 ng/ml $BMDL_{01}$ = 12.3 ng/ml

OEHHA selected the LOAEC of 16.4 ng/ml from the Steenland et al. (2009) study of PFOS and elevated TC in adults from the C8 study area as the basis of its ADD calculations. This study offers the following advantages for dose-response and risk assessment calculations.

Very large sample size (N=46,294). This is by far the largest of the candidate studies OEHHA reviewed for its PFOS POD calculations. The very large sample size helps reduce the probability that findings are due to chance, allows for the detection of relatively subtle effect sizes, and helps to increase the likelihood that study findings are mostly generalizable. In this study, associations were evaluated in PFOS quartiles that included over 10,000 people each. This large size allowed for the calculations of ORs that were much more precise than in any other study.

Valid method for assessing exposure. This study assessed PFOS exposure using a well-accepted and validated method: serum PFOS concentrations. The long half-life of PFOS in serum suggests that a single serum measurement is likely to provide an accurate and precise indication of a person's true long-term exposure. In addition, there was no indication in this study that the serum samples were collected, or that PFOS concentrations were measured, in a way that would likely lead to differential bias and false positive effects. As mentioned for PFOA, non-differential misclassification of exposure can potentially bias ORs in the middle exposure categories (i.e., quartiles 2-3) away from 1.0. However, given the very large sample size of this study and the highly precise ORs that were reported, it is unlikely that this particular bias was large enough to have had a major impact on the statistical significance of the OR at the LOAEC.

Clinically relevant outcome. The cutoff point used to define elevated TC in Steenland et al. (2009) was based on a clinical reference level published by the American Heart Association (Benjamin et al., 2017). A TC level ≥240 mg/dl is a well-known risk factor for heart disease and stroke (Benjamin et al., 2017). The OR at the LOEAC was 1.14 (95% CI, 1.05-1.23). Although ORs can overestimate prevalence ratios when outcomes are common, this OR still likely represents a greater than 10% increase in the number of people with high TC at this exposure level. This is important because exposure levels near the LOAEC (16.4 ng/ml) are common in

the US general population (Dong et al., 2019). As such, this >10% increase likely represents large numbers of people. Overall, while the relatively small changes in mean TC levels seen with increasing PFOS exposure levels may not affect many people on an individual basis, the population effects of these small changes, given that TC is a major risk factor for cardiovascular disease, are likely to be widespread and large.

The two major components of TC are LDL (the "bad" cholesterol) and HDL (the "good" cholesterol). OEHHA selected TC rather than LDL for its critical effect because more studies evaluated TC than LDL, and a somewhat more robust, detailed, and consistent body of evidence was seen for this outcome. Given this, it should be noted that a number of studies have identified associations between PFOS and LDL (Table A7.9; US EPA (2016d)), and these support OEHHA's conclusion that the effects of PFOS on TC are adverse. In Steenland et al. (2009), the most informative results for PFOS and LDL and HDL were presented in the publication's Figures 4 and 5. It can be clearly seen in these figures that PFOS was strongly associated with LDL but not with HDL. This is strong evidence that the effects seen for TC are primarily due to LDL (i.e., its "bad" component) and should therefore be considered adverse.

Consistency of findings. Associations between PFOS and TC were seen both in the C8 study of adults (Steenland et al., 2009) and in the C8 study of children (Frisbee et al., 2009). The results of these two studies, and the PODs derived from them, were almost identical. In addition, the authors of these two studies used multiple different methods to evaluate associations (e.g., linear regression analyses, ORs), and the results of all of these methods were consistent with a statistically significant association between PFOS and TC. The results of these two studies are also consistent with a large number of other studies that have identified associations between PFOS and TC, or related outcomes like LDL, which are reviewed in Section 5.3. A number of studies in non-pregnant adults published after OEHHA's initial literature search (up to January 2, 2020) have also identified evidence of associations between PFOS and increasing TC and LDL (see Appendix 7, Table A7.29). These include three large high quality studies (Canova et al., 2020; Fan et al., 2020; Li et al., 2020). Full references provided in Table A7.29). These studies involved mostly general population-based samples, used serum levels to assess both PFOS and lipid levels, included adjustments for multiple relevant confounders, and involved excellent statistical power. Overall, these studies provide strong additional support that PFOS alters serum lipid levels.

No major confounding identified. The findings in Steenland et al. (2009) were adjusted for a number of factors that could potentially affect TC levels, including age, gender, BMI, socioeconomic status, exercise, smoking, and alcohol consumption. People who were taking cholesterol-lowering medications were excluded from the study. The authors also reported that findings were similar in people who did and did not fast before serum collection. A number of other factors can affect TC levels (e.g., certain genetic disorders, diabetes), but OEHHA found no indication that these were prevalent enough, or related strongly enough to both TC and PFOS, to cause important confounding. Consumption of a high fat diet or high total caloric intake could potentially be related to both elevated TC and higher PFOS exposure, but these could also be in the causal pathway. In addition, they are strongly related to the factors that were controlled for (BMI, smoking, and exercise) and therefore unlikely to have been fully responsible for the PFOS-TC associations reported in this study.

LOAEC unlikely to be affected by PFOA. Potential confounding by PFOA is a concern in this particular study because of the substantial environmental contamination of PFOA that occurred in the study area. While average PFOS serum levels in the study participants were similar to

those seen in NHANES (a mostly general population sample), serum levels of PFOA were markedly higher than general population levels. For example, while the mean (±SD) serum concentrations of PFOA and PFOS in the Steenland et al. (2009) study were 80.3 (±236.1) ng/ml and 22.4 (±14.8) ng/ml, respectively, the corresponding values in US NHANES during approximately the same years were 4.6 and 25.3 ng/ml, respectively (Nelson et al., 2010). Importantly, the correlation between PFOS and PFOA was only modest (R=0.32) in the C8 study area. As noted above, this is markedly lower than the correlations seen in other studies. In addition, the magnitude of the association between PFOS and TC appeared to be generally greater than that for PFOA and TC. For example, the regression coefficients for each lognormal increase in exposure was 0.02660 (SD=0.00140; $p < 0.05$) for PFOS, but >2-times lower for PFOA (β=0.01112; SD=0.00076; $p < 0.05$). Similarly, the OR for elevated TC in 4th versus the 1st quartile of exposure was lower for PFOA than for PFOS (ORs of 1.38 (95% CI, 1.28-1.50) and 1.51 (95% CI, 1.40-1.64), respectively). Overall, the fact that the associations between PFOA and both the exposure of interest here (PFOS) and outcome of interest here (TC) were only modest suggests that PFOA is unlikely to be the sole cause of the elevated ORs seen between PFOS and TC. In fact, the authors did note that, "When both PFOA and PFOS were considered together in the same model for total cholesterol, the effect of each was attenuated (20%–30%), but both continued to show the same monotonic, or nearly monotonic, trend of increasing cholesterol with increasing fluorocarbon." Further details of this particular analysis were not provided. However, given the very large sample size of this study (N=46,294), it is unlikely that a 20-30% reduction in the effect sizes would have had an important change in the statistical significance of the OR reported at the LOAEC.

No obvious selection bias. The C8 study has been estimated to include >80% of all residents of the affected areas. This high participation rate suggests that major errors due to selection bias are highly unlikely. In other studies, participation rates were much lower or participation rates were unclear or not reported, making it more difficult to evaluate the potential impact of selection bias in those studies.

Reverse causality unlikely. The Steenland et al. (2009) analyses were cross-sectional, and a common criticism of cross-sectional studies is the possibility of reverse causation. However, most of the convincing evidence regarding PFAS and reverse causation has involved other health outcomes (e.g., kidney function, time to pregnancy, birth weight, and early menopause) (Fei et al., 2012; Dhingra et al., 2017). With regards to serum lipids, one hypothesis is that higher serum cholesterol levels could lead to higher serum PFAS concentrations through the displacement of protein-bound PFAS from β-lipoprotein or albumin (Kerger et al., 2011). However, while PFAS can bind to human albumin (Han et al., 2003; Chen and Guo, 2009; Kerger et al., 2011), the major transport proteins for cholesterol in blood are lipoproteins, not albumin (Feingold and Grunfeld, 2018). And, while cholesterol binding to β-lipoprotein is significant (e.g., to form LDL), PFAS binding to lipoproteins is fairly minimal (Butenhoff et al., 2012c).

Another hypothesis is that serum lipid and serum PFOS concentrations are related because both bile acids (an important elimination route for cholesterol) and PFOS undergo significant enterohepatic circulation and share the same transport proteins involved in this process (Zhao et al., 2015a). In fact, studies in humans have shown that blocking the enterohepatic circulation of bile acids using cholestyramine, which can increase the fecal elimination of cholesterol, can also increase the fecal elimination of PFOS (Genuis et al., 2010; Genuis et al., 2013). Importantly though, whether this effect can lead to large changes in PFOS serum concentrations or large changes in the body burden of PFOS is unknown. This is important

because the associations seen in Steenland et al. (2009) involved large differences in the serum concentrations of PFOS but only small differences in the serum concentrations of TC. For example, as seen in the publication's Figure 3, an approximately 1,000% increase in serum PFOS was associated with only about a 5% increase in serum TC. This suggests that if enterohepatic circulation was responsible for the Steenland et al. (2009) findings, this mechanism would have to have an extremely large impact on serum PFOS, something that OEHHA was unable to find quantitative evidence to support.

OEHHA identified additional evidence that the associations between PFAS and serum cholesterol levels identified by Steenland et al. (2009) and others are not due to reverse causality. Some of this evidence involves PFOA rather than PFOS. However, the associations between TC and PFOS reported by Steenland et al. (2009) were very similar to those they reported for TC and PFOA. Because of this, and because OEHHA could not find any rational explanation why a true causal association would exist for one of these chemicals but reverse causality would be important for the other, evidence relating to either PFOS or PFOA are presented here.

The evidence that PFAS-TC associations are not due to reverse causality include the following:

- In Everds and Kennedy (2015), PFOA was administered orally to two groups of hamsters, one fed a normal diet and one fed a high fat diet, and serum PFOA concentrations were then compared between these two groups. PFOA was administered in the form of ammonium perfluorooctanoate, at doses of 0.1, 1.0, and 10 mg/kg, for 30 days. The researchers reported that while serum cholesterol levels were about 40-100% higher in the hamsters fed a high fat diet, the differences in serum PFOA concentrations between the hamsters on high fat and normal diets at each PFOA intake level was small (generally <10%). Overall, this finding argues against the hypothesis that increases in serum lipids can cause major increases in serum PFOA.

- Associations between PFOA and TC in the C8 study area have not only been seen in analyses based on actual measured serum PFOA levels, but also in analyses based on modeled PFOA exposures. In Winquist and Steenland (2014), estimates of PFOA concentrations in local air, surface water, and groundwater were generated using an environmental fate and transport model. These were combined with the study participant's residential history, drinking water sources, water consumption rates, and occupational exposures, and entered into a toxicokinetic model to generate yearly PFOA serum concentration estimates for each participant (N=32,254). Participants were defined as having high cholesterol if they reported taking cholesterol-lowering medications, and the year this condition started was defined as the year the participant reported that they were first told by a medical care provider they had high cholesterol. Analyses were adjusted for age, sex, education, race, smoking, and BMI. The researchers found that increasing quintiles of modeled cumulative PFOA exposure were associated with hazard ratios for high cholesterol of 1.00 (reference), 1.24 (95% CI, 1.15-1.33), 1.17 (95% CI, 1.09-1.26), 1.19 (95% CI, 1.11-1.27), 1.19 (95% CI, 1.11-1.28) (p-test for trend=0.005). Overall, because these analyses were based on external metrics of PFOA exposure and not on serum PFOA concentrations, the associations reported here would not be due to any direct effect that serum lipids would have on serum PFOA and therefore would not be due to reverse causality.

- With regards to PFOS, the Steenland et al. (2009) researchers evaluated reverse causality by comparing PFOS serum concentrations in people taking cholesterol-lowering medications to PFOS serum concentrations in people not taking these medications. These analyses were adjusted for gender, age, BMI, race, and other factors. The rationale behind this analysis was that most people who were taking cholesterol-lowering medications would be doing so for reasons unrelated to their PFOS exposure (e.g., elevated cholesterol levels due to diet, lack of exercise, or genetics). Because of this, if higher serum lipid levels did indeed cause higher serum PFOS levels, the use of these medications (and the decreased cholesterol levels that would be associated with this use) would be expected to lead to a major decrease in serum PFOS. However, the authors reported that serum PFOS concentrations were not lower in those taking cholesterol-lowering medications, providing evidence that higher serum lipid levels were not causing higher serum PFOS.

- Finally, perhaps the strongest evidence that reverse causation was not responsible for the effects seen in Steenland et al. (2009), is that an association between PFOS and higher TC levels has also been reported in a prospective study done in the C8 study area. In Fitz-Simon et al. (2013), 560 of the adult participants in the C8 study who provided serum for PFOS and TC measurements in 2005-2006, also provided serum for PFOS and lipid measurements in 2010. Over this time, the geometric mean serum concentration of PFOS in these participants fell from 18.5 ng/ml in 2005-2006 to 8.2 ng/ml in 2010. The authors found that a 50% decrease in serum PFOS over the study period was associated with a 3.2% (95% CI, 1.6-4.8) decrease in serum TC and a 5.0% (95% CI, 2.5-7.4) decrease in serum LDL. A statistically significant change in HDL was not seen. These findings provide strong evidence that reverse causality is not responsible for the PFOS-TC associations seen in the C8 area because there is a well-known reason why the PFOS exposures decreased in this study, and this reason is unrelated to any changes in serum lipids. In fact, serum TC and LDL levels in subjects in the lowest PFOS exposure group actually increased. Rather, the cause of the decreased PFOS levels was the major reductions in the production, use, and exposure to PFOS that took place throughout the US just before and during the years of the study (Dong et al., 2019; ATSDR, 2021). Given these well-known reasons for the declining PFOS exposure, the idea that the decreases in serum PFOS seen in this study were caused by declining TC or LDL levels is implausible.

Overall, based on the findings of Fitz-Simon et al. (2013) and the other data presented above, OEHHA has concluded that it is highly unlikely that reverse causality was responsible for the PFOS-TC associations reported by Steenland et al. (2009) and others.

Appropriate study design. Another criticism of cross-sectional studies is that because they measure exposure and outcome at the same time, the most relevant exposure period might be missed. However, as discussed above for PFOA, the half-life of PFOS is several years. As such, a single serum measurement of PFOS is likely to represent exposure over the long-term. In addition, the half-life of LDL cholesterol in plasma is fairly short, about three days (Daniels et al., 2009), and TC and LDL levels typically fall within days or weeks after beginning cholesterol-lowering medications. Overall, the long half-life of serum PFOS, combined with the short half-life of serum cholesterol suggest that the cross-sectional approach used in Steenland et al. (2009) is a valid method for assessing the dose-response relationship between PFOS and TC.

Acceptable Daily Dose Calculation

The ADD was calculated using the LOAEC of 16.4 ng/ml from the Steenland et al. (2009) study of PFOS and elevated TC in adults from the C8 study area, and applying the clearance factor of 3.9×10^{-4} L/kg-day (or 0.39 ml/kg-day) discussed in Chapter 4. An uncertainty factor (UF) of √10 for intraspecies variation was also applied in these calculations. The Steenland et al. (2009) study involved a very large population of adults and the participation rate was >80%. This large sample size and high participation rate suggests the study participants were a good representation of the study area as a whole, and likely included a diverse group of people in terms of ages, health status, smoking and other chemical exposures, nutrition, socioeconomic status, and other factors. As discussed above, a separate study in this area examined the relationship between PFOS and TC in children (Frisbee et al., 2010), and OEHHA's evaluation found that based on serum levels, the PFOS-TC relationship was essentially the same in children as in adults. A UF of √10 rather than 1 for intraspecies variation was applied because the C8 study population was not diverse in terms of race or ethnicity. In addition, it did not examine other potential susceptibility factors such as obesity or genetics. Some data suggest that obesity or certain genetic polymorphisms might increase susceptibility to PFAS (Ghisari et al., 2017; Jain and Ducatman, 2018b; Jain and Ducatman, 2019; Wen et al., 2019a). However, these data were limited (for a variety of reasons) and OEHHA was unable to identify consistent or convincing evidence that this susceptibility might be greater than a factor of √10.

OEHHA also applied the LOAEC to NOAEC UF of √10 because the Steenland et al. (2009) ORs involved an LOAEC rather than an NOAEC. A full LOAEC to NOAEC UF of 10 was not used for this ADD calculation because the very large sample size and the very precise nature of the ORs allowed the study to detect relatively small effect sizes with excellent statistical power. As such, an LOAEC could be detected at a fairly low PFOS exposure level. In fact, the LOAEC occurred in the second highest PFOS exposure quartile, which had a range of 13.3 to 19.5 ng/ml. These levels are very close to those commonly seen in the general US population (Dong et al., 2019). Overall, given the very high precision of the Steenland et al. (2009) results, it seems highly unlikely that a future study (at least one done in the near future) will identify an NOAEC with good precision that is 10-times lower than the Steenland et al. (2009) LOAEC.

The ADD for PFOS was calculated as:

ADD = (POD × CF) ÷ UF = (16.4 ng/ml × 0.39 ml/kg-day) ÷ 10 = **0.64 ng/kg-day**.

Animal Studies

Similar to PFOA, the major noncancer effects of PFOS in experimental animals are liver, immune system, thyroid, and developmental/reproductive toxicities. Liver, thyroid, and immune system toxicity appear to be the most sensitive endpoints. OEHHA derived ADDs from animal studies for comparison with those derived from human studies. Among the animal studies that exhibited a dose-response, Table 6.1.17 lists candidate critical studies for PFOS that report serum/plasma levels of PFOS and that OEHHA considers are of sufficient quality. BMD modeling was performed on the candidate critical study datasets in Table 6.1.17, using plasma/serum concentration as the dose metric, and the results are presented in Table 6.1.18. As with PFOA, ADDs are not calculated for PFOS due to the availability of human data for derivation of a health-protective concentration.

Table 6.1.17. Candidate critical studies for noncancer effects of PFOS in animals

Reference	Sex/Species	Dose/Route of Exposure/Duration	Endpoint	NOAEL/LOAEL (mg/kg-day)	NOAEL/LOAEL/ BMDL (mg/L)
Liver Toxicity					
Xing et al. (2016)	Male C57BL/6 mice (10/dose)	0, 2.5, 5, or 10 mg/kg-day via gavage for 30 days	↑ relative liver weight	LOAEL: 2.5	LOAEL: 70.2 $BMDL_{1SD}$: 8.9
Lai et al. (2018)	Female CD-1 mice (≥4/dose)	0, 0.3, or 3 mg/kg-day via gavage for 7 weeks	↑ liver triglycerides	LOAEL: 0.3	LOAEL: 33.8 $BMDL_{1SD}$: 11.2
NTP (2019b)	Male and female Sprague Dawley rats (10/dose)	0, 0.312, 0.625, 1.25, 2.5, or 5 mg/kg-day via gavage for 28 days	↑ relative liver weight	LOAEL: 0.312	<u>Males</u> LOAEL: 23.7 $BMDL_{1SD}$: 4.7 <u>Females</u> LOAEL: 30.5 $BMDL_{1SD}$: 44.1
Immunotoxicity					
Zhong et al. (2016)	Pregnant C57BL/6 mice (12/dose)	0, 0.1, 1, or 5 mg/kg-day via gavage from GD 1-17	↓ splenic natural killer cell activity in male pups at 8 weeks of age	NOAEL: 0.1	NOAEL: 3.79
Dong et al. (2009)	Male c57/BL6 mice (10/dose)	0, 0.008, 0.083, 0.417, 0.833, or 2.08 mg/kg-day via gavage for 60 days	↓ plaque forming cell response	NOAEL: 0.008	NOAEL: 0.674 $BMDL_{1SD}$: 5.1 $BMDL_{10}$: 0.75
Thyroid Toxicity					
NTP (2019b)	Male and female Sprague Dawley rats (10/dose)	0, 0.312, 0.625, 1.25, 2.5, or 5 mg/kg-day via gavage for 28 days	↓ free and total T4	LOAEL: 0.312 (both sexes)	<u>Males</u> LOAEL: 23.7 $BMDL_{1SD}$: 4.8 (free T4) 5.2 (total T4)[a] <u>Females</u> LOAEL: 30.5 $BMDL_{1SD}$: 12.1 (free T4)[a] 8.2 (total T4)

GD, gestation day; T4, thyroxine

[a] Highest dose group excluded in BMD modeling

While the NOAEL for immunotoxicty from Zhong et al. (2016) was lower than the BMDLs from the modeled datasets in Table 6.1.17, the reported serum values in pups (measured at 4 weeks and 8 weeks of age) would not be representative of the serum concentrations during the dosing period (GD1-17). Pups at 4 and 8 weeks of age would be expected to have lower serum concentrations than during the period of active dosing.

Table 6.1.18. BMD modeling of noncancer endpoints from candidate PFOS animal studies

Study	Endpoint	Model	Goodness of fit p-value	BMD_{1SD} (mg/L)	$BMDL_{1SD}$ (mg/L)
Xing et al. (2016)	↑ relative liver weight	Linear	0.483	12.8	8.9
Lai et al. (2018)	↑ liver triglycerides	Linear	0.053	15.5	11.2
NTP (2019b)	↑ relative liver weight (females)	Exponential2	0.374	55.1	44.1
NTP (2019b)	↑ relative liver weight (males)	Exponential4	0.679	6.9	4.7
NTP (2019b)	↓ free T4 (males)	Exponential4	0.224	7.5	4.8
NTP (2019b)	↓ total T4 (males)[a]	Exponential4	0.829	7.9	5.2
NTP (2019b)	↓ free T4 (females)[a]	Exponential4	0.330	20.8	12.1
NTP (2019b)	↓ total T4 (females)	Exponential4	0.763	12.5	8.2
Dong et al. (2009)	↓ plaque forming cell response[a]	Exponential5	0.742	10.0	5.1
Dong et al. (2009)	↓ plaque forming cell response[a]	Hill	0.776	1.56[b]	0.750[b]

[a] Highest dose group excluded
[b] Data modeled with a BMR of 10%, therefore these values correspond to BMD_{10} and $BMDL_{10}$

OEHHA identified Dong et al. (2009) as the critical toxicity study for deriving a notification level (NL) recommendation for the noncancer effects of PFOS (OEHHA, 2019). At the time, BMD modeling of the data for plaque forming cell response from (Dong et al., 2009) did not provide any adequate models. Therefore, the NOAEL of 0.008 mg/kg-day, corresponding to a serum concentration of 0.674 mg/L, was selected as the POD. The data are summarized in Table 6.1.19. Here, an additional analysis of this dataset indicates an acceptable model fit can be obtained with BMD modeling when the highest dose is excluded. Excluding the highest dose is appropriate for this dataset since there is a 260-fold difference between the lowest and the highest dose, and inclusion of the highest dose data appeared to compromise the data fit in the low dose range.

Table 6.1.19. Plaque forming cell response in male C57BL/6 mice exposed to PFOS via oral gavage for 60 days (Dong et al., 2009)

Dose (mg/kg-day)	Serum Concentration (mg/L)	Plaque Forming Response[a] (PFC/10^6 spleen cells)
0	0.048 ± 0.014[b]	597 ± 64[b] (202)
0.008	0.674 ± 0.166	538 ± 52 (164)
0.083	7.132 ± 1.039	416 ± 43* (136)
0.417	21.638 ± 4.410	309 ± 27* (85)
0.833	65.426 ± 11.726	253 ± 21* (66)
2.08	120.670 ± 21.759	137 ± 16* (51)

[a] Data taken from New Jersey DWQI (2018). Authors state they received numerical data via personal communication with GH Dong.
[b] Mean ± SEM (N=10/dose); SD in parentheses
* p <0.05, reported by study authors

OEHHA typically uses a BMR of one standard deviation (1 SD) for continuous data when it is uncertain what level of response is biologically significant (US EPA, 2012). This results in a $BMDL_{1SD}$ of 5.1 mg/L (Table 6.1.18). However, when the SDs are relatively large, as for the Dong et al. (2009) dataset (Table 6.1.19), a BMR of 1 SD may represent a much larger change than 10%, which was historically a level to which a 1 SD change was comparable (Davis et al., 2011). Thus, OEHHA also modeled the data using a BMR of 10%, which resulted in a $BMDL_{10}$ of 0.75 mg/L.

6.2. Cancer Dose-Response Analyses and Cancer Potency Derivation

6.2.1. Perfluorooctanoic Acid

Because several high quality human studies were available, dose-response analyses were performed using human rather than animal data. As discussed above, the strongest and most consistent human evidence linking PFOA to cancer involves studies of kidney cancer. Based on evaluations of statistical power, generalizability, potential bias and confounding, and other factors, OEHHA selected the human studies by Shearer et al. (2021) and Vieira et al. (2013) for cancer dose-response analyses. Descriptions of these two studies, and evaluations of their potential strengths and weaknesses are provided in the following sections. Two other epidemiologic studies identified associations between PFOA and kidney cancer (Steenland and Woskie, 2012; Barry et al., 2013). The high exposure occupational study by Steenland and Woskie (2012) was not used for dose-response analysis because information on a range of exposures more relevant to the general population were available from the Shearer et al. (2021) and Vieira et al. (2013). The study by Barry et al. (2013) was not used for dose-response analysis because it was performed in the same study area as the Vieira et al. (2013) study and these two studies likely involved a number of the same participants. Vieira et al. (2013) was selected over the Barry et al. (2013) because it presented dose-response data using a more appropriate exposure metric (discussed in further detail below).

Shearer et al. (2021):

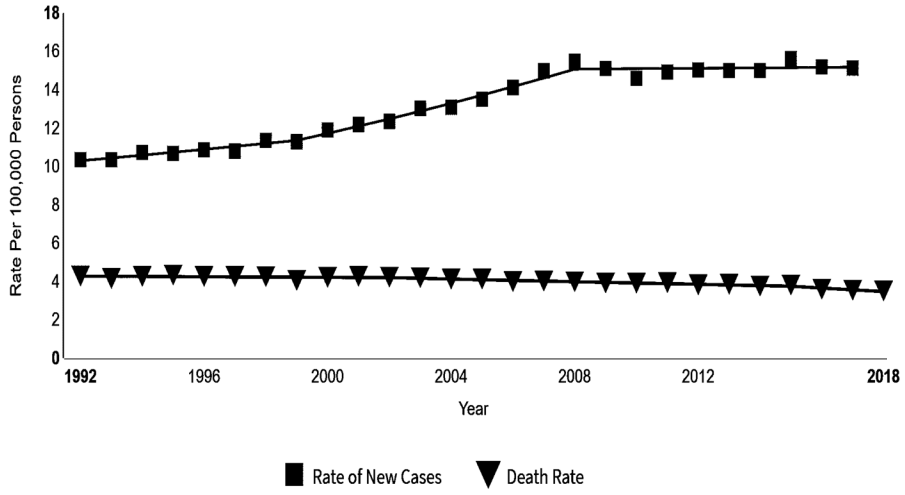
Study design: The study by Shearer et al. (2021) is a case-control study nested within the National Cancer Institute's (NCI) Prostate, Lung, Colorectal, and Ovarian Screening Trial (PLCO). The PLCO is a randomized clinical trial of the use of serum biomarkers for cancer screening. Participants were recruited via convenience sampling from 10 medical centers at various locations throughout the US (Table 6.1.17). The PLCO trial included approximately 150,000 people, with half of all participants randomized to the screening arm, and half randomized to the control arm. Participants in the screening arm had serum samples collected once at baseline during the years 1993-2002. These samples were processed and frozen within two hours of collection and stored at −70°C. A questionnaire was administered at baseline and was used to collect information on demographic variables and other cancer risk factors including smoking, BMI, medical history, and occupations. Participants were followed annually for an average of 8.9 years (range, 2-18 years). Incident cases of RCC (International Classification of Diseases-Oncology (ICD-O)-2 C64.9) were ascertained during the years 1996-2014 by medical record review of suspected cancers reported in the annual questionnaires, or from physicians or relatives, the National Death Index (NDI), or local cancer registries (Liao et al., 2017). The cases in the Shearer et al. (2021) study included all of the participants of the screening arm of the PLCO trial who were newly diagnosed with RCC during the follow-up period (N=326). All cases were histopathologically confirmed. Controls were selected from among participants of the PLCO trial screening arm who had never had RCC. These controls were individually matched to the RCC cases by age at enrollment, sex, race/ethnicity, study center, and year of blood draw. Concentrations of PFOA, PFOS, and other PFAS were measured in the baseline serum samples by the Centers for Disease Control and Prevention in the year 2018 using on-line solid phase extraction liquid chromatography isotope dilution tandem mass spectrometry. Laboratory personnel were blinded to the case-control status of the participants. Two case-control pairs were excluded due to missing PFAS concentrations. Results were presented for total PFOA and total PFOS, which were the sum of the concentrations of their respective isomers (i.e., n-PFOA and sum of the branched PFOA isomers for total PFOA, and n-PFOS and sum of perfluoromethylheptane sulfonic acid isomers for total PFOS). The study was funded by the National Cancer Institute (NCI), the US Department of Defense, and the National Institutes of Health (NIH).

Renal cell carcinoma (RCC): Kidney cancer is among the top ten cancers diagnosed in the US each year (ACS, 2020a). The American Cancer Society (ACS) has estimated that 73,820 new cases of kidney cancer were diagnosed in 2019, and that 14,770 deaths from kidney cancer occurred that year. The 5-year survival rate for kidney cancer is about 75%. RCC is the most common type of kidney cancer, and approximately 90% of all kidney cancers are RCCs. The major risk factors for kidney cancer are excess body weight and smoking, and the ACS has estimated that about half of all kidney cancers could be prevented by eliminating these two risk factors. Other risk factors include high blood pressure, chronic renal failure, and occupational exposure to certain chemicals such as trichloroethylene (TCE).

The incidence rate of kidney cancer in the US increased approximately 1-2% per year from 1992 to 2008. Since 2008, this rate has leveled off (Figure 6.2.1). The same general pattern has been seen for RCC, but with an average annual increase of more than 3% in the 12 years before 2008 (Saad et al., 2019). The exact reasons for the increases prior to 2008 are unknown but may be related to earlier detection through the increased use of abdominal CT scans, increasing obesity, or increases in certain occupational exposures, hypertension, or other risk factors (Saad et al., 2019). The reason for the leveling off after 2008 is also unknown. It is

unlikely that this leveling off is entirely due to changes in smoking or obesity since declines in smoking (and subsequent declines in lung cancer) began decades before 2008, and rates of obesity in the US have continued to increase well beyond 2008. Interestingly, US production and serum levels of PFOA declined sharply 5-10 years prior to the leveling off of RCC rates. Although it is unknown if and how much this leveling off may be due to decreases in PFOA exposure in the US, similar latency patterns following exposure cessation have been seen for other carcinogens, including smoking (Tindle et al., 2018).

Figure 6.2.1. Yearly incidence and death rates from kidney cancer in the US (SEER, 2020)



Demographics of the participants in Shearer et al. (2021): The RCC cases in the Shearer et al. study were unevenly distributed among the various recruitment centers, although no single site contributed more than 26% of all cases (Table 6.2.1). Compared to controls, cases were more likely to be obese (35.5% vs. 23.5%) and more likely to have hypertension (43.5% vs. 33.3%). Similar proportions of cases and controls were never, former or current smokers, and roughly 90% of both cases and controls were either former or never smokers.

Table 6.2.1. Number and percentage of cases and controls in Shearer et al. (2021) and in the PLCO trial by study center

	Shearer et al. (2021)		PLCO trial[1]
Center	**Controls N (%)**	**Cases N (%)**	**All participants N (%)**
Georgetown (Washington, DC)	15 (4.6)	15 (4.6)	8,108 (5.2)
Colorado	20 (6.2)	20 (6.2)	13,165 (8.5)
Hawaii	9 (2.8)	9 (2.8)	10,847 (7.0)
Henry Ford (Michigan)	37 (11.4)	37 (11.4)	24,665 (15.9)
Minnesota	84 (25.9)	84 (25.9)	28,862 (18.6)
Washington University (Missouri)	33 (10.2)	33 (10.2)	15,042 (9.7)
University of Pittsburgh (Pennsylvania)	40 (12.4)	40 (12.4)	16,930 (10.9)
University of Utah	27 (8.3)	27 (8.3)	14,387 (9.3)
Marshfield (Wisconsin)	46 (14.2)	46 (14.2)	16,740 (10.8)
University of Alabama	13 (4.0)	13 (4.0)	6,188 (4.0)

1. Data from Gren et al. (2009)

Exposure levels: The Shearer et al. (2021) serum samples were collected from 1993 to 2002. Geometric mean PFOA levels were somewhat higher in the later years of sample collection (geometric mean PFOA concentrations in the controls were 4.0, 4.3, and 4.6 ng/ml for the years 1993-95, 1996-97, and 1998-2002, respectively). The geometric mean in the 1998-2002 period is very close to the median PFOA level reported for participants age 60 and over in the 1999-2000 NHANES (4.8 ng/ml; 95% CI, 4.3-5.1 ng/ml), the first time PFOA was measured in a US nationwide survey. The similarity of these levels suggest that the Shearer et al. (2021) controls are a good representation of the general US population in terms of PFOA exposure.

Statistical analyses: Associations between PFOA and RCC were analyzed using baseline serum PFOA concentration as either a continuous or a categorical variable. In the categorical analyses, categories of PFOA were based on the quartiles of serum PFOA in the controls, and ORs for RCC were calculated using the lowest PFOA category as the reference group. ORs were adjusted for age, sex, race/ethnicity, study center, year of blood draw, estimated glomerular filtration rate (eGFR), BMI, smoking status, hypertension, prior freeze-thaw cycles of samples, and calendar year of blood draw. Wald tests were used to test for linear trends across the adjusted ORs, and were performed by modeling the within-category median of each PFOA quartile as a continuous variable.

In the analysis of serum PFOA as a continuous variable, PFOA was log2 transformed, and a linear regression analysis was used to calculate the RCC OR for each doubling of serum PFOA. Nonlinear trends were assessed by modeling logOR and the log2-transformed PFOA concentrations using a natural spline with three degrees of freedom.

Results: The ORs for the association between serum PFOA and RCC in the categorical analyses are shown in Table 6.2.2. A statistically significant increase in the odds of RCC was seen when comparing the highest to the lowest exposure category (OR = 2.63; 95% CI, 1.33-5.20). Although the ORs in the 2nd and 3rd highest exposure categories were not statistically significant, they were >1.0 and the p-test for trend across the ORs was statistically significant (p = 0.007).

Table 6.2.2. ORs for the association between PFOA serum concentrations and RCC in Shearer et al. (2021)

Exposure category (ng/ml)	Exposure category midpoint (ng/ml)	OR_a[1]	CI_L	CI_U	Case	Control	OR_u	SE
<4.0	2.0	1.00	Ref		47	81	1.00	
≥4-5.5	4.7	1.47	0.77	2.80	83	79	1.81	0.33
>5.5-7.3	6.4	1.24	0.64	2.41	69	83	1.43	0.34
>7.3-27.2	17.3	2.63	1.33	5.20	125	81	2.66	0.35

Abbreviations: CI_L, lower 95% confidence interval of OR_a; CI_U, upper 95% confidence interval of OR_a; OR_a, adjusted odds ratio; OR_u, unadjusted odds ratio; Ref, reference category; SE, standard error of the logOR
[1] p-trend = 0.007

In the analysis of PFOA as a continuous variable, a statistically significant association was seen between log2 PFOA and the odds of RCC, such that a doubling of PFOA was associated with an RCC OR of 1.71 (95% CI, 1.23-2.37). Analyses of log2-transformed PFOA using natural splines found no evidence of a significant non-linear relationship between PFOA and RCC risk.

The following sections discuss OEHHA's evaluations of the likelihood the Shearer et al. (2021) findings could have been due to bias (exposure misclassification, outcome misclassification, or selection bias) or confounding.

Exposure misclassification: Exposure was based on a single serum PFOA measurement in each participant. Significant bias could occur if people's serum PFOA concentrations change dramatically in a few days or months. As mentioned previously, serum measurements are a commonly used and widely accepted method for assessing PFOA exposure (NTP, 2016), and the half-life for PFOA in serum is in the order of years (Olsen et al., 2007; Mogensen et al., 2015b). This long half-life suggests that a single serum measurement is likely to provide an accurate and precise indication of a person's long-term PFOA exposure. Another important aspect of the Shearer et al. (2021) study is that the serum samples were collected years before cancer diagnoses. This prospective collection of exposure data helps alleviate concerns about reverse causation. In addition, the laboratory personnel measuring the PFOA serum concentrations were blinded to the case or control status of the study participants, which helps reduce concerns about researcher bias. Overall, there is no indication that the serum samples were collected, or that PFOA concentrations were measured, in a way that would have differed between the RCC cases and the controls. Because of this, potential misclassification of PFOA exposure, particularly in the highest exposure category, would most likely be non-differential, and therefore most likely bias results towards the null (i.e., in the direction of finding no association) (Jurek et al., 2005). Non-differential misclassification of exposure could have biased the ORs in the second and third PFOA quartiles away from 1.0. However, there is no indication that this bias would have a major impact on the dose-response slopes.

Outcome misclassification: Under or over-diagnosis of RCC is also possible, but major bias from this is unlikely. Although the Shearer et al. (2021) publication provided little information about case ascertainment, many of the details of this process can be found in other publications on the PLCO trial (Hayes et al., 2000; Prorok et al., 2000; Liao et al., 2017). Over-diagnosis of RCC is unlikely since all of the cases were histologically confirmed. Under-diagnosis (i.e., missed cases) is possible. However, because the serum samples used to measure PFOA were collected before cancer diagnosis, bias from missed cases is most likely non-differential and thus most likely towards the null. In addition, as discussed below in more detail, a comparison of the number of RCC cases in the Shearer et al. (2021) study to the number of cases expected based on US national rates suggests that under-diagnosis of RCC was not a major problem in this study.

Selection bias: The Shearer et al. (2021) study involved a convenience sample of mostly white non-Hispanic participants, and information on household income, education, or other socioeconomic indicators were not presented. While it is possible that the risks of RCC caused by PFOA vary by race, ethnicity, or socioeconomic status, OEHHA was unable to find any evidence showing that this is the case.

Follow-up rates were also not provided in the Shearer et al. (2021) publication. However, one way to evaluate the adequacy of follow-up is to compare the number of RCC cases ascertained in this study to the number of cases expected based on US national rates. Because Shearer et al. (2021) is essentially a population-based study, the PFOA levels in this study should be close to those in the general US population, and the number of cases should be similar to what would be expected based on US national rates.

The RCC rate in the US for the age groups included in the Shearer et al. (2021) study is about 50 cases per 100,000 person-years (Saad et al., 2019; SEER, 2020). Given an underlying cohort of approximately 74,000 people used for the Shearer et al. (2021) study, and an average follow-up period of 8.9 years, the number of cases expected based on US rates is:

74,000 people × 8.9 years × 50 cases per 100,000 person-years = 329 cases.

Although this number is only an estimate, its similarity to the actual number of cases in the Shearer et al. (2021) study (N=326) suggests that under-reporting, under-ascertainment, or poor follow-up were not major problems in this study.

Confounding: The ORs developed by Shearer et al. (2021) were adjusted for all of the important known risk factors for RCC including age, sex, BMI, smoking, and hypertension (IARC, 2019; ACS, 2020b)(. They were also adjusted for eGFR (high vs. low), which is a marker of non-malignant renal disease. The authors also presented a number of subgroup analyses that can be used to evaluate potential confounding or other forms of bias. Here, PFOA-RCC ORs were similar across subgroups of age, gender, BMI, hypertension (yes vs. no), eGFR (high vs. low), time from blood draw to cancer diagnosis (2-8 years vs. ≥8 years), and previous freeze-thaw cycle (none vs. ≥1). The similarity of the ORs within each of these subgroups is evidence that none of these factors caused major confounding. The PFOA-RCC OR was higher in current and former smokers than in never smokers. However, the confidence intervals for these ORs were wide and the difference between them was not statistically significant ($p = 0.24$).

Other known or possible risk factors for kidney cancer include certain chemical or occupational exposures such as arsenic (Ferreccio et al., 2013), TCE (IARC, 2014), asbestos (Smith et al., 1989), and cadmium (IARC, 2012b); medications such as acetaminophen or non-steroidal anti-inflammatory drugs (Karami et al., 2016); or genetic disorders such as von Hippel-Lindau disease or Birt-Hogg-Dube syndrome. Most of these known genetic disorders are too rare to cause important confounding. Arsenic is unlikely to cause confounding since, although it is strongly related to renal pelvis cancer, it is not strongly related to RCC (Ferreccio et al., 2013).

With regards to cadmium and asbestos, several studies have reported associations between these agents and kidney cancer. However, these findings are not consistent across all studies, and IARC has not established cadmium and asbestos as a sufficient cause of kidney cancer (IARC, 2012a; IARC, 2012b). TCE is an established cause of kidney cancer (IARC, 2014). Several studies, have reported an association between acetaminophen use and kidney cancer (Karami et al., 2016). Because there is little to no evidence that PFOA exposure is strongly related to asbestos, cadmium, or TCE exposure or to acetaminophen use, it is very unlikely that these agents could have caused the elevated ORs reported by Shearer et al. (2021). Even if there was some relationship between PFOA and these agents, the magnitude of these relationships would have to be very high, to the point of being implausible, to cause the PFOA-RCC OR of 2.63 reported for the highest PFOA exposure category in Shearer et al. (2021). The reason for this is that most studies linking asbestos, cadmium, TCE, and acetaminophen to kidney cancer report relative risks near 2.0, only in participants with very high exposure levels, levels that do not occur or are very rare outside of certain occupations or unusual medical settings (IARC, 2012a; IARC, 2012b; IARC, 2014). Relative risks for lower exposures to these agents are generally below 2.0. Because the relative risks between these agents and kidney cancer are not very high, and because only a small percentage of the general population are highly exposed to them, it is very unlikely they had important confounding effects (Axelson,

1978). Even if every person in the upper PFOA category was highly exposed to TCE, cadmium, asbestos, or acetaminophen and every person in the lower PFOA reference category was not (an extreme and implausible scenario), it would still be incredibly unlikely that confounding by these agents would cause the OR of 2.63 reported for the higher PFOA category. Smaller differences in TCE, cadmium, asbestos, and acetaminophen use or exposure between people in the higher and lower PFOA categories would only have small impacts on ORs. For example, given a relative risk between asbestos and RCC of 2.0 (Smith et al., 1989) and a prevalence of high asbestos exposure of about 4% in the general population (ATSDR, 2018b), even a 2-fold higher level of asbestos exposure in the highest PFOA exposure quartile would only cause an OR of about 1.04 (Axelson, 1978). This is well below the OR of 2.63 reported by Shearer et al. (2021).

One potential concern is the somewhat high percentage of RCC cases from the Minnesota study site: 25.9% of all RCC cases were from this site. In contrast, only 18.6% of all PLCO study participants were from the Minnesota site. The reason for this difference is unknown. The Minnesota study site is somewhat close to a former PFOA production plant, where high occupational exposures and contamination of local drinking water sources have been well documented (Raleigh et al., 2014; MPCA, 2020). The researchers did not specifically define the catchment area for the Minnesota portion of the study. However, although these exposed areas were nearby, there were many highly populated uncontaminated areas also nearby, and there was no indication that recruitment at this study site was restricted to people living or working in the contaminated area. The Shearer et al. (2021) authors did not report serum PFOA levels for each of the 10 study sites individually, but did report that median levels in participants from the Upper Midwest region (65% of whom were from the Minnesota study site) were somewhat lower than those from the other study regions. That is, the median serum PFOA levels for participants from the Upper Midwest, Western/Southern, and Eastern regions were 3.9, 4.6 and 4.3 ng/ml, respectively. The lower levels in the region containing Minnesota suggests that the large majority of the participants from the Minnesota site were not from the contaminated area and that PFOA exposures directly related to the former PFOA production facility were not a major driver of the Shearer et al. (2021) results.

The high percentage of cases from Minnesota could be due to confounding within the Minnesota site recruitment area, but this is unlikely. Kidney cancer rates for the years 2003-2017 (the years available) in the counties that include or are near the Minnesota study site (Hennepin, Ramsey, Dakota, and Washington Counties) are similar to those in Minnesota and in the US as a whole (Minnesota Department of Health, 2020; SEER, 2020). The fact that kidney cancer rates in these counties were not elevated argues against the presence of an unknown potent and widespread confounder in the Minnesota study area. Because the Shearer et al. (2021) researchers both matched and adjusted results by study site, any RCC risk factor that was greater in the Minnesota area than in the other recruitment areas is unlikely to have caused major confounding (Pearce, 2016). Confounding might still occur if the levels of an RCC risk factor varied within the Minnesota study area. However, this would only cause major confounding if the risk factor was potent (that is, a potent cause of RCC), widespread, strongly correlated with PFOA exposure, and not already adjusted for in the statistical analyses. This is unlikely since the most widespread and potent risk factors for RCC or kidney cancer were already included in the statistical adjustments, including age, sex, obesity, smoking, and hypertension. Major confounding by TCE, cadmium, asbestos, and acetaminophen is also highly unlikely for the same reasons given above.

Potential confounding of the Shearer et al. (2021) results by other PFAS was also evaluated. Serum concentrations of PFOA were correlated with serum concentrations of PFOS and PFHxS (Spearman correlation coefficients of 0.62 and 0.42, respectively). These correlations raise concerns that some of the effect reported for PFOA might actually be due to these other PFAS. To evaluate this possibility, the authors presented PFOA-RCC ORs both adjusted and unadjusted for PFOS and PFHxS. They also presented PFOS-RCC ORs adjusted and unadjusted for PFOA and PFHxS, and PFHxS-RCC ORs adjusted and unadjusted for PFOA and PFOS. The results of these analyses are shown in Table 6.2.3. As seen, the PFOA-RCC ORs changed only slightly with adjustment for PFOS and PFHxS. Although the CIs were wider after adjustment, this was expected given the correlations between these PFAS. The finding that adjusting for PFOS or PFHxS had little effect on the PFOA ORs provides good evidence that the elevated ORs reported for PFOA were not due to these other PFAS.

Table 6.2.3. ORs for serum PFOA, PFOS, PFHxS concentrations and RCC adjusted and unadjusted for other PFAS (Shearer et al., 2021)

				Without PFAS adjustment[a]		With PFAS adjustment[b]	
PFAS	**Exposure**	**Cases**	**Controls**	**OR**	**95% CI**	**OR**	**95% CI**
PFOA	<4.0	47	81	1.00	Ref	1.00	Ref
	≥4.0-5.5	83	79	1.47	0.77-2.80	1.41	0.69-2.90
	>5.5-7.3	69	83	1.24	0.64-2.41	1.12	0.52-2.42
	>7.3-27.2	125	81	2.63	1.33-5.20	2.19	0.86-5.61
	Continuous[c]	324	324	1.71	1.23-2.37	1.68	1.07-2.63
PFOS	≤26.3	60	81	1.00	Ref	1.00	Ref
	>26.3-38.4	82	81	1.67	0.84-3.30	1.24	0.59-2.57
	>38.4-49.9	61	81	0.92	0.45-1.88	0.53	0.22-1.24
	>49.9-154.2	121	81	2.51	1.28-4.92	1.14	0.45-2.88
	Continuous[c]	324	324	1.39	1.04-1.86	0.92	0.60-1.42
PFHxS	≤2.2	75	88	1.00	Ref	1.00	Ref
	>2.2-3.4	74	83	1.41	0.75-2.64	1.28	0.66-2.51
	>3.4-5.5	88	76	1.14	0.59-2.20	0.89	0.43-1.85
	>5.5-37.4	87	77	2.07	1.06-4.04	1.46	0.67-3.18
	Continuous[c]	324	324	1.27	1.03-1.56	1.12	0.88-1.43

Abbreviations: CI, confidence interval; Exposure, serum concentration of each PFAS in ng/ml; OR, odds ratio; Ref, reference category

[a] Adjusted for age, sex, race/ethnicity, study center, year of blood draw, eGFR, BMI, smoking status, history of hypertension, prior freeze-thaw cycles, and calendar year of blood draw

[b] Adjusted for all of the factors listed above plus adjustment for other PFAS (entered as log2-transformed concentrations of PFOA, PFOS, and PFHxS)

[c] Analysis of each PFAS as a continuous variable (entered in the model as log2 PFAS)

Similar findings were seen in analyses stratified by high, medium, and low levels of PFOS and PFHxS. That is, PFOA-RCC ORs comparing the upper tertile (>6 ng/ml) to the lower tertile (≤4 ng/ml) of PFOA were similar in participants with higher and lower levels of these other agents.

CIs widened in these analyses but this was expected given the smaller sample sizes. Overall, these analyses provide additional evidence that the elevated ORs for PFOA were unlikely due to these other agents. The elevated RCC ORs for PFOS and PFHxS mostly disappeared after adjustment for PFOA, suggesting that these other agents are not strongly related to RCC. Several other PFAS were also measured but these were either not associated with RCC or were not strongly correlated with PFOA, and therefore unlikely to have caused major confounding.

Vieira et al. (2013):

Study design: The Vieira et al. (2013) study was a cancer registry-based case-control study that took place in the C8 study area. The cancers of interest included kidney, pancreatic, testicular, and liver cancers. These were selected by the researchers because they had been linked to PFOA in previous animal and human studies. The controls were all other cancer types. The study area encompassed the six contaminated public water districts and the 13 counties in Ohio and West Virginia that surround the DuPont Washington Works PFOA facility. Initially, all incident cancer cases diagnosed from 1996 through 2005 in the Ohio counties of Athens, Meigs, Gallia, Washington, and Morgan and the West Virginia counties of Wood, Mason, Wirt, Putnam, Jackson, Pleasants, Ritchie, and Cabell were obtained from the Ohio Cancer Incidence Surveillance System (OCISS) and the West Virginia Cancer Registry (WVCR), respectively. However, only the OCISS provided the participants addresses, which could be used to develop individual estimates of PFOA exposure.

Initially, the OCISS provided the names of 9,402 cancer cases. According to the authors, 745 cases of oral cavity, pharynx, esophagus, larynx, and stomach cancer, and Hodgkin lymphoma were excluded because there were too few cases for meaningful analysis (<100 cases each), or they had not been previously investigated in relation to PFOA in animal toxicologic studies or occupational mortality studies. Seven hundred and fifty two participants could not be geocoded at the address level and were also excluded. Fifteen cases under the age of 15 years old were also excluded, leaving 7,869 Ohio participants in the study. Information on residences prior to cancer diagnoses were not available.

The exposure assessment in this study was based on estimates of serum PFOA concentrations at the time of diagnosis and 10 years before diagnosis. Because the residences prior to cancer diagnosis were unknown, the latter was done under the assumption that the participant lived at the address at diagnosis for at least ten years prior to diagnosis. Exposure was based on modeled estimates of each participant's annual average PFOA serum concentration. The model used to develop these estimates incorporated information on facility emissions, fate and transport characteristics of PFOA, and hydrogeological properties of the study area. These were then used to estimate yearly PFOA air and water concentrations throughout the exposed water districts. This information was then linked to each participant's residence and to standard assumptions about water intake, body weights, and PFOA half-life, to estimate the yearly average annual PFOA serum concentrations. Information on individual water intake (e.g., the amount of tap water consumed, bottled water use, etc.) or on occupational exposures were not included. These models were only applied to those living in the exposed water districts. Those living in one of the included counties, but outside of an exposed water district, were assigned to the "unexposed" reference category. Cumulative exposure was assessed by summing the yearly serum PFOA exposure estimates for the ten years prior to cancer diagnosis.

Statistical analysis: For participants residing in one of the exposed water districts, PFOA exposure was categorized into groups of "low," "medium," and "high" based on the tertile cutoff points in these participants. Because there was a large break in the distribution at 110 µg/L, participants in the upper 10% of modeled serum PFOA levels were removed from the "high" category and placed into a "very high" category. Logistic regression was used to calculate ORs for PFOA and kidney cancer, using all participants except those with kidney, pancreas, testicular, and liver cancer as controls. Odds ratios were adjusted for age, sex, diagnosis year, smoking status and type of insurance.

Results: The ORs for the association between modeled annual average PFOA serum concentrations 10 years prior to cancer diagnosis and kidney cancer in Vieira et al. (2013) are shown in Table 6.2.4. A statistically significant increase in the odds of kidney cancer was seen when comparing both the high (OR = 2.0; 95% CI, 1.3-3.2) and the very high (OR = 2.0; 95% CI, 1.0-3.9) exposure categories to the unexposed reference population. The corresponding ORs were similar in the high and very high categories of cumulative exposure (2.0 and 2.1, respectively) but were slightly lower (1.8 and 1.7, respectively) in analyses without the 10-year lag. P-values for trends or analyses using continuous estimates of PFOA exposure were not provided.

Table 6.2.4. Adjusted ORs for modeled serum PFOA concentrations and kidney cancer in Vieira et al. (2013)

Category	Serum PFOA	Case	Control[1]	OR	95% CI
"Unexposed"	Unknown	187	5,957	1.0	Ref
Low	3.7-12.8	11	446	0.8	0.4-1.5
Medium	12.9-30.7	17	455	1.2	0.7-2.0
High	30.8-109	22	339	2.0	1.3-3.2
Very High	110-655	9	142	2.0	1.0-3.9

Abbreviations: CI, confidence interval; Serum PFOA, modeled serum PFOA concentration in ng/ml; OR, odds ratio; Ref, reference category
1. Controls were people with cancers other than kidney, pancreatic, testicular, and liver cancer

The following sections discuss OEHHA's evaluations of the likelihood the Vieira et al. (2013) findings could have been due to bias (exposure misclassification, outcome misclassification, or selection bias) or confounding.

Exposure misclassification: A previous validation study found that the measured serum PFOA levels and estimates of serum PFOA based on models similar to those used in Vieira et al. (2013) were reasonably well correlated (Spearman's R = 0.67). This correlation improved only slightly when information on tap water consumption was incorporated (R = 0.69). Overall, the model tended to under-predict the measured serum PFOA concentrations by about 10-20%. One exception involved people from Little Hocking, the water district with the highest PFOA exposures. Here the model appeared to over-estimate measured levels by about 20-30% (Shin et al., 2011).

The likely degree to which exposure misclassification would affect the ORs reported by Vieira et al. (2013) was evaluated using the methods presented by Greenland (1998), with separate analyses run for model sensitivity and model specificity. Since the researchers assessed exposure using the same methods in cases and controls, most errors from exposure

misclassification are likely to be non-differential and therefor have biased the ORs to the null. Analyses using the methods presented by Greenland (1998) show that in most instances, errors in model sensitivity (i.e., those that would be associated with model under-prediction) are likely to have had only small effects on the ORs. For example, correcting for a model sensitivity of 70% would only change the ORs in the very high PFOA category from 2.0 to about 2.1. Errors in model sensitivity would include issues such as occupational exposures that were missed, people who lived in unexposed areas but worked in exposed areas, or people with high PFOA exposures from their diet. Model under-prediction involving participants in the Belpre water district might have biased the OR in the "high" category upwards. However, simulations performed by OEHHA aimed at correcting this show that this bias is also likely to be small.

Errors related to model specificity would occur if participants with lower PFOA exposures were mistakenly assigned to the higher exposure categories (i.e., model over-prediction). This might occur if participants in exposed areas consumed bottled water with low PFOA levels, or if participants moved from an unexposed area to an exposed area just prior to cancer diagnosis. Since there are greater numbers of subjects in the unexposed category than in the higher exposure categories in Vieira et al. (2013), errors related to model specificity can have a greater impact on ORs than errors in model sensitivity. Importantly though, as noted above, the exposure model used in Vieira et al. (2013) tended to under-predict rather than over-predict PFOA exposures, suggesting that errors in specificity are less common than errors in sensitivity. As mentioned, decreases in model specificity could be caused by missing information on tap water source or bottled water use. However, as noted, this information had little effect on model validation results. Model over-prediction did seem to occur in participants from the Little Hocking water district (Shin et al., 2011). However, major bias from this is unlikely since the modeled serum levels in these participants were among the highest in this study, such that the large majority were probably already placed in the highest exposure category.

Outcome misclassification: Reporting of newly diagnosed cancer cases to the OCISS is mandatory in Ohio, and the percentage of all cancers that were kidney cancers in the Vieira et al. (2013) study was similar to the percentage of these cancers in the US as a whole (2.63% vs. 2.58%, respectively, for the years 1996-2005). This suggests that under-reporting or under-ascertainment of kidney cancers was not a major problem in this study.

The Shearer et al. (2021) study provides strong evidence that PFOA is associated with RCC. However, it is unknown whether PFOA is also associated with other, non-RCC, kidney cancer types. Because Vieira et al. (2013) included all kidney cancer types, bias might have occurred if non-RCC kidney cancers are unrelated to PFOA. In other words, ORs could be biased towards the null if kidney cancer types unrelated to PFOA were included in the "case" group. Since it is unknown whether or not PFOA is related to non-RCC kidney cancers, it is unknown whether or not this bias actually occurred. However, this bias, if it did occur, is likely to be small since the large majority (approximately 90%) of all kidney cancers are RCCs. In order to evaluate the likely magnitude of this bias (if it did occur), OEHHA performed simulations in which 10% of all kidney cancers were removed from the Vieira et al. (2013) data, with the percentage removed from each exposure category inversely proportional to the OR for each exposure category. Based on this simulation, correcting for this bias would likely only change the ORs in the high and very high categories from 2.0 to about 2.15.

Selection bias: The use of cancer controls in cancer case-control studies offers several important advantages over the use of noncancer population-based controls (Smith et al., 1988). Regardless, bias of ORs towards the null might occur if some of the control cancers are caused

by PFOA. However, Vieira et al. (2013) excluded participants from the control group who had cancers which have been previously associated with PFOA exposure in animal and human studies, including pancreas, testes, and liver cancers. Sensitivity analyses in which these cancers were included in the control group gave similar results.

As reviewed above, there is some evidence that PFOA may be associated with testicular cancer or certain subtypes of breast cancer. However, there is no evidence that these associations are strong enough or prevalent enough to cause major bias in the Vieira et al. (2013) results.

Confounding: The findings in Vieira et al. (2013) were adjusted for age, sex, diagnosis year, smoking status and type of insurance, the latter being an indicator of socioeconomic status. Data on obesity were not available. However, obesity rates in the C8 study area were similar to those in the region as a whole (Frisbee et al., 2009), and no evidence could be found that modeled estimates of serum PFOA were strongly related to obesity in this area. Similarly, no evidence exists that other possible kidney cancer risk factors like cadmium, arsenic, TCE, asbestos, or acetaminophen are prevalent enough or strongly enough related to PFOA exposure in this area to cause major confounding. With regards to confounding by other PFAS, there is no established association between PFOS or any other PFAS and kidney cancer, based on this review of the literature; correlations between PFOA and other PFAS in the C8 study area were only moderate (Frisbee et al., 2009). As such, important confounding by other PFAS is unlikely. The information presented above for the (Shearer et al., 2021) study also shows that confounding of PFOA-kidney cancer relationships by PFOS and other PFAS is unlikely.

Criteria for causal inference

Overall, the Shearer et al. (2021) and Vieira et al. (2013) studies meet most, if not all, of the criteria commonly used to evaluate causal inference (Hill, 1965).

Chance: Several of the key results in Shearer et al. (2021) are statistically significant, including the OR for the highest PFOA exposure category, the test for trend in the categorical analysis, and the OR in the analysis of PFOA as a continuous variable. The ORs in the two highest exposure categories in the Vieira et al. (2013) study are also statistically significant. The low p-values or confidence intervals excluding 1.0 associated with each of these findings show that the increases in RCC or kidney cancer related to PFOA reported in these studies are unlikely due to chance.

Temporality: The serum samples used to assess PFOA exposure in Shearer et al. (2021) were collected years before the kidney cancers in the study were diagnosed. In Vieira et al. (2013), modeled exposures were estimated for the year ten years prior to cancer diagnosis. Overall, because the exposure assessments in both of these studies were based on PFOA exposures prior to cancer diagnosis, the likelihood of reverse causality (i.e., the likelihood that having kidney cancer might lead to higher serum or modeled PFOA exposures) is small.

Dose-response: Both the linear test for trend in the categorical analysis and the results of the analysis with PFOA as a continuous variable in the Shearer et al. (2021) study are consistent with the presence of a dose-response relationship. While a demonstrated dose-response relationship is not a *sine qua non* for causality, a large number of other established carcinogens exhibit similar dose-response relationships (i.e., increases in cancer risk associated with increasing levels of exposure). Formal analyses of dose-response were not presented in Vieira et al. (2013), although the ORs for the two highest exposure categories were increased and

statistically significant, which is also consistent with a dose-response relationship between PFOA and kidney cancer.

Bias and confounding: The evaluations of information bias (exposure and outcome misclassification) and selection bias discussed above suggest that these potential issues are either minor, or are very unlikely to have caused the positive associations seen in these two studies. Similarly, evaluations of confounding, including potential confounding by all of the known major risk factors for kidney cancer, as well as confounding by other PFAS, show that these factors are also highly unlikely to have caused the Shearer et al. (2021) and Vieira et al. (2013) results.

Biologic plausibility: Findings from a number of other studies support the biologic plausibility of the Shearer et al. (2021) and Vieira et al. (2013) results. This includes the results of studies linking PFOA to non-malignant kidney disease (US EPA, 2016a). These results show that PFOA can not only reach the kidney but can also cause kidney toxicity. The biologic plausibility of the Shearer et al. (2021) and Vieira et al. (2013) findings is also supported by research in laboratory animals, which has shown that, while not specific for kidney cancer, PFOA can cause cancer in mammalian species (Chapter 5). In addition, studies have linked PFOA to mechanisms leading to cancer or key characteristics of carcinogens (Chapter 5 and Appendix 8), which also support the plausibility of the Shearer et al. (2021) and Vieira et al. (2013) findings.

Consistency: Another criterion for causal inference is consistency, both internal and external. The similarity of the Shearer et al. (2021) findings when PFOA was analyzed as either a categorical or a continuous variable, as well as the similarity of the findings across several different subgroups (e.g., subgroups based on age, gender, eGFR, and smoking), all highlight the internal consistency of these results. The similarity of findings in the Vieira et al. (2013) study across different exposure metrics (cumulative vs. annual average, with and without 10-year lags) and with different control groups (i.e., control groups with and without kidney, liver, pancreas, and testicular cancer cases) highlights the internal consistency of the results of this study.

The Shearer et al. (2021) and Vieira et al. (2013) findings are also consistent with two of the other human studies of PFOA and kidney cancer. As described in Chapter 5, OEHHA identified seven human studies of PFOA and kidney cancer. Two of these studies are not informative, either because of the ecologic nature of the exposure data (Mastrontonio et al., 2017), or because of the very small number of cases (Girardi and Merler, 2019). Four of the remaining five studies reported statistically significant associations between PFOA and kidney cancer or RCC incidence (Barry et al., 2013; Vieira et al., 2013; Shearer et al., 2021) or mortality (Steenland and Woskie, 2012). These four studies were very different in terms of study populations, sample sizes, exposure assessment, outcome metrics, statistical analyses, and other study design features. These differences are detailed in Table 6.2.5. Despite all of these differences, each of these four studies identified a strong association between PFOA and kidney cancer.

Table 6.2.5. Differences between the four epidemiologic studies identifying an association between PFOA and kidney cancer

Study criteria	Shearer et al. (2021)	Vieira et al. (2013)	Barry et al. (2013)	Steenland and Woskie (2012)
Study design	Case-control with population controls	Case-control with cancer controls	Retrospective cohort	Retrospective cohort
Population	US population: 10 sites	Exposed population: C8 study area	Exposed population: C8 study area	Occupational: DuPont facility
Sample size	Cases: 324 Controls: 324	Cases: 246 Controls: 7,339	Cases: 105 Cohort: 32,254	Cases: 12 Cohort: 5,791
Exposure assessment	Serum PFOA	Residential exposure model - serum	Residential exposure model - serum	Inhalation exposure model - serum
Exposure categorization	Quartiles and continuous	Contaminated vs. uncontaminated districts; annual average – five categories	Cumulative exposure, continuous and quartiles; 0 and 10-year lags	Job exposure matrix, exposure model cumulative serum
Statistical adjustments	Age, sex, race/ethnicity, study center, year of blood draw, eGFR, smoking, hypertension, freeze-thaw cycles	Age, sex, diagnosis year, smoking, and insurance	Smoking, alcohol, sex, education, and age	Standardized by age and sex
Outcome	Renal cell cancer incidence (ICD-0–2 C64.9)	Kidney cancer incidence (ICD codes not provided)	Kidney cancer incidence (ICD codes not provided)	Kidney cancer mortality (ICD9 189.0–189.2)

eGFR, estimated glomerular filtration rate; ICD, International Classification of Disease

Elevated mortality from kidney cancer was found in the DuPont occupational cohort studied by Steenland and Woskie, 2012 (standardized mortality ratio (SMR) = 2.68; 95% CI, 1.5-5.24). These results were not adjusted for smoking, but major confounding by smoking is unlikely since the SMR for lung cancer was not elevated (SMR = 0.75; 95% CI, 0.48-1.11). TFE was also used at this facility. TFE is classified by IARC as probably carcinogenic to humans (Group 2A), primarily based on increases in kidney cancer, liver cancer, testicular cancer, and leukemia in rodents (IARC, 2017b). It has been hypothesized that because TFE is highly volatile and explosive, it is well controlled and appreciable exposures during normal operations would have been unlikely (Steenland and Woskie, 2012). However, data for any air, biologic, or other monitoring for TFE at the DuPont facility were not located. To date, only one study has investigated the association between TFE and cancer in humans (Consonni et al., 2013). This retrospective cohort study included several facilities in North America and Europe where TFE was used, including the DuPont facility investigated in Steenland and Woskie (2012). In fact, 40% of the Consonni et al. (2013) cohort were DuPont workers. The kidney cancer SMR in the "medium" group of cumulative TFE exposure was elevated (SMR = 2.58; 95% CI, 0.95-5.62, N=6 cases). Risks in the "high" exposure group were below 1.0 (SMR = 0.81; 95% CI, 0.10-2.93) although the numbers of cases was small (N=2). The authors attempted to separate out the individual risks of TFE and PFOA, but the exposures to these agents were too highly correlated to provide meaningful results. Eighty-eight percent of all workers exposed to TFE were also exposed to PFOA, and every worker exposed to PFOA was also exposed to TFE. In the only other occupational study of PFOA and kidney cancer, in a facility where TFE was used infrequently, the relative risk estimates for kidney cancer mortality and incidence were all near or below 1.0 (Raleigh et al., 2014). However, in the two studies of the population surrounding

the DuPont facility, where significant drinking water contamination occurred, some evidence of increased kidney cancer risks were found in both studies. For example, in the cancer registry study by Vieira et al. (2013), kidney cancer ORs of 2.0 (95% CI, 1.3-3.2) and 2.0 (95% CI, 1.0-3.9) were reported in the highest two categories of PFOA exposure. Since TFE is highly volatile, this population is unlikely to have had high and prolonged TFE exposure. In addition, elevated TFE exposure is also unlikely in the population based study by Shearer et al. (2021), which also identified a strong association between PFOA and RCC. Overall, the findings of clear associations between PFOA and kidney cancer in studies where TFE exposures are most likely very low or non-existent suggests that important confounding by TFE is unlikely.

The occupational study by Raleigh et al. (2014) did not find an association between PFOA and kidney cancer. This study examined cancer mortality and incidence in 4,668 workers at a 3M facility in Cottage Grove, Minnesota. This facility produced ammonium perfluorooctanoate (APFO), the ammonium salt of PFOA. SMRs for the period 1960 to 2008 were calculated using Minnesota state rates as the reference. The authors also calculated hazard ratios (HR) for kidney cancer mortality and incidence using non-PFOA exposed workers (N=4,359) from a nearby 3M facility in St. Paul, Minnesota as the reference group. The St. Paul facility manufactured tapes and abrasives. The specific chemicals used to produce these items were not described. Only 200 employees reported for work duties at both locations.

The exact reason why the findings of the Raleigh et al. (2014) study differed from those of most other studies of PFOA and kidney cancer is unknown, but several possibilities exist. One relates to chance and the relatively small numbers of cases in this study. Raleigh et al. (2014) included only six kidney cancer deaths (with only one in the highest exposure category), and only 16 incident kidney cancer cases (with only four in the highest exposure category). For kidney cancer incidence, the HR in the highest exposure category was 0.73 (95% CI, 0.21-2.48). Although this HR was below 1.0, the wide CI shows that this result is imprecise. In fact, the difference between this HR and the relative risk (RR) estimate reported for the most highly exposed workers in Steenland and Woskie (2012), the other occupational study, (SMR = 2.66; 95% CI, 1.15-5.24) was not statistically significant (p = 0.08). Overall, the small numbers of kidney cancer cases, and the imprecise results highlight the possibility that the Raleigh et al. (2014) study could have missed a true association because of chance.

Another possibility is confounding. No data were available from either the Cottage Grove or St. Paul facility on smoking, BMI, or any other known risk factor for kidney cancer except age and sex. Using Minnesota state rates as the reference population, the SMRs in the Cottage Grove facility for all causes (0.85; 95% CI, 0.80-0.90) and for all cancers (0.87; 95% CI, 0.78-0.97) were below 1.0, which likely indicates a significant healthy worker effect. In addition, differences between the SMRs for the Cottage Grove and St. Paul facilities suggest that the latter may not have been an appropriate comparison group. For example, the all cause (0.98; 95% CI, 0.94-1.03 and all cancer (1.04; 95% CI, 0.95-1.13) SMRs for the St. Paul facility were higher than those for Cottage Grove. In fact, the SMRs for almost all individual cancers other than kidney cancer, as well as the SMRs for diabetes, ischemic heart disease, and cerebrovascular disease were all higher for St. Paul than for Cottage Grove. Overall, the higher SMRs seen in the St. Paul workers for outcomes not known to be associated with PFOA show that these workers were generally less healthy than the Cottage Grove workers, and provide evidence that the St. Paul workers were not an appropriate comparison group.

Another reason why the Raleigh et al. (2014) study may have missed a true association could relate to the methods used to assess exposure. Exposure assessment was based on modeled

estimates of PFOA air concentrations in the workplace. Although ground water contamination has been well documented near the Cottage Grove facility (MPCA, 2020), no information was available on non-work related residential exposures. For workers in the APFO production area, the exposure models incorporated industrial hygiene data (205 personal and 659 area samples from 1977-2000), job titles, and the proportion of time spent in the exposed areas. PFOA air levels were expressed as a daily time weighted average (TWA) in mg/m^3 for each job title. Cumulative exposure was then estimated by summing the daily TWAs for all years and all jobs each employee worked at the facility. Exposures prior to 1977 were extrapolated from estimated exposure levels in 1977-2000 using differences in annual APFO production levels. Exposures in non-production areas were based only on expert judgments and physical proximity to the APFO production area. St. Paul workers were assigned an air level one order of magnitude below the non-chemical division workers in Cottage Grove in order to account for non-work related exposure (i.e., residential exposures).

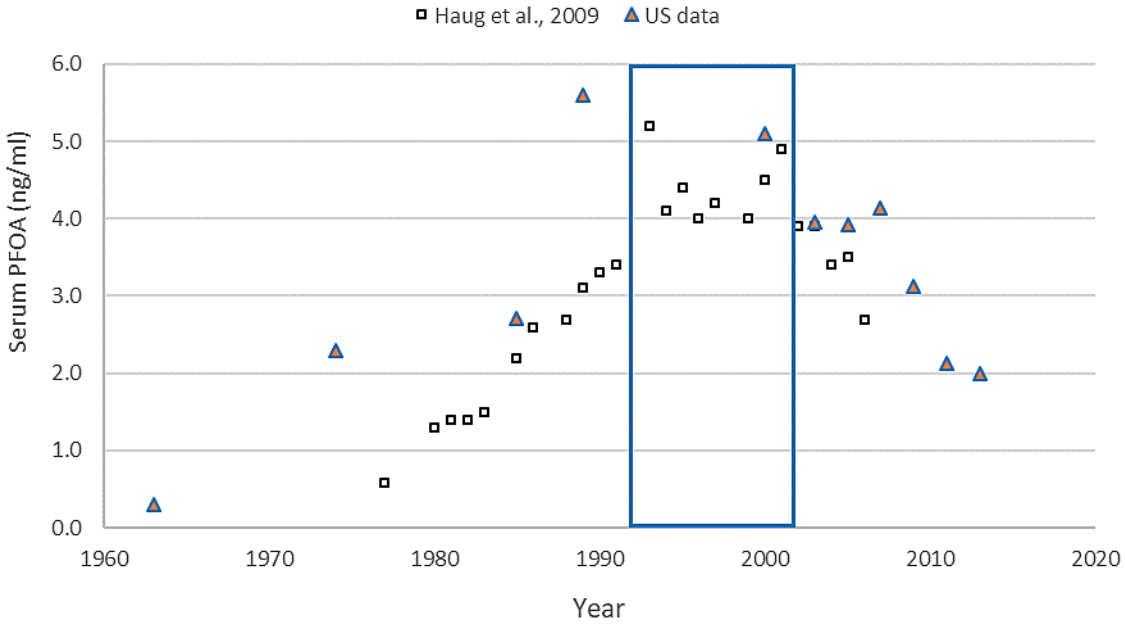
One potential problem with this method of exposure assessment is that little to no information is available on the degree to which inhaled PFOA is absorbed in humans or the inter-individual factors that might affect this absorption. Another potential problem is that the PFOA exposure estimates in both the non-production workers at the Cottage Grove facility and in all of the St. Paul facility workers were not based on actual PFOA measurements. In addition, no validation data were presented showing how well the estimates of airborne PFOA concentrations correlated with actual serum PFOA measurements. Data are presented showing that a sub-sample of Cottage Grove APFO production workers had high levels of serum PFOA (e.g., 282 to 2,538 ng/ml) but these involved a relatively small number of samples (N=148), and they were not used as part of the exposure model or for formal model validation. In contrast, the exposure assessment in the occupational study by Steenland and Woskie (2012), which did find an association between PFOA and kidney cancer, was based on modeled serum (not air) PFOA concentrations, and 2,125 serum measurements in over 1,308 workers were used to develop this model. Overall, the accuracy with which the exposure estimates in the Raleigh et al. (2014) study represent true internal exposure is unknown. This is especially important given the relatively small numbers of kidney cancer cases in this study and the possibility that misclassification of the exposure in only a few workers could have a large impact on study results.

Overview of exposure assessment: peak exposure

As described in this section, the exposure data used in both the Vieira et al. (2013) and Shearer et al. (2021) studies appear to be good indicators of the peak lifetime PFOA exposure for most of the participants in these two studies. As mentioned above, the PFOA serum levels measured in Shearer et al. (2021) were very similar to those in NHANES, suggesting that the exposure patterns in the participants of this study were about the same as those in the US as a whole. US nationwide survey data of PFOA serum levels are not available prior to 1999-2000. However, based on PFOA production levels, data from smaller studies, and subsequent NHANES data, peak serum PFOA levels in the US appeared to have occurred at the about same time that the serum samples were collected in Shearer et al. (2021). That is, PFOA serum levels in the US appeared to have gradually increased from the 1950's until peaking in the early 1990's (Olsen et al., 2005; Calafat et al., 2007a; D'Eon and Mabury, 2011a; Kato et al., 2011; Olsen et al., 2012; Dong et al., 2019). They then remained at these peak levels until about the year 2002, after which they began to steadily decline. Figure 6.2.2 shows serum PFOA concentrations over a 28-year period in 57 pooled serum samples from the Norwegian Institute of Public Health (Haug et al., 2011a) as well as median serum PFOA concentrations

from several studies in the US (Olsen et al., 2005; Calafat et al., 2007a; Kato et al., 2011; Wang et al., 2011; Jain, 2018). These data show that peak PFOA exposure in the US probably occurred from about 1990 to 2002. This period includes the years (1993-2002) in which the serum samples were collected in Shearer et al. (2021). This suggests that the PFOA measurements in the Shearer et al. (2021) study are likely a good representation of the lifetime peak exposure in most of the study's participants. While a few participants may have had higher exposures before or after this period, the large magnitude of this peak, the long half-life of PFOA in serum, and the fact that there are relatively few widespread high exposure sources in the US (e.g., like the C8 area) all suggest that this number is likely to be small.

Figure 6.2.2. PFOA serum levels by year in pooled samples from the Norwegian Institute of Public Health (Haug et al., 2009) and from studies in the US (Olsen et al., 2005; Calafat et al., 2007a; Kato et al., 2011; Wang et al., 2011; Jain, 2018). The blue rectangle area represents the years of serum sample collection in (Shearer et al., 2021)



The exposure data used in Vieira et al. (2013) are also likely a good indicator of the lifetime peak exposure in most of this study's participants. In this study, PFOA exposure was based on modeled estimates occurring 10 years before cancer diagnosis. Since cancers were diagnosed during the years 1996-2005, this exposure period represents the years 1986-1995. Shin et al. (2011) used a retrospective fate and transport model to predict yearly PFOA water concentrations for the six municipal water systems in the C8 area for the years 1951-2010. These models incorporated data on historic emission rates from the local PFOA production facility, physicochemical properties of PFOA, and local geologic and meteorological data. Although actual water measurements were not available for all years, predicted levels correlated well with measured levels for the years these data were available. Overall, these models show that the peak exposure period in the C8 area occurred during the years 1985-2002, a period that includes 1986-1995 exposure period used in Vieira et al. (2013). As such, as with the Shearer et al. (2021) study, the exposure data used Vieira et al. (2013) are probably also a good indicator of the lifetime peak PFOA exposure in the large majority of this study's participants.

Summary of study selection

In conclusion, four human studies (Steenland and Woskie, 2012; Barry et al., 2013; Vieira et al., 2013; Shearer et al., 2021) with adequate data to evaluate an association between PFOA and kidney cancer all reported strong evidence supporting a true causal association between PFOA and this cancer type. Evaluations of chance, bias, confounding, dose-response, consistency, and biologic plausibility all support these findings. There are a number of potential reasons, as detailed above, why a fifth study, the Raleigh et al. (2014) study, could have missed a true effect. Overall, based on these analyses, OEHHA concludes that the positive associations identified in most of the studies of PFOA and kidney cancer are real, and that PFOA is a cause of kidney cancer in humans.

Of the four human studies identifying associations between PFOA and kidney cancer, the Vieira et al. (2013) and Shearer et al. (2021) studies were selected for dose-response assessment and cancer slope factor (CSF) calculations. The study by Steenland and Woskie (2012) was not selected because of its relatively small sample size, and because it involved kidney cancer mortality and very high occupational exposures, exposures that are much higher than those seen in the general population. In contrast, information on kidney cancer incidence and on exposures closer to those seen in the general population were available in the two selected studies. The study by Barry et al. (2013) was not selected because it only presented kidney cancer ORs for categories of cumulative exposure. As discussed above, there was no evidence that cumulative exposure provides a better metric for assessing PFOA-related cancer risks than the metrics of peak exposure used in the Shearer et al. (2021) and Vieira et al. (2013) studies.

Generalizability

Several of the studies identifying links between PFOA and kidney cancer discussed were done in populations that were mostly, although not exclusively, Caucasian. As such, the findings of these studies may not be generalizable to every person in the US. Importantly though, this does not invalidate the elevated risks that were identified in these studies. Currently it is unknown whether PFOA or PFOS cancer risks might vary by race, ethnicity, or any other related factor. There is currently not sufficient data to make conclusive statements about whether the risks in certain groups of people are higher than those currently reported. And if they are higher, there is currently no data available to accurately quantify this increase.

Cancer Slope Factor (CSF) Calculations

Methods

This section describes the method used by OEHHA to calculate CSFs based on data from the Shearer et al. (2021) and Vieira et al. (2013) studies. This method is similar to that used by OEHHA for its PHG for arsenic and to that used by the US EPA for its CSF calculations for TCE (OEHHA, 2004; US EPA, 2011b). Both of these involved data from human case-control studies. The underlying model involves a linear regression between PFOA exposure and cancer relative risk. This model is described in detail below. Other methods considered for calculating CSFs and the cancer health protective concentration are reviewed in Appendix 12.

Calculating the dose-response slope: The dose-response slope between PFOA and cancer for each selected study was calculated using a linear regression analysis, which took the following basic form:

$$RR = bx + 1 \qquad (\textit{Equation 1}).$$

Here, RR is the relative risk between PFOA and kidney cancer (or RCC) in each non-reference category. The variable b is the slope between the excess RR (that is, RR − 1) and PFOA dose (x). This slope, b, can be estimated using the following equation (Rothman, 1986; US EPA, 2011b):

$$b = \frac{\Sigma\, w_j x_j RR_j - \Sigma\, w_j x_j}{\Sigma\, w_j x_j^2} \qquad (\textit{Equation 2})$$

where, x_j is the PFOA exposure level in each non-reference category and w_j is the weight applied to each RR. These weights are the inverse of the variance of each RR_j. Since the incidence of kidney cancer is relatively low and because the cases and controls were matched on age in both studies, the ORs presented in both studies can be used as a good approximation of the underlying RR_js.

The advantages of Equation 2 over a simple linear regression are that each OR is weighted by its precision (i.e., the inverse of its variance), and the precisions of these ORs can be used to calculate a 95% CI around b.

The variances of the ORs were estimated from their CIs using the following equation (US EPA, 2011b):

$$Var(OR_j) = OR_j^2 \times \left(\frac{\ln(CI_{Uj}) - \ln(CI_{Lj})}{2 \times 1.96} \right)^2 \qquad (\textit{Equation 3})$$

where CI_{Uj} and CI_{Lj} are the upper and lower 95% CIs, respectively, of each non-reference category OR.

In Vieira et al. (2013), individual estimates of exposure were only available for the Ohio participants. Because of this, only the dose-response data from the Ohio participants were used in the cancer slope factor calculations for this study. Because both the Shearer et al. (2021) and Vieira et al. (2013) studies presented serum PFOA levels in each exposure category as a range, OEHHA assigned the midpoint of each range to its respective exposure category. However, in Equation 2, the dose in the lowest exposure category is assumed to be zero and is therefore ignored. That is, the intercept is set at a dose of zero and an RR of 1.0. To do this, the midpoint of the lowest exposure category was subtracted from the midpoint of each exposure category for each study. These adjusted dose levels were then used as x_j in the equations above. Because the model is linear and the same value is being subtracted from each dose level, this adjustment will have no effect on b.

The standard error (SE_b) and lower and upper 95% CIs (CI_{Lb}, and CI_{Ub}, respectively) of b were estimated using the following equations:

$$SE_b = \sqrt{}\,(1 \div \Sigma w_j x_j^2) \qquad (\textit{Equation 4})$$

$$95\%\ CI_b = b \pm (1.96 \times SE_b) \qquad (\textit{Equation 5}).$$

Calculating the CSF: For each study, the CSF was first calculated as the excess cancer risk associated with each ng/ml increase in serum PFOA (CSF_{serum}). This was then combined with

the clearance rate (CL) discussed in Chapter 4 to calculate a CSF_{intake}, which is the excess cancer risk associated with each ng/kg-day intake in PFOA.

The CSF_{serum} was calculated by first converting the linear regression model discussed above from the RR scale to the absolute risk scale. This was done by starting with the following two equations:

$RR = bx + 1$ (*Equation 1)*

$RR = (R_E + R_O) \div R_O$ (*Equation 5)*

where R_E is the excess risk associated with a PFOA serum concentration of x, and R_O is the baseline risk, that is, the risk of RCC or kidney cancer in an unexposed or lower exposure reference group.

Combining Equations 1 and 5 gives:

$(R_E + R_O) \div R_O = bx + 1$ (*Equation 6).*

Solving Equation 6 for excess risk per PFOA serum concentration (i.e., solving for $R_E \div x$) gives the equation for CSF_{serum}:

$CSF_{serum} = R_E \div x = bR_O$ (*Equation 7).*

Incorporating information on PFOA clearance (CL) gives the equation for CSF_{intake}:

$CSF_{intake} = CSF_{serum} \div CL$ (*Equation 8)*

where CL is in units of ml/kg-day.

As seen in Equation 7, the decision of what value to use as R_O will have an impact on the CSFs. Commonly, the risk in a study's unexposed or lower exposure reference group is used as the R_O. However, because the studies selected by OEHHA are both case-control studies, direct estimates of absolute risk are not available. Because of this, cancer risks in the general US population were used as the R_Os. For Vieira et al. (2013), the lifetime risk of kidney cancer in US males of 0.0202 was used as the R_O (ACS, 2020a). The lifetime risk in males was used since this is about 2-times higher than that in females (0.0202 vs. 0.0101, respectively) (ACS, 2020a). For Shearer et al. (2021), R_O was the lifetime risk of RCC in US males, which was estimated by multiplying the lifetime risk of kidney cancer in US males by the percentage of all kidney cancers that are the RCC subtype (90%). This gives an R_O of $0.0202 \times 90\% = 0.0182$. These values are likely to overestimate the risk in a truly unexposed population since everyone in the US has had some PFOA exposure (Calafat et al., 2007a) and therefore may have some excess risk from these exposures. However, US lifetime cancer risks have been used in risk assessments of other chemicals that, like PFOA, also have widespread exposure (e.g., arsenic, benzene). In addition, sensitivity analyses show that using lower values for R_O will have only a small effect on the CSFs and therefore only a small effect on the health-protective concentration calculated from these CSFs (Appendix 12).

Integrating the two study CSFs: Because both the Vieira et al. (2013) and Shearer et al. (2021) studies were determined to be of good quality, without evidence for major bias or confounding, an overall CSF_{intake} was calculated by taking the geometric mean of the CSF_{intake}s from each

study. Although the dose-response slopes calculated from these two studies differed, this difference was not statistically significant (discussed in further detail below).

In some cancer risk assessments, the upper 95% CI of the dose-response slopes are used to help account for possible inter-study variance. Here however, the central estimates of the slopes (i.e., the slopes themselves) were used. This is because the results of two separate studies, including one involving ten separate study sites, were combined to develop the final overall CSF. This combination of different studies and different study sites should help account for much of the variance likely to occur across different PFOA-kidney cancer studies.

Results

A simple regression analysis shows that the linear model represented by Equation 1 provides a good fit to the Shearer et al. (2021) data (R^2 = 0.95) (Figure 6.2.3). For Vieira et al. (2013), model fit improved dramatically when the highest dose level was excluded (Figure 6.2.4). Based on this improved fit, and because the PFOA levels in the highest dose category in Vieira et al. (2013) are well above those seen in the very large majority of the US population, the highest dose group in Vieira et al. (2013) was excluded in the subsequent dose-response and CSF calculations.

Figure 6.2.3. Simple linear regression analysis of serum PFOA and RCC ORs from Shearer et al. (2021)

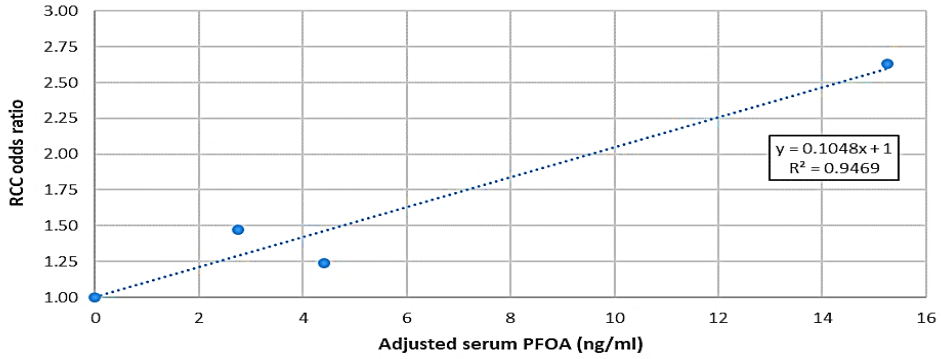
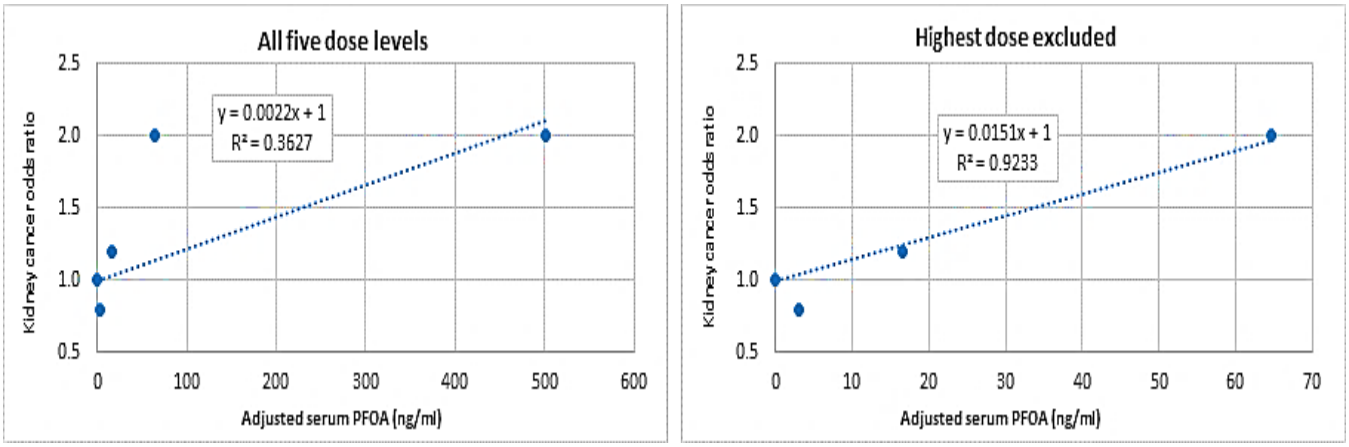


Figure 6.2.4. Simple linear regression analysis of serum PFOA and kidney cancer ORs from Vieira et al. (2013) with and without the highest exposure (dose) category



The key data used in the CSF calculations for both the Vieira et al. (2013) and Shearer et al. (2021) studies are shown in Table 6.2.6, and the results for equations 2, 4, 5, 7, and 8 are shown in Table 6.2.7. The geometric mean of the CSF_{intake} values from the Vieira et al. (2013) and Shearer et al. (2021) studies is **0.0026 (ng/kg-day)$^{-1}$** of PFOA intake.

Table 6.2.6. Data used in the CSF calculations for the Shearer et al. (2021) and Vieira et al. (2013) studies

Study	x_j	OR_j	CI_{Lj}	CI_{Uj}	var(OR_j)	w_j
Shearer et al. (2021)	0.00	1.00	Ref			
	2.75	1.47	0.77	2.80	0.234	4.267
	4.41	1.24	0.64	2.41	0.176	5.685
	15.26	2.63	1.33	5.20	0.837	1.195
Vieira et al. (2013)	0.00	1.0	Ref			
	3.05	0.8	0.4	1.5	0.073	13.743
	16.60	1.2	0.7	2.0	0.103	9.682
	64.70	2.0	1.3	3.2	0.211	4.734

Abbreviations: CI_{Lj}, lower 95% confidence interval of OR_j; CI_{Uj}, upper 95% confidence interval of OR_j; OR_j, odds ratio; Ref, reference group; var(OR_j), variance of OR_j; w_j, the weight applied to each OR_j; x_j, adjusted PFOA serum level in ng/ml

Table 6.2.7. Data and results for Equations 2, 4, 5, 7, and 8

	Shearer et al. (2021)	Vieira et al. (2013)
$\Sigma(w_j x_j OR_j)$	96.32	839.04
$\Sigma(w_j x_j)$	55.06	508.96
$\Sigma(w_j x_j^2)$	421.21	22614.68
b	0.0980	0.0146
SE_b	0.0487	0.0066
CI_{Lb}	0.0025	0.0016
CI_{Ub}	0.1935	0.0276
R_O	0.0182	0.0202
CSF_{serum}	0.00178	0.00029
CL	0.28	0.28
CSF_{intake}	0.00637	0.00105
CSF_{intake} geometric mean = 0.0026		

Abbreviations: b, regression slope from Equation 2; CL, PFOA clearance in mg/kg-day; CL_{Lb}, lower 95% confidence interval of b; CL_{Ub}, upper 95% confidence interval of b; CSF_{intake}, excess cancer risk per ng/kg-day of PFOA intake; CSF_{serum}, excess cancer risk per ng/ml of serum PFOA; OR_j, odds ratio; w_j, the weight applied to each OR_j; x_j, adjusted PFOA serum level in ng/ml

6.2.2. Perfluorooctane Sulfonic Acid

There is sufficient evidence to consider and critically evaluate the liver and pancreatic tumors in male and female rats exposed to PFOS for CSF derivation. First, the two chronic bioassays reported in Butenhoff et al. (2012b) are of sufficient quality (appropriate length, suitable number of animals per dose, adequate reporting, etc.) to warrant consideration as critical studies.

Second, recent studies of PFOA by NTP (2020) provide additional support for considering carcinogenicity as a critical endpoint for PFOS. In its cancer bioassays, NTP (2020) showed that chronic exposure to PFOA led to a significant increase in hepatocellular adenomas and/or carcinomas in male rats (data presented in Table 5.7.3), which is similar to the carcinogenic effects of PFOS reported by Butenhoff et al. (2012b). The similarity in molecular structure between PFOS and PFOA suggests that these chemicals may have comparable biological activities and, in fact, their noncancer toxicity profiles are similar, with the liver, thyroid, and immune and developmental/reproductive systems being major targets of both chemicals. Moreover, International Agency for Research on Cancer (IARC, 2017a) designated PFOA possibly carcinogenic to humans (Group 2B). PFOS also produced a positive trend for pancreatic carcinomas in male rats (Butenhoff et al., 2012b), which is a critical tumor type in PFOA-exposed male rats in the NTP (2020) bioassay (Table 5.7.3. It should be noted that the highest administered dose in the (Butenhoff et al., 2012b) PFOS bioassay (0.984 mg/kg-day) was essentially the same as the lowest administered dose in the NTP (2020) PFOA bioassay (1.0 mg/kg-day). This suggests that the Butenhoff et al. (2012b) studies are less sensitive than the NTP (2020) studies, and that the modest, but significant, tumor incidences observed (when compared against the NTP (2020) PFOA data) are the result of overall lower administered doses.

Third, there is evidence to indicate that PFOS is genotoxic and has multiple key characteristics of carcinogens (see Appendix 8 for greater detail). PFOS likely acts through multiple MOAs, and presently, the evidence does not support excluding genotoxicity as a possible MOA for carcinogenicity. As such, because the carcinogenicity MOA is uncertain, PFOS is evaluated using linear extrapolation.

Hepatocellular tumors in male and female Sprague Dawley rats and pancreatic islet cell tumors in male rats from Butenhoff et al. (2012b) were evaluated for CSF derivation. BMD modeling was conducted using serum concentrations as the dose metric, and results are presented in Appendix 10 and summarized in Table 6.2.8.

Table 6.2.8. BMD modeling results for tumors in male and female Sprague Dawley rats following exposure to PFOS in the diet for two years (Butenhoff et al., 2012b)

	$BMDL_{05(animal)}$ (mg/L)	$BMDL_{05(human)}$ (mg/kg-day)[a]	CSF_{human} $(mg/kg\text{-}day)^{-1}$	Model p-value	Polynomial Degree
Males – liver	24.8	0.0054	9.3	0.1999	1
Males - pancreatic	24.4	0.0053	9.4	0.6540	1
Males – liver and pancreatic combined	14.7	0.0032	**15.6**	-	-
Females - liver	33.5	0.0069	7.2	0.5862	1

[a] Rounded numbers are presented in the table, but the cancer slope factor is derived from unrounded values.

The combined incidence of liver and pancreatic tumors in male rats produced the lowest $BMDL_{05}$ value of 14.7 mg/kg-day. To convert the $BMDL_{05}$ of 14.7 mg/L to an HED, the plasma concentration is first multiplied by a human PFOA clearance factor of 3.9×10^{-4} L/kg-day. The resulting value is 0.0057 mg/kg-day. To estimate an HED from animal data that would result in an equal lifetime risk of cancer, OEHHA uses body weight (BW) scaling to the ¾ power (OEHHA, 2009). This adjustment accounts for interspecies differences in toxicokinetics and toxicodynamics. Because TK differences have already been accounted for by using plasma concentration as the dose metric instead of administered dose, the $BMDL_{05}$ only needs modification for toxicodynamic differences ($BW^{1/8}$ adjustment). The equation is provided below.

$$BMDL_{05(human)} = BMDL_{05(animal)} \times (BW_{animal}/BW_{human})^{1/8}$$

Applying the $BW^{1/8}$ adjustment, where the time-weighted average male rat body weights is 0.687 kg (female rat body weight is 0.412 kg) (from Thomford (2002)), and the adult human body weight is the default of 70 kg, the human $BMDL_{05}$ is 0.0032 mg/kg-day for the combined tumor incidence in males. This BMDL results in a human CSF of **15.6 (mg/kg-day)$^{-1}$**.

7. HEALTH-PROTECTIVE DRINKING WATER CONCENTRATIONS

7.1. Noncancer Health-Protective Drinking Water Concentrations

7.1.1. Perfluorooctanoic Acid

To calculate a health-protective concentration for a chemical, the ADD is converted to a concentration in drinking water that accounts for the total exposure to the chemical that people receive from using tap water. The relative source contribution (RSC) is the proportion of exposures to a chemical attributed to tap water (including inhalation and dermal exposures, e.g., during showering), as part of total exposure from all sources (including food and air pollution). The RSC values typically range from 20 to 80 percent (expressed as 0.20 to 0.80), and are determined based on available exposure data. The default RSC of 0.2 is selected because there is not enough data to determine specific exposure patterns for PFOA (see Appendix 4 for more details). In addition to drinking water, there are several other sources of PFOA that may contribute to exposure in the general population, including air, soil, food, and consumer and industrial products. PFOA released to air may adsorb to airborne particles and travel long distances (US EPA, 2016b). Additionally, the use of PFOA in many consumer products and its environmental persistence has led to the presence of PFOA in indoor air and dust. In fact, (US EPA, 2016b) reports that the most common exposure routes of PFOA are diet and indoor dust. Thus, an RSC of 0.2 is appropriate, and consistent with RSCs used by other agencies, including US EPA and the State of New Jersey.

Oral ingestion is the primary route of exposure for PFOA in drinking water. PFOA is not very volatile in its ionized form (its predominant form in water) (Johansson et al., 2017), so inhalation of PFOA directly from drinking water is not anticipated to be a major route of exposure. Dermal absorption is also not anticipated to be a significant route of exposure from typical household uses of tap water. Ionized PFOA penetrates skin poorly compared to the neutral form, and PFOA should remain ionized in the stratum corneum due to its buffering capacity (Franko et al., 2012).

PFOA can permeate mouse and human skin in vitro, and its absorption following dermal application in mice in vivo was demonstrated by Franko et al. (2012). However, a time-course of >5 hours is needed for PFOA to penetrate full-thickness human skin, and this exposure scenario is unlikely to occur from typical household uses of tap water.

Because oral ingestion is considered to be the only significant route of drinking water exposure, a lifetime average drinking water intake rate of 0.053 L/kg-day (OEHHA, 2012) is used to determine the noncancer health-protective concentration, which is calculated using the following formula:

$$C = ADD \times RSC \div DWI,$$

where:

ADD = acceptable daily dose, in ng/kg-day,

RSC = relative source contribution of 0.2, and

DWI = daily water intake rate of 0.053 L/kg-day.

Thus, based on the increased risk of elevated ALT in humans reported by Gallo et al. (2012), a health-protective concentration is calculated as follows:

C = (0.87 ng/kg-day × 0.2) ÷ 0.053 L/kg-day = 3.28 ng/L or **3 ppt** (rounded).

7.1.2. Perfluorooctane Sulfonic Acid

Calculating the health-protective concentration for PFOS uses the same procedure as for PFOA. The default RSC of 0.2 is selected because there is not enough data to determine specific exposure patterns to PFOS. In addition to drinking water, there are several other sources of PFOS that may contribute to exposure in the general population, including air, soil, food, and consumer and industrial products. PFOS released to air may adsorb to airborne particles and travel long distances (US EPA, 2016d). Additionally, the use of PFOS in many consumer products and its environmental persistence has led to the presence of PFOS in indoor air and dust. As with PFOA, US EPA (2016d) reports that the most common exposure routes of PFOS are diet and indoor dust. Thus, an RSC of 0.2 is appropriate (see Appendix 4 for details), and consistent with RSCs used by other agencies, including US EPA and the State of New Jersey.

Oral ingestion is the primary route of exposure for PFOS in drinking water. Volatilization of the predominant anionic form in water (pKa <1.0) is not expected to occur (HSDB, 2020b).

Dermal absorption is also not anticipated to be a significant route of exposure from typical household uses of tap water, based on its physicochemical similarities to PFOA. However, no specific studies could be identified that addressed absorption of PFOS following dermal exposure. ATSDR (2021) reports the results of an unpublished single-dose dermal absorption study in rabbits, where potassium PFOS or its diethanolamine salt (at doses up to 20 µg/kg) was applied to clipped, intact skin (Johnson et al., 1995a,b, as reported by ATSDR (2021)). Compared to controls, no increase in organic fluoride in the liver was detected, suggesting that PFOS was not absorbed.

Because oral ingestion is considered to be the only significant route of drinking water exposure, a lifetime average drinking rate of 0.053 L/kg-day (Appendix 3 (OEHHA, 2012)) is used to determine the noncancer health-protective concentration. Thus, based on the increased total cholesterol in humans reported by Steenland et al. (2009), a health-protective concentration is calculated as follows:

C = (0.64 ng/kg-day × 0.2) ÷ 0.053 L/kg-day = 2.42 ng/L or **2 ppt** (rounded).

7.2. Cancer Health-Protective Drinking Water Concentrations

7.2.1. Perfluorooctanoic Acid

Because exposure to PFOA is mainly through oral ingestion, the calculation of a cancer-based health-protective concentration is based only on exposure through ingestion of drinking water. Age sensitivity factors were not applied because the NTP (2020) animal bioassay showed no increased risks in combined adenomas and carcinomas from perinatal exposure compared to exposures later in life. The cancer-based health-protective concentration for PFOA can be calculated as:

$C = R \div (CSF_{intake} \times DWI)$,

where:

R = default excess cancer risk level of one in one million, or 10^{-6}

CSF_{intake} = cancer potency in units of excess risk per ng/kg-day intake of PFOA

DWI = time weighted drinking water intake, lifetime average.

For PFOA, the cancer-based health-protective concentration is:

$C = 10^{-6} \div (0.0026\ (ng/kg\text{-}day)^{-1} \times 0.053\ L/kg\text{-}day) = 0.00729$ ng/L, equivalent to **0.007 ppt** (rounded).

The cancer health-protective concentration of 0.007 ppt is selected as the PHG and should protect against the noncancer effects of PFOA since it is lower than the 3 ppt level for noncancer effects.

7.2.2. Perfluorooctane Sulfonic Acid

As described in the noncancer health-protective concentration derivation, oral ingestion is the primary route of exposure to PFOS in drinking water, and inhalation and dermal exposures are considered negligible.

When determining cancer risk, OEHHA typically applies ASFs to account for the increased susceptibility of infants and children to carcinogens (OEHHA, 2009). However, ASFs were not included when deriving the cancer health-protective concentration for PFOA because the NTP (2020) study provided evidence that early-life exposure did not increase tumor incidences later in life. Because it is anticipated that PFOS behaves in a similar manner as PFOA, OEHHA is excluding ASFs in the health-protective concentration derivation for cancer.

Since oral ingestion is considered to be the only significant route of drinking water exposure to PFOS, a lifetime average drinking rate of 0.053 L/kg-day (OEHHA, 2012) is used, along with the CSF of 15.6 $(mg/kg\text{-}day)^{-1}$ determined in Chapter 6, to calculate the health-protective concentration, C, for carcinogenic effects of PFOS as follows:

$C = 10^{-6} \div (15.6\ (mg/kg\text{-}day)^{-1} \times 0.053\ L/kg\text{-}day) = 1.2 \times 10^{-6}$ mg/L

C = 1 ng/L or **1 ppt** (rounded).

The cancer health-protective concentration of 1 ppt is selected as the PHG and should protect against the noncancer effects of PFOS since it is lower than the 2 ppt level for noncancer effects.

8. RISK CHARACTERIZATION

PFOA and PFOS are ubiquitous, and biomonitoring data from California have shown that these compounds are found in the serum samples of nearly all participants examined. Because exposure to these chemicals is so prevalent and elimination times are so long, it is critical to understand the toxicity associated with these compounds, and their impacts on human health.

In the development of the currently proposed PHGs and noncancer health-protective concentrations, OEHHA employed a thorough and methodical approach. OEHHA conducted a comprehensive analysis of the human epidemiology literature, and updated the animal toxicity evaluation to include recent studies and endpoints not captured in the 2019 NL recommendations for these chemicals.

In humans, exposure to environmental levels of PFOA is associated with numerous adverse health effects, including decreased vaccine response, increased liver enzyme levels (indicative of hepatotoxicity), increased cholesterol levels, and kidney cancer. Similarly, PFOS is associated with decreased vaccine response and increased cholesterol levels. The animal toxicity data for PFOA and PFOS support the human epidemiologic studies, with multiple studies reporting immunotoxicity, liver toxicity, and cancer in rodents. Furthermore, animal studies reported several additional toxicity endpoints, including thyroid toxicity, reproductive/developmental toxicity, and, for PFOS, neurotoxicity.

In most cases, humans were more sensitive than animals to PFOA and PFOS, which eliminated the uncertainty in extrapolating animal data to humans. Thus, human data were used to derive the proposed PHG for PFOA and the noncancer health-protective concentrations for PFOA and PFOS. The proposed PHG of 0.007 ppt for PFOA is based on increased risk of kidney cancer in humans (Vieira et al., 2013; Shearer et al., 2021). This level should protect against noncancer toxicity as well, as it is lower than the health-protective concentration of 3 ppt, based on elevated ALT levels in humans. Similarly, the PHG of 1 ppt for PFOS is based on increased liver and pancreatic tumor incidence in a two-year rat study (Butenhoff et al., 2012b). The PHG should be protective for all noncancer toxicity endpoints, as it is lower than the health-protective concentration of 2 ppt, based on elevated total cholesterol in humans.

In this assessment, OEHHA critically evaluated the parameters used to derive the PHGs. These include toxicokinetic parameters, and exposure parameters, such as drinking water intake rates and the relative source contribution. These issues are summarized below:

- **Toxicokinetics.** Due to the long half-lives of PFOA and PFOS in humans, serum concentration is the preferred dose metric for dose-response analysis. TK approaches were developed to convert serum levels of PFOA or PFOS in humans to an external oral dose, and to convert serum concentrations in animals to human equivalent doses, on which the PHGs and health-protective concentrations can be based. OEHHA evaluated several different approaches to address this issue, including multiple PK models, the use of reported serum/plasma concentration as the primary dose metric, and the development of chemical-specific clearance factors. OEHHA's evaluations of PK models indicate that models may produce inaccurate results outside of the optimized range, which may increase uncertainty rather than reduce it. OEHHA determined that using reported serum/plasma concentrations is the least uncertain method and is generally precise when compared against modeled concentrations. To estimate external dose from serum concentration, OEHHA derived human clearance factors for PFOA and

PFOS, based on human exposure studies. OEHHA's clearance factors are larger than the clearance factors derived by US EPA (US EPA, 2016b; US EPA, 2016d). Finally, selecting epidemiologic studies as critical studies for derivation of the noncancer health-protective concentrations bypasses the need for interspecies conversions and additional TK adjustments

- **Relative source contribution.** OEHHA thoroughly evaluated the relative source contribution, following US EPA's Exposure Decision Tree Approach, and determined that there is not enough information to estimate relevant sources of exposure quantitatively, particularly for California residents. Therefore, OEHHA utilized the default RSC of 20% for PFOA and PFOS.
- **Drinking water intake rate.** To derive the PHGs and noncancer health-protective concentrations for PFOA and PFOS, OEHHA used a time-weighted lifetime average drinking water intake rate of 0.053 L/kg-day (OEHHA, 2012). OEHHA evaluated US EPA's recently updated water consumption rates published in the Exposure Factors Handbook (US EPA, 2019). While US EPA's updated water intake rates are based on newer data (NHANES, 2005-2010) than those used by OEHHA (CSFII, 1994-1996, 1998), they do not capture the continued trend of increased water consumption both nationwide and in California. OEHHA's preliminary analysis of newer NHANES data (2015-2016) supports the use of the drinking water intake rates developed previously (OEHHA, 2012). Furthermore, California water consumption rates may differ from national rates due to climate and lifestyle factors. Additionally, OEHHA's drinking water rates are more protective for infants, who may be at greater risk of adverse health effects than the general population due to their greater exposure to drinking water contaminants on a body weight basis.

Mechanistic evidence, some key uncertainties, and other issues also considered in the development of the proposed PHGs and health-protective concentrations for PFOA and PFOS are summarized below:

- **Genotoxicity.** There is some positive evidence of genotoxicity for PFOA and PFOS. For PFOA, the evidence of mutagenicity is limited, but chromosomal effects and DNA damage have been observed both in vivo and in vitro. For PFOS, there is some evidence of mutagenicity, and positive evidence of chromosomal effects and DNA damage. Therefore, genotoxicity cannot be dismissed as a possible mode of action for PFOA and PFOS.
- **Human relevance of PPARα.** PFOA is a known activator of PPARα and there has been considerable discussion in the scientific literature that carcinogenesis in rodents mediated by activation of PPARα is not relevant to humans, as PPARα activation in humans is not known to induce tumors. However, there is evidence in PPARα knockout animals that PFOA induces PPARα-independent toxicity, including carcinogenesis. Additionally, toxicity is observed at doses that do not activate PPARα. This indicates that PFOA acts via multiple mechanisms.
- **Interspecies extrapolation.** To estimate the cancer risk for PFOS based on rodent data, OEHHA would generally use the interspecies scaling factor of body weight to the ¾ power to account for TK and toxicodynamic differences between rodents and humans that might cause differences in tumorigenic response. However, because serum levels of PFOS were used in the cancer dose-response analysis and the chemical is not metabolically active in humans or rodents, the differences in interspecies TK are inherently included. Therefore, only body weight scaling adjustment for toxicodynamics

was applied. The difference between scaled and unscaled CSFs was approximately two-fold for rat-based values.

The California State Water Resources Control Board reported levels of PFOA and PFOS in drinking water from April 2019 to June 2020, with mean values (including non-detects) ranging from 4.79-5.71 ppt for PFOA and 9.7-11.5 ppt for PFOS. These levels are higher than the draft PHGs and HPCs calculated in this draft document from human studies.

Other Regulatory Standards and Advisory Levels

Below is a list of drinking water regulatory standards and advisory levels from US EPA and other states.

Table 8.1. Summary of state and federal drinking water regulations and advisory levels for PFOA and PFOS

State/Organization	Chemical	Regulatory/Advisory Value
US EPA	PFOA (individual and combined sum with PFOS)	HA – 70 ppt
	PFOS (Individual and combined sum with PFOA)	HA – 70 ppt
Connecticut	Sum of PFOA and PFOS (and PFHxS, PFNA, and PFHpA)	AL – 70 ppt
Massachusetts	Sum of PFOA and PFOS (and and PFHxS, PFNA, PFDA and PFHpA)	MCL – 20 ppt
Michigan	PFOA	MCL – 8 ppt
	PFOS	MCL – 16 ppt
Minnesota	PFOA	HRL – 35 ppt
	PFOS	HBV – 15 ppt
New Hampshire	PFOA	MCL – 12 ppt
	PFOS	MCL – 15 ppt
New Jersey	PFOA	MCL – 14 ppt
	PFOS	MCL – 13 ppt
New York	PFOA	MCL – 10 ppt
	PFOS	MCL – 10 ppt
Vermont	Sum of PFOA and PFOS (and PFHxS, PFNA, and PFHpA)	MCL – 20 ppt
Washington	PFOA	Draft AL – 10 ppt
	PFOS	Draft AL – 15 ppt

AL, action level; HA, health advisory; HBV, health based value; HRL, health risk limit; MCL, maximum contaminant level

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APPENDIX 1. LITERATURE SEARCH STRATEGY

Literature search and screening methods

OEHHA's librarian conducted a search of the literature on the toxicity of PFOA and PFOS. The goal was to identify peer-reviewed open-source and proprietary journal articles, print and digital books, reports, and gray literature that potentially reported relevant information on the toxicity of these chemicals. Searches were limited to literature published from January 2016 to the time the searches were executed in September 2019. One animal toxicity study, Blake et al. (2020), was identified after OEHHA's initial literature review (published or identified between January and September 2020). Detailed descriptions of the literature search strategy for epidemiologic studies, based on outcome of interest, can be found in Appendix 7.

Search process

PubMed MeSH browser was used to identify subject headings, other index terms and synonyms for PFOA and PFOS and the concept of toxicity. Preliminary searches were run and results evaluated to identify additional relevant search terms. The resulting search strategy was executed in PubMed.

Toxicity studies

Search as executed in PubMed (PubMed) on September 4, 2019.

Set #	Strategy	Results	Notes
1	("perfluorooctane sulfonic acid"[nm] OR PFOS [Tiab] OR "perfluoroalkyl sulphonate"[tiab] OR "perfluoro-n-octanesulfonic" [Tiab] OR "perfluoroctane sulfonic" [Tiab] OR "perfluorooctane sulfonic" [Tiab] OR perfluoroctanesulfonic [Tiab] OR perfluorooctanesulfonic [Tiab] OR "perfluorooctane sulphonic" [Tiab] OR "perfluoroctane sulphonic" [Tiab] OR perfluoroctanesulphonic [Tiab] OR perfluorooctanesulphonic [Tiab] OR "perfluorooctane sulfonate" [Tiab] OR "perfluoroctane sulfonate" [Tiab] OR perfluorooctanesulfonate [Tiab] OR perfluoroctanesulfonate [Tiab] OR "perfluorooctane sulphonate" [Tiab] OR "perfluoroctane sulphonate" [Tiab] OR perfluoroctanesulphonate [Tiab] OR perfluorooctanesulphonate [Tiab] OR "perfluorooctanyl sulfonate" [Tiab] OR "perfluorooctanyl sulphonate" [Tiab] OR perfluoroctylsulfonic [Tiab] OR "heptadecafluoro-1-octanesulfonic" [Tiab] OR "heptadecafluoro-1-octane sulfonic" [Tiab] OR "heptadecafluorooctane sulfonic" [Tiab] OR "heptadecafluoroctane sulfonic" [Tiab] OR "heptadecafluorooctane sulfonic" [Tiab] OR heptadecafluorooctanesulfonic [Tiab] OR "heptadecafluorooctane-1-sulphonic" [Tiab] OR "heptadecafluorooctane sulphonic" [Tiab] OR "1-octanesulfonic acid" [Tiab] OR "1-octanesulphonic acid" [Tiab] OR "1-perfluoroctanesulfonic" [Tiab] OR "1-perfluorooctanesulfonic [Tiab]" OR "octanesulfonic acid" [Tiab] OR "octanesulphonic acid" [Tiab] OR 1763-23-1 [rn] OR 2795-39-3 [rn] OR 29081-56-9 [rn] OR 29457-72-5 [rn] OR 4021-47-0 [rn] OR 70225-14-8 [rn] OR 307-35-7 [rn]) OR ("perfluorooctanoic acid"[nm] OR PFOA [Tiab] OR PFAA* [Tiab] OR APFO [Tiab] OR "fluorinated surfactants" [Tiab] OR fluorosurfactant* [Tiab] OR "fluorinated polymer*" [Tiab] OR (fluorinated [Tiab] AND (polymer [Tiab] OR polymers [Tiab])) OR (fluorocarbon [Tiab] AND (polymer [Tiab] OR polymers [Tiab])) OR fluoropolymer* [Tiab] OR (fluorinated [Tiab] AND telomer* [Tiab]) OR fluorotelomer* [Tiab] OR fluoro-telomer* [Tiab] OR fluorotelomer	12,393	PFOA & PFAS concepts

	alcohol*[tiab] OR “telomer alcohol*” [Tiab] OR “polyfluoroalkyl* ” [Tiab] OR “N-ethyl perfluorooctanesulfonamido ethanol” [Tiab] OR “N-ethyl perfluorooctanesulfonamidoethanol” [Tiab] OR “N-EtFOSE” [Tiab] OR perfluoroalkyl* [Tiab] OR perfluorocarbon* [Tiab] OR perfluorocarboxyl* [Tiab] OR perfluorochemical* [Tiab] OR “perfluorinated*” [Tiab] OR (perfluorinated [Tiab] AND (C8 [Tiab] OR carboxylic [Tiab] OR chemical* [Tiab] OR compound* [Tiab] OR octanoic [Tiab])) OR (PFO [Tiab] AND (perfluoroalk* [Tiab] OR perfluorocarb* [Tiab] OR perfluorinat* [Tiab] OR perfluorooc* [Tiab])) OR (C8 [Tiab] AND (perfluoroalk* [Tiab] OR perfluorocarb* [Tiab] OR perfluorinat* [Tiab] OR perfluorooc* [Tiab])) OR perfluoroctanoic [Tiab] OR perfluorooctanoic [Tiab] OR “perfluoro octanoic” [Tiab] OR “perfluoro-n-octanoic” [Tiab] OR “perfluorinated octanoic acid” [Tiab] OR perfluorooctanoate [Tiab] OR perfluoroctanoate [Tiab] OR “perfluoro octanoate” [Tiab] OR perfluoroheptanecarboxylic [Tiab] OR “perfluoro-1-heptanecarboxylic” [Tiab] OR perfluorocaprylic [Tiab] OR perfluorocaprilate [Tiab] OR perfluorocaprylate [Tiab] OR perfluoroacrylate [Tiab] OR “perfluorooctanoyl chloride” [Tiab] OR “pentadecafluoro-1-octanoic” [Tiab] OR “pentadecafluoro-n-octanoic” [Tiab] OR pentadecafluorooctanoate* [Tiab] OR pentadecafluoroctanoate* [Tiab] OR pentadecafluoroctanoic [Tiab] OR pentadecafluorooctanoic [Tiab] OR fluorad [Tiab] OR “FC 143” [Tiab] OR FC143 [Tiab] OR 335-67-1 [rn] OR 3825-26-1 [rn] OR 335-95-5 [rn] OR 2395-00-8 [rn])		
2	#1 AND (tox[sb] OR genotox*[tiab] OR toxicity[tiab])	6,230	Toxicity concept
3	#2 AND ("2016/01/01"[PDat] : "3000/12/31"[PDat])	**2,060**	Limit to 2016-present

The PubMed strategy was then tailored for use in additional databases, listed below, according to the search interface and features unique to each resource. For instance, MeSH terms were replaced with Emtree terms for the Embase search strategy and the PubMed toxicology subset search strategy was translated to Embase syntax.

Data sources and results

The following is a list of the databases searched to find information on PFOA and PFOS, and the number of references retrieved from each.

Table A1.1. Database search results

Source	Results
PubMed (National Library of Medicine) (PubMed)	2,060
Embase (Embase)	1,824
Scopus (Scopus)	1,140
TOXLINE (National Library of Medicine): Toxicology Literature Online, (TOXLINE)	27
TOXNET DART (National Library of Medicine): Toxicology Literature Online, DART Subset (TOXNET DART)	0
SciFinder-n (SciFinder-n)	183

After duplicates were removed, the search yielded a total of 2,766 unique references.

Literature screening

Relavant literature was identified though a multi-step screening process outlined in Figure A1.1. Studies from the database search were imported into a web-based systematic review software, DistillerSR, for title/abstract and full-text screening. Both title/abstract and full-text screening were conducted by two independent reviewers. Studies were screened for inclusion or exlcusion based on the Populations, Exposures, Comparator, and Outcome (PECO) criteria outline in Table A1.2. Studies that met PECO criteria during title and abstract screening were considered for full-text screening. At both the title/abstract and full-text review levels, screening conflicts were resolved by discussion.

Figure A1.1. Literature search: recent studies of PFOA or PFOS and animal toxicity

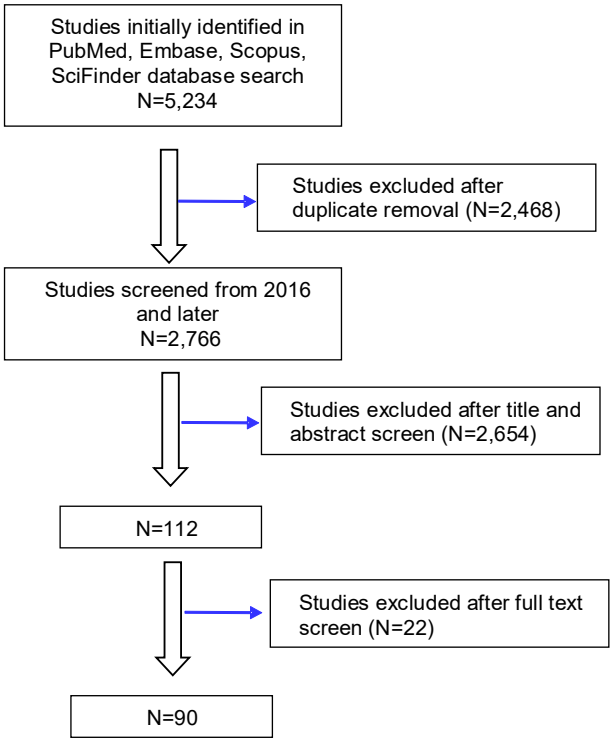


Table A1.2. Populations, exposures, comparators, and outcomes (PECO) criteria

PECO element	Evidence
Populations	**Human:** Studies of any population and lifestage (occupational or general population, including children and other sensitive populations) will be tagged as "potentially relevant supplemental information – human studies". **Exclude**: biomonitoring studies and exposure studies (unless specifically relevant to California). **Animal:** Non-human mammalian animal species of any lifestage (including preconception, in utero, lactation, peripubertal, and adult stages). Zebrafish studies will be tagged as "potentially relevant supplemental information". **Mechanistic:** Studies of any human or animal (mammalian and non-mammalian) cell type, and mechanistic/genomic/in silico data with any biological significance will be tagged as "potentially relevant supplemental information".
Exposures	**Relevant forms:** Perfluorooctanoic acid (CAS 335-67-1), and any salt, and any synonyms. If uncertain about chemical identity, please look it up. Perfluorooctane sulfonic acid (CAS 1763-23-1), any salt, and any synonyms. If uncertain about chemical identity, please look it up. **Human:** Any exposure to PFOS or PFOA via any route. **Animal:** Any exposure to PFOA or PFOS via the oral route. Studies involving intraperitoneal or dermal exposures, or exposure to mixtures will be tagged as "potentially relevant supplemental information". **Mechanistic**: Any cell type exposed to PFOS or PFOA alone. Studies involving exposures to mixtures will be tagged as "potentially relevant supplemental information".
Comparators	**Human:** A comparison or referent population exposed to lower levels (or no exposure/exposure below detection limits) or PFOA or PFOS, or exposure to PFOA or PFOS for shorter periods of time. Case reports and case series will be tracked as "potentially relevant supplemental information." **Animal:** A concurrent control group exposed to vehicle-only treatment or untreated control. **Mechanistic:** A concurrent control group of cells exposed to vehicle-only treatment or untreated control.
Outcomes	All health outcomes (both cancer and noncancer) and toxicokinetics. **Exclude**: ecological studies, animal biomonitoring studies, and reviews

During title/abstract or full-text level screening in DistillerSR, studies that did not meet the PECO criteria, but which could provide supporting information were categorized as supplemental material. Studies that were categorized as supplemental material were not necessarily excluded from consideration in the assessment and may be used as supporting evidence. Studies were tagged as potentially relevant supplemental material if they were:

- Human epidemiology studies[13]
- Mechanistic: cancer and noncancer (including in vitro studies)
- Non-mammalian model
- Non-oral routes of administration

A total of 507 studies at the title/abstract screening level and a total of 5 studies at the full text screening level were tagged as supplemental material.

[13] A separate search was conducted specifically for human epidemiology studies as outlined in Appendix 7.

APPENDIX 2. DEFAULT UNCERTAINTY FACTORS

This appendix describes the default uncertainty factors OEHHA generally uses to calculate the Acceptable Daily Dose when deriving PHGs. When scientific evidence is compelling, these defaults are supplanted by alternative factors or modeled results. Table A2.1 below is adapted from OEHHA's "Technical Support Document for the Development of Noncancer Reference Exposure Levels" (OEHHA, 2008).

Table A2.1. Default uncertainty factors for PHG derivation, adapted from OEHHA (2008)

Uncertainty Factor	Value
Interspecies uncertainty factor (UF_A)	
Combined interspecies uncertainty factor (UF_A):	1 human observation √10 animal observation in nonhuman primates 10 where no data are available on toxicokinetic or toxicodynamic differences between humans and a non-primate test species
Toxicokinetic component (UF_{A-k}) of UF_A:	1 where animal and human PBPK models are used to describe interspecies differences √10 nonprimate studies with no chemical or species specific kinetic data
Toxicodynamic component (UF_{A-d}) of UF_A:	1 where animal and human mechanistic data fully describe interspecies differences (*This is unlikely to be the case.)* 2 for residual susceptibility differences where there are some toxicodynamic data √10 nonprimate studies with no data on toxicodynamic interspecies differences
Intraspecies uncertainty factor (UF_H)	
Toxicokinetic component (UF_{H-k}) of UF_H:	1 human study including sensitive subpopulations (e.g., infants and children), or where a PBPK model is used and accounts for measured interindividual variability √10 for residual susceptibility differences where there are some toxicokinetic data (e.g., PBPK models for adults only) 10 to allow for diversity, including infants and children, with no human kinetic data
Toxicodynamic component (UF_{H-d}) of UF_H:	1 human study including sensitive subpopulations (e.g., infants and children) √10 human studies with normal adult subjects only, but no reason to suspect additional susceptibility of children 10 suspect additional susceptibility of children (e.g., exacerbation of asthma, neurotoxicity)

Uncertainty Factor	Value
LOAEL uncertainty factor (UF_L)	
Values used:	10 LOAEL, any effect 1 NOAEL or BMDL used
Subchronic uncertainty factor (UF_S)[1]	
Values used:	1 study duration >12% of estimated lifetime √10 study duration 8-12% of estimated lifetime 10 study duration <8% of estimated lifetime
Database deficiency factor (UF_D)	
Values used:	1 no substantial data gaps √10 substantial data gaps including, but not limited to, developmental toxicity

[1] Exposure durations of 13 weeks or less are subchronic regardless of species (OEHHA, 2008)

Reference

OEHHA (2008). Air toxics hot spots risk assessment guidelines: technical support document for the derivation of noncancer reference exposure levels. Office of Environmental Health Hazard Assessment, California Environmental Protection Agency, Sacramento, CA.

APPENDIX 3. DRINKING WATER INTAKE RATES

OEHHA's drinking water intake rates were derived from a nationwide survey of food and beverage intake from approximately 20,000 people (US Department of Agriculture's Continuing Survey of Food Intake of Individuals 1994-1996, 1998 dataset), using the 95th percentile values for ages 0<2 (0.196 L/kg-day), 2<16 (0.061 L/kg-day), and 16<70 years (0.045 L/kg-day) as reported in OEHHA (2012). These intake rates were derived from consumers only, using a two-day average for both direct and indirect sources of community water consumption. The lifetime average (0<70 years of age) drinking water intake rate calculated by OEHHA for oral exposure used in the assessment of drinking water contaminants is 0.053 L/kg-day. These rates are discussed in the *Air Toxics Hot Spots Program Risk Assessment Guideline: Technical Support Document for Exposure Assessment and Stochastic Analysis* (OEHHA, 2012).

The US EPA recently updated its chapter on water consumption in the *Exposure Factors Handbook* (EFH) (US EPA, 2019). Their drinking water intake rates, which are lower than those developed by OEHHA in 2012, are based on analyses of the National Health and Nutrition Examination Survey (NHANES) data from 2005 – 2010.

Since then, tap water intake rates have been on the rise. A recent study of trends in tap and bottled water consumption using NHANES 2011 – 2016 data noted, "On the average, about 62% of drinking water came from the tap, a major increase from 56% osbserved in the 2005 – 2010 NHANES database" (Vieux et al., 2020). OEHHA's preliminary analysis of 2015 – 2016 NHANES data (not shown in this document) resulted in estimated drinking water intake rates that are consistent with this increase, and with the intake rates developed previously (OEHHA, 2012).

Furthermore, the two-day average consumer-only 95th percentile intake rate of 0.0439 L/kg-day,[14] derived in the 2019 EFH update for all ages (lifetime), is not different from the value of 0.044 L/kg-day derived for the same parameters in the original EFH (US EPA, 2011). While the weighted average of the 95th percentile lifetime consumption rate (0<70 years old) as calculated from the 2019 EFH intake rates is a bit lower than the value currently used by OEHHA (0.046 L/kg-day[15] compared to 0.053 L/kg-day, for US EPA and OEHHA, respectively), this is partly due to methodological differences in deriving these estimates. The largest difference in water consumption rate was observed in the 0<2 years age range. US EPA's updated water consumption rates for this age group included all infants, while OEHHA's guidance included only infants fed powder formula reconstituted with tap water. The US EPA's inclusion of all infants, particularly those that are exclusively breastfed and drink little or no water, would underestimate drinking water intake rates of infants who are fed reconstituted formula. While OEHHA's approach yielded a higher consumption rate, it is more representative of formula-fed infants. OEHHA is mandated to consider sensitive subgroups, such as children and infants, who may be at greater risk of adverse health effects due to their greater exposure to drinking water contaminants on a body weight basis than the general population.

California-specific consumption rates are likely to be higher than the national average. A variety of other factors, such as geography and resident lifestyles, can contribute to certain subgroups having higher water intake rates. California is diverse in both climate and population and has a

[14] Reported in USEPA (2019) Table 3-21.
[15] The weighted average of the 95th-percentile two-day average values in, and weighted by the number of years in, the 0<2, 2<16, and 16<70 age groups reported in USEPA (2019), Table 3-21.

climate conducive to outdoor activities. California also has a high number of warm, dry days in the warm seasons in much of the state. Extreme heat events in California have increased over the past several years, and are anticipated to continue increasing (OEHHA, 2018). The CDC recommends hydration as a main strategy for combating heat related illnesses,[16] resulting in increased consumption of water during outdoor activities, occupational and recreational, in warm weather.

In 2019, nearly one million Californians were employed in occupations commonly performed outdoors, such as farm work and construction, according to labor statistics released by the US Bureau of Labor Statistics.[17] The California Department of Industrial Relation's Division of Occupational Safety and Health, also known as Cal/OSHA, recommends that farmworkers and other outdoor laborers drink four 8-ounce glasses of water, or a total of one quart, per hour for heat illness prevention.[18] This is equivalent to 7.5 L in an 8 hour shift, twice the 95th percentile consumption rate for adults. A survey of farmworkers in Oregon and Washington found that 78% of participants reported drinking water at least once per hour while working, but did not specify the amount (Bethel, 2018). California has 5.8 times the national average of people employed as farmworkers and laborers.[19] In addition to farmworkers, some construction occupations are two to four times more prevalent in California as well, including solar panel installers, plasterers and stucco masons, and stone masons. Occupational Employment Statistics provided by the US Bureau of Labor Statistics do not specify legal status of workers, so it is possible that undocumented laborers are underrepresented in these numbers, thus the number of workers engaged in outdoor labor in California may be even higher.

Lifestyle and recreation also play a large role in the water consumption habits of Californians. Factors such as the increased use of reusable water bottles, particularly among children, may lead to increased tap water consumption among Californians. Single-use plastic water bottles have been banned in some California cities, and both environmental sustainability and cost effectiveness are possible reasons behind increased sales and use of refillable water bottles state-wide. Water intake in school-aged children is likely higher than current estimates, following the passage of Senate Bill 1413 in 2011, requiring schools to provide access to clean drinking water for children. A survey of 240 California public schools found that 96% allowed student use of reusable water bottles (Altman et al., 2020) which, when coupled with the availability of clean drinking water on school campuses, suggests that water consumption in school-aged children may be higher than estimated by either OEHHA or US EPA, since both values were derived using data collected prior to implementation of SB 1413.

Participation in sports and recreational activities likely also yields increased water consumption in children. In a 2017-2018 survey of California children 6-17 years of age, 58.5% participated in team sports or sports lesson activities after school and on weekends.[20] This was in addition to normal outdoor play and activities on school days. Overall participation in sports and

[16] https://www.cdc.gov/disasters/extremeheat/heattips.html, accessed June 2020.
[17] https://www.bls.gov/oes/current/oes_ca.htm, accessed June 2020.
[18] https://www.dir.ca.gov/dosh/etools/08-006/EWP_water.htm, accessed June 2020.
[19] https://www.bls.gov/oes/current/oes_ca.htm, accessed June 2020.
[20] Child and Adolescent Health Measurement Initiative. 2017-2018 National Survey of Children's Health (NSCH) https://www.childhealthdata.org/browse/survey, accessed June 2020.

exercise is higher in all age groups in western states, with 22.9% of California residents participating in some form of sport or exercise on an average day.[21]

Water consumption during physical activities varies based on environmental conditions and type of activity. A review of athletes' drinking habits during competitive sporting activities evaluated studies involving running (marathon, ultramarathon, triathlon), iron man events, cycling, and indoor and outdoor team sports (soccer, volleyball, and basketball) (Garth, 2013). Mean fluid intake varied considerably, with up to 1.5 L/hour consumed at the high end of the range. The duration of the activity did not seem to relate to the amount of water consumed. Athletes running a half marathon recorded ad libitum fluid intake rate of 0.38 L/hour, while long distance endurance runners consumed between 0.5 and 0.85 L/hour, depending on ambient temperature (Dion, 2013; Hoffman, 2018). A study of endurance athletes running a marathon observed a mean fluid intake rate of 0.5 L/hour (Beis, 2012). Fluid intake for endurance activities may be even higher; for example, a study of ultra-endurance cycling observed fluid intake rates of 0.3 – 1.2 L/hour in a hot climate (Armstrong, 2015). Similar levels of fluid intake were observed for sporting activities with short, intense bursts of activity, such as rugby. Burgh et. al. (2017) recorded 0.88 L intake over a 40-minute period involving six 6-minute games separated by a 2-minute rest period, giving an approximate intake rate of 1.3 L/hour. It is likely that team sports, many of which involve short bursts of intense activity, may last only one hour, whereas endurance sports may last between 4 – 10 hours, occasionally more. Endurance events require extensive training over time, so these elevated water intake rates are not restricted to a single event, but likely persist over longer periods.

Given the scenarios described above, it is unlikely that California's drinking water intake rates have decreased since the publication of OEHHA's guidelines in 2012 (OEHHA, 2012), and the national average presented in US EPA's 2019 Exposure Factors Handbook update may not be representative of this state's water intake rates. Thus, to be protective of all California residents,this assessment uses the age-specific water ingestion estimates based on OEHHA's existing peer-reviewed guidance (OEHHA, 2012).

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APPENDIX 4. RELATIVE SOURCE CONTRIBUTION

In estimating health-protective levels of chemicals in drinking water for noncancer endpoints, OEHHA considers the relative source contribution (RSC), which is the proportion of exposure to a chemical attributed to tap water as part of total exposure from all sources, including food and ambient air. When developing an appropriate RSC value for a chemical, OEHHA follows US EPA's Exposure Decision Tree Approach (US EPA, 2000). This approach takes into account the availability of exposure data, including the levels and relevant sources of exposure, and any other non-water regulatory standards for the chemical (Figure A4.1). In addition, any specific subpopulations of concern are identified and considered in the process. A chemical-specific RSC value can be calculated when adequate data are available for all sources of exposure, including exposures from drinking water. If data are not adequate for one or more of these non-water exposure sources, then default assumptions may be used to ensure a health-protective RSC value (US EPA, 2000). These default values include a floor (or lower-bound estimate) of 20% and a ceiling (or upper-bound estimate) of 80%. However, US EPA indicates that 50% may be used when information is not available to characterize exposures other than that arising from the source of concern, i.e., drinking water.

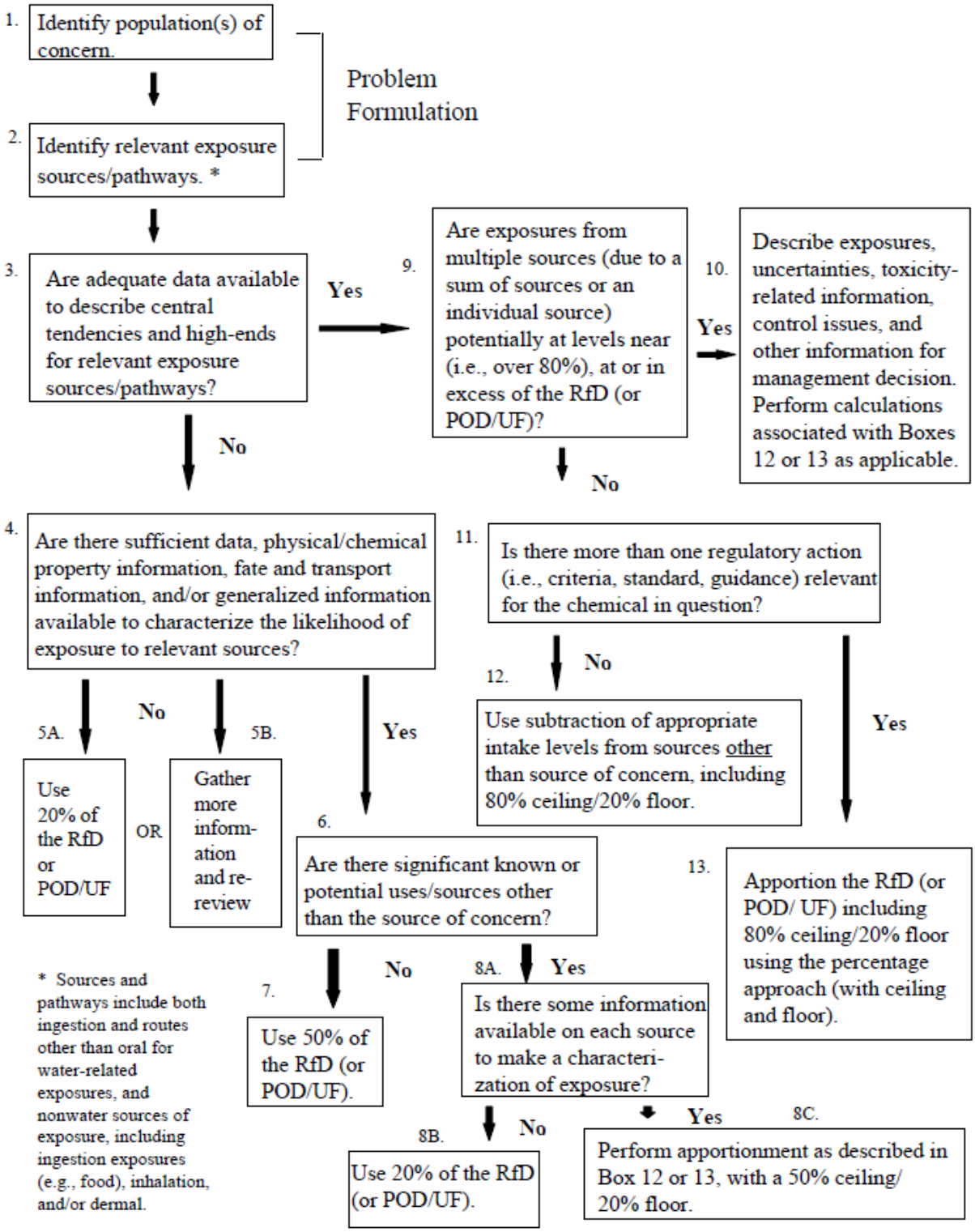
The initial steps in the decision tree, Problem Formulation (Figure A4.1, Boxes 1 and 2), identify populations of concern and relevant sources and pathways of exposure. For PFOA and PFOS, the population of concern is all residents of California. Relevant exposure sources are diet, tap water, household dust, and consumer products (Figure A4.1, Box 2).

Availability of data to describe PFOA and PFOS exposure from these sources is limited. For example, to calculate exposure from dietary sources, a database of PFOA and PFOS levels in individual foods is required, along with dietary consumption rates of the general population. Dietary consumption rates are commonly derived from National Health and Nutrition Examination Survey (NHANES) data; however, a complete dataset for PFOA and PFOS levels in food is lacking. The US Food and Drug Administration (FDA) formed a workgroup to perform ongoing assessments of food contamination related to PFAS.[22] Recently, FDA (2019) released detected levels of 16 PFAS in foods commonly consumed across the US. In the data released thus far, 179 individual food samples from six different food groups were assayed, and PFOS was detected in only two – ground turkey and tilapia (Table A4.1). PFOA was not detected in any of the food samples. The remaining food samples had levels of PFAS below the method detection limit (MDL) for the method used[23] (Table A4.1).

[22] https://www.fda.gov/food/chemicals/and-polyfluoroalkyl-substances-pfas

[23] US FDA Foods Program Compendium of Analytical Laboratory Methods: Chemical Analytical Manual, Method Number: C-010.01. Determination of 16 Perfluoroaklyl and Polyfluoroalkyl Substances (PFAS) in Food using Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS). https://www.fda.gov/media/131510/download

Figure A4.1. US EPA exposure decision tree for defining proposed RfD (or POD/UF) Apportionment (US EPA, 2000)



FDA reported MDLs for the following six food groups: lettuce, salmon, bread, cheese, meat products, and milk (Table A4.1). Of these, the European Food Safety Authority (2018) and Christensen et al. (2017) have indicated that meat products and fish may be important contributors to PFOS exposure, and milk, cheese, and fish may be an important contributor to PFOA exposure. However, due to the FDA's limited data on food in the US, dietary exposures to PFOA and PFOS for the US population cannot be quantified using this data set. OEHHA is not aware of any other US-specific or California-specific data sets for PFOA/PFOS in food that can be used for RSC estimation.

Table A4.1. Detected levels of PFOA and PFOS in six food groups, as reported by FDA (2019)

Food	PFOA Residue or *MDL*[1] (ng/g)	PFOS Residue or *MDL* (ng/g)
Ground Turkey	N/A	0.0857
Tilapia	N/A	0.087
Lettuce	*< 0.020*	*< 0.033*
Salmon	*< 0.090*	*< 0.082*
Meat Products	*< 0.090*	*< 0.082*
Cheese	*< 0.419*	*< 0.344*
Milk	*< 0.042*	*< 0.024*
Bread	*< 0.041*	*< 0.033*

[1] Since no residues were detected in any food samples, the respective MDLs are shown.
N/A, not available

EFSA recently released a draft scientific opinion on the risks to human health related to the presence of PFAS in food (EFSA, 2018). Analytical data on PFAS levels from 11,528 samples of food and beverages were used to estimate total dietary intake across multiple age groups. Of the 17 PFAS assayed, four were included in the risk assessment: PFOA, PFOS, PFHxS, and PFNA. The in-depth analysis estimated significant dietary exposures to adults resulting from consumption of fish and seafood, eggs, meat and meat products, and fruit and fruit products. The mean upper-bound chronic dietary exposure to PFOA and PFOS are 4.18 and 4.47 ng/kg bw per day for adults, respectively. Although the EFSA dietary database is extensive, large dietary differences such as eating habits, foods of choice, and food sources, exist between California and the European countries surveyed in this dataset; thus, this dataset is not appropriate for deriving a dietary intake value for the purposes of deriving a RSC value.

Neither the EFSA nor the FDA data account for exposures from food packaging materials that may be in contact with food items. The FDA uses a market basket approach to sampling, where both packaged and non-packaged foods are included. The amount of PFAS chemicals transferred to food products from packaging may be dependent on food type and the time of contact; thus the contribution of food packaging materials to levels detected in food, and to total PFAS exposure, is uncertain. The EFSA draft dietary risk assessment noted that possible exposures can occur from food packaging materials but their contributions were not included in the assessment. A study in California women observed a correlation between consumption of prepared food in cardboard containers with higher serum levels of PFOA and PFOS (Boronow et al., 2019), suggesting migration of the chemicals from food packaging into the food product. While uses of PFAS in packaging and food contact substances is being phased out, they can still be found in food packaging materials (Schaider et al., 2018).

An additional source of PFAS exposure comes from ingestion of household dust (Karaskova et al., 2016; Knobeloch et al., 2012). This exposure pathway may be important for infants and children, who spend more time in contact with flooring and other surfaces which may harbor household dust (i.e., they are closer to the ground), and have increased hand-to-mouth activity. Although neither PFOA nor PFOS are volatile, inhalation of these chemicals bound to fine particulate matter, such as household dust, may contribute to exposure (Trudel et al. 2008). Inhalation of larger particulate matter (e.g., larger than respirable size or >10 um in diameter) in household dust contributes to the ingestion pathway, since large particles would deposit in the upper airway including nose and mouth and be swallowed. The use of PFAS in carpets and rugs is decreasing; however, PFAS-containing carpets and rugs will likely be found in homes for years to come, making household dust and carpet residues a continuing source of exposure. PFOA and PFOS together accounted for a majority of PFAS detected in dust from US homes, and were significantly higher in homes with carpet and wood, versus other types of floor coverings. Of 14 US homes sampled, the mean values for PFOA and PFOS in house dust were 38.6 ng/g and 42.4 ng/g (Karaskova et al., 2016), respectively.

In addition, there are exposures to PFAS from using consumer products, which is a category of exposure that has not been well characterized. PFOA and PFOS can be found in a wide range of products including but not limited to makeup, hygiene products, cookware, and clothing (Fujii et al., 2013; Hu et al., 2018; Gremmel et al., 2016). Hand-to-mouth exposure stemming from the handling of consumer products in the home is of concern, even for adult populations (Poothong et al., 2019). In addition to uncertainty surrounding quantitative levels of PFAS found in consumer products, there is also uncertainty regarding the extent to which the broad category of consumer products contributes to the overall exposure to PFAS.

As discussed, there is evidence to suggest that there are many sources that may contribute significantly to the total exposure to PFOA and PFOS (Figure A4.1, Boxes 4 and 6). However, there are insufficient data to make a characterization of exposure for each source (Figure A4.1, Box 8A). Thus, OEHHA determined it is appropriate to use an RSC of 20% (Figure A4.1, Box 8B) for tap water.

Of note, US EPA's decision tree presents two approaches for deriving the RSC when sufficient data are available: the subtraction approach and the percentage approach (Figure A4.1, Boxes 12 and 13). The subtraction approach may be used when water is considered the most relevant source of exposure and other sources of exposure can be considered background. The RSC is calculated by subtracting the 'background' level of exposure, i.e., the detected serum levels from biomonitoring studies, from the target serum level. This method allows for the maximum possible contaminant levels in water, after subtracting other sources of exposure. The percentage approach is appropriate when exposure from multiple sources are considered relevant, and appropriate data are available to estimate exposure to a chemical for all water and non-water sources of exposure. The RSC is calculated as a percentage based on the exposure due to drinking water divided by the sum of exposures for all sources. Some states with developed health criteria for PFOA and/or PFOS have utilized these approaches in deriving

their RSC values, including Michigan,[24] Minnesota,[25] and New Hampshire.[26] These RSC values varied from 20% to 50% for both PFOA and PFOS, and relied on state-specific and/or NHANES biomonitoring data. In contrast, other regulatory bodies, including New Jersey, US EPA, and Health Canada, considered a default value of 20% to be appropriate due to a lack of information about sources of exposure and inadequate data to develop chemical-specific values.

The subtraction approach is appropriate for use in areas with high levels of PFOA and/or PFOS in drinking water, relative to other sources. However, data are lacking to suggest that this is the case in California. There are California-specific biomonitoring data on PFOA and PFOS (Table A4.2), showing a decreasing trend of PFOA and PFOS serum levels from 2013 to 2018. For comparison, the 2013-2014 dataset for serum levels in adults from NHANES are also shown (CDC, 2018). When using the subtraction approach, serum levels are assumed to represent background exposure levels (i.e., from all sources other than ingestion of water). However, these California-specific serum levels represent exposure from all sources, including tap water, and there are no data to evaluate the contribution of other sources relative to drinking water; thus, they should not be used to determine the RSC.

Table A4.2. Serum levels of PFOA and PFOS detected in Biomonitoring California[27] and NHANES studies

Study	PFOA (ng/ml)		PFOS (ng/ml)	
	Geometric Mean	95th Percentile	Geometric Mean	95th Percentile
CARE-LA (data collected in 2018)	1.04	3.06	2.13	8.33
BEST Expanded (data collected in 2013)	1.49	4.57	5.21	17.6
NHANES (data collected in 2013-2014)	1.98	5.6	5.22	19.5

The percentage approach requires estimations of exposures to all sources. However, there are extremely limited US-specific or California-specific data on PFOA and PFOS in food groups, indoor dust, and consumer products, as discussed above. Thus, OEHHA determined that the percentage approach cannot be used at this time for estimating RSC values for PFOA and PFOS.

[24] Michigan Department of Public Health and Human Services, Division of Environmental Health, Public health drinking water screening levels for PFAS https://www.michigan.gov/documents/pfasresponse/MDHHS_Public_Health_Drinking_Water_Screening_Levels_for_PFAS_651683_7.pdf

[25] Minnesota Department of Health Toxicological Summary for Perfluorooctanoate and Perfluorooctane sulfonate: https://www.health.state.mn.us/communities/environment/risk/docs/guidance/gw/pfoa.pdf , https://www.health.state.mn.us/communities/environment/risk/docs/guidance/gw/pfos.pdf

[26] Summary Report on the New Hampshire Department of Environmental Services, Development of Maximum Contaminant Levels and Ambient Groundwater Quality Standards for PFOS, PFOA, PFNA, and PFHxS, https://www.des.nh.gov/organization/commissioner/pip/publications/documents/r-wd-19-01.pdf

[27] Biomonitoring California Results Database for PFAS chemicals https://biomonitoring.ca.gov/results/chemical/2183

Due to a lack of information about sources of exposure and inadequate data to develop chemical-specific values, OEHHA is adopting the default value of 20% for PFOA and PFOS in evaluating noncancer endpoints. This determination is consistent with other regulatory bodies, including New Jersey, US EPA, and Health Canada.

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APPENDIX 5. HUMAN EXPOSURE STUDIES AND DATA

Table A5.1. Human exposure studies

Reference	Food	Water	Dust (ingested)	Dermal	Indoor air	Outdoor air	Total
PFOA exposure studies							
Lorber and Egeghy (2011) *young children* % total *adults* % total	*median (range[a])* 8.1 ng/day (1.7-37) 31% 46 ng/day (9.7-208) 72%	 4.8 ng/day (0.72-28) 19% 17 ng/day (2.5-100) 7.5%	 12.6 ng/day (1.0-114) 49% 6.3 ng/day (0.51-55) 19.8%	 0.008 ng/day ($6x10^{-4}$-0.07) <0.1% 0.005 ng/day ($4x10^{-4}$-0.04) <0.1%	 0.26 ng/day (0.12-0.59) 1% 0.61 ng/day (0.26-1.3) 0.4%	 3 pg/day (2-8) <0.1% 4 pg/day (2-9) <0.1%	 *median* 25.8 ng/day *median* 72.2 ng/day
Haug et al. (2011a)[b] *Adult women* % of total *Infants at 6 months* % of total	*median (range[c])* 0.22 ng/kg-day (0.16-0.29) 66% *breastmilk:* 4.1 ng/kg-day (3.5-9.2) 83%	 30 pg/kg-day (27-34) 9.0% -	 49 pg/kg-day (32-81) 14.7% 0.66 ng/kg-day (0.47-1.1) 13%	 - -	 35 pg/kg-day (25-49) 10.5% 0.17 ng/kg-day (0.13-0.20) 3.4%	 - -	*median* *0.33* ng/kg-day *mean* 0.38 ng/kg-day *median* *4.9* ng/kg-day *mean* 14 ng/kg-day
PFOS exposure studies							
Egeghy and Lorber (2011) *young children* % total *adults* % total	*Median (range[a])* 20 ng/day (3.6-97) 42% 112 ng/day (20-550) 71%	 9.3 ng/day (2.7-27) 19% 36 ng/day (11-109) 23%	 17.8 ng/day (0.49-219) 37% 8.6 ng/day (0.25-106) 5.5%	 0.70 ng/day (0.019-8.6) 1.5% 0.29 ng/day (0.008-3.5) 0.2%	 0.10 ng/day (0.047-0.24) 0.2% 0.27 ng/d (0.13-0.6) 0.2%	 2 pg/day (0.9-3) <0.1% 2 pg/day (1-4) <0.1%	 *median* 47.9 ng/day *median* 157 ng/day

Reference	Food	Water	Dust (ingested)	Dermal	Indoor air	Outdoor air	Total
Haug et al. (2011a)[b]	*Median (range[c])*						*median*
Adult women	0. 61 ng/kg-day (0.47-0.88)	4 pg/kg-day (4-5)	11 pg/kg-day (7-22)	-	77 pg/kg-day (36-110)	-	0.70 ng/kg-day *mean* 0.84 ng/kg-day
% of total	87%	0.6%	1.6%		11%		
Infants at 6 months	*breastmilk:* 8.7 ng/kg-day (5.8-9.6)	-	0.09 ng/kg-day (0.07-0.3)	-	0.3 ng/kg-day (0.13-0.43)	-	*median* 9.1 ng/kg-day *mean* 10.0 ng/kg-day
% of total	96%		1%		3.3%		

[a] 5th-95th percentile
[b] Scenario 3 in Haug et al. (2011a) is considered, which included high level of dust exposure (200 mg/kg) and significant contribution of biotransformation of precursors in the indoor air (100% of FOSA/FOSEs to PFOS, and 1.7% of FTOHs to PFOA).
[c] 25th-75th percentile

Table A5.2. PFOA and PFOS trends in serum concentration

Reference	Area	Participants	PFAS	Year-C_{serum}(ng/ml)[#]	Trend
Calafat et al. (2007a)	US, NHANES	General population (≥12 years) (N=2,094)	PFOA	**Total** 1999/2000: 5.2; 2003/04: 3.9 **males** 1999/2000: 5.7; 2003/04: 4.5 **females** 1999/2000: 4.8; 2003/04: 3.6	Slightly **decreased**
			PFOS	**Total** 1999/2000: 30.4; 2003/04: 20.7 **males** 1999/2000: 33.4; 2003/04: 23.3 **females** 1999/2000: 28.0; 2003/04: 18.4	**Decreased**
Kato et al. (2011)	US, NHANES	General population (≥12 years) (N=7,876)	PFOA	1999/00: 5.21 (95th pctl: 11.9) 2003/04: 3.95 (95th pctl: 9.8) 2005/06: 3.92 (95th pctl: 11.3) 2007/08: 4.13 (95th pctl: 9.7)	Slightly **decreased**
			PFOS	1999/00: 30.4 2003/04: 20.7 2005/06: 17.1 2007/08: 13.2	**Decreased**
Wang et al. (2011)	US, Northern California	Pregnant women, archived samples (N=105)	PFOA	1960s[c]: 0.30 1980s: 3.17 2009: 2.21	**Peak** in the 1980s
			PFOS	1960s[c]: 45.90 1980s: 30.60 2009: 9.44	**Decreased**
Olsen et al. (2012)	US, 6 cities (Boston, Charlotte, Hagers-town, Los Angeles, Minnea-polis Saint Paul, Portland)	Red Cross adult plasma samples (N=10/ten-year age interval/sex)	PFOA	**Total** 2000/01: 4.7 (95th pctl: 12) 2006: 3.44 (95th pctl: 7.9) 2010: 2.44 (95th pctl: 6.6) **males** 2000/01: 5.0 (95th pctl: 13.5) 2006: 3.95 (95th pctl: 8.3) 2010: 2.69 (95th pctl: 5.8) **females** 2000/01: 4.4 (95th pctl: 10.7) 2006: 3.0 (95th pctl: 6.7) 2010: 2.22 (95th pctl: 6.8)	Both PFOA and PFOS **decreased**; halving time for PFOS 4.3 years; decreasing trends for both sexes, in all 6 locations
			PFOS	**Total** 2000/01: 34.9 (95th pctl: 75) 2006: 14.5 (95th pctl: 31.5) 2010: 8.3 (95th pctl: 24.9) **males** 2000/01: 37.8 (95th pctl: 80) 2006: 17.1 (95th pctl: 36.5) 2010: 9.7 (95th pctl: 24.9) **females** 2000/01: 32.1 (95th pctl: 74) 2006: 12.3 (95th pctl: 29.7) 2010: 7.2 (95th pctl: 19.6)	

Reference	Area	Participants	PFAS	Year-C_{serum}(ng/ml)[#]	Trend
Gribble et al. (2015)	US, South Carolina	Gullah African Americans (N=71)	PFOA	Median 5.6 at baseline (2010-2013) and decreased by 2.2 (median) over 7.3 years on average	For either PFOA or PFOS, approximately 50% **decrease** over 7 years
			PFOS	Median 41.1 at baseline (2010-2013) and decreased by 21.7 (median) over 7.3 years on average	
Jain (2018)	US, NHANES	Adults (≥20 years), (N=9,650) weighted unadjusted values	PFOA	**Total** 2003/04: 3.99; 2005/06: 4.03 2007/08: 4.17; 2009/10: 3.12 2011/12: 2.13; 2013/14: 2 **males** 2003/04: 4.48; 2005/06: 4.81 2007/08: 4.91; 2009/10: 3.61 2011/12: 2.45; 2013/14: 2.36 **females** 2003/04: 3.57; 2005/06: 3.39 2007/08: 3.56; 2009/10: 2.72 2011/12: 1.88; 2013/14: 1.71	Both PFOA and PFOS **decreased**, on average by 17.8%/2 years and 33.8%/2 years, respectively
			PFOS	**Total** 2003/04: 21.09; 2005/06: 17.7 2007/08: 13.54; 2009/10: 9.7 2011/12: 6.74; 2013/14: 5.27 **males** 2003/04: 23.67; 2005/06: 21.24 2007/08: 17.11; 2009/10: 12.13 2011/12: 8.54; 2013/14: 6.81 **females** 2003/04: 18.81; 2005/06: 14.83 2007/08: 10.79; 2009/10: 7.84 2011/12: 5.4; 2013/14: 4.12	
Kim et al. (2020)	Northern California	Women (mothers) (N=450)	PFOA	2009: 1.66; 2010: 1.37 2011: 1.26; 2012: 1.14 2013: 1.02; 2014: 0.935 2015: 0.801; 2016: 0.675	On average **decreased** by 10.7%/year
			PFOS	2009: 4.86; 2010: 4.1 2011: 3.78; 2012: 3.42 2013: 3.02; 2014: 2.8 2015: 2.42; 2016: 2.12	On average **decreased** by 10.8%/year

Reference	Area	Participants	PFAS	Year-C_{serum}(ng/ml)[#]	Trend
Non-US studies					
Harada et al. (2007a)	Japan (Kyoto)	Adults (N=100)	PFOA	**Males** 1983: 2.52; 1987: 4.4 1991: 5.48; 1995: 6.56 1999: 11.44 **females** 1983: 1.84; 1987: 4.64 1991: 6.36; 1995: 5.52 1999: 8.12	**Increased**
			PFOS	**Males** 1983: 15.1; 1987: 21.9 1991: 15.1; 1995: 18.1 1999: 22.8 **females** 1983: 13.3; 1987: 18.0 1991: 19.1; 1995: 16.4 1999: 18.5	**No trend**
Haug et al. (2009)	Norway	Adult men (N=57, pooled samples)	PFOA	1977: 0.58; 1980: 1.3; 1981: 1.4 1982: 1.4; 1883: 1.5; 1985: 2.2 1986: 2.6; 1988: 2.7; 1989: 3.1 1990: 3.3; 1991: 3.4; 1993: 5.2 1994: 4.1; 1995: 4.4; 1996: 4.0 1997: 4.2; 1999: 4.0; 2000: 4.5 2001: 4.9; 2002: 3.9; 2003: 3.8 2004: 3.4; 2005: 3.5; 2006: 2.7	**Increased** through the 90s, then **decreased**
			PFOS	1977: 3.8; 1980: 6.1; 1981: 9.4 1982: 11; 1983: 10; 1985: 16 1986: 15; 1988: 18; 1989: 22 1990: 20; 1991: 23; 1993: 33 1994: 24; 1995: 31; 1996: 25 1997: 31; 1999: 29; 2000: 30 2001: 27; 2002: 27; 2003: 19 2004: 18; 2005: 21; 2006: 12	**Increased** through the 90s, then **decreased**
Harada et al. (2010)	South Korea	Women, archive samples (N=24-39)	PFOA	**Busan** 1994: 4.1; 2000: 3.7; 2008: 4.5 **Seoul** 1994: 1.1; 2007: 2.7	**No trend to slight increase**
			PFOS	**Busan** 1994: 10.9; 2000: 9.1; 2008: 9.4 **Seoul** 1994: 7.63; 2008: 7.61	**No trend**

Reference	Area	Participants	PFAS	Year-C_{serum}(ng/ml)[#]	Trend
Glynn et al. (2012)	Sweden	Women, 3 weeks after delivery (N= 36, pooled samples)	PFOA	1996: 2.18-2.92; 1997: 2.26-3.07 1998: 2.22-2.66; 1999: 2.38-3.11 2000: 2.50-2.65; 2001: 3.05 2002: 2.17-2.98; 2004: 2.12-2.15 2006: 1.70-2.11; 2007: 1.36-2.42 2008: 1.69-2.01; 2009: 1.54-2.4 2010: 1.39-1.96	**Decreased** an average 3.1 %/year (95% CI: 1.8-4.4 %/year), halving time 22 years
			PFOS	1996: 22.7-27.3; 1997: 20.3-24.8 1998: 20.2-23.1; 1999: 20.0-23.0 2000: 18.7-22.0; 2001: 28.1 2002: 17.0-23.3; 2004: 13.6-16.6 2006: 10.7-16.5; 2007: 8.80-18.3 2008: 9.25-11.1; 2009: 7.14-8.89 2010: 5.11-7.62	**Decreased** an average 8.4 %/year (95% CI: 5.9-11); halving time 8.2 years
Okada et al. (2013)	Japan	Pregnant women (N=150)	PFOA	2003[c]: 2.05; 2005: 1.25 2007: 1.56; 2009: 1.36 2011: 1.42	**Decreased**
			PFOS	2003[c]: 7.76; 2005: 6.20 2007: 6.23; 2009: 4.54 2011: 3.90	**Decreased**
Schröter-Kermani et al. (2013)	Germany	Archived plasma samples (N=260); data also reported separately for men and women	PFOA	1982: 4.0 (range 1.5-7.5) 1986: 7.4 (2.4-15.4) 1989: 6.3 (2.4-11.6) 1992: 5.8 (2.6-9.4) 1995: 5.1 (1.8-8.8) 1998: 5.3 (1.9-10.9) 2001: 5.8 (2.6-10.5) 2003: 4.8 (1.6-9.0) 2005: 5.5 (3.1-12.5) 2006: 4.7 (3.1-9.7) 2007: 5.2 (1.6-10.6) 2008: 4.0 (2.3-6.7) 2010: 3.1 (0.8-8.7)	**Mixed** trend through 2000s
			PFOS	1982: 15.4 (4.6-32.1) 1986: 28.0 (9.5-57.4) 1989: 28.6 (10.3-102.9) 1992: 23.7 (10.4-34.7) 1995: 21.1 (10.1-49.8) 1998: 20.7 (10.3-40.3) 2001: 19.7 (10.3-40.3) 2003: 13.7 (4.9-24.9) 2005: 12.7 (5.7-38.3) 2006: 7.4 (2.4-12.4) 2007: 7.8 (3.4-16.1) 2008: 6.1 (2.6-10.2) 2010: 3.8 (1.9-12.1)	**Peak** in 1989, then **decreased**

Reference	Area	Participants	PFAS	Year-C_{serum}(ng/ml)[#]	Trend
Yeung et al. (2013a)	Germany	Archived serum samples (N=420)	PFOA	**Munster**: concentrations increased from 1981 till 1986 (peak at 10 ng/ml), then steadily decreased through 2010 to approximately 2 ng/ml **Halle**: concentrations decreased 1995-2010	Munster: **peak** (1986), then **decrease** Halle: **decrease**
Yeung et al. (2013b)	Germany	Archived serum samples (N=420)	PFOS	**Munster**: concentrations increased from 1981 till 1986 (peak at approximately 40 ng/ml), then steadily decreased through 2010 to approximately 5 ng/ml **Halle**: concentrations decreased from 1995 through 2010 to approximately 5 ng/ml	Munster: **peak** (1986), then **decrease** Halle: **decrease**
Nøst et al. (2014)	Norway	Adult men (N=53), longitudinal study (same subjects)	PFOA	1979: 0.85; 1986: 2.38 1994: 3.74; 2001: 4.06 2007: 3.00	**Peak** in 2001
			PFOS	1979: 8.94; 1986: 22.7 1994: 36.9; 2001: 43.3 2007: 33.0	**Peak** in 2001
Toms et al. (2014)	Australia	All ages (pooled samples, N=158)	PFOA	2002/03: 10.2; 2006/07: 6.4 2008/09: 5.2; 2010/11: 4.5	**Decreased**
			PFOS	2002/03: 25.9; 2006/07: 15.2 2008/09: 11.9; 2010/11: 10.2	**Decreased**
Bjerregaard-Olesen et al. (2016)	Denmark	Pregnant women (N=1,533)	PFOA	2008[ab]: 0.97; 2009: 0.86 2010: 0.86; 2011: 0.69 2012: 0.57; 2013: 0.51	**Decreased**, adjusted trend: -9.5%/year
			PFOS	2008[ab]: 2.26; 2009: 2.08 2010: 2.01; 2011: 2.00 2012: 1.76; 2013: 1.75	**Decreased**, adjusted trend: -9.3%/year
Eriksson et al. (2017b)	Australia	Adults (N=54, pooled samples)	PFOA	**total[c]** 2002: 8.5; 2004: 6.1; 2006: 6.0 2008: 4.0; 2011: 3.1; 2013: 2.3 **males** 2002: 9.0; 2004: 5.9; 2006: 6.2 2008: 4.3; 2011: 3.4; 2013: 2.3 **females** 2002: 8.0; 2004: 6.4; 2006: 5.7 2008: 3.8; 2011: 2.9; 2013: 2.2	**Decreased** halving time (total): 5.3 years
			PFOS	**total[c]** 2002: 10.9; 2004: 6.9; 2006: 6.6 2008: 3.8; 2011: 2.6; 2013: 1.8 **males** 2002: 11.0; 2004: 7.1; 2006: 6.5 2008: 4.2; 2011: 2.6; 2013: 1.7 **females** 2002: 10.8; 2004: 6.6; 2006: 6.7 2008: 3.3; 2011: 2.3; 2013: 2.1	**Decreased**, halving time (total): 4.2 years

Reference	Area	Participants	PFAS	Year-C_{serum}(ng/ml)[#]	Trend
Seo et al. (2018)	South Korea	Adults (N=786)	PFOA	**Males** 2006: 2.90[c] (range 0.87-8.44) 2007: 5.40 (2.20-9.03) 2008: 4.94 (1.70-11.7) 2009: 5.00 (2.37-9.46) 2011: 4.96 (1.10-13.64) 2013: 4.93 (2.47-9.26) 2014: 12.10 (0.83-31.96) 2015: 4.78 (1.54-8.82) **females** 2006: 2.92[c] (range 0.66-9.99) 2007: 3.96 (1.88-9.34) 2008: 4.68 (1.01-12.37) 2009: 4.25 (1.10-10.46) 2011: 4.51 (0.76-14.17) 2013: 5.50 (1.57-11.71) 2014: 6.25 (0.39-37.79) 2015: 5.48 (0.86-17.18)	**Mixed** trend
			PFOS	**Males** 2006: 11.54 (4.62-29.73) 2007: 14.17 (5.78-29.66) 2008: 16.22 (5.55-50.11) 2009: 15.06 (4.28-31.82) 2011: 16.16 (1.82-40.19) 2013: 32.19 (10.21-70.09) 2014: 9.85 (1.55-29.31) 2015 15.38 (7.37-32.58) **females** 2006: 11.77 (5.90-26.26) 2007: 9.39 (3.35-19.69) 2008: 14.40 (3.51-42.44) 2009: 12.23 (0.69-129.43) 2011: 12.08 (1.17-54.16) 2013: 16.08 (6.07-38.22) 2014: 6.73 (1.04-20.81) 2015: 11.37 (2.04-27.32)	**Mixed** trend
Shu et al. (2019)	Sweden	Pregnant women (N=106-263)	PFOA	2007: 1.81; 2008: 1.65 2009: 1.55; 2010: 1.43	PFOA and PFOS **decreased** by 21% and 31% over 30 months
			PFOS	2007: 6.39; 2008: 5.83 2009: 4.7; 2010: 4.42	

[#]Geometric mean if not indicated otherwise; pctl, percentile
[a] Quantitated from graph using GetData Graph Digitizer 2.26
[b] Median values
[c] Arithmetic mean values

APPENDIX 6. TOXICOKINETICS SUPPLEMENTAL DATA TABLES

Table A6.1. Organ distribution of PFOA and PFOS in rat, mouse, monkey and human

Reference	Species (sex)	PFAS	Dose (mg/kg[a])	Distribution in organs[b]
Johnson et al. (1979) unpublished[c]	rat (m)	PFOS	not reported in Benskin et al. (2009)	**Liver**>plasma>kidney>lung>spleen>bone marrow>red blood cells>adrenals>testes> skin>fat>eye
Ylinen et al. (1990)	rat (m,f)	PFOA	3,10,30 for 28d (oral gavage)	Low dose (f): serum>**liver(0.75)**>lung, spleen, kidney>>brain(nd), ovary(nd) Mid and high doses (f): serum>kidney(0.6-0.9) >**liver(0.3-0.5)**>spleen>lung>ovary>brain; (m): serum>**liver(0.6-0.96)**>kidney(0.5-0.8)> lung>testes>spleen>brain
Vanden Heuvel et al. (1991)	rat (m,f)	PFOA	3.9; single (i.p.)	2h (m): **liver(1)**,plasma>kidney(0.48)>heart> testes, fat>gastrocnemius 2h (f): plasma>kidney(0.8)>**liver(0.6)**>ovaries
Kudo et al. (2001)	rat (m)	PFOA	25; single (i.p.)	5d: **liver(2.1[d])**>serum
Austin et al. (2003)	rat (f)	PFOS	1,10 for 14d (i.p.)	Low dose: **liver(2.5)**>serum>kidney(0.9)>> ovary>adrenal>heart>>brain>spleen High dose: **liver(2.1)**>serum,kidney(1)>heart> spleen, ovary, hypothalamus>brain
Lau et al. (2003)	pregnant rat	PFOS	1,2,3,5 for 20d (GD2-21) (oral gavage)	F1: **liver(1.3,0.98,1.2,1.5[d])**>serum
Seacat et al. (2003)	rat (m,f)	PFOS	(m) 0.05, 0.18, 0.37, 1.51 for 4w; 0.03, 0.13, 0.34, 1.33 for 14w; (f) 0.05, 0.22, 0.47. 1.77 for 4w; 0.04, 0.15, 0.4, 1.56 for 14w in food	4 weeks: **liver(3.7-12.2)**>serum 14 weeks: **liver(4.3-6.8)**>serum
Luebker et al. (2005a)	pregnant rat	PFOS	0.1-3.2 for about 60d (oral gavage)	GD 21 dams: **liver(3.3-4.9)**>serum; pups: at most doses serum>**liver(0.9)** PND 5 dams: **liver(0.6-1.8)**; pups: **liver(1.8-2)**>serum
Hundley et al. (2006)	rat (m)	PFOA	10; single (oral gavage)	120h: **liver(1.7)**>kidney(1)>blood>>lung> heart>skin>testes>muscle>fat>brain

Reference	Species (sex)	PFAS	Dose (mg/kg[a])	Distribution in organs[b]
Kudo et al. (2007)	rat (m)	PFOA	0.041,16.56; single (i.v.)	2h low dose: **liver(2.2)**>kidney(1.1), serum> blood>>lung, heart>spleen, testes, intestine> stomach, fat>brain 2h high dose: serum>**liver(0.83)**, kidney(0.75) > blood>lung>heart>spleen>testes>intestine> fat, stomach>brain
Curran et al. (2008)	rat (m,f)	PFOS	0.14-7.58 for 28d (in diet)	**Liver(13-47)**>>spleen>heart>serum
Benskin et al. (2009)	rat (m)	PFOA	0.4; single (oral gavage)	On day 38: **liver**>blood>kidney>lung>heart> testes>spleen>fat>intestine>muscle>brain
		PFOS	0.27; single (oral gavage)	On day 38: **liver**>lung>kidney>blood>spleen> heart>testes>intestine>muscle>brain>fat
Chang et al. (2009)	pregnant rat	PFOS	0.1,0.3,1 for 21d (GD0-20) (oral gavage)	Dams GD 20: **liver(1.8-4.5)**>serum>>brain Pups GD 20: serum>**liver(0.6-0.8)**>>brain Pups PND 4: **liver(2.2-4.2)**>serum>>brain
Cui et al. (2009)	rat (m)	PFOA	5,20 for 28d (oral gavage)	Low dose: kidney(5.8), **liver(5.6)**>lung>blood, heart>testes>spleen>brain[e] High dose: kidney(3.6), **liver(3.3)**>lung> blood>heart>testes>brain>spleen[e]
		PFOS	5,20 for 28d (oral gavage)	Low dose: **liver(4.8)**>heart>kidney(1.3)>blood >testes, spleen>brain[e] High dose (no blood sample available): **liver**>heart>kidney, lung>spleen>brain>testes
Chang et al. (2012)	rat (m)	PFOS	4.2; single (oral gavage)	89d: **liver(9.3)**>plasma>kidney(0.49),lung>> spleen, red blood cells, testes etc.
	rat (m,f)	PFOS	1,15; single (oral gavage)	10w: **liver(m:5.6-17.8; f:1.8-2.6)**>serum
Gao et al. (2015a)	rat (m,f)	PFOA	0.12 (m), 0.15 (f) for 90d[f] (drinking water)	(f): serum>kidney(0.72)>**liver(0.59)**>lung> spleen>heart>brain (m): serum>kidney(0.71)>lung>**liver(0.22)**> heart>spleen>>brain
		PFOS	0.12 (m), 0.15 (f) for 90d[f] (drinking water)	(f): kidney(1), **liver(1)**, serum>lung>spleen> heart>brain (m): **liver(4.3)**>serum, kidney(0.98)>lung> spleen>heart>brain

Kim et al. (2016b)	rat (m,f)	PFOA	1; single (oral gavage, i.v.)	i.v.(m): **liver(2.3)**>kidney(1.2)>plasma>> lung>heart>spleen;
				i.v.(f): kidney>plasma>**liver(0.8)**> lung>heart,spleen;
				results for oral gavage were reported as similar to i.v.

		PFOS	2; single (oral gavage, i.v.)	i.v.: **liver(2-2.6)**>plasma>kidney(0.2)>lung> spleen, heart
Bagley et al. (2017)	rat (m,f)	PFOS	6 (m), 6.6 (f) for 3w (in diet)	23d (m): **liver(1.5)**>serum: (f): **liver**=serum

Reference	Species (sex)	PFAS	Dose (mg/kg[a])	Distribution in organs[b]
Ishida et al. (2017)	pregnant rat	PFOS	1,2 for 10d (GD11-20) (oral gavage)	PND 4, F0: **liver(4[d])**>serum>>brain PND 4, F1: **liver(2.1-3.2[d])**>serum>>brain
Iwabuchi et al. (2017)	rat (m)	PFOA	0.1 single (oral gavage), or 1,5,25 µg/L[g] for 3 months (drinking water)	28d (single): **liver(1.7)**>>serum>kidney(0.82) >blood>heart>spleen>brain 3 months (high dose): **liver(3.3)**>serum> kidney(0.69)>blood>heart> spleen>>brain
		PFOS	0.1; single (oral gavage), or 1,5,25 µg/L[g] for 3 months (drinking water)	28d (single): **liver(22)**>>kidney(1.6)> serum> blood>spleen>heart,brain 3 months (high dose): **liver(38)**>kidney(1.2)> serum>blood>spleen>heart>brain
Huang et al. (2019a); NTP (2019a)	rat (m,f)	PFOS	2,5; single 2 for 5d (oral gavage)	All doses (single and 5d) (m): **liver(2-29)**>plasma, kidney(~1) >>brain; (f): **liver(1.5-3.7)**>kidney(1.1-1.9)> plasma>>brain
NTP (2019a); Dzierlenga et al. (2020)	rat (m,f)	PFOA	12(m), 80(f); single (oral gavage)	22d (m): **liver(1.3)**>plasma>kidney(0.4)>brain 12h (f): plasma>>**liver**, kidney>>brain
Hundley et al. (2006)	mouse (m)	PFOA	10; single (oral gavage)	120h: **liver(3.1)**>blood>>skin>kidney>fat, lung, heart, muscle>testes>>brain
	mouse (f)	PFOA	10; single (oral gavage)	120h: **liver(4.5)**>blood>kidney>lung, fat> brain>heart>muscle>skin
Chang et al. (2012)	mouse (m,f)	PFOS	1,20; single (oral gavage)	On day 2: **liver(2.2-3.4)**>serum>kidney(0.25-0.30)
Lou et al. (2009)	mouse (m,f)	PFOA	1,10; single (oral gavage)	Males and females: **liver (1.8-2.9)**>plasma> kidney(0.1-0.2) at both doses, at 4h-48d, except low dose (f) at 4h: plasma>liver(0.8)>kidney(0.13)
Minata et al. (2010)	mouse (m)	PFOA	5.2,10.4,21 for 4w (oral gavage)	Low dose: **liver(8.8)**>plasma Mid dose: **liver(4.2)**>plasma High dose: **liver(3.2)**>plasma
Bogdanska et al. (2011)	mouse (m)	PFOS	0.031,23 for 1-5d (oral diet)	Low dose (5d): **liver(5.8)**>lung>bone>blood> kidney(0.9)>pancreas, gut, stomach>skin, spleen, thymus>testes, heart>muscle>brain, fat High dose (5d): **liver(3.6)**>lung>blood> kidney(0.8),skin>bone>pancreas>thyroid>gut, stomach, spleen, thymus>heart, testes>fat, brain, muscle
Macon et al. (2011)	pregnant mouse	PFOA	0.3,1,2 for 17d (GD 1-17) (oral gavage)	Dams PND 7 and PND 14: plasma> **liver (0.25-0.5)**>>brain; Pups, PND 21-84: plasma=**liver**
Fujii et al. (2015)	mouse (m,f)	PFOA	0.13; single (i.v.)	24h (m)[h]: **liver**>serum>>kidney>brain, fat; 24h (f)[h]: serum>**liver**>>kidney>brain, fat

Reference	Species (sex)	PFAS	Dose (mg/kg[a])	Distribution in organs[b]
			1.3; single (oral gavage)	
Yu et al. (2016)	mouse (m)	PFOA	0.5,2.5 for 28d (oral gavage)	Low dose: **liver(1)**=blood High dose: **liver(1.5)**>blood
Zhang et al. (2016c)	mouse (m)	PFOS	0.003-0.012% (in diet) for 2w	**Liver(2.2-3.5[d])**>serum
Lai et al. (2017b)	pregnant mouse	PFOS	0.3 for 19d (GD 1-18.5) (oral gavage)	Dams **liver(4.4[d])**>F1 liver>serum>placenta
Li et al. (2017b)	mouse (m,f)	PFOA	0.05,0.5,2.5 for 28d (oral gavage)	Low dose: **liver**=serum Mid and high doses: **liver(1.5-2[d])**>serum
Zheng et al. (2017)	mouse (m)	PFOA	1.25 for 28d (presumably oral gavage)	**Liver(2.3[d])**>serum
Lai et al. (2018)	mouse (f)	PFOS	0.0003,0.003 for 7w (oral gavage)	Low dose: **liver**=serum High dose: **liver(4.6)**>serum
Guo et al. (2019)	mouse (m)	PFOA	0.4,2,10 for 28d (oral gavage)	**Liver(2)**>serum
Griffith and Long (1980)	monkey (m,f)	PFOA	3,10 for 90d (oral gavage)	Serum>>**liver(0.06-0.2)**
Seacat et al. (2002)	monkey (m,f)	PFOS	0.03,0.15,0.75 for 6 months (oral, capsules)	Low and high doses: **liver(1.7-2.2)**>serum Mid dose: **liver(1.2f)**>serum; serum> **liver(0.9m)**
Butenhoff et al. (2004a)	monkey (m,f)	PFOA	3,10,20(30)[i] for 6 months (oral, capsules)	Serum>>**liver(0.09-0.28)**
Olsen et al. (2003c)	human (post-mortem)	PFOS	unknown	**Liver(1.4)**>serum
Maestri et al. (2006)	human (post-mortem)[j,k]	PFOA	unknown	Lung>kidney(1.2)>**liver(1)**,blood>thyroid> hypophysis>gonads>fat>pancreas>skeletal muscle>brain>basal ganglia
		PFOS	unknown	**Liver(2.7)**>lung>hypophysis>kidney(1.2)> blood>pancreas, gonads>thyroid>fat>brain, basal ganglia>skeletal muscle
Perez et al. (2013), Fabrega et al. (2014)	Human[j] (post-mortem)	PFOA	unknown	Bone>lung>**liver**>>kidney>>brain, fat
		PFOS	unknown	**Liver**>kidney>lung>>brain>>bone, fat

Reference	Species (sex)	PFAS	Dose (mg/kg[a])	Distribution in organs[b]
Mamsen et al. (2019)	human (post-mortem fetus)	PFOA	unknown	1st trimester: maternal serum>>lung>placenta >**liver(0.12)**>heart>>CNS, fat 2nd trimester: maternal serum>**liver(0.34)**> placenta>lung>heart>fat>CNS 3rd trimester: maternal serum>placenta> **liver(0.38)**>lung>fat>heart>CNS
		PFOS	unknown	1st trimester: maternal serum>lung>placenta> heart>**liver(0.10)**>CNS>fat 2nd trimester: maternal serum>**liver(0.71)**>fat> lung>heart>placenta>CNS 3rd trimester: maternal serum>**liver(0.38)**> lung, fat, heart>placenta>CNS

CNS, central nervous system; d, days; f, female; m, male; w, weeks; GD, gestation day; i.p., intraperitoneal injection; i.v., intravenous injection; nd, non-detected; PND, post-natal day;
[a] mg/kg-day for repeated exposures
[b] for the liver and kidney, when available, concentrations are shown as fold relative to plasma/serum
[c] As reported in Benskin et al. (2009)
[d] values digitized from graphical data prior to conversion
[e] Liver and kidney concentrations relative to blood (different from relative to plasma)
[f] Only lowest dose presented here, data were reported for two more doses (Gao et al., 2015a)
[g] Corresponds to 0.077, 0.38 and 1.8 µg/kg-day, respectively (Iwabuchi et al., 2017)
[h] Absolute levels per organ, as reported (Fujii et al., 2015)
[i] In the high dose group, dose was 30 mg/kg-day for 12 days, at which point dosing was discontinued due to toxicity; dosing was resumed at day 22 at 20 mg/kg-day (Butenhoff et al., 2004a)
[j] No blood or serum samples reported
[k] Blood levels of PFOA and PFOS from a similar population were reported in Ericson et al. (2007) and were 1.80 ng/ml and 7.64 ng/ml, respectively

Table A6.2. Protein binding studies for PFOA and PFOS

Reference	Species	Protein or fraction	PFAS	K_d (M) or % binding	Comment
Klevens and Ellenbogen (1954)	bovine	serum albumin	PFOA	3.1×10^{-3}	
Luebker et al. (2002)	rat	L-FABP	PFOA	40% at 10 µM	Percent inhibition of C11 fatty acid binding to L-FABP
			PFOS	70% at 10 µM	
Han et al. (2003)	rat	albumin	PFOA	4×10^{-4}	>90% of PFOA would be bound to serum albumin (prediction)
	human			4×10^{-4}	
Jones et al. (2003)	bird, fish	SHBP (fish)	PFOA	1.7×10^{-5}[b]	Also reported 100% binding of PFOS to BSA in aqueous solution
			PFOS	2.1×10^{-5}[b]	
		CBG (bird)	PFOA	$1.2\text{-}3.1\times10^{-4}$[c]	
			PFOS	$2.5\text{-}2.7\times10^{-4}$[c]	
Kerstner-Wood et al. (2003)[a]	rat, monkey, human	protein fraction of plasma	PFOA	97-100%	
Messina et al. (2005)	human	serum albumin	PFOA	6.9×10^{-6}, 3.2×10^{-5}	Dissociation constants for a two-step binding reaction
Chen and Guo (2009)	human	albumin	PFOA	3.7×10^{-6}	
			PFOS	4.5×10^{-5}	
Li et al. (2009)	human	serum albumin	PFOS	2×10^{-4}	
Weiss et al. (2009)	human	transthyretin	PFOA	0.95×10^{-6}	IC_{50} values measured at 55 nM T4 in vitro
			PFOS	0.94×10^{-6}	
Wu et al. (2009)	human	albumin	PFOA	2.6×10^{-5} 1.3×10^{-3}	Dissociation constants[d] for a two-step binding reaction
Bischel et al. (2010)	human	albumin	PFOA	$0.7\text{-}5\times10^{-6}$	
MacManus-Spencer et al. (2010)	bovine	serum albumin	PFOA	$0.7\text{-}3\times10^{-5}$; 0.01-0.02	Concentration-dependent K_d values for a two-site model
Qin et al. (2010)	bovine	serum albumin	PFOA	2.1×10^{-5}	Detailed thermodynamic study
Salvalaglio et al. (2010)	human	serum albumin (in silico)	PFOA	95%	Predicted binding rate in human blood over range of concentrations
			PFOS	98%	
Woodcroft et al. (2010)	rat	L-FABP	PFOA	13.1×10^{-6} 23.8×10^{-6}	Two binding sites predicted by displacement
			PFOA	3.1×10^{-6} 26.2×10^{-6}	Three binding sites predicted by

Reference	Species	Protein or fraction	PFAS	K_d (M) or % binding	Comment
				52.6×10^{-6}	isothermal titration calorimetry
Zhang et al. (2013a)	human	L-FABP	PFOA	50.4×10^{-6}	
			PFOS	18.5×10^{-6}	
Beesoon and Martin (2015)	human	serum albumin	n-PFOS	8×10^{-8}	Lower binding affinities for branched PFOA and PFOS
			n-PFOA	1×10^{-4}	
Ren et al. (2015)	human	thyroid receptor	PFOA	42×10^{-6}	IC_{50} at 100 nM T3, in competitive binding
			PFOS	16×10^{-6}	
Ren et al. (2016)	human	transthyretin	PFOA	60×10^{-9}	Detected by competitive binding
			PFOS	20×10^{-9}	
		thyroxine-binding globulin	PFOA	ND	
			PFOS	ND	
Sheng et al. (2016)	human	L-FABP	PFOA	$2.4\text{-}6.5\times10^{-6}$	
EFSA (2018)	human	albumin. low-density lipoproteins	PFOS	Strong binding	Referenced unpublished study
		α-globulin, γ-globulin		Medium to weak binding	

BSA, bovine serum albumin; CBG, corticosteroid-binding globulin; K_d, dissociation constant; L-FABP, liver fatty acid-binding protein; M, mol/L; ND, not detectable; n-PFOA, n-PFOS, linear isomers; SHBP, sex-hormone binding protein; T3, triiodothyronine; T4, thyroxine

[a] as reported in US EPA (2016b)

[b] relative to estrogen (Jones et al., 2003)

[c] relative to cortisol (Jones et al., 2003)

[d] dissociation constants are calculated from reported Gibbs energies (Wu et al., 2009; Qin et al., 2010)

Table A6.3. Experimental evidence[a] for PFOA and PFOS transporters

Reference	Transporter	PFAS	K_m[b] or transport	Comment
Kudo et al. (2002)	rat OAT2, OAT3	PFOA	both active	In vivo study, correlation of expression with PFOA clearance
Katakura et al. (2007)	rat OATP1, OAT3	PFOA	both active	Lack of Mrp2 transport: Mrp2-deficient rats had similar renal clearance of PFOA to wild type rat; no transport by rat Npt2
Nakagawa et al. (2008)	human OAT1	PFOA	48 μM	No transport by human or rat OAT2
	human OAT3		49.1 μM	
	rat OAT1		51 μM	
	rat OAT3		80.2 μM	
Nakagawa et al. (2009)	human OAT4	PFOA	active	Uptake was reported but no kinetic characteristics
Yang et al. (2009a)	rat OATP1a1	PFOA	162 μM	Perfluorocarboxylate inhibition activity profile
	rat OAT1	PFOA	43.2 μM	No transport by rat OAT2, URAT1

Reference	Transporter	PFAS	K_m[b] or transport	Comment
Weaver et al. (2010)	rat OAT3		65.7 µM	
	rat OATP1a1		126.5 µM	
Yang et al. (2010)	human OAT4	PFOA	310 µM	No transport by human OATP1A2
	human URAT1		64.1 µM	
Kummu et al. (2015)	human OAT4	PFOA, PFOS	high correlation	Transport in vitro (perfusion); transporter presumed active if transporter protein expression correlated with PFAS transport efficiency
	human ABCG2		no correlation	
Zhao et al. (2015a)	human NTCP	PFOS	130 µM	Also observed transport by human ASBT, and human OSTα/β for PFOS

ASBT, apical sodium-dependent bile salt transporter; ABCG2, ATP-binding cassette transporter G2; K_m, apparent affinity constant; Mrp2, multidrug resistance-associated protein 2; Npt2, type II sodium-dependent phosphate transporter; NTCP, Na^+/taurocholate co-transporting polypeptide; OAT, organic anion transporter; OATP, organic anion-transporting polypeptide; OST, organic solute transporter; URAT, urate transporter (member of OAT family of transporters).

[a] In vitro studies unless indicated otherwise.

[b] Only mean values are presented.

Table A6.4. Estimate of net renal tubular reabsorption of PFOA in different species (adapted from Han et al. (2012))

Reference	Species /Sex	GFR (ml/day-kg)	CL_R (ml/kg-day)	Net reabsorption (ml/kg-day)[a]	% reabsorbed[b]	% excreted
Kudo and Kawashima (2003)	rabbit female	4,000	670	-	not applicable[c]	-
	rabbit male	4,000	640	-	not applicable[c]	-
Kemper (2003, unpublished study)	rat female	14,400	666	-	not applicable[c]	-
	rat male	14,400	18.2	270	93.7%	6.3%
Ohmori et al. (2003)	rat female	14,400	1,009[d]	-	not applicable[c]	-
	rat male	14,400	21.2[d]	267	92.7%	7.3%
Kudo and Kawashima (2003)	dog female	5,300	50.8	55	52%	48%
	dog male	5,300	43	63	59%	41%
Harada et al. (2005a)	Japanese macaque female	8,500	32	138	81.2%	18.8%
	Japanese macaque male	8,500	15	155	91.2%	8.8%
	mouse female	16,700	16	318	95.2%	4.8%

Reference	Species /Sex	GFR (ml/day-kg)	CL_R (ml/kg-day)	Net reabsorption (ml/kg-day)[a]	% reabsorbed[b]	% excreted
Kudo and Kawashima (2003)	mouse male	16,700	10	324	97%	3%
This document (Table 4.5.1	human	2,570	0.06	51.3	99.8%	0.2%

CL_R, renal clearance; GFR, glomerular filtration rate.
[a] Net tubular reabsorption = $f_u \times GFR - CL_R$, where f_u, the unbound fraction is assumed to be 0.02.
[b] Percent reabsorbed = (net reabsorption)/($f_u \times GFR$) $\times 100$
[c] Overall net tubular secretion, and not reabsorption, is observed ($f_u \times GFR < CL_R$).
[d] Values digitized from graph (Ohmori et al., 2003).

Table A6.5. PBPK models for PFOA and PFOS

Reference	Type	Species	PFAS	Optimization data	Validation data	Comment
Andersen et al. (2006)	3-cmpt	monkey	PFOA PFOS	Noker and Gorman (2003, unpublished); Butenhoff et al. (2004a); Seacat et al. (2002)	high dose (20 mg/kg-day) in Butenhoff et al. (2002); Butenhoff et al. (2004a)	First model to incorporate saturable renal reabsorption; over-predicts PFOS plasma concentrations at high dose
Harris and Barton (2008)	8-cmpt	rat	PFOS	Johnson et al. (1979a, b; unpublished); 3M (2002, unpublished)	Same as optimization studies	Time-dependent urinary elimination rate constant and liver-to-plasma partition coefficient; over-predicts plasma concentrations
Tan et al. (2008)	5-cmpt	rat, monkey	PFOA PFOS	Noker and Gorman (2003, unpublished); Kemper (2003, unpublished); Johnson et al. (1979a, b; unpublished)	Kemper (2003, unpublished); Butenhoff et al. (2002)	Time-dependent descriptors for free fraction and V_d; poor prediction for female rat/PFOA; PFOA data used for validation of monkey/PFOS model; derivation of Andersen et al. (2006) model
Lou et al. (2009)	1-cmpt 2-cmpt	mouse	PFOA	Lou et al. (2009)	high dose in Lou et al. (2009)	Unsatisfactory fit with high doses
Rodriguez et al. (2009)	2-cpmt lactation	mouse	PFOA	Lau et al. (2006); Abbott et al. (2007)	Lau et al. (2006); Wolf et al. (2007)	Gestational and lactational model; tendency to overestimate plasma levels
Loccisano et al. (2011)	9-cmpt	monkey, human	PFOA PFOS	Butenhoff et al. (2004a); Emmett et al. (2006); Kudo et al. (2007); Olsen et al. (2007); Calafat et al. (2007a); Calafat et al. (2007b); Hölzer et al. (2008); (Steenland et al., 2009); Bartell et al. (2010)	Emmett et al. (2006)	Derivation of Andersen et al. (2006) and Tan et al. (2008) models; time-dependent free fraction; model predicted a sharper increase in PFOS plasma concentrations than observed
Loccisano et al. (2012a)	8-cmpt	rat	PFOA PFOS	DePierre (2009, unpublished); Kemper (2003, unpublished); Perkins et al. (2004); Kudo et al. (2007)	Same as optimization studies; additional unpublished data (3M); Seacat et al. (2002)	Time-dependent free fraction (for PFOS)

Reference	Type	Species	PFAS	Optimization data	Validation data	Comment
Loccisano et al. (2012b)	14-17 cmpt	rat	PFOA PFOS	Hinderliter et al. (2005)	Lau et al. (2003); Thibodeaux et al. (2003); Hinderliter et al. (2005); Luebker et al. (2005a); Luebker et al. (2005b); Butenhoff et al. (2009); Chang et al. (2009); Yu et al. (2009)	Gestational and lactational models (separate); derivation of Loccisano et al. (2012a) model, with added dynamic changes in gestation and lactation physiology with minimum PFAS-specific optimization; time-dependent free fraction (for PFOS); over-prediction in pup plasma and tissues
Loccisano et al. (2013)	14-cmpt	human	PFOA PFOS	Kärrman et al. (2007); Midasch et al. (2007); Fromme et al. (2010); Haug et al. (2010)	Inoue et al. (2004); Tittlemier et al. (2004); Fei et al. (2007); Monroy et al. (2008); Fromme et al. (2009); Fromme et al. (2010); Hanssen et al. (2010); Liu et al. (2011); Kim et al. (2011a); Kim et al. (2011b)	Gestational and lactational model with many acknowledged limitations, particularly lack of transporter data; derivation of the rat model (Loccisano et al., 2012b) and human model (Loccisano et al., 2011), with added dynamic changes in gestation and lactation physiology with minimum PFAS-specific optimization; under-prediction for PFOA/milk
Wambaugh et al. (2013)	3-cmpt	rat, mouse, monkey	PFOA PFOS	Kemper (2003, unpublished); Seacat et al. (2002); Butenhoff et al. (2004a); DeWitt et al. (2008); Lou et al. (2009); Chang et al. (2012)	Same as optimization studies	Probabilistic derivation of Andersen et al. (2006) model; wide confidence intervals for optimized parameters
Fabrega et al. (2014); Fabrega et al. (2016)	10-cmpt	human	PFOA PFOS	Maestri et al. (2006); Ericson et al. (2007); Fabrega et al. (2014)	Perez et al. (2013)	Derivation of Loccisano et al. (2011) model; underestimation of steady state serum levels; first human model to be optimized at organ level
Worley and Fisher (2015)	8-cmpt	rat	PFOA	Kemper (2003, unpublished); Kudo et al. (2002)	Kemper (2003, unpublished); Kudo et al. (2002)	First model to parameterize renal transport on individual transporter in vitro data; includes in vitro to in vivo extrapolations; only calibrated for single doses
Tarazona et al. (2016)	1-cmpt 2-cmpt	rabbit	PFOS	Tarazona et al. (2016)	Same as optimization data	1-compartment model provided best fit

Reference	Type	Species	PFAS	Optimization data	Validation data	Comment
Verner et al. (2016)	2-cmpt	human	PFOA PFOS	Published parameters ($T_{1/2}$, V_d, placental transfer, lactational transfer)	Magnus et al. (2006); Fromme et al. (2010); Granum et al. (2013)	Gestational and lactational model, assessed variability in a simple mother-child model; predicts significant peak in child/mother ratio at ~6 months
Cheng and Ng (2017)	19-cmpt	rat	PFOA	Model was built without optimization	Kemper (2003, unpublished); Kudo et al. (2007); Kim et al. (2016b)	Permeability-limited model, only single dose prediction, narrow confidence intervals for predicted plasma concentrations.
Worley et al. (2017a)	8-cmpt	human	PFOA	ATSDR (2016)	Bartell et al. (2010)	Derivation of Worley and Fisher (2015) model
Convertino et al. (2018)	2-cmtp	human	PFOA	Elcombe et al. (2013)	Same as optimization data	Stochastic model for dose-response of adverse effects in a pharmacological trial of PFOA in human cancer patients
Chou and Lin (2019)	7-cmpt	rat, mouse, monkey, human	PFOS	Johnson et al. (1979; unpublished); 3M (2002, unpublished); Haug et al. (2009); Chang et al. (2012); Kim et al. (2016b)	Seacat et al. (2002); Olsen et al. (2003b); Olsen et al. (2003c); Olsen et al. (2008); Fabrega et al. (2014)	Derivation of Worley et al. (2017a) model; Bayesian-MCMC simulation; some parameter values sourced from other models such as Loccisano et al. (2011)
Goeden et al. (2019)	2-cmpt	human	PFOA	Summary statistics of published cord-maternal serum and breastmilk transfer studies	Fromme et al. (2010); Mogensen et al. (2015a)	Gestational and lactational model in Excel; predicts a 6-fold increase (relative to mother's serum) in infant PFOA over the first year of life

cmpt, compartment; MCMC, Markov chain Monte Carlo; $T_{1/2}$, half-life; V_d, volume of distribution

Table A6.6. Comparison of reported PFOA serum concentrations (C_{serum}) in subchronic mouse studies to concentrations obtained from PBPK models

Study	Route/duration	Dose (mg/kg-day)	Reported C_{serum} (mg/L)	Calculated[#] C_{serum} Wambaugh et al. (2013) model (mg/L)	Calculated[#] C_{serum} Rodriguez et al. (2009) model (mg/L)
Lau et al. (2006)	Oral gavage 17 days	1 3 5 10 20 40	21.9 40.5 71.9 116 181 271	50.7 52.2 53.8 57.6 65.4 80.8	23.8 57.8 84 136 227 396
Li et al. (2017b)	Oral gavage 28 days	 0.05 0.5 2.5	male/female 1.2/0.97 5.9/2.7 13.5/9.5	not sex-specific 49.9 50.3 53.2	not sex-specific 1.4 12.8 50.4

[#] Value calculated by OEHHA, based on model codes and parameter values obtained from corresponding publications.

APPENDIX 7. HUMAN EPIDEMIOLOGY STUDIES – GENERAL METHODS AND SUMMARY TABLES

General Methods

Literature search

OEHHA attempted to identify all published human epidemiologic literature on the possible health effects of PFOS and PFOA. For most health outcomes, the most recent reviews by the US Environmental Protection Agency (US EPA) were used to identify literature published prior to December 1, 2015, the last day of the literature search performed for these documents. OEHHA searched two electronic databases, PubMed and Embase to identify literature published from December 1, 2015 until January 2, 2020, the last date of the initial full literature review. The exposure portion of the search string included the key words shown below. These keywords are essentially the same as those used by NTP in its recent review of PFOA and PFOS and immunotoxicity (NTP, 2016). The outcome portions of the search string used are given in the sections describing each health outcome.

(perfluoroalkyl*[tiab] OR perfluorocaprylic[tiab] OR perfluorocarbon*[tiab] OR perfluorocarboxyl*[tiab] OR perfluorochemical*[tiab] OR (perfluorinated[tiab] AND (C8[tiab] OR carboxylic[tiab] OR chemical*[tiab] OR compound*[tiab] OR octanoic[tiab])) OR PFAA*[tiab] OR "fluorinated polymer"[tiab] OR "fluorinated polymers"[tiab] OR (fluorinated[tiab] AND (polymer[tiab] OR polymers[tiab])) OR (fluorocarbon[tiab] AND (polymer[tiab] OR polymers[tiab])) OR Fluoropolymer*[tiab] OR (fluorinated[tiab] AND telomer*[tiab]) OR fluorotelomer*[tiab] OR fluoro-telomer*[tiab] OR fluorosurfactant*[tiab] OR "FC 143"[tiab] OR FC143[tiab] OR 335-67-1 [rn] OR Pentadecafluoroctanoate*[tiab] OR Pentadecafluorooctanoate*[tiab] OR pentadecafluoroctanoic[tiab] OR pentadecafluorooctanoic[tiab] OR "pentadecafluoro-1-octanoic"[tiab] OR "pentadecafluoro-n-octanoic"[tiab] OR "perfluoro-1-heptanecarboxylic"[tiab] OR perfluorocaprylic[tiab] OR perfluoroheptanecarboxylic[tiab] OR perfluoroctanoate[tiab] OR perfluorooctanoate[tiab] OR "perfluoro octanoate"[tiab] OR "perfluorooctanoic acid"[nm] OR perfluoroctanoic[tiab] OR perfluorooctanoic[tiab] OR "perfluoro octanoic"[tiab] OR "perfluoro-n-octanoic"[tiab] OR "perfluorooctanoyl chloride"[tiab] OR PFOA[tiab] OR APFO[tiab] OR 1763-23-1[rn] OR 307-35-7[rn] OR "1-octanesulfonic acid"[tiab] OR "1-perfluorooctanesulfonic"[tiab] OR "1-perfluoroctanesulfonic"[tiab] OR "heptadecafluoro-1-octanesulfonic"[tiab] OR "heptadecafluoro-1-octane sulfonic"[tiab] OR "heptadecafluorooctanesulfonic"[tiab] OR "heptadecafluorooctane sulfonic"[tiab] OR "heptadecafluoroctane sulfonic"[tiab] OR "perfluoroalkyl sulphonate"[tiab] OR perfluoroctanesulfonate[tiab] OR perfluorooctanesulfonate[tiab] OR "perfluoroctane sulfonate"[tiab] OR "perfluorooctane sulfonate"[tiab] OR "perfluoro-n-octanesulfonic"[tiab] OR perfluoroctanesulfonic[tiab] OR perfluorooctanesulfonic[tiab] OR "perfluorooctane sulfonic acid"[nm] OR "perfluoroctane sulfonic"[tiab] OR "perfluorooctane sulfonic"[tiab] OR perfluoroctanesulphonic[tiab] OR perfluorooctanesulphonic[tiab] OR "perfluoroctane sulphonic"[tiab] OR "perfluorooctane sulphonic"[tiab] OR perfluoroctylsulfonic[tiab] OR PFOS [tiab])

PubMed and Embase searches were supplemented by also searching the bibliographies of all studies meeting OEHHA's inclusion and exclusion criteria (described below) and all recent relevant review articles. For some outcomes (e.g., immune toxicity), other sources were searched (e.g., the NTP's most recent review of this outcome (NTP, 2016)), and these are noted in the sections below.

OEHHA used the following inclusion and exclusion criteria to identify studies for this review. All human epidemiologic studies of PFOS or PFOA and an adverse human health effect presenting

results as mean differences, regression or correlation coefficients, relative risks, or any other appropriate outcome metrics were eligible for inclusion. No restrictions were placed on the methods used to evaluate PFOA or PFOS exposure levels although almost all studies identified assessed exposure using blood or drinking water concentrations. Studies using modeled intakes of PFOS or PFOA based on drinking water or blood levels, industrial hygiene records, or similar factors were also included.

Studies that involved cohort, case-control, and cross-sectional designs were included. Case-reports were excluded because of the lack of a comparison group. Abstracts and studies without original data (e.g., reviews or editorials) were also excluded. Ecologic studies were considered, although the potential for ecologic fallacy (bias that may occur because an association between variables at an aggregate level may not exist at an individual level) and confounding was evaluated for studies using this design. Cross-sectional studies were initially included although the potential for reverse causation or exposure misclassification (e.g., latency effects) were evaluated. There were no exclusions based on study location, language, or statistical adjustments. Several studies reported results only for multiple PFAS combined. Although the results of these studies were considered in this review, they were not included in OEHHA's detailed evaluations when results were not specifically given for PFOS or PFOA. Studies were initially excluded based on title search, and if needed, then by abstract and full article review.

With the rapid expansion of the PFOA/PFOS database, OEHHA expanded its literature review to a cutoff date of September 20, 2020. This review used the same criteria discussed above. Studies identified after January 2, 2020 that influenced OEHHA's conclusions regarding the adverse health effects of PFOA or PFOS are reviewed here but not included in some of the review tables presented in this document.

Assessment of study methods and quality

OEHHA evaluated the quality of each study identified and the major aspects of causal inference using an updated version of the Hill criteria (Hill, 1965). The criteria used in these evaluations are described below, and are similar to those described in the NTP Risk of Bias Tool (NTP, 2019a).

Study design: When assessing causal inference, evidence from cross-sectional and ecologic studies is sometimes considered to be weaker than evidence from studies using cohort, case-control, or randomized clinical trial designs. For example, in order for an exposure to cause an outcome, the exposure must come before the outcome. However, this temporal relationship can be difficult to establish in some cross-sectional studies. Importantly though, there are many exceptions to this broad generalization. In some cross-sectional studies, such as when a clear mechanism leading from the exposure to outcome has been well established, or when the exposure has been shown to be stable over time, one can sometimes be reasonably confident that the exposure preceded the outcome (Shahar and Shahar, 2013). Common criticisms of ecologic studies include ecologic fallacy (bias that may occur because an association between variables at the aggrefate level may not exist at an individual level) and confounding. However, in some ecologic studies, major errors caused by these factors can be ruled out with good confidence (Smith et al., 2018). OEHHA did not automatically rate cross-sectional and ecologic studies as having weaker study designs because of these issues, but rather evaluated each study on its own merits.

Factors related to bias:
Selection: After reviewing the eligibility criteria for each study (if provided), an attempt was made to evaluate whether all eligible people, or a random selection of all eligible people, were invited to participate in the study. If this did not occur, the possibility of selection bias may be increased.
Participation: Of those who were invited to participate, an attempt was made to evaluate the percentage of people who actually agreed to participate and the percentage of people for whom there were sufficient data to be included in the final study analyses. If this percentage is low (e.g., below 60-70%), this may also increase the possibility of selection bias.
Equal groups: Studies were evaluated for whether there were any major socioeconomic status (SES) or other relevant differences between people with higher or lower PFOA or PFOS levels or between people with or without the outcome of interest. If there were, OEHHA evaluated whether the researchers attempted to account for these differences in the statistical analyses. If major differences occurred, the risk of important selection bias or confounding may be increased.
Blinding: Studies were evaluated for whether the researchers measuring the exposure were blinded to the outcome status of the participants and whether the researchers measuring the outcome were blinded to the exposure status of the participants. If not, the risk of exposure or outcome misclassification may be increased.
Above detection: When this review began, an attempt was made to extract information on the percentage of participants who had detectable levels of PFOA or PFOS. A low percentage could limit the precision of the study findings. However, as this review proceeded, it became clear that the very large majority of participants in all of the studies reviewed had detectable levels of PFOA and PFOS, even in studies that involved participants with no obvious high exposure source. As such, detailed information on this factor is not presented for all health outcomes.
Levels: When possible, data on the distribution of PFOA or PFOS levels among the study participants were extracted. In general, true effects are easier to identify when the contrast in exposure within the study population is large. This criterion was also used to identify a possible source of heterogeneity among studies and to identify studies in which exposure levels may be too low to identify true associations.

Exposure and outcome methods: For each study, the methods used to assess PFOA and PFOS exposure levels and the methods used to evaluate the outcome of interest were evaluated. In general, whether each of these were validated, generally accepted, or otherwise reasonable methods for assessing exposure and outcome was examined. Inaccurate or imprecise methods could lead to exposure or outcome misclassification.

Confounding: Factors most likely to cause confounding were identified. This evaluation considered the following four criteria:

1. What factors are strongly related to the exposure of interest?
2. What factors are strongly related to the outcome of interest?
3. Are these factors in the causal chain linking the exposure and the outcome?
4. Which of the factors are prevalent enough to cause important confounding?

To begin this process OEHHA attempted to identify those factors most commonly associated with higher PFOA and PFOS exposures. Studies from a variety of different countries were included based on the idea that the major sources of PFOA and PFOS exposure are likely to be

different across different study populations. A brief review of the results of a few of the more informative studies is shown in Table A7.1.

Table A7.1. Factors associated with higher PFAS exposure: a review of selected studies

Reference	Where/Who	Factors	
Calafat et al., 2007	US NHANES; age ≥12	+males +non-Hispanics +education	?smoker ?BMI
Coakley et al., 2018	New Zealand; adults	+males +age	
Eriksen et al., 2011	Denmark; men	+never smoker +frying food -BMI	-alcohol +eggs not fish
Harris et al., 2017	Boston; children	+fast food consumption -BMI	-Black -higher income
Jain, 2014	US NHANES; age ≥12	PFOA: -caffeine -milk -dry beans +non-alcohol beverages	PFOS: -caffeine +fish +/-fat +meat -milk +alcohol -cheese
Kang et al., 2018	Korea; children	+breast-feeding duration +fish +non-stick frying pan use*	+waterproof clothing* *(effect sizes are small)
Kato et al., 2009	NHANES; ages 3-11	+non-Mexican Americans	
Kato et al., 2016	Japan; children	-parity	not education, income, smoking, fish
Lee et al., 2017	Korea; adults	+males +plastic wrap +disposable cups	+BMI +exercise
Lee et al., 2018	Korean; women (breast milk samples)	+age +BMI +parity +snacks	+eating out +organochlorines, heptachlor, PCBs, DDE
Manzano-Salgado et al., 2016	Spain; pregnant women	-parity +age	+fish
Nelson et al., 2010	NHANES; ages 12-80	+males +non-Hispanic whites	+SES +/-BMI (depends on age)
Olsen et al., 2017	US Red Cross donors; ages 20-69	+male	
Richterová et al., 2018	Slovakia; pregnant women	+fish +age	+higher education -parity
Skuladottir et al., 2015	Denmark; pregnant women	+meat -vegetables	
Tian et al., 2018	China; pregnant women	+age +parity +education +passive smoking -household income +red meat	+poultry intake +fish +pastries +fried food +tap water consumption
Ye et al., 2018	NHANES; ages 3-11	+males +age	+non-Hispanics

Studies are ordered alphabetically; ages are in years unless otherwise stated
Most studies examined PFOA, PFOS, or combined PFAS serum concentrations where PFOA and PFOS were the predominant agents.
Abbreviations: "+," positively associated with higher PFOS, PFOA, or all PFAS combined concentrations; "-," negatively associated with higher PFOS, PFOA, or all PFAS combined concentrations; "?," data are mixed or unclear; BMI, body mass index; DDE, dichlorodiphenyldichloroethylene; NHANES, US National Health and Nutrition Examination Survey; PCB, polychlorinated biphenyls; SES, socioeconomic status

Interpretation of study results

***Statistical significance*:** Each study was evaluated for whether it found evidence for an association between PFOA or PFOS and the outcome of interest and whether the relevant result was statistically significant. Statistical significance was defined as a p-value <0.05 or 95% confidence intervals (CI) that excluded 1.0 for relative risk estimates or 0 for mean differences or correlation or regression coefficients. OEHHA acknowledges that these definitions are somewhat arbitrary, that some results representing true effects may not meet these definitions, and that some results meeting these definitions may not represent true effects. As such, no conclusions were based solely on statistical significance.

***Magnitude of the association*:** The relative risk estimate (e.g., odds ratio (OR)) was evaluated to determine whether it was greater than 1.2 (or <0.83 if less than 1.0); mean differences were greater than 10%; correlation coefficients were >0.10; or regression coefficients seemed to indicate an effect size >10% between high and low exposure groups. Studies meeting these criteria were labeled as "Large magnitude: yes" in the review tables. This criteron is similar to the "Large magnitude" criterion used by NTP (2019b) and the "Strength of the association" criterion used by AB Hill (1965). In general, small effect sizes (e.g., relative risks close to 1.0 or mean differences close to 0) are more likely to be due to confounding or bias than larger effect sizes (Axelson, 1978; Schlesselman, 1978). Use of these criteria is not meant to imply that all small effect sizes are due to confounding or bias or that all large effect sizes are real. OEHHA also acknowledges that the specific criteria used here are somewhat arbitrary and were sometimes difficult to quantify. This was especially true for linear regression coefficients, where effect sizes were oftentimes difficult to express as a simple single percentage. Importantly though, these criteria were not used as sole determinants of causality, but rather only used to help identify some results that might be especially prone to important bias or confounding.

***Dose-response*:** If an association was identified, the shape of the dose-response curve was evaluated. For many toxic chemical exposures, as the level of exposure increases the degree of toxicity or the numbers of people with the toxic endpoint also increases. Importantly though, dose-response relationships are not always mono-tonic or linear. Here, when statistically significant effects were seen, OEHHA evaluated whether or not a dose-response relationship was seen and attempted to describe the pattern of this relationship. In general, dose-response patterns that are similar across different studies may be more likely to represent real effects. When patterns were not mono-tonic or were not consistent from one study to the next, OEHHA explored whether there might be a potential reason for this.

***Temporal assessment*:** Studies were evaluated as to whether the exposure that was assessed likely occurred before the outcome occurred. Further discussion of this issue is presented under "Study design" above.

***Subgroup only*:** Studies were evaluated for whether statistically significant results were only identified in a specific subgroup of participants. Some studies presented results for all participants combined and separate results for certain subgroups (e.g., males and females). This criterion was considered because increasing the number of subgroups assessed could be helpful for identifying a particularly susceptible group, and evaluating whether a study may have missed an important subgroup. However, examining multiple subgroups in a single study could also increase the chance of false positive results (i.e., the issue of "multiple comparisons").

***Adjustments*:** Studies were evaluated for whether large changes in results were seen following statistical adjustments. These large changes could indicate that a major confounder was present in the original study design. However, they could also be the result of a statistical artifact (e.g., co-variance). In addition, if adjustments move the effect measure closer to the null (e.g., a relative risk of 1.0), a large difference between the adjusted and unadjusted result would raise concerns that any remaining association in the adjusted results might be due to residual confounding. If a large difference between adjusted and unadjusted results was seen, OEHHA evaluated whether the authors provided information that could be used to identify which particular confounder caused this difference. Generally, this involved evaluating information on which potential confounders were strongly associated with both the exposure and the outcome of interest, and information showing that these associations were in the proper direction to cause the potential confounding observed. Overall, unexplained large differences between adjusted and unadjusted results were considered a weakness of the study.

Immunotoxicity

Literature search and methods

OEHHA reviewed a number of previously published review documents on the possible immunotoxicity of PFOA and PFOS, including those published by the NTP (NTP, 2016), US EPA (US EPA, 2016b; US EPA, 2016d) and Agency for Toxic Substances and Disease Registry (ATSDR) (ATSDR, 2018a). OEHHA also identifed and reviewed the human epidemiologic evidence published since the 2016 NTP report (NTP, 2016). To do this OEHHA attempted to identify all human epidemiologic evidence on the immunotoxicological effects of PFOS and PFOA published between May 18, 2016 (the end of the NTP literature review) and September 20, 2020 (the end of this literature review) using the methods described above. The exposure portion of the search string OEHHA used is given in the *General Methods* section above. The outcome portion of the search string used is shown below. This is the same search string used by NTP (2016b).

(immunology[sh] OR immune[tiab] OR immunocomp*[tiab] OR immunogen*[tiab] OR immunolog*[tiab] OR immunotox*[tiab] OR immunotoxins[mh] OR immunity[tiab] OR autoimmun*[tiab] OR "host resistance"[tiab] OR immunocompetence[mh] OR "immune system"[mh] OR spleen[tiab] OR splenic[tiab] OR splenocyt*[tiab] OR thymus[tiab] OR thymic[tiab] OR thymocyt*[tiab] OR leukocyt*[tiab] OR granulocyt*[tiab] OR basophil*[tiab] OR eosinophil*[tiab] OR neutrophil*[tiab] OR lymph[tiab] OR lymphoid*[tiab] OR lymphocyt*[tiab] OR "b-lymphocyte"[tiab] OR "b-lymphocytes"[tiab] OR "t-lymphocyte"[tiab] OR "t-lymphocytes"[tiab] OR "killer cell"[tiab] OR "killer cells"[tiab] OR "NK cell"[tiab] OR "NK-cell"[tiab] OR "NK-cells"[tiab] OR macrophag*[tiab] OR "mast cell"[tiab] OR "mast cells"[tiab] OR monocyt*[tiab] OR phagocyt*[tiab] OR dendrit*[tiab] OR "t-cell"[tiab] OR "t cell"[tiab] OR "t cells"[tiab] OR "t-cells"[tiab] OR "T helper"[tiab] OR "T-helper"[tiab] OR "b-cell"[tiab] OR "b cell"[tiab] OR "b cells"[tiab] OR "b-cells"[tiab] OR antibod*[tiab] OR histamine*[tiab] OR histocompatib*[tiab] OR immunoglobulins[mh] OR immunoglobulin*[tiab] OR "immunoglobulin A"[tiab] OR IgA[tiab] OR "immunoglobulin D"[tiab] OR IgD[tiab] OR "immunoglobulin E"[tiab] OR IgE[tiab] OR "immunoglobulin G"[tiab] OR IgG[tiab] OR "immunoglobulin M"[tiab] OR IgM[tiab] OR "antigens, CD"[mh] OR CD3 [tiab] OR CD4 [tiab] OR CD8 [tiab] OR CD25 [tiab] OR CD27 [tiab] OR CD28 [tiab] OR CD29 [tiab] OR CD45*[tiab] OR cytokines[mh] OR cytokine*[tiab] OR chemokine*[tiab] OR inteferon*[tiab] OR interleukin*[tiab] OR "IL-6"[tiab] OR "IL-8"[tiab] OR lymphokine*[tiab] OR monokine*[tiab] OR ("tumor necrosis"[tiab] AND (factor[tiab] OR factors[tiab])) OR "TNF alpha"[tiab] OR "TNFalpha"[tiab] OR "immune system diseases"[mh] OR autoimmun*[tiab] OR addison[tiab] OR rheumatoid[tiab] OR glomerulonephritis[tiab] OR diabetes[tiab] OR graves[tiab] OR lupus[tiab] OR thyroiditis[tiab] OR hypersensitiv*[tiab] OR sensitization OR hyperresponsiv*[tiab] OR allergy[mh] OR allerg*[tiab] OR atopy[tiab] OR atopic[tiab] OR dermatitis[tiab] OR eczema[tiab] OR otitis[tiab] OR “ear infection”[tiab]

OR “ear inflammation”[tiab] OR Respiratory tract infections[mh] OR (respiratory[tiab] AND infection*[tiab]) OR asthma[tiab] OR bronchitis[tiab] OR pneumonia[tiab] OR bronchiolitis[tiab] OR rhinitis[tiab] OR sinusitis[tiab] OR wheez*[tiab] OR crackle*[tiab] OR cough[mh] OR cough*[tiab] OR dyspnea[tiab] OR gastroenteritis[tiab] OR inflammation[mh] OR inflammat*[tiab] OR pro-inflammat*[tiab] OR anti-inflamm*[tiab] OR "inflammation mediators"[mh] OR autacoid*[tiab] OR eicosanoid*[tiab] OR prostaglandin*[tiab] OR immunomodulation[mh] OR immunomodul*[tiab] OR immunotherap*[tiab] OR vaccin*[tiab] OR immuniz*[tiab] OR immunosuppress*[tiab] OR desensitiz*[tiab] OR immunoproteins[mh] OR immunoprotein*[tiab] OR "c-reactive protein"[tiab] OR CRP[tiab] OR "complement component" [tiab] OR (complement[tiab] AND (C1 OR C2 OR C3 OR C4 OR C5 OR C6 OR C7 OR C8 OR C9)))

OEHHA’s literature search was done as described in the *General Methods* section above. In their review, NTP (2016) divided study results into five major outcome categories. These were antibody response, infectious disease or disease resistance, natural killer cell activity, hypersensitivity, and autoimmunity. Some of the specific diseases, conditions, or effects assessed within each of these categories are shown in Table A7.2. OEHHA classified studies based on these same five categories, plus an additional category labeled “Other,” which includes immune related outcomes not covered under the other five categories. OEHHA excluded studies in which the outcome was telomere length, metabolomics, or Clara cell protein levels. Because the strongest evidence linking PFOA or PFOS to immunotoxicity identified by NTP (2016) was for antibody responses, a number of additional detailed evaluations for this particular outcome are presented.

Table A7.2. Categories of immune toxicity and the specific outcomes in each

Major category	Specific outcomes
Antibody response	IgG levels to vaccines
Infectious disease or disease resistance	Hospitalizations for infectious disease Gastroenteritis Otitis media Colds Influenza
Natural killer cell activity	Multiple
Hypersensitivity	IgE Asthma or related symptoms Eczema Allergy Eosinophils Rhinitis COPD
Autoimmunity	Crohn’s disease Ulcerative colitis Lupus Type 1 diabetes Multiple sclerosis Rheumatoid arthritis IgG Autoantibodies
Other	CRP White blood cell counts Cytokine levels

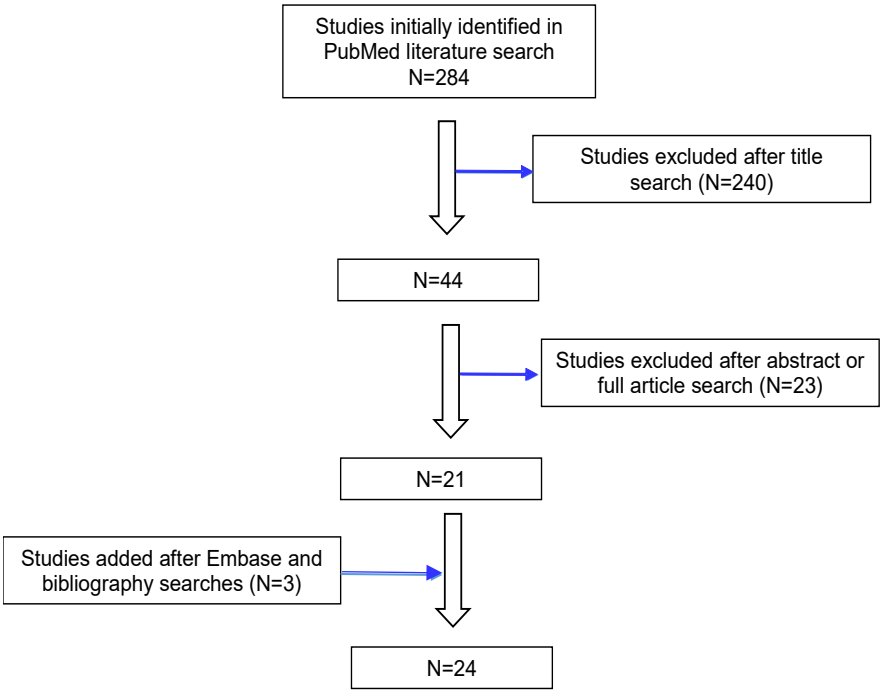
OEHHA evaluated study quality using the factors described above. In the overall evaluation of the evidence, OEHHA considered the possibility that cross-sectional studies might be a weaker

design since certain immunologic outcomes or diseases (or the products associated with their treatment) could potentially lead to increased PFOA or PFOS exposure. In addition, the following factors were identified as those that are especially likely to be potential confounders, that is, prevalent factors that appear to be related both to PFOA or PFOS exposure and immune function (see Table A7.1; de Bruyn 2010; Patin et al., 2018; Van Loveren et al., 2001): age, sex, race/ethnicity, socioeconomic determinants, body mass index (BMI), breast-feeding, and other chemical exposures like polychlorinated biphenyls (PCBs), other PFAS, or organochlorines. These are similar to the potential confounders identified by NTP (2016) in their immunotoxicity review.

Results

A general description of the literature search is provided in Figure A7.1. A list of studies excluded based on OEHHA's abstract or full article review is provided in Table A7.28.

Figure A7.1. Literature search: recent epidemiologic studies of PFOA or PFOS and immunotoxicity*



*This figure is provided to document OEHHA's PubMed, Embase, and bibliography literature searches. It does not include relevant publications identified from other sources such as previously published reviews from other agencies or other authors.

Twenty-four publications published since the 2016 NTP review (NTP, 2016) met the inclusion criteria described above. For each, study descriptions and factors related to OEHHA's evaluations of bias and causality are presented in Tables A7.3 (for PFOA) and A7.4 (for PFOS). A number of publications presented results for several different outcomes, and results for each outcome are presented separately in these tables.

Table A7.3. Recent epidemiologic studies on PFOA and immunotoxicity

Author year	Location Years	Design	Who Ages N	Factors related to bias	Exposure method	Outcome	Outcome method	Results	Comparison	Confounding	Other aspects of causal inference	Notes
Grandjean et al., 2017a	Faroe Islands 1997-2000 and 2007-09 (year of birth)	Prospective cohort and cross-sectional	Who: children Ages: ≤5 N: 275-349	Selection: unclear Participation: unclear Equal groups: unclear Blinded: unclear Above detection: unclear Levels: median (IQR) = 2.8 (2.0-4.5) ng/ml at age 18 months	Serum Near birth, 18 months, age 5	Antibody response: diphtheria	Diphtheria IgG Age 5	**2007-09 cohort:** PFOA IgG %change Birth -18.9 (p=0.03) 18 mo. 4.1 (p=0.63) 5 yr. 18.3 (p=0.24) **Combined cohort:** PFOA IgG %change Birth -17.8 (p=0.009) 18 mo. 5.4 (p=0.52) 5 yr. 3.4 (p=0.73) No major differences between 1997-2000 and 2007-09 cohorts	Percent change for a 2-fold increase in PFOA	Adjusted for age and sex Additional adjustments for PCB concentrations and Cesarean section had little effect	Large magnitude: yes Statistical significance: yes Dose-response: linear Temporal association: yes Subgroup only: no Adjustments: unclear	PFAS concentrations highly correlated with breast-feeding duration Correlation coefficients for PFAS levels up to 0.7 for age 18 months and age 5 years 44% and 36% had IgG below protective levels at age 5 for diphtheria and tetanus, respectively Combined: 2007-09 and 1997-2000 Faroe Islands cohorts combined
Grandjean et al., 2017b	Faroe Islands 1997-2000 (year of birth)	Prospective cohort and cross-sectional	Who: children Ages: ≤13 N: 275-349	Selection: unclear Participation: unclear Equal groups: unclear Blinded: unclear Above detection: unclear Levels: median (IQR) = 4.4 (3.6-5.7) ng/ml	Serum Ages 7 and 13	Antibody response: diphtheria	Diphtheria IgG Age 13	PFOA IgG %change Age 7 -9.2 (p=0.48) Age 13 -25.3 (p=0.03)	Percent change for a 2-fold increase in PFOA	Adjusted for age, sex, booster type at age 5 Additional adjustment for PCBs had little effect	Large magnitude: yes Statistical significance: yes Dose-response: linear Temporal association: see notes Subgroup only: no Adjustments: unclear	PFAS concentrations at age 13 correlated with those at age 7 but not with maternal levels Excludes those with likely booster after age 5 Decline seen prospectively but not statistically significant
Stein et al., 2016a	New York 2010-11	Prospective cohort	Who: adults Ages: 18-49 N: 75	Selection: unclear Participation: unclear Equal groups: yes although analyses limited Blinded: yes Above detection: 100% Levels: geometric mean = 2.28, upper tertile range = 2.8-8.1 ng/ml	Serum Day 0	Antibody response: FluMist	Anti-A H1N1 antibody response by histochem-ical staining (IHC) and hemagglut-ination-inhibition (HAI) Day 30	OR = 6.8 (1.0-48.1) p-trend = 0.07 for HAI OR = 1.8 (0.7-48.1) p-trend=0.27 for IHC	Upper vs. lower tertile of PFOA	Adjusted for age, sex, and race/ethnicity "No covariates… associated with both exposure and outcome"	Large magnitude: yes Statistical significance: borderline Dose-response: linear Temporal association: yes Subgroup only: no Adjustments: unclear	Less than 20 subjects seroconverted
Pilkerton et al., 2018	US NHANES 1999-2000, 2003-04	Cross-sectional	Who: children and adults Ages: ≥12 N: 2,389	Selection: multi-stage cluster sampling Participation: unclear Equal groups: unclear Blinded: likely Above detection: unclear but low LOD Levels: mean = 4.3-6.0 ng/ml	Serum	Antibody response: rubella	Serum IgG	4th vs. 1st quartile: Men: β = -0.4450 (p=0.03) Women: β = -0.1658 (p=0.68) Per quartile increase: For all adults combined: F-value = 6.60 (p=0.002) Children (0-18): no association (p=0.80)	Fourth vs. first quartile of PFOA; linear regression coefficients for increasing PFOA quartile	Adjusted for age, BMI, education, sex, and ethnicity, and parity	Large magnitude: unclear Statistical significance: yes Dose-response: step-shaped Temporal association: no Subgroup only: yes, men Adjustments: unclear	Results for children only and all adults presented only as F-values Gender specific analyses are for adults only

Author year	Location Years	Design	Who Ages N	Factors related to bias	Exposure method	Outcome	Outcome method	Results	Comparison	Confounding	Other aspects of causal inference	Notes
Grandjean et al., 2017a	Faroe Islands 1997-2000 and 2007-09 (year of birth)	Prospective cohort and cross-sectional	Who: children Ages: ≤5 N: 349	Selection: unclear Participation: unclear Equal groups: unclear Blinded: unclear Above detection: unclear Levels: median (IQR) = 2.8 (2.0-4.5) ng/ml at age 18 months	Serum Near birth, 18 months, age 5	Antibody response: tetanus	Tetanus IgG Age 5	**2007-09 cohort:** PFOA IgG %change Birth -22.2 (p=0.007) 18 mo. -16.3 (p=0.03) 5 yr. -25.3 (p=0.03) **Combined cohort:** PFOA IgG %change Birth -17.6 (p=0.007) 18 mo. -16.5 (p=0.03) 5 yr. -18.7 (p=0.02) No major differences between 1997-2000 and 2007-09 cohorts	Percent change for a 2-fold increase in PFOA	Adjusted for age and sex Additional adjustments for PCB concentrations and Cesarean section had little effect	Large magnitude: yes Statistical significance: yes Dose-response: linear Temporal association: yes Subgroup only: no Adjustments: unclear	PFAS concentrations highly correlated with breast-feeding duration Correlation coefficients up to 0.7 for age 18 and age 5 PFAS levels 44% and 36% had IgG below protective levels at age 5 for diphtheria and tetanus, respectively Combined: 2007-09 and 1997-2000 Faroe Islands cohorts combined
Grandjean et al., 2017b	Faroe Islands 1997-2000 (year of birth)	Prospective cohort and cross-sectional	Who: children Ages: ≤13 N: 275-349	Selection: unclear Participation: unclear Equal groups: unclear Blinded: unclear Above detection: unclear Levels: median (IQR) = 4.4 (3.6-5.7) ng/ml	Serum Ages 7 and 13	Antibody response: tetanus	Tetanus IgG Age 13	PFOA IgG %change Age 7 2.9 (p=0.86) Age 13 -5.6 (p=0.71)	Percent change for a 2-fold increase in PFOA	Adjusted for age, sex, booster type at age 5 Additional adjustment for PCBs had little effect	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: unclear	PFAS concentrations at age 13 correlated with those at age 7 but not with maternal levels Excludes those with likely booster after age 5
Hammer et al., 2019	Faroe Islands 1986-2009	Prospective cohort	Who: children and adults Ages: all N: 5,698	Selection: involved in previous cohort studies Participation: 75% of all Faroese births during the recruitment periods Equal groups: unclear Blinded: unclear Above detection: not given Levels: unclear	Serum	Autoimmunity: inflammatory bowel disease	Nationwide Inflammatory Disease database	OR = 0.60 (0.23-1.56)	Upper vs. lower tertile of PFOA	Adjusted for age and calendar period	Large magnitude: yes (below 1.0) Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: not given	Includes six different cohorts and a wide range of follow-up periods 37 cases
Goudarzi et al., 2016	Hokkaido, Japan 2003-09	Prospective cohort	Who: children Ages: 4 N: 1,558	Selection: appears to be all pregnant women in 37 hospitals and clinics in Hokkaido, Japan Participation: <18.9% Equal groups: unclear Blinded: unclear Above detection: 99.9% Levels: median (IQR) = 2.013 (1.314-3.346) ng/ml	Maternal plasma Gestation weeks 28-32	Hypersensitivity: allergy (all)	ISAAC questionnaire Age 4	No association Similar results in males and females	Fourth vs. first quartiles of PFOA	Adjusted for maternal age, siblings, maternal education, parental allergies, infant gender, breast-feeding, day-care attendance, and ETS Additional adjustments for household income, smoking, alcohol, BMI, pets, carpets, heating/cooling systems, and mold or dew condensation in homes had little effect 6.2% of mothers smoked during pregnancy	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: little change	All allergic diseases and related symptoms Combines eczema, wheezing and rhinoconjunctivitis

Author year	Location Years	Design	Who Ages N	Factors related to bias	Exposure method	Outcome	Outcome method	Results	Comparison	Confounding	Other aspects of causal inference	Notes
Timmer-mann et al., 2017	Faroe Islands 1997-2000 (year of birth)	Prospective cohort and cross-sectional	Who: children Ages: 0-13 N: 559	Selection: unclear Participation: unclear Equal groups: see notes Blinded: unclear Above detection: 100% Levels: median (IQR) = 2.0 (1.6-2.5) ng/ml at age 13	Serum Third trimester, and ages 5 and 13	Hyper-sensitivity: allergy	Question-naire and skin prick test Ages 5 and 13	**PFOA in maternal serum:** No association for outcome at ages 5 or 13 **PFOA at age 5:** No association for outcome at ages 5 or 13 **PFOA at age 13 and outcome at age 13:** No association	OR for each 2-fold increase in PFOA	Adjusted for family history of eczema in children, allergic eczema, hay fever, maternal pre-pregnancy BMI, maternal smoking during pregnancy, sex, duration of breast-feeding, fish intake at age 5, number of siblings, and day-care attendance at age 5	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: unclear	Higher PFAS concentrations near birth and at ages 5-7 PFAS concentrations related to breast-feeding, maternal BMI, maternal and paternal smoking, and fish consumption
Averina et al., 2019	Northern Norway 2010-13	Prospective cohort and cross-sectional	Who: children Ages: first year high school students N: 675	Selection: all first year students in eight high schools invited Participation: 60.4% Equal groups: unclear Blinded: unclear Above detection: unclear Levels: geometric mean (IQR) = 2.1 (1.2) ng/ml females, 1.9 (0.7) ng/ml males	Serum Age 16	Hyper-sensitivity: allergy (food)	Self-reported or FX5 food panel IgE Ages 16 (self-reported) and 18 (IgE)	OR = 0.27 (0.12-0.65) (food sensitization, IgE) No association with self-reported food allergies	Above vs. below the median PFOA	Adjusted for sex, age, BMI, physical activity, SES, diet, and allergy medications	Large magnitude: yes Statistical significance: yes Dose-response: not assessed Temporal association: yes Subgroup only: no Adjustments: unclear	Few details provided F/U: 3 years
Impinen et al., 2019	Norway 1999-2008	Prospective cohort	Who: children Ages: 0-7 N: 1,207 at ages 0-3 and 921 at ages 6-7 (same children)	Selection: all pregnant women scheduled for ultrasound Participation: 39% Equal groups: unclear Blinded: unclear Above detection: 100% Levels: median (IQR) = 2.54 (1.86-3.30) ng/ml	Maternal serum, median 18 weeks	Hyper-sensitivity: allergy (food)	Self-reports	ORs = 1.32 (0.92-1.90) for current allergy (higher in boys), near 1.0 for "ever" allergy	OR for one IQR increase in PFOA	Adjusted for maternal age, BMI, education, and smoking Adjusting for nursey school attendance had little impact Stratified by gender	Large magnitude: yes Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: not given	Samples shipped at ambient temperature Cohort members were participants in pre-eclampsia and subfecundity case-control studies Multiple comparisons: many different outcomes assessed Some loss to follow-up
Averina et al., 2019	Northern Norway 2010-13	Cross-sectional	Who: children Ages: first year high school students N: 675	Selection: all students in eight high schools invited Participation: 60.4% Equal groups: unclear Blinded: unclear Above detection: unclear Levels: geometric mean (IQR) = 2.1 (1.2) ng/ml females, 1.9 (0.7) ng/ml males	Serum Age 16	Hyper-sensitivity: allergy (nickel)	Self-reported	No association	Unclear	Unclear	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: unclear	Few details provided OR not given
Averina et al., 2019	Northern Norway 2010-13	Prospective cohort and cross-sectional	Who: children Ages: first year high school students N: 675	Selection: all students in eight high schools invited Participation: 60.4% Equal groups: unclear Blinded: unclear Above detection: unclear Levels: geometric mean (IQR) = 2.1 (1.2) ng/ml females, 1.9 (0.7) ng/ml males	Serum Age 16	Hyper-sensitivity: asthma	MeDALL question-naire Ages 16 and 18	OR = 2.07 (1.01-4.23) cross-sectional study Prospective results not provided	Fourth vs. first quartiles of PFOA	Unclear	Large magnitude: yes Statistical significance: yes Dose-response: unclear Temporal association: no Subgroup only: no Adjustments: unclear	F/U: 3 years Few details provided

Author year	Location Years	Design	Who Ages N	Factors related to bias	Exposure method	Outcome	Outcome method	Results	Comparison	Confounding	Other aspects of causal inference	Notes
Beck et al., 2019	Odense, Denmark 2010-12	Prospective cohort	Who: children Ages: 5 N: 981	Selection: all pregnant women in the municipality Participation: <43% Equal groups: asthma or wheeze greater in boys, smoking parents, and family history of asthma Blinded: unclear Levels: median (IQR) = 1.68 (1.13-2.35) ng/ml	Maternal serum, weeks 8-16	Hyper-sensitivity: asthma	ISSAC question-naire age 5	Doctor diagnosed asthma: Girls: OR = 1.70 (0.63-4.56) Boys: OR = 0.72 (0.46-1.12) Elevated OR for self-reported asthma in boys Wheeze: all ORs near 1.0	OR for a doubling of PFOA concentratio n	Adjusted for parity, education, BMI, asthma predisposition, and sex Also stratified by sex	Large magnitude: yes Statistical significance: no Dose-response: no Temporal association: yes Subgroup only: inconsistent by sex Adjustments: small changes only	Includes asthma and wheezing Samples stored at -80º C Self-reported asthma: only those who did not also report doctor diagnosed asthma Inconsistent results across sexes and self-reports vs. doctor diagnosed asthma
Gaylord et al., 2019	US 2014-16	Cross-sectional	Who: children and adults Ages: 13-22 N: 287	Selection: exposed to the World Trade Center disaster as children and controls Participation: unclear Equal groups: unclear Blinded: unclear Above detection: 100% Levels: mean (SD) = 1.53 (0.65) ng/ml	Serum	Hyper-sensitivity: asthma	Question-naire: similar to NHANES	OR = 1.34 (0.55-3.29)	OR per unit change in log PFOA	Adjusted for sex, race/ethnicity, BMI, and tobacco smoke exposure	Large magnitude: yes Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: yes, unadjusted OR = 1.00	
Goudarzi et al., 2016	Hokkaido, Japan 2003-09	Prospective cohort	Who: children Age: 4 N: 1,558	Selection: appears to be all pregnant women in 37 hospitals and clinics in Hokkaido, Japan Participation: <18.9% Equal groups: unclear Blinded: unclear Above detection: 99.9% Levels: median (IQR) = 2.013 (1.314-3.346) ng/ml	Maternal plasma Gestation weeks 28-32	Hyper-sensitivity: asthma	ISAAC question-naire Age 4	OR = 1.25 (0.71-2.22) in males for wheezing	Fourth vs. first quartiles of PFOA	Adjusted for maternal age, siblings, maternal education, parental allergies, infant gender, breast-feeding, day-care attendance, and ETS Additional adjustments for household income, smoking, alcohol, BMI, pets, carpets, heating/cooling systems, and mold or dew condensation in homes had little effect 6.2% of mothers smoked during pregnancy	Large magnitude: yes, males and wheezing Statistical significance: no Dose-response: no Temporal association: no Subgroup only: males Adjustments: little change	Includes asthma and wheezing Crude OR = 1.37 (0.824-2.28) in males
Impinen et al., 2018	Oslo 1992-93	Prospective cohort	Who: children Ages: 0-10 N: 641	Selection: unclear Participation: unclear Equal groups: unclear Blinded: unclear Above detection: >96% Levels: median (IQR) = 1.6 (1.2-2.1) ng/ml	Cord blood	Hyper-sensitivity: asthma	Parent interview, clinical exam, and spirometry Ages 2-10	No association for asthma OR = 1.27 (0.91-1.78) for wheeze	OR for a doubling of PFOA	Adjusted for sex Birth weight, birth month, breast-feeding, maternal smoking, other smoking, parental asthma and allergies, parental education, and household income not “statistically significant”	Large magnitude: no, for asthma; yes for wheeze Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: unclear	At age 2, subjects included cases with bronchial obstruction and controls; details of selection and recruitment not provided Details of selection and recruitment of subjects at age 10 also not provided Bonferroni correction applied to p-values

Author year	Location Years	Design	Who Ages N	Factors related to bias	Exposure method	Outcome	Outcome method	Results	Comparison	Confounding	Other aspects of causal inference	Notes
Impinen et al., 2019	Norway 1999-2008	Prospective cohort	Who: children Ages: 0-7 N: 1,207 at ages 0-3 and 921 at ages 6-7 (same children)	Selection: all pregnant women scheduled for ultrasound Participation: 39% Equal groups: unclear Blinded: unclear Above detection: 100% Levels: median (IQR) = 2.54 (1.86-3.30) ng/ml	Maternal serum, median 18 weeks	Hyper-sensitivity: asthma	Doctor diagnosed (asthma), self-reports (wheeze)	ORs for asthma and wheeze near 1.0, similar results in boys and girls	OR for one IQR increase in PFOA	Adjusted for maternal age, BMI, education, and smoking Adjusting for nursey school attendance had little impact Stratified by gender	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: not given	Includes asthma and wheezing Samples shipped at ambient temperature Cohort members were participants in pre-eclampsia and subfecundity case-control studies Some loss to follow-up
Kvalem et al., 2020	Oslo, Norway	Prospective cohort and cross-sectional	Who: children Ages: 10 and 16 (age at interview) N: 378	Selection: newborns 1992-3 in Oslo recruited into the original study Participation: unclear but less than 10-15% Equal groups: unclear Blinded: unclear Above detection: 100% Levels: median (IQR) = 4.36 (1.77) ng/ml	Serum, age 10	Hyper-sensitivity: asthma	Questionnaire: based on symptoms, doctor's diagnosis or medication use, up to age 10 (cross-sectional), between ages 10-16, or at age 16 (prospective)	<u>Cross-sectional:</u> OR = 1.29 (lower confidence interval is 0.99, upper is 0.17) in girls Unadjusted OR = 1.39 (1.09-1.77) All other ORs near 1.0	Per IQR increase in PFOA	Adjusted for BMI, puberty, maternal education, and activity	Large magnitude: yes Statistical significance: unknown Dose-response: no Temporal association: no Subgroup only: girls Adjustments: large change	Includes asthma and wheezing F/U: 6 years Environment and Childhood Asthma study Incorrect confidence interval Large change with adjustments Multiple comparisons: many different outcomes and PFAS assessed
Manzano-Salgado et al., 2019	Spain 2003-8	Prospective cohort	Who: children Ages: 1.5-7 N: 1,071-1,188	Selection: giving birth in a participating hospital Participation: unclear Equal groups: unclear Blinded: unclear Above detection: 100% Levels: median (IQR) = 2.35 (1.63-3.30) ng/ml	Maternal plasma, 1st trimester	Hyper-sensitivity: asthma	ISAAC question-naire at ages 1.5, 4 and 7	No association overall and in boys and girls, some decrease at age 7 for wheeze and by breast-feeding status (see notes)	Change in the outcome for a doubling of PFOA	Adjusted for maternal age, parity, breast-feeding, pre-pregnancy BMI, region, and country Little change when adjusted for other PFAS, fish consumption, smoking and maternal education	Large magnitude: inconsistent Statistical significance: no Dose-response: no Temporal association: no Subgroup only: see notes Adjustments: little change	Includes asthma and wheezing Spanish INMA birth cohort Samples stored at -80º C Inconsistent results by breast-feeding status (low ORs with never and longest category of breast-feeding)
Qin et al., 2017	Taiwan 2009-10	Case-control (cross-sectional)	Who: children Ages: 10-15 N: 300	Selection: unclear Participation: <50% Equal groups: similar except higher ETS in non-asthmatics Blinded: unclear Above detection: >96% Levels: median (IQR) = 0.50 (0.43-0.69) ng/ml	Serum	Hyper-sensitivity: asthma	Physician diagnosed	OR = 2.76 (1.82-4.17)	OR for each IQR increase in PFOA	Adjusted for age, sex, BMI, education, exercise, ETS, month of survey Matched on age and sex	Large magnitude: yes Statistical significance: yes Dose-response: unclear Temporal association: no Subgroup only: no Adjustments: little change	Cases from two hospitals in northern Taiwan, controls randomly selected from seven public schools in northern Taiwan

Author year	Location Years	Design	Who Ages N	Factors related to bias	Exposure method	Outcome	Outcome method	Results	Comparison	Confounding	Other aspects of causal inference	Notes
Timmer-mann et al., 2017	Faroe Islands 1997-2000 (year of birth)	Prospective cohort and cross-sectional	Who: children Ages: 0-13 N: 559	Selection: unclear Participation: unclear Equal groups: see notes Blinded: unclear Above detection: 100% Levels: median (IQR) = 2.0 (1.6-2.5) ng/ml at age 13	Serum Third trimester, and ages 5 and 13	Hyper-sensitivity: asthma	Question-naire Ages 5 and 15	**PFOA in maternal serum:** Age OR 5 1.37 (0.81-2.32) 13 1.12 (0.67-188) **PFOA at age 5:** Age MMR OR 5 no 10.4 (1.06-102) 5 yes 0.76 (0.41-1.39) 13 no 9.92 (1.06-93) 13 yes 0.65 (0.35-1.20) **PFOA at age 13 and outcome at age 13:** No association Age column refers to outcome age	OR for each 2-fold increase in PFOA	Adjusted for family history of eczema in children, allergic eczema, hay fever, maternal pre-pregnancy BMI, maternal smoking during pregnancy, sex, duration of breast-feeding, fish intake at age 5, number of siblings, and day-care attendance at age 5, birth weight, and family history of chronic bronchitis/asthma	Large magnitude: yes Statistical significance: yes Dose-response: linear Temporal association: yes Subgroup only: yes Adjustments: unclear	Higher PFAS concentrations near birth and at age 5-7 PFAS concentrations related to breast-feeding, maternal BMI, maternal and paternal smoking, and fish consumption Stratified by MMR vaccination if p-interaction <0.2
Zeng et al., 2019	Shanghai 2012-15	Prospective cohort	Who: children Ages: 0-5 N: 358	Selection: pregnancies at two participating hospitals Participation: <50% Equal groups: unclear Blinded: unclear Above detection: unclear Levels: median and IQR = 7.13 (5.15-9.97) ng/ml boys and 6.51 (4.57-8.73) ng/ml girls	Cord plasma	Hyper-sensitivity: asthma	Pediatrician diagnosis and spirometry	Some ORs above and some below 1.0 based on gender but with very wide confidence intervals	Unclear	Adjusted for child weight, gestational age, breast-feeding, maternal education and BMI, and income	Large magnitude: mixed above and below 1.0 – see results Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: not given	Includes asthma and wheezing F/U: 5 years Samples stored at -80º C
Averina et al., 2019	Northern Norway 2010-13	Cross-sectional	Who: children Ages: first year high school students N: 675	Selection: all students in eight high schools invited Participation: 60.4% Equal groups: unclear Blinded: unclear Above detection: unclear Levels: geometric mean (IQR) = 2.1 (1.2) ng/ml females, 1.9 (0.7) ng/ml males	Serum Age 16	Hyper-sensitivity: eczema	Self-reported doctor diagnosed	No association	Unclear	Unclear	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: unclear	Few details provided
Chen et al., 2018	Shangha i 2012-15	Prospective cohort	Who: children Ages: 0-24 months N: 687	Selection: unclear Participation: 65.1% among those enrolled Equal groups: Eczema and non-eczema cases similar except allergic family history, nulliparous, and breast-feeding greater in eczema cases Blinded: unclear Above detection: >90% Levels: fourth quartile ≥9.42 ng/ml	Cord blood	Hyper-sensitivity: eczema	ISAAC question-naire Ages 6, 12, and 24 months	Females: OR = 2.52 (1.12-5.68) Males: no association	Fourth vs. first quartile of PFOA	Adjusted for maternal age, maternal BMI, gestation week at delivery, birth weight, maternal and paternal education, mode of delivery, family history, family income, ethnicity, paternal smoking, and breast-feeding Less than 2% of mothers drank alcohol or smoked	Large magnitude: yes Statistical significance: yes Dose-response: linear Temporal association: yes Subgroup only: females Adjustments: little change	25.2% of children developed atopic dermatitis by 24 months of age 75.4% delivered by Caesarian section

Author year	Location Years	Design	Who Ages N	Factors related to bias	Exposure method	Outcome	Outcome method	Results	Comparison	Confounding	Other aspects of causal inference	Notes
Goudarzi et al., 2016	Hokkaido, Japan 2003-09	Prospective cohort	Who: children Age: 4 N: 1,558	Selection: appears to be all pregnant women in 37 hospitals and clinics in Hokkaido, Japan Participation: <18.9% Equal groups: unclear Blinded: unclear Above detection: 99.9% Levels: median (IQR) = 2.013 (1.314-3.346) ng/ml	Maternal plasma Gestation weeks 28-32	Hyper-sensitivity: eczema	ISAAC question-naire Age 4	OR = 0.59 (0.32-1.08, p-trend = 0.02) in males OR = 1.21 (0.68-2.17, p-trend = 0.36) in females	Fourth vs. first quartiles of PFOA	Adjusted for maternal age, number of siblings, maternal education, parental allergies, infant gender, breast-feeding, day-care attendance, and ETS Additional adjustments for household income, smoking, alcohol, BMI, pets, carpets, heating/cooling systems, and mold or dew condensation in homes had little effect 6.2% of mothers smoked during pregnancy	Large magnitude: yes Statistical significance: yes Dose-response: non-linear Temporal association: yes Subgroup only: yes, see results Adjustments: little change	Participants had higher maternal education, lower maternal smoking rates, and lower postnatal ETS rates than original cohort
Impinen et al., 2018	Oslo 1992-93	Prospective cohort	Who: children Ages: 0-10 N: 641	Selection: unclear Participation: unclear Equal groups: unclear Blinded: unclear Above detection: >96% Levels: median (IQR) = 1.6 (1.2-2.1) ng/ml	Cord blood	Hyper-sensitivity: eczema	Parent interview and clinical exam Ages 2-10	No association	OR for a doubling of PFOA	Adjusted for sex Birth weight, birth month, breast-feeding, maternal smoking, other smoking, parental asthma and allergies, parental education, and household income not "statistically significant"	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: unclear	At age 2, subjects included cases with bronchial obstruction and controls; details of selection and recruitment not provided Details of selection and recruitment of subjects at age 10 also not provided Bonferroni correction applied to p-values
Impinen et al., 2019	Norway 1999-2008	Prospective cohort	Who: children Ages: 0-7 N: 1,207 at ages 0-3 and 921 at ages 6-7 (same children)	Selection: all pregnant women scheduled for ultrasound Participation: 39% Equal groups: unclear Blinded: unclear Above detection: 100% Levels: median (IQR) = 2.54 (1.86-3.30) ng/ml	Maternal serum, median 18 weeks	Hyper-sensitivity: eczema	Doctor diagnosed	ORs near 1.0, similar results in boys and girls	OR for one IQR increase in PFOA	Adjusted for maternal age, BMI, education, and smoking Adjusting for nursey school attendance had little impact Stratified by gender	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: not given	Samples shipped at ambient temperature Cohort members were participants in pre-eclampsia and subfecundity case-control studies Some loss to follow-up
Kvalem et al., 2020	Oslo, Norway	Prospective cohort and cross-sectional	Who: children Ages: 10 and 16 (age at interview) N: 378	Selection: newborns 1992-3 in Oslo recruited into the original study Participation: unclear but less than 10-15% Equal groups: unclear Blinded: unclear Above detection: 100% Levels: median (IQR) = 4.36 (1.77) ng/ml	Serum, age 10	Hyper-sensitivity: eczema	Questionnaire: based on parent reported eczema up to age 10 (cross-sectional), between ages 10-16, or at age 16 (prospective)	OR = 1.24 (0.86-1.78) for girls ages 10 and16 (prospective) All other ORs closer to 1.0	Per IQR increase in PFOA	Adjusted for BMI, puberty, maternal education, and activity	Large magnitude: yes Statistical significance: no Dose-response: no Temporal association: yes Subgroup only: girls Adjustments: unadjusted OR = 1.18	F/U: 6 years Environment and Childhood Asthma study Some change with adjustments Multiple comparisons: many different outcomes and PFAS assessed

Author year	Location Years	Design	Who Ages N	Factors related to bias	Exposure method	Outcome	Outcome method	Results	Comparison	Confounding	Other aspects of causal inference	Notes
Manzano-Salgado et al., 2019	Spain 2003-8	Prospective cohort	Who: children Ages: 1.5-7 N: 1,071-1,188	Selection: giving birth in a participating hospital Participation: unclear Equal groups: unclear Blinded: unclear Above detection: 100% Levels: median (IQR) = 2.35 (1.63-3.30) ng/ml	Maternal plasma, 1st trimester	Hyper-sensitivity: eczema	ISAAC question-naire at ages 1.5, 4 and 7	No association overall and in boys and girls OR = 0.59 (0.37-0.96) for children of "never" breast-feeding mothers	Change in the outcome for a doubling of PFOA	Adjusted for maternal age, parity, breast-feeding, pre-pregnancy BMI, region, and country Little change when adjusted for other PFAS, fish consumption, smoking and maternal education in overall analysis	Large magnitude: yes (decreased OR) Statistical significance: yes Dose-response: no Temporal association: yes Subgroup only: never breast-feeding mother Adjustments: not given for this subgroup	Spanish INMA birth cohort Samples stored at -80º C
Timmer-mann et al., 2017	Faroe Islands 1997-2000 (year of birth)	Prospective cohort and cross-sectional	Who: children Ages: 0-13 N: 559	Selection: unclear Participation: unclear Equal groups: see notes Blinded: unclear Above detection: 100% Levels: median (IQR) = 2.0 (1.6-2.5) ng/ml at age 13	Serum Third trimester, and ages 5 and 13	Hyper-sensitivity: eczema	ISAAC question-naire Age 13	Prenatal exposure and outcome at age 13: OR = 1.36 (0.85-2.19) No clear association at other ages	OR for each 2-fold increase in PFOA	Adjusted for family history of eczema in children, allergic eczema, hay fever, maternal pre-pregnancy BMI, maternal smoking during pregnancy, sex, duration of breast-feeding, fish intake at age 5, number of siblings, and day-care attendance at age 5	Large magnitude: yes Statistical significance: no Dose-response: no Temporal association: no Subgroup only: yes Adjustments: unclear	Higher PFAS concentrations near birth and at ages 5-7 PFAS concentrations related to breast-feeding, maternal BMI, maternal and paternal smoking, and fish consumption
Wen et al., 2019a	Taiwan 2001-5	Prospective cohort	Who: children Ages: 5 N: 836	Selection: prenatal exams at participating hospital Participation: unclear Equal groups: atopic dermatitis was associated with breast-feeding, parental education, and atopy Blinded: unclear Above detection: 50.75% Levels: median (IQR) = 0.65 (0.23-1.96) ng/ml	Cord plasma	Hyper-sensitivity: eczema	ISAAC question-naire	Hazard ratio= 1.89 (1.10-3.16) Higher risks with GSTT1-null phenotype (Wen et al., 2019b)	Above vs. below the upper quartile PFOA	Adjusted for sex, parental education and atopy, breast-feeding, and maternal age at childbirth	Large magnitude: yes Statistical significance: yes Dose-response: risks increased at upper quartile Temporal association: yes Subgroup only: no Adjustments: little change	F/U: 5 years Median age of atopic dermatitis development was 6 months in the exposed group Prevalence of atopic dermatitis was 7.1% Low exposure levels
Averina et al., 2019	Northern Norway 2010-13	Prospective cohort	Who: children Ages: first year high school students N: 675	Selection: all students in eight high schools invited Participation: 60.4% Equal groups: unclear Blinded: unclear Above detection: unclear Levels: geometric mean (IQR) = 2.1 (1.2) ng/ml females, 1.9 (0.7) ng/ml males	Serum Age 16	Hyper-sensitivity: IgE	Serum Age 18	No association	Unclear	Unclear	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: unclear	Few details provided OR not given

Author year	Location Years	Design	Who Ages N	Factors related to bias	Exposure method	Outcome	Outcome method	Results	Comparison	Confounding	Other aspects of causal inference	Notes
Timmer-mann et al., 2017	Faroe Islands 1997-2000 (year of birth)	Prospective cohort and cross-sectional	Who: children Ages: 0-13 N: 559	Selection: unclear Participation: unclear Equal groups: see notes Blinded: unclear Above detection: 100% Levels: median (IQR) = 2.0 (1.6-2.5) ng/ml at age 13	Serum Third trimester, and ages 5 and 13	Hyper-sensitivity: IgE	Cord blood Serum at age 7	No association	Percent difference for each 2-fold increase in PFOA	Adjusted for family history of eczema in children, allergic eczema, hay fever, maternal pre-pregnancy BMI, maternal smoking during pregnancy, sex, duration of breast-feeding, fish intake at age 5, number of siblings, and day-care attendance at age 5	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: unclear	Higher PFAS concentrations near birth and at ages 5-7 PFAS concentrations related to breast-feeding, maternal BMI, maternal and paternal smoking, and fish consumption
Zeng et al., 2019	Shanghai 2012-15	Prospective cohort	Who: children Ages: 0-5 N: 358	Selection: pregnancies at two participating hospitals Participation: <50% Equal groups: unclear Blinded: unclear Above detection: unclear Levels: median and IQR = 7.13 (5.15-9.97) boys and 6.51 (4.57-8.73) ng/ml girls	Cord plasma	Hyper-sensitivity: IgE Age 5	Serum	Some ORs above and some below 1.0 based on gender and exposure level	Unclear	Adjusted for child weight, gestational age, breast-feeding, maternal education and BMI, and income	Large magnitude: mixed above and below 1.0 – see results Statistical significance: no Dose-response: U-shaped Temporal association: no Subgroup only: no Adjustments: not given	F/U: 5 years Samples stored at -80º C
Averina et al., 2019	Northern Norway 2010-13	Prospective cohort and cross-sectional	Who: children Ages: first year high school students N: 675	Selection: all students in eight high schools invited Participation: 60.4% Equal groups: unclear Blinded: unclear Above detection: unclear Levels: geometric mean (IQR) = 2.1 (1.2) ng/ml females, 1.9 (0.7) ng/ml males	Serum Age 16	Hyper-sensitivity: rhinitis	Self-reported doctor diagnosed Ages 16 and 18	No association	Unclear	Unclear	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: unclear	Allergic rhinitis Few details provided ORs not given
Dalsager et al., 2016	Denmark 2010-12	Prospective cohort	Who: children Ages: 0-3 N: 359	Selection: all pregnant women living in Odense from 2010-12 were invited Participation: <43% Equal groups: PFOA higher in nulliparous, younger maternal age, decreased education, higher BMI, older child and male child Blinded: unclear Above detection: unclear, but LOD was low (0.03 ng/ml) Levels: upper tertile range = 2.04-10.12 ng/ml	Maternal serum Gestation weeks 10-16	Hyper-sensitivity: rhinitis	Mobile phone question-naires on symptoms every 2 weeks for one year Ages 1-3 Proportion of days with symptoms of infectious disease	OR = 1.37 (0.75-2.51)	Upper vs. lower tertile of PFOA	Adjusted for maternal age, maternal education, parity, and child's age Additional adjustments for duration of breast-feeding, day-care, maternal smoking, and child's sex had little effect 3% of mothers smoked during pregnancy	Large magnitude: yes Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustment: little change	Data reported for an average of 86% of days in the year Similar results in those reporting at least 25 of 26 weeks

Author year	Location Years	Design	Who Ages N	Factors related to bias	Exposure method	Outcome	Outcome method	Results	Comparison	Confounding	Other aspects of causal inference	Notes
Goudarzi et al., 2016	Hokkaido, Japan 2003-09	Prospective cohort	Who: children Age: 4 N: 1,558	Selection: appears to be all pregnant women in 37 hospitals and clinics in Hokkaido, Japan Participation: <18.9% Equal groups: unclear Blinded: unclear Above detection: 99.9% Levels: median (IQR) = 2.013 (1.314-3.346) ng/ml	Maternal plasma Gestation weeks 28-32	Hyper-sensitivity: rhinitis	ISAAC question-naire Age 4	OR = 1.27 (0.62-2.61) overall OR higher in females	Fourth vs. first quartiles of PFOA	Adjusted for maternal age, number of siblings, maternal education, parental allergies, infant gender, breast-feeding, day-care attendance, and ETS Additional adjustments for household income, smoking, alcohol, BMI, pets, carpets, heating/cooling systems, and mold or dew condensation in homes had little effect 6.2% of mothers smoked during pregnancy	Large magnitude: yes Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: little change	Outcome is rhinoconjunctivitis Participants had higher maternal education, lower maternal smoking rates, and lower postnatal ETS rates than original cohort
Impinen et al., 2018	Oslo 1992-93	Prospective cohort	Who: children Ages: 0-10 N: 641	Selection: unclear Participation: unclear Equal groups: unclear Blinded: unclear Above detection: >96% Levels: median (IQR) = 1.6 (1.2-2.1) ng/ml	Cord blood	Hyper-sensitivity: rhinitis	Parent interview and clinical exam Ages 2-10	OR = 1.30 (0.97-1.74)	OR for a doubling of PFOA	Adjusted for sex Birth weight, birth month, breast-feeding, maternal smoking, other smoking, parental asthma and allergies, parental education, and household income not "statistically significant"	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: unclear	At age 2, subjects included cases with bronchial obstruction and controls; details of selection and recruitment not provided Details of selection and recruitment of subjects at age 10 also not provided Bonferroni correction applied to p-values
Impinen et al., 2019	Norway 1999-2008	Prospective cohort	Who: children Ages: 0-7 N: 1,207 at ages 0-3 and 921 at ages 6-7 (same children)	Selection: all pregnant women scheduled for ultrasound Participation: 39% Equal groups: unclear Blinded: unclear Above detection: 100% Levels: median (IQR) = 2.54 (1.86-3.30) ng/ml	Maternal serum, median 18 weeks	Hyper-sensitivity: rhinitis	Self-reports	OR = 1.32 (0.86-2.03), similar in boys and girls	OR for one IQR increase in PFOA	Adjusted for maternal age, BMI, education, and smoking Adjusting for nursey school attendance had little impact Stratified by gender	Large magnitude: yes Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: not given	Samples shipped at ambient temperature Cohort members were participants in pre-eclampsia and subfecundity case-control studies Multiple comparisons: many different outcomes assessed Some loss to follow-up
Kvalem et al., 2020	Oslo, Norway	Prospective cohort and cross-sectional	Who: children Ages: 10 and 16 (age at interview) N: 378	Selection: newborns 1992-3 in Oslo recruited into the original study Participation: unclear but less than 10-15% Equal groups: unclear Blinded: unclear Above detection: 100% Levels: median (IQR) = 4.36 (1.77) ng/ml	Serum, age 10	Hyper-sensitivity: rhinitis	Questionnaire: based on parent reported symptoms	Cross-sectional: All ORs near 1.0 Prospective: OR = 1.08 (1.01-1.14), higher in girls	Per IQR increase in PFOA	Adjusted for BMI, puberty, maternal education, and activity	Large magnitude: no Statistical significance: yes Dose-response: no Temporal association: yes Subgroup only: mostly in girls Adjustments: little change	F/U: 6 years Environment and Childhood Asthma study Multiple comparisons: many different outcomes and PFAS assessed Small OR

Author year	Location Years	Design	Who Ages N	Factors related to bias	Exposure method	Outcome	Outcome method	Results	Comparison	Confounding	Other aspects of causal inference	Notes
Timmer-mann et al., 2017	Faroe Islands 1997-2000 (year of birth)	Prospective cohort and cross-sectional	Who: children Ages: 0-13 N: 559	Selection: unclear Participation: unclear Equal groups: see notes Blinded: unclear Above detection: 100% Levels: median (IQR) = 2.0 (1.6-2.5) ng/ml at age 13	Serum Third trimester, and ages 5 and 13	Hyper-sensitivity: rhinitis	ISAAC question-naire Age 13	No association for exposure prenatally or ages 5 or 13	OR for each 2-fold increase in PFOA	Adjusted for family history of eczema in children, allergic eczema, hay fever, maternal pre-pregnancy BMI, maternal smoking during pregnancy, sex, duration of breast-feeding, fish intake at age 5, number of siblings, and day-care attendance at age 5	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: unclear	Outcome is rhinoconjunctivitis Higher PFAS concentrations near birth and at ages 5-7 PFAS concentrations related to breast-feeding, maternal BMI, maternal and paternal smoking, and fish consumption
Goudarzi et al., 2017	Hokkaido, Japan 2003-09	Prospective cohort	Who: children Age: 4 N: 1,558	Selection: appears to be all pregnant women in 37 hospitals and clinics in Hokkaido, Japan Participation: <18.9% Equal groups: unclear Blinded: unclear Above detection: 99.9% Levels: median (IQR) = 2.013 (1.314-3.346) ng/ml	Maternal plasma Gestation weeks 28-32	Infection: any	Self-reported doctor diagnosed otitis media, pneumonia, varicella, or respiratory syncytial virus Age 4	No association in males OR = 1.37 (0.85-2.21) in females	Fourth vs. first quartiles of PFOA	Adjusted for maternal age, number of siblings, maternal education, breast-feeding, and smoking Additional adjustment or stratification for day-care attendance, ETS, pets, carpets, heating/cooling systems, presence of mold or dew condensation in home had little effect	Large magnitude: yes, females Statistical significance: no Dose-response: no Temporal association: no Subgroup only: females Adjustments: unclear	
Impinen et al., 2018	Oslo 1992-93	Prospective cohort	Who: children Ages: 0-10 N: 641	Selection: unclear Participation: unclear Equal groups: unclear Blinded: unclear Above detection: >96% Levels: median (IQR) = 1.6 (1.2-2.1) ng/ml	Cord blood	Infection: colds	Parent interview, clinical exam, and spirometry Ages 0-2	β = -0.04 (-0.08-0.01, p=0.089)	Number of colds age 0-10 per log2 PFOA	Adjusted for sex Birth weight, birth month, breast-feeding, maternal smoking, other smoking, parental asthma and allergies, parental education, and household income not “statistically significant”	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: unclear	At age 2, subjects included cases with bronchial obstruction and controls; details of selection and recruitment not provided Details of selection and recruitment of subjects at age 10 also not provided
Impinen et al., 2019	Norway 1999-2008	Prospective cohort	Who: children Ages: 0-3 N: 1,207	Selection: all pregnant women scheduled for ultrasound Participation: 63% Equal groups: unclear Blinded: unclear Above detection: 100% Levels: median (IQR) = 2.54 (1.86-3.30) ng/ml	Maternal serum, median 18 weeks	Infection: colds	Self-reports	OR below 1.0	OR for one IQR increase in PFOA	Adjusted for maternal age, BMI, education, and smoking Adjusting for nursey school attendance had little impact Stratified by gender	Large magnitude: no Statistical significance: no (below 1.0) Dose-response: no Temporal association: no Subgroup only: no Adjustments: not given	Samples shipped at ambient temperature Cohort members were participants in pre-eclampsia and subfecundity case-control studies Some loss to follow-up
Kvalem et al., 2020	Oslo, Norway	Prospective cohort and cross-sectional	Who: children Ages: 10 and 16 (age at interview) N: 378	Selection: newborns 1992-3 in Oslo recruited into the original study Participation: unclear but less than 10-15% Equal groups: unclear Blinded: unclear Above detection: 100% Levels: median (IQR) = 4.36 (1.77) ng/ml	Serum, age 10	Infection: colds	Questionnaire: parent reported symptoms at ages 10 and 16	ORs above 1.4 in both boys and girls but with wide confidence intervals	Per IQR increase in PFOA	Adjusted for BMI, puberty, maternal education, and activity	Large magnitude: yes Statistical significance: no Dose-response: no Temporal association: yes Subgroup only: no Adjustments: some moderate changes	F/U: 6 years Environment and Childhood Asthma study Some change with adjustments Multiple comparisons: many different outcomes and PFAS assessed

Author year	Location Years	Design	Who Ages N	Factors related to bias	Exposure method	Outcome	Outcome method	Results	Comparison	Confounding	Other aspects of causal inference	Notes
Dalsager et al., 2016	Denmark 2010-12	Prospective cohort	Who: children Ages: 0-3 N: 359	Selection: all pregnant women living in Odense from 2010-12 were invited Participation: <43% Equal groups: PFOA higher in nulliparous, younger maternal age, decreased education, higher BMI, older child and male child Blinded: unclear Above detection: unclear, but LOD was low (0.03 ng/ml) Levels: upper tertile range = 2.04-10.12 ng/ml	Maternal serum Gestation weeks 10-16	Infection: cough	Mobile phone question-naires on symptoms every 2 weeks for one year Ages 1-3 Proportion of days with symptoms of infectious disease	No association	Upper vs. lower tertile of PFOA	Adjusted for maternal age, maternal education, parity, and child's age Additional adjustments for duration of breast-feeding, day-care, maternal smoking, and child's sex had little effect 3% of mothers smoked during pregnancy	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustment: little change	Data reported for an average of 86% of days in the year Similar results in those reporting at least 25 of 26 weeks
Dalsager et al., 2016	Denmark 2010-12	Prospective cohort	Who: children Ages: 0-3 N: 359	Selection: all pregnant women living in Odense from 2010-12 were invited Participation: <43% Equal groups: PFOA higher in nulliparous, younger maternal age, decreased education, higher BMI, older child and male child Blinded: unclear Above detection: unclear, but LOD was low (0.03 ng/ml) Levels: upper tertile range = 2.04-10.12 ng/ml	Maternal serum Gestation weeks 10-16	Infection: fever	Mobile phone question-naires on symptoms every 2 weeks for one year Ages 1-3 Proportion of days with symptoms of infectious disease	OR = 1.97 (1.07-3.62)	Upper vs. lower tertile of PFOA	Adjusted for maternal age, maternal education, parity, and child's age Additional adjustments for duration of breast-feeding, day-care, maternal smoking, and child's sex had little effect 3% of mothers smoked during pregnancy	Large magnitude: yes Statistical significance: yes Dose-response: linear Temporal association: yes Subgroup only: no Adjustment: little change	Data reported for an average of 86% of days in the year Similar results in those reporting at least 25 of 26 weeks
Dalsager et al., 2016	Denmark 2010-12	Prospective cohort	Who: children Ages: 0-3 N: 359	Selection: all pregnant women living in Odense from 2010-12 were invited Participation: <43% Equal groups: PFOA higher in nulliparous, younger maternal age, decreased education, higher BMI, older child and male child Blinded: unclear Above detection: unclear, but LOD was low (0.03 ng/ml) Levels: upper tertile range = 2.04-10.12 ng/ml	Maternal serum Gestation weeks 10-16	Infection: gastro-enteritis (diarrhea, vomiting)	Mobile phone question-naires on symptoms every 2 weeks for one year Ages 1-3 Proportion of days with symptoms of infectious disease	No association	Upper vs. lower tertile of PFOA	Adjusted for maternal age, maternal education, parity, and child's age Additional adjustments for duration of breast-feeding, day-care, maternal smoking, and child's sex had little effect 3% of mothers smoked during pregnancy	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustment: little change	Data reported for an average of 86% of days in the year Similar results in those reporting at least 25 of 26 weeks

Author year	Location Years	Design	Who Ages N	Factors related to bias	Exposure method	Outcome	Outcome method	Results	Comparison	Confounding	Other aspects of causal inference	Notes
Impinen et al., 2019	Norway 1999-2008	Prospective cohort	Who: children Ages: 0-7 N: 1,207 at ages 0-3 and 921 at ages 6-7 (same children)	Selection: all pregnant women scheduled for ultrasound Participation: 63% Equal groups: unclear Blinded: unclear Above detection: 100% Levels: median (IQR) = 2.54 (1.86-3.30) ng/ml	Maternal serum, median 18 weeks	Infection: gastro-enteritis (diarrhea, gastric flu)	Self-reports	OR = 1.48 (1.31-1.67) for ages 6-7, near 1.0 for ages 0-3	OR for one IQR increase in PFOA	Adjusted for maternal age, BMI, education, and smoking Adjusting for nursey school attendance had little impact Stratified by gender	Large magnitude: yes Statistical significance: yes Dose-response: no Temporal association: yes Subgroup only: older age group Adjustments: not given	Samples shipped at ambient temperature Cohort members were participants in pre-eclampsia and subfecundity case-control studies Multiple comparisons: many different outcomes assessed Some loss to follow-up
Impinen et al., 2019	Norway 1999-2008	Prospective cohort	Who: children Ages: 0-7 N: 1,207 at ages 0-3 and 921 at ages 6-7 (same children)	Selection: all pregnant women scheduled for ultrasound Participation: 63% Equal groups: unclear Blinded: unclear Above detection: 100% Levels: median (IQR) = 2.54 (1.86-3.30) ng/ml	Maternal serum, median 18 weeks	Infection: lower respiratory tract infections	Self-reports	OR = 1.27 (1.12-1.43) for ages 0-3, near 1.0 for ages 6-7	OR for one IQR increase in PFOA	Adjusted for maternal age, BMI, education, and smoking Adjusting for nursey school attendance had little impact Stratified by gender	Large magnitude: yes Statistical significance: yes Dose-response: no Temporal association: yes Subgroup only: younger age group Adjustments: not given	Samples shipped at ambient temperature Cohort members were participants in pre-eclampsia and subfecundity case-control studies Multiple comparisons: many different outcomes assessed Some loss to follow-up
Impinen et al., 2018	Oslo 1992-93	Prospective cohort	Who: children Ages: 0-10 N: 641	Selection: unclear Participation: unclear Equal groups: unclear Blinded: unclear Above detection: >96% Levels: median (IQR) = 1.6 (1.2-2.1) ng/ml	Cord blood	Infection: lower respiratory tract infections	Parent interview, clinical exam, and spirometry Ages 0-10	β = 0.28 (0.22-0.35, p <0.001)	Number of infections age 0-10 per log2 PFOA	Adjusted for sex Birth weight, birth month, breast-feeding, maternal smoking, other smoking, parental asthma and allergies, parental education, and household income not “statistically significant”	Large magnitude: unclear Statistical significance: yes Dose-response: linear Temporal association: yes Subgroup only: no Adjustments: unclear	At age 2, subjects included cases with bronchial obstruction and controls; details of selection and recruitment not provided Details of selection and recruitment of subjects at age 10 also not provided Bonferroni correction applied to p-values
Kvalem et al., 2020	Oslo, Norway	Prospective cohort and cross-sectional	Who: children Ages: 10 and 16 (age at interview) N: 378	Selection: newborns 1992-3 in Oslo recruited into the original study Participation: unclear but less than 10-15% Equal groups: unclear Blinded: unclear Above detection: 100% Levels: median (IQR) = 4.36 (1.77) ng/ml	Serum, age 10	Infection: lower respiratory tract infections	Questionnaire: parent reported bronchitis or pneumonia at ages 10 and 16	ORs = 1.49 (1.15-1.92) in girls for ages 10 and 16 ORs closer to 1.0 in boys	Per IQR increase in PFOA	Adjusted for BMI, puberty, maternal education, and activity	Large magnitude: yes Statistical significance: yes Dose-response: no Temporal association: yes Subgroup only: girls Adjustments: little change	F/U: 6 years Environment and Childhood Asthma study Multiple comparisons: many different outcomes and PFAS assessed
Manzano-Salgado et al., 2019	Spain 2003-8	Prospective cohort	Who: children Ages: 1.5-7 N: 1,071-1,188	Selection: giving birth in a participating hospital Participation: unclear Equal groups: unclear Blinded: unclear Above detection: 100% Levels: median (IQR) = 2.35 (1.63-3.30) ng/ml	Maternal plasma, 1st trimester	Infection: lower respiratory tract infections	ISAAC question-naire at ages 1.5, 4 and 7	No association overall and in boys and girls, some decrease at age 7	Change in the outcome for a doubling of PFOA	Adjusted for maternal age, parity, breast-feeding, pre-pregnancy BMI, region, and country Little change when adjusted for other PFAS, fish consumption, smoking and maternal education	Large magnitude: yes, age 7 (OR ≈ 0.65) Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: little change	Spanish INMA birth cohort Samples stored at -80º C

Author year	Location Years	Design	Who Ages N	Factors related to bias	Exposure method	Outcome	Outcome method	Results	Comparison	Confounding	Other aspects of causal inference	Notes
Honda-Kohmo et al., 2019	West Virginia and Ohio 2005-6	Cross-sectional	Who: adults Ages: ≥20 N: 5,270	Selection: unclear Participation: unclear Equal groups: unclear Blinded: unclear Above detection: >90% Levels: mean (SD) = 29.0 (12.7-72.8) ng/ml	Serum	Other: CRP	Serum	OR = 1.05 (1.01-1.09) in those without diabetes Similar result for those with diabetes but wider confidence interval	Per lognormal increase	Unclear	Large magnitude: no Statistical significance: yes Dose-response: no Temporal association: no Subgroup only: no Adjustments: not given	C8 Health Project
Matilla-Santander et al., 2017	Spain 2003-08	Cross-sectional	Who: pregnant women Ages: first trimester N: 651	Selection: "population-based" Participation: 55% Equal groups: unclear Above detection: 100% Blinded: unclear Levels: median (IQR) = 2.35 (1.63-3.30) ng/ml	Plasma	Other: CRP	Serum	No association	Quartiles of PFOA and continuous	Adjusted for sub-cohort, country, BMI, breast-feeding, parity, gestation week, physical activity, and diet	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: little change	
Stein et al., 2016a	New York 2010-11	Cross-sectional	Who: adults Ages: 18-49 N: 75	Selection: unclear Participation: unclear Equal groups: yes although analyses limited Blinded: yes Above detection: 100% Levels: geometric mean = 2.28, upper tertile range = 2.8-8.1 ng/ml	Serum	Other: cytokines	Serum or nasal secretion	No association with IFN-α2, IFN-gamma, TNF-α, IP1-), MCP-1, MIP-1a, G-CSF, IP-10, or mIgA	Upper vs. lower tertile of PFOA	Adjusted for age, sex, and race/ethnicity "No covariates… associated with both exposure and outcome"	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: unclear	See original article for cytokine abbreviations

Rows are sorted by outcome then by first author
Data in parentheses are 95% confidence intervals unless otherwise stated
Ages are in years unless otherwise noted
Abraham et al., 2020 is reviewed below
Abbreviations: β, regression coefficient; BMI, body mass index; CRP, C-reactive protein; ETS, environmental tobacco smoke; HAI, hemagglutination-inhibition; IQR, interquartile range; IHC, histochemical staining; ISAAC, International Study of Asthma and Allergies in Childhood; LOD, limit of detection; MeDALL, Mechanism of Development of Asthma; N, number of participants; NHANES, National Health and Nutrition Examination Survey; OR, odds ratio; PCBs, polychlorinated biphenyls; SES, socioeconomic status

Table A7.4. Recent epidemiologic studies of PFOS and immunotoxicity

Author year	Location Years	Design	Who Ages N	Factors related to bias	Exposure method	Outcome	Outcome method	Results	Comparison	Confounding	Other aspects of causal inference	Notes
Grandjean et al., 2017a	Faroe Islands 2007-09 (year of birth)	Prospective cohort and cross-sectional	Who: children Ages: ≤5 N: 349	Selection: unclear Participation: unclear Equal groups: unclear Blinded: unclear Above detection: unclear Levels: median (IQR) = 7.1 (4.5-10.0) ng/ml at age 18 months	Serum Near birth, 18 months, age 5	Antibody response: diphtheria	Diphtheria IgG Age 5	**2007-09 cohort:** PFOS IgG %change Birth -14.0 (p=0.20) 18 mo. 17.6 (p=0.06) 5 yr. 17.2 (p=0.21) **Combined cohort:** PFOS IgG %change Birth -24.5 (p=0.002) 18 mo. 15.1 (p=0.10) 5 yr. -1.3 (p=0.88)	Percent change for a 2-fold increase in PFOS	Adjusted for age and sex Additional adjustments for PCBs and Cesarean section had little effect	Large magnitude: yes Statistical significance: yes Dose-response: linear Temporal association: yes Subgroup only: no Adjustments: unclear	PFAS concentrations highly correlated with breast-feeding duration Correlation coefficients up to 0.7 for age 18 and age 5 PFAS levels 44% and 36% had IgG below protective levels for diphtheria and tetanus at age 5, respectively Combined: 2007-09 and 1997-2000 Faroe Islands cohorts combined
Grandjean et al., 2017b	Faroe Islands 1997-2000 (year of birth)	Prospective cohort and cross-sectional	Who: children Ages: ≤13 N: 275-349	Selection: unclear Participation: unclear Equal groups: unclear Blinded: unclear Above detection: unclear Levels: median (IQR) = 15.3 (12.4-19.0) ng/ml	Serum Ages 7 and 13	Antibody response: diphtheria	Diphtheria IgG Age 13	PFOS IgG %change Age 7 -25.7 (p=0.06) Age 13 -10.5 (p=0.37)	Percent change for a 2-fold increase in PFOS	Adjusted for age, sex, booster type at age 5 Additional adjustment for PCBs had little effect	Large magnitude: yes Statistical significance: borderline Dose-response: linear Temporal association: no Subgroup only: no Adjustments: unclear	PFAS concentrations at age 13 correlated with those at age 7 but not with maternal levels Excludes those with likely booster after age 5
Stein et al., 2016a	New York 2010-11	Prospective cohort	Who: adults Ages: 18-49 N: 75	Selection: unclear Participation: unclear Equal groups: yes although analyses limited Blinded: yes Above detection: 100% Levels: geometric mean = 5.22 ng/ml, upper tertile range = 7.2-21.4 ng/ml	Serum Day 0	Antibody response: FluMist	Anti-A H1N1 antibody response by histochemical staining (IHC) and hemagglutination-inhibition (HAI) Day 30	OR = 1.3 (0.2-7.3) p-trend=0.81 for HAI OR = 2.4 (0.9-6.6) p-trend=0.12 for IHC	Upper vs. lower tertile of PFOS	Adjusted for age, sex, and race/ethnicity "No covariates… associated with both exposure and outcome"	Large magnitude: yes Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: unclear	Less than 20 subjects seroconverted
Pilkerton et al., 2018	US NHANES 1999-2000, 2003-04	Cross-sectional	Who: children and adults Ages: ≥12 N: 2,389	Selection: multi-stage cluster sampling Participation: unclear Equal groups: unclear Blinded: likely Above detection: unclear but low LOD Levels: mean = 22-28 ng/ml	Serum	Antibody response: rubella	Serum IgG	4th vs. 1st quartile: Men: β = 0.0086 (p=0.97) (β is negative in quartiles 2 and 3) Women: -0.1664 (p=0.73) Per quartile increase: All adults combined: F value = 3.44, p=0.03 Children: no association	Fourth vs. first quartile of PFOS; regression coefficient for per quartile increase in PFOS	Adjusted for age, BMI, education, sex, and ethnicity, and parity	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: unclear	Results for children only and all adults combined presented only as F-values Gender specific analysis includes only adults

Author year	Location Years	Design	Who Ages N	Factors related to bias	Exposure method	Outcome	Outcome method	Results	Comparison	Confounding	Other aspects of causal inference	Notes
Grandjean et al., 2017a	Faroe Islands 2007-09 (year of birth)	Prospective cohort and cross-sectional	Who: children Ages: ≤5 N: 349	Selection: unclear Participation: unclear Equal groups: unclear Blinded: unclear Above detection: unclear Levels: median (IQR) = 7.1 (4.5-10.0) ng/ml at age 18 months	Serum Near birth, 18 months, age 5	Antibody response: tetanus	Tetanus IgG Age 5	**2007-09 cohort:** PFOS IgG %change Birth -10.8 (p=0.30) 18 mo. -7.0 (p=0.40) 5 yr. -9.1 (p=0.43) **Combined cohort:** PFOS IgG %change Birth -10.6 (p=0.20) 18 mo. -7.1 (p=0.39) 5 yr. -10.5 (p=0.18) No major differences between 1997-2000 and 2007-09 cohorts	Percent change for a 2-fold increase in PFOS	Adjusted for age and sex Additional adjustments for PCB concentrations and Cesarean section had little effect	Large magnitude: yes Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: unclear	PFAS concentrations highly correlated with breast-feeding duration Correlation coefficients up to 0.7 for age 18 and age 5 PFAS levels 44% and 36% had IgG below protective levels for diphtheria and tetanus at age 5, respectively Combined: 2007-09 and 1997-2000 Faroe Islands cohorts combined
Grandjean et al., 2017b	Faroe Islands 1997-2000 (year of birth)	Prospective cohort and cross-sectional	Who: children Ages: ≤13 N: 275-349	Selection: unclear Participation: unclear Equal groups: unclear Blinded: unclear Above detection: unclear Levels: median (IQR) = 15.3 (12.4-19.0) ng/ml	Serum Ages 7 and 13	Antibody response: tetanus	Tetanus IgG Age 13	PFOS IgG %change Age 7 2.7 (p=0.85) Age 13 14.8 (p=0.24)	Percent change for a 2-fold increase in PFOS	Adjusted for age, sex, booster type at age 5 Additional adjustment for PCBs had little effect	Large magnitude: yes Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: unclear	PFAS concentrations at age 13 correlated with those at age 7 but not with maternal levels Excludes those with likely booster after age 5
Hammer et al., 2019	Faroe Islands 1986-2009	Prospective cohort	Who: all children and adults Ages: all N: 5,698	Selection: involved in previous cohort studies Participation: 75% of all Faroese births during the recruitment periods Equal groups: unclear Blinded: unclear Above detection: not given Levels: unclear	Serum	Autoimmunity: inflammatory bowel disease	Nationwide Inflammatory Disease database	OR = 0.30 (0.08-1.07) N=37 cases	Upper vs. lower tertile of PFOS	Adjusted for age and calendar period	Large magnitude: yes (OR below 1.0) Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: not given	Includes six different cohorts and a wide range of follow-up periods
Xu et al., 2020	Ronneby, Sweden 1980-2013	Prospective cohort	Who: children and adults Ages: >10 N: 63,074	Selection: all residents of Ronneby and a nearby unexposed town from 1980-2013 Participation: likely close to 100% Equal groups: unclear Blinded: unclear Above detection: 100% Levels: serum median (IQR) = 216 (118-300) ng/ml in a subsample	Exposure to contaminated water based on address and water records	Autoimmunity: inflammatory bowel disease	Swedish National Patient Registry and death records	HR for Crohn's disease = 1.58 (1.00-2.49) for 1985-94 but not for the later years; near 1.0 for ulcerative colitis	Ever exposed to contaminate water source	Adjusted for calendar year, age, and gender	Large magnitude: yes Statistical significance: yes Dose-response: no – see notes Temporal association: yes Subgroup only: no Adjustments: not given	High environmental contamination of PFOS and PFHxS, moderate for PFOA; PFOS, PFOA, and PFHxS highly correlated

Author year	Location Years	Design	Who Ages N	Factors related to bias	Exposure method	Outcome	Outcome method	Results	Comparison	Confounding	Other aspects of causal inference	Notes
Goudarzi et al., 2016	Hokkaido, Japan 2003-09	Prospective cohort	Who: children Age: 4 N: 1,558	Selection: appears to be all pregnant women in 37 hospitals and clinics in Hokkaido, Japan Participation: <18.9% Equal groups: unclear Blinded: unclear Above detection: 100% Levels: median (IQR) = 4.925 (3.667-6.654) ng/ml	Maternal plasma Gestation weeks 28-32	Hyper-sensitivity: allergy (all)	ISAAC question-naire Age 4	No association Similar results in males and females	Fourth vs. first quartiles of PFOS	Adjusted for maternal age, siblings, maternal education, parental allergies, infant gender, breast-feeding, day-care attendance, and ETS Additional adjustments for household income, smoking, alcohol, BMI, pets, carpets, heating/cooling systems, and mold or dew condensation in homes had little effect 6.2% of mothers smoked during pregnancy	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: little change	Includes all allergic diseases and related symptoms Combines eczema, wheezing and rhinoconjunctivitis
Timmer-mann et al., 2017	Faroe Islands 1997-2000 (year of birth)	Prospective cohort and cross-sectional	Who: children Ages: 0-13 N: 559	Selection: unclear Participation: unclear Equal groups: see notes Blinded: unclear Above detection: 100% Levels: median (IQR) = 6.7 (5.2-8.5) ng/ml at age 13	Serum Third trimester, and ages 5 and 13	Hyper-sensitivity: allergy	Question-naire and skin prick test Ages 5 and 13	**PFOS in maternal serum:** Age OR 5 0.73 (0.38-1.41) 13 1.25 (0.75-2.11) **PFOS at age 5:** Age MMR OR 5 no 6.15 (0.77-49.2) 5 yes 0.80 (0.43-1.49) 13 NA 0.76 (0.49-1.18) **PFOS at age 13 and outcome at age 13:** OR = 0.90 (0.63-1.29) Age column refers to outcome age	OR for each 2-fold increase in PFOS	Adjusted for family history of eczema in children, allergic eczema, hay fever, maternal pre-pregnancy BMI, maternal smoking during pregnancy, sex, duration of breast-feeding, fish intake at age 5, number of siblings, and day-care attendance at age 5	Large magnitude: yes Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: unclear	Higher PFAS concentrations near birth and at age 5-7 PFAS concentrations related to breast-feeding, maternal BMI, maternal and paternal smoking, and fish consumption Stratified by MMR vaccination if p-interaction <0.2
Averina et al., 2019	Northern Norway 2010-13	Prospective cohort and cross-sectional	Who: children Ages: first year high school students N: 675	Selection: all students in eight high schools invited Participation: 60.4% Equal groups: unclear Blinded: unclear Above detection: unclear Levels: geometric mean (IQR) = 5.8 (2.7) ng/ml females, 6.8 (3.0) ng/ml males	Serum Age 16	Hyper-sensitivity: allergy (food)	Self-reported or FX5 food panel IgE Ages 16 (self-reported) and 18 (IgE)	No association	Unclear	Adjusted for sex, age, BMI, physical activity, SES, diet, and allergy medications	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: unclear	Authors report “no statistically significant association” but actual OR not given
Impinen et al., 2019	Norway 1999-2008	Prospective cohort	Who: children Ages: 0-7 N: 1,207 at ages 0-3 and 921 at ages 6-7 (same children)	Selection: all pregnant women scheduled for ultrasound Participation: 39% Equal groups: unclear Blinded: unclear Above detection: 100% Levels: median (IQR) = 12.87 (9.92-16.63) ng/ml	Maternal serum, median 18 weeks	Hyper-sensitivity: allergy (food)	Self-reports	ORs near 1.0, similar results in boys and girls	OR for one IQR increase in PFOS	Adjusted for maternal age, BMI, education, and smoking Adjusting for nursey school attendance had little impact Stratified by gender	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: not given	Samples shipped at ambient temperature Cohort members were participants in pre-eclampsia and subfecundity case-control studies Some loss to follow-up

Author year	Location Years	Design	Who Ages N	Factors related to bias	Exposure method	Outcome	Outcome method	Results	Comparison	Confounding	Other aspects of causal inference	Notes
Averina et al., 2019	Northern Norway 2010-13	Cross-sectional	Who: children Ages: first year high school students N: 675	Selection: all students in eight high schools invited Participation: 60.4% Equal groups: unclear Blinded: unclear Above detection: unclear Levels: geometric mean (IQR) = 5.8 (2.7) ng/ml females, 6.8 (3.0) ng/ml males	Serum Age 16	Hyper-sensitivity: allergy (nickel)	Self-reported	OR = 2.23 (1.14-4.35)	Fourth vs. first quartiles of PFOS	Sex and age	Large magnitude: yes Statistical significance: yes Dose-response: step-shaped Temporal association: no Subgroup only: no Adjustments: large change (see notes)	Unadjusted OR = 1.44 (0.76-2.71)
Averina et al., 2019	Northern Norway 2010-13	Prospective cohort and cross-sectional	Who: children Ages: first year high school students N: 675	Selection: all students in eight high schools invited Participation: 60.4% Equal groups: unclear Blinded: unclear Above detection: unclear Levels: geometric mean (IQR) = 5.8 (2.7) ng/ml females, 6.8 (3.0) ng/ml males	Serum Age 16	Hyper-sensitivity: asthma	MeDALL question-naire Ages 16 and 18	OR = 2.11 (1.02-4.37) cross-sectional OR = 1.23 (0.53-2.83) prospective	Fourth vs. first quartiles of PFOS	Adjusted for sex, age, BMI, physical activity, SES, and fish intake	Large magnitude: yes Statistical significance: yes Dose-response: inverted U Temporal association: no Subgroup only: no Adjustments: small change	Unadjusted OR = 2.37 cross-sectional
Beck et al., 2019	Odense, Denmark 2010-12	Prospective cohort	Who: children Ages: 5 N: 981	Selection: all pregnant women in the municipality Participation: <43% Equal groups: asthma or wheeze greater in boys, smoking parents, and family asthma Blinded: unclear Levels: median (IQR) = 7.73 (5.68-10.44) ng/ml	Maternal serum, weeks 8-16	Hyper-sensitivity: asthma	ISSAC question-naire Age 5	Doctor diagnosed asthma: Girls: 1.60 (0.46-5.59) Boys: 0.74 (0.46-1.20) Elevated OR for self-reported asthma in boys Wheeze: all ORs near 1.0	OR for a doubling of PFOS	Adjusted for parity, education, BMI, asthma predisposition, and sex Also stratified by sex	Large magnitude: yes Statistical significance: unclear Dose-response: unclear Temporal association: yes Subgroup only: inconsistent Adjustments: small changes only	Includes asthma and wheezing Samples stored at -80º C Self-reported asthma: only those who did not also report doctor diagnosed asthma Inconsistent results across genders and self-reports with doctor diagnosed
Gaylord et al., 2019	US 2014-16	Cross-sectional	Who: children and adults Ages: 13-22 N: 287	Selection: exposed to the World Trade Center disaster as children and controls Participation: unclear Equal groups: unclear Blinded: unclear Above detection: 100% Levels: mean (SD) = 3.45 (3.30) ng/ml	Serum	Hyper-sensitivity: asthma	Question-naire: similar to NHANES	No association	OR per unit change in log PFOS	Adjusted for sex, race/ethnicity, BMI, tobacco smoke exposure	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: no	

Author year	Location Years	Design	Who Ages N	Factors related to bias	Exposure method	Outcome	Outcome method	Results	Comparison	Confounding	Other aspects of causal inference	Notes
Goudarzi et al., 2016	Hokkaido, Japan 2003-09	Prospective cohort	Who: children Age: 4 N: 1,558	Selection: appears to be all pregnant women in 37 hospitals and clinics in Hokkaido, Japan Participation: <18.9% Equal groups: unclear Blinded: unclear Above detection: 100% Levels: median (IQR) = 4.925 (3.667-6.654) ng/ml	Maternal plasma Gestation weeks 28-32	Hyper-sensitivity: asthma	ISAAC question-naire Age 4	No association Similar results in males and females	Fourth vs. first quartiles of PFOS	Adjusted for maternal age, number of siblings, maternal education, parental allergies, infant gender, breast-feeding, day-care attendance, and ETS Additional adjustments for household income, smoking, alcohol, BMI, pets, carpets, heating/cooling systems, and mold or dew condensation in homes had little effect 6.2% of mothers smoked during pregnancy	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: little change	Includes asthma and wheezing Participants had higher maternal education, lower maternal smoking rates, and lower postnatal ETS rates than original cohort
Impinen et al., 2018	Oslo 1992-93	Prospective cohort	Who: children Ages: 0-10 N: 641	Selection: unclear Participation: unclear Equal groups: unclear Blinded: unclear Above detection: >96% Levels: median (IQR) = 5.2 (4.0-6.6) ng/ml	Cord blood	Hyper-sensitivity: asthma	Parent interview, clinical exam, and spirometry Ages 2 and 10	No association	OR for a doubling of PFOS	Adjusted for sex Birth weight, birth month, breast-feeding, maternal smoking, other smoking, parental asthma and allergies, parental education, and household income not “statistically significant”	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: unclear	At age 2, subjects included cases with bronchial obstruction and controls; details of selection and recruitment not provided Details of selection and recruitment of subjects at age 10 also not provided Bonferroni correction applied to p-values Similar results for wheeze
Impinen et al., 2019	Norway 1999-2008	Prospective cohort	Who: children Ages: 0-7 N: 1,207 at ages 0-3 and 921 at ages 6-7 (same children)	Selection: all pregnant women scheduled for ultrasound Participation: 39% Equal groups: unclear Blinded: unclear Above detection: 100% Levels: median (IQR) = 12.87 (9.92-16.63) ng/ml	Maternal serum, median 18 weeks	Hyper-sensitivity: asthma	Doctor diagnosed (asthma), self-reports (wheeze)	ORs for asthma and wheeze near 1.0, similar results in boys and girls	OR for one IQR increase in PFOS	Adjusted for maternal age, BMI, education, and smoking Adjusting for nursey school attendance had little impact Stratified by gender	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: not given	Samples shipped at ambient temperature Cohort members were participants in pre-eclampsia and subfecundity case-control studies Some loss to follow-up
Kvalem et al., 2020	Oslo, Norway	Prospective cohort and cross-sectional	Who: children Ages: 10 and 16 (age at interview) N: 378	Selection: newborns 1992-3 in Oslo recruited into the original study Participation: unclear but less than 10-15% Equal groups: unclear Blinded: unclear Above detection: 100% Levels: median (IQR) = 19.4 (9.23) ng/ml	Serum, age 10	Hyper-sensitivity: asthma	Questionnaire: based on symptoms, doctor’s diagnosis or medication use, up to age 10 (cross-sectional), between ages 10-16, or at age 16 (prospective)	All ORs near 1.0, similar results in boys and girls	Per IQR increase in PFOS	Adjusted for BMI, puberty, maternal education, and activity	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: little change	Includes asthma and wheezing F/U: 6 years Environment and Childhood Asthma study

Author year	Location Years	Design	Who Ages N	Factors related to bias	Exposure method	Outcome	Outcome method	Results	Comparison	Confounding	Other aspects of causal inference	Notes
Manzano-Salgado et al., 2019	Spain 2003-8	Prospective cohort	Who: children Ages: 1.5-7 N: 1,071-1,188	Selection: giving birth in a participating hospital Participation: unclear Equal groups: unclear Blinded: unclear Above detection: 100% Levels: median (IQR) = 6.06 (4.52-7.82) ng/ml	Maternal plasma, 1st trimester	Hyper-sensitivity: asthma	ISAAC question-naire at ages 1.5, 4 and 7	No clear associations, some ORs below 1.0 but inconsistent by outcome, gender, breast-feeding status, and age	Change in the outcome for a doubling of PFOS	Adjusted for maternal age, parity, breast-feeding, pre-pregnancy BMI, region, and country Little change when adjusted for other PFAS, fish consumption, smoking and maternal education	Large magnitude: inconsistent Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: little change	Includes asthma and wheezing Spanish INMA birth cohort Samples stored at -80º C
Qin et al., 2017	Taiwan 2009-10	Case-control (cross-sectional)	Who: children Ages: 10-15 N: 300	Selection: unclear Participation: <50% Equal groups: similar except higher ETS in non-asthmatics Blinded: unclear Above detection: >96% Levels: median (IQR) = 28.8 (12.4-42.0) ng/ml	Serum	Hyper-sensitivity: asthma	Physician diagnosed	OR = 1.30 (1.00-1.69)	For each IQR increase in PFOS	Adjusted for age, sex, BMI, education, exercise, ETS, and month of survey Matched on age and sex	Large magnitude: yes Statistical significance: borderline Dose-response: unclear Temporal association: no Subgroup only: no Adjustments: little change	Cases from two hospitals in northern Taiwan, controls randomly selected from seven public schools in northern Taiwan Some evidence of interaction with testosterone and estradiol levels (Zhou et al., 2017)
Timmer-mann et al., 2017	Faroe Islands 1997-2000 (year of birth)	Prospective cohort and cross-sectional	Who: children Ages: 0-13 N: 559	Selection: unclear Participation: unclear Equal groups: see notes Blinded: unclear Above detection: 100% Levels: median (IQR) = 6.7 (5.2-8.5) ng/ml at age 13	Serum Third trimester, and ages 5 and 13	Hyper-sensitivity: asthma	Question-naire Age 5	**PFOS in maternal serum:** Age OR 5 1.21 (0.64-2.29) 13 1.61 (0.84-3.08) **PFOS at age 5:** Age MMR OR 5 no 3.96 (0.55-28.4) 5 yes 0.98 (0.55-1.76) 13 no 5.41 (0.62-47.2) 13 yes 0.94 (0.51-1.74) **PFOS at age 13 and outcome at age 13:** OR = 0.63 (0.41-0.97) Age column refers to outcome age	OR for each 2-fold increase in PFOS	Adjusted for family history of eczema in children, allergic eczema, hay fever, maternal pre-pregnancy BMI, maternal smoking during pregnancy, sex, duration of breast-feeding, fish intake at age 5, number of siblings, and day-care attendance at age 5, birth weight, and family history of chronic bronchitis/asthma	Large magnitude: yes Statistical significance: yes Dose-response: linear Temporal association: no Subgroup only: no Adjustments: unclear	Higher PFAS concentrations near birth and at age 5-7 PFAS concentrations related to breast-feeding, maternal BMI, maternal and paternal smoking, and fish consumption Stratified by MMR vaccination if p-interaction <0.2
Zeng et al., 2019	Shangha i 2012-15	Prospective cohort	Who: children Ages: 0-5 N: 358	Selection: pregnancies at two participating hospitals Participation: <50% Equal groups: unclear Blinded: unclear Above detection: unclear Levels: median and IQR = 2.49 (1.81-3.51) boys and 2.38 (1.73-3.13) ng/ml girls	Cord plasma	Hyper-sensitivity: asthma	Pediatrician diagnosis and spirometry	Some ORs above and some below 1.0 based on gender but with very wide confidence intervals	Unclear	Adjusted for child weight, gestational age, breast-feeding, maternal education and BMI, and income	Large magnitude: see results Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: not given	F/U: 5 years Includes asthma and wheezing Samples stored at -80º C PFOA levels higher than PFOS levels

Author year	Location Years	Design	Who Ages N	Factors related to bias	Exposure method	Outcome	Outcome method	Results	Comparison	Confounding	Other aspects of causal inference	Notes
Averina et al., 2019	Northern Norway 2010-13	Cross-sectional	Who: children Ages: first year high school students N: 675	Selection: all students in eight high schools invited Participation: 60.4% Equal groups: unclear Blinded: unclear Above detection: unclear Levels: geometric mean (IQR) = 5.8 (2.7) ng/ml females, 6.8 (3.0) ng/ml males	Serum	Hyper-sensitivity: eczema	Self-reported doctor diagnosed	No association	Unclear	Unclear	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: unclear	Few details provided Authors report “no statistically significant association” but actual OR not given
Chen et al., 2018	Shanghai 2012-15	Prospective cohort	Who: children Ages: 0-24 months N: 687	Selection: unclear Participation: 65.1% among those enrolled Equal groups: Similar except allergic family history, nulliparous, and breast-feeding greater in eczema cases vs. non-cases Blinded: unclear Above detection: 100% Levels: fourth quartile ≥3.22 ng/ml	Cord blood	Hyper-sensitivity: eczema	ISAAC question-naire Ages 6, 12, and 24 months	Females: no association Males: no association	Fourth vs. first quartile of PFOS	Adjusted for maternal age, maternal BMI, gestation week at delivery, birth weight, maternal and paternal education, mode of delivery, family medical history, family income, ethnicity, paternal smoking, and breast-feeding Less than 2% of mothers drank alcohol or smoked	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: little change	25.2% of children developed atopic dermatitis by 24 months of age 75.4% delivered by Caesarian section
Goudarzi et al., 2016	Hokkaido, Japan 2003-09	Prospective cohort	Who: children Age: 4 N: 1,558	Selection: appears to be all pregnant women in 37 hospitals and clinics in Hokkaido, Japan Participation: <18.9% Equal groups: unclear Blinded: unclear Above detection: 100% Levels: median (IQR) = 4.925 (3.667-6.654) ng/ml	Maternal plasma Gestation weeks 28-32	Hyper-sensitivity: eczema	ISAAC question-naire Age 4	No association	Fourth vs. first quartiles of PFOS	Adjusted for maternal age, siblings, maternal education, parental allergies, infant gender, breast-feeding, day-care attendance, and ETS Additional adjustments for household income, smoking, alcohol, BMI, pets, carpets, heating/cooling systems, and mold or dew condensation in homes had little effect 6.2% of mothers smoked during pregnancy	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: little change	Similar results in males and females Participants had higher maternal education, lower maternal smoking rates, and lower postnatal ETS rates than original cohort
Impinen et al., 2018	Oslo 1992-93	Prospective cohort	Who: children Ages: 0-10 N: 641	Selection: unclear Participation: unclear Equal groups: unclear Blinded: unclear Above detection: >96% Levels: median (IQR) = 5.2 (4.0-6.6) ng/ml	Cord blood	Hyper-sensitivity: eczema	Parent interview and clinical exam Ages 2 and 10	No association	OR for a doubling of PFOS	Adjusted for sex Birth weight, birth month, breast-feeding, maternal smoking, other smoking, parental asthma and allergies, parental education, and household income not “statistically significant”	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: unclear	At age 2, subjects included cases with bronchial obstruction and controls; details of selection and recruitment not provided Details of selection and recruitment of subjects at age 10 also not provided Bonferroni correction applied to p-values

Author year	Location Years	Design	Who Ages N	Factors related to bias	Exposure method	Outcome	Outcome method	Results	Comparison	Confounding	Other aspects of causal inference	Notes
Impinen et al., 2019	Norway 1999-2008	Prospective cohort	Who: children Ages: 0-7 N: 1,207 at ages 0-3 and 921 at ages 6-7 (same children)	Selection: all pregnant women scheduled for ultrasound Participation: 39% Equal groups: unclear Blinded: unclear Above detection: 100% Levels: median (IQR) = 12.87 (9.92-16.63) ng/ml	Maternal serum, median 18 weeks	Hyper-sensitivity: eczema	Doctor diagnosed	ORs near 1.0, similar results in boys and girls	OR for one IQR increase in PFOS	Adjusted for maternal age, BMI, education, and smoking Adjusting for nursey school attendance had little impact Stratified by gender	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: not given	Samples shipped at ambient temperature Cohort members were participants in pre-eclampsia and subfecundity case-control studies Some loss to follow-up
Kvalem et al., 2020	Oslo, Norway	Prospective cohort and cross-sectional	Who: children Ages: 10 and 16 (age at interview) N: 378	Selection: newborns 1992-3 in Oslo recruited into the original study Participation: unclear but less than 10-15% Equal groups: unclear Blinded: unclear Above detection: 100% Levels: median (IQR) = 19.4 (9.23) ng/ml	Serum, age 10	Hyper-sensitivity: eczema	Questionnaire: based on symptoms, doctor's diagnosis or medication use, up to age 10 (cross-sectional), between ages 10-16, or at age 16 (prospective)	All ORs near or below 1.0, similar results in boys and girls	Per IQR increase in PFOS	Adjusted for BMI, puberty, maternal education, and activity	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: ORs lower after adjustments	F/U: 6 years Environment and Childhood Asthma study
Manzano-Salgado et al., 2019	Spain 2003-8	Prospective cohort	Who: children Ages: 1.5-7 N: 1,071-1,188	Selection: giving birth in a participating hospital Participation: unclear Equal groups: unclear Blinded: unclear Above detection: 100% Levels: median (IQR) = 6.06 (4.52-7.82) ng/ml	Maternal plasma, 1st trimester	Hyper-sensitivity: eczema	ISAAC question-naire at ages 1.5, 4 and 7	OR = 0.86 (0.75-0.98); greatest effects at ages 4 and 7, and in girls (OR = 0.77 (0.64-0.94)) and with never breast-feeding (OR = 0.44 (0.25-0.78))	Change in the outcome for a doubling of PFOS	Adjusted for maternal age, parity, breast-feeding, pre-pregnancy BMI, region, and country Little change when adjusted for other PFAS, fish consumption, smoking and maternal education	Large magnitude: yes (ORs below 1.0) Statistical significance: yes Dose-response: no Temporal association: yes Subgroup only: yes Adjustments: little change	Spanish INMA birth cohort Samples stored at -80º C
Timmer-mann et al., 2017	Faroe Islands 1997-2000 (year of birth)	Prospective cohort and cross-sectional	Who: children Ages: 0-13 N: 559	Selection: unclear Participation: unclear Equal groups: see notes Blinded: unclear Above detection: 100% Levels: median (IQR) = 6.7 (5.2-8.5) ng/ml at age 13	Serum Third trimester, and ages 5 and 13	Hyper-sensitivity: eczema	ISAAC question-naire Age 13	No association for exposure prenatally or age 5 Age 13 exposure without MMR vaccine: OR = 8.94 (0.27-299)	OR for each 2-fold increase in PFOS	Adjusted for family history of eczema in children, allergic eczema, hay fever, maternal pre-pregnancy BMI, maternal smoking during pregnancy, sex, duration of breast-feeding, fish intake at age 5, number of siblings, and day-care attendance at age 5	Large magnitude: yes Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: unclear	Higher PFAS concentrations near birth and at age 5-7 PFAS concentrations related to breast-feeding, maternal BMI, maternal and paternal smoking, and fish consumption Very wide confidence intervals

Author year	Location Years	Design	Who Ages N	Factors related to bias	Exposure method	Outcome	Outcome method	Results	Comparison	Confounding	Other aspects of causal inference	Notes
Wen et al., 2019a	Taiwan 2001-5	Prospective cohort	Who: children Ages: 5 N: 836	Selection: prenatal exams at participating hospital Participation: unclear Equal groups: atopic dermatitis was associated with breast-feeding, parental education, and atopy Blinded: unclear Above detection: 89.57% Levels: median (IQR) = 2.49 (2.18-5.05) ng/ml	Cord plasma	Hyper-sensitivity: eczema	ISAAC question-naire	HR = 1.43 (0.82-2.43)	Above vs. below the upper quartile of PFOS	Adjusted for sex, parental education and atopy, breast-feeding, and maternal age at childbirth	Large magnitude: yes Statistical significance: no Dose-response: no Temporal association: yes Subgroup only: no Adjustments: little change	F/U: 5 years Prevalence of atopic dermatitis was 7.1%
Averina et al., 2019	Northern Norway 2010-13	Prospective cohort	Who: children Ages: first year high school students N: 675	Selection: all students in eight high schools invited Participation: 60.4% Equal groups: unclear Blinded: unclear Above detection: unclear Levels: geometric mean (IQR) = 5.8 (2.7) ng/ml females, 6.8 (3.0) ng/ml males	Serum Age 16	Hyper-sensitivity: IgE	Serum Age 18	No association	Unclear	Unclear	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: unclear	Few details provided Authors report “no statistically significant association” but actual OR not given
Timmer-mann et al., 2017	Faroe Islands 1997-2000 (year of birth)	Prospective cohort and cross-sectional	Who: children Ages: 0-13 N: 559	Selection: unclear Participation: unclear Equal groups: see notes Blinded: unclear Above detection: 100% Levels: median (IQR) = 6.7 (5.2-8.5) ng/ml at age 13	Serum Third trimester, and ages 5 and 13	Hyper-sensitivity: IgE	Serum Cord blood and age 7	No association	Percent difference for each 2-fold increase in PFOS	Adjusted for family history of eczema in children, allergic eczema, hay fever, maternal pre-pregnancy BMI, maternal smoking during pregnancy, sex, duration of breast-feeding, fish intake at age 5, number of siblings, and day-care attendance at age 5	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: unclear	Higher PFAS concentrations near birth and at age 5-7 PFAS concentrations related to breast-feeding, maternal BMI, maternal and paternal smoking, and fish consumption
Zeng et al., 2019	Shangha i 2012-15	Prospective cohort	Who: children Ages: 5 N: 358	Selection: pregnancies at two participating hospitals Participation: <50% Equal groups: unclear Blinded: unclear Above detection: unclear Levels: median and IQR = 2.49 (1.81-3.51) ng/ml boys, 2.38 (1.73-3.13) ng/ml girls	Cord plasma	Hyper-sensitivity: IgE Age 5	Serum	Some ORs above and some below 1.0 based on gender and exposure levels but with very wide confidence intervals	Unclear	Adjusted for child weight, gestational age, breast-feeding, maternal education and BMI, and income	Large magnitude: see results Statistical significance: no Dose-response: U-shaped Temporal association: no Subgroup only: see results Adjustments: not given	F/U: 5 years Samples stored at -80º C PFOA levels higher than PFOS levels

Author year	Location Years	Design	Who Ages N	Factors related to bias	Exposure method	Outcome	Outcome method	Results	Comparison	Confounding	Other aspects of causal inference	Notes
Averina et al., 2019	Northern Norway 2010-13	Prospective cohort and cross-sectional	Who: children Ages: first year high school students N: 675	Selection: all students in eight high schools invited Participation: 60.4% Equal groups: unclear Blinded: unclear Above detection: unclear Levels: geometric mean (IQR) = 5.8 (2.7) ng/ml females, 6.8 (3.0) ng/ml males	Serum Age 16	Hyper-sensitivity: rhinitis	Self-reported doctor diagnosed Ages 16 and 18	No association	Unclear	Unclear	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: unclear	Outcome is allergic rhinitis Few details provided OR not given
Dalsager et al., 2016	Denmark 2010-12	Prospective cohort	Who: children Ages: 0-3 N: 359	Selection: all pregnant women living in Odense from 2010-12 were invited Participation: <43% Equal groups: PFOS higher in nulliparous, younger maternal age, decreased education, higher BMI, older child and male child Blinded: unclear Above detection: unclear, but LOD was low (0.03 ng/ml) Levels: upper tertile range = 10.19-25.10 ng/ml	Maternal serum Gestation weeks 10-16	Hyper-sensitivity: rhinitis	Mobile phone question-naires on symptoms every 2 weeks for one year Ages 1-3 Proportion of days with symptoms of infectious disease	No association	Upper vs. lower tertile of PFOS	Adjusted for maternal age, maternal education, parity, and child's age Additional adjustments for duration of breast-feeding, day-care, maternal smoking, and child's sex had little effect 3% of mothers smoked during pregnancy	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustment: little change	
Goudarzi et al., 2016	Hokkaido, Japan 2003-09	Prospective cohort	Who: children Age: 4 N: 1,558	Selection: appears to be all pregnant women in 37 hospitals and clinics in Hokkaido, Japan Participation: <18.9% Equal groups: unclear Blinded: unclear Above detection: 100% Levels: median (IQR) = 4.925 (3.667-6.654) ng/ml	Maternal plasma Gestation weeks 28-32	Hyper-sensitivity: rhinitis	ISAAC question-naire Age 4	No association Similar results in males and females	Fourth vs. first quartiles of PFOS	Adjusted for maternal age, siblings, maternal education, parental allergies, infant gender, breast-feeding, day-care attendance, and ETS Additional adjustments for household income, smoking, alcohol, BMI, pets, carpets, heating/cooling systems, and mold or dew condensation in homes had little effect 6.2% of mothers smoked during pregnancy	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: little change	Outcome is rhinoconjunctivitis Participants had higher maternal education, lower maternal smoking rates, and lower postnatal ETS rates than original cohort
Impinen et al., 2018	Oslo 1992-93	Prospective cohort	Who: children Ages: 0-10 N: 641	Selection: unclear Participation: unclear Equal groups: unclear Blinded: unclear Above detection: >96% Levels: median (IQR) = 5.2 (4.0-6.6) ng/ml	Cord blood	Hyper-sensitivity: rhinitis	Parent interview and clinical exam Ages 2 and 10	No association	OR for a doubling of PFOS	Adjusted for sex Birth weight, birth month, breast-feeding, maternal smoking, other smoking, parental asthma and allergies, parental education, and household income not "statistically significant"	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: unclear	At age 2, subjects included cases with bronchial obstruction and controls; details of selection and recruitment not provided Details of selection and recruitment of subjects at age 10 also not provided Bonferroni correction applied to p-values For reported symptoms and IgE

Author year	Location Years	Design	Who Ages N	Factors related to bias	Exposure method	Outcome	Outcome method	Results	Comparison	Confounding	Other aspects of causal inference	Notes
Impinen et al., 2019	Norway 1999-2008	Prospective cohort	Who: children Ages: 0-7 N: 1,207 at ages 0-3 and 921 at ages 6-7 (same children)	Selection: all pregnant women scheduled for ultrasound Participation: 39% Equal groups: unclear Blinded: unclear Above detection: 100% Levels: median (IQR) = 12.87 (9.92-16.63) ng/ml	Maternal serum, median 18 weeks	Hyper-sensitivity: rhinitis	Self-reports	ORs near 1.0, similar results in boys and girls	OR for one IQR increase in PFOS	Adjusted for maternal age, BMI, education, and smoking Adjusting for nursey school attendance had little impact Stratified by gender	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: not given	Samples shipped at ambient temperature Cohort members were participants in pre-eclampsia and subfecundity case-control studies Some loss to follow-up
Kvalem et al., 2020	Oslo, Norway	Prospective cohort and cross-sectional	Who: children Ages: 10 and 16 (age at interview) N: 378	Selection: newborns 1992-3 in Oslo recruited into the original study Participation: unclear but less than 10-15% Equal groups: unclear Blinded: unclear Above detection: 100% Levels: median (IQR) = 19.4 (9.23) ng/ml	Serum, age 10	Hyper-sensitivity: rhinitis	Questionnaire: based on parent reported symptoms at age 10 or 16	All ORs near 1.0, similar results in boys and girls	Per IQR increase in PFOS	Adjusted for BMI, puberty, maternal education, and activity	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: little change	F/U: 6 years Environment and Childhood Asthma study
Timmer-mann et al., 2017	Faroe Islands 1997-2000 (year of birth)	Prospective cohort and cross-sectional	Who: children Ages: 0-13 N: 559	Selection: unclear Participation: unclear Equal groups: see notes Blinded: unclear Above detection: 100% Levels: median (IQR) = 6.7 (5.2-8.5) ng/ml at age 13	Serum Third trimester, and ages 5 and 13	Hyper-sensitivity: rhinitis	ISAAC question-naire Age 13	No association for exposure prenatally or age 5 or 13	OR for each 2-fold increase in PFOS	Adjusted for family history of eczema in children, allergic eczema, hay fever, maternal pre-pregnancy BMI, maternal smoking during pregnancy, sex, duration of breast-feeding, fish intake at age 5, number of siblings, and day-care attendance at age 5	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: unclear	Outcome is rhinoconjunctivitis Higher PFAS concentrations near birth and at age 5-7 PFAS concentrations related to breast-feeding, maternal BMI, maternal and paternal smoking, and fish consumption
Goudarzi et al., 2017	Hokkaido, Japan 2003-09	Prospective cohort	Who: children Age: 4 N: 1,558	Selection: appears to be all pregnant women in 37 hospitals and clinics in Hokkaido, Japan Participation: <18.9% Equal groups: unclear Blinded: unclear Above detection: 100% Levels: median (IQR) = 4.925 (3.667-6.654) ng/ml	Maternal plasma Gestation weeks 28-32	Infection: any	Self-reported doctor diagnosed otitis media, pneumonia, varicella, or respiratory syncytial virus Age 4	OR = 1.61 (1.18-2.21)	Fourth vs. first quartiles of PFOS	Adjusted for maternal age, siblings, maternal education, breast-feeding, and smoking Additional adjustment or stratification for day-care attendance, ETS, pets, carpets, heating/cooling systems, presence of mold or dew condensation in home had little effect	Large magnitude: yes Statistical significance: yes Dose-response: step-shaped Temporal association: yes Subgroup only: no Adjustments: unclear	Similar results in males and females Participants had higher maternal education and lower maternal smoking rates than original cohort
Impinen et al., 2018	Oslo 1992-93	Prospective cohort	Who: children Ages: 0-10 N: 641	Selection: unclear Participation: unclear Equal groups: unclear Blinded: unclear Above detection: >96% Levels: median (IQR) = 5.2 (4.0-6.6) ng/ml	Cord blood	Infection: colds	Parent interview, clinical exam, and spirometry Ages 0-2	No association	Number of colds ages 0-10 per log2 PFOS	Adjusted for sex Birth weight, birth month, breast-feeding, maternal smoking, other smoking, parental asthma and allergies, parental education, and household income not “statistically significant”	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: unclear	At age 2, subjects included cases with bronchial obstruction and controls; details of selection and recruitment not provided Details of selection and recruitment of subjects at age 10 also not provided

Author year	Location Years	Design	Who Ages N	Factors related to bias	Exposure method	Outcome	Outcome method	Results	Comparison	Confounding	Other aspects of causal inference	Notes
Impinen et al., 2019	Norway 1999-2008	Prospective cohort	Who: children Ages: 0-3 N: 1,207	Selection: all pregnant women scheduled for ultrasound Participation: 63% Equal groups: unclear Blinded: unclear Above detection: 100% Levels: median (IQR) = 12.87 (9.92-16.63) ng/ml	Maternal serum, median 18 weeks	Infection: colds	Self-reports	OR below 1.0	OR for one IQR increase in PFOS	Adjusted for maternal age, BMI, education, and smoking Adjusting for nursey school attendance had little impact Stratified by gender	Large magnitude: no Statistical significance: no (below 1.0) Dose-response: no Temporal association: no Subgroup only: no Adjustments: not given	Samples shipped at ambient temperature Cohort members were participants in pre-eclampsia and subfecundity case-control studies Multiple comparisons: many different outcomes assessed Some loss to follow-up
Kvalem et al., 2020	Oslo, Norway	Prospective cohort and cross-sectional	Who: children Ages: 10 and 16 (age at interview) N: 378	Selection: newborns 1992-3 in Oslo recruited into the original study Participation: unclear but less than 10-15% Equal groups: unclear Blinded: unclear Above detection: 100% Levels: median (IQR) = 19.4 (9.23) ng/ml	Serum, age 10	Infection: colds	Questionnaire: based on parent reported symptoms at age 10 or 16	Some ORs above and some below 1.0 but very inconsistent by age and gender or very wide confidence intervals	Per IQR increase in PFOS	Adjusted for BMI, puberty, maternal education, and activity	Large magnitude: see results Statistical significance: see results Dose-response: no Temporal association: see results Subgroup only: see results Adjustments: some moderate changes	F/U: 6 years Environment and Childhood Asthma study Multiple comparisons: many different outcomes and PFAS assessed
Dalsager et al., 2016	Denmark 2010-12	Prospective cohort	Who: children Ages: 0-3 N: 359	Selection: all pregnant women living in Odense from 2010-12 were invited Participation: <43% Equal groups: PFOS higher in nulliparous, younger maternal age, decreased education, higher BMI, older child and male child Blinded: unclear Above detection: unclear, but LOD was low (0.03 ng/ml) Levels: upper tertile range = 10.19-25.10 ng/ml	Maternal serum Gestation weeks 10-16	Infection: cough	Mobile phone question-naires on symptoms every 2 weeks for one year Ages 1-3 Proportion of days with symptoms of infectious disease	No association	Upper vs. lower tertile of PFOS	Adjusted for maternal age, maternal education, parity, and child's age Additional adjustments for duration of breast-feeding, day-care, maternal smoking, and child's sex had little effect 3% of mothers smoked during pregnancy	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustment: little change	Data reported for an average of 86% of days in the year Similar results in those reporting at least 25 of 26 weeks
Dalsager et al., 2016	Denmark 2010-12	Prospective cohort	Who: children Ages: 0-3 N: 359	Selection: all pregnant women living in Odense from 2010-12 were invited Participation: <43% Equal groups: PFOS higher in nulliparous, younger maternal age, decreased education, higher BMI, older child and male child Blinded: unclear Above detection: unclear, but LOD was low (0.03 ng/ml) Levels: upper tertile range = 10.19-25.10 ng/ml	Maternal serum Gestation weeks 10-16	Infection: fever	Mobile phone question-naires on symptoms every 2 weeks for one year Ages 1-3 Proportion of days with symptoms of infectious disease	OR = 2.35 (1.34-4.11)	Upper vs. lower tertile of PFOS	Adjusted for maternal age, maternal education, parity, and child's age Additional adjustments for duration of breast-feeding, day-care, maternal smoking, and child's sex had little effect 3% of mothers smoked during pregnancy	Large magnitude: yes Statistical significance: yes Dose-response: linear Temporal association: yes Subgroup only: no Adjustment: little change	Data reported for an average of 86% of days in the year Similar results in those reporting at least 25 of 26 weeks

Author year	Location Years	Design	Who Ages N	Factors related to bias	Exposure method	Outcome	Outcome method	Results	Comparison	Confounding	Other aspects of causal inference	Notes
Dalsager et al., 2016	Denmark 2010-12	Prospective cohort	Who: children Ages: 0-3 N: 359	Selection: all pregnant women living in Odense from 2010-12 were invited Participation: <43% Equal groups: PFOS higher in nulliparous, younger maternal age, decreased education, higher BMI, older child and male child Blinded: unclear Above detection: unclear, but LOD was low (0.03 ng/ml) Levels: upper tertile range = 10.19-25.10 ng/ml	Maternal serum Gestation weeks 10-16	Infection: gastro-enteritis (diarrhea, vomiting)	Mobile phone question-naires on symptoms every 2 weeks for one year Ages 1-3 Proportion of days with symptoms of infectious disease	No association	Upper vs. lower tertile of PFOS	Adjusted for maternal age, maternal education, parity, and child's age Additional adjustments for duration of breast-feeding, day-care, maternal smoking, and child's sex had little effect 3% of mothers smoked during pregnancy	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustment: little change	
Impinen et al., 2019	Norway 1999-2008	Prospective cohort	Who: children Ages: 0-7 N: 1,207 at ages 0-3 and 921 at ages 6-7 (same children)	Selection: all pregnant women scheduled for ultrasound Participation: 63% Equal groups: unclear Blinded: unclear Above detection: 100% Levels: median (IQR) = 12.87 (9.92-16.63) ng/ml	Maternal serum, median 18 weeks	Infection: gastro-enteritis (diarrhea, gastric flu)	Self-reports	OR = 1.12 (1.01-1.24) at ages 6-7, near 1.0 at ages 0-3	OR for one IQR increase in PFOS	Adjusted for maternal age, BMI, education, and smoking Adjusting for nursey school attendance had little impact Stratified by gender	Large magnitude: no Statistical significance: see notes Dose-response: no Temporal association: yes Subgroup only: older age group Adjustments: not given	Samples shipped at ambient temperature Cohort members were participants in pre-eclampsia and subfecundity case-control studies Multiple comparisons: many different outcomes assessed Some loss to follow-up Not statistically significant when adjusted for multiple comparisons
Impinen et al., 2018	Oslo 1992-93	Prospective cohort	Who: children Ages: 0-10 N: 641	Selection: unclear Participation: unclear Equal groups: unclear Blinded: unclear Above detection: >96% Levels: median (IQR) = 5.2 (4.0-6.6) ng/ml	Cord blood	Infection: lower respiratory tract infections	Parent interview, clinical exam, and spirometry Ages 0-10	β = 0.50 (0.42-0.57, p <0.001)	Number of infections ages 0-10 per log2 PFOS	Adjusted for sex Birth weight, birth month, breast-feeding, maternal smoking, other smoking, parental asthma and allergies, parental education, and household income not "statistically significant"	Large magnitude: unclear Statistical significance: yes Dose-response: linear Temporal association: yes Subgroup only: no Adjustments: unclear	At age 2, subjects included cases with bronchial obstruction and controls; details of selection and recruitment not provided Details of selection and recruitment of subjects at age 10 also not provided Bonferroni correction applied
Impinen et al., 2019	Norway 1999-2008	Prospective cohort	Who: children Ages: 0-7 N: 1,207 at ages 0-3 and 921 at ages 6-7 (same children)	Selection: all pregnant women scheduled for ultrasound Participation: 63% Equal groups: unclear Blinded: unclear Above detection: 100% Levels: median (IQR) = 12.87 (9.92-16.63) ng/ml	Maternal serum, median 18 weeks	Infection: lower respiratory tract infections	Self-reports	OR = 1.20 (1.07-1.34) for ages 0-3, near 1.0 for ages 6-7	OR for one IQR increase in PFOS	Adjusted for maternal age, BMI, education, and smoking Adjusting for nursey school attendance had little impact Stratified by gender	Large magnitude: borderline Statistical significance: yes Dose-response: no Temporal association: yes Subgroup only: younger age group Adjustments: not given	Samples shipped at ambient temperature Cohort members were participants in pre-eclampsia and subfecundity case-control studies Multiple comparisons: many different outcomes assessed Some loss to follow-up
Kvalem et al., 2020	Oslo, Norway	Prospective cohort and cross-sectional	Who: children Ages: 10 and 16 (age at interview) N: 378	Selection: newborns 1992-3 in Oslo recruited into the original study Participation: unclear but less than 10-15% Equal groups: unclear Blinded: unclear Above detection: 100% Levels: median (IQR) = 19.4 (9.23) ng/ml	Serum, age 10	Infection: lower respiratory tract infections	Questionnaire: based on parent reported bronchitis or pneumonia at age 10 or 16	OR = 1.34 (1.17-1.55) overall at ages 10 to 16 mostly in boys OR near 1.0 at age 16 (last 12 months)	Per IQR increase in PFOS	Adjusted for BMI, puberty, maternal education, and activity	Large magnitude: yes Statistical significance: yes Dose-response: no Temporal association: yes Subgroup only: higher in boys Adjustments: little change	F/U: 6 years Environment and Childhood Asthma study Inconsistent results by age Multiple comparisons: many different outcomes and PFAS assessed

Author year	Location Years	Design	Who Ages N	Factors related to bias	Exposure method	Outcome	Outcome method	Results	Comparison	Confounding	Other aspects of causal inference	Notes
Manzano-Salgado et al., 2019	Spain 2003-8	Prospective cohort	Who: children Ages: 1.5-7 N: 1,071-1,188	Selection: giving birth in a participating hospital Participation: unclear Equal groups: unclear Blinded: unclear Above detection: 100% Levels: median (IQR) = 6.06 (4.52-7.82) ng/ml	Maternal plasma, 1st trimester	Infection: lower respiratory tract infections	ISAAC question-naire at ages 1.5, 4 and 7	No association overall and by gender, some decrease with increasing age (OR = 0.83 (0.57-1.20) at age 7) and with never breast-feeding (OR = 0.66 (0.42-1.05))	Change in the outcome for a doubling of PFOS	Adjusted for maternal age, parity, breast-feeding, pre-pregnancy BMI, region, and country Little change when adjusted for other PFAS, fish consumption, smoking and maternal education	Large magnitude: yes (ORs below 1.0) Statistical significance: no Dose-response: no Temporal association: yes Subgroup only: yes Adjustments: little change	Spanish INMA birth cohort Samples stored at -80º C
Ammitz-boll et al., 2019	Denmark Years unknown	Cross-sectional	Who: adults Ages: unclear N: 79	Selection: unclear Participation: unclear Equal groups: PFOS increased with age and in men, decreased with years menstruation, number of children, and breast-feeding Blinded: unclear Levels: median (IQR) = 9.41 (6.41-13.05) ng/ml	Serum PFOS	Multiple immune cell types	Serum	Associations with CD27 and plasmablasts. In the CD4- CD8+ T cell category: associations with T central memory cells, T naïve, T effector memory, and CXCR3hi cells (all p <0.05)	Spearman correlation coefficients	Appears unadjusted	Large magnitude: yes Statistical significance: yes Dose-response: unclear Temporal association: no Subgroup only: no Adjustments: not given	May have included roughly 50% multiple sclerosis patients Results for PFOA not given Clinical relevance is unknown Multiple comparisons issues: 50 cell types tested
Honda-Kohmo et al., 2019	West Virginia and Ohio 2005-6	Cross-sectional	Who: adults Ages: ≥20 N: 5,270	Selection: unclear Participation: unclear Equal groups: unclear Blinded: unclear Above detection: >90% Levels: median (IQR) = 21.1 (13.7-31.3) ng/ml	Serum	Other: CRP	Serum	OR = 1.05 (1.01-1.09) in those without diabetes Similar results in those with diabetes but with wider confidence interval	Per lognormal increase	Unclear	Large magnitude: no Statistical significance: yes Dose-response: no Temporal association: no Subgroup only: no Adjustments: not given	C8 Health Project Same result as for PFOA
Matilla-Santan-der et al., 2017	Spain 2003-08	Cross-sectional	Who: pregnant women Ages: first trimester N: 1,240	Selection: "population-based" Participation: <55% Equal groups: unclear Above detection: 100% Blinded: unclear Levels: median (IQR) = 6.05 (4.51-7.81) ng/ml	Plasma	Other: CRP	Serum	No association	Quartiles of PFOS and continuous	Adjusted for sub-cohort, country, BMI, breast-feeding, parity, gestation week, physical activity, and diet	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: little change	
Stein et al., 2016a	New York 2010-11	Cross-sectional	Who: adults Ages 18-49 N: 75	Selection: unclear Participation: unclear Equal groups: yes although analyses limited Blinded: yes Above detection: 100% Levels: geometric mean = 5.22 ng/ml, upper tertile range = 7.2-21.4 ng/ml	Serum	Other: cytokines	Serum or nasal secretion	No association with IFN-α2, IFN-gamma, TNF-α, IP1-), MCP-1, MIP-1a, G-CSF, IP-10, or mIgA	Upper vs. lower tertile of PFOS	Adjusted for age, sex, and race/ethnicity "No covariates… associated with both exposure and outcome"	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: unclear	Less than 20 subjects seroconverted Please see original article for cytokine abbreviations

Rows are sorted by outcome then by first author
Data in parentheses are 95% confidence intervals unless otherwise stated
Ages are in years unless otherwise noted
Abraham et al., 2020 is reviewed below
Abbreviations: β, regression coefficient; BMI, body mass index; CRP, C-reactive protein; ETS, environmental tobacco smoke; HAI, hemagglutination-inhibition; IQR, interquartile range; IHC, histochemical staining; ISAAC, International Study of Asthma and Allergies in Childhood; LOD, limit of detection; MeDALL, Mechanism of Development of Asthma; N, number of participants; NA, given available; OR, odds ratio; PCBs, polychlorinated biphenyls; SES, socioeconomic status

Liver Toxicity

Literature search and methods

In addition to reviewing the results of previous reviews by the US EPA (US EPA, 2016b; 2016d), ATSDR (2018), and others, OEHHA searched for all new human epidemiologic studies on PFOA or PFOS and liver toxicity published since the 2016 US EPA reviews. Briefly, PubMed and Embase were searched for all human studies on PFOA or PFOS and liver toxicity published between December 2015 (the end of the US EPA literature searches) and September 20, 2020 (the end of OEHHA's literature search). The outcome portion of the search string used is shown below.

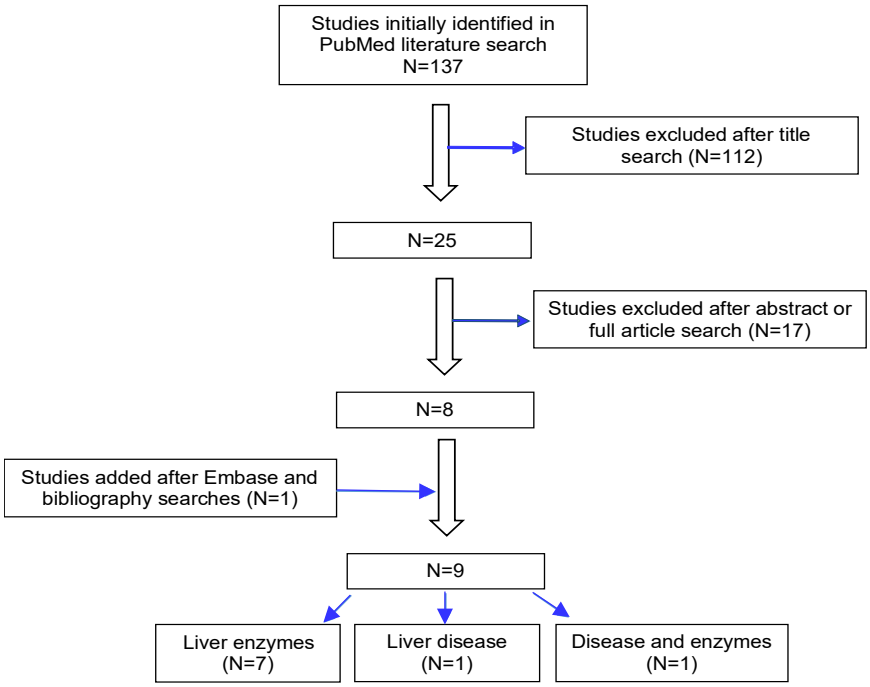
(liver[tiab] OR hepati[tiab] OR cirrhosis OR alanine transaminase OR ALT OR aspartate transaminase OR AST OR alkaline phosphatase OR ALP OR gamma-glutamyl transpeptidase OR GGT OR bilirubin*) NOT (mice[tiab] OR mouse[tiab] OR rat[tiab] OR rats[tiab] OR zebrafish[tiab])*

These key words were based on the outcomes that were assessed in the studies identified by US EPA (2016b and 2016b) and on the key words of articles OEHHA identified in a preliminary literature search.

Results

A general description of OEHHA's literature search is shown in Figure A7.2. A list of studies excluded based on OEHHA's abstract or full article review is provided in Table A7.28.

Figure A7.2. Literature search: recent epidemiologic studies of PFOA or PFOS and liver toxicity*



*This figure is provided to document OEHHA's PubMed, Embase, and bibliography literature searches. It does not include relevant publications identified from other sources such as previously published reviews from other agencies or other authors.

OEHHA identified nine studies of PFOA or PFOS and liver toxicity published since December 2015 or that were otherwise not included in the most recent US EPA (2016a; 2016b) reviews (Tables A7.5 and A7.6). Two of these provided only results for PFOA while the others provided information on both PFOA and PFOS. Five studies used a cross-sectional design, two used prospective cohort designs, one used a retrospective cohort design, and one involved both prospective and cross-sectional analyses. Six of these studies were done in the US, two were done in Europe, and one was done in Asia. One study involved highly exposed workers, two involved residents in highly exposed communities, and the remainder were general population studies with no known high exposure source. Seven studies reported information only on liver enzymes, one on liver disease mortality, and one study reported information on both liver enzymes and liver disease. Further details of the methods and results of these studies are shown in Tables A7.5 and A7.6.

Table A7.5. Recent epidemiologic studies on PFOA and liver toxicity

Author Year	Location Years	Design	Who Ages N	Factors related to bias	Exposure method	Outcome	Outcome method	Results	Compari-son	Confounding	Other aspects of causal inference	Notes
Girardi and Merler, 2019	Where: Trissino, Veneto, Italy Years: 1960-2018	Occupation-al Retro-spective cohort	Who: worked at least 1 day from 1948-2002 Ages: adults N: 462 chemical or office workers, 1,383 railroad workers	Selection: all male workers, at least 6 months, from 1960-2008 Participation: unclear Equal groups: unclear Blinded: unclear Above detection: not given Levels: geometric mean = 4.048 µg/ml	Job activities and tasks based on records and some interviews used to classify workers as ever, never exposed, or office workers. Jobs, years worked, and serum levels in a subsample (N=120 workers) used to model cumulative exposure	Liver cirrhosis mortality	Local and national death records	SMR = 1.71 (0.77-3.81) RR = 3.87 (1.18-12.7) N=6 exposed cases	Regional mortality rates (for SMRs), and local railroad workers (for RRs); tertiles of cumulative exposure	Adjusted for age Males only	Large magnitude: yes Statistical significance: yes Dose-response: plateau Temporal association: yes Subgroup only: no Adjustments: not given	F/U 1970 to 2018, average 31.7 years Average length of employment = 17.1 years Other chemicals produced at the plant include fluoroaromatics, benzotrofluorides, and PFOS (mean serum PFOS = 0.148 µg/ml, correlation with PFOA = 0.59) Elevated SMRs for suicides
Darrow et al., 2016	Parkers-burg, West Virginia 2005-06	Prospective cohort	Who: adults Ages: ≥20 N: 30,723	Selection: unclear Participation: unclear Equal groups: unclear Blinded: unclear Above detection: unclear Levels: medians ranged from 11.9 to 25.7 ng/ml depending on age group	Environmental fate and transport model used to produce yearly and cumulative serum levels - see notes	ALT, GGT, direct bilirubin, liver disease	Serum (liver enzymes) and self-reported and adjudicated (liver disease)	<u>Regression coefficients:</u> ALT: 0.012 (0.008 to 0.016) GGT: 0.003 (-0.003 to 0.008) Direct bilirubin: -0.005 (-0.008 to -0.002) An estimated 6 percent (95% CI, 4-8 percent) increase in ALT level was seen between the fifth and first quartiles of PFOA exposure Liver disease: all hazard ratios near 1.0 Also associated with CK18 M30, a marker of liver cell apoptosis (see Bassler et al., 2019)	Linear regression (liver enzymes) Unknown for liver disease	Adjusted for age, sex, BMI, alcohol consumption, regular exercise, smoking, education, insulin resistance, fasting, occupational exposure, and race	Large magnitude: no Statistical significance: yes Dose-response: inverted U Temporal association: yes Subgroup only: no Adjustments: yes (increased)	Groundwater contamination of PFOA from a nearby chemical manufacturing plant Exposure models included information on PFOA water concentrations, residential history, water sources, water consumption rates and job, department, and work histories if occupationally exposed. Correlation coefficient between modeled serum estimates and measured serum concentrations in was 0.71 The percentage change in liver function biomarkers for a given change in PFOA can be calculated as [exp(β)–1] × 100, where β is the linear regression coefficient. For continuous ln PFOA, this would represent the percent change in the biomarker for a 1 ln unit increase in PFOA

Author Year	Location Years	Design	Who Ages N	Factors related to bias	Exposure method	Outcome	Outcome method	Results	Compari-son	Confounding	Other aspects of causal inference	Notes
Nian et al., 2019	Shen-yang, China	Cross-sectional 2015-16	Who: adults Ages: ≥35 N: 1,605	Selection: government workers, retirees, and others who were long-term residents of the city Participation: 93% Equal groups: unclear Blinded: unclear Above detection: 99.9% Levels: median (IQR) = 6.19 (4.08-9.31) ng/ml	Serum	AST, ALT, ALP, TB, GGT	Serum	Regression coefficients: ALT: 7.4 (3.9-11.0) AST: 2.9 (0.7-5.2) ALP: -1.1 (-2.9-0.8) GGT: 8.6 (4.9-12.3) TB: 1.6 (-1.1-4.2) Results are for total PFOA; associations less strong for iso-PFOA than n-PFOA	Percent change in linear regression coefficients of lognormal liver biomarkers per each one lognormal increase in PFOA	Adjusted for age, sex, career, income, education, alcohol, smoking, giblet and seafood consumption, exercise, and BMI Similar results when those taking relevant medications, smokers, and alcohol drinkers are excluded	Large magnitude: no Statistical significance: yes Dose-response: linear or plateau Temporal association: no Subgroup only: no Adjustments: not given	The city is one of the largest fluoropolymer manufacturing centers in China Fasting samples PFAS highly correlated
Attanasio, 2019	US NHANES 2013-16	Cross-sectional	Who: adolescents Ages: 12-19 N: 354 males and 305 females	Selection: random cluster sampling Participation: unclear Equal groups: unclear Blinded: likely Levels: geometric mean (SE) = 1.50 (0.06) and 1.22 (0.06) in males and females, respectively	Serum	ALT, AST, GGT, TB	Serum	Regression coefficients: Males: ALT: -0.11 (-0.21 to -0.01) (p-trend = 0.09) AST: no association GGT: -0.13 (-0.25-0.003) (p-trend = 0.08) TB: 0.19 (0.08-0.30) (p-trend <0.01) ORs for an elevated ALT and AST are below 0.5 Females: ALT: 0.20 (0.07-0.34) (p-trend <0.01)* AST: 0.11 (0.00-0.21) (p-trend = 0.03)* GGT: 0.19 (0.02-0.35) (p-trend = 0.02)* TB: 0.10 (-0.01-0.20) (p-trend = 0.09)* ORs for an elevated ALT, AST, and GGT are above 1.5.	Difference in log liver outcome between the 4th and 1st quartiles of PFOA; logistic regression	Adjusted for age, race, BMI, education, poverty index, smoking, and menarche	Large magnitude: yes Statistical significance: yes Dose-response: linear or plateau Temporal association: no Subgroup only: mostly in women Adjustments: *large changes with adjustments	Opposite effects in males and females
Gleason et al., 2015	US NHANES 2007-10	Cross-sectional	Who: children and adults Ages: ≥12 N: 4,333	Selection: random cluster sampling Participation: unclear Equal groups: unclear Blinded: likely Levels: median (IQR) = 3.7 (2.5-5.2)	Serum	ALT, AST, GGT, , ALP, TB	Serum	Regression coefficients: ALT: 0.038 (0.014-0.062) GGT: 0.058 (0.021-0.096) AST: 0.025 (0.007-0.043) ALP: 0.003 (-0.023-0.016) TB: 0.048 (0.016-0.081)	Linear regression for ln PFOA and ln liver enzyme	Adjusted for age, gender, race/ethnicity, BMI, poverty, and alcohol	Large magnitude: unclear Statistical significance: yes Dose-response: mixed, see their Figure 1 Temporal association: no Subgroup only: no Adjustments: reduced magnitude with adjustments	SAS surveyreg used Not in US EPA 2016a or 2016b

Author Year	Location Years	Design	Who Ages N	Factors related to bias	Exposure method	Outcome	Outcome method	Results	Compari-son	Confounding	Other aspects of causal inference	Notes
Jain and Ducatman, 2018a	US NHANES 2011-14	Cross-sectional	Who: adults Ages: ≥20 N: 3,573	Selection: random cluster sampling Participation: unclear Equal groups: unclear Blinded: likely Above detection: 99.4% Levels: geometric means and 95% CIs = 2.2 (2.0 - 2.3) and 2.0 (1.8 - 2.1) ng/ml in non-obese and obese participants, respectively	Serum	AST, ALT, GGT, ALP, and TB	Serum	Regression coefficients: **For obese participants:** ALT: 0.07065, p <0.01 AST: 0.0744, p=0.03 GGT: 0.07422, p=0.03 TB: 0.06023, p=0.03 **Non-obese subjects:** no associations A 10 percent increase in PFOA was associated with a 0.68 percent increase in ALT, a 0.71 percent increase in GGT, a 0.49 percent increase in AST, a 0.13 percent increase in ALP, and a 0.58 percent increase in TB among obese participants	Linear regression, see notes	Adjusted for gender, race/ethnicity, age, poverty income ratio, physical activity, BMI, and serum cotinine	Large magnitude: unclear Statistical significance: yes Dose-response: yes Temporal association: no Subgroup only: yes, obese Adjustments: unclear	Regression coefficients appear to represent linear associations between log10 PFAS and log10 liver enzyme levels Associations varied by GFR but not clearly consistent with confounding (Jain, 2019)
Khalil et al., 2018	Dayton, Ohio 2016	Cross-sectional	Who: obese children Ages: 8-12 N: 48	Selection: unclear Participation: unclear Equal groups: unclear Blinded: unclear Above detection: unclear but low detection limits Levels: median (IQR) = 0.99 (0.45) ng/ml	Serum	ALT, AST	Serum	ALT: no association AST: no association	Linear regression	Adjusted for age, sex, and ethnicity	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: unclear	
Mora et al., 2018	Boston, Massa-chusetts 1999-2002	Prospective cohort and cross-sectional	Who: maternal offspring pairs Ages: 6-11 (children) N: 653	Selection: unclear Participation: 38% Equal groups: unclear Blinded: unclear Above detection: >99% Levels: median (IQR) = 5.4 (3.9-7.6) ng/ml	Plasma Maternal (gestation week 7) and child average age 7 years	ALT	Plasma Child Average age 7 years	Cohort: no associations Cross-sectional: β = -0.7 (-1.4 to 0.0) Similar results in boys and girls	Change per interquartile increase in PFOA	Adjusted for maternal education, prenatal smoking, gestational age at blood draw, child's sex, race/ethnicity, and age at ALT measurement Additional adjustments for household income, marital status, maternal plasma albumin concentrations and GFR during pregnancy, breast-feeding duration; and child's physical activity, screen time, and fast food and soda consumption did not materially change PFAS coefficients	Large magnitude: unclear Statistical significance: borderline Dose-response: yes Temporal association: no Subgroup only: no Adjustments: unclear	Difference between prospective vs. cross-sectional results

Author Year	Location Years	Design	Who Ages N	Factors related to bias	Exposure method	Outcome	Outcome method	Results	Compari-son	Confounding	Other aspects of causal inference	Notes
Salihovic et al., 2018	Sweden 2001-14	Prospective cohort	Who: adults Ages: 75-80 N: 1,002	Selection: randomly selected from general population registers Participation: unclear Equal groups: unclear Blinded: unclear Above detection: unclear but low detection limits Levels: median (IQR) = 3.31 (2.52-4.39) ng/ml	Serum or plasma	ALT, ALP, GGT, TB	Serum	Regression coefficients: TB: -1.39 (p <0.001) ALT: 0.04 (p <0.001) ALP: 0.11 (p <0.001) GGT: 0.07 (p=0.01) No interaction with BMI	Change in lipid level per ln increase in PFOA	Adjusted for sex, LDL and HDL cholesterol, serum triglycerides, BMI, fasting glucose levels, statin use, and smoking Similar results following adjustment for only sex or only sex and GFR	Large magnitude: unclear Statistical significance: yes Dose-response: yes Temporal association: yes Subgroup only: no Adjustments: no	Ten year follow-up Correlation between PFOS and PFOA = 0.45

Data in parentheses are 95% confidence intervals unless otherwise stated
High exposure occupational and community studies are listed first, then rows are sorted alphabetically by first author
Ages are in years unless otherwise noted
Abbreviations: β, regression coefficient; ALT, alanine aminotransferase, ALP, alkaline phosphatase; AST, aspartate; BMI, body mass index; F/U, follow-up; GFR, glomerular filtration rate; GGT, γ-glutamyl transferase; HDL, high density lipoprotein; IQR, interquartile range; LDL, low density lipoprotein; N, number of participants; OR, odds ratio; RR, rate ratio; SE, standard error; SMR, standardized mortality ratio; TB, total bilirubin

Table A7.6. Recent epidemiologic studies on PFOS and liver toxicity

Author Year	Location Years	Design	Who Ages N	Factors related to bias	Exposure method	Outcome	Outcome method	Results	Compari-son	Confounding	Other aspects of causal inference	Notes
Nian et al., 2019	Shen-yang, China	Cross-sectional 2015-16	Who: adults Ages: ≥35 N: 1,605	Selection: government workers, retirees, and others who were long-term residents of the city Participation: 93% Equal groups: unclear Above detection: 100% Blinded: unclear Levels: median (IQR) = 27.39 (18.05-40.62) ng/ml	Serum	AST, ALT, ALP, TB, GGT	Serum	Regression coefficients: ALT: 4.1 (0.6-7.7) AST: 2.0 (-0.3-4.3) ALP: no association GGT: 2.8 (-0.8-6.5) TB: 2.4 (-0.3-6.5) Results are for total PFOS; associations less strong for branched chain PFOS than n-PFOS except for TB (β = 2.5 (0.1-4.9))	Percent change in linear regression coefficients of lognormal liver biomarkers per each one lognormal increase in PFOS	Adjusted for age, sex, career, income, education, alcohol, smoking, giblet and seafood consumption, exercise, and BMI Mixed results when those taking relevant medications, smokers, and alcohol drinkers are excluded, some associations still seen	Large magnitude: no Statistical significance: yes Dose-response: plateau Temporal association: no Subgroup only: no Adjustments: not given	The city is one of the largest fluoropolymer manufacturing centers in China Fasting samples PFAS highly correlated
Attanasio, 2019	US NHANES 2013-16	Cross-sectional	Who: adolescents Ages: 12-19 N: 354 males and 305 females	Selection: random cluster sampling Participation: unclear Equal groups: unclear Blinded: likely Levels: geometric mean (SE) = 3.68 (0.12) and 2.76 (0.14) in males and females, respectively	Serum	ALT, AST, GGT, TB	Serum	Regression coefficients: Males: ALT: no association AST: no association GGT: no associationa TB: 0.14 (0.02-0.27) (p-trend <0.01) ORs for an elevated ALT, AST, and GGT are near 1.0 Females: ALT: 0.15 (0.02-0.29) (p-trend = 0.13)* AST: 0.14 (0.04-0.23) (p-trend = 0.04)* GGT: 0.11 (-0.02-0.24) (p-trend = 0.34) TB: 0.10 (0.02-0.19) (p-trend <0.01)* ORs for an elevated ALT, AST, and GGT are above 1.5.	Difference in log liver outcome between the 4th and 1st quartiles of PFOS; logistic regression	Adjusted for age, race, BMI, education, poverty index, smoking, and menarche	Large magnitude: yes Statistical significance: yes Dose-response: linear or plateau Temporal association: no Subgroup only: mostly in women Adjustments: *large changes with adjustments	Some different effects in males and females

Author Year	Location Years	Design	Who Ages N	Factors related to bias	Exposure method	Outcome	Outcome method	Results	Compari-son	Confounding	Other aspects of causal inference	Notes
Gleason et al., 2015	US NHANES 2007-10	Cross-sectional	Who: children and adults Ages: ≥12 N: 4,333	Selection: random cluster sampling Participation: unclear Equal groups: unclear Blinded: likely Levels: median (IQR) = 11.3 (7.0-18.0) ng/ml	Serum	ALT, AST, GGT, , ALP, TB	Serum	ALT: no association GGT: no association AST: no association ALP: no association TB: no association	Linear regression for ln PFOS and ln liver enzyme	Adjusted for age, gender, race/ethnicity, BMI, poverty, and alcohol	Large magnitude: unclear Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: reduced magnitude with adjustments	SAS surveyreg used Not in US EPA 2016a or b
Jain and Ducatman, 2018a	US NHANES 2011-14	Cross-sectional	Who: adults Ages: ≥20 N: 3,573	Selection: random cluster sampling Participation: unclear Equal groups: unclear Blinded: likely Above detection: 99.4% Levels: geometric means and 95% CIs = 6.3 (5.8 - 6.8) and 5.5 (5.0 - 6.0) ng/ml in non-obese and obese participants, respectively	Serum	AST, ALT, GGT, ALP, and TB	Serum	No associations in obese or non-obese participants	Linear regression	Adjusted for gender, race/ethnicity, age, poverty income ratio, physical activity, BMI, and serum cotinine	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: unclear	Associations varied by GFR but not clearly consistent with confounding (Jain, 2019)
Khalil et al., 2018	Dayton, Ohio 2016	Cross-sectional	Who: obese children Ages: 8-12 N: 48	Selection: unclear Participation: unclear Equal groups: unclear Blinded: unclear Above detection: unclear but low detection limits Levels: median (IQR) = 2.79 (2.10) ng/ml	Serum	ALT, AST	Serum	ALT: no association AST: no association	Linear regression	Adjusted for age, sex, and ethnicity	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: unclear	
Mora et al., 2018	Boston, Massa-chusetts 1999-2002	Prospective cohort and cross-sectional	Who: maternal offspring pairs Ages: 6-11 (children) N: 653	Selection: unclear Participation: 38% Equal groups: unclear Blinded: unclear Above detection: >99% Levels: median (IQR) = 24.6 (17.9–34) ng/ml	Plasma Maternal (gestation week 7) and child average age 7 years	ALT	Plasma Child Average age 7 years	Prospective cohort: no association Cross-sectional: no association Similar results in boys and girls	Change per interquartile increase in PFOS	Adjusted for maternal education, prenatal smoking, gestational age at blood draw, child's sex, race/ethnicity, and age at ALT measurement Additional adjustments for household income, marital status, maternal plasma albumin concentrations and GFR during pregnancy, breast-feeding duration; and child's physical activity, screen time, and fast food and soda consumption did not materially change coefficients	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: unclear	
Salihovic et al., 2018	Sweden 2001-14	Prospective cohort	Who: adults Ages: 75-80 N: 1,002	Selection: randomly selected from general population registers Participation: unclear Equal groups: unclear Blinded: unclear Above detection: unclear but low detection limits Levels: median (IQR) = 13.2 (9.95-17.8) ng/ml	Serum or plasma	ALT, ALP, GGT, TB	Serum	Regression coefficients: TB: -0.58 (p <0.001) ALT: 0.03 (p <0.001) ALP: 0.02 (p=0.37) GGT: 0.02 (p=0.40)	Change in lipid level per ln increase in PFOS	Adjusted for sex, LDL and HDL cholesterol, serum triglycerides, BMI, fasting glucose levels, statin use, and smoking	Large magnitude: unclear Statistical significance: yes Dose-response: yes Temporal association: yes Subgroup only: no Adjustments: unclear	Ten year follow-up Correlation between PFOS and PFOA = 0.45

Thyroid Toxicity

Literature search and methods

In addition to reviewing the results of previous reviews by the US EPA (US EPA, 2016b; 2016d), ATSDR (2018a), and others, OEHHA searched for all new human epidemiologic studies on PFOA or PFOS and thyroid toxicity published since the 2016 US EPA reviews. PubMed and Embase were searched for all human studies on PFOA or PFOS and thyroid disease or thyroid hormones published between December 2015 (the end of the US EPA literature search) and Septmber 20, 2020 (the end of OEHHA's literature search). The outcome portion of the search string used is shown below.

(thyro OR goiter OR Grave* OR hyperthyro* OR hypothyro* OR diiodotyrosine OR triiodothyronine OR TSH OR T4 OR TT4 OR FT4 OR T3 OR Hashimoto*)*

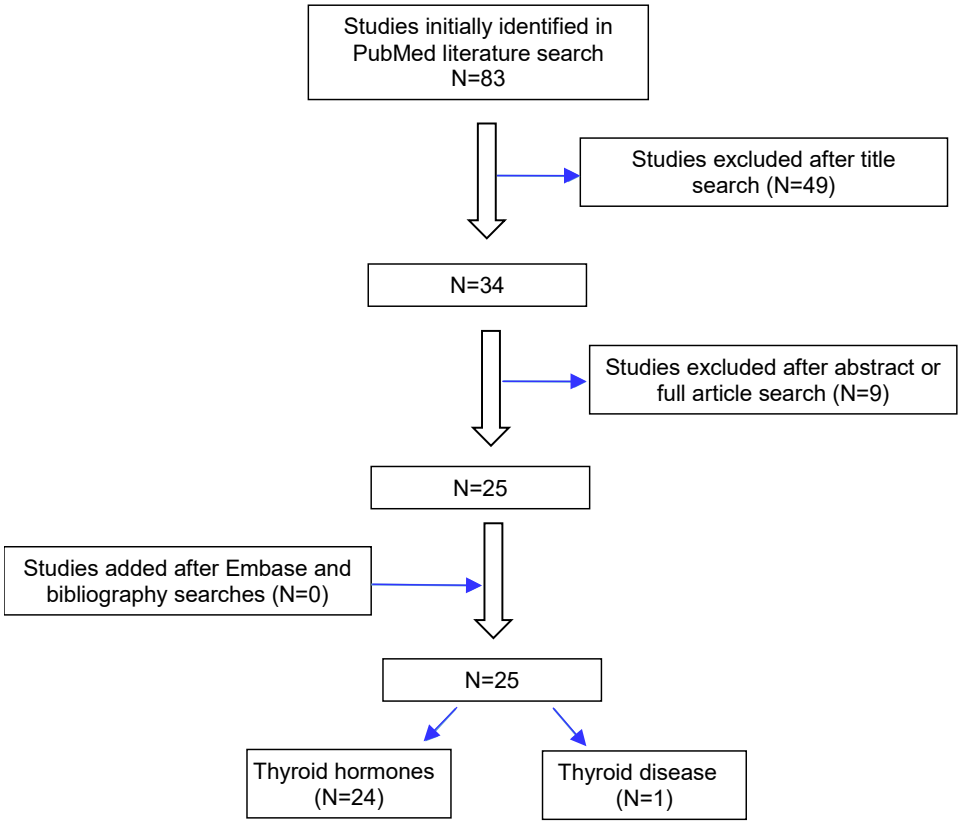
The key words in this search string were based on the outcomes identified by US EPA and on preliminary searches OEHHA performed in PubMed.

In the large majority of studies OEHHA identified, and in the large majority of studies identified by US EPA (2016a, b), the outcomes assessed were thyroid hormone levels and not outcomes involving more overt thyroid disease. In order to identify trends in the literature overall, the results across all studies were compared regardless of the date published. This assessment included the studies identified by three sources: 1. those identified by US EPA (2016a, b); 2. those identified in a 2017 meta-analysis of PFOA or PFOS and thyroid hormones in children and pregnant women (Ballesteros et al., 2017); and 3. the more recent studies that OEHHA identified in this literature search. The studies OEHHA identified from these three sources did not present results using the same scales for exposure and outcome, which made it difficult to directly and quantitatively compare results across all studies. For example, some studies reported only linear regression coefficients while others reported only correlation coefficients. In some studies, regression coefficients were calculated for the change in thyroid hormone levels associated with each log-normal increase in PFOA or PFOS, while in others this was for each log10 increase or each interquartile increase. Because of this heterogeneity, OEHHA initially sought to identify trends across studies by simply evaluating whether or not study results were consistent with either a positive association (e.g., a positive regression or correlation coefficient) or an inverse association (e.g., a negative regression or correlation coefficient) between PFOA or PFOS and thyroid hormone levels, and whether or not these associations were statistically significant. Since clear trends were not seen when OEHHA examined all studies together, the studies were then sub-categorized based on various aspects of study quality or study design and were evaluated for whether trends might be seen within these subcategories. OEHHA also attempted to identify trends in study results across various sex and age groups by categorizing results as being in only men or only women, or only in newborns, young children (generally 0-5 years old), older children (generally 10-19 years old), or adults (most subjects ≥19 years old). Because clear trends could not be identified in any of these categories, studies and their results and key design features are shown in summary form only.

Results

A general description of OEHHA's literature search is shown in Figure A7.3. A list of studies excluded after abstract and title search is provided in Table A7.28.

Figure A7.3. Literature search: recent epidemiologic studies of PFOA or PFOS and thyroid toxicity*



*This figure is provided to document OEHHA's PubMed, Embase, and bibliography literature searches. It does not include relevant publications identified from other sources such as previously published reviews from other agencies or other authors.

Overall, OEHHA found 25 publications that examined associations between PFOA or PFOS and thyroid hormone levels (Table A7.7). A number of these studies presented results for different demographic groups, and overall there were a total of 67 sets of results for PFOA or PFOS. Twenty of these were in non-pregnant adults, 11 were in pregnant females, 20 were in newborns, six were in young children, nine were in older children, and two involved children with a wide range of ages. The studies took place in a wide variety of locations throughout Europe, Asia, and the US. Of the 67 sets of results, 16 involved prospective analyses and 51 were cross-sectional. Sample sizes ranged from 29 to 7,020 participants. In all but one study (Lopez-Espinosa et al., 2012), the outcome and exposure were assessed using PFOA or PFOS and thyroid hormone concentrations in serum or plasma. In Lopez-Espinosa et al. (2012), in utero PFOA serum concentrations were estimated using models that included information on PFOA releases from a nearby chemical plant, environmental characteristics of the region, and participant information on residential history, water sources, water intake, and TK data. Few studies presented detailed information on subject recruitment or participation rates and it appears that most studies involved convenience samples. Studies were adjusted for a variety of different potential confounding variables and these are shown in Table A7.7.

Table A7.7 Studies reporting regression or correlation coefficients between PFOA or PFOS and thyroid hormone levels: all years[a]

Study	Source[b]	Location	Design	N	Exp	Group	Sex	PFAS	TH	PFOA[c]			PFOS[c]			Adjustments	Notes
										TSH	T4	fT4	TSH	T4	fT4		
Blake et al., 2018	New	Fernald, OH	Pros	122	Low	Adults	All	Adult	Adult	↓	↓	0	↑↑	↓	0	Age, year, sex, educ, income, marital, BMI	Living near a uranium processing facility
Bloom et al., 2010	USEPA	New York	CS	31	Low	Adults	All	Adult	Adult	↓	0	↓	↑	0	↑	Unknown	In anglers
Byrne et al., 2018	New	Alaska	CS	85	Low	Adults	All	Adult	Adult	↑↑	↑	↓	↓	↓	↑	Age, sex, smok, other PFAS	Native Alaskans
Dallaire et al., 2009	USEPA	Canada	CS	623	High	Adults	All	Adult	Adult	0	0	0	↓↓	0	↑↑	Age, sex, BMI, educ, lipids, smok	Inuit population
Emmett et al., 2006	USEPA	Southeast Ohio	CS	371	High	Adults	All	Adult	Adult	↑	0	0	0	0	0	Unclear	High environmental exposure
Jain &Ducatman 2019a	New	US	CS	7,020	Low	Adults	All	Adult	Adult	--	--	--	--	--	--	Gender, race, age, BMI, fasting, poverty, smok, calories, others	NHANES 2007-12, inconsistent by GFR
Li et al., 2017	New	China	CS	202	Low	Adults	All	Adult	Adult	↑	0	↓	↑↑	0	↓↓	Unadjusted	
Liu et al., 2018a	New	Boston	CS	621	Low	Adults	All	Adult	Adult	↓	↑	↑	↓	↑↑	↑	Age, race, sex, educ, smok, ETOH, exercise, meno, diet	Overweight and obese adults
Olsen et al., 2007	USEPA	Antwerp, Belgium and Decatur, AL	CS	506	High	Adults	All	Adult	Adult	↑	↓	↓↓	ns	ns	0	Age, BMI, ETOH	Occupational exposure
Shrestha et al., 2015	USEPA	Hudson River Valley	CS	87	Low	Adults	All	Adult	Adult	↑	↑	↑	↑	↑↑	↑↑	Age, sex, educ, PCBs	
Webster et al., 2016	USEPA	US	CS	1,525	Low	Adults	All	Adult	Adult	↑	↓↑	↑	↓	↑	↑	Age, race, cotinine, sex, parity, pregnancy, meno	NHANES 2007-8, assoc. with anti-TPO
Crawford et al., 2017	New	North Carolina	CS	99	Low	Adults	Female	Adult	Adult	↑	↑	↑	↓	↑	↑	Age	
Heffernan et al., 2018	New	UK	CS	30	Low	Adults	Female	Adult	Adult	↓	0	↓	↑	0	↓	Age, BMI, GFR, albumin, meds	POS case control study: controls
Heffernan et al., 2018	New	UK	CS	29	Low	Adults	Female	Adult	Adult	↑↑	0	↑	↑	0	↓	Age, BMI, GFR, albumin, meds	POS case control study: cases
Seo et al., 2018	New	S Korea	CS	357	Low	Adults	Female	Adult	Adult	ns	0	↑	ns	0	↑↑	Unk	
Wen et al., 2013	USEPA	US	CS	509	Low	Adults	Female	Adult	Adult	↓	↑	↓	↑	↑	↑	Age, race, ETOH, smok, iodine	NHANES 2007-8, 2009-10
Zhang et al., 2018	New	China	CS	120	Low	Adults	Female	Adult	Adult	↑↑	0	↓↓	↑↑	0	↓↓	Age, BMI, educ, income, sleep, parity	Premature ovarian insufficiency study
Seo et al., 2018	New	S Korea	CS	Unk	Low	Adults	Male	Adult	Adult	ns	0	ns	ns	0	↑↑	Unk	
Wen et al., 2013	USEPA	US	CS	672	Low	Adults	Male	Adult	Adult	↑	↑↓	↓	↓	↓	↓	Age, race, ETOH, smok, iodine	NHANES 2007-8, 2009-10
Caron-Beaudoin et al., 2019	New	Quebec	CS	89	Low	Child: all	Female	Child	Child	↑↓	0	↑↓	↑↓	0	↑↓	Age, BMI, ethnicity	Some differences by tribe and antibody status
Caron-Beaudoin et al., 2019	New	Quebec	CS	95	Low	Child: all	Male	Child	Child	↑↓	0	↑↓	↑↓	0	↑↓	Age, BMI, ethnicity	
Kim et al., 2011	Balles	S Korea	CS	31	Low	Child: newborn	All	Cord	Cord	↑	↓	0	↓	↓	0	Age, BMI, gest	
Kim et al., 2011	Balles	S Korea	Pros	29	Low	Child: newborn	All	Preg	Cord	↑↑	↓	0	↑	↓	0	Age, BMI, gest	
Wang et al., 2014	Balles	Taiwan	Pros	114	Low	Child: newborn	All	Preg	Cord	↓	↑	↓	↓	↑	↑	Age, educ, parity, neonatal sex, cesarean	

Study	Source[b]	Location	Design	N	Exp	Group	Sex	PFAS	TH	PFOA[c]			PFOS[c]			Adjustments	Notes
										TSH	T4	fT4	TSH	T4	fT4		
Yang et al., 2016	New	China	CS	157	Low	Child: newborn	All	Preg	Cord	↓	↓	↓	↓↓	↓	↓	Age, BMI, income, parity, delivery	
Aimuzi et al., 2019	New	Shanghai	CS	262	Low	Child: newborn	Female	Cord	Cord	ns	0	ns	↓↓	0	ns	Age, fish, parity, gest, BMI	All had Cesarean sections
de Cock et al., 2014	Balles	Netherlands	Pros	31	Low	Child: newborn	Female	Cord	Newborn	0	↑↑	0	0	↑	0	Age, weight, gest, parity, smok, ETOH, BMI	
Dufour et al., 2018	New	Belgium	CS	101	Low	Child: newborn	Female	Cord	Newborn	↓	0	0	↓	0	0	Parity, gest	
Shah-Kulkarni et al., 2016	New	S Korea	CS	147	Low	Child: newborn	Female	Cord	Cord	↓	↑	0	↓	↑	0	Age, educ, BMI, ETOH, parity, gest	
Tsai et al., 2017	New	Taiwan	CS	54	Low	Child: newborn	Female	Cord	Cord	↑	↑	0	↑	↑	0	Age, BMI, educ, gest, cesarean	
Preston et al., 2018	New	Boston	Pros	229	Low	Child: newborn	Female	Preg	Newborn	0	↓	0	0	↓	0	Age, race, smok, parity, gest, cesarean	
Xiao et al., 2019	New	Faroe Islands	Pros	172	Low	Child: newborn	Female	Preg	Cord	↑	↑	↑↓	↑	↑	↑	Gest, educ, ETOH, BMI, parity, smok, Hg, PCBs	
Aimuzi et al., 2019	New	Shanghai	CS	305	Low	Child: newborn	Male	Cord	Cord	ns	0	↑↑	ns	0	ns	Age, fish, parity, gest, BMI	All had Cesarean sections
de Cock et al., 2014	Balles	Netherlands	Pros	52	Low	Child: newborn	Male	Cord	Newborn	0	↑	0	0	↓	0	Age, weight, gest, parity, smok, ETOH, BMI	
Dufour et al., 2018	New	Belgium	CS	113	Low	Child: newborn	Male	Cord	Newborn	↓	0	0	↓	0	0	Parity, gest	
Shah-Kulkarni et al., 2016	New	S Korea	CS	132	Low	Child: newborn	Male	Cord	Cord	↓	↓	0	↑	↑	0	Age, educ, BMI, ETOH, parity, gest	
Tsai et al., 2017	New	Taiwan	CS	64	Low	Child: newborn	Male	Cord	Cord	↑	↓	0	↑↑	↓↓	0	Age, BMI, educ, gest, cesarean	
Itoh et al., 2019	New	Japan	Pros	259	Low	Child: newborn	Male	Preg	Cord	↑↓	0	↑↓	↑↑	0	↑↓	Age, parity, educ, ETOH, smok, BMI	
Preston et al., 2018	New	Boston	Pros	236	Low	Child: newborn	Male	Preg	Newborn	0	↓	0	0	↓↓	0	Age, race, smok, parity, gest, cesarean	
Xiao et al., 2019	New	Faroe Islands	Pros	172	Low	Child: newborn	Male	Preg	Cord	↑	↓	↑↓	↑	↑↓	↑	Gest, educ, ETOH, BMI, parity, smok, Hg, PCBs	
Kang et al., 2018	New	S Korea	CS	147	Low	Child: older	All	Child	Child	↓	0	↑	↓	0	↑↓	Age, sex, BMI, income, ETS	
Khalil et al., 2018	New	Ohio	CS	48	Low	Child: older	All	Child	Child	↓	0	↓	↓	0	↓	Age, sex, race	Obese children
Lopez-Espinoza et al., 2012	Balles	Mid-Ohio Valley	Pros	2,741	High	Child: older	All	Preg	Child	↑	↓	0	0	0	0	Age, sex, and month of sampling	High environmental exposure, modeled
Lewis et al., 2015	Balles	US	CS	145	Low	Child: older	Female	Child	Child	↓↓	↑	↑	↓	↓	↑	Age, BMI, income, race, cotinine	NHANES
Lin et al., 2013	Balles	Taipei	CS	144	Low	Child: older	Female	Child	Child	↓	0	↓	↓	0	↑	Age, sex, smok, ETOH	
Lopez-Espinoza et al., 2012	Balles	Mid-Ohio Valley	CS	3,062	High	Child: older	Female	Child	Child	↑	↓	0	↑	↑↑	0	Age, sex, and month of sampling	High environmental exposure
Lewis et al., 2015	Balles	US	CS	158	Low	Child: older	Male	Child	Child	↑	↓	↓	↑↑	↓	↑	Age, BMI, income, race, cotinine	NHANES
Lin et al., 2013	Balles	Taipei	CS	65	Low	Child: older	Male	Child	Child	↓	0	↓	↑↓	0	↓	Age, sex, smok, ETOH	
Lopez-Espinoza et al., 2012	Balles	Mid-Ohio Valley	CS	3,328	High	Child: older	Male	Child	Child	↑	↑	0	↑	↑↑	0	Age, sex, and month of sampling	High environmental exposure
Kim et al., 2016	New	S Korea	CS	40	Low	Child: young	All	Child	Child	ns	0	ns	ns	0	ns	Unadjusted	

Study	Source[b]	Location	Design	N	Exp	Group	Sex	PFAS	TH	PFOA[c]			PFOS[c]			Adjustments	Notes
										TSH	T4	fT4	TSH	T4	fT4		
Lopez-Espinoza et al., 2012	Balles	Mid-Ohio Valley	Pros	476	High	Child: young	All	Preg	Child	↓	↑↑	0	0	0	0	Age, sex, and month of sampling	High environmental exposure, modeled
Lopez-Espinoza et al., 2012	Balles	Mid-Ohio Valley	CS	500	High	Child: young	Female	Child	Child	↓↓	↓	0	↑	↑	0	Age, sex, and month of sampling	High environmental exposure
Kato et al., 2016	New	Japan	Pros	212	Low	Child: young	Female	Preg	Child	↑	0	↓	↑↑	0	↓	Age, parity, anti-TPO, sampling, gest, weight, cesarean	
Lopez-Espinoza et al., 2012	Balles	Mid-Ohio Valley	CS	471	High	Child: young	Male	Child	Child	↓	↑	0	↑	↑	0	Age, sex, and month of sampling	High environmental exposure
Kato et al., 2016	New	Japan	Pros	180	Low	Child: young	Male	Preg	Child	↓	0	↑	↑↑	0	↓	Age, parity, TPO, sampling, gest, weight, cesarean	
Itoh et al., 2019	New	Japan	Pros	240	Low	Child: newborn	Female	Preg	Cord	↑↓	0	↑↓	↑↓	0	↑↓	Age, parity, educ, ETOH, smok, BMI	Similar results by thyroid antibody status
Berg et al., 2015	Balles	Norway	CS	375	Low	Pregnant	Female	Preg	Preg	↑	0	0	↑↑	0	0	Age, parity, BMI, thyroxine binding capacity	
Inoue et al., 2019	New	Denmark	CS	1,366	Low	Pregnant	Female	Preg	Preg	↑	0	↑↓	↑	0	↓	Age, SES, BMI, parity, birth year	Inconsistent by gestational week
Itoh et al., 2019	New	Japan	CS	701	Low	Pregnant	Female	Preg	Preg	↑↓	0	↑↓	↓	0	↑	Age, parity, BMI, educ, ETOH, smok	Small differences by thyroid antibody status
Kato et al., 2016	New	Japan	CS	392	Low	Pregnant	Female	Preg	Preg	↑	0	↑	↓↓	0	↑	Age, parity, educ, anti-TPO, diet, sampling	
Preston et al., 2018	New	Boston	CS	718	Low	Pregnant	Female	Preg	Preg	↑	↑	↓↓	↓	↑	↓	Age, race, smok, diet, parity, gest	
Reardon et al., 2019	New	Calgary	Pros	494	Low	Pregnant	Female	Preg	Preg	↑↓	0	0	↑↓	0	0	Age, ethnicity, smok, thyroid condition, ETOH, drug use	No interaction with gestational age or TPO
Wang et al., 2013	Balles	Norway	CS	903	Low	Pregnant	Female	Preg	Preg	↓	0	0	↑↑	0	0	Age, HDL chol, diet, parity, gest	
Wang et al., 2014	Balles	Taiwan	CS	283	Low	Pregnant	Female	Preg	Preg	↑	↑	↓	↓	↑	↑	Age, educ, parity, neonatal sex, cesarean	
Webster et al., 2014	Balles	Vancouver	CS	151	Low	Pregnant	Female	Preg	Preg	↑	↓	↓	↑	↓	↑	Age, sampling time, anti-TPO	Association seen with anti-TPO
Yang et al., 2016	New	China	CS	157	Low	Pregnant	Female	Preg	Preg	↓	↑	↑↓	↓↓	↑	↓	Age, BMI, income, delivery	
Xiao et al., 2019	New	Faroe Islands	CS	172	Low	Pregnant	Female	Preg	Preg	↑	↑↓	↑↓	↑	↑↓	↑↓	Gest, educ, ETOH, BMI, parity, smok, Hg, PCBs	

[a] Rows are sorted by Group then by Sex

[b] Source: results are from US EPA (2016a and b), Ballesteros et al. (2017)("Balles"), or OEHHA's updated literature review ("New")

[c] Codes to the results:

↑, positive association, not statistically significant
↑↑, positive association, statistically significant
↓, inverse association, not statistically significant
↓↓, inverse association, statistically significant
↑↓, regression or correlation coefficient is zero
0, data not provided
ns, result not statistically significant but actual coefficient not given

Abbreviations: anti-TPO, anti-thyroid peroxidase antibodies; BMI, body mass index; chol, cholesterol; CS, cross sectional study design; educ, education; ETOH, alcohol use; ETS, environmental tobacco smoke; exp, high or low exposure scenario; gest, gestational age; GFR, glomerular filtration rate; Hg, mercury; marital, marital status; meds, medication use; meno, menopause; N, number of participants; NHANES, US National Health and Nutrition Examination Survey; PCBs, polychlorinated biphenyls; PFAS, period when PFOA or PFOS were measured; POS, polycystic ovarian syndrome; Preg, pregnant; Pros, prospective study design; smok, smoking; TH, period when thyroid hormone levels were measured; TPO, thyroid peroxidase antibody; Unk, unknown

Serum Lipid Concentrations

Literature search and methods

In addition to reviewing the results of previous reviews by the US EPA (US EPA, 2016b; 2016d), ATSDR (2018a), and others, OEHHA searched for all human epidemiologic studies on PFOA or PFOS and lipid levels or lipid specific disorders (e.g., hypercholesterolemia) published since the 2016 US EPA reviews, and up to September 20, 2020. The general methods described above were used. Because the studies identified by US EPA mostly involved total cholesterol, LDL, HDL, and TG levels, OEHHA's focus was also on these particular outcomes, although other lipid-related outcomes were also considered. The outcome portion of the search string used is shown below. The key words in this string were based on the outcomes in the studies identified by US EPA and on the key words and medical subject headings listed in articles identified in a preliminary search OEHHA performed in PubMed.

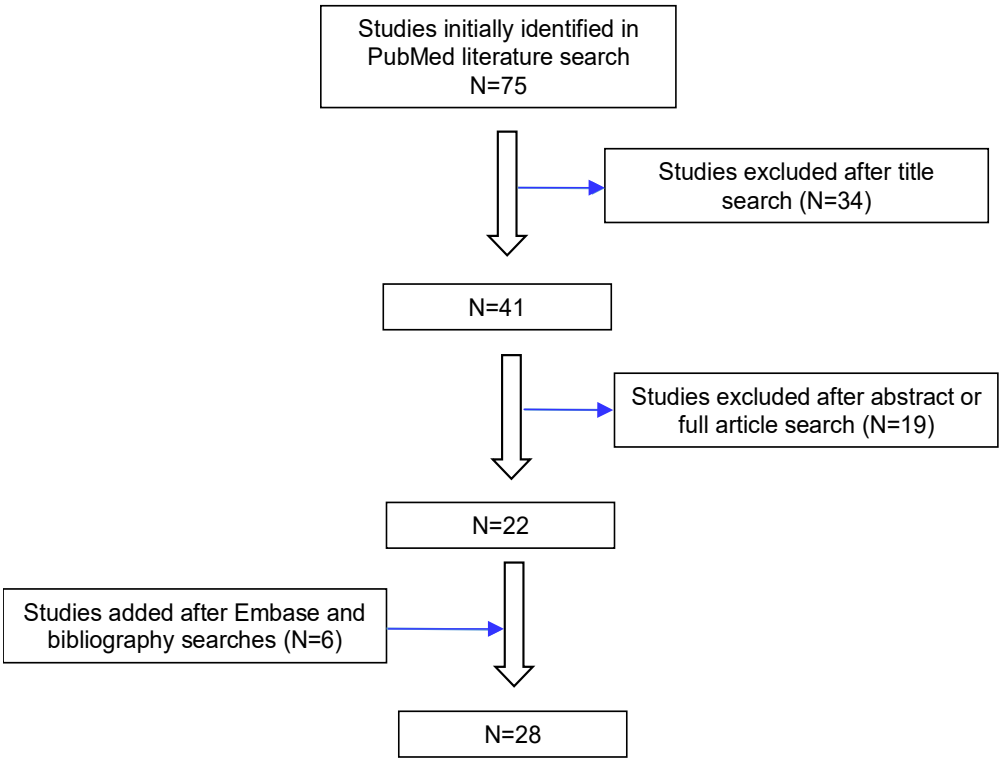
(cholesterol[tiab] OR lipid[tiab] OR LDL[tiab] HDL[tiab] OR VLDL[tiab] OR lipoprotein*[tiab] OR apolipoprotein*[tiab] OR triglyceride*[tiab] OR hypercholesterol*[tiab] OR hypocholesterol*[tiab] OR hyperlipid*[tiab] OR hypolipid*[tiab] OR dyslipidemia*[tiab] OR hyertriglyceridemia[tiab])*

Results

OEHHA identified 28 human epidemiologic studies of PFOA and PFOS and lipid levels published since (or otherwise not included in) the 2016 US EPA reviews. Details for each study are provided in Tables A7.8 and A7.9. A general description of the literature search is provided in Figure A7.4. A list of studies excluded after abstract and title search is provided in Table A7.28. A brief summary of the results of each study and quality ratings given to each study are shown in Tables A7.11 and A7.12.

Twenty-seven of 28 studies provided results for both PFOA and PFOS, with one only providing results for PFOA (Starling et al., 2017). Eighteen studies were done primarily in adults, 10 were done primarily in children. Most studies were done in the US, but several included study populations from Asia and Europe. Seven studies were based on information provided as part of US NHANES. All used blood concentrations to assess exposure and outcome. In the large majority of studies, fasting blood samples were used. Five of the studies used prospective cohort designs, the rest were cross-sectional studies. The overall quality of the studies done in adults appeared to be fairly high, with overall quality scores ≥8 in most studies (see Tables A7.11 and A7.12). Study quality tended to be lower in studies done in children.

Figure A7.4. Literature search: recent epidemiologic studies of PFOA or PFOS and lipid levels*



*This figure is provided to document OEHHA's PubMed, Embase, and bibliography literature searches. It does not include relevant publications identified from other sources such as previously published reviews from other agencies or other authors.

Table A7.8. Recent epidemiologic studies of PFOA and lipid levels

Reference	Location Years	Design	Who Ages N	Factors related to bias	Exposure method	Outcome method	Results	Comparison	Confounding	Other aspects of causal inference	Notes
Christen-sen et al., 2019	Location: US NHANES Years: 2007-14	Cross-sectional	Who: adults Ages: ≥20 N: 2,975	Selection: randomized cluster sample Participation: unclear Equal groups: unclear Blinded: yes Levels: median = 2.8 ng/ml	Serum, fasting	Blood, fasting, and use of lipid lowering medication	TC: NA LDL: NA HDL: OR for low HDL = 1.26 (0.73-2.16) TG: OR for high TG = 1.27 (0.73-2.22) Other: NA	OR for 4th vs. 1st quartile of PFOA	Adjusted for survey year, age, sex, race/ethnicity, income, alcohol, and smoking	Large magnitude: yes for HDL and TG Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: not given	Overlap with other NHANES studies Presents results adjusted and unadjusted for NHANES sampling weights "High triglycerides" if TG >150 mg/dl or medication use; "Low HDL" if HDL <30 mg/dl or medication use Potential weaknesses: Low statistical power Cross-sectional: reverse causation
Dong et al., 2019	Location: US NHANES Years: 2003-14	Cross-sectional	Who: adults Ages: 20-80 N: 8,948	Selection: randomized cluster sample Participation: unclear Equal groups: unclear Blinded: yes Levels: median = 3.0 ng/ml	Serum, fasting	Blood, fasting	TC: β = 1.48 (0.2-2.8), means for each quintile are given in their Figure S4 LDL: small increase (figure form only) HDL: no association TG: NA Other: NA See separate entry for results in adolescents	Change in lipid level (mg/dl) per ng/ml increase in PFOA (most data in figure form)	Adjusted for age, gender, race, BMI, income, waist circumference, activity, diabetes, smoking, and alcohol	Large magnitude: no Statistical significance: yes for TC Dose-response: unclear Temporal association: no Subgroup only: no Adjustments: not given	Overlap with other NHANES studies PFOA and PFOS levels decreased over time PFOA and PFOS correlation = 0.69 Reference doses and benchmark doses also calculated Potential weaknesses: Cross-sectional: reverse causation Unclear if adequately adjusted for survey year
He et al., 2018	Location: US NHANES Years: 2003-12	Cross-sectional	Who: adults Ages: ≥20 N: 7,904	Selection: randomized cluster sample Participation: unclear Equal groups: unclear Blinded: yes Levels: mean = 3.46-4.50 ng/ml	Serum, likely fasting	Serum, likely fasting	TC: 1.43% (0.62-2.34%) increase in men and 1.07% (0.27-1.97%) increase in women LDL: 1.25% increase in women, p=0.128 for quartile 4 vs. 1, no increase in men HDL: no association TG: 1.61 to 2.06% increase in men and women for quartile 4 vs. 1, p >0.05 Other: NA	Percent change in lipid level per interquartile increase in PFOA	Adjusted for age, race, BMI, education, alcohol, income, and activity Stratified by gender	Large magnitude: no Statistical significance: yes for TC Dose-response: inverse U in women, linear in men Temporal association: no Subgroup only: for TC strongest associations in men ages 40-60 and women ages 60-70 Adjustments: not given	Overlap with other NHANES studies Potential weaknesses: Small effect size Cross-sectional: reverse causation Not adjusted for survey year
Huang et al., 2018	Location: US NHANES Years: 1999-2014	Cross-sectional	Who: adults Ages: ≥20 N: 10,859	Selection: randomized cluster sample Participation: unclear Equal groups: data only for total PFAS Blinded: yes Levels: median = 3.17 ng/ml	Serum, likely fasting	Serum, likely fasting	TC: R = 0.092 LDL: R = 0.086 HDL: R = -0.037 TG: R = 0.086 Other: NA All p-values <0.01	Spearman correlation coefficients (R)	Results appear to be unadjusted	Large magnitude: no Statistical significance: yes for all Dose-response: unclear Temporal association: no Subgroup only: no Adjustments: not given	Overlap with other NHANES studies Correlation with PFOS = 0.69 PFOA and PFOS levels declined over time Potential weaknesses: Relatively small effect sizes Limited co-variates including survey year Some lipid levels have also declined over time Cross-sectional: reverse causation

Reference	Location Years	Design	Who Ages N	Factors related to bias	Exposure method	Outcome method	Results	Comparison	Confounding	Other aspects of causal inference	Notes
Jain and Ducatman, 2019b	Location: US NHANES Years: 2005-14	Cross-sectional	Who: adults Ages: ≥20 N: 3,629	Selection: randomized cluster sample Participation: unclear Equal groups: unclear Blinded: yes Levels: geometric mean = 2.9 ng/ml	Serum, fasting	Blood, fasting	TC: 0.50% (p=0.03) in obese men, no clear association in non-obese men or in women LDL: 0.79% (p=0.01) in obese men, no clear association in non-obese men or in women HDL: no association TG: no association Other: NA	Percent increase in lipid level for each 10% increase in PFOA	Adjusted for race/ethnicity, smoking, age, income, fasting time, lipid lowering medication, activity, survey year, and diet Stratified by gender	Large magnitude: no Statistical significance: yes for TC and LDL Dose-response: unclear Temporal association: no Subgroup only: yes, obese men Adjustments: not given	Overlap with other NHANES studies Stratified by obesity and gender Potential weaknesses: Cross-sectional: reverse causation
Liu et al., 2018b	Location: US NHANES Years: 2013-4	Cross-sectional	Who: adults Ages: ≥18 N: 1,871	Selection: randomized cluster sample Participation: unclear Equal groups: mostly, some differences in PFOA by age, race, income, and other factors Blinded: yes Levels: geometric mean = 1.86 ng/ml	Serum, likely fasting	Blood, likely fasting	TC: β = 5.58 (p <0.05) LDL: β = 4.47 (p >0.05) HDL: β = 1.93 (p <0.01) TG: β = -0.08 (p >0.05) Other: apolipoprotein B no association	Change in lipid level (in mg/dl) for each log increase in PFOA	Adjusted for age, gender, ethnicity, smoking, alcohol, income, waist circumference and relevant medications	Large magnitude: no Statistical significance: yes for TC and HDL Dose-response: log-linear Temporal association: no Subgroup only: no Adjustments: some decrease for HDL, small change for TC	Overlap with other NHANES studies Potential weaknesses: Cross-sectional: reverse causation
Chen et al., 2019	Location: Hvar, Croatia Years: 2007-8	Cross-sectional	Who: adults Ages: 44-56 N: 122	Selection: unclear Participation: unclear Equal groups: PFOA higher with lower education, otherwise similar Blinded: unclear Levels: geometric mean = 2.87 ng/ml	Plasma, fasting	Plasma, fasting	TC: no association in regression analyses; OR = 1.60 (0.64-4.00) for high TC (or lipid lowering medication use) in logistic regression LDL: no association HDL: no association TG: no association Other: NA	Change in lipid levels per one unit increase in ln PFOA; logistic regression	Adjusted for age, sex, SES, smoking, dietary pattern, and activity	Large magnitude: yes for TC Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: not given	Potential weaknesses: Low statistical power Cross-sectional: reverse causation
Christen-sen et al., 2016	Location: Wisconsin Years: 2012-3	Cross-sectional	Who: male fishermen Ages: ≥50 N: 154	Selection: "interested in future studies" per online survey; flyers Participation: unclear Equal groups: unclear Blinded: unclear Levels: median = 2.50 ng/ml	Serum, fasting unknown	"have you ever been told by a doctor you have…high cholesterol"	TC: OR = 1.12 (0.85-1.50) for "high cholesterol" LDL: NA HDL: NA TG: NA Other: NA	Answered yes vs. no	Adjusted for age, BMI, work status, and alcohol consumption Males only	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: not given	Samples stored at -20º C Potential weaknesses: Self-reported non-specific outcome

Reference	Location Years	Design	Who Ages N	Factors related to bias	Exposure method	Outcome method	Results	Comparison	Confounding	Other aspects of causal inference	Notes
Donat-Vargas et al., 2019	Location: Sweden Years: 1990-2013	Cohort F/U: approxi-mately 10 years	Who: adults Ages: 40-60 at baseline N: 187	Selection: controls in a diabetes case-control study Participation: 56% Equal groups: unclear Blinded: unclear Levels: median = 2.9 ng/ml	Blood, fasting	Plasma, fasting	TC: no clear association in prospective analysis, possibly some decrease in repeated measures analysis LDL: NA HDL: NA TG: β = -0.10 mmol/L (-0.22-0.02) decrease for each one SD increase in PFOA Other: NA	Change in lipid level for tertiles of PFOA or per each SD increase in PFOA	Adjusted for gender, age, education, sample year, BMI, alcohol, exercise, energy intake, cotinine, and diet	Large magnitude: no, about a 7-8% decrease for TG Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: not given	PFOA and PFOS levels decreased over time PFOA and PFOS highly correlated Potential weaknesses: Some inconsistency between repeated measures and prospective analysis
Graber et al., 2019	Location: New Jersey Years: 2016-17	Cross-sectional	Who: residents of an area contaminated with PFAS, likely from a nearby manufacturing facility Ages: ≥19 N: 105	Selection: claimants in a class action lawsuit, flers to all local residents Participation: unclear but likely low Equal groups: unclear Blinded: unclear Levels: geometric mean (95% CI) = 3.03 (2.70-3.40) ng/ml	Serum, fasting unknown	Self-reported physician diagnosed "high cholesterol"	TC: NA LDL: NA HDL: NA TG: NA Other: self-reported "high cholesterol" OR = 1.12 (0.94, 1.35)	Unclear	Adjusted for age and BMI	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: OR = 1.03 (0.94, 1.14) after adjustment for other PFAS	PFNA levels in water and serum were high Serum samples were collected and measured as part of a lawsuit, not collected or measured by the researchers Potential weaknesses: Methods are unclear Outcome not verified
Kishi et al., 2015	Location: Hokkaido, Japan Years: 2002-5	Cross-sectional	Who: pregnant women Ages: unknown N: 306	Selection: pregnant women, preterm care and delivery at participating hospital Participation: 28.6% Equal groups: higher PFOA associated with lower parity, lower caffeine intake, and male offspring Blinded: unclear Levels: median = 1.40 ng/ml	Serum, 23-35 weeks gestation, non-fasting	Blood, non-fasting	TC: No association Others not assessed	Linear regression with log10 PFOA and log10 TC	Adjusted for age, smoking, alcohol, income, parity, and gestation week	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: little change	Sample stored at -80º C Potential weaknesses:

Reference	Location Years	Design	Who Ages N	Factors related to bias	Exposure method	Outcome method	Results	Comparison	Confounding	Other aspects of causal inference	Notes
Lin et al., 2019	Location: 27 US medical centers Years: 1996-2014	Cohort and cross-sectional	Who: adults Ages: ≥25 N: 888	Selection: pre-diabetic adults Participation: unclear Equal groups: unclear Blinded: unclear Levels: median = 4.9 ng/ml	Plasma at baseline 1996-99, fasting unclear	Blood at baseline and annually, fasting	**Cross-sectional:** TC: β = 6.09 (3.14-9.04) LDL: β = 2.93 (0.22-5.63) HDL: β = -0.49 (-1.38-0.40) TG: β = 17.75 (9.77-25.74) Other: VLDL β = 3.66 (2.18-5.15) No effect modification by treatment group **Prospective:** Hypercholesterolemia: OR = 1.29 (1.05-1.57) per doubling of PFOA Greater effect in the placebo group Hypertriglyceridemia: OR = 1.48 (1.21-1.81) per doubling of PFOA Greater effect in the placebo group	Change in lipid level (mg/dl) per doubling of plasma PFOA; ORs	Adjusted for age, sex, race/ethnicity, marital status, education, smoking, alcohol, fat intake, fiber intake, activity, and waist circumference Cross-sectional: excluded people taking lipid lowering medications	Large magnitude: yes for multiple outcomes Statistical significance: yes for multiple outcomes Dose-response: linear Temporal association: yes Subgroup only: those not on special intervention Adjustments: not given	Randomized to lifestyle intervention or placebo Hypercholesterolemia defined by high TC, high LDL, or use of lipid lowering medication F/U: 15 years Potential weaknesses: Intervention may have altered results
Lin et al., 2020	Location: Taiwan Years: 2009-11	Cross-sectional	Who: adults Ages: 22-63 N: 597	Selection: controls in a coronary heart disease case-control study, recruited from a bulletin board announcement Participation: unclear Equal groups: PFOA higher in men, higher BMI, higher alcohol consumption, otherwise similar Blinded: unclear Levels: geometric mean = 3.77 ng/ml	Blood, fasting unknown	Blood, fasting unknown	TC: NA LDL: β = 6.12 (p=0.017) HDL: no association TG: β = 0.13 (p=0.004) Other: NA	Change in lipid level for each one unit ln increase in PFOA	Adjusted for age, gender, smoking alcohol, education, hypertension, BMI, and diabetes	Large magnitude: unclear Statistical significance: yes for LDL and TG Dose-response: possibly log-linear Temporal association: no Subgroup only: no Adjustments: little change	PFOA higher with higher BMI Potential weaknesses: Fasting status unknown Cross-sectional: reverse causation
Liu et al., 2018a	Location: Boston, MA Years: 2004-7	Cross-sectional	Who: participants in a weight loss program at baseline Ages: 30-70 N: 621	Selection: convenience sample Participation: unclear Equal groups: unclear Blinded: unclear Levels: median = 4.1 and 5.2 ng/ml in men and women, respectively	Plasma, fasting	Plasma, fasting	TC: R = 0.02 (p >0.05) LDL: R = 0.06 (p >0.05) HDL: R = -0.10 (p <0.05) TG: R = 0.08 (p <0.05) Other: NA	Partial Spearman correlation coefficients (R)	Adjusted for age, sex, race, education, smoking, alcohol, activity, menopause, hormone replacement, and dietary intervention group	Large magnitude: yes for HDL Statistical significance: yes for HDL and TG Dose-response: unclear Temporal association: no Subgroup only: no Adjustments: not given	Change in lipid levels during weight loss also given but difficult to interpret Potential weaknesses: Cross-sectional: reverse causation

Reference	Location Years	Design	Who Ages N	Factors related to bias	Exposure method	Outcome method	Results	Comparison	Confounding	Other aspects of causal inference	Notes
Matilla-Santander et al., 2017	Location: Spain Years: 2003-8	Cross-sectional	Who: pregnant women Ages: first trimester N: 1,194	Selection: "population-based" Participation: <55% Equal groups: unclear Above detection: 100% Blinded: unclear Levels: median (IQR) = 2.35 (1.63-3.30) ng/ml	First trimester plasma, non-fasting	First trimester serum, non-fasting	TC: β = 1.26 (0.01-2.54) 3.15 mg/dl difference between quartile 1 and 4 LDL: NA HDL: NA TG: β = -2.78 (-6.15-1.42) with inverted U-shapted dose-response Other: NA	Change in lipid level (mg/dl) per log10 increase in PFOA; PFOA quartiles	Adjusted for sub-cohort, place of birth, BMI, breast-feeding, parity, collection time, activity, and diet	Large magnitude: no Statistical significance: yes for TC Dose-response: log-linear (continuous), flattened (categorical) Temporal association: no Subgroup only: no Adjustments: mixed, effect size decreases in categorical analysis and increases in linear regression	Samples stored at -80º C Potential weaknesses: Inconsistent impact with adjustments Cross-sectional: reverse causation Little increase in effect after quartile 2 Non-fasting
Seo et al., 2018	Location: Korea Years: 2006-15	Cross-sectional	Who: adults Ages: 40-60 N: 786	Selection: residents of Seoul Participation: unclear Equal groups: PFOA and PFOS generally increased with age, and higher in males, lower in obese Blinded: unclear Levels: median = 4.06 ng/ml	Serum, appears non-fasting	Not discussed	*Most findings given in figure form* TC: increase from quartile 1 to 2 then flat (p-trend = 0.29) LDL: inverted U-shaped dose-response (p=0.27) HDL: no association TG: small increase across quartiles (p=0.12) Other: NA	Quartiles of PFOA	Unadjusted	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: unadjusted only	Samples frozen at -20º C and analyzed within 30 days Potential weaknesses: Limited covariates Cross-sectional: reverse causation Fasting unknown
Starling et al., 2017	Location: Colorado Years: 2010-14	Cross-sectional	Who: pregnant women Ages: ≥16 N: 628	Selection: convenience sample from pregnancies at the University of Colorado Hospital Participation: <50% Equal groups: higher PFOA in non-Hispanic whites, higher education, higher income, and fewer prior pregnancies but otherwise similar Blinded: yes Levels: median = 1.1 ng/ml	Serum, fasting	Plasma, fasting	TC: 209.5; 213.4; 215.2 LDL: NA HDL: 62.2; 64.1; 65.3 TG: 159.6; 167.4; 157.8 Other: NA	Mean lipid levels (mg/dl) from 1st to 3rd tertile of PFOA	Unadjusted	Large magnitude: no Statistical significance: no Dose-response: possible for TC and HDL Temporal association: no Subgroup only: no Adjustments: not given	Samples collected at the 24th-30th week of gestation Samples stored at -80º C PFAS highly correlated No lipid data for PFOS Potential weaknesses: Adjusted results not found in article or supplement

Reference	Location Years	Design	Who Ages N	Factors related to bias	Exposure method	Outcome method	Results	Comparison	Confounding	Other aspects of causal inference	Notes
Yang et al., 2018	Location: China Years: 2015	Cross-sectional	Who: males, cases and controls in a study of metabolic syndrome Ages: 19-60 N: 145	Selection: unclear Participation: unclear Equal groups: unclear Blinded: unclear Levels: median = 1.90 ng/ml	Serum, fasting unknown	Blood, fasting unknown	**With metabolic syndrome:** TC: R = -0.052 LDL: R = -0.094 HDL: R = -0.082 TG: R = 0.133 Other: NA **Without metabolic syndrome:** TC: R = -0.272* LDL: R = -0.223 HDL: R = -0.058 TG: R = 0.001 Other: NA * p <0.05, all others >0.05	Spearman correlation coefficients (R)	Unadjusted	Large magnitude: yes for TC in those without metabolic syndrome Statistical significance: yes see above Dose-response: unclear Temporal association: no Subgroup only: without metabolic syndrome Adjustments: not given	Samples stored at -80º C Potential weaknesses: Limited co-variates Fasting unknown
Dong et al., 2019	Location: US NHANES Years: 2003-14	Cross-sectional	Who: adolescents Ages: 12-19 N: 2,947	Selection: randomized cluster sample Participation: unclear Equal groups: unclear Blinded: yes Levels: median = 2.9 ng/ml	Serum, fasting	Blood, fasting	TC: no association LDL: no association HDL: no association TG: NA Other: NA See other table entry for results in adults	Change in lipid level (mg/dl) per ng/ml increase in PFOA (most data in figure form only)	Adjusted for age, gender, race, BMI, income, waist circumference, activity, diabetes, smoking, and alcohol	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: not given	Overlap with other NHANES studies PFOA and PFOS levels decreased over time PFOA and PFOS correlation = 0.69 Reference doses and benchmark doses also calculated Potential weaknesses: Cross-sectional: reverse causation Unclear if adequately adjusted for survey year
Jain and Ducatman (2018a)	Location: US NHANES Years: 2013-4	Cross-sectional	Who: children Ages: 6-11 N: 458	Selection: randomized cluster sample Participation: unclear Equal groups: unclear Blinded: yes Levels: geometric mean = 1.78 ng/ml	Serum, likely fasting	Serum, likely fasting	TC: no association LDL: NA HDL: no association TG: NA Other: non-HDL (TC – HDL), no association	Linear regression with log-lipid	Adjusted for gender, race, age, income, BMI, fasting time, and second hand smoke Little change when adjusted for other PFAS	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: not given	Potential weaknesses: Low lipid levels in children
Domazet et al., 2016	Location: Denmark Years: 1997-2009	Cohort	Who: children and young adults Ages: 9-21 N: 444	Selection: unclear Participation: 48% Equal groups: unclear Blinded: unclear Levels: medians = 2.7-9.7 ng/ml	Plasma, fasting	Plasma, fasting	TC: NA LDL: NA HDL: NA TG: no association Other: NA	Change in outcome for each 10 ng/ml increase in PFOA	Adjusted for age, sex, outcome level at baseline, and ethnicity	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: not given	F/U: 12 years PFOA measured at ages 9 and 15; TG measured at ages 15 and 21 Samples stored at -80º C Tracking coefficients over time: 0.07-0.50 for PFOA Potential weaknesses: Low lipid levels in children

Reference	Location Years	Design	Who Ages N	Factors related to bias	Exposure method	Outcome method	Results	Comparison	Confounding	Other aspects of causal inference	Notes
Fassler et al., 2019	Location: Cincinnati, OH Years: 2004-6	Cross-sectional	Who: girls Ages: 8 N: 353	Selection: recruited from schools and the breast cancer registry (family members) Participation: unclear Equal groups: unclear Blinded: unclear Levels: median = 7.7 ng/ml	Serum, fasting	Plasma, fasting	TC: no association LDL: NA HDL: β = 0.0442 (p=0.0046) No association with structural equation modeling TG: no association Other: NA	Regression coefficient for log PFOA (ng/ml) and log lipid levels (units not given)	Initial models adjusted for age and race; structural equation modeling adjusted for multiple other factors	Large magnitude: no Statistical significance: possible for HDL Dose-response: unclear Temporal association: no Subgroup only: no Adjustments: not given	Samples stored at -80º C Potential weaknesses: Structural equation modeling difficult to understand Inconsistent results between structural equation modeling and linear regressions Cross-sectional: reverse causation Low lipid levels in children
Kang et al., 2018	Location: Korea Years: 2012-4	Cross-sectional	Who: children Ages: 3-18 N: 150	Selection: subgroup from a nationwide survey Participation: unclear Equal groups: unclear Blinded: unclear Levels: median = 1.88 ng/ml	Serum, fasting	Serum, fasting	TC: no association LDL: no association HDL: NA TG: no association Other: NA	Linear regression	Adjusted for age, sex, BMI, second hand smoke, and income	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: not given	Stored at -70º C PFOA highly correlated with other PFAS Potential weaknesses: Low lipid levels in children
Khalil et al., 2018	Location: Dayton, OH Years: 2016	Cross-sectional	Who: obese children Ages: 8-12 N: 48	Selection: Lipid Clinic Dayton Children's Hospital Participation: unclear Equal groups: unclear Blinded: unclear Levels: median = 0.99 ng/ml	Blood, fasting	Blood, fasting	TC: β = 39.9 (8.00-65.7) LDL: β = 35.1 (8.57-61.6) HDL: no association TG: no association Other: NA	Linear regression, not log transformed	Adjusted for age, sex, and ethnicity	Large magnitude: unclear Statistical significance: yes for TC and LDL Dose-response: yes Temporal association: no Subgroup only: no Adjustments: not given	Potential weaknesses: Limited co-variates Cross-sectional: reverse causation
Koshy et al., 2017	Location: New York Years: 2001-12	Cross-sectional	Who: children and adolescents Ages: 10-19 N: 402	Selection: World Trade Center exposure ("cases") and matched controls Participation: <48% Equal groups: unclear Blinded: unclear Levels: 1.39 and 1.81 ng/ml in controls and cases, respectively	Blood, 6 hour fast	Blood, 6 hour fast	TC: β = 0.09 (p <0.001) (9.2% increase) LDL: β = 0.11 (p=0.006) (11.5% increase) HDL: no association TG: β = 0.14 (p=0.03) (15% change) Other: NA	Change in lipid levels and percent changes for each log increase in PFOA	Adjusted for sex, race, calories, activity, cotinine, and BMI	Large magnitude: yes for LDL and TG Statistical significance: yes for TC, LDL, and TG Dose-response: linear Temporal association: no Subgroup only: no Adjustments: only small changes with adjustments	Potential weaknesses: Cross-sectional: reverse causation Low lipid levels in children
Manzano-Salgado et al., 2017	Location: Spain Years: 2003-12	Cohort	Who: children Ages: 4 N: 627	Selection: pregnant women recruited at baseline, methods unclear Participation: <51% Equal groups: unclear Blinded: unclear Levels: geometric mean = 2.32 ng/ml	First trimester maternal plasma, likely non-fasting	Children's blood, non-fasting	TC: no association LDL: no association HDL: see notes TG: no association Other: NA	SDs of lipid level per doubling of PFOA	Adjusted for region, birth place, prior breast-feeding, age, sex, parity, and BMI	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: not given	F/U: 4 years Samples stored at -80º C HDL results for boys are unusual, CI does not include the effect estimate Potential weaknesses: In utero exposure, relevance unknown Non-fasting Low lipid levels in children

Reference	Location Years	Design	Who Ages N	Factors related to bias	Exposure method	Outcome method	Results	Comparison	Confounding	Other aspects of causal inference	Notes
Mora et al., 2018	Location: Boston, MA Years: 1999-2010	Cohort and cross-sectional	Who: children Ages: 7 N: 682	Selection: prenatal visits at a medical group in Boston Participation: <38% Equal groups: unclear Blinded: unclear Levels: median = 5.4 ng/ml	First trimester maternal plasma, non-fasting	Children's fasting plasma	**Prospective (prenatal):** TC: no association LDL: no association HDL: no association TG: no association Other: NA **Cross-sectional:** TC: β = 2.6 (-0.5-5.7) LDL: no association HDL: β = 1.5 (0.1-2.9) TG: no association Other: NA No effect modification by sex	Change in lipid level for each interquartile increase in PFOA	Adjusted for education, smoking, gestational age, sex, race, and child's age Adjusting for income, albumin, marital status, breast-feeding, activity, fast food and soda consumption did not change results	Large magnitude: no Statistical significance: possible for HDL Dose-response: linear Temporal association: no Subgroup only: no Adjustments: not given	F/U: 7 years Potential weaknesses: In utero exposure, relevance unknown Low lipid levels in children
Spratlen et al., 2020	Location: New York Years: 2001-2	Cross-sectional	Who: neonates Ages: 0 N: 222	Selection: World Trade Center cohort Participation: unclear Equal groups: higher total PFAS in Asians, lower education Blinded: unclear Levels: median = 2.46	Cord blood, likely non-fasting	Cord blood, likely non-fasting	TC: no association LDL: NA HDL: NA TG: 0.256% (0.129-0.383) Other: NA	Percent increase in lipid levels per 1% increase in PFOA	Adjusted for maternal age, child sex, maternal education, race, parity, BMI, marital status, smoking, and gestational age	Large magnitude: no Statistical significance: yes for TG Dose-response: unclear Temporal association: no Subgroup only: no Adjustments: not given	Pregnant women 18-39 years old Potential weaknesses: Non-fasting Cross-sectional: reverse causation Low lipid levels in children

Rows are sorted by adult vs. children studies, then by whether the study was in NHANES or not, then by first author
Numbers in parentheses are 95% confidence intervals unless otherwise noted
Ages are in years unless otherwise noted
Abbreviations: β, regression coefficient; BMI, body mass index; CI, confidence interval; F/U: follow-up period; HDL, high density lipoprotein; LDL, low density lipoprotein; N, number of participants; NA, not assessed; NHANES, US National Health and Nutrition Examination Survey; OR, odds ratio; PFAS, per-and polyfluorinated substances; perfluorononanoic acid (PFNA); R, correlation coefficient; SD, standard deviation; SES, socioeconomic status; TC, total cholesterol; TG, triglycerides; VLDL, very low density lipoprotein

Table A7.9. Recent epidemiologic studies of PFOS and lipid levels

Reference	Location Years	Design	Who Ages N	Factors related to bias	Exposure method	Outcome method	Results	Comparison	Confounding	Other aspects of causal inference	Notes
Christen-sen et al., 2019	Location: US NHANES Years: 2007-14	Cross-sectional	Who: adults Ages: ≥20 N: 2,975	Selection: randomized cluster sample Participation: unclear Equal groups: unclear Blinded: yes Levels: median = 8.04 ng/ml	Blood, fasting	Blood, fasting, and use of lipid lowering medication	TC: NA LDL: NA HDL: OR for low HDL = 1.33 (0.80-2.21) TG: OR for high TG = 0.64 (0.37-1.08) Other: NA	ORs for 4th vs. 1st quartile of PFOS	Adjusted for survey year, age, sex, race/ethnicity, income, alcohol, and smoking	Large magnitude: yes for HDL and TG Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: not given	Overlap with other NHANES studies Large reduction in PFOS levels from 2007-8 to 2013-4 Presents results adjusted and unadjusted for NHANES sampling weights "High triglycerides" if TG >150 mg/dl or medication use; "Low HDL" if HDL <30 mg/dl or medication use Potential weaknesses: Low statistical power Cross-sectional: reverse causation
Dong et al., 2019	Location: US NHANES Years: 2003-14	Cross-sectional	Who: adults Ages: 20-80 N: 8,948	Selection: randomized cluster sample Participation: unclear Equal groups: unclear Blinded: yes Levels: median = 10.9 ng/ml	Serum, fasting	Blood, fasting	TC: β = 0.4 (0.06-0.6); means for each quintile are given in their Figure S4 LDL: small increase (figure form only) HDL: no association TG: NA Other: NA See separate entry for results in adolescents	Change in lipid level (mg/dl) per ng/ml increase in PFOS (most data in figure form only)	Adjusted for age, gender, race, BMI, income, waist circumference, activity, diabetes, smoking, and alcohol	Large magnitude: no Statistical significance: yes for TC Dose-response: unclear Temporal association: no Subgroup only: adults (see separate entry for children) Adjustments: not given	Overlap with other NHANES studies PFOA and PFOS levels decreased over time PFOA and PFOS correlation = 0.69 Reference doses and benchmark doses also calculated Potential weaknesses: Cross-sectional: reverse causation Unclear if adequately adjusted for survey year
He et al., 2018	Location: US NHANES Years: 2003-12	Cross-sectional	Who: adults Ages: ≥20 N: 7,904	Selection: randomized cluster sample Participation: unclear Equal groups: unclear Blinded: yes Levels: mean = 14.51-20.80 ng/ml in females and males, respectively	Serum, likely fasting	Serum, likely fasting	TC: 2.28% (0.68-3.90%) and 1.36% (-0.11-2.85%) increase in men and women, respectively LDL: no clear association HDL: 2.51% and 3.08% increase (p <0.05) in men and women, respectively TG: no association Other: NA	Percent change in lipid level for quartile 4 vs. 1 of PFOS	Adjusted for age, race, BMI, education, alcohol, income, and activity Stratified by gender	Large magnitude: no Statistical significance: yes for TC and HDL Dose-response: yes Temporal association: no Subgroup only: no Adjustments: not given	Overlap with other NHANES studies Potential weaknesses: Cross-sectional: reverse causation Not adjusted for survey year
Huang et al., 2018	Location: US NHANES Years: 1999-2014	Cross-sectional	Who: adults Ages: ≥20 N: 10,859	Selection: randomized cluster sample Participation: unclear Equal groups: data only for total PFAS Blinded: yes Levels: median = 12.40 ng/ml	Serum, likely fasting	Serum, likely fasting	TC: R = 0.105 LDL: R = 0.091 HDL: R = -0.013 TG: R = 0.075 Other: NA All p-values <0.01 except HDL	Spearman correlation coefficients (R)	Results appear to be unadjusted	Large magnitude: yes for TC Statistical significance: yes for all except HDL Dose-response: unclear Temporal association: no Subgroup only: no Adjustments: not given	Overlap with other NHANES studies Correlation with PFOA = 0.69 NHANES survey weights used PFOA and PFOS levels declined over time Potential weaknesses: Relatively small effect sizes Limited covariates including survey year Some lipid levels have also declined over time Cross-sectional: reverse causation

Reference	Location Years	Design	Who Ages N	Factors related to bias	Exposure method	Outcome method	Results	Comparison	Confounding	Other aspects of causal inference	Notes
Jain and Ducatman, 2019b	Location: US NHANES Years: 2005-14	Cross-sectional	Who: adults Ages: ≥20 N: 3,629	Selection: randomized cluster sample Participation: unclear Equal groups: unclear Blinded: yes Levels: geometric mean = 9.3 ng/ml	Blood, fasting	Blood, fasting	TC: no association LDL: 0.36% (p=0.04) in obese women, no clear association in non-obese women or in men HDL: no association TG: -0.87% (p <0.01) in obese women, no association in non-obese women or in men Other: NA	Percent change in lipid level for each 10% change in PFOS	Adjusted for race/ethnicity, smoking, age, income, fasting time, lipid lowering medication, activity, survey year, and diet Stratified by obesity and gender	Large magnitude: no Statistical significance: yes for LDL and TG Dose-response: unclear Temporal association: no Subgroup only: yes, obese women Adjustments: not given	Overlap with other NHANES studies Potential weaknesses: Cross-sectional: reverse causation
Liu et al., 2018b	Location: US NHANES Years: 2013-4	Cross-sectional	Who: adults Ages: ≥18 N: 1,871	Selection: randomized cluster sample Participation: unclear Equal groups: mostly, some differences in PFOS by age, race, income, and other factors Blinded: yes Levels: geometric mean = 5.28 ng/ml	Serum, likely fasting	Blood, likely fasting	TC: no association LDL: no association HDL: no association TG: no association Other: apolipoprotein B no association	Change in lipid level (in mg/dl) for each log increase in PFOS	Adjusted for age, gender, ethnicity, smoking, alcohol, income, waist circumference and relevant medications	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: relatively small changes only except decrease for HDL	Overlap with other NHANES studies Potential weaknesses: Cross-sectional: reverse causation
Chen et al., 2019	Location: Hvar, Croatia Years: 2007-8	Cross-sectional	Who: adults Ages: 44-56 N: 122	Selection: unclear Participation: unclear Equal groups: PFOS higher with lower education, otherwise similar Blinded: unclear Levels: geometric mean = 8.91 ng/ml	Plasma, fasting	Plasma, fasting	TC: no association LDL: no association HDL: no association TG: no association Other: NA	Change in lipid levels per one unit increase in ln PFOS, levels unclear	Adjusted for age, sex, SES, smoking, dietary pattern, and activity	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: not given	Potential weaknesses: Low statistical power
Christian-sen et al., 2016	Location: Wisconsin Years: 2012-3	Cross-sectional	Who: male fishermen Ages: ≥50 N: 154	Selection: "interested in future studies" per online survey; flyers Participation: unclear Equal groups: unclear Blinded: unclear Levels: median = 19.00 ng/ml	Serum, fasting unknown	Self-report, "have you ever been told by a doctor you have…high cholesterol"	TC: NA LDL: NA HDL: NA TG: NA Other: OR = 1.02 (1.00-1.04) for ever told by a doctor the participant had high cholesterol	Answered yes vs. no	Adjusted for age, BMI, work status, and alcohol consumption	Large magnitude: no Statistical significance: unclear Dose-response: no Temporal association: no Subgroup only: no Adjustments: not given	Samples stored at -20° C Potential weaknesses: Self-reported non-specific outcome Unusually narrow CI

Reference	Location Years	Design	Who Ages N	Factors related to bias	Exposure method	Outcome method	Results	Comparison	Confounding	Other aspects of causal inference	Notes
Donat-Vargas et al., 2019	Location: Sweden Years: 1990-2013	Cohort	Who: adults Ages: 40-60 at baseline N: 187	Selection: controls in a diabetes case-control study Participation: 56% Equal groups: unclear Blinded: unclear Levels: median = 20 ng/ml	Blood, fasting	Plasma, fasting	TC: no clear association in prospective analysis, possibly some decrease in repeated measures analysis but strong U-shaped dose-response curve LDL: NA HDL: NA TG: β = -0.14 mmol/L (-0.27 to -0.02) decrease for each one SD increase in PFOA Other: NA	Change in lipid level by tertile or per SD increase in PFOS	Adjusted for gender, age, education, sample year, BMI, smoking, alcohol, exercise, and diet	Large magnitude: about a 10% decrease for TG Statistical significance: yes for TG Dose-response: U-shaped Temporal association: yes Subgroup only: no Adjustments: not given	F/U: approximately 10 years PFOA and PFOS levels decreased over time PFOA and PFOS highly correlated Potential weaknesses: Some inconsistency between repeated measures and prospective analysis
Graber et al., 2019	Location: New Jersey Years: 2016-17	Cross-sectional	Who: residents of an area contaminated with PFAS, likely from a nearby manufacturing facility Ages: ≥19 N: 105	Selection: claimants in a class action lawsuit, flers to all local residents Participation: unclear but likely low Equal groups: unclear Blinded: unclear Levels: geometric mean (95% CI) = 5.37 (4.75-6.06) ng/ml	Serum, fasting unknown	Self-reported physician diagnosed "high cholesterol"	TC: NA LDL: NA HDL: NA TG: NA Other: self-reported "high cholesterol" OR = 1.08 (0.98, 1.21)	Unclear	Adjusted for age and BMI	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: OR = 1.10 (0.95, 1.27) after adjustment for other PFAS	PFNA levels in water and serum were high Serum samples were collected and measured as part of a lawsuit, not collected or measured by the researchers Potential weaknesses: Methods are unclear Outcome not verified
Kishi et al., 2015	Location: Hokkaido, Japan Years: 2002-5	Cross-sectional	Who: pregnant women Ages: unknown N: 306	Selection: pregnant women, preterm care and delivery at participating hospital Participation: 28.6% Equal groups: higher PFOS associated with younger age, lower parity, non-smoking, and earlier sampling week Blinded: unclear Levels: median = 5.60 ng/ml	Serum, 23-35 weeks gestation, fasting	Blood, non-fasting	TC: β = -0.130 (-0.253—0.011) Others not assessed	Linear regression with log10 PFOS and log10 TC	Adjusted for age, smoking, alcohol, income, parity, and gestation week	Large magnitude: unclear Statistical significance: yes (decrease) Dose-response: somewhat linear Temporal association: no Subgroup only: no Adjustments: small decrease in magnitude	Sample stored at -80º C Potential weaknesses:

Reference	Location Years	Design	Who Ages N	Factors related to bias	Exposure method	Outcome method	Results	Comparison	Confounding	Other aspects of causal inference	Notes
Lin et al., 2019	Location: 27 US medical centers Years: 1996-2014	Cohort and cross-sectional	Who: adults Ages: ≥25 N: 888	Selection: pre-diabetic adults Participation: unclear Equal groups: unclear Blinded: unclear Levels: median = 27.2 ng/ml	Plasma at baseline 1996-99, fasting unknown	Blood at baseline and annually, fasting	**Cross-sectional:** TC: β = 2.53 (-0.10–5.16) LDL: β = 1.38 (-1.02-3.77) HDL: β = -0.40 (-1.19-0.39) TG: β = 7.75 (0.63-14.88) Other: VLDL β = 1.57 (0.24-2.89) No effect modification by treatment group **Prospective:** Hypercholesterolemia: No association Hypertriglyceridemia: OR = 1.23 (1.03-1.46) per doubling of PFOS Greater effect in the placebo group	Change in lipid level (mg/dl) per doubling of PFOS; ORs	Adjusted for age, sex, race/ethnicity, marital status, education, smoking, alcohol, fat intake, fiber intake, activity, and waist circumference Cross-sectional: excluded people taking lipid lowering medications	Large magnitude: yes for TG Statistical significance: yes for TG and VLDL Dose-response: linear Temporal association: yes Subgroup only: those not on special intervention Adjustments: not given	F/U: 15 years Randomized to lifestyle intervention or placebo Hypercholesterolemia defined by high TC, high LDL, or use of lipid lowering medication Potential weaknesses:
Lin et al., 2020	Location: Taiwan Years: 2009-11	Cross-sectional	Who: adults Ages: 22-63 N: 597	Selection: controls in a coronary heart disease case-control study, recruited from a bulletin board announcement Participation: unclear Equal groups: PFOS higher in men, older age, less education, higher alcohol consumption, otherwise similar Blinded: unclear Levels: geometric mean = 3.77 ng/ml	Blood, fasting unknown	Blood, fasting unknown	TC: NA LDL: β = 5.90 (p=0.002) HDL: β = 1.31 (p=0.023) TG: no association Other: NA	Change in lipid level for each one unit ln increase in PFOS; levels unclear	Adjusted for age, gender, smoking alcohol, education, hypertension, BMI, and diabetes	Large magnitude: unclear Statistical significance: yes for LDL and HDL Dose-response: possibly log-linear Temporal association: no Subgroup only: no Adjustments: little change	Somewhat greater effect sizes in subjects with elevated levels of oxidative stress biomarkers Potential weaknesses: Fasting unknown Cross-sectional: reverse causation
Liu et al., 2018a	Location: Boston, MA Years: 2004-7	Cross-sectional	Who: participants in a weight loss program at baseline Ages: 30-70 N: 621	Selection: convenience sample Participation: unclear Equal groups: unclear Blinded: unclear Levels: median = 27.2 and 22.3 ng/ml in men and women, respectively	Plasma, fasting	Plasma, fasting	TC: R = 0.04 (p >0.05) LDL: R = 0.09 (p <0.05) HDL: R = 0.01 (p >0.05) TG: R = -0.02 (p >0.05) Other: NA	Partial Spearman correlation coefficients (R)	Adjusted for age, sex, race, education, smoking, alcohol, activity, menopause, hormone replacement, and dietary intervention group	Large magnitude: no Statistical significance: yes for LDL Dose-response: unclear Temporal association: no Subgroup only: no Adjustments: not given	Change in lipid levels during weight loss also given but difficult to interpret Potential weaknesses: Cross-sectional: reverse causation
Matilla-Santander et al., 2017	Location: Spain Years: 2003-8	Cross-sectional	Who: pregnant women Ages: ≥16 N: 1,194	Selection: "population-based" Participation: <55% Equal groups: unclear Above detection: 100% Blinded: unclear Levels: geometric mean = 5.77 ng/ml	First trimester plasma, non-fasting	First trimester serum, non-fasting	TC: no association LDL: NA HDL: NA TG: β = -5.86 (-9.91 to -1.63) Other: NA	Change in lipid level (mg/dl) per log10 increase in PFOS; PFOS quartiles	Adjusted for sub-cohort, place of birth, BMI, breast-feeding, parity, collection time, activity, and diet	Large magnitude: no Statistical significance: yes for TG Dose-response: log-linear (continuous), flattened (categorical) Temporal association: no Subgroup only: no Adjustments: small decrease	Samples stored at -80º C Potential weaknesses: Cross-sectional: reverse causation Little increase in effect after quartile 2 Non-fasting

Reference	Location Years	Design	Who Ages N	Factors related to bias	Exposure method	Outcome method	Results	Comparison	Confounding	Other aspects of causal inference	Notes
Seo et al., 2018	Location: Korea Years: 2006-15	Cross-sectional	Who: adults Ages:40-60 N: 786	Selection: residents of Seoul Participation: unclear Equal groups: PFOA and PFOS generally increased with age, and higher in males, lower in obese Blinded: unclear Levels: median = 12.43 ng/ml	Serum, appears non-fasting	Blood, appears non-fasting	*Most findings given in figure form* TC: increase across quartiles (p-trend = 0.05) LDL: increase but mostly from quartile 1 to 2 (p-trend = 0.06) HDL: no association TG: increase across quartiles (p-trend = 0.08) Other: NA	PFOS quartiles	Unadjusted	Large magnitude: unclear Statistical significance: borderline for TC and LDL Dose-response: see results Temporal association: no Subgroup only: no Adjustments: not given	Samples frozen at -20° C and analyzed within 30 days Potential weaknesses: Limited covariates Cross-sectional: reverse causation Non-fasting
Yang et al., 2018	Location: China Years: 2015	Cross-sectional	Who: males, cases and controls in a study of metabolic syndrome Ages: 19-60 N: 144	Selection: unclear Participation: unclear Equal groups: unclear Blinded: unclear Levels: median = 3.00 ng/ml	Serum, fasting unknown	Blood, fasting unknown	**With metabolic syndrome:** TC: R = -0.004 LDL: R = -0.03 HDL: R = 0.02 TG: R = 0.140 Other: NA **Without metabolic syndrome:** TC: R = 0.097 LDL: R = 0.068 HDL: R = 0.142 TG: R = 0.030 Other: NA all p >0.05	Spearman correlation coefficients	Unadjusted	Large magnitude: yes for HDL and TG by subgroup Statistical significance: no Dose-response: unclear Temporal association: no Subgroup only: by metabolic syndrome Adjustments: not given	Samples stored at -80° C Potential weaknesses: Limited co-variates Subgroup inconsistencies Fasting unknown
Dong et al., 2019	Location: US NHANES Years: 2003-14	Cross-sectional	Who: adolescents Ages: 12-19 N: 2,947	Selection: randomized cluster sample Participation: unclear Equal groups: unclear Blinded: yes Levels: median = 9.4 ng/ml	Serum, fasting	Blood, fasting	TC: no association LDL: no association HDL: no association TG: NA Other: NA See separate entry for results in adults	Change in lipid level (mg/dl) per ng/ml increase in PFOS (most data in figure form)	Adjusted for age, gender, race, BMI, income, waist circumference, activity, diabetes, smoking, and alcohol	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: not given	Overlap with other NHANES studies PFOA and PFOS levels decreased over time PFOA and PFOS correlation = 0.69 Reference doses and benchmark doses also calculated Potential weaknesses: Cross-sectional: reverse causation Unclear if adequately adjusted for survey year

Reference	Location Years	Design	Who Ages N	Factors related to bias	Exposure method	Outcome method	Results	Comparison	Confounding	Other aspects of causal inference	Notes
Jain and Ducatman, 2018b	Location: US NHANES Years: 2013-14	Cross-sectional	Who: children Ages: 6-11 N: 458	Selection: randomized cluster sample Participation: unclear Equal groups: unclear Blinded: yes Levels: geometric mean = 2.67 ng/ml	Serum, likely fasting	Serum, likely fasting	TC: β = 0.02738 (p=0.03) β = 0.0439 (p=0.03) when adjusted for other PFAS LDL: NA HDL: no association TG: NA Other: non-HDL (TC – HDL), no association	Linear regression with log-lipid level	Gender, race, age, income, BMI, fasting time, and second hand smoke; other PFAS	Large magnitude: no Statistical significance: yes for TC Dose-response: yes Temporal association: no Subgroup only: no Adjustments: some change when adjusted for other PFAS	Overlap with other NHANES studies Potential weaknesses: Low lipid levels in children Small effect size: β = 0.02738 is a 0.03% change per 10% change in PFOS Concerns about collinearity in model adjusted for other PFAS Cross-sectional: reverse causation
Domazet et al., 2016	Location: Denmark Years: 1997-2009	Cohort	Who: children and young adults Ages: 9-21 N: 444	Selection: unclear Participation: 48% Equal groups: unclear Blinded: unclear Levels: median = 9.1-44.5 ng/ml (medians vary by sex and age)	Plasma, fasting	Plasma, fasting	TC: NA LDL: NA HDL: NA TG: no association Other: NA	Change in lipid level for each 10 ng/ml increase in PFOS	Adjusted for age, sex, outcome level at baseline, and ethnicity	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: not given	F/U: 12 years PFOS measured at ages 9 and 15 TGs measured at ages 15 and 21 Samples stored at -80º C Tracking coefficients over time: 0.43-0.69 Potential weaknesses: Low lipid levels in children
Fassler et al., 2019	Location: Cincin-nati, OH Years: 2004-06	Cross-sectional	Who: girls Ages: 8 N: 353	Selection: recruited from schools and the breast cancer registry (family members) Participation: unclear Equal groups: unclear Blinded: unclear Levels: median = 13.6 ng/ml	Serum, fasting	Plasma, fasting	TC: no association LDL: NA HDL: no association TG: no association Other: NA Actual coefficients not given	Linear regression coefficients	Initial models adjusted for age and race. Structural equation modeling adjusted for multiple other factors	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: not given	Samples stored at -80º C Potential weaknesses: Structural equation modeling difficult to understand Cross-sectional: reverse causation Low lipid levels in children
Kang et al., 2018	Location: Korea Years: 2012-14	Cross-sectional	Who: children Ages: 3-18 N: 150	Selection: subgroup from a nationwide survey Participation: unclear Equal groups: unclear Blinded: unclear Levels: median = 5.68 ng/ml	Serum, fasting	Serum, fasting	TC: no association LDL: no association HDL: NA TG: no association Other: NA	Linear regression	Adjusted for age, sex, BMI, second hand smoke, and income	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: not given	Stored at -70º C PFOA highly correlated with other PFAS Potential weaknesses: Low lipid levels in children
Khalil et al., 2018	Location: Dayton, OH Years: 2016	Cross-sectional	Who: obese children Ages: 8-12 N: 48	Selection: Lipid Clinic Dayton Children's Hospital Participation: unclear Equal groups: unclear Blinded: unclear Levels: median = 2.79 ng/ml	Blood, fasting	Blood, fasting	TC: β = 5.63 (-0.11 to 11.4) LDL: β = 5.70 (0.458 to 10.9) HDL: no association TG: no association Other: NA	Linear regression, not log transformed	Adjusted for age, sex, and ethnicity	Large magnitude: unclear Statistical significance: yes for LDL Dose-response: yes Temporal association: no Subgroup only: no Adjustments: not given	Potential weaknesses: Other potential confounders Cross-sectional: reverse causation

Reference	Location Years	Design	Who Ages N	Factors related to bias	Exposure method	Outcome method	Results	Comparison	Confounding	Other aspects of causal inference	Notes
Koshy et al., 2017	Location: New York Years: 2001-12	Cross-sectional	Who: children and adolescents Ages:10-19 N: 402	Selection: World Trade Center exposure ("cases") and matched controls Participation: <48% Equal groups: unclear Blinded: unclear Levels: 2.78 and 3.72 ng/ml in controls and cases, respectively	Blood, 6 hour fast	Blood, 6 hour fast	TC: β = 0.08 (p <0.001) (8.5% increase) LDL: β = 0.10 (p <0.001) (10.7% increase) HDL: β = 0.06 (p=0.04) (6.6% increase) TG: no association Other: NA	PFOS and lipids were log transformed; percent change in lipid level for each log increase in PFOS	Adjusted for sex, race, calories, activity, cotinine, and BMI	Large magnitude: yes for LDL Statistical significance: yes for TC, LDL, and HDL Dose-response: linear Temporal association: no Subgroup only: no Adjustments: only small changes with adjustments	Potential weaknesses: Cross-sectional: reverse causation Low lipid levels in children
Manzano-Salgado et al., 2017	Location: Spain Years: 2003-12	Cohort	Who: children Ages: 4 N: 627	Selection: pregnant women recruited at baseline, methods unclear Participation: <51% Equal groups: unclear Blinded: unclear Levels: geometric mean = 5.80 ng/ml	First trimester maternal plasma, likely non-fasting	Children's blood, non-fasting	TC: no association LDL: no association HDL: no association TG: no association Other: NA	SD of lipid level per doubling of PFOS	Adjusted for region, birth place, prior breast-feeding, age, sex, parity, and BMI	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: not given	Samples stored at -80º C Potential weaknesses: In utero exposure, relevance unknown Non-fasting Low lipid levels in children
Mora et al., 2018	Location: Boston, MA Years: 1999-2010	Cohort and cross-sectional	Who: children Ages: 7 N: 682	Selection: prenatal visits at a medical group in Boston Participation: <38% Equal groups: unclear Blinded: unclear Levels: median = 5.4 ng/ml	First trimester maternal plasma, non-fasting	Children's fasting plasma	**Prospective (prenatal):** TC: no association LDL: no association HDL: no association TG: no association Other: NA **Cross-sectional:** TC: β = 1.8 (-0.2-3.7) LDL: no association HDL: β = 1.5 (0.4-2.5) TG: β = -2.5 (-4.3 to – 0.6) Other: NA No effect modification by sex	Change in lipid level for each interquartile increase in PFOS	Adjusted for education, smoking, gestational age, sex, race, and child's age Adjusting for income, albumin, marital status, breast-feeding, activity, fast food and soda consumption did not change results	Large magnitude: no Statistical significance: yes for HDL and TG in cross-sectional analysis Dose-response: linear Temporal association: no Subgroup only: no Adjustments: not given	Potential weaknesses: In utero exposure, relevance unknown Low lipid levels in children Some differences in cross-sectional and prospective results
Spratlen et al., 2020	Location: New York Years: 2001-2	Cross-sectional	Who: neonates Ages: 0 N: 222	Selection: World Trade Center cohort Participation: unclear Equal groups: higher total PFAS in Asians, lower education Blinded: unclear Levels: median = 2.46	Cord blood, likely non-fasting	Cord blood, likely non-fasting	TC: no association LDL: NA HDL: NA TG: no association Other: NA	Percent increase in lipid level per 1% increase in PFOS	Adjusted for maternal age, child sex, maternal education, race, parity, BMI, marital status, smoking, and gestational age	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: not given	Pregnant women 18-39 years old Potential weaknesses: Non-fasting Low lipid levels in children

Numbers in parentheses are 95% confidence intervals unless otherwise noted
Ages are in years unless otherwise noted
Abbreviations: β, regression coefficient; BMI, body mass index; CI, confidence interval; F/U: follow-up period; HDL, high density lipoprotein; LDL, low density lipoprotein; N, number of participants; NA, not assessed; NHANES, US National Health and Nutrition Examination Survey; OR, odds ratio; PFAS, per-and polyfluorinated substances; perfluorononanoic acid (PFNA); R, correlation coefficient; SD, standard deviation; SES, socioeconomic status; TC, total cholesterol; TG, triglycerides; VLDL, very low density lipoprotein

As shown in Tables A7.8 and A7.9, there is a relatively large number of studies in children, as well as a relatively large number of studies that used data from NHANES. Overall, the results of these studies appeared to be somewhat heterogeneous. In order to help explore and identify the possible sources of this heterogeneity, these studies were divided into several categories (i.e., children vs. adults, NHANES vs. non-NHANES studies), and study quality summary tables (Tables A7.11 and A7.12) were created, with key study characteristics, results, and factors potentially related to study quality for each of these categories. The characteristics and study quality factors, as well as the codes used in these summary tables are shown in Table A7.10.

Table A7.10. Characteristics and codes for the study quality summary tables of recent epidemiologic studies of PFOA or PFOS and lipids

Category	Label	Definition	Codes
General	Age	Ages of the study participants	A for adults, otherwise age in years
	N	Number of participants in the study	
Results	TC	Result for total cholesterol	+: Statistically significant positive association[1] (+): Positive association but not statistically[2] significant -: Statistically significant negative association[3] (-): Negative association but not statistically significant[2] 0: No association U: unclear
	LDL	Result for low density lipoprotein	Same as above
	HDL	Result for high density lipoprotein	Same as above
	TG	Result for triglycerides	Same as above
Quality scoring factors	Design	Study design	XS: Cross-sectional CO: Prospective cohort study
	Selection	Participant selection	1: Population based selection 0: Convenience sample or not clear
	Demo	Was demographic information compared and mostly similar across PFOA/PFOS levels?	1: Yes or differences were adjusted for 0: No
	Blind	Were the study personnel assessing the exposures or the outcomes blinded?	1: Yes, reported 0: No or not reported
	Range	Range of PFOA or PFOS levels	1: Wide range of PFOA or PFOS exposure levels (this includes occupational studies or communities with known industrial contamination) 0: Population based studies with no known high exposure source
	Exposure	How was PFOA or PFOS exposure assessed?	SB: A single blood measurement MB: Multiple blood measurements U: Unknown or not mentioned 0: Other metrics including modeling of environmental exposure or ecologic assessment
	Outcome	How was the outcome (lipid levels) assessed?	SB: A single blood measurement MB: Multiple blood measurements M: Use of a lipid lowering medication or physician's diagnosis of abnormal lipid levels U: Unknown or not mentioned 0: Other

Category	Label	Definition	Codes
	Fasting	If serum, were samples collected after fasting?	1: Yes, fasting blood lipid level, or exposure assessed by a method not impacted by fasting (e.g., modeled PFOA serum levels) 0: No or not mentioned
	Age/Sex	Were the results adjusted for or otherwise controlled for age and sex?	1: Yes 0: No
	Diet/BMI	Were the results adjusted for some aspect of diet or BMI?	1: Yes (e.g., BMI, waist circumference, calorie intake, diet patterns, fat intake) 0: No
	SES	Were the results adjusted for some aspect of socioeconomic status?	1: Yes, including poverty indices, income, or education 0: No
	Year	If a cross-sectional study >3 years, were the results adjusted for possible changes in PFAS and lipid levels over time?	1: Yes, cross-sectional and not >3 years, or prospective design 0: No Note: this primarily applies to multi-year NHANES studies
	Clear Res	Were the results presented clearly and were they consistent across different analyses?	1: Yes 0: No
	Overall	Overall quality score (range 0-13)	Sum of the individual quality scoring factors listed above with prospective cohort studies receiving a score of 1 and cross-sectional studies receiving a score of 0; and exposure and outcome ratings of MB or SB receiving a quality score of 1 and all other metrics a quality score of 0
Overlap		Did participants of the study overlap with those of another study?	Y: Yes N: No

1. A statistically significant association between increasing PFOA or PFOS exposures and increasing lipid levels
2. Generally includes results that are not statistically significant but where effect sizes are >10 percent
3. A statistically significant association between increasing PFOA or PFOS exposures and decreasing lipid levels

Table A7.11. Study quality summary of recent epidemiologic studies of PFOA and lipid levels

General			Results				Quality scoring															
Reference	Age	N	TC	LDL	HDL	TG	Design	Selection	Demo	Blind	Range	Exposure	Outcome	Fasting	Age/Sex	Diet/BMI	SES	Year	Clear res	Overall	Overlap	Notes
ADULTS NHANES:																						
Christensen et al., 2019	A	2,975			(-)	(+)	XS	1	0	1	L	SB	SB, M	1	1	0	1	1	1	9	Y	NHANES 2007-14
Dong et al., 2019	A	8,948	+	(+)	0		XS	1	0	1	L	SB	SB	1	1	1	1	0	1	9	Y	NHANES 2003-14
He et al., 2018	A	7,904	+	(+)	0	(+)	XS	1	0	1	L	SB	SB	1	1	1	1	0	1	9	Y	NHANES 2003-12
Huang et al., 2018	A	10,859	+	+	-	+	XS	1	0	1	L	SB	SB	1	0	0	0	0	1	6	Y	NHANES 1999-2014; correlations
Jain & Ducatman, 2019b	A	3,629	+	+	0	0	XS	1	0	1	L	SB	SB	1	1	1	1	1	1	10	Y	NHANES 2005-14; in obese women
Liu et al., 2018b	A	1,871	+	(+)	+	0	XS	1	1	1	L	SB	SB	1	1	1	1	1	1	11	Y	NHANES 2013-4
ADULTS OTHER:																						
Chen et al., 2019	A	122	U	0	0	0	XS	0	1	0	L	SB	SB	1	1	1	1	1	0	8	N	Differences in ORs vs. regressions
Christiansen et al., 2016	A	154	U	U	U	U	XS	0	0	0	L	SB	M	0	1	1	0	1	0	4	N	Self-reported high cholesterol: no association
Donat-Vargas et al., 2019	A	187	U			(-)	CO	0	0	0	L	SB	SB	1	1	1	1	1	0	8	N	Differences prospective vs. repeated measures
Graber et al., 2019	A	105	U				XS	0	0	0	L	SB	M	0	0	0	0	1	0	3	N	Self-reported high cholesterol: no association
Kishi et al., 2015	A	306	0				XS	0	1	0	L	SB	SB	0	1	0	1	1	1	5	N	
Lin et al., 2019	A	888	+	+	(-)	+	CO	0	0	0	L	SB	SB, M	1	1	1	1	1	1	9	N	Similar CO and XS results
Lin et al., 2020	A	597		+	0	+	XS	0	1	0	L	SB	SB	0	1	1	1	1	1	8	N	
Liu et al., 2018a	A	621	0	0	-	+	XS	0	0	0	L	SB	SB	1	1	U	1	1	1	7	N	Correlations
Matilla-Santander et al., 2017	A	1,194	+			U	XS	1	0	0	L	SB	SB	0	0	1	0	1	0	5	N	U-shaped dose-response for TGs
Seo et al., 2018	A	786	U	U	0	(+)	XS	0	0	0	L	SB	U	0	0	0	0	0	0	1	N	Unusual dose-response
Starling et al., 2017	A	628	(+)		0	0	XS	0	0	1	L	SB	SB	1	0	0	0	1	0	5	N	Pregnant women
Yang et al., 2018	A	145	-	(-)	0	(+)	XS	0	0	0	L	SB	SB	0	0	0	0	1	0	3	N	In subgroups only
CHILDREN NHANES:																						
Dong et al., 2019	12-19	2,947	0	0	0		XS	1	0	1	L	SB	SB	1	1	1	1	0	1	9	Y	NHANES 2003-14
Jain & Ducatman, 2018b	6-11	458	0		0		XS	1	0	1	L	SB	SB	1	1	1	1	1	1	10	Y	NHANES 2013-14
CHILDREN OTHER:																						
Domazet et al., 2016	9-21	444				0	CO	0	0	0	L	SB	SB	1	1	0	0	1	1	7	N	
Fassler et al., 2019	8	353	0		U	0	XS	0	0	0	L	SB	SB	1	1	1	1	1	0	7	N	Differences with structural equation models
Kang et al., 2018	3-18	150	0	0		0	XS	1	0	0	L	SB	SB	1	1	1	1	1	1	9	N	
Khalil et al., 2018	8-12	48	+	+	0	0	XS	0	0	0	L	SB	SB	1	1	0	0	1	1	6	N	
Koshy et al., 2017	10-19	402	+	+	0	+	XS	0	0	0	L	SB	SB	1	0	1	0	1	1	6	N	
Manzano-Salgado et al., 2017	4	627	0	0	U	0	CO	0	0	0	L	SB	SB	0	1	1	0	1	1	7	N	
Mora et al., 2018	7	682	U	0	U	0	CO	0	0	0	L	SB	SB	1	1	1	1	1	0	8	N	XS results: positive for TC and HDL
Spratlen et al., 2020	0	222	0			+	XS	0	1	0	L	SB	SB	0	1	1	1	1	1	8	N	

See Table A7.10 for abbreviations
Empty cells (darkened) indicate that no data was available for this variable

Table A7.12. Study quality summary of recent epidemiologic studies of PFOS and lipid levels

General			Results				Quality scoring															
Reference	Age	N	TC	LDL	HDL	TG	Design	Selection	Demo	Blind	Range	Exposure	Outcome	Fast	Age/Sex	Diet/BMI	SES	Year	Clear res	Overall	Overlap	Notes
ADULTS NHANES:																						
Christensen et al., 2019	A	2,975			(+)	(-)	XS	1	0	1	L	SB	SB, M	1	1	0	1	1	1	9	Y	NHANES 2007-14
Dong et al., 2019	A	8,948	+	(+)	0		XS	1	0	1	L	SB	SB	1	1	1	1	0	1	9	Y	NHANES 2003-14
He et al., 2018	A	7,904	+	0	+	0	XS	1	0	1	L	SB	SB	1	1	1	1	0	1	9	Y	NHANES 2003-12
Huang et al., 2018	A	10,859	+	+	0	+	XS	1	0	1	L	SB	SB	1	0	0	0	0	1	6	Y	NHANES 1999-2014; correlations
Jain & Ducatman, 2019b	A	3,629	0	+	0	-	XS	1	0	1	L	SB	SB	1	1	1	1	1	1	10	Y	NHANES 2005-14; in obese women
Liu et al., 2018b	A	1,871	0	0	0	0	XS	1	1	1	L	SB	SB	1	1	1	0	1	1	10	Y	NHANES 2013-4
ADULTS OTHER:																						
Chen et al., 2019	A	122	0	0	0	0	XS	0	1	0	L	SB	SB	1	1	1	1	1	0	8	N	Differences in ORs vs. regressions
Christiansen et al., 2016	A	154	U	U	U	U	XS	0	0	0	L	SB	M	0	1	1	0	1	0	4	N	Self-reported high cholesterol: unusual results
Donat-Vargas et al., 2019	A	187	U			-	CO	0	0	0	L	SB	SB	1	1	1	1	1	0	8	N	Differences prospective vs. repeated measures
Graber et al., 2019	A	105	U				XS	0	0	0	L	SB	M	0	0	0	0	1	0	3	N	Self-reported high cholesterol
Kishi et al., 2015	A	306	-				XS	0	1	0	L	SB	SB	0	1	0	1	1	1	5	N	
Lin et al., 2019	A	888	(+)	(+)	(-)	+	CO	0	0	0	L	SB	SB, M	1	1	1	1	1	1	9	N	Some differences between CO and XS results
Lin et al., 2020	A	597		+	+	0	XS	0	1	0	L	SB	SB	0	1	1	1	1	1	8	N	
Liu et al., 2018a	A	621	0	+	0	0	XS	0	0	0	L	SB	SB	1	1	U	1	1	1	7	N	Correlations
Matilla-Santander et al., 2017	A	1,194	0			-	XS	1	0	0	L	SB	SB	0	0	1	0	1	0	5	N	Unusual dose-response
Seo et al., 2018	A	786	+	(+)	0	(+)	XS	0	0	0	L	SB	U	0	0	0	0	0	0	1	N	
Yang et al., 2018	A	145	0	0	(+)	(+)	XS	0	0	0	L	SB	SB	0	0	0	0	1	0	3	N	In subgroups only
CHILDREN NHANES:																						
Dong et al., 2019	12-19	2,947	0	0	0		XS	1	0	1	L	SB	SB	1	1	1	1	0	1	9	Y	NHANES 2003-14
Jain and Ducatman, 2018b	6-11	458	+		0		XS	1	0	1	L	SB	SB	1	1	1	1	1	1	10	Y	NHANES 2013-14
CHILDREN OTHER:																						
Domazet et al., 2016	9-21	444				0	CO	0	0	0	L	SB	SB	1	1	0	0	1	1	7	N	
Fassler et al., 2019	8	353	0		0	0	XS	0	0	0	L	SB	SB	1	1	1	1	1	0	7	N	Differences with structural equation models
Kang et al., 2018	3-18	150	0	0		0	XS	1	0	0	L	SB	SB	1	1	1	1	1	1	9	N	
Khalil et al., 2018	8-12	48	(+)	+	0	0	XS	0	0	0	L	SB	SB	1	1	0	0	1	1	6	N	
Koshy et al., 2017	10-19	402	+	+	+	0	XS	0	0	0	L	SB	SB	1	0	1	0	1	1	6	N	
Manzano-Salgado et al., 2017	4	627	0	0	0	0	CO	0	0	0	L	SB	SB	0	1	1	0	1	1	7	N	
Mora et al., 2018	7	682	0	0	U	U	CO	0	0	0	L	SB	SB	1	1	1	1	1	0	8	N	XS results: positive for HDL, negative for TG
Spratlen et al., 2020	0	222	0			0	XS	0	1	0	L	SB	SB	0	1	1	1	1	1	8	N	

See Table A7.10 for abbreviations
Empty cells (darkened) indicate that no data was available for this variable

Developmental and Reproductive Toxicity

Literature search and methods

In addition to reviewing the results of previous reviews by the US EPA (US EPA, 2016b; 2016d), ATSDR (2018a), and others, OEHHA searched for all new human epidemiologic studies on PFOA or PFOS and developmental and reproductive toxicity (DART) published since the 2016 US EPA reviews. The search for literature on epidemiologic studies of DART effects of PFOA and PFOS exposure was performed sequentially in four bibliographic databases: PubMed, Embase, Scopus and SciFinder. The search in each database was limited to 1/1/2016 to 12/31/19. In PubMed the DART[sb] search (shown below) strategy was used to restrict to DART studies. The DART[sb] strategy was translated to the appropriate syntax for use in the other databases.

PubMed Search Strategy

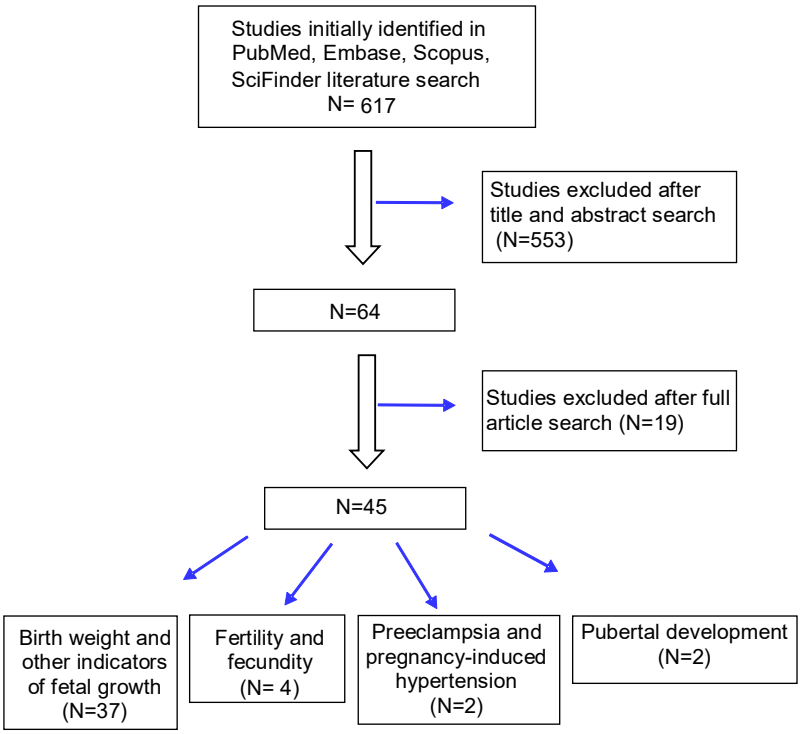
1	(“perfluorooctane sulfonic acid”[nm] OR PFOS [Tiab] OR “perfluoroalkyl sulphonate”[tiab] OR “perfluoro-n-octanesulfonic” [Tiab] OR “perfluoroctane sulfonic” [Tiab] OR “perfluorooctane sulfonic” [Tiab] OR perfluoroctanesulfonic [Tiab] OR perfluorooctanesulfonic [Tiab] OR “perfluorooctane sulphonic” [Tiab] OR “perfluoroctane sulphonic” [Tiab] OR perfluoroctanesulphonic [Tiab] OR perfluorooctanesulphonic [Tiab] OR “perfluorooctane sulfonate” [Tiab] OR “perfluoroctane sulfonate” [Tiab] OR perfluorooctanesulfonate [Tiab] OR perfluoroctanesulfonate [Tiab] OR “perfluorooctane sulphonate” [Tiab] OR “perfluoroctane sulphonate” [Tiab] OR perfluoroctanesulphonate [Tiab] OR perfluorooctanesulphonate [Tiab] OR “perfluorooctanyl sulfonate” [Tiab] OR “perfluorooctanyl sulphonate” [Tiab] OR perfluoroctylsulfonic [Tiab] OR “heptadecafluoro-1-octanesulfonic” [Tiab] OR “heptadecafluoro-1-octane sulfonic” [Tiab] OR “heptadecafluorooctane sulfonic” [Tiab] OR “heptadecafluoroctane sulfonic” [Tiab] OR “heptadecafluorooctane sulfonic” [Tiab] OR heptadecafluorooctanesulfonic [Tiab] OR “heptadecafluorooctane-1-sulphonic” [Tiab] OR “heptadecafluorooctane sulphonic” [Tiab] OR “1-octanesulfonic acid” [Tiab] OR “1-octanesulphonic acid” [Tiab] OR “1-perfluoroctanesulfonic” [Tiab] OR “1-perfluorooctanesulfonic [Tiab]” OR “octanesulfonic acid” [Tiab] OR “octanesulphonic acid” [Tiab] OR "ammonium perfluorosulfonate"[tiab] OR 1763-23-1 [rn] OR 2795-39-3 [rn] OR 29081-56-9 [rn] OR 29457-72-5 [rn] OR 4021-47-0 [rn] OR 70225-14-8 [rn] OR 307-35-7 [rn] OR 56773-42-3[rn] OR 251099-16-8[rn]) OR (“perfluorooctanoic acid”[nm] OR PFOA [Tiab] OR PFAA* [Tiab] OR APFO [Tiab] OR “fluorinated surfactants” [Tiab] OR fluorosurfactant* [Tiab] OR “fluorinated polymer*” [Tiab] OR (fluorinated [Tiab] AND (polymer [Tiab] OR polymers [Tiab])) OR (fluorocarbon [Tiab] AND (polymer [Tiab] OR polymers [Tiab])) OR fluoropolymer* [Tiab] OR (fluorinated [Tiab] AND telomer* [Tiab]) OR fluorotelomer* [Tiab] OR fluoro-telomer* [Tiab] OR fluorotelomer alcohol*[tiab] OR “telomer alcohol*” [Tiab] OR “polyfluoroalkyl* ” [Tiab] OR “N-ethyl perfluorooctanesulfonamido ethanol” [Tiab] OR “N-ethyl perfluorooctanesulfonamidoethanol” [Tiab] OR “N-EtFOSE” [Tiab] OR perfluoroalkyl* [Tiab] OR perfluorocarbon* [Tiab] OR perfluorocarboxyl* [Tiab] OR perfluorochemical* [Tiab] OR “perfluorinated*” [Tiab] OR (perfluorinated [Tiab] AND (C8 [Tiab] OR carboxylic [Tiab] OR chemical* [Tiab] OR compound* [Tiab] OR octanoic [Tiab])) OR (PFO [Tiab] AND (perfluoroalk* [Tiab] OR perfluorocarb* [Tiab] OR perfluorinat* [Tiab] OR perfluorooc* [Tiab])) OR (C8 [Tiab] AND (perfluoroalk* [Tiab] OR perfluorocarb* [Tiab] OR perfluorinat* [Tiab] OR perfluorooc* [Tiab])) OR perfluoroctanoic [Tiab] OR perfluorooctanoic [Tiab] OR “perfluoro octanoic” [Tiab] OR “perfluoro-n-octanoic” [Tiab] OR “perfluorinated octanoic acid” [Tiab] OR perfluorooctanoate [Tiab] OR perfluoroctanoate [Tiab] OR “perfluoro octanoate” [Tiab] OR perfluoroheptanecarboxylic [Tiab] OR “perfluoro-1-heptanecarboxylic” [Tiab] OR perfluorocaprylic [Tiab] OR perfluorocaprilate [Tiab] OR perfluorocaprylate [Tiab] OR	PFOS & PFOA terms

	perfluoroacrylate [Tiab] OR "perfluorooctanoyl chloride" [Tiab] OR "pentadecafluoro-1-octanoic" [Tiab] OR "pentadecafluoro-n-octanoic" [Tiab] OR pentadecafluorooctanoate* [Tiab] OR pentadecafluoroctanoate* [Tiab] OR pentadecafluoroctanoic [Tiab] OR pentadecafluorooctanoic [Tiab] OR fluorad [Tiab] OR "FC 143" [Tiab] OR FC143 [Tiab] OR 335-67-1 [rn] OR 3825-26-1 [rn] OR 335-95-5 [rn] OR 2395-00-8 [rn] OR 90480-56-1[rn] OR 335-93-3[rn])	
2	dart[sb]	DART terms
3	#1 AND #2	Combine Chemicals & DART
4	limit to 2016 to present	Add Date Limit

Results

A general description of OEHHA's literature search is shown in Figure A7.5. A list of studies excluded based on OEHHA's abstract or full article review is provided in Table A7.28.

Figure A7.5. Literature search: recent epidemiologic studies of PFOA or PFOS and developmental and reproductive toxicity*



*This figure is provided to document OEHHA's PubMed, Embase, Scopus, SciFinder. It does not include relevant publications identified from other sources such as previously published reviews from other agencies or other authors.

OEHHA identified 45 studies of PFOA or PFOS and developmental and reproductive toxicity published since January 2016. Two studies reported information on pubertal development, two on preecamplsia and pregnancy-induced hypertension, four on fertility and fecundity, and 37 studies reported information on birth weight and other indicators of fetal growth. Further details of the methods and results of these studies are shown in Tables A7.13-20.

Table A7.13. Recent epidemiologic studies on PFOA and preeclampsia and gestational hypertension

Author Year	Location Years	Design	Who N	Factors related to bias	Exposure method	Outcome	Outcome method	Results	Comparison	Confounding	Other aspects of causal inference	Notes
Wikstrom et al., 2019	Sweden 2007-2010	Prospective cohort	N=1,773 pregnant women from Varmland County (SELMA Pregnancy Cohort) N=64 cases (3.6%) N=42 cases among nulliparous	Selection: unclear Participation: unclear Equal groups: unclear Blinded: unclear Levels: median (IQR): 1.61 (1.12-2.31) ng/ml	Serum PFAS in early pregnancy (median 10 weeks gestation)	Preeclamp-sia	National birth registry	OR=2.40 (0.95-6.06) for highest quartile of serum PFOA compared to lowest All women OR=1.31 (0.93-1.87) Per unit increase in PFOA (log base 2)* Nulliparous women OR=1.38 (0.90-2.21) for serum PFOA (log base 2)* *one unit increase in log base 2 concentrations is a doubling of exposure	OR for highest vs. lowest exposure quartile OR per doubling in PFOA exposure	Adjusted for parity (except when stratified by parity), age, weight, smoking (serum cotinine) Twin pregnancies, education level, and month of sampling were not confounders	Magnitude: yes Statistical significance: no Dose-response: risk increased to a similar degree in 2nd-4th quartiles Temporal association: yes Subgroup only: no Adjustments: little change in nulliparous women. Unadjusted OR for preeclampsia in all women was 1.53 (1.13- 2.07).	Analyses were for individual PFAS exposures. Correlation between PFOA (log base 2) and PFOS (log base 2): r=0.60; correlations between PFOA and PFNA and PFDA were 0.66 and 0.72, respectively. PFNA was associated with preeclampsia: All women OR=1.38 (1.01-1.89) Nulliparous women OR=1.50 (1.04-2.16)
Huang et al., 2019	Shanghai, China 2011-2012	Cross-sectional	N=674 Women with singleton pregnancies who came to one of two hospitals to deliver	Selection: unclear Participation: unclear Equal groups: unclear Blinded: unclear Levels: median (IQR): 6.98 (4.95-9.54) ng/ml Sample selection was not described	Cord blood samples at delivery	Gestational hyper-tension Preeclamp-sia	Medical records	Preeclampsia ORs Tertile 2: 2.23 (0.67-7.44) Tertile 3: 1.41 (0.38-5.14) Continuous*: 1.12 (0.68-1.84) Gestational hypertension ORs Tertile 2: 0.33 (0.10-1.11) Tertile 3: 0.77 (0.30-2.01) Continuous*: 0.95 (0.61-1.48) PFOA was not selected in elastic net regression to select exposures related to preeclampsia or gestational hypertension *Continuous exposure: ln-transformed, centered, and standardized with 1 SD	ORs for 2nd and 3rd tertile of exposure compared to 1st tertile ORs per unit increase in ln-transformed, centered, and standardized exposure	Selected covariates based on a directed acyclic graph: age, education level, parity, pre-pregnancy BMI	Preeclampsia Magnitude: yes Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: unclear Gestational hypertension Magnitude: yes Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: unclear	Prevalence of smoking was 1.5% before and 0.4% during pregnancy, so was not included as a confounder (authors did not state whether based on self-report) Overall risk of preeclampsia was 2.8% and gestational hypertension 3.3% Correlation between PFOA and PFOS was 0.33, and with other PFAS ranged from 0.12 to 0.61

Abbreviations and units: β, regression coefficient; BMI, body mass index (kilogram/meter^2); IQR, interquartile range; ln, natural logarithm; N, number of participants; ng/ml, nanograms/milliliter; OR, odds ratio; PFAS, per-and polyfluoroalkyl substances; PFDA, perfluorodecanoic acid; PFNA, perfluorononanoic acid; r, correlation coefficient; SD, standard deviation; SES, socioeconomic status

Table A7.14. Recent epidemiologic studies on PFOS and preeclampsia and gestational hypertension

Author Year	Location Years	Design	Who N	Factors related to bias	Exposure method	Outcome	Outcome method	Results	Comparison	Confounding	Other aspects of causal inference	Notes
Wikstrom et al., 2019	Sweden 2007-2010	Prospective cohort	N=1,773 pregnant women from Varmland County (SELMA Pregnancy Cohort) N=64 cases (3.6%) N=42 cases among nulliparous	Selection: unclear Participation: unclear Equal groups: unclear Blinded: unclear Levels: Median (IQR) 5.39 (3.95-7.61) ng/ml	Serum PFAS in early pregnancy (median 10 weeks gestation)	Preeclamp-sia	National birth registry	OR=2.68 (1.17-6.12) for highest quartile of serum PFOS compared to lowest All women OR=1.53 (1.07-2.20) for serum PFOS (log base 2*) Nulliparous women OR=2.02 (1.26-3.29) for serum PFOS (log base 2*) *one unit increase in log base 2 concentrations is a doubling of exposure	OR for highest vs. lowest exposure quartile OR per doubling in PFOA exposure	Adjusted for parity (except when stratified by parity), age, weight, smoking (serum cotinine) Twin pregnancies, education level, and month of sampling were not confounders	Magnitude: yes Statistical significance: yes Dose-response: unclear Temporal association: yes Subgroup only: no Adjustments: little change in nulliparous women. Unadjusted OR for preeclampsia in all women was 1.74 (1.23-2.46).	Analyses were for individual PFAS exposures. Correlation between PFOA (log base 2) and PFOS (log base 2): r=0.60; Correlations between PFOS and PFNA and PFDA were 0.55 and 0.57, respectively. PFNA was associated with preeclampsia: All women OR=1.38 (1.01–1.89) Nulliparous women 1.50 (1.04–2.16)
Huang et al., 2019	Shanghai, China 2011-2012	Cross-sectional	Women with singleton pregnancies who came to one of two hospitals to deliver N=674	Selection: unclear Participation: unclear Equal groups: unclear Blinded: unclear Levels: Median (IQR) 2.38 (1.81-3.23) ng/ml	Cord blood samples at delivery	Preeclamp-sia Gestational hypertensi on	Medical records	<u>Preeclampsia ORs</u> Tertile 2: 0.59 (0.19-1.87) Tertile 3: 0.70 (0.23-2.08) Continuous*: 0.83 (0.52-1.32) <u>Gestational hypertension ORs</u> Tertile 2: 0.54 (0.17-1.66) Tertile 3: 0.95 (0.36-2.49) Continuous*: 0.87 (0.57-1.34) *Continuous exposure: ln-transformed, centered, and standardized with 1 SD PFOS was not selected in elastic net regression to select exposures related to preeclampsia or gestational hypertension	ORs for 2nd and 3rd tertile of exposure compared to 1st tertile ORs per unit increase in ln-transformed, centered, and standardized exposure	Selected covariates based on a directed acyclic graph: age, education level, parity, pre-pregnancy BMI	<u>Preeclampsia</u> Magnitude: yes Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: unclear <u>Gestational hypertension</u> Magnitude: yes Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: unclear	Prevalence of smoking was 1.5% before and 0.4% during pregnancy, so was not included as a confounder. (authors did not state whether based on self-report) Overall risk of pre-eclampsia was 2.8% and gestational hypertension 3.3% Correlation between PFOA and PFOS was 0.33, and with other PFAS ranged from 0.01 to 0.87.

Abbreviations and units: β, regression coefficient; BMI, body mass index (kilogram/meter^2); IQR, interquartile range; ln, natural logarithm; N, number of participants; ng/ml, nanograms/milliliter; OR, odds ratio; PFAS, per-and polyfluoroalkyl substances; PFDA, perfluorodecanoic acid; PFNA, perfluorononanoic acid; r, correlation coefficient; SD, standard deviation; SES, socioeconomic status

Table A7.15. Recent epidemiologic studies on PFOA and indicators of fetal growth (see footnote for abbreviations and units)

Author Year	Location Years	Design	Who N	Factors related to bias	Exposure method	Outcome	Outcome method	Results	Comparison	Confounding	Other aspects of causal inference	Notes
Alkhalawi et al., 2016	Duisberg, Germany 2000-2002	Retrospective cohort	N=148 mother-child pairs from the Duisberg Cohort	Selection: of 196 mothers who met inclusion criteria, sufficient blood/plasma for analysis (including imputation for missing data) was available for 148 Participation: unclear Equal groups: unclear Blinded: unclear Levels: geometric mean (range) Cord plasma 1.75 (<0.4-6.54) Maternal plasma 2.44 (<0.4-9.2)	Maternal plasma at 32 weeks Cord blood or plasma collected at delivery See notes	Birth weight PI BL	"Child examination booklets"	BW β = –0.025 (–0.090 - 0.040) PI β = –0.412 (–0.788 - –0.037) kg/m^3 BL β = 0.152 (–0.199 - 0.503)	Per quartile increase in maternal plasma PFOA Quartiles (ng/ml): 1: <0.4–1.97 2: 1.99–2.73 3: 2.75–3.48 4: 3.52–9.20	Adjusted for pregnancy duration, pre-pregnancy BMI, maternal height, lead in maternal blood, gender, mode of delivery, mother born in Germany, and smoking during pregnancy	Magnitude: no Statistical significance: yes (PI) Dose-response: no Temporal association: yes Subgroup only: no Adjustments: unclear	Missing values for PFOA, PFOS, and PFHxS in maternal and cord plasma were replaced by calculating conversion factors from geometric mean ratios of available paired cord plasma and cord whole blood samples, and paired cord plasma and maternal plasma samples. A factor of 2 was calculated to convert cord plasma to cord whole blood for all three PFAS; calculated conversion factors of 0.35 for PFOS and 0.76 for PFOA were used for cord plasma to maternal plasma. No mention of correlations among three PFAS studied. Associations between PFHxS and birth size were in the same direction as PFOA and PFOS.
Ashley-Martin 2017	10 cities in Canada 2008-2011	Prospective cohort	N=1,705 women ≥18 years old, with no history of pregnancy complications or known fetal or chromo-somal anomaly in the current pregnancy, and their live born singletons	Selection: unclear Participation: unclear Equal groups: unclear Blinded: unclear Levels: median (IQR), (range) 1.7, (1.2-2.4), (LOD-16)	First trimester maternal plasma	BW	Medical charts	BW *z*-score β = −0.10 (95% credible interval [CrI], −0.34 - 0.13)	Per log-unit increase in PFOA	Adjusted for: PFOS, PFHxS, maternal age, pre-pregnancy BMI, parity, income, and maternal smoking	Magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: no	Pearson correlation coefficients for correlations between log-transformed PFAS ranged from r = 0.5 (PFOA and PFHxS) to r = 0.6 (PFOA and PFOS).
Bach et al., 2016	Aarhus, Denmark 2008-2013	Prospective cohort	N=1,507 women and their infants from the Aarhus Birth Cohort	Selection: random sample of eligible women (nulliparous, donated blood sample between 9-20 weeks gestation and gave birth to live singleton) Participation: unclear Equal groups: unclear Blinded: unclear Levels: median (IQR) 2.0 (1.5-2.6)	Maternal serum at 12 weeks (96% within 13 weeks gestation)	BW BW *z*-scores, calculated by standardi-zation of BW for GA according to recent Scandi-navian reference BL HC	Collected by clinical staff immediately after delivery; after January 2013, registry records were used	BW Overall: β = 21 (–1 - 44) Adjusting for GA or restricting to term births attenuated this association Boys: β = 31 (4 - 59) Girls: β = 4 (–34 - 42) No associations with BW *z*-scores, BL, or HC	Per IQR of PFOA exposure	Adjusted for maternal age, pre-pregnancy BMI, education Some BW analyses adjusted for GA, were restricted to term infants, or were stratified by sex	Magnitude: no Statistical significance: yes (BW – boys) Dose-response: no Temporal association: no Subgroup only: BW- boys Adjustments: little change	Of 16 PFAS measured, 7 that were quantifiable in >50% of the study sample were presented. Spearman correlations among PFAS ranged from 0.14 to 0.85. The correlation for PFOS and PFOA was moderate, r = 0.40. The highest correlation for PFOA was with PFNA, r = 0.82.

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Bell et al., 2018	New York State (excluding New York City) 2008-2010	Cross-sectional	N=6,171 infants born to 5,034 women in the upstate KIDS cohort study	Selection: Infants were sampled based on infertility treatment field on birth certificates, frequency matched 1:3 with infants conceived without infertility treatment by region; all mothers of twins were recruited Participation: unclear Equal groups: unclear Blinded: unclear Levels: median (IQR) All 1.07 (0.67-1.60) Singletons 1.10 (0.69-1.63) Twins 1.01 (0.63-1.53)	Newborn dried blood spots from heel sticks, captured onto filter paper cards	BW BL HC PI	BW: birth certificate data BL, HC: maternal question-naire	No significant associations between PFOA and birth size (BW, BL, HC, or PI) when parity was included in the models.	Per increase in log-transformed and scaled PFOA concentration	Adjusted for maternal age, BMI, education, infertility treatment, parity	Magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: no	Correlation between PFOS and PFOA r = 0.32. No other PFAS were considered. The addition of PFOS and BPA to the models did not alter the results.
Buck Louis et al., 2018	12 clinical sites in the US 2009-2013	Prospective cohort (National Institute for Child Health and Human Development Fetal Growth Studies)	N=2,106 healthy women with low-risk pregnancies who delivered live singleton infants and had plasma samples available.	Selection: unclear Participation: unclear Equal groups: non-Hispanic white women had higher PFOA concentrations (median 2.9); mean age and BMI of mothers varied by race Blinded: unclear Levels: median (IQR) 1.985 (1.297-3.001)	Maternal plasma at 10-13 weeks gestation	BW BL Upper arm length Upper thigh length Head circumference Umbilical circumference	Trained nurses completed the neonatal anthropo-metric assessment	<u>BL</u> Overall β = −0.23 (−0.35 - −0.10) Black women β = –0.47 (–0.73 - –0.21) <u>Upper thigh length</u> Overall β = −0.19 (−0.26-−0.12) No consistent, statistically significant associations with other measures of fetal growth	Per log (PFOA+1), scaled by SD	Adjusted for maternal age, race, education, gender, serum cotinine, delivery mode (in head circumference models), interaction of chemical and race/ethnicity	Magnitude: no Statistical significance: yes (BL, upper thigh length) Dose-response: no Temporal association: yes Subgroup only: no Adjustments: unclear	Cohort was racially/ethnically diverse, mostly married, and had a high education level
Callan et al., 2016	Western Australia 2008-2011	Cross-sectional	N=98 pregnant women >18 years	Selection: unclear Participation: unclear Equal groups: unclear Blinded: unclear Levels: Mean ± SD, median (range) 1.00 ± 0.60, 0.86 (0.21-3.1)	Maternal whole blood collected ~2 weeks before due date	BW Proportion of optimal BW (POBW; see notes) BL Proportion of optimal BL (POBL; see notes) HC Proportion of optimal HC (POHC; see notes) PI (100*BW g /BL cm^3)	Participant question-naire	<u>BW</u> β = −48 (−203 - 108) <u>HC</u> β = −0.40 (−0.96 - 0.16) PFOA was not associated with BL, PI, POBW, POBL, POHC A possible trend for lower BW and POBW with increasing PFOA was visible among girls, but no statistics were reported.	Per ln-unit increase in PFOA (equivalent to a ~2.7-fold increase)	BW, BL, HC models adjusted for GA, maternal height, pre-pregnancy BMI, weight gain during pregnancy, and sex of infant. POBW, POBL, POHC: adjusted for weight gain during pregnancy, maternal age (except POHC), and annual household income.	Magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: little change	Pearson's correlations among PFAS ranged from 0.20 to 0.84. For PFOS and PFOA, r = 0.75. PFOA was also highly correlated with PFNA, r = 0.84 and PFDA, r = 0.79. POBW, POBL, POHC calculations incorporate adjustments for GA, maternal height, parity, sex of infant, maternal age (for POHC only) based on a large cohort of Caucasian single births in Western Australia. Authors state that multiplication of the concentrations by 2 to account for the dilution of whole blood has been reported as an acceptable means of estimating serum concentrations.

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Cao et al., 2018	Zhoukou City, China 2013-2015	Cross-sectional	N=337 mother-infant pairs	Selection: native Chinese mothers who had lived in the local residence for >1 year and their live singleton infant Participation: unclear Equal groups: unclear Blinded: unclear Levels: median (IQR) 1.25 (0.87-1.82)	Cord serum	BL BW PI	Hospital birth records	BW, PI: no associations BL, by tertile 2: β = –0.21 (–0.56 - 0.14) 3: β = –0.45 (–0.79 - –0.10) *p trend* = 0.01 Girls 2: β = –0.16 (–0.68 - 0.37) 3: β = –0.58 (–1.12 - –0.04) *p trend* = 0.04 Boys 2: β = –0.22 (–0.68 - 0.23) 3: β = –0.36 (–0.80 - 0.09) *p trend* = 0.11	Compared to 1st tertile of exposure Exposure tertiles (ng/ml): 1: <0.99 2: 0.99 – 1.59 3: >1.59	Adjusted for gender, maternal age, household income, paternal drinking, parity. Birth weight and PI analyses also included maternal education and paternal smoking.	Magnitude: no Statistical significance: yes (BL) Dose-response: yes, trend of shorter BL with higher PFOA exposure Temporal association: no Subgroup only: no Adjustments: unclear	The sum of all 11 measured PFAS was not associated with birth outcomes. PFNA, PFDA, and PFUnDA appeared to be positively associated with fetal growth. Correlations among PFAS were not reported.
Chen et al., 2017	Taipei and New Taipei, Taiwan 2004-2005	Cross-sectional	N=429 mother-infant pairs from the Taiwan Birth Panel Study. Mothers were non-smokers.	Selection: unclear Participation: unclear Equal groups: unclear Blinded: unclear Levels: median (IQR) 1.9 (2.7)	Cord plasma	Age-specific *z*-scores for BW, BL, BMI	Medical records	BW *z*-score β = –0.07 (–0.18 - 0.03) BL *z*-score β = −0.04 (−0.16 - 0.08) BMI *z*-score β = −0.09 (−0.20 - 0.02)	Per ln-unit increase in PFOA	Adjusted for maternal age, pre-pregnancy BMI, education, infant sex, PTB, history of breastfeeding, ln-cotinine	Magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: no	Spearman correlation coefficient for PFOA and PFOS r = 0.025 (p = 0.61)
Costa et al., 2019	Gipuzkoa, Sabadell, and Valencia, Spain 2004-2008	Prospective cohort	N=1,230 mothers with exposure, ultrasound, and delivery data from the INMA (Infancia y Medio Ambiente) Project	Selection: unclear (see note) Participation: unclear Equal groups: unclear Blinded: unclear Levels: median (IQR) 2.35 (1.63-3.30)	Maternal plasma at end of 1st trimester (mean ± SD 13.5 ± 1.7 weeks)	Abdominal circumfer-ence (AC) Biparietal diameter (BPD) Femur length (FL) Estimated fetal weight (EFW)	Ultrasound scans performed by specialized obstetricians at 12, 20, and 34 weeks gestation.	No evidence of association between log_2 PFOA concentrations and fetal growth, except among smokers. Among smokers (N=382): FL at week 20: –6.8% (–12.4 - –1.0) EFW at week 20: –5.7% (–11.4 - –0.1) AC, BPD: no associations No associations at 12 or 34 weeks.	% change in growth per log_2-unit increase in PFOA	Adjusted for cohort (city), parity, maternal age and country of birth, smoking at week 12. Estimated glomerular filtration rate, plasma albumin, other PFAS, and other variables were evaluated but found to not be confounders.	Magnitude: no Statistical significance: yes (FL, EFW) Dose-response: no Temporal association: yes Subgroup only: yes, smokers Adjustments: little change	Women who were excluded based on missing data were somewhat less likely to have been born in Spain, more likely to smoke, and be of lower SES. Excluded women's children had small, non-significant increases in growth parameters, while children of included women had small, mostly non-significant decreases in growth parameters. PFAS compounds were correlated, with highest correlations between PFOA and PFNA (Pearson's partial correlation r = 0.74) and PFOS and PFHxS (r = 0.58) Results for PFNA were similar to those for PFOA

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de Cock et al. 2016	Netherlands 2011-2013	Cross-sectional	N=62 mother-child pairs with PFAS data Women were recruited at first antenatal visit. Children were singletons without major congenital anomalies.	Selection: unclear Participation: only 62 of 148 participants are included in unadjusted models (fewer in adjusted models), with explanation for only 57 of the 86 missing subjects Equal groups: unclear Blinded: unclear Levels: mean, median (range) 0.93, 0.87 (0.20-2.70)	Umbilical cord plasma	BW	Midwife registries	No associations with BW in boys or girls.	Compared to lowest exposure quartile Quartiles (ng/ml): 1: <0.591 2: 0.591 - 0.870 3: 0.871 - 1.150 4: ≥1.511	"Partially adjusted" models included: GA, maternal BMI, height, age, gestational weight gain, parity. "Fully adjusted" models additionally included smoking, alcohol intake, paternal BMI and height, fish and folic acid intake	Magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: no	No mention of correlations between PFOA and PFOS or with other chemicals examined (DDE, PCB-153, and three phthalate metabolites). 68% of women had bachelor's degree or higher education, compared with 28% in the general Dutch population. Male/female ratio of offspring was 1.7.
Govarts et al., 2016	Flanders, Belgium 2008-2009	Cross-sectional	N=213 mother-infant pairs recruited from general population Uncompli-cated live-born singletons	Selection: multi-stage sampling Participation: unclear Equal groups: unclear Blinded: unclear Levels: geometric mean (IQR) 1.52 (1.10-2.10)	Cord plasma	BW	Medical records	PFOA was not significantly associated with BW in adjusted single exposure models. PFOA appeared to contribute to reduced BW of female infants in multi-exposure models, but measures of association for PFOA in these models were not reported.	Per unit (1 ng/ml) increase in PFOA	Adjusted for GA, sex, smoking during pregnancy, parity, pre-pregnancy BMI. Considered but not included: maternal age stress/pres-sure during pregnancy, education, smoking before pregnancy, alcohol use before/during pregnancy, maternal height, income, infections/ complications, folic acid use, cesarean section. Sex and smoking status were evaluated for effect modification.	Magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: unclear	Focus was on using cord blood vs. maternal blood biomarkers, and identifying effects of multiple simultaneous exposures PFOA and PFOS correlation: r = 0.50 PFOA was one of four chemicals that "enhanced the association" between arsenic and lower birth weight when both sexes were included.

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Govarts et al., 2018	Europe (Belgium, Norway, Slovakia, Netherlands) 2002-2012	Pooled analysis of cross-sectional data from 4 cohorts with PFOA exposure data	N=693 women and their live-born singletons	Selection: unclear Participation: unclear Equal groups: unclear Blinded: unclear Levels: cord serum combined median (IQR): 0.550 (0.299-1.200) (medians for 4 cohorts ranged from 0.312-1.500)	Cord serum, observed and estimated based on breast milk concentrations Conversion factor: cord serum level = 13 x breast milk level	SGA, defined as BW <10th percentile for each week of pregnancy and each country and sex-specific reference weight curve	Medical records (BW) and question-naires or ultrasound (GA)	OR = 1.64 (0.97 - 2.76) Mothers who smoked during pregnancy: OR = 2.18 (1.02 - 4.64) Nonsmokers: OR = 1.51 (0.87 - 2.63) p-interaction = 0.33 Sex was not an effect modifier.	Per IQR increase in cord serum PFOA	Adjusted for sex, maternal height, pre-pregnancy BMI, education, age, parity, smoking during pregnancy. Effect varied by cohort (p-interaction <0.05), but the direction of the estimates was the same.	Magnitude: yes Statistical significance: yes (smokers) Dose-response: unclear Temporal association: no Subgroup only: stronger association in smokers Adjustments: unclear	For women in the Norwegian (N=196) and Slovakian (N=207) cohorts, cord serum PFOA levels were estimated based on breast milk samples. For women in the Dutch cohort (N=80), levels were a combination of observed and estimated PFOA concentrations. Correlation between PFOA and PFOS: r = 0.47, and between PFOA and *p,p'*-DDE: r = 0.59. The Belgian cohort (N=208) is included in the Govarts et al., 2016 study of BW.
Gyllen-hammar et al., 2018	Uppsala County, Sweden 1996-2011	Cross-sectional	N=381 first-time, Swedish-born mothers of singletons within the Persistent Organic Pollutants in Uppsala Primiparas (POPUP) study	Selection: unclear Participation: unclear Equal groups: unclear Blinded: unclear Levels: median (IQR): 2.3 (1.6-3.0) ng/g	Maternal serum, sampled 3 weeks after delivery	Standard deviation scores (SDS) for BW, BL, HC (SDSs corrected for GA)	Swedish Medical Birth Register	PFOA exposure was not associated with BW, BL, or HC SDS. Adjustment for maternal estimated glomerular filtration rate (eGFR) did not markedly influence associations between PFAS and birth weight.	Per IQR increase in PFOA	Adjusted for sampling year, maternal age, pre-pregnancy BMI, maternal weight gain during pregnancy, maternal weight loss after delivery, education, smoking during pregnancy, total fish consumption	Magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: no	eGFR was estimated using creatinine and cystatin C (GFR_{cc}) in serum at 3 weeks post-pregnancy. Both measures were associated with PFOA. GFR_{cc} was inversely associated with gestation length, but not birth weight SDS. Correlations between PFOA levels in 3rd trimester and 3 weeks after delivery in a sample of 20: r = 0.94, p <0.001

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Hjermit-slev et al., 2020	Greenland 2010-2011 and 2013-2015	Prospective cohort (authors called it cross-sectional but measure-ments were taken during pregnancy) 2 groups, recruited 2010-2011 and 2013-2015	N=482 pregnant Inuit women	Selection: unclear Participation: unclear Equal groups: unclear Blinded: unclear Levels: median (range), 1.06 (0.10-7.26)	Maternal serum collected at mean 26.2 (range 7-40) weeks gestation for 1st group and before end of gestation week 13 for 2nd group	BW BL HC LBW	Assessed by midwives and data were obtained from the Greenland Doctors Office	Results are adjusted for GA except as noted <u>BW</u> All β = –119 (–202 - –36.6) Females β = –161 (–283 - –40.1) Males β = –81.2 (–194 - 31.2) All (no adjustment for GA) β = –27.3 (–127 - 72.0) Restricting analysis to term births did not change GA-adjusted association with BW. <u>BL</u> All β = –0.37 (–0.76 - 0.02) <u>HC</u> All β = –0.35 (–0.59 - –0.10) Females β = –0.51 (–0.88 - –0.15) Males β = –0.22 (–0.56 - 0.12) <u>LBW</u>: no associations	Per unit increase in PFOA	"Core" adjustments: maternal age, pre-pregnancy BMI, parity, smoking status, alcohol use during pregnancy; additional adjustment: GA Stratification by gender	Magnitude: yes (BW) Statistical significance: yes (BW, HC) Dose-response: unclear Temporal association: yes Subgroup only: effects were larger for females Adjustments: core adjustments diminished the association and statistical significance for BW. Adjustment for GA increased the magnitude and statistical significance of the inverse associations between PFOA and BW, HC, and BL.	Generalizability may be limited due to unique population of Inuit women with high smoking rates and possibly high exposure to persistent organic pollutants through traditional diet. PFOA was statistically significantly associated with older GA at birth for both boys (0.42 weeks) and girls (0.48 weeks).
Kwon et al., 2016	Seoul, South Korea 2006-2010	Cross-sectional	N=268 pregnant women enrolled in the Ewha Birth & Growth Retrospective Cohort (EBGRC) study	Selection: unclear Participation: unclear Equal groups: unclear Blinded: unclear Levels: median (range) 0.91 (0.68-1.15)	Cord serum	BW Effect modification by glutathione S-transferase M1 (GSTM1) and cytochrome P4501A1 (CYP1A1) polymor-phisms	Medical records	<u>BW</u> β = −77.93 (−153.56-−2.30) <u>Effect modification</u> For mothers with *CYP1A1 Val* variant, β = −128.89 (−265.71-7.93) *CYP1A1 Ile/Ile* variant, β = −13.15 (−119.37, 93.07) Association between PFOA and BW was not statistically significantly modified by GSTM1 or CYP1A1.	Per log-unit increase in PFOA	Adjusted for maternal age, pre-pregnancy BMI, history of alcohol consumption, GA, gender, and parity. Results were similar with adjustment for history of smoking.	Magnitude: yes Statistical significance: yes Dose-response: unclear Temporal association: no Subgroup only: no Adjustments: adjustment resulted in slightly larger coefficient. Unadjusted β = −47.04 (−121.22, 27.14).	Spearman's rank correlations were high for PFOA and PFNA (r = 0.78) and PFOS and PFNA (r = 0.60). Correlations for PFOA and PFOS were not reported.

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Lauritzen et al., 2017	Norway and Sweden 1986-1988	Case-cohort: the study sample comprised a 10% random sample and SGA cases from a high risk group* from a population-based cohort study. *defined as any of the following: prior SGA or LBW child, cigarette smoker, pre-pregnancy weight <50kg, previous perinatal death, chronic disease	N=424 pairs of parous women and children 143 SGA cases; 281 randomly sampled non-SGA controls Norway N=265 Sweden N=159	Selection: based on availability of maternal serum samples Participation: unclear Equal groups: see notes Blinded: unclear Levels: median, arithmetic mean (SD), Sweden: 2.33, 2.42 (1.00) Norway: 1.62, 1.83 (1.00)	Second trimester maternal serum	SGA (BW <10th percentile adjusted for GA, parity, and sex) BW BL HC	Measured and recorded at birth	In pooled analyses with data from both Norway and Sweden, and from Norway alone, there were no significant associations between PFOA and indicators of fetal growth after adjustment for important covariates. In the Swedish cohort: SGA Boys OR = 6.55 (1.14 - 37.5) Girls OR = 4.73 (0.79 - 28.3) All OR = 5.25 (1.68 - 16.4) BW Boys β = –526 (–828 - – 222) Girls β = –156 (–541 - 228) All β = –359 (–596 - – 122) BL Boys β = –1.6 (–2.9 - – 0.4) Girls β = –0.8 (–2.4 - 0.8) All β = –1.3 (–2.3 - –0.3) HC Boys β = –0.6 (–1.3 - 0.1) Girls β = –0.1 (–1.0 - 0.7) All β = –0.4 (–1.0 - 0.1)	Per ln-unit increase in PFOA	Adjusted for maternal age, height, pre-pregnancy BMI, education, smoking status, inter-pregnancy interval, parity (except SGA analyses). Adjustment for infant sex, hexachloro-benzene, PCB-153, alcohol, or weight gain did not change estimates.	Magnitude: yes Statistical significance: yes Dose-response: unclear Temporal association: yes Subgroup only: Swedish women, stronger effects in boys Adjustments: unclear	Correlation between PFOA and PFOS, r = 0.56-0.73 Swedish women had slightly higher pre-pregnancy BMI, were less likely to smoke, and had higher PFAS and organochlorine levels than Norwegian women. Swedish offspring were also longer at birth.
Lee et al., 2016	Seoul, South Korea 2008	Cross-sectional	N=85 newborns delivered by cesarean section	Selection: unclear Participation: unclear Equal groups: participants treated by one gynecologist had greater mean GA and BW (3.33 kg, vs. 2.72 kg). A variable representing treatment by this clinician was included in models. Blinded: unclear Levels: mean ± SD (IQR) 1.11 ± 0.48 (0.83-1.29)	Cord serum	BW	Medical records	β = –0.03 (–0.25 - 0.18)	Per ln-unit increase in PFOA	Adjusted for GA, maternal age, infant gender, and clinician	Magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: no	Concentrations of the 6 studied PFAS were correlated with each other. Spearman's rank correlations: PFOS and PFOA r = 0.5290 PFOS and PFNA r = 0.7181 PFOA and PFNA r = 0.6830

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Lenters et al., 2016	Greenland, Poland, Ukraine 2002-2004	Prospective cohort INUENDO Cohort	N=1,250 mother-child pairs. Women ≥18 years old and born in the country of study were enrolled at a prenatal visit. Infants were term singletons with complete exposure data.	Selection: unclear Participation: Ukraine 26%, Greenland 90%, Poland 68% Equal groups: unclear Blinded: unclear Levels: 5th, 50th, 95th percentiles Greenland 0.78, 1.84, 3.55 Poland 1.34, 2.51, 4.36 Ukraine 0.45, 0.96, 2.10	Maternal serum during pregnancy (timing of serum samples varied widely by location and overall)	BW	Hospital records	PFOA was selected by elastic net regression modeling as a predictor of BW in term infants. β = –42.77 (–108.19 - 22.65) Smokers: β = –80.35 (–211.94 - 51.23) Non-smokers: β = –36.93 (–109.75 - 35.88) p-interaction = 0.03 "Further adjusted" model: β = –63.77 (–122.83 - –4.71) g.	Per 2-SD increase in ln-transformed PFOA (1.18 ng/ml)	"Minimal sufficient adjustment set": study population (location), maternal age, pre-pregnancy BMI, parity. "Further adjusted models" also included: GA, sex, maternal height, alcohol consumption near conception, maternal serum cotinine, maternal serum vitamin D, ln-mono(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP), ln-mono(4-methyl-7-oxo-octyl) phthalate (MOiNP), and ln-*p,p'*-DDE.	Magnitude: no Statistical significance: yes Dose-response: unclear Temporal association: yes Subgroup only: no Adjustments: further adjusted model suggested a stronger relationship between PFOA and birth weight	Elastic net regression modeling was used to select covariates and consistently selected PFOA. Spearman correlation between PFOA and PFOS r = 0.61; all other correlations with PFOA were lower. Authors note that there is no consensus on adjustment for GA, and that "further adjusted models" might over-adjust or adjust unnecessarily.
Li et al., 2017	Guangzhou, China 2013	Cross-sectional	N=317 mother-infant pairs Guangzhou Birth Cohort Study	Selection: unclear Participation: unclear Equal groups: unclear Blinded: unclear Levels: median (IQR) Total PFOA 1.2 (0.9-1.8) n-PFOA (linear PFOA) 1.0 (0.7-1.6)	Cord serum	BW LBW	Medical records	BW Total PFOA β = –112.7 (–171.9 - –53.5) n-PFOA β = –85.0 (–133.6 - –36.5) LBW results not reported	Per ln-unit increase in PFOA isomers	Adjusted for GA (BW analyses), BW (GA analyses), delivery, education, parity, infant sex, maternal age, PIH, GDM, anemia.	Magnitude: yes Statistical significance: yes Dose-response: unclear Temporal association: no Subgroup only: no Adjustments: adjustment for maternal education and maternal age changed some coefficients substantially	Electrochemical fluorination, used in the manufacture of the majority of PFOS, PFHxS, and PFOA, and phased out in 2001, results in 20–30% branched isomers. The telomerization method for PFOA manufacturing produces isomers that are almost completely linear. Spearman correlations PFOA and PFOS r = 0.65
Lind et al., 2017	Odense, Denmark 2010-2012	Prospective cohort	N=638 pregnant women and singleton children Odense Child Cohort	Selection: all pregnant women in Odense were invited. Women were recruited at ultrasound information meeting or first antenatal visit. Sample for present study included singleton children of 200 women randomly selected in 2010, and 449 women selected from 2011-2012 with adequate data. Participation: 70.5% for the cohort. Equal groups: unclear Blinded: yes Levels: median (IQR) 1.7 (1.1-2.3)	Maternal serum collected at recruitment (median 10 weeks gestation, range 5-12 weeks)	BW HC Abdominal circum-ference	Birth records	BW Boys β = –5 (–92 - 82) Girls β = 6 (–90 - 102) Effects of PFAS on BW were modified by sex, though no β coefficients for PFOA (continuous or quartiles) and BW were statistically significant. PFOA was not associated with HC or abdominal circumference.	Per ln-unit increase in PFOA	Adjusted for GA, parity, maternal smoking during pregnancy, pre-pregnancy BMI. Maternal ethnicity and education were considered but were not associated with outcomes.	Magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: unclear	Correlations among PFAS not reported.

Author Year	Location Years	Design	Who N	Factors related to bias	Exposure method	Outcome	Outcome method	Results	Comparison	Confounding	Other aspects of causal inference	Notes
Manzano-Salgado et al., 2017	Gipuzkoa, Sabadell, and Valencia, Spain 2003-2008	Prospective cohort	N=1,202 mother-child pairs from the INMA cohort Mothers were >16 years old, without communication barriers and reproductive assistance, delivering in a reference hospital.	Selection: unclear Participation: participants were more educated, less likely to come from Gipuzkoa, and had slightly longer infants than excluded women Equal groups: yes Blinded: unclear Levels: median ± SD 2.35 ± 1.25	Maternal plasma collected at mean ± SD 12.3 ± 5.6 weeks gestation	BW, BL, HC (size measurements were standardized to week 40 of gestation) LBW SGA (below 10th percentile for GA and sex according to national references)	Infant size was measured by midwives and nurses GA was calculated based on last menstrual period	<u>BW</u> All: β = –9.33 (–38.81 - 20.16) Boys: β = –24.75 (–66.71 - 17.22) Girls: β = 13.81 (-26.67 - 54.30) [Interaction term for sex, p = 0.25] <u>SGA</u> All: OR = 0.92 (0.72 - 1.19) Boys: OR = 1.18 (0.82 - 1.69) Girls: OR = 0.72 (0.50 - 1.04) [Interaction term for sex, p = 0.08] LBW associations were similar to those for SGA. No statistically significant associations with BL and HC	Per doubling of PFOA	Adjusted for maternal age, parity, pre-pregnancy BMI, fish intake, type of delivery (HC only). Region was included as a random effect. Adjusting for smoking during pregnancy did not substantially change results.	Magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: little change	Maternal GFR was calculated from plasma-creatinine measurements in the 1st trimester. GFR did not confound associations. Increasing PFAS concentrations were associated with lower BW in Sabadell and Valencia, and higher birth weight in Gipuzkoa. Region-specific PFOA concentrations were not reported. Spearman correlation coefficients for PFOS, PFOA, PFHxS, and PFNA ranged between 0.43 and 0.68. When all PFAS were included in a model, betas for the continuous outcomes were close to the null.
Marks et al., 2019	Former Avon Region in South West England 1991-1992	Prospective cohort	N=457 mother-son pairs enrolled in the Avon Longitudinal Study of Parents and Children (ALSPAC).	Selection: unclear; women were selected for the present analysis to maximize data on puberty and dual energy X-ray absorptiometry (DXA) scans. Participation: unclear Equal groups: "no…strong associations between PFAS and maternal characteristics. There was some evidence that mothers who reported taking folic acid had higher PFAS concentrations" Blinded: unclear Levels: median (IQR) 3.0 (2.3-3.8)	Maternal serum collected at median 30 weeks gestation	BW Crown to heel length (BL) HC	Medical records for BW BL and HC measured by study staff	PFOA was not associated with birth size outcomes in boys.	Per unit increase in PFOA	Adjusted for pregnancy weight gain, pre-pregnancy BMI, education level, vitamin use, folic acid use, smoking during pregnancy, parity, GA at sample collection. BW analyses also adjusted for maternal age and alcohol use during pregnancy.	Magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: no	Authors previously published a study of PFAS and birth size in girls. In a sensitivity analysis including only 1st trimester samples (N=115), associations were consistent with entire study sample. This sample of the ALSPAC cohort was disproportionately white, educated, older, and nonsmoking compared to the overall ALSPAC cohort. PFOS, PFHxS, and PFNA were also analyzed. PFAS concentrations were strongly correlated; PFOA and PFOS (r = 0.63) and PFOS and PFNA (r = 0.60) were most strongly correlated. BMI, parity, and education did not interact with PFAS' effects on birth size.

Author Year	Location Years	Design	Who N	Factors related to bias	Exposure method	Outcome	Outcome method	Results	Comparison	Confounding	Other aspects of causal inference	Notes
Meng et al., 2018	Denmark 1996-2002	Prospective cohort	N=3,507 mother and singleton infant pairs from the Danish National Birth Cohort Three samples from sub-studies that had measured PFAS within the same source population were included.	Selection: unclear Participation: about 60% of invited women accepted Equal groups: unclear Blinded: yes Levels: median (IQR) 4.6 (3.3-6.0)	Maternal plasma in 1st (92%) and 2nd trimester Sample 1 plasma was measured at 3M for only PFOS and PFOA in 2007. Samples 2 and 3 were analyzed at Aarhus University in 2011 and 2014, respectively.	BW LBW	Hospital discharge register	BW, by quartile 2: β = –20.4 (–70.0 - 29.2) 3: β = –25.9 (–77.7 - 25.9) 4: β = –117.0 (–172.3 - –61.6) Continuous β = –35.6 (–66.3 - –5.0) LBW, by quartile 2: OR = 1.5 (0.8 - 3.1) 3: OR = 1.2 (0.5 - 2.5) 4: OR = 1.5 (0.7 - 3.3) Continuous OR = 1.0 (0.7 - 1.5)	Compared to 1st quartile of exposure, or per doubling of PFOA exposure Quartiles (ng/ml): 1: ≤3.3 2: >3.3 and ≤4.6 3: >4.6 and ≤6.0 4: >6.0	Adjusted for infant sex, birth year, maternal age, parity, socio-occupational status, pre-pregnancy BMI, smoking during pregnancy, alcohol use during pregnancy, gestational week of blood draw.	Magnitude: no Statistical significance: yes (BW) Dose-response: yes (BW) Temporal association: yes Subgroup only: no Adjustments: for BW, unadjusted continuous β = –90.1 (–117.9 - –62.2)	6 PFAS were analyzed in two different labs, with results differing by lab, though correlations of PFOS and PFOA concentrations measured in both labs were high (r = 0.94 for PFOS and r = 0.95 for PFOA) Correlations among PFAS: PFOS and PFOA r = 0.66 PFOS and PFHpS r = 0.89 PFOA and PFHpS r = 0.67 3 of the other 4 PFAS evaluated were associated with BW. Adjustment for other PFAS changed the direction of the association between PFOA and BW, though the associations were not statistically significant.
Minatoya et al., 2017	Hokkaido, Japan 2002-2005	Prospective cohort	N=168 pairs of mothers and their term singletons Sapporo Cohort	Selection: unclear Participation: unclear Equal groups: unclear Blinded: unclear Levels: median (IQR) 1.4 (0.9-2.2)	Maternal serum, collected between 23 weeks gestation and delivery	BW PI	Birth records	BW β = –197 (–391 - –3) PI β = –1.32 (–2.66 - 0.02) kg/m^3 No interaction with sex for BW and PI.	Per log-unit increase in PFOA	Adjusted for BMI, maternal smoking during pregnancy, parity, gestation weeks at blood sampling, infant sex, GA	Magnitude: yes (BW) Statistical significance: yes (BW) Dose-response: yes (BW, p-trend <0.05) Temporal association: yes Subgroup only: no Adjustments: unclear	PFOA and PFOS were modestly correlated, Spearman r = 0.287 Adjustment for PFOS did not change coefficients
Sagiv et al., 2018	Boston, MA 1999-2002	Prospective cohort	N=1,645 women with singleton live births	Selection: unclear Participation: 78% Equal groups: unclear Blinded: unclear Levels: median (IQR) 5.8 (3.8)	Maternal plasma at median 9 weeks gestation	Birth weight-for-gestational-age (BW-for-GA) *z*-score Term BW	Medical records (US national reference for BW-for-GA and BW-for-sex *z*-score)	BW-for-GA *z*-score β = –0.02 (–0.08 - 0.03) The effect was entirely among girls, though not statistically significant (data presented graphically). Additional adjustments for eGFR and albumin did not substantially change the estimate. Term BW β = −18.5 (−45.4 - 8.3)	Per IQR increase in PFOA	Adjusted for mother's age, race/ethnicity, education, prenatal smoking, parity, history of breastfeeding, pre-pregnancy BMI, GA at blood draw, sex, paternal education, household income. Plasma albumin concentration and estimated glomerular filtration rate (eGFR) were also included in some models.	Magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: little change	Highly educated, high-income population. Authors state PFAS were "moderately correlated"; PFOS and PFOA Spearman r = 0.72 An objective of this study was to evaluate whether adjusting for pregnancy hemodynamics affected associations between PFAS and birth outcomes.

Author Year	Location Years	Design	Who N	Factors related to bias	Exposure method	Outcome	Outcome method	Results	Comparison	Confounding	Other aspects of causal inference	Notes
Shi et al., 2017	Beijing, China 2012	Cross-sectional	N=170 women who gave birth to singletons without congenital anomalies were recruited from one hospital	Selection: unclear Participation: unclear Equal groups: unclear Blinded: unclear Levels: mean ± SD, median (IQR) 1.285 ± 0.721, 1.097 (0.817-1.443)	Cord serum	BW BL PI	Medical records	BW β = 163.28 (–127.66 - 454.23) BL β = 0.38 (–0.41 - 1.17) PI β = 0.06 (–0.10 - 0.22)	Per log-unit increase in PFOA	Adjusted for maternal age, pre-pregnancy BMI, parity, GA, gender, and height (birth length only). Participants were non-smokers and did not drink alcohol during pregnancy.	Magnitude: yes (BW) Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: large difference between crude and adjusted coefficients for BW	The sample included 1 LBW infant
Shoaff et al., 2018	Cincinnati, OH 2003-2006	Prospective cohort	N=345 mother-child pairs Eligibility criteria: ≥18 years old, recruited at 16 ± 3 weeks gestation, living in Cincinnati area in a home built before 1978, no history of HIV, no medication for seizure or thyroid disorders, singleton pregnancy	Selection: unclear Participation: unclear Equal groups: unclear Blinded: yes Levels: median (IQR) 5.5 (3.8-7.7)	Maternal serum at ~16 weeks gestation if available (86%), at 26 weeks gestation (9%), or within 48 hours of delivery (5%) if neither earlier sample was available.	BW *z*-scores, standard-ized for gestational age using US reference data	Hospital records	Continuous β = –0.03 (–0.17 - 0.10) By tertiles: 2: β = 0.18 (–0.06 - 0.42) 3: β = –0.15 (–0.40 - 0.10) Sex did not modify associations between PFAS and BW *z*-scores.	Change in BW *z*-score per doubling of serum PFOA, or compared to 1st tertile of PFOA concentration Tertiles (ng/ml): 1: 0.5-4.3 2: 4.4-6.7 3: 6.8-26.4	Adjusted for maternal age, race, marital status, insurance status, income, education, parity, serum cotinine, depressive symptoms, mid-pregnancy BMI, food security, fruit/vegetable consumption during pregnancy, fish consumption during pregnancy, prenatal vitamin use.	Magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: unclear	PFAS were moderately correlated, the highest correlation was for PFOS and PFOA, r = 0.60. Including all measured PFAS (PFOS, PFNA, PFHxS) in the model attenuated the association toward the null.
Starling et al., 2017	Colorado 2010-2014	Prospective cohort	N=628 participants selected based on availability of maternal serum and cord blood, from women ≥16 years old with singleton pregnancies	Selection: unclear Participation: 11 of 1,410 enrolled dropped out and the 628 included participants did not meaningfully differ from the larger cohort of 1,410 Equal groups: unclear Blinded: yes Levels: median (IQR) 1.1 (0.7-1.6)	Maternal serum from fasting blood collected at median 27 weeks gestation (range 20-34 weeks)	BW	Weighed by clinical personnel	Continuous β = –51.4 (–97.2 - –5.7) By tertiles: 2: β = −15.9 (−84.9 - 53.2) 3: β = −92.4 (−166.2 - – 18.5) In sensitivity analyses to evaluate possible confounding by other PFAS, PFOA remained inversely associated with lower BW, but the coefficients were smaller and not statistically significant. No significant interactions between PFOA and sex.	Per ln-unit increase in PFOA, or compared to 1st tertile Tertiles (ng/ml): 1: 0.1-0.8 2: 0.9-1.4* 3: 1.4-17.0 * Study reported 1.4 in both 2nd and 3rd tertiles.	Adjusted for maternal age, pre-pregnancy BMI, race/ethnicity, education, parity, smoking during pregnancy, gestational weight gain, sex, GA, gestational age at maternal blood draw	Magnitude: yes Statistical significance: yes Dose-response: yes Temporal association: yes Subgroup only: no Adjustments: yes, unadjusted continuous β = –9.9 g	PFAS concentrations showed moderate to high correlations; the highest Spearman's rank correlation was for PFOA and PFOS, r = 0.76. PFOA level was lower than among NHANES females (NHANES 2011-2012 geometric mean = 1.84) Authors estimated that maternal glucose mediated 3.0% of the effect of PFOA on BW.

Author Year	Location Years	Design	Who N	Factors related to bias	Exposure method	Outcome	Outcome method	Results	Comparison	Confounding	Other aspects of causal inference	Notes
Valvi et al., 2017	Faroe Islands 1997-2000	Prospective cohort	N=604 mother-child pairs recruited at 34 weeks' gestation with singleton pregnancies	Selection: unclear Participation: 92% of 656 recruited mother-child pairs with complete data on key variables were included Equal groups: unclear Blinded: yes Levels: median (IQR) 3.31 (2.54-3.99)	Maternal serum at gestation week 34	BW BL HC	Unclear for BW, HC BL was measured by midwife at 14 days postpartum	<u>BW</u> β = –11 (–88 - 67) Although not statistically significant, PFOA was slightly positively associated with BW in girls, and inversely associated for boys (data presented graphically only). <u>BL</u> β = –0.28 (–0.60 - 0.03) <u>HC</u> β = 0.00 (–0.22 - 0.23) Sex was not a modifier of associations with length.	Per doubling of PFOA	Adjusted for maternal age, education, parity, pre-pregnancy BMI, smoking during pregnancy, and sex Evaluated, but not included: gestational weight gain, family history of diabetes, vitamin D concentration, docosahexa-enoic acid concentration, and GA	Magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: no	Authors were interested in gestational diabetes as a possible mediator of the association between environmental pollutants and offspring size, but did not find that it modified or mediated the associations with birth size.
Wang et al., 2016	Taiwan 2000-2001	Prospective cohort	N=223 mothers and term infants	Selection: unclear Participation: unclear Equal groups: unclear Blinded: unclear Levels: median (IQR) Female 2.34 (1.57-3.43) Male 2.37 (1.35-3.47)	Maternal serum in 3rd trimester	BW BL HC SGA	Measure-ments were taken by clinic nurses	<u>BW</u> Girls β = –0.08 (–0.18 - 0.01) Boys β = 0.04 (–0.05 - 0.12) PFOA was not associated with BL, HC, or SGA.	Per ln-unit increase in PFOA	Adjusted for maternal age, education, previous live births, pre-pregnancy BMI, and income. No adjustment for smoking and alcohol consumption due to low prevalence (2% and 1%, respectively). GA and maternal weight gain were not confounders.	Magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: unclear	PFOA was not highly correlated with other perfluorocarboxylic acids (r≤0.34).

Author Year	Location Years	Design	Who N	Factors related to bias	Exposure method	Outcome	Outcome method	Results	Comparison	Confounding	Other aspects of causal inference	Notes
Wang et al., 2019	Hebei Province, China 2013	Cross-sectional	N=424 mother-infant pairs selected from healthy 20-40-year-old women recruited in first 12 weeks of pregnancy	Selection: unclear Participation: unclear Equal groups: unclear Blinded: unclear Levels: Mean ± SD, median (IQR) 2.64 ± 2.41, 1.99 (1.22-3.11)	Cord serum	BW BL HC PI	Measured at birth by obstetric nurses	No associations with BW or BL. HC (mm) β = –3.87 (–6.96 - –0.77) [possible error: the study's text shows β = –3.87 but Table 2 shows β = –3.70 (–7.00 - –0.40)] PI β = –0.05 (–0.10 - 0.01)	Per ln-unit increase in PFOA	Adjusted for: age, family income, maternal education, maternal career, husband's smoking, daily energy intake, daily physical activity, GA, parity, pre-pregnancy BMI, GDM, sex, delivery mode, gestational weight gain. No women smoked during pregnancy.	Magnitude: no Statistical significance: yes (HC) Dose-response: no Temporal association: no Subgroup only: no Adjustments: no large changes evident	Authors were also interested in effects of PFAS on estrogen homeostasis and role of estrogen as a mediator between PFAS and birth size.
Wikstrom et al., 2019	Sweden 2007-2010	Prospective cohort	N=1,533 mother and singleton infant pairs with complete data from the SELMA (Swedish Environ-mental, Longitudinal, Mother and child, Asthma and allergy) pregnancy cohort	Selection: unclear Participation: unclear Equal groups: unclear Blinded: unclear Levels: median (IQR) 1.61 (1.11-2.30)	Maternal serum (measured at median 10 weeks gestation)	BW Sex- and GA-specific birth weight standard deviation score (BW-SDS) SGA	Swedish Medical Birth Register	BW All children β = –68 (–112 - –24) Girls β = –86 (–145 - –26) Boys β = –49 (–113 - 15) SGA All children OR = 1.43 (1.03 - 1.99) Girls OR = 1.96 (1.18 - 3.28) Boys OR = 1.16 (0.75 - 1.78) BW-SDS All children β = –0.152 (–0.251 - –0.052) Girls β = –0.191 (–0.325 - –0.057) Boys β = –0.111 (–0.258 - 0.036) Restricting analyses to 1st trimester serum samples did not change regression coefficients. GA did not mediate associations between PFAS and BW.	Per ln-unit increase in PFOA (correspond-ing to an increase from 25th to 75th percentile of exposure)	Adjusted for parity, maternal weight, cotinine concentration, sex (unless stratified by sex), GA (for BW only). Maternal age, education level, and week of sampling were not confounders.	BW Magnitude: yes overall and for girls Statistical significance: yes overall and for girls Dose-response: no Temporal association: yes Subgroup only: no Adjustments: unclear SGA Magnitude: yes overall and for girls Statistical significance: yes overall and for girls Dose-response: yes, larger OR with higher exposure quartile Temporal association: yes Subgroup only: stronger association for girls Adjustments: unclear BW-SDS Magnitude: no Statistical significance: yes overall and for girls Dose-response: no Temporal association: yes Subgroup only: stronger association for girls Adjustments: unclear	No reporting of correlations among different PFAS. BW was inversely associated with PFOS, PFNA, PFDA, and PFUnDA. The finding of significance in girls, not boys, was also consistent with the same PFAS.

Author Year	Location Years	Design	Who N	Factors related to bias	Exposure method	Outcome	Outcome method	Results	Comparison	Confounding	Other aspects of causal inference	Notes
Woods et al., 2017	9 counties in Cincinnati, OH area 2003-2006	Prospective cohort	N=272 pregnant women enrolled in the Health Outcomes and Measures of Environment (HOME) Study	Selection: Women >18 years of age were recruited from prenatal clinics at 13 to <19 weeks pregnancy. Women who were free of specified health conditions and living in residences built before 1978 to select for lead exposure were included. Black women were oversampled. Participation: unclear Equal groups: unclear Blinded: unclear Levels: median (IQR) 5.4 (3.8-8.1)	Maternal serum at ~16 and ~26 weeks gestation	BW	Birth records	PFOA was associated with a small (~15 g) decrease in BW but the confidence interval was wide and included 0 (data presented graphically; statistics were not reported).	Per log-unit increase in PFOA (correspond-ing to a 10-fold increase)	Adjusted for maternal age, race, marital status, employment status, income, insurance, education level, tobacco use, gender, BMI, 56 other chemicals from 5 classes of potential endocrine disrupting chemicals, organo-chlorine pesticides, and heavy metals	Magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: no	All PFAS were associated with small, non-statistically significant decreases in BW. .
Workman et al., 2019	Winnipeg, Manitoba, Canada 2010-2011	Prospective cohort	N=414 mother-infant pairs with prenatal samples from the Canadian Healthy Infant Longitudinal Development Study Cohort	Selection: unclear Participation: unclear Equal groups: unclear Blinded: unclear Levels: median (range) 0.89 (0.16-7.1)	Maternal prenatal plasma collected at 16 and 26 weeks	BW BL HC PI	Not reported	No significant associations between PFOA and size parameters (statistics not reported). Result for PI was not reported.	Regression coefficients were not reported	Adjusted for maternal age, smoking during pregnancy, hypertension during pregnancy, diabetes during pregnancy, parity, sex, GA, method of delivery (for HC)	Magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: unclear	
Xu et al., 2019	Hangzhou, China 2016-2017	Cross-sectional	N=98 mother-infant pairs Women with serious illnesses and clinical symptoms, multiple births, infants with congenital diseases were excluded.	Selection: unclear Participation: unclear Equal groups: unclear Blinded: unclear Levels: median (IQR) 1.05 (0.779-1.33)	Umbilical cord serum	BW LBW SGA HC PI	Hospital birth records	<u>BW</u> β = 315.5 (–159.7 - 790.7) <u>SGA</u> OR = 0.790 (0.319 - 1.959) No associations with HC or PI. Because there were only 3 LBW infants, authors did not analyze LBW.	Per log-unit increase in PFOA	Adjusted for maternal age, GA (except in SGA analyses), abortion history, parity, pre-pregnancy BMI, pregnancy weight gain, education, job type, gender, tap vs. filtered drinking water	Magnitude: yes Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: large changes with adjustment for BW: Unadjusted β = 165.6 (–285.8 - 617.1)	Correlation between PFOS and PFOA, r=0.35. Correlations between PFOA and other PFAS, |r| ≤ 0.35 Low exposure contrast (small range of exposure)

Studies are in alphabetical order by first author and year

For BW, a change of >50 g was considered "large magnitude"

Numbers in parentheses are 95% confidence intervals unless otherwise noted

PFOA concentrations are in ng/ml unless otherwise noted

Abbreviations and units: β, regression coefficient; BL, birth (body) length (cm unless otherwise noted); BMI, body mass index (kg/m^2); BW, birth weight (g unless otherwise notes); BW-SDS, sex- and gestational age-specific birth weight standard deviation score; CI, confidence interval; cm, centimeter; CrI, credible interval; *p,p'*-DDE, dichlorodiphenyldichloroethylene; g, gram; GA, gestational age; GDM, gestational diabetes mellitus; GFR, glomerular filtration rate; GSTM 1, glutathione S-transferase M1; HC, head circumference (cm unless otherwise noted); IQR, interquartile range; kg, kilogram; LBW, low birth weight (birth weight <2,500 g); ln, natural logarithm; LOD, limit of detection; m, meter; N, number of participants; ng/ml, nanograms/milliliter; NHANES, US National Health and Nutrition Examination Survey; OR, odds ratio; PCB-153, polychlorinated biphenyls; PFAS, per-and polyfluoroalkyl substances; PFDA, perfluorodecanoic acid; PFHpS, perfluoroheptane sulfonic acid; PFHxS, perfluorohexane sulfonic acid; PFNA,

perfluorononanoic acid; PFUnDA, perfluoroundecanoic acid PI, ponderal index (calculated as [1000*BW/BL] g/cm^3 or [100*BW/BL] g/cm^3 unless otherwise noted); PIH, pregnancy-induced hypertension; PTB, preterm birth; r, correlation coefficient; SD, standard deviation; SES, socioeconomic status; SGA, small for gestational age (<10th percentile for age and sex unless otherwise noted)

Table A7.16. Recent epidemiologic studies on PFOS and indicators of fetal growth (see footnote for abbreviations and units)

Author year	Location Years	Design	Who N	Factors related to bias	Exposure method	Outcome	Outcome method	Results	Comparison	Confounding	Other aspects of causal inference	Notes
Alkhalawi et al., 2016	Duisberg, Germany 2000-2002	Retrospective cohort	N=148 mother–child pairs from the Duisberg Cohort	Selection: of 196 mothers who met inclusion criteria, sufficient blood/plasma for analysis (including imputation for missing data) was available for 148 Participation: unclear Equal groups: unclear Blinded: unclear Levels: geometric mean (range) Cord plasma 2.83 (0.53-11.7) Maternal plasma 9.04 (1.70-21.93 [possible error: 29.2 in text]	Maternal plasma at 32 weeks gestation Cord blood or plasma collected at delivery See notes	BW PI BL	"Child examination booklets"	BW β = –0.030 (–0.091 - 0.030) PI β = –0.355 (–0.702 - –0.008) kg/m^3 BL β = 0.103 (–0.221-0.428)	Per quartile increase in maternal plasma PFOS Quartiles (ng/ml): 1: 1.70-6.98 2: 7.02-9.31 3: 9.33-11.80 4: 11.86-21.93	Adjusted for pregnancy duration, pre-pregnancy BMI, maternal height, lead in maternal blood, sex, mode of delivery, mother born in/outside Germany, and smoking during pregnancy	Magnitude: no Statistical significance: yes (PI) Dose-response: no Temporal association: yes Subgroup only: no Adjustments: unclear	Missing values for PFOA, PFOS, and PFHxS in maternal and cord plasma were replaced by calculating conversion factors from geometric mean ratios of available paired cord plasma and cord whole blood samples, and paired cord plasma and maternal plasma samples. A factor of 2 was calculated to convert cord plasma to cord whole blood for all three PFAS and a factor of 0.35 for PFOS for cord plasma to maternal plasma. Using these calculations, 81 maternal and 83 cord plasma samples were increased to 186 ng/ml each. There was an unexplained discrepancy in sample size between the publication text and Table 2 No mention of correlations among three PFAS studied. Associations between PFHxS and birth size were in the same direction as PFOA and PFOS.
Ashley-Martin 2016	10 cities in Canada 2008-2011	Prospective cohort	N=1,705 women ≥18 years old, with no history of pregnancy complications or known fetal or chromo-somal anomaly in the current pregnancy, and their live born singletons	Selection: unclear Participation: unclear Equal groups: unclear Blinded: unclear Levels: median (IQR), (range) 4.6 (3.2-6.8), (LOD-36)	First trimester maternal plasma	BW	Medical charts	BW z-score β = 0.05 (95% credible interval [CrI], −0.18 - 0.29)	Per log-unit increase in PFOS	Adjusted for: PFOA, PFHxS, maternal age, pre-pregnancy BMI, parity, income, and maternal smoking. Gestational weight gain was not a confounder or effect modifier.	Magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: no	Pearson correlation coefficients for correlations between log-transformed PFAS ranged from r = 0.5 (PFOA and PFHxS) to r = 0.6 (PFOA and PFOS)

Author year	Location Years	Design	Who N	Factors related to bias	Exposure method	Outcome	Outcome method	Results	Comparison	Confounding	Other aspects of causal inference	Notes
Bach et al., 2016	Aarhus, Denmark 2008-2013	Prospective cohort	N=1,507 women and their infants from the Aarhus Birth Cohort	Selection: random sample of eligible women (nulliparous, donated blood sample between 9-20 weeks gestation and gave birth to live singleton) Participation: unclear Equal groups: unclear Blinded: unclear Levels: median (IQR) 8.30 (6.03-10.80)	Maternal serum at 12 weeks (96% within 13 weeks gestation)	BW BW *z*-scores, calculated by standard-ization of BW for GA according to recent Scandi-navian reference BL HC GA PTB	Collected by clinical staff immediate-ly after delivery; in 2013, registry records were used	BW All births By PFOS exposure quartile, compared to lowest quartile: 2: β = –86 (–159 - –13) 3: β = –21 (–91 - 48) 4: β = –50 (–123 - 23) Per IQR (4.8 ng/ml): All: β = –2 (–30 - 26) Boys: β = 26 (–13 - 65) Girls: β = –32 (–71 - 7) Term births By exposure quartile: 2: β = –93 (–157 - –29) 3: β = –50 (–113 - 13) 4: β = –62 (–126 - 3) Per IQR: β = –14 (–40 - 11) PFOS was not associated with BW *z*-scores, BL, HC, GA, or PTB.	Compared to lowest exposure quartile, or per IQR of PFOS exposure Quartiles (ng/ml): 1: <6.02 2: 6.03-8.29 3: 8.30-10.80 4: 10.81-36.0	Adjusted for maternal age, pre-pregnancy BMI, education. Some BW analyses adjusted for GA, were restricted to term infants, or were stratified by sex.	Magnitude: yes Statistical significance: yes Dose-response: no Temporal association: yes Subgroup only: no Adjustments: little change	Of 16 PFAS measured, 7 that were quantifiable in >50% of the study sample were presented. Spearman correlations among PFAS ranged from r = 0.14 to r = 0.85. The correlation for PFOS and PFOA was moderate, r = 0.40. The highest correlation for PFOS was with PFNA, r = 0.46.
Bell et al., 2018	New York State (excluding New York City) 2008-2010	Cross-sectional	N=6,171 infants born to 5,034 women in the upstate KIDS cohort study. Infants were sampled based on infertility treatment field on birth certificates, frequency matched 1:3 with infants conceived without infertility treatment by region. All mothers of twins were recruited.	Selection: possible, based on infertility treatment; twins Participation: unclear Equal groups: unclear Blinded: unclear Levels: median (IQR) All 1.69 (1.12-2.40) Singletons 1.72 (1.14-2.44) Twins 1.64 (1.09-2.33)	Newborn dried blood spots from heel sticks, captured onto filter paper cards	BW BL HC PI	BW: birth certificate data BL, HC: maternal question-naire	No significant associations with BW, BL, or HC when parity was included in the models. Models remained non-significant with the addition of PFOA and BPA.	Per increase in log-transformed and scaled PFOS concentration	Adjusted for maternal age, BMI, education, infertility treatment, parity	Magnitude: no Statistical significance: yes Dose-response: no Temporal association: no Subgroup only: singletons Adjustments: little change	Correlation between PFOS and PFOA r = 0.32. No other PFAS were considered.

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Buck Louis et al., 2018	12 clinical sites in the US 2009-2013	Prospective cohort (National Institute for Child Health and Human Develop-ment Fetal Growth Studies)	N=2,106 healthy women with low-risk pregnancies who delivered live singleton infants and had plasma samples available	Selection: unclear Participation: unclear Equal groups: unclear Blinded: unclear Levels: median (IQR) 5.133 (3.39-7.981)	Maternal plasma at 10-13 weeks gestation	BW BL Upper arm length Upper thigh length HC Umbilical circum-ference	Trained nurses completed the neonatal anthropo-metric assess-ment	No consistent statistically significant associations with measures of fetal growth	Per log (PFOS+1), scaled by SD	Adjusted for maternal age, race, education, gender, serum cotinine, delivery mode (in HC models), interaction of chemical and race/ethnicity	Magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: unclear	Cohort was racially/ethnically diverse, mostly married, and had a high education level.
Callan et al., 2016	Western Australia 2008-2011	Cross-sectional	N=98 pregnant women >18 years old	Selection: unclear Participation: unclear Equal groups: unclear Blinded: unclear Levels: median (range), 1.99 (0.45-8.1); mean ± SD, 2.32 ± 1.42	Maternal whole blood collected ~2 weeks before due date	BW Proportion of optimal BW (POBW; see notes) BL Proportion of optimal BL (POBL; see notes) HC Proportion of optimal HC (POHC; see notes) PI (g/cm^3 *100)	Participant question-naire	BW: −69 (−231 - 94) HC: −0.39 (−0.98 - 0.20) PFOS was not associated with BL, PI, POBW, POBL, POHC. A possible trend for lower BW and POBW with increasing PFOS was visible among girls, but no statistics were reported.	Per ln-unit increase in PFOS (equivalent to a ~2.7-fold increase)	BW, BL, HC models adjusted for GA, maternal height, pre-pregnancy BMI, weight gain during pregnancy and sex of infant. POBW, POBL, POHC models adjusted for weight gain during pregnancy, maternal age (except POHC), and income.	Magnitude: yes (BW) Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: little change	Pearson correlations among PFAS ranged from r = 0.20 tor = 0.84. For PFOS and PFOA, r = 0.75. POBW, POBL, POHC calculations incorporate adjustments for GA, maternal height, parity, sex of infant, maternal age (for POHC only) based on a large cohort of Caucasian single births in Western Australia. Authors state that multiplication of the concentrations by 2 to account for the dilution of whole blood has been reported as an acceptable means of estimating serum concentrations.
Cao et al., 2018	Zhoukou City, China 2013-2015	Cross-sectional	N=337 mother-infant pairs	Selection: native Chinese mothers who had lived in the local residence for >1 year and their live singleton infant Participation: unclear Equal groups: unclear Blinded: unclear Levels: median (IQR) 1.01 (0.60-1.76)	Cord serum	BW BL PI	Hospital birth records	PFOS was not associated with BW, BL, or PI.	Compared to 1st tertile of exposure Tertiles (ng/ml): 1: <0.74 2: 0.74-1.52 3: >1.52	Adjusted for gender, maternal age, household income, paternal drinking, parity. BW and PI analyses also included maternal education and paternal smoking.	Magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: unclear	The sum of all 11 measured PFAS was not associated with birth outcomes. PFNA, PFDA, and PFUndA appeared to be positively associated with fetal growth. Correlations among PFAS were not reported.

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Chen et al., 2017	Taipei and New Taipei, Taiwan 2004-2005	Cross-sectional	N=429 mother-infant pairs from the Taiwan Birth Panel Study. Mothers were non-smokers.	Selection: unclear. Participation: unclear Equal groups: unclear Blinded: unclear Levels: median (IQR) 5.7 (5.0)	Cord plasma	Age-specific *z*-scores for BW, BL, BMI	Medical records	BW *z*-score β = −0.14 (−0.26 - −0.01) BL *z*-score β = −0.16 (−0.31 - −0.02) BMI *z*-score β = −0.11 (−0.25 - 0.02)	Per ln-unit increase in PFOS	Adjusted for maternal age, pre-pregnancy BMI, education, infant sex, preterm birth, breastfeeding, ln-cotinine	Magnitude: no Statistical significance: yes (BW *z*-score, BL *z*-score) Dose-response: greater exposure appeared to be associated with strongest effects on BW, BL, BMI Temporal association: no Subgroup only: no Adjustments: little change	Spearman correlation coefficient for PFOA and PFOS r = 0.025 (p =0.61).
Costa et al., 2019	Spain (3 locations) 2004-2008	Prospective cohort	N=1,230 mothers with exposure, ultrasound, and delivery data from the INMA (Infancia y Medio Ambiente) Project	Selection: unclear Participation: unclear Equal groups: unclear Blinded: unclear Levels: median (IQR) 6.05 (4.52-7.82)	Maternal plasma at end of 1st trimester (mean ± SD 13.5 ± 1.7 weeks)	Fetal growth parameters: Abdominal circum-ference (AC) Biparietal diameter (BPD) Femur length (FL) Estimated fetal weight (EFW)	Ultrasound scans performed by specialized obstetri-cians at 12, 20, and 34 weeks gestation	No evidence of association between log_2 PFOS concentrations and fetal growth, except among smokers. Among smokers, PFOS was associated with 6.3 % (0.1 - 12.3) increase in BPD at week 34.	% change in growth per log_2 unit increase in PFOS	Adjusted for cohort (city), parity, maternal age and country of birth, smoking at week 12 (PFOS and BPD), estimated glomerular filtration rate (eGFR), plasma albumin; other variables, including other PFAS, were evaluated but found to not be confounders	Magnitude: no Statistical significance: yes (BPD at 34 weeks) Dose-response: no Temporal association: yes Subgroup only: smokers Adjustments: little change	PFAS were correlated, with highest correlations between PFOA and PFNA (Pearson's partial correlation r = 0.74) and PFOS and PFHxS (r = 0.58). The correlation between PFOA and PFOA was 0.5.
de Cock et al. 2016	Nether-lands 2011-2013	Cross-sectional	N=62 mother-child pairs with PFAS data Women were recruited at first antenatal visit. Children were singletons without major congenital anomalies.	Selection: unclear Participation: only 62 of 148 participants are included in unadjusted models (fewer in adjusted models), with explanation for only 57 of the 86 missing subjects Equal groups: unclear Blinded: unclear Levels: mean, median (range) 1.60, 1.60 (0.57-3.20)	Umbilical cord plasma	BW	Midwife registries	PFOS exposure was not associated with BW in boys. For girls in the highest exposure quartile: Unadjusted β = 65.6 (–431.46 - 562.71) "Partially adjusted" β = 224.3 (–193.07 - 641.68) "Fully adjusted" β = 595.9 (88.77 - 1103.04) No other PFOS exposure quartiles were significantly associated with BW in girls.	Compared to lowest exposure quartile. Quartiles (ng/ml): 1: <0.996 2: 0.996 - 1.600 3: 1.601 - 2.000 4: ≥2.001	"Partially adjusted" models included: GA, maternal BMI, height, age at birth, gestational weight gain, parity. "Fully adjusted" models additionally included smoking, alcohol intake, paternal BMI and height, fish and folic acid intake.	Magnitude: yes (girls, "fully adjusted" model) Statistical significance: yes for "fully adjusted" model Dose-response: unclear Temporal association: no Subgroup only: girls Adjustments: large changes with adjustment	No mention of correlations between PFOA and PFOS or with other chemicals examined (DDE, PCB-153, and three phthalate metabolites). 68% of women had bachelor's degree or higher education, compared with 28% in the general Dutch population. Male/female ratio of offspring was 1.7.

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Govarts et al., 2016	Flanders, Belgium 2008-2009	Cross-sectional	N=213 mother-infant pairs recruited from general population Uncompli-cated live-born singletons	Selection: multi-stage sampling Participation: unclear Equal groups: unclear Blinded: unclear Levels: geometric mean (IQR) 2.63 (1.70-3.80)	Cord plasma	BW	Medical records	PFOS was not significantly associated with BW in adjusted single exposure models. Measures of association for PFOS in multi-exposure models were not reported. PFOS appeared to contribute to reduced BW of female infants in multi-exposure models.	Per unit (1 ng/ml) increase in PFOS	Adjusted for GA, sex, smoking during pregnancy, parity, pre-pregnancy BMI. Considered but not included: maternal age, stress during pregnancy, education, smoking before pregnancy, alcohol use before/during pregnancy, maternal height, income, infections/compli-cations, folic acid use, cesarean section. Sex and smoking status were evaluated for effect modification.	Magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: unclear	Focus was on using cord blood vs. maternal blood biomarkers, and identifying effects of multiple simultaneous exposures PFOA and PFOS correlation: r = 0.50
Govarts et al., 2018	Europe (Belgium, Norway, Slovakia, Nether-lands) 2002-2012	Pooled analysis of cross-sectional data from 4 cohorts with PFOS exposure data	N=688 women and their live-born singletons	Selection: unclear Participation: unclear Equal groups: unclear Blinded: unclear Levels: cord serum PFOS combined median (IQR): 1.984 (1.200-3.008) (medians for 4 cohorts ranged from 0.960-2.700)	Cord serum, observed and estimated based on breast milk concentra-tions Conversion factor: cord serum level = 32 x breast milk level	SGA, defined as BW <10th percentile for each week of pregnancy and each country and sex-specific reference weight curve	Medical records (BW) and question-naires or ultrasound (GA)	Mothers who smoked during pregnancy: OR = 1.63 (1.02 - 2.59) Nonsmokers: OR = 0.66 (0.61 - 0.72) p-interaction = 0.0004 Sex was not an effect modifier.	Per IQR increase in cord serum PFOS	Adjusted for sex, maternal height, pre-pregnancy BMI, education, age, parity, smoking during pregnancy. Sex and smoking status were evaluated for effect modification.	Magnitude: yes, in different directions for smokers and nonsmokers Statistical significance: yes Dose-response: unclear Temporal association: no Subgroup only: yes Adjustments: unclear	For women in the Norwegian (N=196) and Slovakian (N=204) cohorts, cord serum PFOS levels were estimated based on breast milk samples. For women in the Dutch cohort (N=80), levels were a combination of observed and estimated PFOS concentrations. Correlation between PFOA and PFOS: r = 0.47, and between PFOA and *p,p'*-DDE: r = 0.59. The Belgian cohort (N=208) is included in the Govarts et al., 2016 study of BW.
Gyllen-hammar et al., 2018	Uppsala County, Sweden 1996-2011	Cross-sectional	N=381 first-time, Swedish-born mothers of singletons within the Persistent Organic Pollutants in Uppsala Primiparas (POPUP) study	Selection: unclear Participation: unclear Equal groups: unclear Blinded: unclear Levels: Median (IQR): 13 (7.4-19) ng/g	Maternal serum, sampled 3 weeks after delivery	Standard deviation scores (SDS) for BW, BL, and HC (SDSs corrected for GA)	Swedish Medical Birth Register	PFOS exposure was not associated with BW, BL, or HC SDS. Adjustment for maternal estimated glomerular filtration rate (eGFR) did not markedly influence association between PFOS and BW.	Per IQR increase in PFOS	Adjusted for sampling year, maternal age, pre-pregnancy BMI, maternal weight gain during pregnancy, maternal weight loss after delivery, education, smoking during pregnancy, total fish consumption	Magnitude: no Statistical significance: no Dose response: no Temporal association: no Subgroup only: no Adjustments: no	eGFR was estimated using creatinine or cystatin C (GFR_{cc}) in serum at 3 weeks post-pregnancy. Neither was associated with maternal serum PFOS 3 weeks after delivery. GFR_{cc} was inversely associated with gestation length, but not BW SDS. Correlation between PFOS levels in 3rd trimester and 3 weeks after delivery in a sample of 20: r = 0.82, p <0.001 No mention of correlations among different PFAS.

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Hjermits-lev et al., 2020	Greenland 2010-2011 and 2013-2015	Prospective cohort (authors called it cross-sectional but measure-ments were taken during pregnancy) 2 groups, recruited 2010-2011 and 2013-2015	N=482 pregnant Inuit women	Selection: unclear Participation: unclear Equal groups: unclear Blinded: unclear Blinded: unclear Levels: median (range) 8.99 (1.50-61.3)	Maternal serum collected at mean 26.2 (range 7-40) weeks gestation for 1st group and before end of gestation week 13 for 2nd group.	BW BL HC LBW	Assessed by midwives and data were obtained from the Greenland Doctors Office	(Adjusted for GA) BW β = –5.47 (–12.6 - 1.67) BL β = –0.009 (–0.04 - 0.03) HC: β = –0.01 (–0.03 - 0.01) LBW: no associations; data not reported	Per unit increase in PFOS	"Core" adjustments: maternal age, pre-pregnancy BMI, parity, smoking status, alcohol use during pregnancy. Additional adjustment: GA. Stratification by gender.	Magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: little change	Generalizability may be limited due to unique population of Inuit women with high smoking rates and possibly high exposure to persistent organic pollutants through traditional diet.
Kwon et al., 2016	Seoul, South Korea 2006-2010	Cross-sectional	N=268 pregnant women enrolled in the Ewha Birth & Growth Retrospec-tive Cohort study	Selection: unclear Participation: unclear Equal groups: unclear Blinded: unclear Levels: median (range) 0.64 (0.29-1.09)	Cord serum	BW Effect modifica-tion by glutathione S-transferase M1 (GSTM1) and cytochrome P4501A1 (CYP1A1) polymorph-isms	Medical records	β = −49.41 (−95.57 - −3.25) No effect modification by GSTM1 or CYP1A1	Per log-unit increase in PFOs	Adjusted for maternal age, pre-pregnancy BMI, history of alcohol consumption, GA, gender, and parity. Results were similar with adjustment for history of smoking.	Magnitude: no Statistical significance: yes Dose-response: unclear Temporal association: no Subgroup only: no Adjustments: Adjustment resulted in slightly larger coefficients. Unadjusted β = −22.26 (−68.76-24.24)	Spearman's rank correlations were high for PFOA and PFNA (r = 0.78) and PFOS and PFNA (r = 0.60). Correlations for PFOA and PFOS were not reported.
Lauritzen et al. 2017	Norway and Sweden 1986-1988	Case-cohort: study sample comprised a 10% random sample and SGA cases from a high risk group* from a population-based cohort study. *defined as any of the following: prior SGA or LBW child, cigarette smoker, pre-pregnancy weight <50kg, previous perinatal death, chronic disease	N=424 pairs of parous women and children 143 SGA cases; 281 randomly sampled non-SGA controls Norway N=265 Sweden N=159	Selection: based on availability of maternal serum samples Participation: unclear Equal groups: see notes Blinded: unclear Levels: median, arithmetic mean (SD) Sweden: 16.4, 17.3 (7.45) Norway: 9.74, 11.3 (7.02)	Second trimester maternal serum	SGA (BW below the 10th percentile adjusted for GA, parity, and sex) BW BL HC	Measured and recorded at birth	In pooled analyses with data from both Norway and Sweden, no significant associations between PFOS and indicators of fetal growth after adjustment for important covariates. PFOS was not associated with indicators of fetal growth in the Norwegian cohort. In the Swedish cohort: SGA OR = 2.51 (0.93 - 6.77) BW β = –292 (–500 - –84) BL β = –1.2 (–2.1 - –0.3) HC β = –0.4 (–0.9 - 0.04)	Per ln-unit increase in PFOS	Adjusted for maternal age, height, pre-pregnancy BMI, education, smoking status, inter-pregnancy interval, parity (except SGA analyses). Alcohol and weight gain did not change estimates in sensitivity analyses.	Magnitude: yes (SGA, BW) Statistical significance: yes (BW, BL) Dose-response: unclear Temporal association: yes Subgroup only: yes, Swedish women Adjustments: unclear	Correlations between PFOA and PFOS: Norway r = 0.56, Sweden r = 0.73 Swedish women had slightly higher pre-pregnancy BMI, were less likely to smoke, and had higher PFAS and organochlorine levels than Norwegian women. Swedish offspring were also longer at birth.

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Lee et al., 2016	Seoul, South Korea 2008	Cross-sectional	N=85 newborns delivered by cesarean section	Selection: unclear Participation: unclear Equal groups: participants treated by one gynecologist had greater mean GA and BW (3.33 kg vs. 2.72 kg). A variable representing treatment by this clinician was included in models. Blinded: unclear Levels: mean ± SD (IQR) 0.87 ± 0.46 (0.56-1.02)	Cord serum	BW	Medical records	β = –0.14 (–0.33 - 0.03)	Per ln-unit increase in PFOA	Adjusted for GA, maternal age, infant gender, and clinician	Magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: no	Concentrations of the 6 studied PFAS were correlated with each other. Spearman's rank correlations: PFOS and PFOA r = 0.5290 PFOS and PFNA r = 0.7181 PFOA and PFNA r = 0.6830
Lenters et al., 2016	Greenland, Poland, Ukraine 2002-2004	Prospective cohort INUENDO Cohort	N=1,250 mother-child pairs with singleton, term infants with complete exposure data	Selection: unclear Participation: Ukraine 26%, Greenland 90%, Poland 68% Equal groups: unclear Blinded: unclear Levels: 5^{th}, 50^{th}, 95^{th} percentiles Greenland 10.23, 20.09, 49.47 Poland 4.38, 7.81, 12.40 Ukraine 2.27, 5.04, 9.48	Maternal serum during pregnancy (timing of serum samples varied widely by location and overall)	BW	Hospital records	PFOS was not selected in elastic net regression, and associations decreased markedly when modeled with other exposures, suggesting absence of an independent relationship with BW. The pooled relationship between PFOS and BW was non-linear, which appeared to be due to different exposure ranges and a slight positive slope for Poland. For the Greenland sample, which had higher PFOS concentrations, the slope was highly negative (i.e., PFOS was associated with lower BW).	Per 2-SD increase in ln-transformed PFOS (1.60 ng/ml)	"Minimal sufficient adjustment set:" study population (location), maternal age, pre-pregnancy BMI, parity. "Further adjusted models" add: GA, sex, maternal height, alcohol consumption near conception, maternal serum cotinine, maternal serum vitamin D. Multiple exposure models included ln-mono(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP), ln-mono(4-methyl-7-oxo-octyl) phthalate (MOiNP), ln-dichlorodiphenyl-dichloroethylene (*p,p'*-DDE;).	Magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: unclear	Elastic net regression modeling was used to select covariates and consistently selected PFOA, but not PFOS. Spearman correlation between PFOA and PFOS r = 0.61 Timing of serum samples varied widely by population/location and overall. Authors note that there is no consensus on adjustment for GA, and that "further adjusted models" might over-adjust or adjust unnecessarily.

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Li et al., 2017	Guang-zhou, China 2013	Cross-sectional	N=317 mother-infant pairs Guangzhou Birth Cohort Study	Selection: unclear Participation: unclear Equal groups: unclear Blinded: unclear Levels: median (IQR) Total PFOS 3.0 (1.7, 4.6) n-PFOS (linear PFOS) 2.1 (1.2, 3.6) Total branched (Br) PFOS 0.7 (0.4, 1.3) 1m-PFOS 0.1 (0.1-0.2) iso-PFOS 0.2 (0.1-0.3) 3 + 4 + 5m-PFOS 0.4 (0.2-0.7) See notes for isomer nomenclature details.	Cord serum	BW LBW	Medical records	BW Total PFOS Boys β = –150.6 (–225.4 - –75.7) Girls β = –26.6 (–125.1 - 71.8) n-PFOS Boys β = –100.2 (–160.5 - –39.9) Girls β = –10.0 (–83.0 - 63.0) Total Br-PFOS Boys β = –190.8 (–277.04 - 104.46) Girls β = –48.0 (–168.4 - 72.5) 1m-PFOS Boys β = –167.3 (–263.02 —71.64) Girls β = –15.0 (–139.1 - 109.2) Iso-PFOS Boys β = –156.1 (–231.91 - –80.37) Girls β = –66.9 (–168.5 - 34.8) 3 + 4 + 5m-PFOS Boys β = –184.7 (–269.9 - –99.5) Girls β = –38.7 (–156.9 - 79.5) PTB and LBW results not reported	Per ln-unit increase in PFOS isomers	Adjusted for GA, delivery, education, parity, infant sex, maternal age, PIH, GDM, anemia.	Magnitude: yes Statistical significance: yes (boys) Dose-response: unclear Temporal association: no Subgroup only: boys Adjustments: adjustment for maternal education and maternal age changed some coefficients substantially	Isomer nomenclature for branched PFOS: the abbreviations denote the structure of each isomer in relation to the position of the substituted perfluoromethyl, “m.” The number preceding “m” indicates the carbon position of the branching point. E.g., sodium perfluoro-1-methylheptanesulfonate is 1m-PFOS, sodium perfluoro-3-methylheptanesulfonate is 3m-PFOS. Sodium perfluoro-6-methylheptanesulfonate is iso-PFOS. Spearman correlations PFOA and PFOS r = 0.65 PFOS and PFOS isomers: all r>0.97. Correlations with other PFAS were much lower.
Lind et al., 2017	Odense, Denmark 2010-2012	Prospective cohort	N=638 pregnant women and singleton children Odense Child Cohort	Selection: all pregnant women in Odense were invited. Women were recruited at ultrasound information meeting or first antenatal visit. Sample for present study included singleton children of 200 women randomly selected in 2010, and 449 women selected from 2011-2012 with adequate data. Participation: 70.5% for the cohort. Equal groups: unclear Blinded: yes Levels: median (IQR) 8.1 (6.0-11.0)	Maternal serum collected at recruitment (median 10 weeks gestation, range 5-12 weeks)	BW HC Abdominal circum-ference	Birth records	BW Girls β = 92 (–15 - 199) Boys β = –17 (–130 - 97) Effects of PFAS on BW were modified by sex, though no β coefficients for PFOS and BW were statistically significant. No associations with HC or abdominal circumference.	Per ln-unit increase in PFOS	Adjusted for GA, parity, maternal smoking during pregnancy, pre-pregnancy BMI. Maternal ethnicity and education were considered but were not associated with outcomes.	Magnitude: yes (girls) Statistical significance: no Dose-response: no Temporal association: yes Subgroup only: tendency towards reduced BW in boys and greater BW in girls Adjustments: unclear	Correlations among PFAS not reported.

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Manzano-Salgado et al., 2017	Gipuz-koa, Sabadell, and Valencia, Spain 2003-2008	Prospective cohort	N=1,202 mother-child pairs from the INMA cohort Mothers were >16 years old, without communicati on barriers and reproductive assistance, delivering in a reference hospital.	Selection: unclear Participation: participants were more educated, less likely to come from Gipuzkoa, and had slightly longer infants than excluded women Equal groups: yes Blinded: unclear Levels: median ± SD 6.05 ± 2.74	Maternal plasma collected at mean ± SD 12.3 ± 5.6 weeks	BW, BL, HC (size measure-ments were standard-ized to week 40 of gestation) LBW SGA (below 10th percentile for GA and sex according to national references)	Infant size was measured by midwives and nurses GA was calculated based on last menstrual period	PFOS was not statistically significantly associated with BW, BL, HC, GA, SGA, and PTB. <u>LBW</u> Boys OR = 1.90 (0.98 - 3.68) Girls OR = 0.73 (0.46 - 1.19); [Interaction term for sex, p = 0.01]	Per doubling of PFOS	Adjusted for maternal age, parity, pre-pregnancy BMI, fish intake, type of delivery (HC only). Region was included as a random effect. Adjusting for smoking during pregnancy did not substantially change results.	Magnitude: yes (LBW) Statistical significance: no Dose-response: no Temporal association: yes Subgroup only: no Adjustments: little change	Maternal GFR was calculated from plasma-creatinine measurements in the 1st trimester. GFR did not confound associations. Increasing PFAS concentrations were associated with lower BW in Sabadell and Valencia, and higher BW in Gipuzkoa. Region-specific PFOS concentrations were not reported. Spearman correlation coefficients for PFOS, PFOA, PFHxS, and PFNA ranged between 0.43 and 0.68. When all PFAS were included in a model, betas for the continuous outcomes were close to the null.
Marks et al., 2019	Former Avon Region in South West England 1991-1992	Prospective cohort	N=457 mother-son pairs enrolled in the Avon Longitudinal Study of Parents and Children (ALSPAC).	Selection: unclear; women were selected for the present analysis to maximize data on puberty and dual energy X-ray absorptiometry (DXA) scans. Participation: unclear Equal groups: unclear Blinded: unclear Levels: median (IQR) 13.8 (11.0-7.7)	Maternal serum at median 30 weeks gestation (IQR 12-33 weeks)	BW Crown to heel length (BL) HC	Medical records for BW Length and HC measured by study staff	<u>BW</u> β = −8.50 (−15.93 - −1.07) <u>BL</u> β = −0.04 (−0.08 - −0.01) <u>HC</u> β = −0.02 (−0.04 - −0.002)	Per unit increase in PFOS	Adjusted for pregnancy weight gain, maternal age, pre-pregnancy BMI, education level, vitamin use, folic acid use, smoking during pregnancy, alcohol use during pregnancy, parity, GA at sample collection. BW analyses also adjusted for maternal age and alcohol use during pregnancy.	Magnitude: no Statistical significance: yes Dose-response: no Temporal association: yes Subgroup only: boys Adjustments: unclear	Authors previously published a study of PFAS and birth size in girls. In a sensitivity analysis including only 1st trimester samples (N=115), associations were consistent with entire study sample. This sample of the ALSPAC cohort was disproportionately white, educated, older, and nonsmoking compared to the overall ALSPAC cohort. PFOA, PFHxS, and PFNA were also analyzed. PFAS concentrations were strongly correlated; PFOA and PFOS (r = 0.63) and PFOS and PFNA (r = 0.60) were most strongly correlated. BMI, parity, and education did not interact with PFAS effects on birth size.

Author year	Location Years	Design	Who N	Factors related to bias	Exposure method	Outcome	Outcome method	Results	Comparison	Confounding	Other aspects of causal inference	Notes
Meng et al., 2018	Denmark 1996-2002	Prospective cohort	N=3,535 mother and singleton infant pairs from the Danish National Birth Cohort Three samples from sub-studies within the same source population were included.	Selection: unclear Participation: about 60% of invited women accepted Equal groups: unclear Blinded: yes Levels: median (IQR) 30.1 (22.9-39.0)	Maternal plasma in 1st (92%) and 2nd trimester Sample 1 plasma was measured at 3M for only PFOS and PFOA in 2007. Plasma from Samples 2 and 3 was analyzed at Aarhus University in 2011 and 2014, respectively, and included 16 PFAS.	BW LBW	Hospital discharge register	BW β = –45.2 (–76.8 - –13.6) LBW OR = 1.3 (0.9 - 2.0)	Per doubling of PFOS exposure	Adjusted for infant sex, birth year, maternal age, parity, socio-occupational status, pre-pregnancy BMI, smoking during pregnancy, alcohol use during pregnancy, gestational week of blood draw.	Magnitude: yes (LBW) Statistical significance: BW-yes, LBW-no Dose-response: no Temporal association: yes Subgroup only: no Adjustments: little change	6 PFAS were analyzed in two different labs, with results differing by lab, though correlations of PFOS and PFOA concentrations measured in both labs were high (r = 0.94 for PFOS and r = 0.95 for PFOA). Adjustment for other PFAS augmented the association between PFOS and BW, though the associations were not statistically significant. Correlations among PFAS: PFOS and PFOA r = 0.66, PFOS and PFHpS r = 0.89, PFOA and PFHpS r = 0.67. 3 of the other 4 PFAS evaluated were associated with BW.
Minatoya et al., 2017	Hokkaido, Japan 2002-2005	Prospective cohort	N=168 pairs of mothers and their term singletons Sapporo Cohort .	Selection: unclear Participation: unclear Equal groups: unclear Blinded: unclear Levels: median (IQR) 5.1 (3.7-6.7)	Maternal serum, collected between 23 weeks gestation and delivery	BW PI	Birth records	BW All: β = –29 (–289 - 232) Girls: β = –251 (–645 - 143) Boys: β = 190 (–162 - 543) PI β = –2.25 kg/m^3 (– 4.01 - –0.50)	Per log-unit increase in PFOS	Adjusted for BMI, maternal smoking during pregnancy, parity, gestation weeks at blood sampling, infant sex, GA. Analyses were conducted for male and female infants combined and separately.	Magnitude: yes (BW - girls and boys) Statistical significance: yes (PI) Dose-response: no Temporal association: yes Subgroup only: no Adjustments: unclear	PFOA and PFOS were modestly correlated, Spearman r = 0.287
Rokoff et al., 2018	Eastern Massa-chusetts 1999-2002	Prospective cohort (Project Viva)	N=1,597 pregnant women recruited at first prenatal visit. Exclusions: multiple gestation, inability to answer in English, gestation >22 weeks at recruitment, plans to move from the area.	Selection: unclear Participation: unclear Equal groups: unclear Blinded: unclear Levels: Mean ± SD 29.1± 16.5 ng/ml	Maternal plasma at first prenatal visit (median 9.9 weeks gestation)	Sex-specific BW for GA (BW/GA) *z*-score based on US national reference data	Hospital medical record	β = –0.03 (–0.08 - 0.02) from best-fitting model (multipollutant additive model with no interaction terms)	Per IQR increase in PFOS (16.1 ng/ml)	Adjusted for maternal age, education, race/ethnicity, parity, pre-pregnancy body mass index, and season of birth, date of birth. Smoking and black carbon exposure in 1st trimester were also added in some models. GFR, plasma albumin, and week of gestation did not significantly change estimates.	Magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: little change	Authors were interested in interactions of early pregnancy exposures to multiple pollutants and associations with BW/GA. PFOA was not evaluated in this study because authors had found null associations with BW/GA in this cohort. Included women were more likely to be white, but were otherwise similar to excluded women.

Author year	Location Years	Design	Who N	Factors related to bias	Exposure method	Outcome	Outcome method	Results	Comparison	Confounding	Other aspects of causal inference	Notes
Sagiv et al., 2018	Boston, MA 1999-2002	Prospective cohort	N=1,645 women with singleton live births	Selection: unclear Participation: 78% Equal groups: unclear Blinded: unclear Levels: median (IQR) 25.7 (16.0)	Maternal plasma at median 9 weeks gestation	BW for GA (BW/GA) *z*- score Term BW	Medical records (US national reference for BW/GA and BW for sex *z*-score)	BW/GA *z*-score β = –0.04 (–0.08 - 0.01) Additional adjustments for eGFR and albumin did not substantially change the estimate. Term BW β = −17.9 (−40.9 - 5.1)	Per IQR increase in PFOS	Adjusted for mother's age, race/ethnicity, education, prenatal smoking, parity, history of breastfeeding, pre-pregnancy BMI, GA at blood draw, sex, paternal education, household income. Plasma albumin concentration and estimated glomerular filtration rate (eGFR) using plasma creatinine values were also included in some models.	Magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: first adjustments (primarily parity) attenuated estimates, but addition of eGFR and plasma albumin concentration did not	Highly educated, high-income population. PFAS were "moderately correlated;" PFOS and PFOA Spearman correlation was 0.72. An objective of this study was to evaluate whether adjusting for pregnancy hemodynamics affected associations between PFAS and birth outcomes.
Shi et al., 2017	Beijing, China 2012	Cross-sectional	N=170 women who gave birth to singletons without congenital anomalies, recruited from one hospital	Selection: unclear Participation: unclear Equal groups: unclear Blinded: unclear Levels: geometric mean ± SD, median (IQR) 1.228 ± 0.899, 0.974 (0.626-1.584)	Cord serum	BW BL PI	Medical records	BW β = 160.45 (–11.85 - 332.75) BL β = 0.33 (–0.14 - 0.79) PI β = 0.07 (–0.03 - 0.16)	Per log-unit increase in PFOS	Models included maternal age, pre-pregnancy BMI, parity, GA, gender, and height (BL only). Participants were non-smokers and did not drink alcohol during pregnancy.	Magnitude: yes (BW) Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: no	The sample included 1 LBW infant.
Shoaff et al., 2018	Cincin-nati, OH 2003-2006	Prospective cohort	N=345 mother-child pairs Eligibility criteria: ≥18 years old, recruited at 16 ± 3 weeks gestation, living in Cincinnati area in a home built before 1978, no history of HIV, no medication for seizure or thyroid disorders, singleton pregnancy.	Selection: unclear Participation: unclear Equal groups: unclear Blinded: yes Levels: median (IQR) 14 (9.6-18)	Maternal serum at ~16 weeks gestation if available (86%), or 26 weeks gestation (9%), or within 48 hours of delivery (5%) if neither earlier sample was available.	BW *z*-scores, standard-ized for GA using US reference data	Hospital records	Continuous β = –0.06 (–0.16 - 0.04) By tertiles: 2: β = –0.05 (–0.29 - 0.19) 3: β = –0.12 (–0.36 - 0.13) p trend <0.36 Sex did not modify associations between PFAS and BW *z*-scores.	Change in BW *z*-score per doubling of serum PFOS, or compared to 1st tertile of PFOS concen-tration PFOS tertiles (ng/ml): 1: 0.4-10.8 2: 10.9-16.5 3: 16.6-57.2	Adjusted for maternal age, race, marital status, insurance status, income, education, parity, serum cotinine, depressive symptoms, mid-pregnancy BMI, food security, fruit/vegetable consumption during pregnancy, fish consumption during pregnancy, prenatal vitamin use	Magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: unclear	PFAS were moderately correlated, the highest correlation was for PFOS and PFOA, r = 0.60. Including all measured PFAS (PFOA, PFNA, PFHxS) in the model attenuated the association toward the null.

Author year	Location Years	Design	Who N	Factors related to bias	Exposure method	Outcome	Outcome method	Results	Comparison	Confounding	Other aspects of causal inference	Notes
Starling et al., 2017	Colorado 2010-2014	Prospective cohort	N=628 participants selected based on availability of maternal serum and cord blood, from women ≥16 years old with singleton pregnancies	Selection: unclear Participation: 11 of 1,410 enrolled dropped out and the 628 included participants did not meaningfully differ from the larger cohort of 1,410. Equal groups: unclear Blinded: yes Levels: median (Q1, Q3) 2.4 (1.5-3.7)	Maternal serum from fasting blood collected at median 27 weeks gestation (range 20-34 weeks)	BW	Weighed by clinical personnel	β = –13.8 (–53.8 - 26.3) No significant interactions between PFAS and sex.	Per ln-unit increase in PFOS	Adjusted for maternal age, pre-pregnancy BMI, race/ethnicity, education level, parity, smoking during pregnancy, gestational weight gain, sex, GA at birth, GA at maternal blood draw	Magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: no	PFAS concentrations showed moderate to high correlations; the highest Spearman rank correlation was for PFOA and PFOS, ρ=0.76. PFOS level was lower than among NHANES females (NHANES geometric mean=5.10).
Valvi et al., 2017	Faroe Islands 1997-2000	Prospective cohort	N=604 mother-child pairs recruited at 34 weeks gestation with singleton	Selection: unclear Participation: 92% of 656 recruited mother-child pairs with complete data on key variables were included Equal groups: n/a Blinded: yes Levels: median (IQR) 27.2 (23.1-33.1)	Maternal serum at week 34	BW BL HC	Unclear for BW and HC BL was measured by midwife at 14 days postpartum	<u>BW</u> All children: β = –81 (–173 - 11) Boys: β = ~–150 p <0.05 (presented graphically) <u>BL</u> β = 0.05 (–0.33 - 0.43) Sex was not a modifier of associations with length <u>HC</u> All children: β = 0.00 (–0.28 - 0.27) Girls: β = ~0.5 cm, p <0.05 Boys: β = ~–0.3 cm, p >0.05 (presented graphically)	Per doubling of PFOS	Adjusted for maternal age, education, parity, pre-pregnancy BMI, smoking during pregnancy, and sex. Evaluated, but not included: gestational weight gain, family history of diabetes, vitamin D concentration, docosahexaenoic acid concentration, and GA.	Magnitude: yes (BW) Statistical significance: yes (BW - boys, and HC - girls) Dose-response: no Temporal association: yes Subgroup only: yes (BW and boys, and HC and girls) Adjustments: no change	Authors were interested in GDM as a possible mediator of the association between environmental pollutants and offspring size, but did not find that it modified or mediated the associations with birth size. Among the PFAS, the highest correlation was between PFOS and PFNA, r=0.63. High PFOS levels. Among the 5 PFAS presented, PFOS appeared to have the strongest effects.
Wang et al., 2019	Hebei Province, China 2013	Cross-sectional	N=424 mother-infant pairs, from 924 healthy 20-40-year-old women recruited in first 12 weeks of pregnancy	Selection: unclear Participation: unclear Equal groups: unclear Blinded: unclear Levels: mean ± SD, median (IQR) 1.10 ± 1.34, 0.65 (0.40-1.19)	Cord blood serum	BW BL HC PI	Measured at birth by obstetric nurses	<u>PI</u> β = –0.04 (–0.09 - 0.001) No associations with BW, BL, or HC	Per ln-unit increase in PFOS	Adjusted for maternal age, family income, maternal education, maternal career, husband's smoking, daily energy intake, daily physical activity, GA, parity, pre-pregnancy BMI, GDM, sex, delivery mode, gestational weight gain. Only 0.5% smoked before pregnancy and none smoked during pregnancy.	Magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: little change	Authors were also interested in effects of PFAS on estrogen homeostasis and role of estrogen as a mediator between PFAS and birth size. Ln PFOS was positively associated with estrone and estriol. Estriol mediated the association between ln PFOS and reduced BW.

Author year	Location Years	Design	Who N	Factors related to bias	Exposure method	Outcome	Outcome method	Results	Comparison	Confounding	Other aspects of causal inference	Notes
Wikstrom et al., 2019	Sweden 2007-2010	Prospective cohort	N=1,533 mother and singleton infant pairs with complete data from the SELMA (Swedish Environ-mental, Longitudinal, Mother and child, Asthma and allergy) pregnancy cohort	Selection: unclear Participation: unclear Equal groups: unclear Blinded: unclear Levels: median (IQR) 5.38 (3.97-7.60)	Maternal serum (measured at median 10 weeks gestation)	BW Sex- and GA-specific BW standard deviation score (BW-SDS) SGA	Swedish Medical Birth Register	BW All children β = –46 (–88 - –3) Girls β = –85 (–145 - –25) Boys β = –13 (–73 - 47) SGA All children OR = 1.19 (0.87 - 0.64) Girls OR = 1.40 (0.83 - 2.35) Boys OR = 1.08 (0.72 - 1.63) BW-SDS All children β = –0.100 (–0.197 - –0.004) Girls β = –0.167 (–0.301 - –0.034) Boys β = –0.027 (–0.166 - 0.112) p-interaction for sex and BW was not significant (p=0.06)	Per ln-unit increase in PFOS (correspond-ing to an increase from 25th to 75th percentile of exposure)	Adjusted for parity, maternal weight, cotinine concentration, sex (unless stratified by sex), GA (BW only). Maternal age, education level, and week of sampling were not confounders.	Magnitude: yes (BW - girls, SGA - girls) Statistical significance: yes (BW and BW-SDS overall, and for girls) Dose-response: yes (BW and BW-SDS for girls: larger change with higher exposure quartile) Temporal association: yes Subgroup only: yes - girls Adjustments: unclear	Restricting analyses to serum samples collected in the first trimester did not change regression coefficients. Correlations among PFAS not reported. BW was inversely associated with PFOS, PFNA, PFDA, and PFUnDA. The finding of significance for girls but not boys was also consistent with the same PFAS. GA did not mediate associations between PFOS and BW.
Woods et al., 2017	9 counties in Cincinnati, OH area. 2003-2006	Prospective cohort	N=272 pregnant women enrolled in the Health Outcomes and Measures of Environment (HOME) Study	Selection: women >18 years old were recruited from prenatal clinics at 13 to <19 weeks pregnancy. Women who were free of specified health conditions and living in residences built before 1978 to select for lead exposure were included. Black women were oversampled. Participation: unclear Equal groups: unclear Blinded: unclear Levels: median (IQR) 14.4 (10-17.9)	Maternal serum at ~16 and ~26 weeks gestation (log_{10} transformed and averaged if multiple measure-ments were available)	BW	Birth records	PFOS was associated with a small (~10 g) decrease in BW but the confidence interval was wide and included 0 (data presented graphically; statistics were not reported).	Per log-unit increase in PFOS (10-fold increase)	Adjusted for maternal age, race, marital status, employment status, income, insurance, education level, tobacco use, gender, BMI, 56 other chemicals from 5 classes of potential endocrine disrupting chemicals, organochlorine pesticides, and heavy metals	Magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: no	All PFAS compounds were associated with small, non-statistically significant decreases in BW. PFOA and PFOS were dropped from the LASSO (least absolute shrinkage and selection operator) and elastic net regression models.
Workman et al., 2019	Winnipeg, Manitoba, Canada 2010-2011	Prospective cohort (subset of Canadian Healthy Infant longitudinal Develop-ment Study cohort)	N=414 mother-infant pairs with prenatal samples from the Canadian Healthy Infant Longitudinal Developmen t Study Cohort	Selection: unclear Participation: unclear Equal groups: unclear Blinded: unclear Levels: median (range) in prenatal plasma 2.2 (0.18-21)	Maternal prenatal plasma collected at 16 and 26 weeks gestation	BW BL HC PI	Not reported	No significant associations between PFOS and size parameters (statistics not reported). Result for PI was not reported.	Regression coefficients were not reported	Adjusted for maternal age, smoking during pregnancy, hypertension during pregnancy, diabetes during pregnancy, parity, sex, GA, method of delivery (for HC)	Magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: unclear	

Author year	Location Years	Design	Who N	Factors related to bias	Exposure method	Outcome	Outcome method	Results	Comparison	Confounding	Other aspects of causal inference	Notes
Xu et al., 2019	Hangzhou, China 2016-2017	Cross-sectional	N=98 mother-infant pairs Women with serious illnesses and clinical symptoms, multiple births, infants with congenital diseases were excluded.	Selection: unclear Participation: unclear Equal groups: n/a Blinded: unclear Levels: median (IQR), mean ± SD 4.07 (2.86-8.05), 5.69 ± 4.19	Umbilical cord serum	BW LBW SGA HC PI	Hospital birth records	BW β = –417.3 (–742.1 - –92.4) PI β = –0.005 (–0.008 - –0.002) BL β = 1.101 (–0.042 - 2.159) SGA OR = 4.138 (1.072 - 15.977) HC No association Because there were only 3 LBW infants, authors did not analyze LBW.	Per log-unit increase in PFOS	Adjusted for maternal age, GA (not in SGA analyses), abortion history, parity, pre-pregnancy BMI, pregnancy weight gain, education, job type, infant gender, tap vs. filtered drinking water.	Magnitude: yes (BW, SGA) Statistical significance: yes (BW, PI, SGA) Dose-response: no Temporal association: no Subgroup only: no Adjustments: large changes with adjustment for BW (unadjusted β = –334.1 (–636.5 - –31.6)) and SGA (unadjusted OR = 1.642 (0.699 - 3.856))	PFOS associated with tap (not filtered) water consumption, overweight (pre-pregnancy BMI>22.5). Correlation between PFOS and PFOA, r=0.35. Correlations between PFOS and other PFAS, |r| ≤ 0.60

Studies are in alphabetical order by first author and year
For BW, a change of >50 g was considered "large magnitude"
Numbers in parentheses are 95% confidence intervals unless otherwise noted
PFOS concentrations are in ng/ml unless otherwise noted
Abbreviations and units: β, regression coefficient; BL, birth (body) length (cm unless otherwise noted); BMI, body mass index (kg/m^2); BW, birth weight (grams unless otherwise notes); BW-SDS, sex- and gestational age-specific birth weight standard deviation score; CI, confidence interval; cm, centimeter; CrI, credible interval; *p,p'*-DDE, dichlorodiphenyldichloroethylene; g, gram; GA, gestational age; GDM, gestational diabetes mellitus; GFR, glomerular filtration rate; GSTM 1, glutathione S-transferase M1; HC, head circumference (cm unless otherwise noted); IQR, interquartile range; kg, kilogram; LBW, low birth weight (birth weight <2,500 g); ln, natural logarithm; LOD, limit of detection; m, meter; N, number of participants; ng/ml, nanograms/milliliter; NHANES, US National Health and Nutrition Examination Survey; OR, odds ratio; PCB-153, polychlorinated biphenyls; PFAS, per-and polyfluoroalkyl substances; PFDA, perfluorodecanoic acid; PFHpS, perfluoroheptane sulfonic acid; PFHxS, perfluorohexane sulfonic acid; PFNA, perfluorononanoic acid; PFUnDA, perfluoroundecanoic acid PI, ponderal index (calculated as [1000*BW/BL] g/cm^3 or [100*BW/BL] g/cm^3 unless otherwise noted); PIH, pregnancy-induced hypertension; PTB, preterm birth; r, correlation coefficient; SD, standard deviation; SES, socioeconomic status; SGA, small for gestational age (<10th percentile for age and sex unless otherwise noted)

Table A7.17. Recent epidemiologic studies on PFOA and pubertal development

Author Year	Location Years	Design	Who N	Factors related to bias	Exposure method	Outcome	Outcome method	Results	Comparison	Confounding	Other aspects of causal inference	Notes
Ernst et al., 2019	Denmark 2000-2003	Retrospective cohort	N=1,167 children from the Danish National Birth Cohort (DNBC) Sample 1: N=722 from the Puberty Cohort Sample 2: N=445 children from another study within the DNBC	Selection: unclear Participation: 66% of invited children from the Puberty Cohort (nested within the DNBC) responded. Children from the larger cohort who had responded to the 11-year questionnaire were also included, which increased the participation to 71% of the Puberty Cohort. Equal groups: unclear Blinded: yes Levels: Median (10^{th}, 90^{th} percentile) ng/ml Sample 1 Boys 5.1 (2.8, 8.3) Girls 4.8 (2.7, 8.2) Sample 2 Boys 4.3 (2.2, 6.7) Girls 4.1 (2.3, 6.4)	First trimester maternal plasma (median 9 gestational weeks, IQR 8-11 weeks).	Age at menarche, voice break, first ejaculation, and Tanner stages 2-5 for pubic hair, breast, genital develop-ment, and a combined puberty indicator	Self-assessment through web-based question-naires administered biannually from age 11 years	No consistent pattern of associations between PFOA concentrations and markers of male or female pubertal development, or the combined puberty indicator.	Change in onset of puberty indicators compared to lowest exposure tertile	Adjusted for: highest social class of parents, maternal age at menarche, maternal age at delivery,parity, pre-pregnancy BMI, and smoking during first trimester	Magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: unclear Magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: unclear	Sample 1 and Sample 2 were analyzed by different labs, with some overlap. Correlations between lab measurements were 0.95 for PFOA and 0.92 for PFOS.
Di Nisio et al., 2020	Veneto Region, Italy 2018-2019	Retrospective cohort with ecologic exposure	N=1,226 female high school students (mean age 18.1 years) who were lifelong residents of the area. 146 were considered "exposed" and 1,080 were "controls" based on residence.	Selection: unclear Participation: 88.5% in the "exposed" group and 90.0% in the "control" group Equal groups: yes Blinded: unclear Levels: median (IQR) ng/ml, based on a subset of 68 "exposed" and 56 "control" participants "exposed" 28.71 (12.36-46.21) "control" 2.59 (1.81-4.75)	Residence as a proxy for exposure	Age at menarche	Question-naire	Mean age at menarche was 164 days later in the exposed group (12.72 vs. 12.27 years), p <0.001	"Exposed" vs. "control" area of residence	Age at survey, BMI	Magnitude: yes Statistical significance: yes Dose-response: no Temporal association: no Subgroup only: no Adjustments: unclear	Authors express interest in in utero exposure, but do not mention any effort to determine whether participants' mothers lived in the area while pregnant. Thus exposure may have represented postnatal exposure more than in utero exposure. Authors report that previous research showed concordance between geographic selection criteria and PFAS exposure patterns, and consistent exposure in all subjects from the area.

Numbers in parentheses are 95% confidence intervals unless otherwise noted
Abbreviations and units: β, regression coefficient; BMI, body mass index; CI, confidence interval; DNBC, Danish National Birth Cohort; IQR, interquartile range; N, number of participants; ng/ml, nanograms/milliliter; OR, odds ratio; PFAS, per-and polyfluoroalkyl substances

Table A7.18. Recent epidemiologic studies on PFOS and pubertal development

Author Year	Location Years	Design	Who N	Factors related to bias	Exposure method	Outcome	Outcome method	Results	Comparison	Confounding	Other aspects of causal inference	Notes
Ernst et al., 2019	Denmark 2000-2003	Retrospective cohort	N=1,167 children from the Danish National Birth Cohort (DNBC) Sample 1: N=722 from the Puberty Cohort Sample 2: N=445 children from another study within the DNBC	Selection: unclear Participation: 66% of invited children from the Puberty Cohort (nested within the DNBC) responded. Children from the larger cohort who had responded to the 11-year questionnaire were also included, which increased the participation to 71% of the Puberty Cohort. Equal groups: unclear Blinded: yes Levels: Median (10^{th}, 90^{th} percentile) ng/ml Sample 1 Boys 31.9 (19.2, 51.2) Girls 32.3 (19.3, 50.8) Sample 2 Boys 27.2 (16.7, 45.2) Girls 27.9 (16.5, 42.2)	First trimester maternal plasma (median 9 gestational weeks, IQR 8-11 weeks).	Age at menarche, voice break, first ejaculation, and Tanner stages 2-5 for pubic hair, breast, genital develop-ment, and a combined puberty indicator	Self-assessment through web-based question-naires	In girls, the middle tertile of PFOS exposure was associated with earlier onset for all individual outcomes except acne; CIs excluded null for Tanner breast stages 2-4. The middle tertile was also associated with earlier puberty based on the combined puberty indicator, β=−3.73 (−6.59-−0.87) months. The highest tertile of PFOS exposure had weaker associations and CIs included the null for all Tanner breast stages, and were mostly null or nonsignificantly associated with later onset for other indicators. In boys, mean ages at onset of Tanner stages of genital development and voice break were earlier with similar differences across the middle and highest tertiles, but only Tanner genital stage 2 for the middle tertile was statistically significant. Tanner pubic hair, axillary hair, acne, and first ejaculation were similar across exposure tertiles.	Change in onset of puberty indicators compared to lowest exposure tertile Middle exposure tertiles, ng/ml: Sample 1: 28.1-38.4 Sample 2: 23.3-31.5	Adjusted for: highest social class of parents, maternal age at menarche, maternal age at delivery, parity, pre-pregnancy BMI, and smoking during first trimester	Girls Magnitude: no Statistical significance: yes (Tanner breast stages 2-4 only) Dose-response: non-monotonic Temporal association: yes Subgroup only: no Adjustments: unclear Boys Magnitude: no Statistical significance: no (except Tanner genital stage 2) Dose-response: no Temporal association: yes Subgroup only: no Adjustments: unclear	Sample 1 and Sample 2 were analyzed by different labs, with some overlap. Correlations between lab measurements were 0.95 for PFOA and 0.92 for PFOS.

Numbers in parentheses are 95% confidence intervals unless otherwise noted
PFOS concentrations are in ng/ml unless otherwise noted
Abbreviations and units: β, regression coefficient; BMI, body mass index; CI, confidence interval; DNBC, Danish National Birth Cohort; IQR, interquartile range; N, number of participants; ng/ml, nanograms/milliliter

Table A7.19. Recent epidemiologic studies on PFOA and fertility and fecundability

Author Year	Location Years	Design	Who N	Factors related to bias	Exposure method	Outcome	Outcome method	Results	Comparison	Confounding	Other aspects of causal inference	Notes
Bach et al., 2018	Denmark 1996-2002	Cross-sectional	N=1,251 pregnant women 613 parous women, 638 nulliparous Sample of women from the Lifestyle During Pregnancy Study nested in the Danish National Birth Cohort Women had a planned pregnancy, a live birth and information on time to pregnancy (TTP)	Selection: unclear Participation: unclear Equal groups: unclear Blinded: unclear Levels: median (IQR) Nulliparous women 5.03 (4.02 - 6.13) Parous women 3.43 (2.60 - 4.53)	Plasma sampled in early pregnancy, median (IQR) 8 (7-10) weeks gestation	Fecunda-bility (probability of pregnancy per cycle), indicated by TTP	Woman's information on TTP	For nulliparous women, PFOA was not associated with fecundability (Model A*). For parous women, Model A fecundability ratio (FR) = 0.63 (0.47-0.86) Model B FR = 0.79 (0.75 - 0.83) Model C FR = 0.85 (0.62 - 1.16) * see Confounding column for adjustments in the different models	Fecundability in highest quartile of plasma PFOA compared to lowest quartile Exposure quartiles: Nulliparous women 1: 1.21-4.02 2: 4.03-5.03 3: 5.0-6.13 4: 6.14-13.80 Parous women 1: 0.61-2.60 2: 2.61-3.43 3: 3.44-4.53 4: 4.54-15.00	Model A adjustments: age, SES; pre-pregnancy BMI Parous women: Model B adjustments: Model A adjustments plus interpreg-nancy interval Model C: Model A adjustments using PFOA quartiles corrected to median interpreg-nancy interval (2.6 years)	Parous women: Magnitude: yes Statistical significance: yes Dose-response: no Temporal association: no Subgroup only: yes Adjustments: substantial changes; see Models B and C vs. Model A results	Most women who had a second pregnancy became pregnant again within 5 years with a peak at approximately 2.5 years. Spearman's correlation between PFOA levels and the interpregnancy interval, r=0.32 PFOA concentrations were higher in women with longer interpregnancy intervals. Used directed acyclic graphs and sensitivity analyses to evaluate unmeasured confounding in parous women. Censoring at 6 months instead of 12 months resulted in similar associations for nulliparous women and slightly attenuated associations for parous women. Authors concluded that associations observed "in parous women may be biased by confounders related to previous pregnancies and exposure measurement error."
Crawford et al., 2017	North Carolina 2008-2009	Prospective cohort	N=99 women attempting to conceive for three months or less, ages 30-44 years, and English-speaking. Women with history of infertility, polycystic ovarian disease, pelvic inflammatory disease, endometriosis, pelvic radiation, or with a partner with a history of infertility were excluded.	Selection: unclear Participation: unclear Equal groups: unclear Blinded: unclear Levels: geometric mean (95% CI) 2.79 (2.48 - 3.16)	Serum from initial study visit	Fecunda-bility using cycle-specific and day-specific models Anti-mullerian hormone (AMH) as an indicator of ovarian reserve	Blood samples and daily diaries Pregnancy was defined by a positive home pregnancy test	PFOA was not associated with fecundability or ovarian reserve. FR Cycle-specific FR = 1.15 (0.66 - 2.01) Day-specific FR = 0.96 (0.31 - 1.94) AMH β = –0.56 (p=0.75)	Highest quartile compared to three lower quartiles of exposure (quartile cut points were not reported)	Adjusted for maternal age (dichotomized at 35 years) and mean cycle length (cycle-specific models only)	Magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: no	Women in the highest combined exposure group (sum of PFOA, PFOS, PFNA, and PFHxS levels) "were more likely to have longer mean cycle lengths (30.7 versus 28.7 days, p=0.02) and less likely to achieve pregnancy at study end (54% versus 75%, p=0.04) as compared to women with lower PFC exposures." Comparing highest quartile with lowest quartile may have yielded different results. Authors found no significant differences in fecundability by parity. Correlations: PFOA and PFOS, r = 0.37 PFOA and other PFAS, r ≤ 0.55 No correlation between PFOA and thyroid stimulating hormone or AMH. PFOA was positively correlated with triiodothyronine (r = 0.23, p = 003). Authors hypothesized that greater exposure to PFAS could be associated with thyroid dysfunction and decreased ovarian reserve, which would result in lower fecundability.

Author Year	Location Years	Design	Who N	Factors related to bias	Exposure method	Outcome	Outcome method	Results	Comparison	Confounding	Other aspects of causal inference	Notes
McCoy et al. 2017	South Carolina 2013-2014	Prospective cohort	N=34 women undergoing in vitro fertilization (IVF) at a fertility clinic, from whom follicular fluid and stimulatory phase plasma samples were collected. No exclusions were applied.	Selection: unclear Participation: of 50 women enrolled, baseline and stimulatory phase and follicular fluid samples were collected from only 26 women. Baseline and stimulatory plasma samples were collected from 8 additional women. No explanation for the absence of information on the remaining women. Equal groups: unclear Blinded: unclear Levels: mean (± SEM) plasma 2.44 (± 0.30) ng/g; follicular fluid 1.94 (± 0.20) ng/g	Plasma at baseline (prior to gonadotropin stimulation phase) Follicular fluid collected at oocyte retrieval	Ovarian function Ovarian response to administration of human chorionic gonadotropin Fertilization	Correlation between plasma and follicular fluid and the following measures: estradiol, follicle count, change in peak 17β-estradiol, change in follicle number, number of oocytes retrieved, fertilization rate, blastocyst conversion (see Notes)	No statistically significant association between plasma or follicular fluid PFOA and any outcomes	Correlations and partial correlations	Corrected for age	Magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: unclear	Correlations among PFAS: <u>Follicular fluid</u> PFOA and PFOS r=0.524 All: range, r = 0.276 - 0.850 <u>Plasma</u> PFOA and PFOS, r=0.540 All: range, r = 0.173 - 0.814 PFOA levels in follicular fluid were similar to those in plasma and highly correlated (r = 0.96) Blastocyst conversion, an important measure of IVF cycle quality, was defined as the number of blastocysts generated by day 6 of development divided by the number of embryos cultured past day 3 of development.
Wang et al., 2017	China 2014-2015	Case-control	Cases N=157 women diagnosed with endometriosis-related infertility. Controls N=178 women with no reproductive endocrine disorders seeking infertility treatment because of male reproductive dysfunction	Selection: unclear Participation: unclear Equal groups: cases were more educated than controls; household income data were missing for 51.6% of cases and 75.3% of controls. Blinded: unclear Levels: median (IQR) cases 14.67 (7.32 - 23.73) controls 12.09 (7.33 - 22.59)	Plasma	Endometriosis-related infertility Infertility was defined as having unprotected intercourse for 12 months without conceiving spontaneously	Diagnosed at infertility clinic	PFOA was not associated with endometriosis-related infertility	Compared to lowest tertile Exposure tertiles: 1: 1.52-8.74 2: >8.74-19.6 3: >19.6-72.1	Adjusted for age, BMI, household income, education No adjustment for alcohol and tobacco use because the vast majority of women were non-smokers and non-drinkers.	Magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: 3rd tertile unadjusted OR = 1.36 (0.80–2.30), adjusted OR = 1.05 (0.58–1.91)	Imputed values were used for missing household income and education. Sensitivity analyses including only nulliparous women and only women without relevant gynecologic disorders as the reference group did not materially change results. Controls did not undergo laparoscopic examination, so some might have had asymptomatic endometriosis. PFBS was associated with endometriosis-related infertility. Correlations among PFAS: PFOA and PFOS, r = 0.31 PFOA and PFBS, r = 0.30 PFOA and other PFAS, range, r=0.21 - 0.50

Studies are in alphabetical order by first author and year
Numbers in parentheses are 95% confidence intervals unless otherwise noted
PFOA concentrations are in ng/ml unless otherwise noted
FRs greater than 1.2 or less than 0.83 were considered large magnitude and labeled "yes" for magnitude in the table.
Abbreviations and units: AMH, Anti-mullerian hormone; β, regression coefficient; BMI, body mass index (kg/m^2); CI, confidence interval; FR, fecundability ratio; IQR, interquartile range; IVF, in vitro fertilization; N, number of participants; ng/ml, nanograms/milliliter; OR, odds ratio; PFBS, perfluorobutanesulfonic acid; PFHxS, perfluorohexane sulfonic acid; PFNA, perfluorononanoic acid; r, correlation coefficient; SEM, standard error of the mean; SES, socioeconomic status; TTP, time to pregnancy

Table A7.20. Recent epidemiologic studies on PFOS and fertility and fecundability

Author Year	Location Years	Design	Who N	Factors related to bias	Exposure method	Outcome	Outcome method	Results	Comparison	Confounding	Other aspects of causal inference	Notes
Bach et al., 2018	Denmark 1996-2002	Cross-sectional	N=1,251 pregnant women 613 parous women, 638 nulliparous Sample of women from the Lifestyle During Pregnancy Study nested in the Danish National Birth Cohort. Women had a planned pregnancy and information on time to pregnancy (TTP).	Selection: unclear Participation: unclear Equal groups: unclear Blinded: unclear Levels: median (IQR) Nulliparous women 30.2 (23.4-38.0) Parous women 26.0 (20.7-33.6)	Plasma sampled in early pregnancy, median (IQR) 8 (7-10) weeks gestation	Fecunda-bility, indicated by TTP	Woman's information on TTP	For nulliparous women, PFOS was not associated with fecundability (Model A). For parous women, Model A fecundability ratio (FR) = 0.60 (0.44 - 0.82) Model B FR = 0.69 (0.66 - 0.73) Model C FR= 0.84 (0.61 - 1.15) * see Confounding column for adjustments in the different models	Fecundability in highest quartile of plasma PFOS compared to lowest quartile Exposure quartiles: Nulliparous women 1: 6.7-23.4 2: 23.5-30.2 3: 30.3-38.0 4: 38.1-117.0 Parous women 1: 6.3-20.7 2: 20.8-26.0 3: 26.1-33.6 4: 33.7-127.0	Model A adjustments: age, SES; pre-pregnancy BMI Parous women: Model B adjustments: Model A adjustments plus interpreg-nancy interval Model C: Model A adjustments using PFOS quartiles corrected to the median interpregnancy interval (2.6 years)	Parous women: Magnitude: yes Statistical significance: yes Dose-response: unclear Temporal association: no Subgroup only: yes Adjustments: substantial change; see Model C vs. Model A results	Most women who had a second pregnancy became pregnant again within 5 years with a peak at approximately 2.5 years. Spearman's correlation between PFOS levels and the interpregnancy interval r=0.13. PFOS concentrations were higher in women with longer interpregnancy intervals. Used directed acyclic graphs (DAGs) and sensitivity analyses to evaluate unmeasured confounding in parous women. Authors concluded that associations observed "in parous women may be biased by confounders related to previous pregnancies and exposure measurement error." Censoring at 6 months instead of 12 months resulted in similar associations for nulliparous women and slightly attenuated associations for parous women.
Crawford et al., 2017	North Carolina 2008-2009	Prospective cohort	N=99 women attempting to conceive for three months or less, ages 30-44 years, and English-speaking. Women with history of infertility, polycystic ovarian disease, pelvic inflammatory disease, endometriosis, pelvic radiation, or with a partner with a history of infertility were excluded.	Selection: unclear Participation: unclear Equal groups: unclear Blinded: unclear Levels: geometric mean (95% CI) 9.29 (8.31 - 10.38)	Serum sampled at initial study visit	Fecund-ability using a cycle-specific and day-specific model Anti-mullerian hormone (AMH) as an indicator of ovarian reserve	Blood samples and daily diaries Pregnancy was defined by a positive home pregnancy test	PFOS was not associated with fecundability or ovarian reserve. FR Cycle-specific FR = 0.89 (0.49 - 1.60) Day-specific FR = 0.99 (0.28 - 2.32) AMH β = 0.07 (p = 0.73) .	Highest quartile compared to three lower quartiles of exposure (quartile cut points were not reported)	Adjusted for maternal age (dichotomized at 35 years), and mean cycle length (cycle-specific models only)	Magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: no	Women in the highest combined exposure group (sum of PFOA, PFOS, PFNA, and PFHxS levels) "were more likely to have longer mean cycle lengths (30.7 versus 28.7 days, p=0.02) and less likely to achieve pregnancy at study end (54% versus 75%, p=0.04) as compared to women with lower PFC exposures." Comparing highest quartile with lowest quartile may have yielded different results. Correlations: PFOA and PFOS, r = 0.37 PFOS and other PFAS, r ≤0.36 PFOS and TSH, r = 0.07 PFOS and AMH, r = -0.01 Authors found no significant differences in fecundability by parity.

Author Year	Location Years	Design	Who N	Factors related to bias	Exposure method	Outcome	Outcome method	Results	Comparison	Confounding	Other aspects of causal inference	Notes
McCoy et al. 2017	South Carolina 2013-2014	Prospective cohort	N=34 women undergoing IVF at a fertility clinic, from whom follicular fluid and stimulatory phase plasma samples were collected. No exclusions were applied.	Selection: unclear Participation: of 50 women enrolled, baseline and stimulatory phase and follicular fluid samples were collected from only 26 women. Baseline and stimulatory plasma samples were collected from 8 additional women. No explanation for the absence of information on the remaining women. Equal groups: unclear Blinded: unclear Levels: mean (± SEM) plasma 6.52 (± 0.50) ng/g; follicular fluid 5.33 (± 0.42) ng/g	Plasma at baseline (prior to gonadotropin stimulation phase) Follicular fluid collected at oocyte retrieval	Ovarian function Ovarian response to administration of human chorionic gonadotropin Fertilization	Correlation between plasma and follicular fluid and the following measures: estradiol, follicle count, change in peak 17β-estradiol, change in follicle number, number of oocytes retrieved, fertilization rate, blastocyst conversion (see Notes)	In plasma PFOS was negatively correlated with plasma estradiol, r = –0.47 pg/ml ($p < 0.05$) No other statistically significant associations with outcomes	Correlations and partial correlations	Corrected for age	Magnitude: yes (estradiol) Statistical significance: yes (estradiol) Dose response: unclear Temporal association: yes Subgroup only: no Adjustments: unclear	Correlations among PFAS: <u>Follicular fluid</u> PFOA and PFOS, r = 0.524 All: range, r = 0.276 - 0.850 <u>Plasma</u> PFOA and PFOS, r = 0.540 All: range, r = 0.173 - 0.814 PFOS levels in follicular fluid were similar to those in plasma and correlated (r = 0.81) Blastocyst conversion, an important measure of IVF cycle quality, was defined as the number of blastocysts generated by day 6 of development divided by the number of embryos cultured past day 3 of development.
Wang et al., 2017	China 2014-2015	Case-control	Cases N=157 women diagnosed with endometriosis-related infertility. Controls N=178 women without reproductive endocrine disorders seeking infertility treatment because of male reproductive dysfunction	Selection: unclear Participation: unclear Equal groups: cases were more educated than controls; household income data were missing for 51.6% of cases and 75.3% of controls. Blinded: unclear Levels: median (IQR) cases 6.40 (4.02 - 11.42) controls 6.60 (3.92 - 13.54)	Plasma	Endometriosis-related infertility	Diagnosed at infertility clinic	ORs by exposure tertile: <u>Entire sample</u> 2: OR = 1.11 (0.61 - 1.99) 3: OR = 0.66 (0.36 - 1.21) <u>Sensitivity analyses</u> Women without pregnancy history: 2: OR = 1.00 (0.45 - 2.21) 3: OR = 0.62 (0.26 - 1.46) Women without other gynecologic pathology: 2: OR = 0.87 (0.44 - 1.73) 3: OR = 0.47 (0.22 - 0.99)	Compared to lowest tertile Exposure tertiles: 1: 0.54-4.70 2: >4.70-9.36 3: >9.36-138	Adjusted for age, BMI, household income, education	Magnitude: yes Statistical significance: no Dose-response: yes (women without other gynecologic pathology) Temporal association: no Subgroup only: no Adjustments: unadjusted entire sample, 3rd tertile OR = 0.88 (0.52 - 1.50)	Imputed values were used for missing household income and education. When analyses did not use imputed values for missing data, associations were attenuated. Controls did not undergo laparoscopic examination, so some might have had asymptomatic endometriosis. PFBS was associated with endometriosis-related infertility Correlations among PFAS: PFOS and PFOA, r = 0.31 PFOS and PFBS, r = 0.13 PFOS and other PFAS range, r=0.17 - 0.81

Studies are in alphabetical order by first author and year
Numbers in parentheses are 95% confidence intervals unless otherwise noted
PFOS concentrations are in ng/ml unless otherwise noted
FRs greater than 1.2 or less than 0.83 were considered large magnitude and labeled "yes" for magnitude in the table.
Abbreviations and units: AMH, Anti-mullerian hormone; β, regression coefficient; BMI, body mass index (kg/m^2); CI, confidence interval; FR, fecundability ratio; IQR, interquartile range; IVF, in vitro fertilization; ln, natural logarithm; N, number of participants; ng/ml, nanograms/milliliter; OR, odds ratio; PFBS, perfluorobutanesulfonic acid; PFHxS, perfluorohexane sulfonic acid; PFNA, perfluorononanoic acid; pg/ml, picograms/ml; r, correlation coefficient; SEM, standard error of the mean; SES, socioeconomic status; TTP, time to pregnancy

Cancer

Literature search and methods

In addition to reviewing the results of previous reviews by the US EPA (US EPA, 2016b; 2016d), ATSDR (2018a), and others, OEHHA reviewed the epidemiologic literature on PFOA and PFOS and cancer, with a focus on cancer of the kidney, testis, bladder, breast, prostate, and pancreas. All cancer types were considered but these types were selected for greater focus because they were the cancers of concern noted in previous reviews and because at least some positive associations were seen for these types in OEHHA's preliminary review of the literature. This literature search included relevant review articles and documents (Chang et al., 2014; IARC, 2018a; ATSDR 2018a; US EPA 2016b and d), and the bibliographies of all study publications meeting OEHHA's inclusion and exclusion criteria. PubMed and Embase searches were also performed for articles published since these reviews (i.e., those published from December 2016 to September 20, 2020). The outcome portion of the search string used is shown below and was derived from the NTP's Draft Handbook for Preparing Report on Carcinogens Monographs (NTP 2016b).

("angiogenesis inducing agents"[mh] OR "myelodysplastic-myeloproliferative diseases"[mh] OR "neoplasms"[mh] OR "carcinogenicity tests"[mh] OR "carcinogens"[mh] OR (sentinel-lymph-node[tiab] NOT biopsy[tiab]) OR (ASCO[tiab] NOT fungi[tiab]) OR (WAGR[tiab] AND syndrome[tiab]) OR 5q-syndrome[tiab] OR leukostasis[tiab] OR acanthoma[tiab] OR acanthomas[tiab] OR acrochordon[tiab] OR acrochordons[tiab] OR acrospiroma[tiab] OR acrospiromas[tiab] OR adamantinoma[tiab] OR adamantinomas[tiab] OR adenoacanthoma[tiab] OR adenoacanthomas[tiab] OR adenoameloblastoma[tiab] OR adenoameloblastomas[tiab] OR adenocanthoma[tiab] OR adenocanthomas[tiab] OR adenocarcinoma[tiab] OR adenocarcinomas[tiab] OR adenofibroma[tiab] OR adenofibromas[tiab] OR adenolipoma[tiab] OR adenolipomas[tiab] OR adenolymphoma[tiab] OR adenolymphomas[tiab] OR adenoma[tiab] OR adenomas[tiab] OR adenomatosis[tiab] OR adenomatous[tiab] OR adenomyoepithelioma[tiab] OR adenomyoepitheliomas[tiab] OR adenomyoma[tiab] OR adenomyomas[tiab] OR adenosarcoma[tiab] OR adenosarcomas[tiab] OR adenosis[tiab] OR aesthesioneuroblastoma[tiab] OR aesthesioneuroblastomas[tiab] OR ameloblastoma[tiab] OR ameloblastomas[tiab] OR amyloidoses[tiab] OR amyloidosis[tiab] OR anaplasia[tiab] OR androblastoma[tiab] OR androblastomas[tiab] OR angioblastoma[tiab] OR angioblastomas[tiab] OR angioendothelioma[tiab] OR angioendotheliomas[tiab] OR angioendotheliomatosis[tiab] OR angiofibroma[tiab] OR angiofibromas[tiab] OR angiofibrosarcoma[tiab] OR angiokeratoma[tiab] OR angiokeratomas[tiab] OR angioleiomyoma[tiab] OR angioleiomyomas[tiab] OR angiolipoma[tiab] OR angiolipomas[tiab] OR angioma[tiab] OR angiomas[tiab] OR angiomatosis[tiab] OR angiomyolipoma[tiab] OR angiomyolipomas[tiab] OR angiomyoma[tiab] OR angiomyomas[tiab] OR angiomyxoma[tiab] OR angiomyxomas[tiab] OR angioreticuloma[tiab] OR angioreticulomas[tiab] OR angiosarcoma[tiab] OR angiosarcomas[tiab] OR apudoma[tiab] OR apudomas[tiab] OR argentaffinoma[tiab] OR argentaffinomas[tiab] OR arrhenoblastoma[tiab] OR arrhenoblastomas[tiab] OR astroblastoma[tiab] OR astroblastomas[tiab] OR astrocytoma[tiab] OR astrocytomas[tiab] OR astroglioma[tiab] OR astrogliomas[tiab] OR baltoma[tiab] OR basiloma[tiab] OR basilomas[tiab] OR blastoma[tiab] OR blastomas[tiab] OR Buschke-Lowenstein[tiab] OR cancer[tiab] OR cancerous[tiab] OR cancers[tiab] OR carcinogen[tiab] OR carcinogenesis[tiab] OR carcinogenic[tiab] OR carcinogenicity-test*[tiab] OR carcinogens[tiab] OR carcinoid[tiab] OR carcinoma[tiab] OR carcinomas[tiab] OR carcinomatosis[tiab] OR carcinosarcoma[tiab] OR carcinosarcomas[tiab] OR cavernoma[tiab] OR cavernomas[tiab] OR cementoma[tiab] OR cementomas[tiab] OR ceruminoma[tiab] OR ceruminomas[tiab] OR chemodectoma[tiab] OR chemodectomas[tiab] OR cherubism[tiab] OR chloroma[tiab] OR chloromas[tiab] OR cholangiocarcinoma[tiab] OR cholangiocarcinomas[tiab] OR cholangiohepatoma[tiab] OR cholangioma[tiab] OR cholangiomas[tiab] OR cholangiosarcoma[tiab] OR cholesteatoma[tiab] OR

cholesteatomas[tiab] OR chondroblastoma[tiab] OR chondroblastomas[tiab] OR chondroma[tiab] OR chondromas[tiab] OR chondrosarcoma[tiab] OR chondrosarcomas[tiab] OR chordoma[tiab] OR chordomas[tiab] OR chorioadenoma[tiab] OR chorioadenomas[tiab] OR chorioangioma[tiab] OR chorioangiomas[tiab] OR choriocarcinoma[tiab] OR choriocarcinomas[tiab] OR chorioepithelioma[tiab] OR chorioepitheliomas[tiab] OR chorionepithelioma[tiab] OR chorionepitheliomas[tiab] OR choristoma[tiab] OR choristomas[tiab] OR chromaffinoma[tiab] OR chromaffinomas[tiab] OR CIN[tiab] OR CLL[tiab] OR cocarcinogenesis[tiab] OR collagenoma[tiab] OR collagenomas[tiab] OR comedocarcinoma[tiab] OR comedocarcinomas[tiab] OR condyloma[tiab] OR condylomas[tiab] OR corticotropinoma[tiab] OR corticotropinomas[tiab] OR craniopharyngioma[tiab] OR craniopharyngiomas[tiab] OR cylindroma[tiab] OR cylindromas[tiab] OR cystadenocarcinoma[tiab] OR cystadenocarcinomas[tiab] OR cystadenofibroma[tiab] OR cystadenofibromas[tiab] OR cystadenoma[tiab] OR cystadenomas[tiab] OR cystoma[tiab] OR cystomas[tiab] OR cystosarcoma[tiab] OR cystosarcomas[tiab] OR dentinoma[tiab] OR dentinomas[tiab] OR dermatofibroma[tiab] OR dermatofibromas[tiab] OR dermatofibrosarcoma[tiab] OR dermatofibrosarcomas[tiab] OR dermoid[tiab] OR dictyoma[tiab] OR dysgerminoma[tiab] OR dysgerminomas[tiab] OR dyskeratoma[tiab] OR dyskeratomas[tiab] OR dysmyelopoiesis[tiab] OR ectomesenchymoma[tiab] OR ectomesenchymomas[tiab] OR elastofibroma[tiab] OR elastofibromas[tiab] OR enchondroma[tiab] OR enchondromas[tiab] OR enchondromatosis[tiab] OR endothelioma[tiab] OR endotheliomas[tiab] OR ependymoblastoma[tiab] OR ependymoblastomas[tiab] OR ependymoma[tiab] OR ependymomas[tiab] OR epidermoid[tiab] OR epithelioma[tiab] OR epitheliomas[tiab] OR erythroleukaemia[tiab] OR erythroleukaemias[tiab] OR erythroleukemia[tiab] OR erythroleukemias[tiab] OR erythroplakia[tiab] OR erythroplakias[tiab] OR erythroplasia[tiab] OR essential-thrombocythemia[tiab] OR esthesioneuroblastoma[tiab] OR esthesioneuroblastomas[tiab] OR esthesioneuroepithelioma[tiab] OR esthesioneuroepitheliomas[tiab] OR exostosis[tiab] OR fibroadenoma[tiab] OR fibroadenomas[tiab] OR fibroadenosarcoma[tiab] OR fibroadenosis[tiab] OR fibrochondrosarcoma[tiab] OR fibroelastoma[tiab] OR fibroelastomas[tiab] OR fibroepithelioma[tiab] OR fibroepitheliomas[tiab] OR fibrofolliculoma[tiab] OR fibrofolliculomas[tiab] OR fibroid[tiab] OR fibroids[tiab] OR fibrolipoma[tiab] OR fibrolipomas[tiab] OR fibroliposarcoma[tiab] OR fibroma[tiab] OR fibromas[tiab] OR fibromatosis[tiab] OR fibromyoma[tiab] OR fibromyomas[tiab] OR fibromyxolipoma[tiab] OR fibromyxoma[tiab] OR fibromyxomas[tiab] OR fibroodontoma[tiab] OR fibroodontomas[tiab] OR fibrosarcoma[tiab] OR fibrosarcomas[tiab] OR fibrothecoma[tiab] OR fibrothecomas[tiab] OR fibroxanthoma[tiab] OR fibroxanthomas[tiab] OR fibroxanthosarcoma[tiab] OR fibroxanthosarcomas[tiab] OR ganglioblastoma[tiab] OR ganglioblastomas[tiab] OR gangliocytoma[tiab] OR gangliocytomas[tiab] OR ganglioglioma[tiab] OR gangliogliomas[tiab] OR ganglioneuroblastoma[tiab] OR ganglioneuroblastomas[tiab] OR ganglioneurofibroma[tiab] OR ganglioneurofibromas[tiab] OR ganglioneuroma[tiab] OR ganglioneuromas[tiab] OR gastrinoma[tiab] OR gastrinomas[tiab] OR germinoma[tiab] OR germinomas[tiab] OR glioblastoma[tiab] OR glioblastomas[tiab] OR gliofibroma[tiab] OR gliofibromas[tiab] OR glioma[tiab] OR gliomas[tiab] OR gliomatosis[tiab] OR glioneuroma[tiab] OR glioneuromas[tiab] OR gliosarcoma[tiab] OR gliosarcomas[tiab] OR glomangioma[tiab] OR glomangiomas[tiab] OR glomangiomatosis[tiab] OR glomangiomyoma[tiab] OR glomangiomyomas[tiab] OR glomangiosarcoma[tiab] OR glomangiosarcomas[tiab] OR glucagonoma[tiab] OR glucagonomas[tiab] OR gonadoblastoma[tiab] OR gonadoblastomas[tiab] OR gonocytoma[tiab] OR gonocytomas[tiab] OR granuloma[tiab] OR granulomas[tiab] OR granulomatosis[tiab] OR gynaecomastia[tiab] OR gynandroblastoma[tiab] OR gynecomastia[tiab] OR haemangioblastoma[tiab] OR haemangioblastomas[tiab] OR haemangioma[tiab] OR haemangiomas[tiab] OR haemangiopericytoma[tiab] OR haemangiopericytomas[tiab] OR haemangiosarcoma[tiab] OR haemangiosarcomas[tiab] OR hamartoma[tiab] OR hamartomas[tiab] OR hemangioblastoma[tiab] OR hemangioblastomas[tiab] OR hemangioendothelioma[tiab] OR hemangioendotheliomas[tiab] OR hemangioendotheliosarcoma[tiab] OR hemangioendotheliosarcomas[tiab] OR hemangioma[tiab] OR hemangiomas[tiab] OR hemangiomatosis[tiab] OR hemangiopericytoma[tiab] OR hemangiopericytomas[tiab] OR hemangioperithelioma[tiab] OR hemangiosarcoma[tiab] OR

hemangiosarcomas[tiab] OR hepatoblastoma[tiab] OR hepatoblastomas[tiab] OR hepatocarcinoma[tiab] OR hepatocarcinomas[tiab] OR hepatocholangiocarcinoma[tiab] OR hepatocholangiocarcinomas[tiab] OR hepatoma[tiab] OR hepatomas[tiab] OR hibernoma[tiab] OR hibernomas[tiab] OR hidradenoma[tiab] OR hidradenomas[tiab] OR hidrocystoma[tiab] OR hidrocystomas[tiab] OR histiocytoma[tiab] OR histiocytomas[tiab] OR hodgkin[tiab] OR hodgkins[tiab] OR hydradenoma[tiab] OR hydradenomas[tiab] OR hypernephroma[tiab] OR hypernephromas[tiab] OR immunocytoma[tiab] OR immunocytoma[tiab] OR insulinomas[tiab] OR keratoacanthoma[tiab] OR keratoacanthomas[tiab] OR leiomyoblastoma[tiab] OR leiomyoblastomas[tiab] OR leiomyofibroma[tiab] OR leiomyofibromas[tiab] OR leiomyoma[tiab] OR leiomyomas[tiab] OR leiomyomatosis[tiab] OR leiomyosarcoma[tiab] OR leiomyosarcomas[tiab] OR leukaemia[tiab] OR leukaemias[tiab] OR leukemia[tiab] OR leukemias[tiab] OR leukoplakia[tiab] OR leukoplakias[tiab] OR lipoadenoma[tiab] OR lipoadenomas[tiab] OR lipoblastoma[tiab] OR lipoblastomas[tiab] OR lipoblastomatosis[tiab] OR lipoma[tiab] OR lipomas[tiab] OR lipomatosis[tiab] OR liposarcoma[tiab] OR liposarcomas[tiab] OR luteinoma[tiab] OR luteoma[tiab] OR luteomas[tiab] OR lymphangioendothelioma[tiab] OR lymphangioendotheliomas[tiab] OR lymphangioleiomyomatosis[tiab] OR lymphangioma[tiab] OR lymphangiomas[tiab] OR lymphangiomatosis[tiab] OR lymphangiomyoma[tiab] OR lymphangiomyomas[tiab] OR lymphangiomyomatosis[tiab] OR lymphangiosarcoma[tiab] OR lymphangiosarcomas[tiab] OR lymphoepithelioma[tiab] OR lymphoepitheliomas[tiab] OR lymphoma[tiab] OR lymphomas[tiab] OR lymphoproliferation[tiab] OR lymphoproliferations[tiab] OR lymphoproliferative[tiab] OR lymphoscintigraphic[tiab] OR lymphoscintigraphy[tiab] OR macroglobulinemia[tiab] OR macroglobulinemias[tiab] OR macroprolactinoma[tiab] OR malignancies[tiab] OR malignancy[tiab] OR malignant[tiab] OR maltoma[tiab] OR maltomas[tiab] OR masculinovoblastoma[tiab] OR mastocytoma[tiab] OR mastocytomas[tiab] OR mastocytosis[tiab] OR medulloblastoma[tiab] OR medulloblastomas[tiab] OR medullocytoma[tiab] OR medullocytomas[tiab] OR medulloepithelioma[tiab] OR medulloepitheliomas[tiab] OR medullomyoblastoma[tiab] OR medullomyoblastomas[tiab] OR meigs-syndrome[tiab] OR melanoacanthoma[tiab] OR melanoacanthomas[tiab] OR melanoameloblastoma[tiab] OR melanocytoma[tiab] OR melanocytomas[tiab] OR melanoma[tiab] OR melanomas[tiab] OR melanomatosis[tiab] OR meningioblastoma[tiab] OR meningioma[tiab] OR meningiomas[tiab] OR meningiomatosis[tiab] OR mesenchymoma[tiab] OR mesenchymomas[tiab] OR mesonephroma[tiab] OR mesonephromas[tiab] OR mesothelioma[tiab] OR mesotheliomas[tiab] OR metaplasia[tiab] OR microglioma[tiab] OR microgliomas[tiab] OR micrometastases[tiab] OR micrometastasis[tiab] OR mycosis-fungoides[tiab] OR myelodysplasia[tiab] OR myelodysplasias[tiab] OR myelodysplastic[tiab] OR myelofibrosis[tiab] OR myelolipoma[tiab] OR myelolipomas[tiab] OR myeloma[tiab] OR myelomas[tiab] OR myelomatosis[tiab] OR myeloproliferation[tiab] OR myeloproliferations[tiab] OR myeloproliferative[tiab] OR myelosuppression[tiab] OR myoblastoma[tiab] OR myoblastomas[tiab] OR myoepithelioma[tiab] OR myoepitheliomas[tiab] OR myofibroblastoma[tiab] OR myofibroblastomas[tiab] OR myofibroma[tiab] OR myofibromas[tiab] OR myofibromatosis[tiab] OR myofibrosarcoma[tiab] OR myofibrosarcomas[tiab] OR myolipoma[tiab] OR myolipomas[tiab] OR myoma[tiab] OR myomas[tiab] OR myosarcoma[tiab] OR myosarcomas[tiab] OR myxofibroma[tiab] OR myxofibromas[tiab] OR myxolipoma[tiab] OR myxolipomas[tiab] OR myxoliposarcoma[tiab] OR myxoma[tiab] OR myxomas[tiab] OR neoplasia[tiab] OR neoplasia[tiab] OR neoplasm[tiab] OR neoplasms[tiab] OR neoplastic[tiab] OR nephroblastoma[tiab] OR nephroblastomas[tiab] OR neurilemmoma[tiab] OR neurilemmomas[tiab] OR neurilemmomatosis[tiab] OR neurilemoma[tiab] OR neurilemomas[tiab] OR neurinoma[tiab] OR neurinomas[tiab] OR neuroblastoma[tiab] OR neuroblastomas[tiab] OR neurocytoma[tiab] OR neurocytomas[tiab] OR neuroepithelioma[tiab] OR neuroepitheliomas[tiab] OR neurofibroma[tiab] OR neurofibromas[tiab] OR neurofibromatosis[tiab] OR neurofibrosarcoma[tiab] OR neurofibrosarcomas[tiab] OR neurolipocytoma[tiab] OR neuroma[tiab] OR neuromas[tiab] OR neuronevus[tiab] OR neurothekeoma[tiab] OR neurothekeomas[tiab] OR nonhodgkin[tiab] OR nonhodgkins[tiab] OR nonseminoma[tiab] OR nonseminomas[tiab] OR nonseminomatous[tiab] OR NSCLC[tiab] OR odontoameloblastoma[tiab] OR odontoma[tiab] OR oligoastrocytoma[tiab] OR oligoastrocytomas[tiab] OR oligodendroglioma[tiab] OR oligodendrogliomas[tiab] OR

oncocytoma[tiab] OR oncocytomas[tiab] OR opsoclonus-myoclonus[tiab] OR orchioblastoma[tiab] OR orchioblastomas[tiab] OR osteoblastoma[tiab] OR osteoblastomas[tiab] OR osteochondroma[tiab] OR osteochondromas[tiab] OR osteochondrosarcoma[tiab] OR osteochondrosarcomas[tiab] OR osteoclastoma[tiab] OR osteoclastomas[tiab] OR osteofibrosarcoma[tiab] OR osteoma[tiab] OR osteomas[tiab] OR osteosarcoma[tiab] OR osteosarcomas[tiab] OR pancreatoblastoma[tiab] OR pancreatoblastomas[tiab] OR papillomas[tiab] OR papillomata[tiab] OR papillomatosis[tiab] OR parachordoma[tiab] OR parachordomas[tiab] OR paraganglioma[tiab] OR paragangliomas[tiab] OR paraneoplastic[tiab] OR perineurioma[tiab] OR perineuriomas[tiab] OR peutz-jeghers[tiab] OR phaeochromocytoma[tiab] OR phaeochromocytomas[tiab] OR pheochromoblastoma[tiab] OR pheochromoblastomas[tiab] OR pheochromocytoma[tiab] OR pheochromocytomas[tiab] OR pilomatricoma[tiab] OR pilomatricomas[tiab] OR pilomatrixoma[tiab] OR pilomatrixomas[tiab] OR pinealblastoma[tiab] OR pinealoblastoma[tiab] OR pinealoblastomas[tiab] OR pinealoma[tiab] OR pinealomas[tiab] OR pineoblastoma[tiab] OR pineoblastomas[tiab] OR pineocytoma[tiab] OR pineocytomas[tiab] OR plasmacytoma[tiab] OR plasmacytomas[tiab] OR pneumoblastoma[tiab] OR pneumoblastomas[tiab] OR pneumocytoma[tiab] OR polyembryoma[tiab] OR polyembryomas[tiab] OR polyhistioma[tiab] OR polyhistiomas[tiab] OR polyposis[tiab] OR porocarcinoma[tiab] OR porocarcinomas[tiab] OR poroma[tiab] OR poromas[tiab] OR precancer[tiab] OR precancerous[tiab] OR reninoma[tiab] OR reninomas[tiab] OR reticuloendothelioma[tiab] OR reticuloendotheliomas[tiab] OR reticulohistiocytoma[tiab] OR reticulohistiocytomas[tiab] OR retinoblastoma[tiab] OR retinoblastomas[tiab] OR rhabdomyoma[tiab] OR rhabdomyomas[tiab] OR rhabdomyosarcoma[tiab] OR rhabdomyosarcomas[tiab] OR rhabdosarcoma[tiab] OR rhabdosarcomas[tiab] OR sarcoma[tiab] OR sarcomas[tiab] OR sarcomatosis[tiab] OR schwannoma[tiab] OR schwannomas[tiab] OR schwannomatosis[tiab] OR seminoma[tiab] OR seminomas[tiab] OR seminomatous[tiab] OR sezary-syndrome[tiab] OR somatostatinoma[tiab] OR somatostatinomas[tiab] OR somatotropinoma[tiab] OR somatotropinomas[tiab] OR spermatocytoma[tiab] OR spiradenoma[tiab] OR spiradenomas[tiab] OR spongioblastoma[tiab] OR spongioblastomas[tiab] OR steatocystoma[tiab] OR steatocystomas[tiab] OR struma-ovarii[tiab] OR subependymoma[tiab] OR subependymomas[tiab] OR syringadenoma[tiab] OR syringadenomas[tiab] OR syringocystadenoma[tiab] OR syringocystadenomas[tiab] OR syringoma[tiab] OR syringomas[tiab] OR teratocarcinoma[tiab] OR teratocarcinomas[tiab] OR teratoma[tiab] OR teratomas[tiab] OR thecoma[tiab] OR thecomas[tiab] OR thymolipoma[tiab] OR thymolipomas[tiab] OR thymoma[tiab] OR thymomas[tiab] OR trichilemmoma[tiab] OR trichilemmomas[tiab] OR trichoadenoma[tiab] OR trichoblastoma[tiab] OR trichoblastomas[tiab] OR trichodiscoma[tiab] OR trichodiscomas[tiab] OR trichoepithelioma[tiab] OR trichoepitheliomas[tiab] OR trichofolliculoma[tiab] OR trichofolliculomas[tiab] OR tricholemmoma[tiab] OR tricholemmomas[tiab] OR tumor[tiab] OR tumorgenesis[tiab] OR tumorgenic[tiab] OR tumorigenesis[tiab] OR tumorigenic[tiab] OR tumor-marker*[tiab] OR tumorogenesis[tiab] OR tumorogenic[tiab] OR tumors[tiab] OR tumour[tiab] OR tumours[tiab] OR vipoma[tiab] OR vipomas[tiab] OR waldenstrom[tiab] OR waldenstroms[tiab] OR xanthoastrocytoma[tiab] OR xanthoastrocytomas[tiab] OR xanthofibroma[tiab] OR xanthofibromas[tiab] OR xanthogranuloma[tiab] OR xanthogranulomas[tiab] OR xanthoma[tiab] OR xanthomas[tiab] OR xanthosarcoma[tiab] OR xanthosarcomas[tiab] OR oncogene-fusion[tiab])

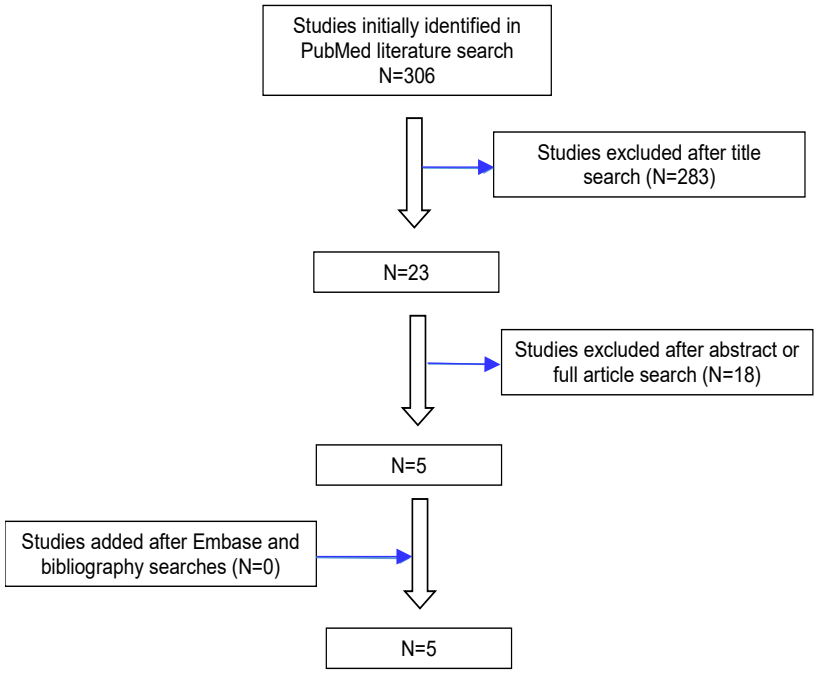
A number of studies of PFOA and PFOS and cancer were cross-sectional, that is, serum PFOA or PFOS levels were measured at or near the time of cancer diagnosis. If the latency period between exposure and cancer development and diagnosis is fairly short, PFOA or PFOS levels measured during these times could potentially provide an accurate metric for assessing the relevant exposure periods since the half-life of PFOA and PFOS in human serum is several years (Olsen et al., 2007). Currently however, the latency period for PFOA or PFOS associated cancer is unknown. Overall, although the results of some cross-sectional studies are discussed in this review, OEHHA excluded cross-sectional studies of cancer from its main analyses given the long latency usually associated with environmentally caused cancer, and the possibility that

cancer diagnosis or treatment could lead to medication use or a change in behaviors that could change PFOA or PFOS exposure levels. The study by Consonni et al. (2013) was reviewed but was excluded from OEHHA's main analyses because of the significant co-exposures to tetrafluoroethylene (TFE), which has been linked to kidney cancer, liver cancer, testicular cancer, and leukemia in rodents (IARC, 2018b). OEHHA included studies of cancer mortality and incidence, with the understanding that mortality studies may be less informative for cancer types with low mortality rates. Studies of cancer mechanisms or biomarkers (e.g., DNA methylation, prostate specific antigen) were not included in this section. In several instances there were multiple publications involving the same study population. When this occurred, a greater emphasis was usually placed on results from the most recent update, although all results were considered.

Results

A general description of the literature search is provided in Figure 7.6. A list of studies excluded after abstract and title search is provided in Table A7.28. Detailed reviews of the studies meeting OEHHA's inclusion criteria are shown in Tables A7.21-27.

Figure A7.6. Literature search: recent epidemiologic studies of PFOA or PFOS and cancer*



*This figure is provided to document OEHHA's PubMed, Embase, and bibliography literature searches. It does not include relevant publications identified from other sources such as previously published reviews from other agencies or other authors.

Table A7.21. Epidemiologic studies of PFOA and bladder cancer: all years

Author Year	Location Years	Design	Who N	Factors related to bias	Exposure method	Outcome	Outcome method	Results	Compari-son	Confound-ing	Other aspects of causal inference	Notes
Steen-land and Woskie, 2012 Steen-land et al., 2015	Where: DuPont, West Virginia Years: 1948-2008	Occupational Retrospective cohort F/U: mean 30 years (mortality)	Who: worked at least 1 day from 1948-2002 Ages: adults N: 5,791 (mortality), 3,713 (incidence)	Selection: all workers Participation: 5,791 of 6,027 (96%) with sufficient records Equal groups: likely Blinded: unclear Levels: mean serum 0.113 µg/ml (incidence) and 0.35 µg/ml (mortality)	Modeled serum levels based on serum from 1,308 workers (2004) and job categories	Bladder incidence and mortality	Incidence: self-reports Mortality: NDI, death certificates	Incidence: RR = 0.23 (0.05-0.93) N=29 cases Similar results with 10-year lag Mortality: SMR = 0.36 (0.10-2.01) for the 4th quartile (≥2,700 ppm-years) N=1 case in the highest category Note: SMR = 2.49 (0.97-5.78) in 2nd vs. 1st quartile N=6 cases, 904-<1,520 ppm-years)	Other DuPont workers in the Appalachian region; quartiles	Adjusted for age and gender	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: not given	Follow-up of Leonard et al., 2008 Mean employment: 19 years F/U through 2008 Peak PFOA usage in the 1990s 19% women and 5% non-whites Likely overlap with other C8 area studies Potential weaknesses: TFE exposure possible but levels unknown Mesothelioma SMRs were above 1.0 suggesting asbestos exposure Modeled exposures Limited information on confounding Possible short duration of exposure in some workers
Raleigh et al., 2014	Where: 3M plants in Minnesota Years: 1960-2008	Occupational Retrospective cohort F/U: 34 years (mean ages 29 to 63 years at start and end, respectively)	Who: all workers at two facilities who worked at least 1 year Ages: adults N: 4,668 (Cottage Grove), 4,359 (Saint Paul)	Selection: all workers Participation: follow-up of approximately 89% Equal groups: likely Blinded: unclear Levels: air levels up to 0.4 mg/m^3 (vs. 1 x 10^{-6} mg/m^3 in unexposed workers), geometric mean serum PFOA concentration of 2538 ng/ml (95% CI, 1626-3961 ng/ml) in those only working in the PFOA area	IH monitoring data and work records used to estimate air levels for all workers	Bladder incidence and mortality	Incidence: Minnesota and Wisconsin cancer registries (mandatory reporting) Mortality: Social Security, NDI	Incidence: HRs of 1.00 (ref), 0.81, 0.78, 1.50, and 1.66 by exposure quartiles HR = 1.66 (0.86-3.18) in the highest quartile (>7.9 x 10^{-4} $µg/m^3$-years) N=12 cases p-trend not given Mortality: Similar results	Minnesota cancer rates and unexposed workers (3M workers in Saint Paul); quartiles	Adjusted for age and sex	Large magnitude: yes Statistical significance: no Dose-response: possible Temporal association: yes Subgroup only: no Adjustments: not given	Follow-up of Lundin et al., 2009 and Gilliland and Mandel, 1993 Cottage Grove plant used PFOA. The Saint Paul plant did not Saint Paul workers were 9 years older 21% women F/U: 1988 through 2008 (incidence) and 1960-2008 (mortality) No TFE exposure Potential weaknesses: Some out-migration (e.g., 31%) in lower exposed Cottage Grove workers Limited information on confounding
Barry et al., 2013	Where: West Virginia and Ohio (DuPont) Years: 1952-2011	Retrospective cohort F/U: average 33 years	Who: living near DuPont plant and DuPont workers Ages: average age 53 N: 32,254	Selection: all current residents and all workers Participation: about 55% Equal groups: likely Blinded: unclear Levels: mean serum 32.91 ng/ml (Frisbee et al., 2009)	Estimated yearly serum levels for 1952-2011 based on plant emissions, wind patterns, river and ground water flow, workplace exposure (see Steenland and Woskie, 2012), drinking water source, and water consumption	Bladder incidence	Self-reports ("Have you ever been told by a doctor…you had cancer…"), with confirmation by cancer registries or medical records	HR = 1.00 (0.89-1.12) for each log increase Similar results for 10- and 20-year lag Similar results for community members and workers	Subjects without the cancer of interest; log increase in PFOA	Adjusted for smoking, alcohol, sex, educa-tion, and age	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: not given	Cohort assembled in 2005-6 and re-interviewed in 2008-11 Followed from age 20 or year 1952, whichever was later Likely overlap with other C8 area studies 30% of cancers reported could not be confirmed (but similar results when these were included) Potential weaknesses: Self-reported cancer Modeled exposures

Author Year	Location Years	Design	Who N	Factors related to bias	Exposure method	Outcome	Outcome method	Results	Compari-son	Confound-ing	Other aspects of causal inference	Notes
Vieira et al., 2013	Where: West Virginia and Ohio (DuPont) Years: 1996-2005	Cancer registry study	Who: cancer cases diagnosed 1996-2005 living near DuPont plant at time of diagnosis Ages: adults N: 25,107	Selection: all cancer cases in registry Participation: 100% Equal groups: unclear Blinded: unclear Levels: serum 5.3 to 125 ng/ml	Median serum levels by water district (both states) or individual modeled estimated serum levels (Ohio only) (see Shin et al., 2011 for details)	Bladder incidence	West Virginia and Ohio cancer registries	OR = 0.60 (0.2-1.5) for the highest category (30.8-109 ng/ml) of modeled serum levels N=4 cases (69 exposed cases overall)	Other cancers except kidney, pancreas, testicular and liver	Adjusted for age, sex, diagno-sis year, smoking, and insur-ance	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: not given	Some analyses assumed a 10-year latency Median residency duration of 17 years for participants of the C8 Health Project Model validation R = 0.82 Likely overlap with other C8 area studies <u>Potential weaknesses:</u> Only includes residence at the time of cancer diagnosis; some analyses assumed a 10-year residency Other cancer cases used as controls Partially ecologic Few highly exposed cases
Mastran-tonio et al., 2017	Where: Veneto, Italy Years: 1980-2013	Ecologic F/U: 31 years	Who: all residents of non-urban areas in Veneto Region Ages: all N: 143,605 residents in the contami-nated areas, 588,012 in the uncon-taminated areas	Selection: all residents of non-urban areas in Veneto Region Participation: 100% Equal groups: the groups being compared were similar in terms of deprivation indices (socioeconomic status) and prevalence of smoking Blinded: unclear Levels: water levels >500 ng/L	Contaminated vs. non-contaminated areas. Contamination defined as at least one exceedance of over 500 ng/L in 2013-2015 in water	Bladder mortality	Death records for 1980-2013	RR = 1.04 (0.91-1.19) N=282 exposed cases RRs of 1.12 and 1.15 in men and women	Communi-ties in the Veneto Region with water PFAS levels <10 ng/L. RR is the ratio of SMRs in the contami-nated vs. uncontami-nated areas	Age and sex stratified. Similar in SES and smoking	Large magnitude: no Statistical significance: no Dose-response: not assessed Temporal association: no Subgroup only: no Adjustments: NA	Local water sources contaminated by a chemical manufacturing plant operating since 1964 Urban areas were excluded <u>Potential weaknesses:</u> Ecologic design Limited information on confounding Mortality only Multiple PFAS, but it appears the highest exposures were for PFOA
Eriksen et al., 2009	Where: Denmark Years: 1993-2006	Nested case-control F/U: 12-13 years	Who: general population Ages: 50-64 N: 332 cases, 772 controls	Selection: all people ages 50-64, born in Denmark, no diagnosis of cancer and living in the Copenhagen, Frederiksberg, Aarhus, Hinnerup or Hørning municipalities Participation: approximately 40% (see Tjonneland et al., 2007) Equal groups: likely Blinded: yes Levels: median serum = 6.9 ng/ml	Serum PFOA collected at recruitment	Bladder incidence	Danish Cancer Registry 1993-2006 ("virtually complete ascertain-ment")	IRRs by quartile all near or below 1.0 No effect modification by sex	Randomly sampled cohort members without cancer; quartiles	Adjusted for age, smoking, educa-tion, occupa-tional expo-sures Matched on sex	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: not given	Samples stored at -150° C Information on confounders from detailed questionnaires at baseline (i.e.,the beginning of the follow-up period) PFOA and PFOS serum concentrations highly correlated (R = 0.70) <u>Potential weaknesses:</u> Single measurement

For all cancer tables:
Rows are sorted by exposure level, from high (top) to low (bottom)
Data in parentheses are 95% confidence intervals unless otherwise stated
Ages are in years unless otherwise stated
Abbreviations: F/U, follow-up; HR, hazard ratio; IH, industrial hygiene; IRR, incidence rate ratio; NA, not applicable; NDI, National Death Index; OR, odds ratio; PFAS, per- and polyfluoroalkyl substances; PFHxS, perfluorohexane sulfonate; PFOSA, perfluorooctanesulfonamide; R, correlation coefficient; RR, rate ratio; SMR, standardized mortality ratio

Table A7.22. Epidemiologic studies of PFOA and breast cancer: all years

Author Year	Location Years	Design	Who N	Factors related to bias	Exposure method	Outcome	Outcome method	Results	Compari-son	Confound-ing	Other aspects of causal inference	Notes
Steen-land and Woskie, 2012	Where: DuPont plant, West Virginia Years: 1948-2008	Occupational Retrospective cohort F/U: mean 30 years	Who: worked at least 1 day in 1948-2002 Ages: adults N: 5,791	Selection: all workers Participation: 5,791 of 6,027 (96%) with sufficient records Equal groups: likely Blinded: unclear Levels: mean serum = 0.35 µg/ml	Modeled serum levels based on serum from 1,308 workers (2004) and job categories	Breast mortality	NDI, death certificates	Only 3 cases, no SMRs reported	Other DuPont workers in the Appalachian region	NA	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: NA	Follow-up of Leonard et al., 2008 Mean employment: 19 years F/U through 2008 Incidence data not given for breast cancer Peak PFOA usage in the 1990s 19% women and 5% non-whites Likely overlap with other C8 area studies <u>Potential weaknesses:</u> TFE exposure possible but levels unknown Mesothelioma SMRs were above 1.0 suggesting asbestos exposure Modeled exposures Limited information on confounding Breast cancer subtypes not examined Possible short duration of exposure in some workers
Raleigh et al., 2014	Where: 3M plants in Minnesota Years: 1960-2008	Occupational Retrospective cohort F/U: 34 years (mean ages 29 to 63 years at start and end, respectively)	Who: all workers at two facilities who worked at least 1 year Ages: adults N: 4,668 (Cottage Grove), 4,359 (Saint Paul)	Selection: all workers Participation: follow-up of approximately 89% Equal groups: likely Blinded: unclear Levels: air levels up to 0.4 mg/m^3 (vs. 1 x 10^{-6} mg/m^3 in unexposed workers), geometric mean serum PFOA concentration of 2,538 ng/ml (95% CI, 1626-3,961 ng/ml) in those only working in the PFOA area	IH monitoring data and work records used to estimate air levels for all workers	Breast incidence and mortality	Incidence: Minnesota and Wisconsin cancer registries (mandatory reporting) Mortality: Social Security, NDI	<u>Mortality:</u> HR = 0.54 (0.15-1.94) for the 3rd and 4th quartile combined (>1.5 x 10^{-4} $µg/m^3$-years) quartile N not given <u>Incidence:</u> HR = 0.85 (0.29-2.46) for the 4th quartile (>7.9 x 10^{-4} $µg/m^3$-years) N=55 cases Note: HR = 1.47 (0.77-2.80) for the 3rd quartile N=14 cases	Minnesota cancer rates and unexposed workers (3M workers in Saint Paul); quartiles	Adjusted for age	Large magnitude: no (but see 3rd quartile) Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: not given	Follow-up of Lundin et al., 2009 and Gilliland and Mandel, 1993 Cottage Grove plant used PFOA. The Saint Paul plant did not Saint Paul workers were 9 years older 21% women F/U: 1988 through 2008 (incidence) and 1960-2008 (mortality) No TFE exposure <u>Potential weaknesses:</u> Some out-migration (e.g., 31%) in lower exposed Cottage Grove workers Limited information on confounding Breast cancer subtypes not examined
Barry et al., 2013	Where: West Virginia and Ohio (DuPont) Years: 1952-2011	Retrospective cohort F/U: average 33 years	Who: living near DuPont plant and DuPont workers Ages: average age 53 N: 32,254	Selection: all current residents and all workers Participation: about 55% Equal groups: likely Blinded: unclear Levels: mean serum = 32.91 ng/ml (Frisbee et al., 2009)	Estimated yearly serum levels for 1952-2011 based on plant emissions, wind patterns, river and ground water flow, workplace exposure (see Steenland and Woskie, 2012), drinking water source, and water consumption	Breast incidence	Self-reports ("Have you ever been told by a doctor…you had cancer…"), with confirmation by cancer registries or medical records	HR = 0.94 (0.89-1.00) for each log increase Similar results for 10- and 20-year lag Similar results for community members and workers	Subjects without the cancer of interest; log increase in PFOA	Adjusted for smoking, alcohol, educa-tion, and age	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: not given	Cohort assembled in 2005-6, and re-interviewed in 2008-11 Followed from age 20 or year 1952, whichever was later (no childhood exposure information) Likely overlap with other C8 area studies 30% of cancers reported could not be confirmed (but similar results when these were included) <u>Potential weaknesses:</u> Self-reported cancer Modeled exposures Breast cancer subtypes not examined

Author Year	Location Years	Design	Who N	Factors related to bias	Exposure method	Outcome	Outcome method	Results	Compari-son	Confound-ing	Other aspects of causal inference	Notes
Vieira et al., 2013	Where: West Virginia and Ohio (DuPont) Years: 1996-2005	Cancer registry study	Who: cancer cases diagnosed from 1996-2005 living near DuPont plant at time of diagnosis Ages: adults N: 25,107	Selection: all cancer cases in registry Participation: 100% Equal groups: unclear Blinded: unclear Levels: estimated serum by city = 5.3 to 125 ng/ml	Median serum levels by water district (both states) or individual modeled estimated serum levels (Ohio only) (see Shin et al., 2011 for details)	Breast incidence	West Virginia and Ohio cancer registries	OR = 1.4 (0.9-2.3) for the highest category (30.8-109 ng/ml) of modeled serum levels N=29 cases ORs near 1.0 for all other categories	Other cancers except kidney, pancreas, testicular and liver	Adjusted for age, diagno-sis year, smoking, and insur-ance	Large magnitude: yes Statistical significance: no Dose-response: not linear Temporal association: yes Subgroup only: no Adjustments: not given	Some analyses assumed a 10-year latency Median residency duration of 17 years for participants of the C8 Health Project Model validation R = 0.82 Likely overlap with other C8 area studies <u>Potential weaknesses:</u> Only includes residence at the time of cancer diagnosis; some analyses assumed a 10-year residency Other cancer cases used as controls Partially ecologic Breast cancer subtypes not examined
Mastran-tonio et al., 2017	Where: Veneto, Italy Years: 1980-2013	Ecologic F/U: 31 years	Who: all residents of non-urban areas in Veneto Region Ages: all N: 143,605 residents in the contami-nated areas, 588,012 in the uncontami-nated areas	Selection: all residents of non-urban areas in Veneto Region Participation: 100% Equal groups: the groups being compared were similar in terms of deprivation indices (socioeconomic status) and prevalence of smoking Blinded: unclear Levels: water levels >500 ng/L	Contaminated vs. non-contaminated areas. Contamination defined as at least one exceedance of 500 ng/L in 2013-2015 in water	Breast mortality	Death records for 1980-2013	RR = 1.11 (1.02-1.20) N=809 exposed cases	Communi-ties in the Veneto Region with water PFAS levels <10 ng/L. RR is the ratio of SMRs in the contamin-ated vs. uncontamin-ated areas	Age and sex stratified. Similar in SES and smoking	Large magnitude: no Statistical significance: yes Dose-response: not assessed Temporal association: unclear Subgroup only: no Adjustments: NA	Local water sources contaminated by a chemical manufacturing plant operating since 1964 Urban areas were excluded <u>Potential weaknesses:</u> Ecologic design Limited information on confounding Mortality only Small increase in RR Breast cancer subtypes not examined Multiple PFAS, but it appears the highest exposures were for PFOA
Mancini et al., 2019	Where: France Years: 1990-2013	Nested case-control F/U: to 2013	Who: had national health insurance for workers Ages: 40-65 at enrollment N: 194 cases, 194 controls	Selection: unclear Participation: >97% follow-up Equal groups: yes Blinded: unclear Levels: serum median = 6.64 ng/ml	Blood collected in 1993-4	Breast incidence	Self-reports and NDI	No clear increase overall ORs of 3-7 for estrogen (ER) or progesterone receptor (PR) negative cases but p-trend = 0.59 and 0.90 respectively	Controls without breast cancer randomly selected from the cohort	Adjusted for lipids, BMI, smoking, exercise, educa-tion, family history, and many other known risk factors Matched by age, meno-pause status, BMI, and year of blood collection	Large magnitude: mixed Statistical significance: no Dose-response: no Temporal association: no Subgroup only: elevated risks with ER or PR negative cases Adjustments: little change	Information on confounders collected at baseline <u>Potential weaknesses:</u>

Author Year	Location Years	Design	Who N	Factors related to bias	Exposure method	Outcome	Outcome method	Results	Compari-son	Confound-ing	Other aspects of causal inference	Notes
Bonefeld-Jorgen-sen et al., 2014 Ghisari et al., 2017	Where: Denmark Years: 1996-2010	Nested case-control F/U: 10-15 years	Who: pregnant women Ages: adults N: 250 breast cancer cases and 233 controls	Selection: Danish National Birth Cohort recruitment in 1996-2002 Participation: ½ of all Danish pregnant women invited, about 60% agreed Equal groups: yes (see their Table 1) Blinded: unclear Levels: mean serum = 5.2 ng/ml	Blood at 1st and 2nd trimester of pregnancy	Breast incidence	Cancer registry	All RRs near 1.0 Highest category: >6.53 ng/ml Some RRs above 1.0 in women >40 years old, but unusual dose-response pattern	Other randomly selected members of the cohort	Adjusted for age, BMI, parity, oral contra-ceptive use, men-arche, smoking, alcohol, educa-tion, exercise Matched on age and parity	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: little change	Samples stored at -80º C F/U through 2010 Mostly premenopausal women Questionnaires administered during pregnancy and 6 months post-partum Correlation between PFOA and PFOS = 0.69 Positive association seen for PFOSA, negative association seen for PFHxS Possible interaction with CYP19 CC genotype but multiple comparisons (Ghisari et al., 2017) <u>Weaknesses:</u> Only 1 or 2 serum samples Breast cancer subtypes not examined
Cohn et al., 2019	Where: California Years: 1959-2013	Nested case-control F/U: 54 years, through 2013	Who: Kaiser members, in utero at time of recruitment (1959-67) Ages: adults N: 102 cases and 310 controls	Selection: >99% of eligible women enrolled Participation: <50% Equal groups: unclear Blinded: yes Levels: median serum = 0.4 ng/ml	Perinatal serum, mostly 1-3 days after delivery	Breast incidence	Vital status records, cancer registry (>99% complete), and self-reports	"no association"; actual results not given	Controls randomly selected from the cohort	Adjusted for choles-terol, DDT, age, race, BMI, parity, maternal history of breast cancer Matched on birth year and trimester of blood draw	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: not given	Samples stored at -20º C Includes mothers and their daughters Questionnaire information collected at baseline (i.e., at the start of the follow-up period) <u>Weaknesses:</u> Single measurement in mother Limited results Breast cancer subtypes not examined

Table A7.23. Epidemiologic studies of PFOA and kidney cancer: all years

Author Year	Location Years	Design	Who N	Factors related to bias	Exposure method	Outcome	Outcome method	Results	Compari-son	Confound-ing	Other aspects of causal inference	Notes
Girardi and Merler, 2019	Where: Trissino, Veneto, Italy Years: 1960-2018	Occupational Retrospective cohort F/U: average of 31.7 years	Who: worked at least 1 day from 1948-2002 Ages: adults N: 462 chemical or office workers, 1,383 railroad workers	Selection: all male workers, at least 6 months, from 1960-2008 Participation: unclear Equal groups: unclear Blinded: unclear Levels: geometric mean = 4.048 µg/ml	Job activities and tasks based on records and some interviews used to classify workers as ever, never exposed, or office workers. Jobs, years worked, and serum levels in a subsample (N = 120 workers) used to model cumulative exposure	Kidney mortality	Local and national death records	Two cases, SMRs not provided	Regional mortality rates (for SMRs), and local railroad workers (for RRs); tertiles of cumulative exposure	Adjusted for age Males only	Large magnitude: not given Statistical significance: not given Dose-response: unknown Temporal association: not given Subgroup only: no Adjustments: not given	F/U 1970 to 2018 Average length of employment = 17.1 years Other chemicals produced at the plant include fluoroaromatics, benzotrifluorides, and PFOS (mean serum PFOS = 0.148 µg/ml, correlation with PFOA = 0.59) Elevated SMRs for suicides Potential weaknesses: Small number of cases No measure of association provided
Steen-land and Woskie, 2012	Where: DuPont plant, West Virginia Years: 1948-2008	Occupational Retrospective cohort F/U: mean 30 years	Who: worked at least 1 day 1948-2002 Ages: adults N: 5,791	Selection: all workers Participation: 5,791 of 6,027 (96%) with sufficient records Equal groups: likely Blinded: unclear Levels: mean serum = 0.35 µg/ml	Modeled serum levels based on serum from 1,308 workers (2004) and job categories	Kidney mortality	NDI, death certificates	SMR = 2.68 (1.15-5.24) for the 4th quartile (≥2,700 ppm-years) N=8 cases p-trend = 0.02 SMR increases somewhat with 10- and 20-year lags	Other DuPont workers in the Appalachian region; quartiles	Adjusted for age and gender	Large magnitude: yes Statistical significance: yes Dose-response: linear trend Temporal association: yes Subgroup only: no Adjustments: not given	Follow-up of Leonard et al., 2008 Mean employment: 19 years F/U through 2008 Incidence data not given for kidney cancer Peak PFOA usage in the 1990s 19% women and 5% non-whites Likely overlap with other C8 area studies Potential weaknesses: TFE exposure possible but levels unknown Mesothelioma SMRs were above 1.0 suggesting asbestos exposure Modeled exposures Limited information on confounding Exposure duration could be short in some workers Possible short duration of exposure in some workers
Raleigh et al., 2014	Where: 3M plants in Minnesota Years: 1960-2008	Occupational Retrospective cohort F/U: 34 years (mean ages 29 to 63 years at start and end, respectively)	Who: all workers at two facilities who worked at least 1 year Ages: adults N: 4,668 (Cottage Grove), 4,359 (Saint Paul)	Selection: all workers Participation: follow-up of approximately 89% Equal groups: likely Blinded: unclear Levels: air levels up to 0.4 mg/m^3 (vs. 1 x 10^{-6} mg/m^3 in unexposed workers), geometric mean serum PFOA concentration of 2538 ng/ml (95% CI, 1626-3961 ng/ml) in those only working in the PFOA area	IH monitoring data and work records used to estimate air levels for all workers	Kidney incidence and mortality	Incidence: Minnesota and Wisconsin cancer registries (mandatory reporting) Mortality: Social Security, NDI	Incidence: No increase N=16 cases Little change with 10-year lag Highest category: >7.9 x 10^{-4} $µg/m^3$-years Mortality: No increase	Minnesota cancer rates and unexposed workers (3M workers in Saint Paul); categorical	Adjusted for age and sex	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: not given	Follow-up of Lundin et al., 2009 and Gilliland and Mandel, 1993 Cottage Grove plant used PFOA. The Saint Paul plant did not Saint Paul workers were 9 years older 21% women F/U: 1988 through 2008 (incidence) and 1960-2008 (mortality) No TFE exposure Potential weaknesses: Some out-migration (e.g., 31%) in lower exposed Cottage Grove workers Limited information on confounding

Author Year	Location Years	Design	Who N	Factors related to bias	Exposure method	Outcome	Outcome method	Results	Compari-son	Confound-ing	Other aspects of causal inference	Notes
Barry et al., 2013	Where: West Virginia and Ohio (DuPont) Years: 1952-2011	Retrospective cohort F/U: average 33 years	Who: living near DuPont plant and DuPont workers Ages: average age 53 N: 32,254	Selection: all current residents and all workers Participation: about 55% Equal groups: likely Blinded: unclear Levels: mean serum = 32.91 ng/ml (Frisbee et al., 2009)	Estimated yearly serum levels for 1952-2011 based on plant emissions, wind patterns, river and ground water flow, workplace exposure (see Steenland and Woskie, 2012), drinking water source, and water consumption	Kidney incidence	Self-reports ("Have you ever been told by a doctor…you had cancer…"), with confirmation by cancer registries or medical records	HRs = 1.00, 1.23, 1.48, and 1.58 by quartile of cumulative exposure HR = 1.58 (0.88-2.84) for the 4th quartile N=105 cases overall p-trend = 0.18 Similar results for 10- and 20-year lag Workers: HRs of 1.00, 0.84 (0.21-3.40), 4.20 (1.07-16.44), and 0.83 (0.20-3.55) by quartiles N=18 cases p-trend = 0.54	Subjects without the cancer of interest; quartiles	Adjusted for smoking, alcohol, sex, educa-tion, and age	Large magnitude: yes Statistical significance: not in highest quartile Dose-response: possible Temporal association: yes Subgroup only: no Adjustments: not given	Cohort assembled in 2005-6, and re-interviewed in 2008-11 Followed from age 20 or year 1952, whichever was later (no childhood exposure information) Likely overlap with other C8 area studies 30% of cancers reported could not be confirmed (but similar results when these were included) Potential weaknesses: Self-reported cancer Modeled exposures
Vieira et al., 2013	Where: West Virginia and Ohio (DuPont) Years: 1996-2005	Cancer registry study	Who: cancer cases diagnosed 1996-2005 living near DuPont plant at time of diagnosis Ages: adults N: 25,107	Selection: all cancer cases in registry Participation: 100% Equal groups: unclear Blinded: unclear Levels: estimated mean serum by city = 5.3 to 125 ng/ml	Median serum levels by water district (both states) or individual modeled estimated serum levels (Ohio only) (see Shin et al., 2011 for details)	Kidney incidence	West Virginia and Ohio cancer registries	OR = 0.8, 1.2, 2.0 (1.3-3.2), and 2.0 (1.0-3.9) by categories of exposure vs. unexposed (modeled serum levels) N=9 cases in the highest category (30.8-109 ng/ml) of modeled serum levels OR in women = 3.5 (1.4-8.3)	Other cancers except kidney, pancreas, testicular and liver; categorical	Adjusted for age, sex, diagno-sis year, smoking, and insur-ance	Large magnitude: yes Statistical significance: yes Dose-response: plateau Temporal association: yes Subgroup only: highest OR in women Adjustments: not given	Some analyses assumed a 10-year latency Median residency duration of 17 years for participants of the C8 Health Project Model validation R = 0.82 Likely overlap with other C8 area studies Potential weaknesses: Only includes residence at the time of cancer diagnosis; some analyses assumed a 10-year residency Other cancer cases used as controls Partially ecologic
Mastran-tonio et al., 2017	Where: Veneto, Italy Years: 1980-2013	Ecologic F/U: 31 years	Who: all residents of non-urban areas in Veneto Region Ages: all N: 143,605 residents in the contami-nated areas, 588,012 in the uncon-taminated areas	Selection: all residents of non-urban areas in Veneto Region Participation: 100% Equal groups: the groups being compared were similar in terms of deprivation indices (socioeconomic status) and prevalence of smoking Blinded: unclear Levels: water levels >500 ng/L	Contaminated vs. non-contaminated areas. Contamination defined as at least one exceedance of 500 ng/L in 2013-2015 in water	Kidney mortality	Death records for 1980-2013	RR = 1.07 (0.90-1.28) in men N=225 exposed cases RR = 1.32 (1.06-1.65) in women N=103 exposed cases	Communi-ties in the Veneto Region with water PFAS levels <10 ng/L. RR is the ratio of SMRs in the contami-nated vs. uncontami-nated areas	Age and sex stratified. Similar in SES and smoking	Large magnitude: yes Statistical significance: yes Dose-response: not assessed Temporal association: unclear Subgroup only: women Adjustments: NA	Local water sources contaminated by a chemical manufacturing plant operating since 1964 Urban areas were excluded Potential weaknesses: Ecologic design Limited information on confounding Mortality only Multiple PFAS, but it appears the highest exposures were for PFOA

Author Year	Location Years	Design	Who N	Factors related to bias	Exposure method	Outcome	Outcome method	Results	Compari-son	Confound-ing	Other aspects of causal inference	Notes
Shearer et al., 2021	Where: US (10 sites) Years: 1993-2014	Nested case-control F/U: 8.9 years on average	Who: Adults Ages: 55-74 at baseline N: 324 cases; 324 controls	Selection: convenience sample Participation: unclear Equal groups: yes Blinded: yes Levels: median serum levels of 5.5 ng/ml	Serum	Renal cell carcinoma	Annual question-naires, physicians or relatives, the National Death Index, or local cancer registries	Adjusted ORs of 1.00 (ref), 1.47 (0.77-2.80), 1.24 (0.64-2.41), and 2.63 (1.33-5.20) by quartile if exposure in controls	Lowest quartile	Adjusted for age, sex, race/ ethnicity, study center, year of blood draw, eGFR, BMI, smoking, hyperten sion, freeze-thaw cycles of samples, and calendar year of blood draw	Large magnitude: yes Statistical significance: yes Dose-response: yes Temporal association: yes Subgroup only: no Adjustments: little change	<u>Potential weaknesses:</u> Single blood sample

Table A7.24. Epidemiologic studies of PFOA and liver cancer: all years

Author Year	Location Years	Design	Who N	Factors related to bias	Exposure method	Outcome	Outcome method	Results	Compari-son	Confound-ing	Other aspects of causal inference	Notes
Girardi and Merler, 2019	Where: Trissino, Veneto, Italy Years: 1960-2018	Occupational Retrospective cohort F/U: average of 31.7 years	Who: worked at least 1 day from 1948-2002 Ages: adults N: 462 chemical or office workers, 1,383 railroad workers	Selection: all male workers, at least 6 months, from 1960-2008 Participation: unclear Equal groups: unclear Blinded: unclear Levels: geometric mean = 4.048 µg/ml	Job activities and tasks based on records and some interviews used to classify workers as ever, never exposed, or office workers. Jobs, years worked, and serum levels in a subsample (N = 120 workers) used to model cumulative exposure	Liver mortality	Local and national death records	SMR = 4.71 (1.52-14.6) for those ever working at the PFAS facility N=3 cases (7 cases total) SMRs of 1.02 (0.12-7.21), 2.76 (0.69-11.0), and 3.07 (1.15-8.18) by tertile of cumulative exposure p-trend = 0.027 Higher mortality RRs when compared to railroad workers	Regional mortality rates (for SMRs), and local railroad workers (for RRs); tertiles of cumulative exposure	Adjusted for age Males only	Large magnitude: yes Statistical significance: yes Dose-response: yes Temporal association: yes Subgroup only: no Adjustments: not given	F/U 1970 to 2018 Average length of employment = 17.1 years Other chemicals produced at the plant include fluoroaromatics, benzotrifluorides, and PFOS (mean serum PFOS = 0.148 µg/ml, correlation with PFOA = 0.59) Elevated SMRs for suicides <u>Potential weaknesses:</u> Increased SMR in those never working in the PFAS area (SMR = 2.71; 95% CI, 1.02-7.22) (geometric mean serum levels = 977 ng/ml; range = 19–15,786 ng/ml) Limited information on confounding Small numbers of cases

Author Year	Location Years	Design	Who N	Factors related to bias	Exposure method	Outcome	Outcome method	Results	Compari-son	Confound-ing	Other aspects of causal inference	Notes
Steen-land and Woskie, 2012	Where: DuPont plant, West Virginia Years: 1948-2008	Occupational Retrospective cohort F/U: mean 30 years	Who: worked at least 1 day from 1948-2002 Ages: adults N: 5,791	Selection: all workers Participation: 5,791 of 6,027 (96%) with sufficient records Equal groups: likely Blinded: unclear Levels: mean serum = 0.35 µg/ml	Modeled serum levels based on serum from 1,308 workers (2004) and job categories	Liver mortality	NDI, death certificates	SMRs of 2.39 (0.65-6.13), 0 (0-1.81), 2.01 (0.65-4.68), and 0.32 (0.01-1.76) by quartile of exposure vs. the referent population N=1 case in the highest quartile and 10 cases overall	Other DuPont workers in the Appalachian region; quartiles	Adjusted for age and gender	Large magnitude: unclear Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: not given	Follow-up of Leonard et al., 2008 Mean employment: 19 years F/U through 2008 Incidence data not given for liver cancer Peak PFOA usage in the 1990s 19% women and 5% non-whites Likely overlap with other C8 area studies <u>Potential weaknesses:</u> TFE exposure possible but levels unknown Mesothelioma SMRs were above 1.0 suggesting asbestos exposure Modeled exposures Limited information on confounding Small numbers of cases Possible short duration of exposure in some workers
Raleigh et al., 2014	Where: 3M plants in Minnesota Years: 1960-2008	Occupational Retrospective cohort F/U: 34 years (mean ages 29 to 63 years at start and end, respectively)	Who: all workers at two facilities who worked at least 1 year Ages: adults N: 4,668 (Cottage Grove), 4,359 (Saint Paul)	Selection: all workers Participation: follow-up of approximately 89% Equal groups: likely Blinded: unclear Levels: air levels up to 0.4 mg/m^3 (vs. 1 x 10^{-6} mg/m^3 in unexposed workers), geometric mean serum PFOA concentration of 2538 ng/ml (95% CI, 1626-3961 ng/ml) in those only working in the PFOA area	IH monitoring data and work records used to estimate air levels for all workers	Liver incidence and mortality	Incidence: Minnesota and Wisconsin cancer registries (mandatory reporting) Mortality: Social Security, NDI	<u>Incidence:</u> Few cases, HRs not calculated <u>Mortality:</u> HRs of 1.00 (ref), 2.09 (0.69-6.31), and 0.67 (0.14-3.27) for quartile 1-2 combined, and quartile 3-4 combined, respectively N=8 cases total in exposed quartiles 1-4	Minnesota cancer rates and unexposed workers (3M workers in Saint Paul); quartiles	Adjusted for age and sex	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: not given	Follow-up of Lundin et al., 2009 and Gilliland and Mandel, 1993 Cottage Grove plant used PFOA. The Saint Paul plant did not Saint Paul workers were 9 years older 21% women F/U: 1988 through 2008 (incidence) and 1960-2008 (mortality) No TFE exposure <u>Potential weaknesses:</u> Some out-migration (e.g., 31%) in lower exposed Cottage Grove workers Limited information on confounding Small numbers of cases
Barry et al., 2013	Where: West Virginia and Ohio (DuPont) Years: 1952-2011	Retrospective cohort F/U: average 33 years	Who: living near DuPont plant and DuPont workers Ages: average age 53 N: 32,254	Selection: all current residents and all workers Participation: about 55% Equal groups: likely Blinded: unclear Levels: mean serum = 32.91 ng/ml (Frisbee et al., 2009)	Estimated yearly serum levels for 1952-2011 based on plant emissions, wind patterns, river and ground water flow, workplace exposure (see Steenland and Woskie, 2012), drinking water source, and water consumption	Liver incidence	Self-reports ("Have you ever been told by a doctor…you had cancer…"), with confirmation by cancer registries or medical records	HR = 0.73 (0.43-1.23) for each log increase N=9 cases overall Similar results for 10- and 20-year lag Only 1 occupationally exposed case	Subjects without the cancer of interest; log increase in PFOA	Adjusted for smoking, alcohol, sex, educa-tion, and age	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: not given	Cohort assembled in 2005-6, and re-interviewed in 2008-11 Followed from age 20 or year 1952, whichever was later Likely overlap with other C8 area studies 30% of cancers reported could not be confirmed (but similar results when these were included) <u>Potential weaknesses:</u> Self-reported cancer Modeled exposures Small number of cases

Author Year	Location Years	Design	Who N	Factors related to bias	Exposure method	Outcome	Outcome method	Results	Compari-son	Confound-ing	Other aspects of causal inference	Notes
Vieira et al., 2013	Where: West Virginia and Ohio (DuPont) Years: 1996-2005	Cancer registry study	Who: cancer cases diagnosed 1996-2005 living near DuPont plant at time of diagnosis Ages: adults N: 25,107	Selection: all cancer cases in registry Participation: 100% Equal groups: unclear Blinded: unclear Levels: estimated mean serum by city = 5.3 to 125 ng/ml	Median serum levels by water district (both states) or individual modeled estimated serum levels (Ohio only) (see Shin et al., 2011 for details)	Liver incidence	West Virginia and Ohio cancer registries	OR = 1.0 (0.3-3.1) for the "high" category (30.8-109 ng/ml) of modeled serum levels N=3 cases (11 exposed cases overall)	Other cancers except kidney, pancreas, testicular and liver; categorical	Adjusted for age, sex, diagno-sis year, smoking, and insur-ance	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: not given	Some analyses assumed a 10-year latency Median residency duration of 17 years for participants of the C8 Health Project Model validation R = 0.82 Likely overlap with other C8 area studies <u>Potential weaknesses:</u> Only includes residence at the time of cancer diagnosis; some analyses assumed a 10-year residency Other cancer cases used as controls Partially ecologic Few highly exposed cases
Mastran-tonio et al., 2017	Where: Veneto, Italy Years: 1980-2013	Ecologic F/U: 31 years	Who: all residents of non-urban areas in Veneto Region Ages: all N: 143,605 residents in the contami-nated areas, 588,012 in the uncontam-inated areas	Selection: all residents of non-urban areas in Veneto Region Participation: 100% Equal groups: the groups being compared were similar in terms of deprivation indices (socioeconomic status) and prevalence of smoking Blinded: unclear Levels: water levels >500 ng/L	Contaminated vs. non-contaminated areas. Contamination defined as at least one exceedance of 500 ng/L in 2013-2015 in water	Liver mortality	Death records for 1980-2013	RR = 0.89 (0.78-1.03) N=242 exposed cases Similar RRs in men and women	Communi-ties in the Veneto Region with water PFAS levels <10 ng/L. RR is the ratio of SMRs in the contami-nated vs. uncontami-nated areas	Age and sex stratified. Similar in SES and smoking	Large magnitude: no Statistical significance: no Dose-response: not assessed Temporal association: no Subgroup only: no Adjustments: NA	Local water sources contaminated by a chemical manufacturing plant operating since 1964 Urban areas were excluded <u>Potential weaknesses:</u> Ecologic design Limited information on confounding Mortality only Multiple PFAS, but it appears the highest exposures were for PFOA
Eriksen et al., 2009	Where: Denmark Years: 1993-2006	Nested case-control F/U: 12-13 years	Who: general population Ages: 50-64 N: 67 cases, 772 controls	Selection: all people ages 50-64, born in Denmark, no diagnosis of cancer and living in the Copenhagen, Frederiksberg, Aarhus, Hinnerup or Hørning municipalities Participation: approximately 40% (see Tjonneland et al., 2007) Equal groups: likely Blinded: yes Levels: median serum = 6.9 ng/ml	Serum PFOA collected at recruitment	Liver incidence	Danish Cancer Registry 1993-2006 ("virtually complete ascertain-ment")	IRRs by quartile all near or below 1.0 N=67 cases total No effect modification by sex	Randomly sampled cohort members without cancer; quartiles	Adjusted for age, smoking, educa-tion, occupa-tional expo-sures Matched on sex	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: not given	Samples stored at -150° C Information on confounders from detailed questionnaires at baseline (i.e.,the beginning of the follow-up period) PFOA and PFOS serum concentrations highly correlated (R = 0.70) <u>Potential weaknesses:</u> Single measurement

Table A7.25. Epidemiologic studies of PFOA and pancreatic cancer: all years

Author Year	Location Years	Design	Who N	Factors related to bias	Exposure method	Outcome	Outcome method	Results	Compari-son	Confound-ing	Other aspects of causal inference	Notes
Steen-land and Woskie, 2012	Where: DuPont plant, West Virginia Years: 1948-2008	Occupational Retrospective cohort F/U: mean 30 years	Who: worked at least 1 day 1948-2002 Ages: adults N: 5,791	Selection: all workers Participation: 5,791 of 6,027 (96%) with sufficient records Equal groups: likely Blinded: unclear Levels: mean serum = 0.35 µg/ml	Modeled serum levels based on serum from 1,308 workers (2004) and job categories	Pancreas mortality	NDI, death certificates	SMR = 0.92 (0.30-2.16) for the 4th quartile (≥2,700 ppm-years) N=5 cases in the highest quartile	Other DuPont workers in the Appalachian region; quartiles	Adjusted for age and gender	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: not given	Follow-up of Leonard et al., 2008 Mean employment: 19 years F/U through 2008 Incidence data not given for pancreatic cancer Peak PFOA usage in the 1990s 19% women and 5% non-whites Likely overlap with other C8 area studies Potential weaknesses: TFE exposure possible but levels unknown Mesothelioma SMRs were above 1.0 suggesting asbestos exposure Modeled exposures Limited information on confounding Possible short duration of exposure in some workers
Raleigh et al., 2014	Where: 3M plants in Minnesota Years: 1960-2008	Occupational Retrospective cohort F/U: 34 years (mean ages 29 to 63 years at start and end, respectively)	Who: all workers at two facilities who worked at least 1 year Ages: adults N: 4,668 (Cottage Grove), 4,359 (Saint Paul)	Selection: all workers Participation: follow-up of approximately 89% Equal groups: likely Blinded: unclear Levels: air levels up to 0.4 mg/m^3 (vs. 1 x 10^{-6} mg/m^3 in unexposed workers), geometric mean serum PFOA concentration of 2538 ng/ml (95% CI, 1626-3961 ng/ml) in those only working in the PFOA area	IH monitoring data and work records used to estimate air levels for all workers	Pancreas incidence and mortality	Incidence: Minnesota and Wisconsin cancer registries (mandatory reporting) Mortality: Social Security, NDI	Incidence: HR = 1.36 (0.59-3.11) for the highest two quartiles combined (>1.5 x 10^{-4} $µg/m^3$-years) N=9 cases in quartiles 3-4 p-trend not given Mortality: Similar results	Minnesota cancer rates and unexposed workers (3M workers in Saint Paul); quartiles	Adjusted for age and sex	Large magnitude: yes Statistical significance: no Dose-response: no Temporal association: yes Subgroup only: no Adjustments: not given	Follow-up of Lundin et al., 2009 and Gilliland and Mandel, 1993 Cottage Grove plant used PFOA. The Saint Paul plant did not Saint Paul workers were 9 years older 21% women F/U: 1988 through 2008 (incidence) and 1960-2008 (mortality) No TFE exposure Potential weaknesses: Some out-migration (e.g., 31%) in lower exposed Cottage Grove workers Limited information on confounding
Barry et al., 2013	Where: West Virginia and Ohio (DuPont) Years: 1952-2011	Retrospective cohort F/U: average 33 years	Who: living near DuPont plant and DuPont workers Ages: average age 53 N: 32,254	Selection: all current residents and all workers Participation: about 55% Equal groups: likely Blinded: unclear Levels: mean serum = 32.91 ng/ml (Frisbee et al., 2009)	Estimated yearly serum levels for 1952-2011 based on plant emissions, wind patterns, river and ground water flow, workplace exposure (see Steenland and Woskie, 2012), drinking water source, and water consumption	Pancreas incidence	Self-reports ("Have you ever been told by a doctor…you had cancer…"), with confirmation by cancer registries or medical records	HR = 1.00 (0.89-1.12) for each log increase N=24 cases overall Similar results for 10- and 20-year lag Similar results for community members and workers	Subjects without the cancer of interest; log increase in PFOA	Adjusted for smoking, alcohol, sex, educa-tion, and age	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: not given	Cohort assembled in 2005-6, and re-interviewed in 2008-11 Followed from age 20 or year 1952, whichever was later (no childhood exposure information) Likely overlap with other C8 area studies 30% of cancers reported could not be confirmed (but similar results when these were included) Potential weaknesses: Self-reported cancer Modeled exposures

Author Year	Location Years	Design	Who N	Factors related to bias	Exposure method	Outcome	Outcome method	Results	Compari-son	Confound-ing	Other aspects of causal inference	Notes
Vieira et al., 2013	Where: West Virginia and Ohio (DuPont) Years: 1996-2005	Cancer registry study	Who: cancer cases diagnosed 1996-2005 living near DuPont plant at time of diagnosis Ages: adults N: 25,107	Selection: all cancer cases in registry Participation: 100% Equal groups: unclear Blinded: unclear Levels: estimated mean serum by city = 5.3 to 125 ng/ml	Median serum levels by water district (both states) or individual modeled estimated serum levels (Ohio only) (see Shin et al., 2011 for details)	Pancreas incidence	West Virginia and Ohio cancer registries	OR = 0.6 (0.1-2.5) for the "very high" category (30.8-109 ng/ml) of modeled serum levels N=2 cases in this category	Other cancers except kidney, pancreas, testicular and liver; categorical	Adjusted for age, sex, diagno-sis year, smoking, and insur-ance	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: not given	Some analyses assumed a 10-year latency Median residency duration of 17 years for participants of the C8 Health Project Model validation R = 0.82 Likely overlap with other C8 area studies <u>Potential weaknesses:</u> Only includes residence at the time of cancer diagnosis; some analyses assumed a 10-year residency Other cancer cases used as controls Partially ecologic
Mastran-tonio et al., 2017	Where: Veneto, Italy Years: 1980-2013	Ecologic F/U: 31 years	Who: all residents of non-urban areas in Veneto Region Ages: all N: 143,605 residents in the contami-nated areas, 588,012 in the uncontami-nated areas	Selection: all residents of non-urban areas in Veneto Region Participation: 100% Equal groups: the groups being compared were similar in terms of deprivation indices (socioeconomic status) and prevalence of smoking Blinded: unclear Levels: water levels >500 ng/L	Contaminated vs. non-contaminated areas. Contamination defined as at least one exceedance of 500 ng/L in 2013-2015 in water	Pancreas mortality	Death records for 1980-2013	RR = 1.11 (0.99-1.25) in men N=361 exposed cases RR = 0.99 (0.87-1.12) in women N=302 exposed cases	Communi-ties in the Veneto Region with water PFAS levels <10 ng/L. RR is the ratio of SMRs in the contami-nated vs. uncontami-nated areas	Age and sex stratified. Similar in SES and smoking	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: NA	Local water sources contaminated by a chemical manufacturing plant operating since 1964 Urban areas were excluded <u>Potential weaknesses:</u> Ecologic design Limited information on confounding Mortality only Multiple PFAS, but it appears the highest exposures were for PFOA
Eriksen et al., 2009	Where: Denmark Years: 1993-2006	Nested case-control F/U: 12-13 years	Who: general population adults Ages: 50-64 N: 128 cases, 772 controls	Selection: all people ages 50-64, born in Denmark, no diagnosis of cancer and living in the Copenhagen, Frederiksberg, Aarhus, Hinnerup or Hørning municipalities Participation: approximately 40% (see Tjonneland et al., 2007) Equal groups: likely Blinded: yes Levels: median serum = 6.9 ng/ml	Serum PFOA collected at recruitment	Pancreas incidence	Danish Cancer Registry 1993-2006 ("virtually complete ascertain-ment")	IRR by quartile of 1.00, 0.88, 1.33, 1.55 (0.85-2.80) Quartile cut-off points not given N=32 cases in the highest quartile Trend per 1 ng/ml IRR = 1.03 (0.98-1.10)	Randomly sampled cohort members without cancer; quartiles	Adjusted for age, smoking, diet Matched on sex	Large magnitude: yes Statistical significance: no Dose-response: possible linear Temporal association: yes Subgroup only: no Adjustments: not given	Samples stored at -150º C Information on confounders from detailed questionnaires at baseline (i.e.,the beginning of the follow-up period) PFOA and PFOS serum concentrations highly correlated (R = 0.70) No effect modification by sex <u>Potential weaknesses:</u> Single measurement

Table A7.26. Epidemiologic studies of PFOA and prostate cancer: all years

Author Year	Location Years	Design	Who N	Factors related to bias	Exposure method	Outcome	Outcome method	Results	Compari-son	Confound-ing	Other aspects of causal inference	Notes
Steen-land and Woskie, 2012 Steen-land et al., 2015	Where: DuPont plant, West Virginia Years: 1948-2008	Occupational Retrospective cohort F/U: mean 30 years	Who: worked at least 1 day 1948-2002 Ages: adults N: 5,791 (mortality), 3,713 (incidence)	Selection: all workers Participation: 5,791 of 6,027 (96%) with sufficient records Equal groups: likely Blinded: unclear Levels: mean serum = 0.113 µg/ml (incidence) and 0.35 µg/ml (mortality)	Modeled serum levels based on serum from 1,308 workers (2004) and job categories	Prostate incidence and mortality	Incidence: self-reports Mortality: NDI, death certificates	Incidence: RR = 1.00, 1.81, 2.45, and 1.88 by quartiles RR = 1.88 (0.72-4.88) for the highest quartile N=129 cases overall Mortality: SMR = 0.57 (0.16-1.46) for the 4th quartile (≥2,700 ppm-years) N=4 cases in the highest category	Other DuPont workers in the Appalachian region; quartiles	Adjusted for age	Large magnitude: yes Statistical significance: no Dose-response: plateau Temporal association: yes Subgroup only: no Adjustments: not given	Follow-up of Leonard et al., 2008 Mean employment: 19 years F/U through 2008 Peak PFOA usage in the 1990s 19% women and 5% non-whites Likely overlap with other C8 area studies Potential weaknesses: TFE exposure possible but levels unknown Mesothelioma SMRs were above 1.0 suggesting asbestos exposure Modeled exposures Limited information on confounding Possible short duration of exposure in some workers
Raleigh et al., 2014	Where: 3M plants in Minnesota Years: 1960-2008	Occupational Retrospective cohort F/U: 34 years (mean ages 29 to 63 years at start and end, respectively)	Who: all workers at two facilities who worked at least 1 year Ages: adults N: 4,668 (Cottage Grove), 4,359 (Saint Paul)	Selection: all workers Participation: follow-up of approximately 89% Equal groups: likely Blinded: unclear Levels: air levels up to 0.4 mg/m^3 (vs. 1 x 10^{-6} mg/m^3 in unexposed workers), geometric mean serum PFOA concentration of 2538 ng/ml (95% CI, 1626-3961 ng/ml) in those only working in the PFOA area	IH monitoring data and work records used to estimate air levels for all workers	Prostate incidence and mortality	Incidence: Minnesota and Wisconsin cancer registries (mandatory reporting) Mortality: Social Security, NDI	Mortality: HR = 1.32 (0.61-2.84) for the 4th quartile (>7.9 x 10^{-4} $\mu g/m^3$-years) N not given Incidence: HR = 1.11 (0.82-1.49) for the 4th quartile (>7.9 x 10^{-4} $\mu g/m^3$-years) N=55 cases	Minnesota cancer rates and unexposed workers (3M workers in Saint Paul); quartiles	Adjusted for age	Large magnitude: yes Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: not given	Follow-up of Lundin et al., 2009 and Gilliland and Mandel, 1993 Cottage Grove plant used PFOA. The Saint Paul plant did not Saint Paul workers were 9 years older 21% women F/U: 1988 through 2008 (incidence) and 1960-2008 (mortality) No TFE exposure Potential weaknesses: Some out-migration (e.g., 31%) in lower exposed Cottage Grove workers Limited information on confounding
Barry et al., 2013	Where: West Virginia and Ohio (DuPont) Years: 1952-2011	Retrospective cohort F/U: average 33 years	Who: living near DuPont plant and DuPont workers Ages: average age 53 N: 32,254	Selection: all current residents and all workers Participation: about 55% Equal groups: likely Blinded: unclear Levels: mean serum = 32.91 ng/ml (Frisbee et al., 2009)	Estimated yearly serum levels for 1952-2011 based on plant emissions, wind patterns, river and ground water flow, workplace exposure (see Steenland and Woskie, 2012), drinking water source, and water consumption	Prostate incidence	Self-reports ("Have you ever been told by a doctor…you had cancer…"), with confirmation by cancer registries or medical records	HR = 0.99 (0.93-1.04) for each log increase Similar results for 10- and 20-year lag Similar results for community members and workers	Subjects without the cancer of interest; log increase in PFOA	Adjusted for smoking, alcohol, educa-tion, and age	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: not given	Cohort assembled in 2005-6, and re-interviewed in 2008-11 Followed from age 20 or year 1952, whichever was later (no childhood exposure information) Likely overlap with other C8 area studies 30% of cancers reported could not be confirmed (but similar results when these were included) Potential weaknesses: Self-reported cancer Modeled exposures

Author Year	Location Years	Design	Who N	Factors related to bias	Exposure method	Outcome	Outcome method	Results	Compari-son	Confound-ing	Other aspects of causal inference	Notes
Vieira et al., 2013	Where: West Virginia and Ohio (DuPont) Years: 1996-2005	Cancer registry study	Who: cancer cases diagnosed 1996-2005 living near DuPont plant at time of diagnosis Ages: adults N: 25,107	Selection: all cancer cases in registry Participation: 100% Equal groups: unclear Blinded: unclear Levels: estimated mean serum by city = 5.3 to 125 ng/ml	Median serum levels by water district (both states) or individual modeled estimated serum levels (Ohio only) (see Shin et al., 2011 for details)	Prostate incidence	West Virginia and Ohio cancer registries	OR = 1.5 (0.9-2.5) for the highest category (30.8-109 ng/ml) of modeled serum levels N=31 cases ORs near 1.0 in all other categories	Other cancers except kidney, pancreas, testicular and liver; categorical	Adjusted for age, diagno-sis year, smoking, and insur-ance	Large magnitude: yes Statistical significance: no Dose-response: no Temporal association: yes Subgroup only: no Adjustments: not given	Some analyses assumed a 10-year latency Median residency duration of 17 years for participants of the C8 Health Project Model validation R = 0.82 Likely overlap with other C8 area studies <u>Potential weaknesses:</u> Only includes residence at the time of cancer diagnosis; some analyses assumed a 10-year residency Other cancer cases used as controls Partially ecologic
Mastran-tonio et al., 2017	Where: Veneto, Italy Years: 1980-2013	Ecologic F/U: 31 years	Who: all residents of non-urban areas in Veneto Region Ages: all N: 143,605 residents in the contami-nated areas, 588,012 in the uncontam-inated areas	Selection: all residents of non-urban areas in Veneto Region Participation: 100% Equal groups: the groups being compared were similar in terms of deprivation indices (socioeconomic status) and prevalence of smoking Blinded: unclear Levels: water levels >500 ng/L	Contaminated vs. non-contaminated areas. Contamination defined as at least one exceedance of 500 ng/L in 2013-2015 in water	Prostate mortality	Death records for 1980-2013	RR = 1.00 (0.90-1.12) in the contaminated area N=401 exposed cases	Communi-ties in the Veneto Region with water PFAS levels <10 ng/L. RR is the ratio of SMRs in the contami-nated vs. uncontam-inated areas	Age. Similar in SES and smoking	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: NA	Local water sources contaminated by a chemical manufacturing plant operating since 1964 Urban areas were excluded <u>Potential weaknesses:</u> Ecologic design Limited information on confounding Mortality only Multiple PFAS, but it appears the highest exposures were for PFOA
Eriksen et al., 2009	Where: Denmark Years: 1993-2006	Nested case-control F/U: 12-13 years	Who: general population adults Ages: 50-64 N: 713 cases, 772 controls	Selection: all people ages 50-64, born in Denmark, no diagnosis of cancer and living in the Copenhagen, Frederiksberg, Aarhus, Hinnerup or Hørning municipalities Participation: approximately 40% (see Tjonneland et al., 2007) Equal groups: likely Blinded: yes Levels: median serum = 6.9 ng/ml	Serum PFOA collected at recruitment	Prostate incidence	Danish Cancer Registry 1993-2006 ("virtually complete ascertain-ment")	IRR by quartile all near 1.0 OR = 1.38 (0.99-1.93) for the upper 3 quartiles vs. lowest quartile, but no clear dose-response relationship (see notes)	Randomly sampled cohort members without cancer, matched on sex; quartiles	Adjusted for age, educa-tion, BMI, and diet	Large magnitude: yes Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: not given	Samples stored at -150º C Information on confounders from detailed questionnaires at baseline (i.e.,the beginning of the follow-up period) PFOA and PFOS serum concentrations highly correlated (R = 0.70) No effect modification by sex <u>Potential weaknesses:</u> Single measurement OR of 1.38 could be due to lower risk in lower quartile caused by chance

Table A7.27. Epidemiologic studies of PFOA and testicular cancer: all years

Author Year	Location Years	Design	Who N	Factors related to bias	Exposure method	Outcome	Outcome method	Results	Compari-son	Confound-ing	Other aspects of causal inference	Notes
Steen-land and Woskie, 2012	Where: DuPont plant, West Virginia Years: 1948-2008	Occupa-tional Retrospec-tive cohort F/U: mean 30 years	Who: worked at least one day 1948-2002 Ages: adults N: 5,791	Selection: all workers Participation: 5,791 of 6,027 (96%) with sufficient records Equal groups: likely Blinded: unclear Levels: mean serum = 0.35 µg/ml	Modeled serum levels based on serum from 1,308 workers (2004) and job categories	Testicular mortality	NDI, death certificates	SMR = 1.81 (0.05-10.03) for all exposed quartiles combined (0 to ≥2,700 ppm-years) N=1 case	Other DuPont workers in the Appalachian region; quartiles	Adjusted for age and gender	Large magnitude: yes Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: not given	Follow-up of Leonard et al., 2008 Mean employment: 19 years F/U through 2008 Incidence data not given for testicular cancer Peak PFOA usage in the 1990s 19% women and 5% non-whites Likely overlap with other C8 area studies Potential weaknesses: TFE exposure possible but levels unknown Mesothelioma SMRs were above 1.0 suggesting asbestos exposure Modeled exposures Limited information on confounding Possible short duration of exposure in some workers
Raleigh et al., 2014	Where: 3M plants in Minnesota Years: 1960-2008	Occupa-tional Retrospec-tive cohort F/U: 34 years (mean ages 29 to 63 years at start and end, respective-ly)	Who: all workers at two facilities who worked at least 1 year Ages: adults N: 4,668 (Cottage Grove), 4,359 (Saint Paul)	Selection: all workers Participation: follow-up of approximately 89% Equal groups: likely Blinded: unclear Levels: air levels up to 0.4 mg/m^3 (vs. 1 x 10^{-6} mg/m^3 in unexposed workers), geometric mean serum PFOA concentration of 2538 ng/ml (95% CI, 1626-3961 ng/ml) in those only working in the PFOA area	IH monitoring data and work records used to estimate air levels for all workers	Testicular incidence and mortality	Incidence: Minnesota and Wisconsin cancer registries (mandatory reporting) Mortality: Social Security, NDI	Few cases (N=5), SMRs and RRs not given	Minnesota cancer rates and unexposed workers (3M workers in Saint Paul)	Adjusted: NA	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: NA	Follow-up of Lundin et al., 2009 and Gilliland and Mandel, 1993 Cottage Grove plant used PFOA. The Saint Paul plant did not Saint Paul workers were 9 years older 21% women F/U: 1988 through 2008 (incidence) and 1960-2008 (mortality) No TFE exposure Potential weaknesses: Some out-migration (e.g., 31%) in lower exposed Cottage Grove workers Limited information on confounding
Barry et al., 2013	Where: West Virginia and Ohio (DuPont) Years: 1952-2011	Retrospec-tive cohort F/U: average 33 years	Who: living near DuPont plant and DuPont workers Ages: average age 53 N: 32,254	Selection: all current residents and all workers Participation: about 55% Equal groups: likely Blinded: unclear Levels: mean serum = 32.91 ng/ml (Frisbee et al., 2009)	Estimated yearly serum levels for 1952-2011 based on plant emissions, wind patterns, river and ground water flow, workplace exposure (see Steenland and Woskie, 2012), drinking water source, and water consumption	Testicular incidence	Self-reports ("Have you ever been told by a doctor…you had cancer…"), with confirmation by cancer registries or medical records	HRs = 1.00, 1.04, 1.91, and 3.17 by quartile of cumulative exposure HR = 3.17 (0.75-13.45) for the 4th quartile N=17 cases overall p-trend = 0.04 Similar results for 10- and 20-year lag Only 2 cases in the worker cohort	Subjects without the cancer of interest; quartiles	Adjusted for smoking, alcohol, sex, educa-tion, and age	Large magnitude: yes Statistical significance: yes Dose-response: yes Temporal association: yes Subgroup only: no Adjustments: not given	Cohort assembled in 2005-6, and re-interviewed in 2008-11 Followed from age 20 or year 1952, whichever was later (no childhood exposure information) Likely overlap with other C8 area studies 30% of cancers reported could not be confirmed (but similar results when these were included) Potential weaknesses: Self-reported cancer Modeled exposures

Author Year	Location Years	Design	Who N	Factors related to bias	Exposure method	Outcome	Outcome method	Results	Compari-son	Confound-ing	Other aspects of causal inference	Notes
Vieira et al., 2013	Where: West Virginia and Ohio (DuPont) Years: 1996-2005	Cancer registry study	Who: cancer cases diagnosed 1996-2005 living near DuPont plant at time of diagnosis Ages: adults N: 25,107	Selection: all cancer cases in registry Participation: 100% Equal groups: unclear Blinded: unclear Levels: estimated mean serum by city = 5.3 to 125 ng/ml	Median serum levels by water district (both states) or individual modeled estimated serum levels (Ohio only) (see Shin et al., 2011 for details)	Testicular incidence	West Virginia and Ohio cancer registries	OR = 2.8 (0.8-9.2) for the highest category (30.8-109 ng/ml) of modeled serum levels N=6 cases ORs below 1.0 in all other categories OR = 5.1 (1.6-15.6) in the highest exposed water district	Other cancers except kidney, pancreas, testicular and liver; categorical	Adjusted for age, sex, diagno-sis year, smoking, and insur-ance	Large magnitude: yes Statistical significance: no for the analyses of modeled serum, yes for district water concentrations Dose-response: no Temporal association: yes Subgroup only: no Adjustments: not given	Some analyses assumed a 10-year latency Median residency duration of 17 years for participants of the C8 Health Project Model validation R = 0.82 Likely overlap with other C8 area studies <u>Potential weaknesses:</u> Only includes residence at the time of cancer diagnosis; some analyses assumed a 10-year residency Other cancer cases used as controls Partially ecologic
Mastran-tonio et al., 2017	Where: Veneto, Italy Years: 1980-2013	Ecologic F/U: 31 years	Who: all residents of non-urban areas in Veneto Region Ages: all N: 143,605 residents in the contami-nated areas, 588,012 in the uncontami-nated areas	Selection: all residents of non-urban areas in Veneto Region Participation: 100% Equal groups: the groups being compared were similar in terms of deprivation indices (socioeconomic status) and prevalence of smoking Blinded: unclear Levels: water levels >500 ng/L	Contaminated vs. non-contaminated areas. Contamination defined as at least one exceedance of 500 ng/L in 2013-2015 in water	Testicular mortality	Death records for 1980-2013	RR = 1.86 (0.81-4.27) for the contaminated area N=8 exposed cases	Communi-ties in the Veneto Region with water PFAS levels <10 ng/L. RR is the ratio of SMRs in the contami-nated vs. uncontami-nated areas	Age and sex stratified. Similar in SES and smoking	Large magnitude: yes Statistical significance: no Dose-response: not assessed Temporal association: unclear Subgroup only: no Adjustments: NA	Local water sources contaminated by a chemical manufacturing plant operating since 1964 Urban areas were excluded <u>Potential weaknesses:</u> Ecologic design Limited information on confounding Mortality only Multiple PFAS, but it appears the highest exposures were for PFOA

Excluded Studies

Table A7.28. Studies excluded after abstract or full article review and reasons for exclusion

Study reference	Health outcome	Reason for exclusion[1]
Bonefeld-Jorgensen EC, Long M, Bossi R, Ayotte P, Asmund G, Krüger T, Ghisari M et al. (2011). Perfluorinated compounds are related to breast cancer risk in Greenlandic Inuit: a case control study. Environ Health. 10:88	Cancer	Cross-sectional: problems assessing temporal association; exposure data collected at or near the time of outcome diagnosis or assessment
Cordiano V., Bai E., Crosignani P., Mastrantonio M. Increased mortality from diabetes mellitus, acute myocardial infarction, genitourinary tract diseases in a community heavily exposed since 1960s to drinking water contaminated with perfluorinated substances a class of endocrine disruptors and carcinogens in the Veneto Region. Italian Journal of Medicine 2016 10 Supplement 2 (30-)	Cancer	Overlap with Mastrantonio et al., 2017
Costa G, Sartori S, Consonni D (2009). Thirty years of medical surveillance in perfluorooctanoic acid production workers. J Occup Environ Med. 51(3):364-72.	Cancer	No cancer data (cholesterol and exposure data)
Fry K., Power M.C. Persistent organic pollutants and mortality in the United States, NHANES 1999-2011. Environmental Health: A Global Access Science Source 2017 16:1	Cancer	No PFOS or PFOA and cancer data
Ghisari M, Eiberg H, Long M, Bonefeld-Jørgensen EC (2014). Polymorphisms in phase I and phase II genes and breast cancer risk and relations to persistent organic pollutant exposure: a case-control study in Inuit women. Environ Health. 13(1):19.	Cancer	Cross-sectional: problems assessing temporal association; exposure data collected at or near the time of outcome diagnosis or assessment
Girardi P and Merler E (2017). Mortality study of a cohort of chemical workers producing perfluorinated derivatives and other chemicals. Occupational and Environmental Medicine 74(Suppl 1): A12-A	Cancer	Abstract; overlap with Girardi and Merler, 2019
Giuliani J., Bonetti A. The exposure to perfluoroalkyl substances (PFAS) and the development of cancer. Recenti Progressi in Medicina 2019 110:7-8 (368-370)	Cancer	Review
Grandjean P. Health Status of Workers Exposed to Perfluorinated Alkylate Substances. J Occup Environ Med. 2018 Oct;60(10):e562.	Cancer	Letter to the Editor
Hardell, E., A. Kärrman, B. van Bavel, J. Boa, M. Carlberg, and L. Hardell. 2014. Case-control study on perfluorinated alkyl acids (PFAAs) and the risk of prostate cancer. Environment International 63:35–39.	Cancer	Cross-sectional: problems assessing temporal association; exposure data collected at or near the time of outcome diagnosis or assessment
Hocevar B.A., Kamendulis L.M. Promotion of pancreatic cancer by perfluorooctanoic acid (PFOA). Cancer Research 2018 78:13 Supplement 1	Cancer	Mice
Hurley S, Goldberg D, Wang M, Park JS, Petreas M, Bernstein L, Anton-Culver H, Nelson DO, Reynolds P. Breast cancer risk and serum levels of per- and poly-fluoroalkyl substances: a case-control study nested in the California Teachers Study. Environ Health. 2018 Nov 27;17(1):83.	Cancer	Serum collected 35 months after breast cancer diagnosis
Innes KE, Wimsatt JH, Frisbee S, Ducatman AM (2014). Inverse association of colorectal cancer prevalence to serum levels of perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA) in a large Appalachian population. BMC Cancer. 14:45	Cancer	Cross-sectional: problems assessing temporal association; exposure data collected at or near the time of outcome diagnosis or assessment

Study reference	Health outcome	Reason for exclusion[1]
Roswall N., Larsen S.B., Sørensen M., Tjønneland A., Raaschou-Nielsen O. Perfluorooctanoate and Perfluorooctanesulfonate plasma concentrations and survival after prostate and bladder cancer in a population-based study. Environmental Epidemiology 2018 2:3 Article Number e018	Cancer	Cancer survival
Tsai MS, Chang SH, Kuo WH, et al. A case-control study of perfluoroalkyl substances and the risk of breast cancer in Taiwanese women. Environ Int. 2020;142:105850.	Cancer	Cross-sectional: problems assessing temporal association; exposure data collected at or near the time of outcome diagnosis or assessment
Ubel FA, Sorenson SD, Roach DE. Health status of plant workers exposed to fluorochemicals--a preliminary report. Am Ind Hyg Assoc J. 1980 Aug;41(8):584-9.	Cancer	More detailed data in Raleigh et al., 2014
Vassiliadou, I., D. Costopoulou, A. Ferderigou, and L. Leondiadis. 2010. Levels of perfluorooctanesulfonate (PFOS) and perfluorooctanoate (PFOA) in blood samples from different groups of adults living in Greece. Chemosphere 80:1199–1206.	Cancer	Cross-sectional: problems assessing temporal association; exposure data collected at or near the time of outcome diagnosis or assessment
Wielsøe M, Kern P, Bonefeld-Jørgensen EC. Serum levels of environmental pollutants is a risk factor for breast cancer in Inuit: a case control study. Environ Health. 2017 Jun 13;16(1):56.	Cancer	Cross-sectional: problems assessing temporal association; exposure data collected at or near the time of outcome diagnosis or assessment
Wielsøe M., Eiberg H., Ghisari M., Kern P., Lind O., Bonefeld-Jørgensen E.C. Genetic Variations, Exposure to Persistent Organic Pollutants and Breast Cancer Risk – A Greenlandic Case–Control Study. Basic and Clinical Pharmacology and Toxicology 2018 123:3 (335-346)	Cancer	Cross-sectional: problems assessing temporal association; exposure data collected at or near the time of outcome diagnosis or assessment
Yeung LW, Guruge KS, Taniyasu S, Yamashita N, Angus PW, Herath CB. Profiles of perfluoroalkyl substances in the liver and serum of patients with liver cancer and cirrhosis in Australia. Ecotoxicol Environ Saf. 2013 Oct;96:139-46.	Cancer	Cross-sectional: problems assessing temporal association; exposure data collected at or near the time of outcome diagnosis or assessment
Yeung LW, Robinson SJ, Koschorreck J, Mabury SA. Part II. A temporal study of PFOS and its precursors in human plasma from two German cities in 1982-2009. Environ Sci Technol. 2013 Apr 16;47(8):3875-82.	Cancer	Exposure and half-life study
Yeung, L.W.Y., K.S. Guruge, S. Taniyasu, N. Yamashita, P.W. Angus, and C.B. Herath. 2013. Profiles of perfluoroalkyl substances in the liver and serum of patients with liver cancer and cirrhosis in Australia. Ecotoxicology & Environmental Safety 96:139–146.	Cancer	Cross-sectional: problems assessing temporal association; exposure data collected at or near the time of outcome diagnosis or assessment
Bjerregaard-Olesen C, Bach CC, Long M, Wielsøe M, Bech BH, Henriksen TB, et al. (2019). Associations of fetal growth outcomes with measures of the combined xenoestrogenic activity of maternal serum perfluorinated alkyl acids in danish pregnant women. Environ Health Perspect 127:17006-17006.	DART - fetal growth	PFAS mixtures (effects of PFOA and PFOS not separated)
Kishi R, Araki A, Minatoya M, Hanaoka T, Miyashita C, Itoh S, et al. (2017). The Hokkaido birth cohort study on environment and children's health: Cohort profile-updated 2017. Environ Health Prev Med 22:46-46.	DART - fetal growth	Review of Hokkaido Cohort studies
Liew Z, Goudarzi H, Oulhote Y. (2018). Developmental exposures to perfluoroalkyl substances (PFASs): An update of associated health outcomes. Curr Environ Health Rep 5:1-19.	DART - fetal growth	Review
Liu J, Liu G, Li Z. (2017). Importance of metabolomics analyses of maternal parameters and their influence on fetal growth. Experimental and Therapeutic Medicine 14:467-472.	DART - fetal growth	Review

Study reference	Health outcome	Reason for exclusion[1]
Ma RCW, Tam CHT, Cheung GPY, Lowe W, Metzger BE, Tam WH, et al. (2018). Maternal exposure to perfluorooctane sulfonate (PFOS) is associated with maternal hyperglycaemia and adverse neonatal and childhood outcomes. Diabetes 67:LB46.	DART - fetal growth	Conference abstract
Malits J, Blustein J, Trasande L, Attina TM. (2018). Perfluorooctanoic acid and low birth weight: Estimates of US attributable burden and economic costs from 2003 through 2014. Int J Hyg Environ Health 221:269-275.	DART - fetal growth	Attributable cost study
Negri E, Metruccio F, Guercio V, Tosti L, Benfenati E, Bonzi R, et al. (2017). Exposure to PFOA and PFOS and fetal growth: A critical merging of toxicological and epidemiological data. Crit Rev Toxicol 47:482-508.	DART - fetal growth	Review
Nicolle-Mir L. (2018). Perfluorinated compounds and child's health: Review of the epidemiological literature. Environnement, Risques et Sante 17:97-98.	DART - fetal growth	Review
Nicolle-Mir L. (2019). Influence of in utero exposure to organochlorines and perfluorochemicals on the risk of low birth weight: Pooled analysis of seven European cohorts. Environnement, Risques et Sante 18:14-15.	DART - fetal growth	Brief taken from Govarts et al. (2018)
Steenland K, Barry V, Savitz D. (2018). Serum perfluorooctanoic acid and birthweight: An updated meta-analysis with bias analysis. Epidemiology 29:765-776.	DART - fetal growth	Review
Tsai M-S, Chen M-H, Lin C-C, Ng S, Hsieh C-J, Liu C-Y, et al. (2017). Children's environmental health based on birth cohort studies of Asia. Sci Total Environ 609:396-409.	DART - fetal growth	Review
Tsuda S. (2016). Differential toxicity between perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA). J Toxicol Sci 41:SP27-SP36.	DART - fetal growth	Discussion of US EPA and IARC documents
Vrijheid M, Casas M, Gascon M, Valvi D, Nieuwenhuijsen M. (2016). Environmental pollutants and child health-a review of recent concerns. Int J Hyg Environ Health 219:331-342.	DART - fetal growth	Review
Wang A, Padula A, Sirota M, Woodruff TJ. (2016). Environmental influences on reproductive health: The importance of chemical exposures. Fertil Steril 106:905-929.	DART - fetal growth	Review
Zlatnik MG. 2016. Endocrine-disrupting chemicals and reproductive health. J Midwifery Womens Health 61:442-455.	DART - fetal growth	Review
Bach CC, Vested A, Jørgensen KT, Bonde JPE, Henriksen TB, Toft G. (2016). Perfluoroalkyl and polyfluoroalkyl substances and measures of human fertility: A systematic review. Crit Rev Toxicol 46:735-755.	DART- fertility and fecundity	Review
Karwacka A, Zamkowska D, Radwan M, Jurewicz J. (2019). Exposure to modern, widespread environmental endocrine disrupting chemicals and their effect on the reproductive potential of women: An overview of current epidemiological evidence. Human Fertility 22:2-25	DART- fertility and fecundity	Review
Avanasi R, Shin H-M, Vieira VM, Bartell SM. (2016a). Impacts of geocoding uncertainty on reconstructed pfoa exposures and their epidemiological association with preeclampsia. Environ Res 151:505-512.	DART - preeclampsia and gestational hypertension	Re-examination of previously published data
Avanasi R, Shin H-M, Vieira VM, Savitz DA, Bartell SM. (2016b). Impact of exposure uncertainty on the association between perfluorooctanoate and preeclampsia in the c8 health project population. Environ Health Perspect 124:126-132.	DART - preeclampsia and gestational hypertension	Re-examination of previously published data

Study reference	Health outcome	Reason for exclusion[1]
Casas M., Manzano-Salgado C.B., Granum B., Lopez-Espinosa M.-J., Ballester F., Iniguez C., Gascon M., Martinez D., Guxens M., Basterretxea M., Zabaleta C., Schettgen T., Sunyer J., Vrijheid M.Prenatal exposure to perfluoroalkyl substances and immune and respiratory outcomes. European Respiratory Journal 2018 52 Supplement 62	Immunotoxicity	Conference abstract (limited information)
Conway BN, Badders AN, Costacou T, Arthur JM, Innes KE. Perfluoroalkyl substances and kidney function in chronic kidney disease, anemia, and diabetes. Diabetes Metab Syndr Obes. 2018 Nov 15;11:707-716.	Immunotoxicity	Diabetes, CRP as a confounding variable only
DeWitt JC, Blossom SJ, Schaider LA. Exposure to per-fluoroalkyl and polyfluoroalkyl substances leads to immunotoxicity: epidemiological and toxicological evidence. J Expo Sci Environ Epidemiol. 2019 Mar;29(2):148-156	Immunotoxicity	Review
Hammer T, Lophaven SN, Nielsen KR, von Euler-Chelpin M, Weihe P, Munkholm P, Burisch J, Lynge E. Inflammatory bowel diseases in Faroese-born Danish residents and their offspring: further evidence of the dominant role of environmental factors in IBD development. Aliment Pharmacol Ther. 2017 Apr;45(8):1107-1114.	Immunotoxicity	No PFOA or PFOS data
Honda-Kohmo K., Hutcheson R., Innes K.E., Conway B.N. Perfluoroalkyl substances are inversely associated with coronary heart disease in adults with diabetes. Journal of Diabetes and its Complications 2019 33:6 (407-412)	Immunotoxicity	Conorary heart diseaese
Huang H, Wang Q, He X, Wu Y, Xu C. Association between polyfluoroalkyl chemical concentrations and leucocyte telomere length in US adults. Sci Total Environ. 2018 Oct 30;653:547-553.	Immunotoxicity	Telomere length
Kingsley S.L., Walker D.I., Calafat A.M., Chen A., Papandonatos G.D., Xu Y., Jones D.P., Lanphear B.P., Pennell K.D., Braun J.M. Metabolomics of childhood exposure to perfluoroalkyl substances: a cross-sectional study. Metabolomics 2019 15:7	Immunotoxicity	Metabolomics
Knudsen AS, Long M, Pedersen HS, Bonefeld-Jørgensen EC. Persistent organic pollutants and haematological markers in pregnant women: the ACCEPT sub-study. Int J Circumpolar Health. 2018 Dec;77(1):1456303.	Immunotoxicity	Only gives data for the sum of all PFAS
Lai KP, Ng AH, Wan HT, Wong AY, Leung CC, Li R, Wong CK. Dietary Exposure to the Environmental Chemical, PFOS on the Diversity of Gut Microbiota, Associated With the Development of Metabolic Syndrome. Front Microbiol. 2018 Oct 24;9:2552.	Immunotoxicity	Mice
Lee JK, Lee S, Baek MC, Lee BH, Lee HS, Kwon TK, Park PH, Shin TY, Khang D, Kim SH. Association between perfluorooctanoic acid exposure and degranulation of mast cells in allergic inflammation. J Appl Toxicol. 2017 May;37(5):554-562.	Immunotoxicity	In vitro study
Liu H, Chen Q, Lei L, Zhou W, Huang L, Zhang J, Chen D. Prenatal exposure to perfluoroalkyl and polyfluoroalkyl substances affects leukocyte telomere length in female newborns. Environ Pollut. 2018 Apr;235:446-452.	Immunotoxicity	Telomere length
Liu J, Liu S, Huang Z, et al. Associations between the serum levels of PFOS/PFOA and IgG N-glycosylation in adult or children. Environ Pollut. 2020;265(Pt A):114285	Immunotoxicity	IgG glycolsylation
Lowe AJ, Dharmage SC, Abramson MJ, Vijayasarathy S, Erbas B, Mueller JF, Lodge CJ. Cord-serum per- and poly-fluoroalkyl substances and atopy and eczema at 12-months. Allergy. 2019 Apr;74(4):812-815.	Immunotoxicity	Diagnosis of atopic eczema is unclear

Study reference	Health outcome	Reason for exclusion[1]
Luo Y, Deji Z, Huang Z. Exposure to perfluoroalkyl substances and allergic outcomes in children: A systematic review and meta-analysis [published online ahead of print, 2020 Aug 30]. Environ Res. 2020;191:110145.	Immunotoxicity	Meta-analysis
Oulhote Y, Shamim Z, Kielsen K, Weihe P, Grandjean P, Ryder LP, Heilmann C. Children's white blood cell counts in relation to developmental exposures to methylmercury and persistent organic pollutants. Reprod Toxicol. 2017 Mar;68:207-214.	Immunotoxicity	Only gives data for the sum of all PFAS
Panikkar B, Lemmond B, Allen L, DiPirro C, Kasper S. Making the invisible visible: results of a community-led health survey following PFAS contamination of drinking water in Merrimack, New Hampshire. Environ Health. 2019 Aug 30;18(1):79.	Immunotoxicity	Multiple PFAS combined
Pennings JL, Jennen DG, Nygaard UC, Namork E, Haug LS, van Loveren H, Granum B. Cord blood gene expression supports that prenatal exposure to perfluoroalkyl substances causes depressed immune functionality in early childhood. J Immunotoxicol. 2016;13(2):173-80.	Immunotoxicity	Gene expression; mechanism
Rainieri S, Conlledo N, Langerholc T, Madorran E, Sala M, Barranco A. Toxic effects of perfluorinated compounds at human cellular level and on a model vertebrate. Food Chem Toxicol. 2017 Jun;104:14-25.	Immunotoxicity	In vitro
Salihovic S, Fall T, Ganna A, Broeckling CD, Prenni JE, Hyötyläinen T, Kärrman A, Lind PM, Ingelsson E, Lind L. Identification of metabolic profiles associated with human exposure to perfluoroalkyl substances. J Expo Sci Environ Epidemiol. 2018 Sep 5.	Immunotoxicity	Metabolomics
Steenland K, Kugathasan S, Barr DB. PFOA and ulcerative colitis. Environ Res. 2018 Aug;165:317-321.	Immunotoxicity	Overlap with previous study
Suo C, Fan Z, Zhou L, Qiu J. Perfluorooctane sulfonate affects intestinal immunity against bacterial infection. Sci Rep. 2017 Jul 12;7(1):5166.	Immunotoxicity	Mice
Wang J, Pan Y, Cui Q, Yao B, Wang J, Dai J. Penetration of PFASs Across the Blood Cerebrospinal Fluid Barrier and Its Determinants in Humans. Environ Sci Technol. 2018 Nov 20;52(22):13553-13561.	Immunotoxicity	Levels in cerebrospinal fluid
Wang X, Liu L, Zhang W, Zhang J, Du X, Huang Q, Tian M, Shen H. Serum metabolome biomarkers associate low-level environmental perfluorinated compound exposure with oxidative /nitrosative stress in humans. Environ Pollut. 2017 Oct;229:168-176.	Immunotoxicity	Metabolomics
Wikström S, Lin PI, Lindh CH, Shu H, Bornehag CG. Maternal serum levels of perfluoroalkyl substances in early pregnancy and offspring birth weight. Pediatr Res. 2019 Dec 13. doi: 10.1038/s41390-019-0720-1.	Immunotoxicity	No immune, liver, thyroid
Yarahalli Jayaram V, Baggavalli S, Reddy D, Sistla S, Malempati R. Effect of endosulfan and bisphenol A on the expression of SUMO and UBC9. Drug Chem Toxicol. 2018 Nov 14:1-8.	Immunotoxicity	In vitro
Zeng XW, Bloom MS, Dharmage SC, Lodge CJ, Chen D, Li S, Guo Y, Roponen M, Jalava P, Hirvonen MR, Ma H, Hao YT, Chen W, Yang M, Chu C, Li QQ, Hu LW, Liu KK, Yang BY, Liu S, Fu C, Dong GH. Prenatal exposure to perfluoroalkyl substances is associated with lower hand, foot and mouth disease viruses antibody response in infancy: Findings from the Guangzhou Birth Cohort Study. Sci Total Environ. 2019 May 1;663:60-67.	Immunotoxicity	Antibodies to hand, foot, and mouth disease; exposure to hand, foot, and mouth disease antigen is unknown

Study reference	Health outcome	Reason for exclusion[1]
Zeng XW, Li QQ, Chu C, Ye WL, Yu S, Ma H, Zeng XY, Zhou Y, Yu HY, Hu LW, Yang BY, Dong GH. Alternatives of perfluoroalkyl acids and hepatitis B virus surface antibody in adults: Isomers of C8 Health Project in China. Environ Pollut. 2019 Dec 27;259:113857.	Immunotoxicity	Hepatitis B antibody levels
Zeng Z, Song B, Xiao R, Zeng G, Gong J, Chen M, Xu P, Zhang P, Shen M, Yi H. Assessing the human health risks of perfluorooctane sulfonate by in vivo and in vitro studies. Environ Int. 2019 May;126:598 610.	Immunotoxicity	Review
Zhong SQ, Chen ZX, Kong ML, Xie YQ, Zhou Y, Qin XD, Paul G, Zeng XW, Dong GH. Testosterone-Mediated Endocrine Function and TH1/TH2 Cytokine Balance after Prenatal Exposure to Perfluorooctane Sulfonate: By Sex Status. Int J Mol Sci. 2016;17(9).	Immunotoxicity	Mice
Zhou Y, Bao WW, Qian ZM, Dee Geiger S, Parrish KL, Yang BY, Lee YL, Dong GH. Perfluoroalkyl substance exposure and urine CC16 levels among asthmatics: A case-control study of children. Environ Res. 2017;159:158-163.	Immunotoxicity	Clara cell protein (CC16) levels
Alderete T.L., Jin R., Walker D.I., Valvi D., Chen Z., Jones D.P., Peng C., Gilliland F.D., Berhane K., Conti D.V., Goran M.I., Chatzi L. Perfluoroalkyl substances, metabolomic profiling, and alterations in glucose homeostasis among overweight and obese Hispanic children: A proof-of-concept analysis. Environment International 2019 126 (445-453)	Lipids	No lipid data
Ashley-Martin J, Dodds L, Arbuckle TE, Bouchard MF, Fisher M, Morriset AS, Monnier P, Shapiro GD, Ettinger AS, Dallaire R, Taback S, Fraser W, Platt RW. Maternal Concentrations of Perfluoroalkyl Substances and Fetal Markers of Metabolic Function and Birth Weight. Am J Epidemiol. 2017 Feb 1;185(3):185-193.	Lipids	Leptin and adiponectin
Bi X, Tey SL, Loo YT, Henry CJ. 2017. Central adiposity-induced plasma-free amino acid alterations are associated with increased insulin resistance in healthy Singaporean adults. Eur J Clin Nutr 71:1080-1087.	Lipids	Not PFOA or PFOS
Braun JM, Chen A, Romano ME, Calafat AM, Webster GM, Yolton K, Lanphear BP. Prenatal perfluoroalkyl substance exposure and child adiposity at 8 years of age: The HOME study. Obesity (Silver Spring). 2016 Jan;24(1):231-7.	Lipids	No lipid data
Cardenas A, Gold DR, Hauser R, Kleinman KP, Hivert MF, Calafat AM, Ye X, Webster TF, Horton ES, Oken E. Plasma Concentrations of Per- and Polyfluoroalkyl Substances at Baseline and Associations with Glycemic Indicators and Diabetes Incidence among High-Risk Adults in the Diabetes Prevention Program Trial. Environ Health Perspect. 2017 Oct 2;125(10):107001.	Lipids	Diabetes
Cardenas A, Hauser R, Gold DR, Kleinman KP, Hivert MF, Fleisch AF, Lin PD, Calafat AM, Webster TF, Horton ES, Oken E. Association of Perfluoroalkyl and Polyfluoroalkyl Substances With Adiposity. JAMA Netw Open. 2018 Aug 3;1(4):e181493.	Lipids	No lipid data
Convertino M, Church TR, Olsen GW, Liu Y, Doyle E, Elcombe CR, et al. 2018. Stochastic Pharmacokinetic-Pharmacodynamic Modeling for Assessing the Systemic Health Risk of Perfluorooctanoate (PFOA). Toxicol Sci 163:293-306.	Lipids	PBPK modeling; cancer patients given extremely high PFOA doses
Conway B, Innes KE, Long D. Perfluoroalkyl substances and beta cell deficient diabetes. J Diabetes Complications. 2016 Aug;30(6):993-8.	Lipids	No lipid data

Study reference	Health outcome	Reason for exclusion[1]
Das KP, Wood CR, Lin MT, Starkov AA, Lau C, Wallace KB, et al. 2017. Perfluoroalkyl acids-induced liver steatosis: Effects on genes controlling lipid homeostasis. Toxicology 378:37-52	Lipids	Mice
Donat-Vargas C, Bergdahl IA, Tornevi A, Wennberg M, Sommar J, Kiviranta H, Koponen J, Rolandsson O, Åkesson A. Perfluoroalkyl substances and risk of type II diabetes: A prospective nested case-control study. Environ Int. 2019 Feb;123:390-398.	Lipids	No lipid data
Fai Tse WK, Li JW, Kwan Tse AC, Chan TF, Hin Ho JC, Sun Wu RS, Chu Wong CK, Lai KP. Fatty liver disease induced by perfluorooctane sulfonate: Novel insight from transcriptome analysis. Chemosphere. 2016 Sep;159:166-177.	Lipids	Zebrafish
Fleisch AF, Rifas-Shiman SL, Mora AM, Calafat AM, Ye X, Luttmann-Gibson H, Gillman MW, Oken E, Sagiv SK. Early-Life Exposure to Perfluoroalkyl Substances and Childhood Metabolic Function. Environ Health Perspect. 2017 Mar;125(3):481-487.	Lipids	No lipid data
Hartman TJ, Calafat AM, Holmes AK, Marcus M, Northstone K, Flanders WD, Kato K, Taylor EV. Prenatal Exposure to Perfluoroalkyl Substances and Body Fatness in Girls. Child Obes. 2017 Jun;13(3):222-230.	Lipids	No lipid data
Heffernan A.L., Cunningham T.K., Drage D.S., Aylward L.L., Thompson K., Vijayasarathy S., Mueller J.F., Atkin S.L., Sathyapalan T. Perfluorinated alkyl acids in the serum and follicular fluid of UK women with and without polycystic ovarian syndrome undergoing fertility treatment and associations with hormonal and metabolic parameters. Int J Hyg Environ Health 2018 221:7 (1068-1075)	Lipids	Polycystic ovarian syndrome patients only (used for thyroid)
Hui Z, Li R, Chen L. 2017. The impact of exposure to environmental contaminant on hepatocellular lipid metabolism. Gene 622:67-71.	Lipids	Mice
Hutcheson R, Innes K, Conway B. 2019. Perfluoroalkyl substances and likelihood of stroke in persons with and without diabetes. Diab Vasc Dis Res:1479164119892223.	Lipids	Stroke
Kingsley SL, Walker DI, Calafat AM, Chen A, Papandonatos GD, Xu Y, et al. 2019. Metabolomics of childhood exposure to perfluoroalkyl substances: a cross-sectional study. Metabolomics 15:95.	Lipids	Metabolomics
Lai K.P., Ng A.H.-M., Wan H.T., Wong A.Y.-M., Leung C.C.-T., Li R., Wong C.K.-C. Dietary exposure to the environmental chemical, PFOS on the diversity of gut microbiota, associated with the development of metabolic syndrome. Frontiers in Microbiology 2018 9:OCT	Lipids	Mice
Lind L, Salihovic S, Lampa E, Lind PM. Mixture effects of 30 environmental contaminants on incident metabolic syndrome-A prospective study. Environ Int. 2017 Oct;107:8-15.	Lipids	Metabolic syndrome; chemical mixtures
Lind PM, Salihovic S, Stubleski J, Karrman A, Lind L. 2018. Changes in plasma levels of perfluoroalkyl substances (PFASs) are related to increase in carotid intima-media thickness over 10 years - a longitudinal study. Environ Health 17:59.	Lipids	Intima media thickness
Lind PM, Salihovic S, van Bavel B, Lind L. Circulating levels of perfluoroalkyl substances (PFASs) and carotid artery atherosclerosis. Environ Res. 2017 Jan;152:157-164.	Lipids	Carotid artery atherosclerosis
Liu W, Qin H, Pan Y, Luo F, Zhang Z. 2019. Low concentrations of perfluorooctane sulfonate repress osteogenic and enhance adipogenic differentiation of human mesenchymal stem cells. Toxicol Appl Pharmacol 367:82-91.	Lipids	In vitro

Study reference	Health outcome	Reason for exclusion[1]
Lu Y., Gao K., Li X., Tang Z., Xiang L., Zhao H., Fu J., Wang L., Zhu N., Cai Z., Liang Y., Wang Y., Jiang G.Mass Spectrometry-Based Metabolomics Reveals Occupational Exposure to Per-And Polyfluoroalkyl Substances Relates to Oxidative Stress, Fatty Acid β-Oxidation Disorder, and Kidney Injury in a Manufactory in China. Environmental Science and Technology 2019 53:16 (9800-9809)	Lipids	Metabolomics
Ma Y, Yang J, Wan Y, Peng Y, Ding S, Li Y, et al. 2018. Low-level perfluorooctanoic acid enhances 3T3-L1 preadipocyte differentiation via altering peroxisome proliferator activated receptor gamma expression and its promoter DNA methylation. J Appl Toxicol 38:398-407.	Lipids	In vitro
Mattsson K, Rignell-Hydbom A, Holmberg S, Thelin A, Jönsson BA, Lindh CH, Sehlstedt A, Rylander L. Levels of perfluoroalkyl substances and risk of coronary heart disease: Findings from a population-based longitudinal study. Environ Res. 2015 Oct;142:148-54.	Lipids	Coronary heart disease
McGlinchey A, Sinioja T, Lamichhane S, et al. Prenatal exposure to perfluoroalkyl substances modulates neonatal serum phospholipids, increasing risk of type 1 diabetes [published online ahead of print, 2020 Jul 4]. Environ Int. 2020;143:105935	Lipids	Lipid metabolomics
Minatoya M, Itoh S, Miyashita C, Araki A, Sasaki S, Miura R, Goudarzi H, Iwasaki Y, Kishi R. Association of prenatal exposure to perfluoroalkyl substances with cord blood adipokines and birth size: The Hokkaido Study on environment and children's health. Environ Res. 2017 Jul;156:175-182.	Lipids	Adipokines
Mitro SD, Sagiv SK, Fleisch AF, et al. Pregnancy Per- and Polyfluoroalkyl Substance Concentrations and Postpartum Health in Project Viva: A Prospective Cohort. J Clin Endocrinol Metab. 2020;105(9):dgaa431.	Lipids	No lipid information
Mora AM, Oken E, Rifas-Shiman SL, Webster TF, Gillman MW, Calafat AM, Ye X, Sagiv SK. Prenatal Exposure to Perfluoroalkyl Substances and Adiposity in Early and Mid-Childhood. Environ Health Perspect. 2017 Mar;125(3):467-473.	Lipids	No lipid data
More VR, Campos CR, Evans RA, Oliver KD, Chan GN, Miller DS, et al. 2017. PPAR-alpha, a lipid-sensing transcription factor, regulates blood-brain barrier efflux transporter expression. J Cereb Blood Flow Metab 37:1199-1212.	Lipids	Rats
Parikh A, Vacek TP. 2018. PFO closure in high-risk patient with paradoxical arterial embolism, deep vein thrombosis, pulmonary embolism and factor V Leiden genetic mutation. Oxf Med Case Reports 2018:omx105.	Lipids	Case report
Qi W, Clark JM, Timme-Laragy AR, Park Y. 2018. Perfluorobutanesulfonic acid (PFBS) potentiates adipogenesis of 3T3-L1 adipocytes. Food Chem Toxicol 120:340-345.	Lipids	In vitro
Qiu T, Chen M, Sun X, Cao J, Feng C, Li D, et al. 2016. Perfluorooctane sulfonate-induced insulin resistance is mediated by protein kinase B pathway. Biochem Biophys Res Commun 477:781-785.	Lipids	In vitro
Raymond M.R., Christensen K.Y., Thompson B.A., Anderson H.A. Associations between fish consumption and contaminant biomarkers with cardiovascular conditions among older male anglers in Wisconsin. Journal of Occupational and Environmental Medicine 2016 58:7 (676-682)	Lipids	Overlap with Christensen et al., 2016

Study reference	Health outcome	Reason for exclusion[1]
Rosen MB, Das KP, Rooney J, Abbott B, Lau C, Corton JC. 2017. PPARalpha-independent transcriptional targets of perfluoroalkyl acids revealed by transcript profiling. Toxicology 387:95-107.	Lipids	Mice
Sakuma A, Wasada Ochi H, Yoshioka M, Yamanaka N, Ikezawa M, Guruge KS. 2019. Changes in hepato-renal gene expression in microminipigs following a single exposure to a mixture of perfluoroalkyl acids. PLoS One 14:e0210110.	Lipids	Pigs
Salihovic S., Fall T., Ganna A., Broeckling C.D., Prenni J.E., Hyötyläinen T., Kärrman A., Lind P.M., Ingelsson E., Lind L. Identification of metabolic profiles associated with human exposure to perfluoroalkyl substances. [In Process] Journal of exposure science & environmental epidemiology 2019 29:2 (196-205)	Lipids	Metabolomics
Shapiro GD, Dodds L, Arbuckle TE, Ashley-Martin J, Ettinger AS, Fisher M, Taback S, Bouchard MF, Monnier P, Dallaire R, Morisset AS, Fraser W. Exposure to organophosphorus and organochlorine pesticides, perfluoroalkyl substances, and polychlorinated biphenyls in pregnancy and the association with impaired glucose tolerance and gestational diabetes mellitus: The MIREC Study. Environ Res. 2016 May;147:71-81.	Lipids	No lipid data
Su TC, Kuo CC, Hwang JJ, Lien GW, Chen MF, Chen PC. Serum perfluorinated chemicals, glucose homeostasis and the risk of diabetes in working-aged Taiwanese adults. Environ Int. 2016 Mar;88:15-22.	Lipids	No lipid data
Sunderland EM, Hu XC, Dassuncao C, Tokranov AK, Wagner CC, Allen JG. 2019. A review of the pathways of human exposure to poly- and perfluoroalkyl substances (PFASs) and present understanding of health effects. J Expo Sci Environ Epidemiol 29:131-147.	Lipids	Review
Tang W., He X., Liu Y., Xu B., Gu L. Blood perfluorooctanoate is associated with diabetes and metabolic alteration. Diabetes/Metabolism Research and Reviews 2017 33 Supplement 1	Lipids	Abstract (poster) only
Valvi D, P Weihe, P Grandjean. Cardiometabolic Risk in Young Adults Exposed to Perfluoroalkyl Substances during Critical Developmental Periods - ISEE Conference Abstracts, 2016	Lipids	No lipid data
Wang X, Liu L, Zhang W, Zhang J, Du X, Huang Q, Tian M, Shen H. Serum metabolome biomarkers associate low-level environmental perfluorinated compound exposure with oxidative /nitrosative stress in humans. Environ Pollut. 2017 Oct;229:168-176.	Lipids	Metabolomics
Yang C., Lee H.K., Kong A.P.S., Lim L.L., Cai Z., Chung A.C.K. Early-life exposure to endocrine disrupting chemicals associates with childhood obesity. Ann Ped Endocrinol Metabol 2018 23:4 182-195	Lipids	Review
Zeng Z., Song B., Xiao R., Zeng G., Gong J., Chen M., Xu P., Zhang P., Shen M., Yi H. Assessing the human health risks of perfluorooctane sulfonate by in vivo and in vitro studies. Environment International 2019 126 (598-610)	Lipids	Review
Abe T, Takahashi M, Kano M, Amaike Y, Ishii C, Maeda K, Kudoh Y, Morishita T, Hosaka T, Sasaki T, Kodama S, Matsuzawa A, Kojima H, Yoshinari K. Activation of nuclear receptor CAR by an environmental pollutant perfluorooctanoic acid. Arch Toxicol. 2017 91(6):2365-2374.	Liver	Mice
Attanasio R. Association between perfluoroalkyl acids and liver function: Data on sex differences in adolescents. Data Brief. 2019 Oct 5;27:104618.	Liver	Same as Attanasio, 2019

Study reference	Health outcome	Reason for exclusion[1]
Beggs KM, McGreal SR, McCarthy A, Gunewardena S, Lampe JN, Lau C, Apte U. The role of hepatocyte nuclear factor 4-alpha in perfluorooctanoic acid- and perfluorooctanesulfonic acid-induced hepatocellular dysfunction. Toxicol Appl Pharmacol. 2016 304:18-29.	Liver	In vitro
Chen Y, Hu W, Huang C, Hua S, Wei Q, Bai C, Chen J, Norris MB, Winn R, Yang D, Dong Q. Subchronic perfluorooctanesulfonate (PFOS) exposure induces elevated mutant frequency in an in vivo λ transgenic medaka mutation assay. Sci Rep. 2016 6:38466.	Liver	Fish
Das KP, Wood CR, Lin MT, Starkov AA, Lau C, Wallace KB, Corton JC, Abbott BD. Perfluoroalkyl acids-induced liver steatosis: Effects on genes controlling lipid homeostasis. Toxicology. 2017 378:37-52.	Liver	Mice
Deierlein AL, Rock S, Park S. Persistent endocrine-disrupting chemicals and fatty liver disease. Curr Environ Health Rep. 2017 4(4):439-449.	Liver	Review
Du G, Sun J, Zhang Y. Perfluorooctanoic acid impaired glucose homeostasis through affecting adipose AKT pathway. Cytotechnology. 2018 70(1):479-487.	Liver	Mice
Fai Tse WK, Li JW, Kwan Tse AC, Chan TF, Hin Ho JC, Sun Wu RS, Chu Wong CK, Lai KP. Fatty liver disease induced by perfluorooctane sulfonate: Novel insight from transcriptome analysis. Chemosphere. 2016 159:166-177.	Liver	Zebrafish
Fleisch AF, Rifas-Shiman SL, Mora AM, Calafat AM, Ye X, Luttmann-Gibson H, Gillman MW, Oken E, Sagiv SK. Early-life exposure to perfluoroalkyl substances and childhood metabolic function. Environ Health Perspect. 2017 125(3):481-487.	Liver	No liver outcomes
Foulds CE, Treviño LS, York B, Walker CL. Endocrine disrupting chemicals and fatty liver disease. Nat Rev Endocrinol. 2017 Aug;13(8):445-457.	Liver	Review
Gomis MI, Vestergren R, Borg D, Cousins IT. Comparing the toxic potency in vivo of long-chain perfluoroalkyl acids and fluorinated alternatives. Environ Int. 2018 113:1-9.	Liver	Rats
Han R, Hu M, Zhong Q, Wan C, Liu L, Li F, Zhang F, Ding W. Perfluorooctane sulphonate induces oxidative hepatic damage via mitochondria-dependent and NF-κB/TNF-α -mediated pathway. Chemosphere. 2018 Jan;191:1056-1064.	Liver	Rats
Jin R, McConnell R, Catherine C, Xu S, Walker DI, Stratakis N, Jones DP, Miller GW, Peng C, Conti DV, Vos MB, Chatzi L. Perfluoroalkyl substances and severity of nonalcoholic fatty liver in Children: An untargeted metabolomics approach. Environ Int. 2020 Jan;134:105220.	Liver	Only includes children with non-alcoholic fatty liver disease
Lai KP, Li JW, Cheung A, Li R, Billah MB, Chan TF, Wong CKC. Transcriptome sequencing reveals prenatal PFOS exposure on liver disorders. Environ Pollut. 2017 223:416-425.	Liver	Transcriptome sequencing
Liu WS, Chan HL, Lai YT, Lin CC, Li SY, Liu CK, Tsou HH, Liu TY. Dialysis membranes influence perfluorochemical concentrations and liver function in patients on hemodialysis. Int J Environ Res Public Health. 2018 15(11) (no page numbers)	Liver	Associations before and after dialysis
Liu, W. S.,Lai, Y. T.,Chan, H. L.,Li, S. Y.,Lin, C. C.,Liu, C. K.,Tsou, H. H.,Liu, T. Y.. Associations between perfluorinated chemicals and serum biochemical markers and performance status in uremic patients under hemodialysis. PLoS One. 2018. 13:e0200271	Liver	Dialysis patients

Study reference	Health outcome	Reason for exclusion[1]
Rantakokko P, Männistö V, Airaksinen R, Koponen J, Viluksela M, Kiviranta H, Pihlajamäki J. Persistent organic pollutants and non-alcoholic fatty liver disease in morbidly obese patients: a cohort study. Environ Health. 2015 Sep 29;14:79.	Liver	Bariatric surgery patients
VoPham T. Environmental risk factors for liver cancer and nonalcoholic fatty liver disease. Curr Epidemiol Rep. 2019 Mar;6(1):50-66.	Liver	Review
Yamakado M, Tanaka T, Nagao K, Imaizumi A, Komatsu M, Daimon T, Miyano H, Tani M, Toda A, Yamamoto H, Horimoto K, Ishizaka Y. Plasma amino acid profile associated with fatty liver disease and co-occurrence of metabolic risk factors. Sci Rep. 2017 7(1):14485.	Liver	No data on PFAS
Yan S, Zhang H, Guo X, Wang J, Dai J. High perfluorooctanoic acid exposure induces autophagy blockage and disturbs intracellular vesicle fusion in the liver. Arch Toxicol. 2017 91(1):247-258.	Liver	Mice
Berg V, Nøst TH, Pettersen RD, Hansen S, Veyhe AS, Jorde R, Odland JØ, Sandanger TM. Persistent Organic Pollutants and the Association with Maternal and Infant Thyroid Homeostasis: A Multipollutant Assessment. Environ Health Perspect. 2017 Jan;125(1):127-133.	Thyroid	Overlap with Berg et al., 2015
Deng M, Wu Y, Xu C, Jin Y, He X, Wan J, Yu X, Rao H, Tu W. Multiple approaches to assess the effects of F-53B, a Chinese PFOS alternative, on thyroid endocrine disruption at environmentally relevant concentrations. Sci Total Environ. 2018 May 15;624:215-224.	Thyroid	Zebrafish and rat study
Gaberščček S, Zaletel K. Epidemiological trends of iodine-related thyroid disorders: an example from Slovenia. Arh Hig Rada Toksikol. 2016 Jun 1;67(2):93-8.	Thyroid	Review
Gaudino R., Beccherle F., Cavarzere P., Lauriola S., Camilot M., Teofoli F., Vincenzi M., Rizzoli C., Antoniazzi F. Neonatal screening for congenital hypothyroidism: Analysis of a large cohort of affected patients (1987-2017) and relationship with perfluoroalkylated substances (PFAS) in Northeastern Italy. Hormone Research in Paediatrics 2019 91 Supplement 1 (94-)	Thyroid	Abstract only, limited information
Goudarzi H, Araki A, Itoh S, Sasaki S, Miyashita C, Mitsui T, Nakazawa H, Nonomura K, Kishi R. The Association of Prenatal Exposure to Perfluorinated Chemicals with Glucocorticoid and Androgenic Hormones in Cord Blood Samples: The Hokkaido Study. Environ Health Perspect. 2017 Jan;125(1):111-118.	Thyroid	No thyroid hormone data
Kim H.Y., Kim K.-N., Lee Y.A., Lim Y.-H., Kim J.I., Kim B.-N., Oh S.-Y., Hong Y.-C., Shin C.H. The relationship between perfluoroalkyl compounds concentrations at ages 2, 4, and 6 years and thyroid function in early childhood: A prospective cohort study. Hormone Research in Paediatrics 2019 91 Supplement 1 (343-)	Thyroid	Abstract only, limited information
Kishi R, Araki A, Minatoya M, Hanaoka T, Miyashita C, Itoh S, Kobayashi S, Ait Bamai Y, Yamazaki K, Miura R, Tamura N, Ito K, Goudarzi H; members of The Hokkaido Study on Environment and Children's Health. The Hokkaido Birth Cohort Study on Environment and Children's Health: cohort profile-updated 2017. Environ Health Prev Med. 2017 May 18;22(1):46.	Thyroid	Review
Liew Z, Goudarzi H, Oulhote Y. Developmental Exposures to Perfluoroalkyl Substances (PFASs): An Update of Associated Health Outcomes. Curr Environ Health Rep. 2018 Mar;5(1):1-19.	Thyroid	Review

Study reference	Health outcome	Reason for exclusion[1]
Lopez-Espinosa MJ, Mondal D, Armstrong BG, Eskenazi B, Fletcher T. Perfluoroalkyl Substances, Sex Hormones, and Insulin-like Growth Factor-1 at 6-9 Years of Age: A Cross-Sectional Analysis within the C8 Health Project. Environ Health Perspect. 2016 Aug;124(8):1269-75.	Thyroid	No thyroid hormone data
Shrestha S, Bloom MS, Yucel R, Seegal RF, Rej R, McCaffrey RJ, Wu Q, Kannan K, Fitzgerald EF. Perfluoroalkyl substances, thyroid hormones, and neuropsychological status in older adults. Int J Hyg Environ Health. 2017 Jun;220(4):679-685.	Thyroid	Same as Shrestha et al., 2015 reviewed and cited in US EPA 2016a and 2016b
Silva AV, Ringblom J, Lindh C, Scott K, Jakobsson K, Öberg M. A Probabilistic Approach to Evaluate the Risk of Decreased Total Triiodothyronine Hormone Levels following Chronic Exposure to PFOS and PFHxS via Contaminated Drinking Water [published correction appears in Environ Health Perspect. 2020 Aug;128(8):89001]. Environ Health Perspect. 2020;128(7):76001.	Thyroid	Risk assessment
Yu N, Wang X, Zhang B, Yang J, Li M, Li J, Shi W, Wei S, Yu H. Distribution of perfluorooctane sulfonate isomers and predicted risk of thyroid hormonal perturbation in drinking water. Water Res. 2015 Jun 1;76:171-80.	Thyroid	Risk assessment

Studies Identified After Initial Literature Review

Table A7.29. Studies identified after OEHHAs initial literature review (published or identified between January 2, 2020 and December 31, 2020)

Reference	Major finding	Risk assessment implications
Abraham K, Mielke H, Fromme H, et al (2020). Internal exposure to perfluoroalkyl substances (PFASs) and biological markers in 101 healthy 1-year-old children: associations between levels of perfluorooctanoic acid (PFOA) and vaccine response. Arch Toxicol 94(6):2131-47.	PFOA associated with decreases in antibody response to tetanus, diphtheria, and influenza vaccine in 1 year old children; further described in the main PHG document	Used to develop PODs for PFOA
Aimuzi R, Luo K, Huang R, et al. (2020). Perfluoroalkyl and polyfluroalkyl substances and maternal thyroid hormones in early pregnancy. Environ Pollut 264:114557	1,885 pregnant women from the Shanghai Birth Cohort; cross-sectional analyses of serum PFAS and serum thyroid hormones collected prior to 16 weeks gestation; PFOA was positively associated with FT4; no association with TSH or FT3. No associations seen for PFOS overall, although some decrease in TSH in TPO antibody positive women	No change
Ait Bamai Y, Goudarzi H, Araki A, et al. (2020). Effect of prenatal exposure to per- and polyfluoroalkyl substances on childhood allergies and common infectious diseases in children up to age 7 years: The Hokkaido study on environment and children's health [published online ahead of print, 2020 Jul 24]. Environ Int 143:105979	Continuation of the Hokkaido study (Goudarzi et al., 2016; Goudarzi et al., 2017; Kishi et al., 2015); prospective study; 2,689 mother-child pairs; maternal serum PFAS and allergy or immune-related symptoms at age 7 (questionnaire); PFOA and PFOS inversely associated with eczema. PFOS inversely associated with respiratory syncytial virus. Possible association between PFOA and respiratory syncytial virus and pneumonia. No association with rhinoconjunctivitis, wheeze, or chickenpox.	Some evidence to support immune-related effects of PFOA and PFOS, but results are mixed overall
Chen Z, Yang T, Walker DI, et al. (2020). Dysregulated lipid and fatty acid metabolism link perfluoroalkyl substances exposure and impaired glucose metabolism in young adults [published online ahead of print, 2020 Sep 3]. Environ Int 145:106091	102 mostly overweight or obese young adults from Southern California; cross-sectional analysis of serum PFAS and serum lipids; no association between PFOA and TC, LDL or TG; inverse association between PFOA and HDL; no associations with PFOS.	Small study, mixed results overall
Cohn BA, La Merrill MA, Krigbaum NY, et al. (2020). In utero exposure to poly- and perfluoroalkyl substances (PFASs) and subsequent breast cancer. Reprod Toxicol 92:112-9	Perinatal PFAS and breast cancer risk; 54 year follow-up; nested case-control (102 cases and 310 controls); elevated maternal N-ethyl-perfluorooctane sulfonamido acetic acid, a PFOS precursor, in combination with high maternal TC associated with a 3.6-fold increased risk of breast cancer (p-interaction<0.05); PFOS associated with decreased breast cancer risk; no associations seen for PFOA.	No change
Dzierlenga MW, Moreau M, Song G, et al. (2020). Quantitative bias analysis of the association between subclinical thyroid disease and two perfluoroalkyl substances in a single study. Environ Res 182:109017	Quantitative analysis of bias using physiologically-based pharmacokinetic modeling and simulations; results suggest that some links between PFOA or PFOS and hypothyroidism may be due to reverse causality (i.e., the effects of hypothyroidism on GFR and PFAS excretion).	No change

Reference	Major finding	Risk assessment implications
Fan Y, Li X, Xu Q, et al. (2020). Serum albumin mediates the effect of multiple per- and polyfluoroalkyl substances on serum lipid levels. Environ Pollut 266(Pt 2):115138	1,067 adults from NHANES 2001-2014; cross-sectional analyses of serum lipids and serum PFAS; associations seen between PFOA, PFOS, and PFNA and increased LDL and TC; effect for PFOS less at higher exposure levels when adjusted for other PFAS; PFNA and PFOS highly correlated; in weighted quantile sum regression analyses PFNA and PFOS appear to have the greatest impacts on LDL and TC; evidence of some modest mediation (<30%) of these associations by serum albumin.	Some findings support an association between PFOS and TC and LDL
Huang H, Yu K, Zeng X, et al. (2020). Association between prenatal exposure to perfluoroalkyl substances and respiratory tract infections in preschool children [published online ahead of print, 2020 Aug 29]. Environ Res 110156	344 children from the Shanghai Prenatal Cohort; cord blood PFAS and respiratory tract infections (face to face interviews/medical records, yearly follow-up) in the first five years of life; no association between PFOA or PFOS and respiratory tract infections or serum IgE levels; similar results when stratified by age and sex.	No change
Jackson-Browne MS, Eliot M, Patti M, Spanier AJ, Braun JM (2020). PFAS (per- and polyfluoroalkyl substances) and asthma in young children: NHANES 2013-2014. Int J Hyg Environ Health 229:113565	607 children ages 3-11 in the 2013-14 US NHANES; serum PFAS and parent-reported, doctor-diagnosed, asthma using a standardized questionnaire; no association between PFOA or PFOS and asthma overall; OR = 1.7 (1.0-3.0) for PFOS in children age 3-5.	Possible effect modification of PFOS and asthma association by age
Jensen RC, Andersen MS, Larsen PV, et al. (2020). Prenatal exposures to perfluoroalkyl acids and associations with markers of adiposity and plasma lipids in infancy: an Odense Child Cohort Study. Environ Health Perspect 128(7):77001	Maternal PFAS serum levels and non-fasting lipids in the offspring at ages 3 months (n=262) and 18 months (n=198); dropout rate >50%; no associations seen between PFOA or PFOS and TC and HDL at 3 or 18 months; association between PFOA and decreased LDL and increased TG in boys at 18 months but not in girls and not at 3 months.	Mostly null results; in children
Kim HY, Kim KN, Shin CH, et al. (2020). The relationship between perfluoroalkyl substances concentrations and thyroid function in early childhood: a prospective cohort study [published online ahead of print, 2020 Jun 2]. Thyroid 10.1089/thy.2019.0436	Approximately 600 children from South Korea; serum PFAS and thyroid hormones measured at ages 2, 4, and 6; no association between PFOA or PFOS and TSH after adjustment for iodine intake; association between PFOA and fT4 at age 6 in boys; association between PFOS and triiodothyronine (T3) at age 6 in boys.	Mixed results
Lebeaux RM, Doherty BT, Gallagher LG, et al. (2020). Maternal serum perfluoroalkyl substance mixtures and thyroid hormone concentrations in maternal and cord sera: The HOME Study. Environ Res. 185:109395	Pregnant women and their children from the greater Cincinnati, Ohio region; maternal (n=185) and cord blood (n=256) PFAS and thyroid hormone measurements; no clear association between PFOA or PFOS and TSH or FT4 overall; PFOS associated with increased TSH and decreased FT4 in maternal samples, although a large number of comparisons.	Mixed results
Li Y, Barregard L, Xu Y, et al. (2020). Associations between perfluoroalkyl substances and serum lipids in a Swedish adult population with contaminated drinking water. Environ Health. 2020;19(1):33	1,945 adults aged 20–60 from Ronneby, Sweden, a municipality with environmental contamination to PFOS (median serum level = 157 ng/ml) and PFHxS (median =136 ng/ml); these two chemicals were highly correlated (R=0.9); associations were reported between PFOS and PFHxS with increased TC and LDL; these associations were seen using both serum levels as well as exposure estimates based on residential history; some dose-response data.	Supports an association between PFOS and TC and LDL; similarity of findings based on serum levels and residential history argues against reverse causality related to bile acids

Reference	Major finding	Risk assessment implications
Lin TW, Chen MK, Lin CC, et al. (2020). Association between exposure to perfluoroalkyl substances and metabolic syndrome and related outcomes among older residents living near a Science Park in Taiwan [published online ahead of print, 2020 Sep 9]. Int J Hyg Environ Health 230:113607	397 adults living near an industrial park in Taiwan with environmental PFAS contamination; cross-sectional analysis of serum lipids and serum PFAS; for PFOS, ORs for an elevated LDL are above 1.0 but plateau after quartile 2 (p-trend = 0.03); corresponding ORs are also elevated for PFOA but are not statistically significant.	Supports an association between PFOS and LDL
Liu G, Zhang B, Hu Y, et al. (2020). Associations of perfluoroalkyl substances with blood lipids and apolipoproteins in lipoprotein subspecies: the POUNDS-lost study. Environ Health 19(1):5	326 participants in a weight loss trial; cross-sectional and prospective (2-year follow-up); serum PFOS associated with 5-6% increase in TC at baseline, but not statistically significant (p=0.21); no association seen for PFOA; PFOA associated with 15-20% increase in TG but not statistically significant (p=0.06); no association between TG and PFOS; PFOA associated with subspecies of intermediate density lipoprotein, LDL, and HDL that contain apolipoprotein C-III.	Supports an association between PFOS and TC. Similar effect size as Steenland et al., 2009.
Preston EV, Webster TF, Claus Henn B, et al. (2020). Prenatal exposure to per- and polyfluoroalkyl substances and maternal and neonatal thyroid function in the Project Viva Cohort: A mixtures approach. Environ Int 139:105728	726 mothers and 465 neonates in Boston, Massachusetts area; PFAS and thyroid hormones measured in maternal plasma samples, and T4 in neonatal heel stick samples; statistical analyses involved weighted quantile sum (WQS) regression and Bayesian kernel machine regression (BKMR); PFAS mixture associated with lower maternal fT4, with 2-(N-ethylperfluorooctane sulfonamido) acetate, and 2-(N-methyl-perfluorooctane sulfonamido) acetate, PFOA, and PFHxS contributing most to the overall mixture effect; no association with T4 or TSH; in infants, PFAS mixture was associated with lower T4 levels, primarily in males, with PFHxS and MeFOSAA contributing most in the WQS regression, and PFHxS contributing most in the BKMR analysis.	Possible mixture effects with thyroid hormone
Salihović S, Dickens AM, Schoultz I, et al. (2020). Simultaneous determination of perfluoroalkyl substances and bile acids in human serum using ultra-high-performance liquid chromatography-tandem mass spectrometry. Anal Bioanal Chem 412(10):2251-9	A new method was used to simultaneously measure PFAS and bile acids in plasma; in 20 participants, PFAS were negatively associated with most bile acids.	Findings are consistent with other research showing that several PFAS, including PFOS, can suppress CYP7A1, an enzyme that controls the rate-limiting step in bile acid formation from cholesterol. These findings argue against the hypothesis that PFOS-TC associations are due to similarities in enterohepatic circulation.

Reference	Major finding	Risk assessment implications
Salihovic S, Lind L, Larsson A, Lind PM (2020). Plasma perfluoroalkyls are associated with decreased levels of proteomic inflammatory markers in a cross-sectional study of an elderly population [published online ahead of print, 2020 Sep 8]. Environ Int 145:106099	N=965, all age 70 from Sweden; proteomic analysis; PFOA and PFOS associated with several protein markers of inflammation; not associated with CRP after adjustments for sex, sample storage time, smoking, exercise, education, energy and alcohol intake, BMI, GFR, corticoid and COX-inhibitor treatment.	May provide some biologic plausibility or mechanistic information for associations between PFAS and immune function
Stratakis N, Conti DV, Jin R, et al. (2020). Prenatal exposure to perfluoroalkyl substances associated with increased susceptibility to liver injury in children [published online ahead of print, 2020 Aug 1]. Hepatology 10.1002/hep.31483	1,105 mothers and their children (median age 8 years) in Europe; PFAS measured in maternal blood and liver enzymes in child serum; analyses based on BKMR; PFAS mixture during pregnancy was associated with higher liver enzyme levels (ALT, AST, GGT) in children; effects seem to be primarily due to PFOA and PFNA.	Supports the association between PFOA and increased liver enzymes
Timmermann CAG, Jensen KJ, Nielsen F, et al. (2020). Serum perfluoroalkyl substances, vaccine responses, and morbidity in a cohort of Guinea-Bissau children. Environ Health Perspect 128(8):87002	PFOA and PFOS associated with reductions in measles antibody levels in 237 children age 9 months from Guinea-Bissau, West Africa. ORs elevated for some respiratory/infectious symptoms	Supports a causal association between PFOA and PFOS and decreases in antibody response
Xiao C, Grandjean P, Valvi D, et al. (2020). Associations of exposure to perfluoroalkyl substances With thyroid hormone concentrations and birth size. J Clin Endocrinol Metab 105(3):735-45	Faroe Islands; PFAS measured in maternal serum at 34 weeks gestation in 172 mother-child pairs; thyroid hormones measured in maternal and cord serum; maternal serum PFOS and PFOA associated with a 53% (95% CI, 18-99%) and 40% (95% CI, 8-81%) increase in cord TSH; most other results null or not statistically significant	No change
Yang Q, Guo X, Chen Y, et al. (2020). Blood levels of perfluoroalkyl substances (PFASs), elements and their associations with metabolic syndrome (MetS) in Chinese male adults mediated by metabolic-related risk factors. Sci Total Environ 742:140595	80 adult males with metabolic syndrome and 64 males without; cross-sectional analysis of serum PFAS and thyroid hormones; authors report that no associations seen with thyroid hormones although actual results are not presented	No change
Zeng XW, Li QQ, Chu C, et al. (2020). Alternatives of perfluoroalkyl acids and hepatitis B virus surface antibody in adults: Isomers of C8 Health Project in China. Environ Pollut. 259:113857	Association between PFOA and PFOS and lower hepatitis B antibody levels in 605 adults from the Isomer of C8 Health Project in China	Supports a causal association between PFOA and PFOS and decreased antibody response to vaccines
BA Cohn, MA La Merrill, NY Krigbaum, M Wang, JS Park, M Petreas, G Yeh, RC Hovey, L Zimmermann, PM Cirillo (2020). In utero exposure to poly- and perfluoroalkyl substances (PFASs) and subsequent breast cancer. Reproductive toxicology (Elmsford, N.Y.), 92	Maternal perinatal serum levels measured in 1959-67; 102 cases in daughters and 310 controls; interaction between a PFOS precursor and cholesterol reported; decreased risk with maternal PFOS. PFAS levels not measured in daughters	Mixed results overall

Reference	Major finding	Risk assessment implications
R Jin, R McConnell, C Catherine, S Xu, DI Walker, N Stratakis, DP Jones, GW Miller, C Peng, DV Conti, MB Vos, L Chatzi (2020). Perfluoroalkyl substances and severity of nonalcoholic fatty liver in Children: An untargeted metabolomics approach. Environment international, 134	74 children with non-alcoholic fatty liver disease; no healthy comparison group.; increased odds of nonalcoholic steatohepatitis compared to children with steatosis alone with increased PFOS; no association with PFOA	PFOS may be associated with increased liver disease severity; no change
P Dufour, C Pirard, P Petrossians, A Beckers, C Charlier (2020). Association between mixture of persistent organic pollutants and thyroid pathologies in a Belgian population. Environmental research, 181	Belgian adults; case-control study of hypo- and hyper- thyroidism; 79 cases and 160 controls; PFOS and PFOA associated with lower ORs of both hypo- and hyper- thyroidism	Mixed results
G Liu, B Zhang, Y Hu, J Rood, L Liang, L Qi, GA Bray, L DeJonge, B Coull, P Grandjean, JD Furtado, Q Sun (2020). Associations of Perfluoroalkyl substances with blood lipids and Apolipoproteins in lipoprotein subspecies: the POUNDS-lost study. Environmental health : a global access science source, 19(1)	326 men and women from the Prevention of Obesity Using Novel Dietary Strategies Lost randomized trial; serum PFOS associated with some increase in TC (p=0.21); no association with PFOA	Supports association between PFOS and lipid alterations
RM Lebeaux, BT Doherty, LG Gallagher, RT Zoeller, AN Hoofnagle, AM Calafat, MR Karagas, K Yolton, A Chen, BP Lanphear, JM Braun, ME Romano (2020). Maternal serum perfluoroalkyl substance mixtures and thyroid hormone concentrations in maternal and cord sera: The HOME Study. Environmental research, 185	468 pregnant women and their children in the greater Cincinnati, Ohio region; serum PFAS concentrations during pregnancy and maternal (n = 185) and cord (n = 256) thyroid hormone levels; generally null results although some indication of effect modification by anti-thyroid antibodies for both PFOA and PFOS and decreased free T4	No change
EV Preston, TF Webster, B Claus Henn, MD McClean, C Gennings, E Oken, SL Rifas-Shiman, EN Pearce, AM Calafat, AF Fleisch, SK Sagiv (2020). Prenatal exposure to per- and polyfluoroalkyl substances and maternal and neonatal thyroid function in the Project Viva Cohort: A mixtures approach. Environment international, 139	726 mothers and 465 neonates from the Boston, Massachusetts area; maternal plasma PFAS and thyroid hormones collected during early pregnancy, and neonatal postpartum heel stick thyroxine levels; overlap with Preston et al (2018); focus on PFAS mixtures; overall mixed results	No change
HY Kim, KN Kim, CH Shin, YH Lim, JI Kim, BN Kim, YC Hong, YA Lee (2020). The Relationship Between Perfluoroalkyl Substances Concentrations and Thyroid Function in Early Childhood: A Prospective Cohort Study. Thyroid : official journal of the American Thyroid Association, 30(11),	Serum PFAS and TSH in 660 children at 2, 4, or 6 years of age; inverse association between PFOA and TSH in boys although somewhat reduced after adjustment for iodine intake	No change
R Aimuzi, K Luo, R Huang, X Huo, M Nian, F Ouyang, Y Du, L Feng, W Wang, J Zhang, (2020). Perfluoroalkyl and polyfluroalkyl substances and maternal thyroid hormones in early pregnancy. Environmental pollution (Barking, Essex : 1987), 264	1885 pregnant women in the Shanghai Birth Cohort; PFAS and thyroid hormones in maternal blood collected prior to 16 weeks of gestation; PFOA positively associated with free T4; no clear associations for PFOS	No change

Reference	Major finding	Risk assessment implications
RC Jensen, MS Andersen, PV Larsen, D Glintborg, C Dalgård, CAG Timmermann, F Nielsen, MB Sandberg, HR Andersen, HT Christesen, P Grandjean, TK Jensen (2020). Prenatal Exposures to Perfluoroalkyl Acids and Associations with Markers of Adiposity and Plasma Lipids in Infancy: An Odense Child Cohort Study. Environmental health perspectives, 128(7)	649 women and children from the Odense Cohort study; serum lipids measured at 3 and 18 months of age; maternal PFAS measured at various times in pregnancy; mostly no associations	Study in children; no change
Y Fan, X Li, Q Xu, Y Zhang, X Yang, X Han, G Du, Y Xia, X Wang, C Lu (2020). Serum albumin mediates the effect of multiple per- and polyfluoroalkyl substances on serum lipid levels. Environmental pollution (Barking, Essex : 1987), 266(Pt 2)	NHANES 2011-14; 1,067 adults; PFOS and PFOA associated with HDL, LDL, and TC with associations greater for PFOS and PFHxS; evidence of some mediation (16-27%) by serum albumin	Supports association between PFOS and serum lipids; no new data for dose-response
C Canova, G Barbieri, M Zare Jeddi, M Gion, A Fabricio, F Daprà, F Russo, T Fletcher, G Pitter (2020). Associations between perfluoroalkyl substances and lipid profile in a highly exposed young adult population in the Veneto Region. Environment international, 145	Serum PFAS and lipids in 15,720 adults from a regional health study in Veneto region, Italy, an area with water contamination by PFOA; associations identified between PFOA and PFOS with TC, LDL, and HDL; greater effects for PFOS than PFOA	Supports PFOS and lipid associations; dose response data by deciles are available although no obvious major advantage over Steenland et al. (2009); evidence that associations for PFOS not solely due to PFOA
H Gardener, Q Sun, P Grandjean (2021). PFAS concentration during pregnancy in relation to cardiometabolic health and birth outcomes. Environmental research, 192	433 pregnant women enrolled in the US Vanguard Pilot Study of the National Children's Study; PFAS, TC, and TG in third trimester serum; PFOS associated with increased TC and TG; PFOA associated with increased TG but not TC	Supports PFOS-TC association; dose-response data in figure form only
J Yang, H Wang, H Du, H Fang, M Han, L Xu, S Liu, J Yi, Y Chen, Q Jiang, G He (2020). Serum perfluoroalkyl substances in relation to lipid metabolism in Chinese pregnant women. Chemosphere	436 pregnant women in Tangshan City, North China; serum levels of PFAS in first term, serum lipids in third term; possible decrease in LDL/HDL ratio for PFOA otherwise no clear associations	Findings in pregnant women, no change
OE Omoike, RP Pack, HM Mamudu, Y Liu, S Strasser, S Zheng, J Okoro, L Wang (2020). Association between per and polyfluoroalkyl substances and markers of inflammation and oxidative stress. Environmental research	NHANES 2005-2012; 6,652 adults; associations between PFOS and PFOA with some inflammatory markers including neutrophil and lymphocyte count but not CRP	Supports association between PFOA and PFOS and immune toxicity; no change
P Grandjean, CAG Timmermann, M Kruse, F Nielsen, PJ Vinholt, L Boding, C Heilmann, K Mølbak (2020). Severity of COVID-19 at elevated exposure to perfluorinated alkylates. medRxiv : the preprint server for health sciences	Plasma PFAS and health registry data in 323 adults from Denmark with known Covid-19 infection; no increase in infection severity with increased PFOS or PFOA	No change
Y Tian, M Miao, H Ji, X Zhang, A Chen, Z Wang, W Yuan, H Liang (2021). Prenatal exposure to perfluoroalkyl substances and cord plasma lipid concentrations. Environmental pollution (Barking, Essex : 1987), 268(Pt A)	Maternal plasma PFAS at 12-16 weeks and cord blood lipid levels; PFOS associated with decreased TC; no clear association with PFOA	Findings are in neonates; no change

Reference	Major finding	Risk assessment implications
H Liang, Z Wang, M Miao, Y Tian, Y Zhou, S Wen, Y Chen, X Sun, W Yuan (2020). Prenatal exposure to perfluoroalkyl substances and thyroid hormone concentrations in cord plasma in a Chinese birth cohort. Environmental health : a global access science source, 19(1)	300 mother-infant pairs from the Shanghai-Minhang Birth Cohort Study; PFAS and thyroid hormones in maternal plasma at 12-16 weeks gestation; mixed and inconsistent effects overall	No change
M van Gerwen, N Alpert, M Alsen, K Ziadkhanpour, E Taioli, E Genden (2020). The Impact of Smoking on the Association between Perfluoroalkyl Acids (PFAS) and Thyroid Hormones: A National Health and Nutrition Examination Survey Analysis. Toxics, 8(4)	NHANES 2011-12; 1,325 adults; positive association between PFOS and free T4, primarily in non-smokers; no associations for PFOA	No change
Y Li, Y Xu, T Fletcher, K Scott, C Nielsen, D Pineda, CH Lindh, DS Olsson, EM Andersson, K Jakobsson (2020). Associations between perfluoroalkyl substances and thyroid hormones after high exposure through drinking water. Environmental research, 194	3,297 participants from Ronneby, a municipality with drinking water highly contaminated by PFAS and a reference group (n=226) from a nearby municipality with non-contaminated drinking water supply; overall no clear associations except PFOS and PFOA associated with increased free T4 in males over 50 years old	No change
T Dalla Zuanna, DA Savitz, G Barbieri, G Pitter, M Zare Jeddi, F Daprà, ASC Fabricio, F Russo, T Fletcher, C Canova (2021). The association between perfluoroalkyl substances and lipid profile in exposed pregnant women in the Veneto region, Italy. Ecotoxicology and environmental safety, 209	319 pregnant women from Veneto region, Italy, an area with PFOA drinking water contamination; serum PFAS and lipids collected at various times during pregnancy; fairly large increases in TC and LDL from first to third trimester; PFOS was positively associated with TC in the first trimester, mixed results otherwise; an inverse relationship seen between PFOA both TC and LDL-C in the third trimester	Findings in pregnant women; no change
N Li, Y Liu, GD Papandonatos, AM Calafat, CB Eaton, KT Kelsey, KM Cecil, HJ Kalkwarf, K Yolton, BP Lanphear, A Chen, JM Braun (2021). Gestational and childhood exposure to per- and polyfluoroalkyl substances and cardiometabolic risk at age 12 years. Environment international, 147	PFAS in serum during pregnancy, at birth, and at ages 3, 8, and 12 years from 221 mother-child pairs in the HOME Study; 2003-06, Cincinnati, Ohio; HDL, TGs and other biomarkers measured in children at age 12 years old; positive association between PFOS at 8 and 12 years old and HDL; no clear associations with TGs or with PFOA	Findings are in children; TC or LDL not measured; no change
M Averina, J Brox, S Huber, AS Furberg (2021). Exposure to perfluoroalkyl substances (PFAS) and dyslipidemia, hypertension and obesity in adolescents. The Fit Futures study. Environmental research, 195	940 adolescents in Norway; serum PFAS and lipids; positive association between PFOS and TC and LDL; overlap with Averina et al (2019)	Supports association between PFOS and lipid alterations
J Guo, J Zhang, Z Wang, L Zhang, X Qi, Y Zhang, X Chang, C Wu, Z Zhou (2021). Umbilical cord serum perfluoroalkyl substance mixtures in relation to thyroid function of newborns: Findings from Sheyang Mini Birth Cohort Study. Chemosphere, 273	490 mother-newborn pairs from the Sheyang Mini Birth Cohort Study, recruited between June 2009 and January 2010; PFAS and thyroid hormones measured in cord blood; PFOS associated with increased total T4 and contributed 46% to the PFAS mixture effect; PFOA also associated with free T4 and contributed 29% to the PFAS mixture effect	PFOS associated with increased total and free T4 in newborns; no change

Reference	Major finding	Risk assessment implications
L Dalsager, N Christensen, U Halekoh, CAG Timmermann, F Nielsen, HB Kyhl, S Husby, P Grandjean, TK Jensen, HR Andersen (2021). Exposure to perfluoroalkyl substances during fetal life and hospitalization for infectious disease in childhood: A study among 1,503 children from the Odense Child Cohort. Environment international, 149	PFAS serum levels in first trimester pregnant women from the Odense Child Cohort (Denmark) were measured in 2010-2012; data on child hospitalizations at ages 0-4 years old for infectious disease obtained from the Danish National Patient Register; appears to be an expansion of Dalsager et al (2016); 1,503 mother-child pairs; a doubling in maternal PFOS was associated with a 23% increase in the risk of hospitalization due to any infection (hazard ratio (HR): 1.23 (95% CI: 1.05, 1.44); a doubling of PFOA or PFOS increased the risk of lower respiratory tract infection by 27% (HR: 1.27 (1.01, 1.59)) and 54% (HR: 1.54 (1.11, 2.15)), respectively	Supports the association between PFOS and PFOA and immune toxicity
AL Bjorke-Monsen, K Varsi, M Averina, J Brox, S Huber (2020). Perfluoroalkyl substances (PFASs) and mercury in never-pregnant women of fertile age: association with fish consumption and unfavorable lipid profile. BMJ nutrition, prevention & health, 3(2)	158 Norwegian women (not pregnant) ages 18-39 years old; serum PFAS and lipids; PFOS associated with increased TC and LDL; no associations seen for PFOA	Supports the association between PFOS and lipid alterations; no new data for dose-response
CM Bulka, V Avula, RC Fry (2021). Associations of exposure to perfluoroalkyl substances individually and in mixtures with persistent infections: Recent findings from NHANES 1999-2016. Environmental pollution (Barking, Essex : 1987), 275	8,778 individuals (3,189 adolescents; 5,589 adults) in NHANES 1999-2016; serum concentrations of PFAS and antibodies to cytomegalovirus, Epstein Barr virus, hepatitis C and E, herpes simplex 1 and 2, HIV, T. gondii, and Toxocara spp; seropositivity summed to calculate a pathogen burden score; both PFOA and PFOS associated with a higher pathogen burden scores	Supports the association between PFOS and PFOA and immune toxicity

APPENDIX 8. KEY CHARACTERISTICS OF CARCINOGENS

The key characteristics (KCs) of carcinogens (Smith et al., 2016; IARC, 2020) were used to organize the mechanistic data relevant to carcinogenicity from studies of PFOA and PFOS. OEHHA utilized the KCs concept to systematically identify, organize, and summarize mechanistic information. Human carcinogens often share one or more KCs, and act through multiple mechanisms. Therefore, the KCs approach allows for a broader consideration of possible mechanistic pathways and hypotheses based on the available evidence. In the case of the mechanistic data currently available for PFOA and PFOS, OEHHA reviewed the evidence identified through literature searches on five of the KCs (2, 5, 7, 8, and 10).

KC2: Is genotoxic

Genotoxicity refers to the ability of a chemical or other type of agent or biological process to damage DNA or induce changes in the DNA sequence. The link between genotoxicity and carcinogenesis is well established (Smith et al., 2016; Smith et al., 2020). Changes in the DNA sequence include gene or point mutations, such as base substitutions, frameshifts and small deletions or insertions, and chromosomal effects, such as chromosomal aberrations, micronuclei, and aneuploidy. Examples of DNA damage include DNA adducts, DNA strand breaks, and DNA-DNA and DNA-protein crosslinks.

Genotoxicity studies of PFOA and PFOS have been reviewed and summarized in detail by IARC (2017a) for PFOA and by the European Food Safety Authority (EFSA, 2008; EFSA, 2018) for PFOA and PFOS. IARC (2017a) stated that “PFOA is not DNA-reactive,” based on negative findings in a large number of assays assessing direct genotoxic activity, while noting that “some studies [of PFOA] indicate that indirect DNA damage may result from induction of oxidative stress.” In reviewing the genotoxicity studies for PFOA and PFOS, EFSA (2018) concluded “the available data are inconclusive,” noting that there is “some evidence that the observed effects [genotoxicity] are related to oxidative stress” and “[f]rom in vitro and in vivo genotoxicity studies, there is no evidence for a direct genotoxic mode of action for both PFOS and PFOA, however, genotoxicity cannot be excluded.”

OEHHA identified additional studies relevant to the genotoxicity of PFOA that were not included in the IARC (2017a) and EFSA (2008, 2018) evaluations, and additional studies relevant to the genotoxicity of PFOS that were not included in the EFSA (2008, 2018) assessments. These additional studies are discussed separately below, first for PFOA and then for PFOS. Findings from all of the studies relevant to genotoxicity are summarized in Tables A8.1, A8.2, and A8.3 for PFOA, and in Tables A8.4, A8.5, and A8.6 for PFOS.

PFOA

OEHHA identified seven additional PFOA studies and reports that were not included in the IARC (2017a) and EFSA reviews (Governini et al., 2015; Lu et al., 2016; Franken et al., 2017; Peropadre et al., 2018; Crebelli et al., 2019; Li et al., 2019; NTP, 2019a). Two of these studies are of PFOA exposed humans (Governini et al., 2015; Franken et al., 2017), and a third is in a human cell line (Peropadre et al., 2018). One study in exposed humans examined associations between a number of chemicals and reported a statistically significant association between serum PFOA concentrations and increased DNA damage (single- and double-strand breaks and alkali-labile sites, measured in the alkaline comet assay) in the peripheral blood cells of Flemish teenagers (14-15 years old) (Franken et al., 2017). This association was no longer significant

after correction for multiple hypothesis testing. No association was observed between serum PFOA and oxidative damage to DNA bases in peripheral blood cells, measured by the formamidopyrimidine DNA glycosylase (FPG)-modified comet assays, or between serum PFOA and urinary levels of the oxidized DNA base 8-OHdG. Regression models were adjusted for sex, age, smoking status, maximum body temperature seven days before sample collection (alkaline comet assay only), BMI (FPG-modified comet assay only), and highest education level of family (FPG-modified comet assay only).

The second additional genotoxicity study in exposed humans, Governini et al. (2015), examined chromosomal effects, namely disomy and aneuploidy of chromosomes 18, X and Y in human sperm obtained from patients at a fertility clinic in Italy, associated with the presence of PFOA and/or PFOS in seminal plasma and whole blood. Study participants with detectable levels of either PFOA or PFOS in blood and seminal plasma (58% of the study population) were considered "PFC [perfluorinated compound] positive." Limits of detection for PFOA and PFOS were reported as 3 ng/g and 1.5 ng/g, respectively. In men with detectable levels of these compounds in seminal plasma, mean values were 7.68 ± 0.78 ng/g for PFOA and 5.37 ± 0.45 ng/g for PFOS. In men with detectable levels of these compounds in whole blood, mean values were 8.03 ± 1.04 ng/g for PFOA and 7.07 ± 0.66 ng/g for PFOS. The authors reported increased aneuploidy, chromosome 18 disomy, and total disomies in the "PFC positive" group, compared to the PFC negative group. Because this study did not report results specific to either PFOA or PFOS, it is not included in Table A8.1.

In a study using a p53-deficient human skin cell line (HaCaT keratinocytes), PFOA at 50 µM induced DNA damage, as detected by immunofluorescence staining of γ-H2AX foci. Increased staining for γ-H2AX was observed immediately after the 24-hour exposure period, and persisted for 8 days post-exposure, during which time the cells were cultured in PFOA-free medium (Peropadre et al., 2018). Although several other studies (see Tables A8.1 and A8.2) have reported that PFOA may induce DNA damage as a result of increased oxidative stress, Peropadre et al. (2018) noted, "Our results were not entirely consistent with this assumption because DNA damage [increased staining for γ-H2AX] found in HaCaT cells immediately after a moderate treatment with PFOA was not concomitant with significant oxidative stress, as determined by 8-OHdG immunostaining." An increase in 8-OHdG staining, a biomarker of oxidative damage to DNA, was observed 8 days post-exposure, but not immediately after the 24-hour exposure period (Peropadre et al., 2018).

Three additional publications report genotoxicity findings observed in rodents exposed to PFOA (Crebelli et al., 2019; Li et al., 2019; NTP, 2019a). In the NTP studies, a statistically significant increase in micronucleated reticulocytes was observed in the peripheral blood of male Sprague Dawley rats treated with PFOA via gavage, at a dose range of 0.625 to 10 mg/kg-day for 28 days (dose-related trend, $p \leq 0.025$), but no effects were observed in female Sprague Dawley rats (NTP, 2019a). The lack of effects in female rats might be attributed to the much faster PFOA clearance rate compared to male rats (see Table A6.4). No effect was observed in immature erythrocytes of either sex. The authors note that the increase in micronucleated reticulocytes is significant compared to concurrent controls, but within historical control data. NTP (2019a) also reported that PFOA did not induce bacterial reverse mutations in *S. typhimurium* TA98, TA100, or in *E. coli* (WP2 uvRA pKM101). A second publication reported that gavage exposure of pregnant Kunming mice to PFOA at daily doses of 0, 1, 2.5, 5, or 10 mg/kg body weight from GD 1-17 resulted in a dose-dependent increase in 8-OHdG in the liver of female offspring, when assessed on PND 21 (Li et al., 2019). A third publication reported negative genotoxicity results in mice given PFOA in drinking water for five weeks (Crebelli et al.,

2019). Specifically, no increases in MN formation were observed in splenocytes or reticulocytes, and no increases in DNA strand breaks (assessed by the comet assay) were observed in the liver or testis.

The seventh additional genotoxicity study used a cell-free system to measure DNA charge transfer in 15-base pair double-stranded (ds) DNA oligonucleotides in the presence and absence of PFOA (Lu et al., 2016). PFOA increased the DNA charge transfer resistance in this system, and the authors interpreted this as resulting from a loosening of the duplex DNA structure and a change in the DNA base pair stacking by PFOA. The authors postulate that the perfluorinated alkyl carbon chain of PFOA binds to the groove in the DNA double helix and disrupts the hydrogen binding of G to C and A to T by forming hydrogen bonds with fluorine (F) (Lu et al., 2016).

Findings from studies relevant to the genotoxicity of PFOA, including those reviewed by IARC and EFSA (see IARC (2017a): Table 4.2 on pages 78-79, EFSA (2018): Table 18 on pages 118-119, and EFSA (2008): pages 88-89) are presented in Tables A8.1, A8.2, and A8.3 below.

Table A8.1. Genotoxicity studies of PFOA in humans and other mammals

Test endpoint	Species assayed	Route, duration, dosing regimen	Results	Reference
Micronuclei	Mouse	5,000 mg/kg-day	No significant changes in polychromatic erythrocytes in bone marrow	Murli (1995) as reported by IARC (2017a)[1]
Micronuclei	Mouse	A single dose of 950 mg/kg via oral gavage	No significant changes in polychromatic erythrocytes in bone marrow	Murli (1996) as reported by IARC (2017a) and (EFSA, 2008)[2]
Micronuclei	Mouse: male C57BL/6 (6-8/dose)	0, 0.55, 5.5, or 28 mg/L in drinking water for 5 weeks (0, 0.1, 1, or 5 mg/kg-day)	No significant changes in reticulocytes or splenocytes	Crebelli et al. (2019)
Micronuclei	Rat: female Sprague Dawley (10/dose)	0, 6.25, 12.5, 25, 50, or 100 mg/kg-day via oral gavage for 28 days	No significant changes in reticulocytes or immature erythrocytes	NTP (2019a)
Micronuclei	Rat: male Sprague Dawley (10/dose)	0, 0.625, 1.25, 2.5, 5, or 10 mg/kg-day via oral gavage for 28 days	Increased micronucleated reticulocytes in a dose-dependent manner; no significant changes in immature erythrocytes	NTP (2019a)

Test endpoint	Species assayed	Route, duration, dosing regimen	Results	Reference
DNA strand breaks (alkaline comet assay, and FPG-modified comet assay)	Human: 14- to 15-year-old Flemish subjects (N=196 for alkaline comet assay; N=193 for FPG-modified comet assay)	Exposure effect estimate in blood: 1.090 (95% CI, 1.015-1.170) µg/L for alkaline comet assay; 1.012 (95% CI, 0.871-1.175) µg/L for FPG-modified comet assay	Increased serum PFOA levels associated with increased DNA damage in peripheral blood cells, as measured by alkaline comet assay; not significant after correction for multiple hypothesis testing. No association with breaks caused by oxidative DNA damage, as measured by FPG-modified comet assay.	Franken et al. (2017)
DNA strand breaks (comet assay)	Mouse: male C57BL/6 (6-8/dose)	0, 0.55, 5.5, or 28 mg/L in drinking water for 5 weeks (0, 0.1, 1, or 5 mg/kg-day)	No significant changes in liver or testis	Crebelli et al. (2019)
Oxidative damage to DNA (8-OHdG in urine)	Human: 14- to 15-year-old Flemish subjects (N=195)	Exposure effect estimate in blood: 1.053 (95% CI, 0.968-1.147) µg/L	No significant association with PFOA	Franken et al. (2017)
Oxidative damage to DNA (8-OHdG in liver)	Mice: female Kunming offspring (6/dose)	Pregnant dams treated with 0, 1, 2.5, 5, or 10 mg/kg via gavage from GD 1-17; offspring assessed at PND 21	Dose-dependent and significant increases in liver 8-OHdG in 2.5, 5 and 10 mg/kg groups	Li et al. (2019)
Oxidative damage to DNA (8-OHdG in liver)	Rat: male Fischer 344 (5/dose/time point)	A single i.p. injection of 100 mg/kg; samples were collected after 1, 3, 5, and 8 days	Significant increases of 8-OHdG in liver after 3, 5, and 8 days	Takagi et al. (1991), also reviewed in IARC (2017a) and EFSA (2008)
Oxidative damage to DNA (8-OHdG in kidney)	Rat: male Fischer 344 (5/dose/time point)	A single i.p. injection of 100 mg/kg; samples were collected after 1, 3, 5, and 8 days	No increase of 8-OHdG in kidney	Takagi et al. (1991), also reviewed in IARC (2017a)
Oxidative damage to DNA (8-OHdG in liver)	Rat: male Fischer 344 (5/dose)	0.02% in the diet for 2 weeks	Significant increases of 8-OHdG in liver	Takagi et al. (1991), also reviewed in IARC (2017a) and EFSA (2008)
Oxidative damage to DNA (8-OHdG in kidney)	Rat: male Fischer 344 (5/dose)	0.02% in the diet for 2 weeks	No increase of 8-OHdG in kidney	Takagi et al. (1991), also reviewed in IARC (2017a)

8-OHdG, 8-hydroxydeoxyguanosine; GD, gestation day; i.p., intraperitoneal; PND, postnatal day

[1] OEHHA has no access to Murli (1995), which was summarized by IARC (2017a). The IARC (2017a) summary of the study did not include information on study duration or the number of animals per treatment group.
[2] OEHHA has no access to Murli (1996), which was summarized by EFSA (2008) and IARC (2017a). Neither the EFSA (2008) summary nor the IARC (2017a) summary of the study included information on the number of animals per treatment group.

Table A8.2. Genotoxicity studies of PFOA in mammalian cell lines

Test endpoint	Species/ cell line	Concentration/ duration	Results/comments	Reference
Mutation (CD59 gene locus)	Human-hamster hybrid (A_L) cells	0-200 µM, 16 days	Positive at 200 µM for 16 days; negative at 0-200 µM for 1-8 days	Zhao et al. (2011), also reviewed in IARC (2017a) and EFSA (2018)
Mutation (CD59 gene locus)	Mitochondrial DNA-deficient human-hamster hybrid (ρ^0A_L) cells	0-200 µM, 16 days	Negative	(Zhao et al., 2011), also reviewed in IARC (2017a) and EFSA (2018)
Mutation (*Hprt* locus)	K-1 Chinese hamster ovary cells	Up to 94 µM (-S9, +S9)	Negative with or without S9	Sadhu (2002), as reported by IARC (2017a) and EFSA (2008)[1]
Micronuclei	Human hepatoma HepG2 cells	0, 50-400 µM, 24 hours	Positive at ≥100 µM	Yao and Zhong (2005), also reviewed in IARC (2017a) and EFSA (2018)
Micronuclei	Human hepatoma HepG2 cells	0, 50-400 µM, 1 or 24 hours	Negative; significant cytotoxicity at ≥200 µM	Florentin et al. (2011), also reviewed in IARC (2017a) and EFSA (2018)
Micronuclei	Chinese hamster V79 lung cells	10 µM (-S9, +S9), 3 hours (+21 hours post-incubation)	Negative	Buhrke et al. (2013), also reviewed in IARC (2017a) and EFSA (2018)
Chromosomal aberrations	Human lymphocytes	Up to 3,640 µM (-S9, +S9)	Negative with or without S9	Murli (1996a), as reported by IARC (2017a)[2]

Test endpoint	Species/ cell line	Concentration/ duration	Results/comments	Reference
Chromosomal aberrations	Chinese hamster ovary cells	Up to 2,000 µM	Positive at 2,000 µM in 3-hour treatment with S9 and harvest time 20 hours after initiation of treatment	Murli (1996b), as reported by IARC (2017a) and EFSA (2008)[3]
Chromosomal aberrations	Chinese hamster ovary cells	Up to 3,740 µM	Positive at 3,740 µM in 3-hour treatment with S9 and harvest time 20 hours after initiation of treatment	Murli (1996c), as reported by IARC (2017a) and EFSA (2008)[4]
Polyploidy	Chinese hamster ovary cells	Up to 2,000 µM	Positive at 2,000 µM in 3-hour treatment with S9 and harvest time 44 hours after initiation of treatment	Murli (1996b), as reported by IARC (2017a) and EFSA (2008)[3]
Polyploidy	Chinese hamster ovary cells	Up to 3,740 µM (-S9); 4,970 µM (+S9)	Positive at 3,740 µM in 3-hour treatment without S9 (4,970 µM (+S9)) and harvest time 44 hours after initiation of treatment	Murli (1996c), as reported by IARC (2017a) and EFSA (2008)[4]
DNA strand breaks (comet assay)	Human hepatoma HepG2 cells	0, 50-400 µM, 1 hour	Positive at ≥50 µM	Yao and Zhong (2005), also reviewed in IARC (2017a) and EFSA (2018)
DNA strand breaks and FPG-sensitive sites (comet assay)	Human hepatoma HepG2 cells	0, 100, 400 µM, 24 hours	Negative; all tested doses were not cytotoxic	Eriksen et al. (2010), also reviewed in IARC (2017a) and EFSA (2018)
DNA strand breaks (comet assay)	Human hepatoma HepG2 cells	0, 50-400 µM, 1 or 24 hours	Negative; significant cytotoxicity at ≥200 µM	Florentin et al. (2011), also reviewed in IARC (2017a) and EFSA (2018)
DNA strand breaks (comet assay)	Human hepatoma HepG2 cells	0, 0.2-20 µM, 24 hours	Positive at ≥10 µM	Wielsoe et al. (2015), also reviewed in EFSA (2018)
DNA strand breaks (comet assay)	Human lymphoblastoid (TK6) cells	0, 125, 250, 500 µg/ml, 2 hours	Positive at ≥250 µg/ml; the authors stated that cells were viable (as measured by trypan blue)	Yahia et al. (2014), also reviewed in EFSA (2018)

Test endpoint	Species/ cell line	Concentration/ duration	Results/comments	Reference
DNA strand breaks (comet assay)	Syrian hamster embryo (SHE) cells	0, 0.00037-300 µM (0-124 µg/ml), 5 or 24 hours	Negative	Jacquet et al. (2012b), also reviewed in EFSA (2018)
DNA damage (γ-H2AX)	Human epidermal p53-deficient (HaCaT) keratinocytes	50 µM for 24 hours	Positive at 50 µM for 24 hours; increased staining for γH2AX persisted for 8 days	Peropadre et al. (2018)
Oxidative damage to DNA (8-OHdG)	Human hepatoma HepG2 cells	0, 50-400 µM, 3 hours	Positive at ≥100 µM	Yao and Zhong (2005), also reviewed in (IARC, 2017a) and EFSA (2018)
Oxidative damage to DNA (8-OHdG)	Human lymphoblastoid (TK6) cells	0, 125, 250, 500 µg/ml, 2 hours	Positive at ≥250 µg/ml; the authors stated that cells were viable (as measured by trypan blue)	Yahia et al. (2014), also reviewed in EFSA (2018)
Oxidative damage to DNA (8-OHdG)	Human epidermal p53-deficient (HaCaT) keratinocytes	50 µM for 24 hours	Negative at 24 hours; positive at 8 days	Peropadre et al. (2018)

[1] OEHHA has no access to Sadhu (2002), which was summarized by EFSA (2008) and IARC (2017a). Neither the EFSA (2008) summary nor the IARC (2017a) summary of the study included information on treatment duration.
[2] OEHHA has no access to Murli (1996a), which was summarized by IARC (2017a). The IARC (2017a) summary of the study did not include information on treatment duration.
[3] OEHHA has no access to Murli (1996b), which was summarized by EFSA (2008) and IARC (2017a). Neither the EFSA (2008) summary nor the IARC (2017a) summary of the study included information on treatment duration.
[4] OEHHA has no access to Murli (1996c), which was summarized by EFSA (2008) and IARC (2017a). Neither the EFSA (2008) summary nor the IARC (2017a) summary of the study included information on treatment duration.

Table A8.3. Genotoxicity studies of PFOA in non-mammalian systems

Test endpoint	Test system	Concentration	Results/comments	Reference
Mutation	*Saccharomyces cerevisiae*	Up to 500 µg/plate	Negative with or without S9	Griffith and Long (1980), also reviewed in IARC (2017a)
Reverse mutation assay (Ames test)	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538	Up to 1,000 µg/plate	Negative with or without S9	Griffith and Long (1980), also reviewed in IARC (2017a)

Test endpoint	Test system	Concentration	Results/comments	Reference
Reverse mutation assay (Ames test)	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538	Up to 5,000 µg/plate	Negative with or without S9	Lawlor (1995, 1996), as reported by IARC (2017a) and EFSA (2008)
Reverse mutation assay (Ames test)	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538	5 µmol/plate	Negative with or without S9	Buhrke et al. (2013), also reviewed in IARC (2017a) and EFSA (2018)
Reverse mutation assay (Ames test)	*S. typhimurium* TA98, TA100, TA102, TA104	100, 500 µM (up to 207 µg/plate)	Negative with or without S9	Fernández Freire et al. (2008), also reviewed in IARC (2017a) and EFSA (2018)
Reverse mutation assay (Ames test)	*S. typhimurium* TA98 and TA100	0-1,000 µg/plate without 10% rat liver S9; 0-5,000 µg/plate with 10% rat liver S9	Negative in TA100 with or without S9; negative in TA98 with S9; equivocal in TA98 without S9	NTP (2019a)
Reverse mutation assay (*umu* test)	*S. typhimurium* TA1535/pSK1002 (*hisG46, rfa, uvrB*)	Up to 414 µg/plate	Negative with or without S9	Oda et al. (2007), as reported by IARC (2017a)
Reverse mutation assay	*E. coli* (WP2 *uvrA*)	Up to 5,000 µg/plate	Negative with or without S9	Lawlor (1995, 1996), as reported by IARC (2017a) and EFSA (2008)
Reverse mutation assay	*E. coli* (WP2 uvRA pKM101)	0-1,000 µg/plate without 10% rat liver S9; 0-10,000 µg/plate with 10% rat liver S9	Negative with or without S9	NTP (2019a)
DNA strand breaks (alkali-labile sites, apurinic/ apyrimidinic (AP) sites) (comet assay, pH ≥13)	*Paramecium caudatum*	0, 10, 30, 100 µM (1, 3, 24 hours); 0, 100 µM (6, 12 hours)	Positive at 100 µM for 12 and 24 hours	Kawamoto et al. (2010), also reviewed in IARC (2017a) and EFSA (2018)

Test endpoint	Test system	Concentration	Results/comments	Reference
DNA strand breaks (comet assay, pH=12.1)	*Paramecium caudatum*	0, 10, 30, 100 µM (24 hours)	Negative	Kawamoto et al. (2010), also reviewed in IARC (2017a) and EFSA (2018)
DNA strand breaks (comet assay)	Green mussel (*Perna viridis*)	0, 0.01-1,000 µg/L (7 days)	Positive at ≥1,000 µg/L	Liu et al. (2014), also reviewed in EFSA (2018)
Oxidative damage to DNA (8-OHdG)	*Paramecium caudatum*	0, 10, 30, 100 µM (1, 3 hours); 0, 10, 30 µM (24 hours)	Negative	Kawamoto et al. (2010), also reviewed in IARC (2017a) and EFSA (2018)
Altered DNA structure (DNA charge transfer)	Cell-free; synthesized double-stranded (ds) DNA oligonucleotides (15-mers)	1.00×10^{-8} µM to 100 µM	Increased DNA charge transfer resistance with a positive linear dose-response (R=0.996) between 10^{-8} to 100 µM. Authors interpret this as an indication of the loosening of duplex DNA structure and a change in DNA base pair stacking.	Lu et al. (2016)

As shown in Table A8.3, PFOA is not mutagenic in bacterial assays conducted in multiple strains of *S. typhimurium* and *E. coli*, or in the yeast *S. cerevisae*. PFOA induced mutations at the *CD59* gene locus in human-hamster hybrid (A_L) cells (with normal levels of mitochondrial DNA) after long-term (16 days) exposure (Zhao et al., 2011), but not in K-1 Chinese hamster ovary (CHO) cells at the *Hprt* locus (Table A8.2).

In studies detecting chromosomal effects (Tables A8.2 and A8.3), PFOA increased micronuclei (MN) in vivo in male rat reticulocytes (NTP, 2019a), but not in female rat reticulocytes or in mouse reticulocytes, splenocytes, or bone marrow. PFOA increased MN in human hepatoma HepG2 cells in one study (Yao and Zhong, 2005), but not another (Florentin et al., 2011) and no increase was observed in Chinese hamster lung V79 cells (Buhrke et al., 2013; IARC, 2017a). PFOA induced chromosomal aberrations (CA) (and polyploidy) in CHO cells in two studies, and did not increase CA in human lymphocytes exposed in vitro (IARC, 2017a).

In several studies, PFOA induced DNA damage, measured as increases in DNA strand breaks, γ-H2AX, and 8-OHdG (IARC, 2017a; EFSA, 2018). In one study in humans, increased serum levels of PFOA were associated with increased levels of DNA strand breaks as measured in the alkaline comet assay, although the authors reported that the association was no longer significant after correction for multiple hypothesis testing (Franken et al., 2017). No increase in

DNA strand breaks was observed in the liver or testis of mice exposed to PFOA (Crebelli et al., 2019). Among four studies conducted in human hepatoma HepG2 cells, DNA strand breaks were assessed at 1 hour in one study and at 24 hours in three studies (Yao and Zhong, 2005; Eriksen et al., 2010; Florentin et al., 2011; Wielsoe et al., 2015). Increases were observed in the study that assessed DNA strand breaks in HepG2 cells at 1 hour, and in one of the three studies that assessed DNA strand breaks in HepG2 cells at 24 hours. The lowest concentration tested in the two studies that did not observe increases in DNA strand breaks at 24 hours was higher than the highest concentration tested in the study that did observe increases. PFOA also increased DNA strand breaks in human lymphoblastoid (TK6) cells (Yahia et al., 2014), but not in Syrian hamster embryo cells (Jacquet et al., 2012b). In non-mammalian systems, PFOA induced DNA strand breaks in green mussels and *Paramecium caudatum* (Kawamoto et al., 2010; Liu et al., 2014). PFOA increased staining for γ-H2AX, another marker of DNA damage, in HaCaT keratinocytes, and this effect persisted for 8 days following cessation of exposure (Peropadre et al., 2018). With regard to 8-OHdG, no association was observed between PFOA serum levels and urinary levels of 8-OHdG in one study in humans (Franken et al., 2017). In rodents exposed to PFOA, levels of 8-OHdG were increased in the liver of female Kunming mice exposed in utero (Li et al., 2019), and in the liver but not the kidney of rats exposed either via a single i.p. injection, or via the diet for two weeks (Takagi et al., 1991). PFOA increased 8-OHdG levels in human HepG2 and TK6 cells (Yao and Zhong, 2005; Yahia et al., 2014). 8-OHdG was not increased in human HaCaT keratinocytes immediately after a 24-hour PFOA exposure, but levels were increased 8 days later (Peropadre et al., 2018). No increase in 8-OHdG was observed in *Paramecium caudatum* (Liu et al., 2014).

PFOS

OEHHA identified four genotoxicity publications on PFOS that were not included in the EFSA (2008, 2018) reviews (Lu et al., 2012; Chen et al., 2016; Eke et al., 2017; NTP, 2019b). One fish study showed that a 30-day exposure to PFOS, followed by a 15-day exposure to clean water induced mutations in a target gene present in the liver of transgenic medaka fish (Chen et al., 2016). These authors noted that PFOS induced “a distinct mutational spectrum dominated by +1 frameshift mutations” in the target gene in the liver of exposed fish. One rat study reported that PFOS administered at doses of 0, 0.6, 1.25, or 2.5 mg/kg every 48 hours via oral gavage over a four-week period increased MN and DNA damage (DNA strand breaks, as measured by the comet assay) in a dose-dependent manner in liver hepatocytes of male rats (Eke et al., 2017). A set of studies conducted by NTP reported findings from bacterial mutagenicity assays and 28-day MN studies in rats exposed by gavage (NTP, 2019b). PFOS was not mutagenic in the two strains of *Salmonella* in which it was tested (TA98, TA100) or in the *E. coli* strain WP2 *uvrA*/pkM101, in either the presence or absence of metabolic activation (S9). A statistically significant increase in MN was observed in polychromatic erythrocytes in the peripheral blood of female rats in the high dose group, with a significant dose-related trend, following exposure to PFOS (NTP, 2019b). However, the increases in MN were within the historical control range, and thus NTP considered the findings in female rats to be equivocal. No increases in MN were observed in similarly exposed male rats (NTP, 2019b). Dose dependent decreases in the percentages of polychromatic erythrocytes in peripheral blood were observed in rats of both sexes following 28-day administration of PFOS, suggesting that the bone marrow is a target of PFOS cytotoxicity (NTP, 2019b). In addition, one electrochemical study of the interaction of PFOS with calf thymus DNA immobilized on a specially prepared carbon electrode found that PFOS binds to the groove in the DNA double helix, intercalates into the DNA, and forms hydrogen bonds with DNA bases, perturbing base pair stacking and reducing DNA charge transport (Lu et al., 2012).

Findings from studies relevant to the genotoxicity of PFOS, including those reviewed by EFSA (see EFSA (2008): page 73, and EFSA (2018): Table 17 on pages 116-117) are presented in Tables A8.4, A8.5, and A8.6 below.

Table A8.4. Genotoxicity studies of PFOS in mammals

Test endpoint	Species assayed	Route, duration, dosing regimen	Results	Reference
Mutation	Mouse: male *gpt* delta transgenic (6/dose)	0, 1.5, 4, or 10 mg/kg via gavage for 28 days	Increase of the *red/gam* locus mutation frequencies in the liver at ≥4 mg/kg	Wang et al. (2015), also reviewed in EFSA (2018)
Micronuclei	Mouse: male and female	A single oral dose of 237.5, 450, or 950 mg/kg, with sampling at 24, 48, or 72 hours	Negative	Corning Hazleton, Inc. (1993), as reported by EFSA (2008)[1]
Micronuclei	Mouse: male *gpt* delta transgenic (6/dose)	0, 1.5, 4, or 10 mg/kg via gavage for 28 days	Non-significant increase of MN frequency in the liver at ≥4 mg/kg	Wang et al. (2015), also reviewed in EFSA (2018)
Micronuclei	Rat: male Swiss albino (Wistar) (6/dose)	0, 0.6, 1.25, or 2.5 mg/kg via gavage every 48 hours over a 4-week period	Increased MN frequency of polychromatic erythrocytes in bone marrow at ≥1.25 mg/kg	Celik et al. (2013), also reviewed in EFSA (2018)
Micronuclei	Rat: male Swiss albino (Wistar) (6/dose)	0, 0.6, 1.25, or 2.5 mg/kg via gavage every 48 hours over a 4-week period	Increased MN frequency in peripheral blood cells at ≥0.6 mg/kg	Eke and Celik (2016), also reviewed in EFSA (2018)
Micronuclei	Rat: male Swiss albino (Wistar) (6/dose)	0, 0.6, 1.25, or 2.5 mg/kg via gavage every 48 hours over a 4-week period	Dose-dependent increases in MN frequency in hepatocytes at ≥0.6 mg/kg	Eke et al. (2017)
Micronuclei	Rat: female Sprague Dawley (10/dose)	0, 0.312, 0.625, 1.25, 2.5, or 5 mg/kg-day via gavage for 28 days	Dose-dependent increases in MN in polychromatic erythrocytes in peripheral blood; increases are within historical control range. Also reported a dose-dependent decrease in percentage of polychromatic erythrocytes in peripheral blood.	NTP (2019b)
Micronuclei	Rat: male Sprague Dawley (10/dose)	0, 0.312, 0.625, 1.25, 2.5, or 5 mg/kg-day via gavage for 28 days	No increase in MN in polychromatic erythrocytes in peripheral blood; dose-dependent decrease in percentage of polychromatic erythrocytes in peripheral blood	NTP (2019b)
DNA strand breaks (comet assay)	Rat: male Swiss albino (Wistar) (6/dose)	0, 0.6, 1.25, or 2.5 mg/kg via gavage every 48 hours over a 4-week period	Increased DNA damage (strand breaks) in bone marrow at ≥0.6 mg/kg	Celik et al. (2013), also reviewed in EFSA (2018)

Test endpoint	Species assayed	Route, duration, dosing regimen	Results	Reference
DNA strand breaks (comet assay)	Rat: male Swiss albino (Wistar) (6/dose)	0, 0.6, 1.25, or 2.5 mg/kg via gavage every 48 hours over a 4-week period	Increased DNA damage (strand breaks) in peripheral blood cells at ≥0.6 mg/kg	Eke and Celik (2016), also reviewed in EFSA (2018)
DNA strand breaks (comet assay)	Rat: male Swiss albino (Wistar) (6/dose)	0, 0.6, 1.25, or 2.5 mg/kg via gavage every 48 hours over a 4-week period	Increased DNA damage (strand breaks) in hepatocytes at ≥0.6 mg/kg	Eke et al. (2017)

[1] OEHHA has no access to Corning Hazleton, Inc. (1993), which was summarized by EFSA (2008). The EFSA (2008) summary of the study did not include information on the number of animals per treatment group.

Table A8.5. Genotoxicity studies of PFOS in mammalian cell lines

Test endpoint	Species/cell line	Concentration/ duration	Results/comments	Reference
Mutation (*redBA/gam* gene locus (Spi$^-$ assay))	*gpt* delta transgenic mouse embryonic fibroblasts	0, 0-20 µM, 24 hours	Positive at ≥10 µM	Wang et al. (2015), also reviewed in EFSA (2018)
Micronuclei	Human hepatoma HepG2 cells	0, 5-300 µM, 24 hours	Negative	Florentin et al. (2011), also reviewed in EFSA (2018)
Chromosomal aberrations	Human peripheral blood lymphocytes	Up to 599 µg/ml (-S9); up to 449 µg/ml (+S9)	Negative with or without S9	Cifone (1999), as reported by EFSA (2008)[1]
DNA strand breaks (comet assay)	Human hepatoma HepG2 cells	0, 5-300 µM, 24 hours	Negative	Florentin et al. (2011), also reviewed in EFSA (2018)
DNA strand breaks (comet assay)	Human hepatoma HepG2 cells	0, 0.2-20 µM, 24 hours	Positive at ≥0.02 µM	Wielsoe et al. (2015), also reviewed in EFSA (2018)
DNA strand breaks and FPG-sensitive sites (comet assay)	Human hepatoma HepG2 cells	0, 100, 400 µM, 24 hours	Negative	Eriksen et al. (2010), also reviewed in EFSA (2018)
DNA strand breaks (comet assay)	Syrian hamster embryo cells	0, 0.00037-93 µM, 5 or 24 hours	Negative	Jacquet et al. (2012a), also reviewed in EFSA (2018)
DNA damage (γ-H2AX)	*gpt* delta transgenic mouse embryonic fibroblasts	0, 0-20 µM, 24 hours	Positive at 20 µM	Wang et al. (2015), also reviewed in EFSA (2018)

Test endpoint	Species/cell line	Concentration/ duration	Results/comments	Reference
Unscheduled DNA synthesis	Primary cultured rat liver cells	Up to 4,000 µg/ml	Negative	Cifone (1999), as reported by EFSA (2008)[1]

[1] OEHHA has no access to Cifone (1999), which was summarized by EFSA (2008). The EFSA (2008) summary of the study did not report the numeric results for chromosomal aberrations or unscheduled DNA synthesis observed at each treatment concentration.

Table A8.6. Genotoxicity studies of PFOS in non-mammalian systems

Test endpoint	Test system	Concentration	Results/comments	Reference
Mutation (c*II* gene locus)	Fish: λ transgenic medaka (7-12/conc.)	0, 6.7, 27.6, or 87.6 µg/L in water for 30 days	Dose-dependent increase in mutations in liver, as measured in the *cII* transgene, with a distinct mutational spectrum dominated by +1 frameshift mutations at ≥6.7 µg/L	Chen et al. (2016)
Reverse mutation assay (Ames test)	*S. typhimurium* TA98, TA100, TA1535, TA1537	Up to 5,000 µg/plate	Negative with or without S9	Mecchi (1999), as reported by EFSA (2008)[1]
Reverse mutation assay (Ames test)	*S. typhimurium* TA09, TA100, TA1535, TA1537, TA1538	0.01-500 µg/plate (-S9); 0.1-500 µg/plate (+S9)	Negative with or without S9	Litton Bionetics, Inc. (1978), as reported by EFSA (2008)[2]
Reverse mutation assay (Ames test)	*S. typhimurium* TA98 and TA100	0-10,000 µg/plate with or without 10% rat liver S9	Negative with or without S9	NTP (2019b)
Reverse mutation assay	*E. coli (WP2 uvRA pKM101)*	0-5,000 µg/plate with or without 10% rat liver S9	Negative with or without S9	NTP (2019b)
Reverse mutation assay	*E. coli* (WP2 uvRA)	Up to 5,000 µg/plate	Negative with or without S9	Mecchi (1999), as reported by EFSA (2008)[1]
Mitotic recombination	*Saccharomyces cerevisiae* (D4)	Not reported	Negative	Litton Bionetics, Inc. (1978), as reported by EFSA (2008)[2]
Micronuclei	Zebrafish	0, 0.4-1.6 mg/L, 30 days incubation of embryos	Positive in peripheral blood cells at ≥0.8 mg/L	Du et al. (2014), also reviewed in EFSA (2018)
DNA strand breaks (comet assay)	Zebrafish	0, 0.4-1.6 mg/L, 30 days incubation of embryos	Positive in peripheral blood cells at ≥0.4 mg/L	Du et al. (2014), also reviewed in EFSA (2018)

Test endpoint	Test system	Concentration	Results/comments	Reference
DNA strand breaks (comet assay)	Gull eggs (*Larus michahellis*)	0, 100, 200 ng/g egg (injected)	Negative	Parolini et al. (2016), also reviewed in EFSA (2018)
DNA strand breaks (comet assay)	Green mussel (*Perna viridis*)	0, 0.01-1,000 µg/L for 7 days	Positive at ≥1,000 µg/L	Liu et al. (2014), also reviewed in EFSA (2018)
DNA strand breaks (comet assay)	Earthworms (*E. foetida*)	0, 0.25-8 µg/cm^3, 48 hours	Positive at ≥0.25 µg/cm^3	Xu et al. (2013), also reviewed in EFSA (2018)
DNA strand breaks (comet assay)	*Paramecium caudatum*	0, 10, 30, 100 µM (1, 3 hours); 0, 10, 30 µM (24 hours)	Negative	Kawamoto et al. (2010), also reviewed in EFSA (2018)
DNA damage (Hus-1: GFP Focus)	*Caenorhabditis elegans*	0, 0.25-25µM, 12-60 hours	Positive at ≥0.25 µM, 24 hours, in germ cell nuclei	Guo et al. (2016), also reviewed in EFSA (2018)
Altered DNA structure (DNA charge transfer)	Cell-free; calf thymus DNA	10 µmol/L, 30 min, 37°C	Positive; increased DNA charge transfer resistance. Authors interpret this as an indication of the loosening of duplex DNA structure and change in DNA base pair stacking	Lu et al. (2012)

[1] OEHHA has no access to Mecchi (1999), which was summarized by EFSA (2008). The EFSA (2008) summary of the studies did not report the numeric results for mutations observed at each treatment concentration.
[2] OEHHA has no access to Litton Bionetics, INC. (1978), which was summarized by EFSA (2008). The EFSA (2008) summary of the studies did not report the numeric results for mutations or recombination events at each treatment concentration.

As shown in Tables A8.6, PFOS was not mutagenic in bacterial assays conducted in multiple strains of *S. typhimurium* and *E. coli*. PFOS induced mutations in the liver of gpt delta transgenic mice at the *redBA/gam* locus (Wang et al., 2015) and transgenic fish at the c*II* gene locus (Chen et al., 2016) after long-term exposure (28 and 30 days, respectively), and at the *redBA/gam* locus in *gpt* delta transgenic mouse embryonic fibroblast cells in vitro (Wang et al., 2015).

With regard to chromosomal effects, a number of studies in rodents and one study in fish (Table A8.4) have reported increases in MN following long-term exposure (28 or 30 days) to PFOS. A 28-day exposure to PFOS increased MN in male Wistar rat bone marrow polychromatic erythrocytes (Celik et al., 2013), peripheral blood cells (Eke and Celik, 2016), and hepatocytes (Eke et al., 2017), and in female Sprague Dawley rat polychromatic erythrocytes (in a dose-dependent manner, although the levels were within the historical control range) but not in male

Sprague Dawley rat polychromatic erythrocytes (NTP, 2019b). A 28-day exposure to PFOS increased MN in hepatocytes of male transgenic mice, although the increase did not reach statistical significance (Wang et al., 2015), whereas a single oral dose of PFOS did not increase MN in mice (EFSA, 2008). A 30-day exposure to PFOS increased MN in peripheral blood cells of zebrafish (Du et al., 2014). PFOS did not increase MN in human hepatoma HepG2 cells (Yao and Zhong, 2005), CA in human peripheral blood lymphocytes exposed in vitro (Buhrke et al., 2013; IARC, 2017a), or mitotic recombination in *S. cerevisae*.

In several studies, PFOS induced DNA damage, measured as increases in DNA strand breaks, γ-H2AX, and foci of Hus-1 (Eke et al., 2017; EFSA, 2018). In male Wistar rats exposed for 28 days, PFOS significantly increased DNA strand breaks as measured in the comet assay in bone marrow (Celik et al., 2013), peripheral blood cells (Eke and Celik, 2016), and hepatocytes (Eke et al., 2017). PFOS also increased DNA strand breaks as measured in the comet assay in the peripheral blood cells of zebrafish following a 30-day exposure, and in green mussels and earthworms, but not in gull eggs or *Paramecium caudatum* (EFSA, 2018). In vitro, PFOS increased DNA strand breaks in one of three studies conducted in human hepatoma HepG2 cells (Table A8.5). The lowest concentration tested in the two HepG2 studies (Eriksen et al., 2010; Florentin et al., 2011) that did not observe increases in DNA strand breaks was higher than the highest concentration tested in the one HepG2 study (Wielsoe et al., 2015) that did observe increases. PFOS did not increase DNA strand breaks in Syrian hamster embryo cells (Jacquet et al., 2012a). PFOS increased γ-H2AX, a marker for DNA damage, in transgenic mouse embryonic fibroblasts (Wang et al., 2015), and increased the number of foci of the DNA damage checkpoint protein Hus-1 in germ cells of *C. elegans* (strain *hus-1:gfp*) (Guo et al., 2016). No increase in unscheduled DNA synthesis was observed in primary liver cell cultures (EFSA, 2008).

Summary of evidence

PFOA has been tested in many genotoxicity test systems that have assessed numerous endpoints indicative of either mutagenicity, chromosomal effects, or DNA damage. Several studies provide evidence that PFOA causes DNA damage, measured as increases in DNA strand breaks (in human cell lines and in non-mammalian species), γ-H2AX (in a human cell line), and 8-OHdG (in human cell lines and in rodent liver). Some studies provide evidence that PFOA may have chromosomal effects, while others do not, and several studies provide evidence that PFOA is not mutagenic.

PFOS has also been tested in many genotoxicity test systems that have assessed numerous endpoints indicative of either mutagenicity, chromosomal effects, or DNA damage. Some studies provide evidence that PFOS is mutagenic (in transgenic mice and fish and transgenic mouse cells), several studies provide evidence of chromosomal effects (e.g., induction of MN in rodents and zebrafish), and several studies provide evidence of DNA damage (e.g., induction of DNA strand breaks in rats, zebrafish, and other non-mammalian species).

KC5: Induces oxidative stress

Oxidative stress refers to a condition of an imbalance between the production and elimination of reactive oxygen and nitrogen species (ROS, RNS). Oxidative stress may contribute to carcinogenic processes by causing DNA mutations, chromosomal damage, genomic instability, and altered cell cycle regulation (Reuter et al., 2010).

There are a number of studies conducted in either whole animals or a variety of in vitro systems that have investigated whether PFOA and PFOS induce oxidative stress. Findings from several of these studies are briefly summarized here, including studies that have looked at 8-OHdG, a marker of oxidative damage to DNA that is linked to mutagenesis and carcinogenesis, and other oxidative damage to DNA, ROS or RNS production, malondialdehyde (MDA, a marker of lipid peroxidation), total antioxidant capacity (TAC), antioxidant enzyme activities (e.g., superoxide dismutase (SOD), catalase (CAT)), and glutathione status (e.g., reduced glutathione (GSH), glutathione disulfide (GSSG), GSH/GSSG ratios). Studies reporting on PFOA and PFOS induced oxidative DNA damage, e.g., 8-OHdG formation, have also been discussed under KC2.

PFOA

Studies assessing the effect of PFOA on levels of 8-OHdG provide evidence relevant to both KC5 and KC2 and are included in Tables A8.1, A8.2, and A8.3 (see discussion of KC2, above). A brief summary of the findings from these studies of PFOA on 8-OHdG levels follows. In one study in humans, no associations were observed between PFOA serum levels and either urinary levels of 8-OHdG or oxidative damage to DNA in peripheral blood cells (measured in the FPG-modified comet assay) (Franken et al., 2017). Increased levels of 8-OHdG were observed in the liver of PND 21 female Kunming mice exposed in utero from GD 1-17 at maternal PFOA doses of 2.5 mg/kg-day and above (Li et al., 2019) and in the liver, but not the kidney of rats exposed either via a single i.p. injection of 100 mg/kg, or via the diet (0.02%) for two weeks (Takagi et al., 1991). PFOA increased 8-OHdG levels in human liver HepG2 cells following exposure to 250 µg/ml and above for two hours (Yahia et al., 2014) and human lymphoblastoid (TK6) cells following exposure to 100 µM and above for three hours (Yao and Zhong, 2005). 8-OHdG was not increased in human epidermal p53-deficient keratinocytes immediately after a 24-hour PFOA exposure, but levels were increased eight days later (Peropadre et al., 2018). No increase in 8-OHdG was observed in *Paramecium caudatum* (Liu et al., 2014).

PFOA significantly increased hepatic levels of ROS, measured as hydrogen peroxide, in male Kunming mice exposed to 5 or 10 mg/kg-day PFOA for 14 days (Yang et al., 2014). The ability of PFOA to significantly increase ROS in HepG2 cells was observed in four studies (at concentrations as low as 0.2 µM in one study and 0.4 µM in another) (Panaretakis et al., 2001; Hu and Hu, 2009; Eriksen et al., 2010; Wielsoe et al., 2015), but not in a fifth (at concentrations up to 400 µM) (Florentin et al., 2011). Exposure of human-hamster hybrid (A_L) cells to PFOA for one day at 100 µM and above increased intracellular levels of ROS and RNS, and similar increases were also observed after longer exposures (4 and 16 days) (Zhao et al., 2011). Significant increases in intracellular ROS were also observed in mouse Leydig tumor cells (mLTC-1) after exposure to PFOA at 50 µM and above for 24 hours (Zhao et al., 2017) and in *Paramecium caudatum* after exposure at 100 µM for one hour and 10 µM for 24 hours (Kawamoto et al., 2010).

PFOA has been observed to increase lipid peroxidation, measured as MDA, in the liver of male Kunming mice exposed orally for 14 consecutive days at doses of 1.5, 5, or 10 mg/kg-day (Yang et al., 2014). An increase in MDA was observed in human erythrocytes exposed for 3 hours to 100 µM PFOA (Pan et al., 2018). In addition, PFOA was observed to increase MDA in rat PC12 cells (an adrenal pheochromocytoma-derived, neuronotypic cell line) at a concentration of 10 µM in undifferentiated cells, and at concentrations of 10 µM and 250 µM in differentiating cells (Slotkin et al., 2008; Pan et al., 2018).

Increased levels of the antioxidant enzymes SOD and CAT were observed in the liver of PND 21 female Kunming mice exposed in utero from GD 1-17 at maternal doses of 2.5 mg/kg-day and above for SOD, and 5 mg/kg-day and above for CAT (Li et al., 2019). In human erythrocytes exposed for 3 hours, GSH levels were decreased at PFOA concentrations of 10 µM and above and CAT and glutathione peroxidase activities were decreased at 100 µM, while no change in SOD activity was observed (Pan et al., 2018). The effect of PFOA on antioxidant enzyme activity in HepG2 cells has been reported in two studies. In the first, PFOA exposure for 48 hours resulted in decreases in GSH levels and glutathione peroxidase activity at concentrations of 100 µM and above, and increases in SOD, CAT, and glutathione reductase activities at concentrations of 150 µM and above (Hu and Hu, 2009). In the second, PFOA exposure for 24 hours decreased measures of TAC at concentrations of 0.02 µM and above (Wielsoe et al., 2015). Exposure of male Japanese medaka fish *Oryzias latipes* to PFOA at concentrations of 50 or 100 mg/L for 7 days decreased hepatic CAT activity, but had no effect on SOD or glutathione peroxidase activity (Yang, 2010).

PFOS

PFOS increased intracellular hepatic levels of ROS in a dose-dependent manner in male Sprague Dawley rats exposed to 1 or 10 mg/kg-day PFOS for 28 days (Han et al., 2018). The ability of PFOS to significantly increase ROS in HepG2 cells was observed in three studies (at concentrations as low as 0.2 µM in one study and 0.4 µM in another) (Hu and Hu, 2009; Eriksen et al., 2010; Wielsoe et al., 2015). In a study of isolated rat hepatocytes, ROS formation was increased after incubation with 25 µM PFOS for one hour (Khansari et al., 2017). Similar to PFOA, significant increases in intracellular ROS were observed in mLTC-1 (mouse Leydig tumor) cells after exposure to PFOS at 50 µM and above for 24 hours (Zhao et al., 2017). Additionally, ROS levels were increased in a dose-dependent manner in *C. elegans* exposed to PFOS concentrations of 0.25, 2.5, and 25 µM (Guo et al., 2016).

PFOS has been observed to increase lipid peroxidation, measured as MDA, in the liver of male Sprague Dawley rats exposed to 1 or 10 mg/kg-day PFOS for 28 days (Han et al., 2018). PFOS also increased levels of MDA (or thiobarbituric acid-reactive substances) in undifferentiated and differentiating rat PC12 cells at concentrations of 10 µM and above, and 50 µM and above, respectively (Slotkin et al., 2008) and in isolated rat hepatocytes following a 3-hour incubation with 25 µM PFOS (Khansari et al., 2017).

In male Sprague Dawley rats exposed to PFOS orally (1 or 10 mg/kg-day) for 28 days, alterations in the liver content and form of glutathione were observed, as well as alterations in liver antioxidant enzyme activities (Han et al., 2018). Specifically, decreases in GSH, increases in GSSG, and decreases in the ratio of GSH:GSSG were observed in both the 1 and 10 mg/kg-day dose groups, along with decreases in CAT activity in both dose groups and decreases in SOD activity in the 10 mg/kg-day dose group (Han et al., 2018). The effect of PFOS on antioxidant enzyme activity in HepG2 cells has been investigated in two studies. In the first, PFOS exposure for 48 hours resulted in decreases in GSH and glutathione peroxidase activity at concentrations of 100 µM and above, decreases in glutathione-S-transferase activity at 200 µM, and increases in SOD, CAT, and glutathione reductase activities at concentrations of 150 µM and above (Hu and Hu, 2009). In the second, PFOS exposure for 24 hours slightly and non-statistically significantly decreased measures of TAC at concentrations of 0.2-20 µM (Wielsoe et al., 2015).

Summary of Evidence

A number of studies indicate that PFOA may cause oxidative stress. Two studies in rodents and two studies in human cells have shown that PFOA led to increased 8-OHdG, a marker of oxidative DNA damage, while one study in human cells reported mixed results and two studies (one in exposed humans and one in a unicellular organism) found no effect. Several studies, including one in mice, four in human HepG2 cells, and one in a mouse cell line, have shown that PFOA increased intracellular production of ROS, while a study in human-hamster hybrid cells showed increased intracellular production of both ROS and RNS. Increased lipid peroxidation was observed in mice, in human erythrocytes exposed in vitro, and in a rat cell line. PFOA also has been shown to alter TAC, antioxidant enzyme content or activity, and glutathione levels in mice, fish, and in human erythrocytes and HepG2 cells.

A number of studies indicate that PFOS may cause oxidative stress. Several studies, including one in rats, three in human HepG2 cells, two in rodent cells or cell lines, and one in *C. elegans*, have shown that PFOS increased intracellular production of ROS. Increased lipid peroxidation was observed in one study in rats and two studies in rat cells or cell lines. PFOS also has been shown to alter antioxidant enzyme activity and glutathione levels in one study in rats and two studies in human HepG2 cells.

KC7: Is immunosuppressive

Immunosuppression can result in a reduction in the capacity of the immune system to respond effectively to tumor cells. Immunosuppression may allow neoplastic cells to escape immune surveillance and permit the survival and replication of these cells to form tumors (Smith et al., 2020). Both the innate and adaptive parts of the immune system participate in immune surveillance, i.e., recognition and removal of malignant cells. The innate immune system is the first line of defense, and key components of the innate (or natural) immune system include natural immunoglobulin M (IgM) antibody-producing B1 or $CD5^+$ cells, macrophages, mast cells, dendritic cells, and natural killer (NK) cells (Vollmers and Brandlein, 2009). The adaptive immune system consists of a heterogeneous population of infiltrating lymphocytes such as T cells and other immune cells to modulate the anti-tumor response (Neeve et al., 2019).

Both natural IgM (produced by B1 cells and marginal zone cells) and adaptive IgM (synthesized by B2 cells) play important roles in the cancer immune response. Natural IgM eliminates tumor cells when they begin to transform; adaptive IgM eliminates tumor cells during growth (Diaz-Zaragoza et al., 2015). Natural IgM antibodies recognize and bind to tumor-specific surface antigens and induce apoptosis via induction of cellular stress, for example by cross-linking of modified anti-complement receptors, blocking of growth-factor receptors, or by increasing the intracellular level of neutral lipids (Vollmers and Brandlein, 2009). NK cells are effector lymphocytes that control several types of tumors and microbial infections by limiting their spread and subsequent tissue damage. Functions of NK cells, including the control of tumor development, can be dependent on their interaction with dendritic cells, macrophages, T cells and endothelial cells (Vivier et al., 2008). NK cells have been observed to induce tumor cell apoptosis through interferon gamma (IFN-γ) and perforin release (Neeve et al., 2019). Dendritic cells are antigen-presenting cells (i.e., they capture tumor antigen) and are capable of activating naive T cells to differentiate into tumor antigen-specific $CD4^+$ helper T cells or to $CD8^+$ cytotoxic T cells. Neutrophils are key cellular mediators of the innate immune response (Qazi et al., 2009a). Results from various studies suggest that tumor-associated neutrophils have anti-tumor properties, including the ability to induce cytotoxicity and inhibit metastasis. Conversely,

other studies point to a tumor-supporting role of neutrophils (Uribe-Querol and Rosales, 2015; Shaul and Fridlender, 2019).

This section summarizes data for immunosuppressive effects of PFOA and PFOS that are relevant to carcinogenesis. Some studies discussed below have also been summarized earlier in Section 5.1 of this document.

PFOA

Effects on T cell dependent and independent antibody response (TDAR/TIAR)

The T cell dependent antibody response (TDAR) assesses immune function in rodents. TDAR focuses on the humoral arm of adaptive immunity and a response requires antigen recognition and presentation, T and B cell signaling, and class switching (DeWitt et al., 2012). TDAR can detect immunosuppression across a range of cell types and signals, usually measured as changes in levels of IgM or IgG production (DeWitt et al., 2012).

The response of IgM, which peaks at days 7 to 14, precedes the response of IgG, which peaks at days 14 to 21 (Lebrec et al., 2011). Assessing IgM TDAR to an antigen is a sensitive measure of immune function, as it requires T cells, B cells, and antigen-presenting cells to function properly to elicit an antibody response (DeWitt et al., 2008). T cell dependent release of IgM (also known as adaptive IgM) has been associated with recognition of breast cancer antigens and priming of the subsequent adaptive immune response (Diaz-Zaragoza et al., 2015). While TDAR measures antibody production resulting from the combined action of T and B cells, another assay, T cell independent IgM antibody response (TIAR), assesses B cell-specific antibody production (DeWitt et al., 2012).

Here, relevant results from animal studies using TDAR and TIAR to assess immune function after PFOA administration are briefly described.

- Suppression of TDAR: Reductions of IgM (but not IgG) titers in adult female C57BL/6J mice (30 mg/kg-day PFOA for 10 or 15 days via gavage), and dose-dependent reduction of IgM by 7-29% in adult female C57BL/6N mice (3.75, 7.5, 15 or 30 mg/kg-day PFOA for 15 days in drinking water) in the presence of antigen challenge with sheep red blood cells (SRBCs) (DeWitt et al., 2008).
- Suppression of TDAR: Reductions of IgM (but not IgG) titers in adult female C57BL/6N wild-type and PPARα knockout mice exposed to 30 mg/kg-day PFOA for 15 days (no effect at 7.5 mg/kg-day) and antigen challenge with SRBCs (DeWitt et al., 2016).
- Suppression of TIAR: Reductions of IgM in female BALB/c mice following gavage treatment with PFOA (20 mg/kg-day) and antigen challenge with trinitrophenol (TNP), and inhibition of general immunoglobulin formation following challenge with ovalbumin (Vetvicka and Vetvickova, 2013).
- Suppression of TIAR: Suppression of IgM in all but the lowest dose in female C57BL/6N mice exposed to 0.94, 1.88, 3.75, or 7.5 mg/kg-day PFOA for 15 days and immunized with a T cell independent antigen dinitrophenyl-ficoll (DNP) on day 11, with sera collected 7 days later (DeWitt et al., 2016).

Effects on T cell and B cell cellularity or proliferation

PFOA has been shown to reduce the number and proliferation of thymocytes and splenocytes in mice in multiple studies. Reduced spleen and thymus weights can be reflective of reductions in splenocytes and thymocytes, respectively, although moderate caloric restriction is also known to reduce thymic and splenic weight and cellularity (Qazi et al., 2009a; Qazi et al., 2009b).

- Reduction of thymocytes in female BALB/c mice following gavage treatment with PFOA (20 mg/kg-day for 7 days) and reduced mitogen-stimulated proliferation of T cells and B cells (Vetvicka and Vetvickova, 2013).
- Reduction of thymocytes and splenocytes by 85% and 80%, respectively, in male C57BL/6 mice exposed via diet (0.02% PFOA weight/weight) for 7 days; altered ratio of thymocyte subpopulations, with 95% decrease in the number of $CD4^+CD8^+$ cells; 64% and 72% decrease in the number of $CD4^+CD8^-$ and $CD4^-CD8^+$ cells, respectively; and 57% decrease in the number of $CD4^-CD8^-$ cells (Yang et al., 2000).
- Decreased relative spleen (17%) and thymus (14%) weights in female C57BL/6N mice following exposure to 7.5 mg/kg-day PFOA in drinking water for 15 days (DeWitt et al., 2016).
- Decreased absolute and relative spleen weight in male Sprague Dawley rats at 16 weeks following post-weaning exposure to 150 or 300 ppm PFOA in the diet (NTP, 2020).

Effects on Neutrophils

- Reduced numbers of circulating neutrophils in male C57BL/6 mice following 10 days of dietary exposure to 0.02% (weight/weight) PFOA (Qazi et al., 2009a).

PFOS

Effects on T cell dependent and independent antibody response (TDAR/TIAR)

TDAR-IgM and TIAR-IgM responses were suppressed in several studies following administration of PFOS and subsequent antigen challenge. One study reported IgM suppression without antigen challenge.

- Suppression of TDAR: Suppression (by 53%) of SRBC-specific IgM production in male B6C3F1 pups following maternal exposure to PFOS (5 mg/kg-day) during gestation and challenge with SRBCs; no suppression was observed in female pups (Keil et al., 2008).
- Suppression of TDAR/TIAR: Dose-dependent suppression of IgM production in male and female B6C3F1 mice exposed to PFOS daily via gavage for 28 days (0, 0.005, 0.05, 0.1, 0.5, 1, or 5 mg/kg) and challenged with SRBC (males responded at lower doses), and suppression of TNP-specific IgM following a challenge with TNP conjugated to LPS (T cell independent) (Peden-Adams et al., 2008).
- Suppression of TIAR: Suppression of total immunoglobulin formation (measured as response to ovalbumin) and IgM formation (measured as TNP response) in female BALB/c mice exposed to PFOS via gavage (20 mg/kg-day) and challenged with ovalbumin or TNP (Vetvicka and Vetvickova, 2013).

- IgM suppression without antigen challenge: Dose-dependent suppression of total IgM in adult male C57BL/6 mice (oral gavage for 7 days; 0, 5, or 20 mg/kg-day); no consistent effect on IgG levels (Zheng et al., 2011).

Effects on T cell and B cell cellularity or proliferation

PFOS has been shown to reduce the number and proliferation of thymocytes and splenocytes in mice in multiple studies.

- Reduced total number of thymocytes and splenocytes, as well as the ratio of thymocytes expressing both CD4 and CD8 to total thymocytes in male C57BL/6 mice following exposure via diet to PFOS (0.02%) (Qazi et al., 2009b).
- Reduced proliferation of T cells and decreased cellularity in the thymus (by more than 50%) and in the spleen in BALB/c mice following exposure to PFOS via gavage (20 mg/kg-day) (Vetvicka and Vetvickova, 2013).
- Reductions in splenic and thymic cellularity by up to 51% and 61%, respectively, in male C57BL/6 mice treated with 20 and 40 mg/kg-day PFOS; and decreases of 28% and 21%, respectively, in the relative $CD4^+CD8^-$ and $CD4^-CD8^+$ populations in the highest dose group (Zheng et al., 2009).
- Modulation of T cell subpopulations in male B6C3F1 mice, with increased $CD4^-CD8^-$ subpopulation at all doses; minimal alteration of splenic T cell immune phenotypes in females and no alteration of lymphocyte proliferation in either sex following exposure up to 5 mg/kg-day PFOS via gavage (Peden-Adams et al., 2008).
- Decreased splenic B220 cells at 4 weeks (but not 8 weeks) in female (but not male) B6C3F1 pups exposed to 5 mg/kg-day PFOS in utero; however, male pups exposed to this dose in utero had a 25% decrease in $CD3^+$ and a 28% decrease in $CD4^+$ thymocytes at 8 weeks), and a reduction in thymocyte CD4:CD8 ratio to 3.5 (as compared to 4.2 in controls) (Keil et al., 2008).

Additional evidence of PFOS's effects on immune system cellularity is described in the immunotoxicity section of this document, and includes decreased absolute spleen weight (Xing et al., 2016; Zhong et al., 2016) and decreased absolute thymus weight and cellularity (Zhong et al., 2016) in C57BL/6 mice, decreased relative thymus weight in Sprague Dawley rats (NTP, 2019b), and decreased white blood cells, neutrophils, and eosinophils in male Sprague Dawley rats (NTP, 2019b).

Effects on NK cells

NK cell activity was assessed in one human in vitro study and in four animal studies. The human study and three animal studies reported decreases in NK cell activity following exposure to PFOS. The fourth animal study reported an increase in NK cell activity in male but not female mice.

- Decreased NK cell activity in human peripheral blood mononuclear cells cultured for 24 hours in the presence of PFOS (Brieger et al., 2011).
- Suppressed NK cell activity in 8-week-old male and female B6C3F1 pups following maternal oral exposure to PFOS at the two highest doses (1 and 5 mg/kg-day) (Keil et al., 2008).

- Decreased NK cell activity in adult male C57BL/6 mice at the two highest doses (20 and 40 mg/kg-day) (Zheng et al., 2009).
- Decreased splenic NK cell activity in C57BL/6 mice of both sexes exposed in utero *(*1.0 and 5.0 mg/kg-day maternal dose) (Zhong et al., 2016).
- A 2–2.5-fold increase in splenic NK activity in male (but not female) B6C3F1 mice treated via gavage (at 0.5, 1, and 5 mg/kg total administered dose); no change in lymphocyte proliferation in either sex (Peden-Adams et al., 2008).

Summary of evidence

Several animal studies have shown that PFOA suppresses TDAR/TIAR IgM production, reduces cellularity and proliferation of T cells and B cells, and reduces the number of neutrophils. Similarly, several animal studies have shown that PFOS suppresses TDAR/TIAR IgM production and reduces cellularity of T cells and B cells. PFOS also suppresses NK cell activity, including one study in cultured human blood cells and three studies in mice, although one other mouse study reported an increase in NK cell activity. The preponderance of evidence shows that PFOA and PFOS can suppress the immune system in ways that would allow neoplastic cells to escape immune surveillance, survive, and replicate to form tumors.

KC8: Modulates receptor-mediated effects

Chemicals may modulate receptor-mediated effects in a variety of ways, including binding to and either activating or inactivating a receptor, altering receptor levels or function, altering levels of endogenous ligands that are available to bind to the receptor, or otherwise altering receptor-mediated gene expression or intracellular signaling. Many cellular receptors regulate critical cellular pathways, such as those involved in differentiation and proliferation, the disruption of which can contribute to carcinogenic processes. For example, activation of certain growth factor receptors, or estrogen, androgen, or progesterone receptors can lead to the development of various types of cancer in humans and animals. As another example, activation of the aryl hydrocarbon receptor (AhR) is involved in the development of a number of different types of cancers in humans and animals. Activation of other nuclear receptors, including peroxisome proliferator activated receptor alpha (PPARα) and the constitutive androstane receptor (CAR), also has been associated with carcinogenesis (Smith et al., 2020).

PFOA

Estrogen receptor

One study compared the gene expression profiles in liver of wild-type mice treated with 3 mg/kg PFOA by gavage for seven days to those of wild-type or ERα -null mice treated with a known ERα agonist. The gene expression profile in the liver of PFOA-treated wild-type mice was similar to that of wild-type mice treated with a known ERα agonist, and different from the gene expression profile in the liver of ERα-null mice treated with the known ERα agonist, indicating that PFOA's effects on gene expression in the liver are mediated through ERα (Rosen et al., 2017).

One study reported that PFOA increased ERα reporter activity in human embryonic kidney (HEK293T) cells transfected with a human ERα reporter gene and two studies reported that PFOA induced estrogenic effects in human breast cancer (MCF-7) cells (Maras et al., 2006;

Benninghoff et al., 2011; Henry and Fair, 2013). PFOA induced dose-dependent activation of human ERα reporter gene transcription at concentrations ranging from 1-1,000 nM in HEK293T cells, with a 2- to 2.5-fold induction above control levels at concentrations of 100 and 1,000 nM (Benninghoff et al., 2011). Analyses using an in silico computational model indicate that PFOA can efficiently dock with human ERα in the ligand-binding domain and form a hydrogen bond at residue Arg394 in a manner similar to that of estrogens (Benninghoff et al., 2011). Henry and Fair (2013) reported that PFOA induced significantly positive estrogenic responses in human MCF-7 cells (E-SCREEN) at all concentrations tested (0.01 to 30 µg/ml, or 0.024 to 72.5 µM), and anti-estrogenic activity when cells were co-exposed to PFOA and estradiol (E2). E-SCREEN is an assay designed to use the estrogen sensitivity of phosphate-buffered saline pre-incubated MCF-7 cells to determine effects of exogenous agents on estrogen receptor dependent-cell proliferation. Also using E-SCREEN, Maras et al. (2006) reported that while PFOA did not induce proliferation in MCF-7 cells at a concentration of 50 µM, it induced a small but significant up-regulation in gene expression of two estrogen-responsive genes, trefoil peptide gene (*TFF1*) and estrogen receptor α gene (*ESR1*), and a small but significant downregulation of another estrogen-responsive gene, erb-b2 receptor tyrosine kinase 2 (*ERBB2*).

ER-mediated effects of PFOA have been observed in studies in rainbow trout in vivo and in vitro (Benninghoff et al., 2011). Specifically, a significantly dose-dependent increase in the level of the estrogen-responsive biomarker protein, vitellogenin (Vtg) in plasma was observed in fish administered diets containing 0, 5, 50, or 250 ppm of PFOA for 2 weeks (5 days/week). PFOA competitively binds to trout liver ER, albeit weakly, with a half-maximal inhibitory concentration (IC_{50}) value of 1.82 mM in liver cytosol homogenates. In toxicogenomic studies in rainbow trout, the hepatic gene expression profile of fish exposed to PFOA (2,000 ppm) for two weeks in the diet was similar overall to that of fish exposed to E2 for two weeks (Benninghoff et al., 2012).

In addition, a liver tumor promotion study in rainbow trout suggests that PFOA may cause effects that are mediated through the ER, since PFOA and E2 had similar tumor promoting activity in fish exposed to the liver tumor initiator aflatoxin B1 (Benninghoff et al., 2012).

PPARα

Several rodent studies have shown that PFOA alters gene expression in the liver through PPARα mediated effects (Rosen et al., 2008a; Rosen et al., 2008b; Elcombe et al., 2010; Rosen et al., 2017; NTP, 2019a). The gene expression profiles of livers from wild-type or PPARα-null mice exposed to PFOA (1 or 3 mg/kg-day) by gavage for seven days were compared to those exposed to 50 mg/kg-day Wy-14,643, a PPARα agonist (Rosen et al., 2008a; Rosen et al., 2008b; Rosen et al., 2017). The data showed that the expression of most genes was altered by PFOA through PPARα mediated pathways in wild-type mice. However, in PPARα-null mice, the expression of a subset of genes appeared to be altered through PPARα-independent pathways. In two other studies, male Sprague Dawley rats exposed to PFOA (up to 5 mg/kg-day) by gavage (NTP, 2019a) or 300 ppm in the diet (Elcombe et al., 2010) for 28 days exhibited significant increases in the expression of *Acox1* and *Cyp4a1 (NTP, 2019a)* or just *Cyp4a1* (Elcombe et al., 2010), which are markers of PPARα activity.

Bjork et al. (2011) showed that PFOA at a concentration of 25 µM can induce a number of PPARα-related genes in primary rat hepatocytes and to a lesser extent in primary human hepatocytes. In human HepG2 cells, PFOA at concentrations of 30 and 100 µM induced a significant increase in activity in a PPARα reporter gene assay (Rosenmai et al., 2018).

Additionally, Corsini et al. (2012) reported that PFOA at a concentration of 25 μM significantly increased PPARα-driven transcription in a human promyelocytic cell line (THP-1 cells) transiently transfected with a luciferase reporter plasmid construct containing the ligand binding domain of PPARα.

Other nuclear receptors, i.e., PPARγ, PXR, CAR, and PPARβ/δ

Besides effects on PPARα, several studies show that PFOA can modulate other receptors such as PPARγ, pregnane X receptor (PXR), CAR, and possibly PPARβ/δ, by measuring increased expression of target gene and/or protein levels (cytochrome P450 isoforms) from translation of downstream regulated genes in rodents (Takacs and Abbott, 2007; Rosen et al., 2008a; Rosen et al., 2008b; Elcombe et al., 2010; Rosen et al., 2017; NTP, 2019a). In both PPARα-null mice and wild-type mice treated with PFOA, microarray data revealed increased gene expression of PPARγ and CAR (as well as ERα) transcriptional targets, as compared to mice that received no treatment (Rosen et al., 2017). In other studies in PPARα-null mice, PFOA increased expression of genes regulated by either PPARγ, PXR, or CAR (Rosen et al., 2008a; Rosen et al., 2008b). Additionally, male Sprague Dawley rats exposed to PFOA by gavage (NTP, 2019a) or 300 ppm in diet (Elcombe et al., 2010) for 28 days exhibited significant increases in the expression of *Cyp2b1*, and *Cyp2b2*, which are markers of CAR activity (NTP, 2019a) or significantly increased expression of *Cyp3a1*, which is the marker of PXR activity (Elcombe et al., 2010). Finally, in studies conducted in Cos-1 cells (a monkey kidney fibroblast-like cell line) transfected with reporter genes, PFOA transactivated mouse PPARβ/δ, but not human PPARβ/δ or mouse or human PPARγ (Takacs and Abbott, 2007).

Modulation of endogenous hormones and other receptor ligands

Estradiol and progesterone in females

Exposure of female rodents to PFOA during critical periods of development has been observed to alter serum levels of either estradiol or progesterone. In one study in female Sprague Dawley rats, neonatal PFOA exposure (0.1 and 1 mg/kg-day via subcutaneous injection during PND 1-5) increased serum estradiol levels, while exposure during PND 26-30 had no effect (Du et al., 2019). In a study in C57BL/6 mice, prenatal exposure to PFOA (0.01 to 1.0 mg/kg-day between GD 1-17) increased serum estradiol in female offspring (assessed on PND 61), but had no effect on serum progesterone (Tucker et al., 2015). In another study in female C57BL/6 mice, peripubertal (3-7 weeks of age) exposure to PFOA (5 mg/kg-day for 4 weeks via gavage) significantly increased serum progesterone levels about three-fold, but had no effect on serum estradiol levels (Zhao et al., 2010).

In female mice, peripubertal (3-7 weeks of age) exposure to PFOA has been found to alter development of reproductive tissues, including the mammary gland (e.g., the growth of terminal end buds (TEBs) and terminal ducts (TDs)) and uterus (e.g., relative uterine weight) (Yang et al., 2009; Zhao et al., 2010; White et al., 2011). In general, TEBs are sensitive to chemical carcinogen treatment and persistent proliferation of TEBs has been positively associated with the development of mammary tumors in rodent bioassays. The peripubertal period, when TEBs are proliferating, may be an important window of mammary gland susceptibility to environmental exposures that may affect breast cancer risk later in life (Russo and Russo, 1996; Fenton, 2006). In one study, Yang et al. (2009) treated 21-day-old female C57BL/6 and Balb/c mice with PFOA by oral gavage at 0, 1, 5, or 10 mg/kg-day, 5 days per week for 4 weeks, and examined developmental effects in the mammary gland and uterus. Yang et al. (2009) reported

that exposure to PFOA induced stimulatory effects on the development of the mammary gland (measured as increased number of TEBs and stimulated TDs) and the uterus at the lower dose (5 mg/kg) and inhibitory effects at the higher dose (10 mg/kg) in female C57BL/6 mice. However, in female Balb/c mice PFOA exposure inhibited development of both organs at the two higher doses (5 and 10 mg/kg). The basis for the strain-specific differences in these organs' responses to PFOA is unknown.

Zhao et al. (2010) compared the stimulatory effects of peripubertal exposure to PFOA (5 mg/kg-day, 5 days/week for 4 weeks) via gavage on the development of the mammary gland in female C57BL/6 wild-type and PPARα-null mice. Besides effects on TEBs and TDs, Zhao et al. (2010) also reported significant increases in the levels of the steroid hormone synthetic enzymes in the ovaries, serum progesterone, and growth factors in the mammary glands of PFOA treated C57BL/6 wild-type mice. A subset of these effects were also observed in PFOA treated PPARα-null mice. These effects of PFOA were not seen in ovariectomized mice. Therefore, the authors hypothesized the underlying mechanism by which PFOA stimulates mammary gland development might be through indirect ER-mediated effects by promoting steroid hormone production in ovaries and increasing the levels of growth factors.

Estradiol and testosterone in males

In one experiment, Biegel et al. (1995) treated adult male CD rats with PFOA (ammonium salt) by oral gavage at 0 or 25 mg/kg-day for 14 days, and observed a statistically significant 4.5-fold increase in hepatic aromatase activity in treated rats, compared to pair-fed controls. Aromatase converts testosterone to estradiol. In another experiment, the same treatment of adult male CD rats with PFOA (ammonium salt) resulted in an increase in serum and testicular interstitial fluid estradiol levels and an increase in transforming growth factor α (TGF α) in testicular interstitial fluid, all compared to pair-fed controls (Biegel et al., 1995). Increased levels of serum estradiol were also observed in male CD rats fed 300 ppm PFOA (ammonium salt) in the diet during the first 12 months of a two-year study; increases in Leydig cell hyperplasia and adenoma were also observed in the PFOA treatment group, compared to pair fed and ad libitum controls, respectively (Biegel et al., 2001). An earlier study of adult male CD rats employing a broader range of PFOA (ammonium salt) doses (0, 1, 10, 25, or 50 mg/kg-day for 14 days) resulted in significant increases in serum estradiol levels in the 10, 25 and 50 mg/kg-day dose groups, compared to either pair-fed or ad libitum controls (Cook et al., 1992). A decrease in serum testosterone, which was significant by trend (using ad libitum, but not pair-fed controls), was also observed by Cook et al. (1992). PFOA-treated male CD rats had a significantly reduced response (i.e., an increase in serum testosterone) to a human chorionic gonadotropin (hCG) challenge as compared to untreated controls (Cook et al., 1992). These investigators proposed that PFOA's induction of hepatic aromatase activity increases estradiol levels in the serum and in the testicular interstitial fluid, which can lead to altered function and responses of Leydig cells, resulting in effects on testosterone production and testicular TGF α levels, all of which may play a role in the induction of Leydig cell tumors by PFOA (Cook et al., 1992; Biegel et al., 1995).

Thyroid hormones

The effect of PFOA on thyroid hormones has been discussed in detail in Section 5.4 of this document. In the general human population, while several studies report statistically significant findings, OEHHA did not identify consistent associations between PFOA in serum and levels of TSH or thyroid hormones. However, in pregnant women with anti-TPO antibodies, an association between PFOA and TSH has been observed (Webster et al., 2014). In animals

exposed to PFOA, changes in levels of thyroid hormones have been observed in rodents (Martin et al., 2007; NTP, 2019a) and in non-human primates (Butenhoff et al., 2002). Overall, there are consistent trends showing PFOA can decrease thyroid hormone levels in animals, although the study numbers are limited.

PFOS

Estrogen receptor

One study compared the gene expression profiles in liver of wild-type mice treated with 10 mg/kg PFOS by gavage for seven days to those of wild-type or ERα-null mice treated with a known ERα agonist. The gene expression profile in the liver of PFOS-treated wild-type mice was similar to that of wild-type mice treated with a known ERα agonist, and different from the gene expression profile in the liver of ERα-null mice treated with the known ERα agonist, indicating that the effects of PFOS on gene expression in the liver are mediated through ERα (Rosen et al., 2017).

One study reported that PFOS increased ERα reporter activity in human embryonic kidney (HEK293T) cells transfected with a human ERα reporter gene and multiple studies reported that PFOS induced estrogenic effects in human breast cell lines (Maras et al., 2006; Benninghoff et al., 2011; Henry and Fair, 2013; Pierozan and Karlsson, 2018). PFOS induced dose-dependent activation of human ERα reporter gene transcription at concentrations ranging from 1-1,000 nM in HEK293T cells, with a greater than 2-fold induction above control levels at concentrations of 100 and 1,000 nM (Benninghoff et al., 2011). Analyses using an in silico computational model indicate that PFOS can efficiently dock with human ERα in the ligand-binding domain and form a hydrogen bond at residue Arg394 in a manner similar to that of estrogens (Benninghoff et al., 2011). PFOS increased cell proliferation and cell cycle progression in human breast epithelial MCF-10A cells at concentrations of 1 or 10 µM after 72 hours of treatment, and had no effect on cellular protein levels of either ERα or ERβ (Pierozan and Karlsson, 2018). PFOS-induced cell proliferation was partially blocked by the ER antagonist ICI 182,780, indicating that the effect of PFOS on cell proliferation was partially due to activation of ER (Pierozan and Karlsson, 2018). Henry and Fair (2013) reported that PFOS induced significantly positive estrogenic responses in human MCF-7 cells (E-SCREEN) at 0.01 µg/ml (~0.025 µM) and 30 µg/ml (~75 µM), but not at concentrations in between, and anti-estrogenic activity at all concentrations tested when cells were co-exposed to PFOS and E2. Also using E-SCREEN, Maras et al. (2006) reported that while PFOS did not induce proliferation in MCF-7 cells at a concentration of 50 µM, it induced a small but significant downregulation in gene expression of two estrogen-responsive genes, *TFF1* and *ESR1*.

In studies with rainbow trout, PFOS in the diet at 250 ppm for two weeks (5 days/week) had no effect on plasma levels of Vtg, the estrogen-responsive biomarker protein. However, PFOS competitively binds, albeit weakly (IC_{50} value of 1.82 mM) to trout liver ER in studies with liver cytosol homogenates (Benninghoff et al., 2011). In toxicogenomic studies in rainbow trout, the hepatic gene expression profile of fish exposed to PFOS (200 ppm) for two weeks in the diet was similar overall to that of fish exposed to E2 for two weeks (Benninghoff et al., 2012).

Other studies in rats and rainbow trout suggest that PFOS causes effects that are mediated through the ER. In female Sprague Dawley rats, daily administration of PFOS (at doses of 1 or 10 mg/kg bw by i.p. injection for two weeks) resulted in dose-dependent changes in estrous cyclicity (irregular cycles/persistent diestrus) that were statistically significant at the high dose

(Austin et al., 2003). In liver tumor promotion studies in rainbow trout initiated with either aflatoxin B1 or N-methyl-N'-nitrosoguanidine, PFOS promoted liver tumors in a manner similar to that of E2 (Benninghoff et al., 2012).

Androgen receptor (AR)

PFOS significantly antagonized dihydrotestosterone-induced AR activity in a concentration-dependent manner in a Chinese hamster ovary cell line (CHO-K1) transfected with AR and a reporter vector (Kjeldsen and Bonefeld-Jorgensen, 2013).

PPARα

Several rodent studies have shown that PFOS alters gene expression in the liver through PPARα mediated effects (Elcombe et al., 2012a; Elcombe et al., 2012b; Rosen et al., 2017; NTP, 2019b). The gene expression profiles of livers from wild-type or PPARα-null mice exposed to PFOS (10 mg/kg-day) by gavage for seven days were compared to those exposed to 50 mg/kg-day Wy-14,643, a PPARα agonist (Rosen et al., 2017). The data showed that the expression of most genes was altered by PFOS through PPARα mediated pathways in wild-type mice. However, in PPARα-null mice, the expression of a subset of genes appeared to be altered through PPARα-independent pathways. In another study, male Sprague Dawley rats exposed to PFOS (up to 5 mg/kg-day) by gavage for 28 days exhibited significant increases in the expression of *Acox1* and *Cyp4a1*, which are markers of PPARα activity (NTP, 2019b). In a study of male Sprague Dawley rats exposed to PFOS in the diet at concentrations of 0, 20 or 100 ppm for 1, 7 or 28 days, significant increases in liver palmitoyl CoA oxidase (ACOX) activity, a marker of PPARα activation, were observed in animals exposed to 20 ppm for 28 days and in animals exposed to 100 ppm for 1 or 28 days. Increases in hepatic lauric acid 12-hydroxylation activity (a marker for CYP4A, another indicator of PPARα activation) were observed in animals exposed to 20 or 100 ppm for 7 days and increases in hepatic CYP4A1 protein levels were observed in both dose groups at each time point (Elcombe et al., 2012a). In another study, male Sprague Dawley rats were exposed to PFOS in the diet at concentrations of 0, 20 or 100 ppm for 7 days, and then fed control diet for up to 84 days. Increases in ACOX and lauric acid 12-hydroxylase activities were observed in the 100 ppm PFOS dose group through recovery day 56 (no increase at recovery day 84) (Elcombe et al., 2012b). No increases were seen in the 20 ppm dose group at any recovery time point (Elcombe et al., 2012b). Increased expression of PPARα was observed in the liver of male adult Sprague Dawley rats treated with 100 mg/kg PFOS by i.p. injection, while no increase was seen in the liver of neonatal rats exposed to PFOS in utero (dams were treated at 3 mg/kg-day via gavage on GD 2-20) (Bjork et al., 2008).

In human HepG2 cells transfected with a reporter gene, PFOS at concentrations up to 100 µM did not induce PPARα activity (Rosenmai et al., 2018). However, cellular uptake of PFOS by the HepG2 cells was less than 0.05% (approximately 6-fold lower than the cellular uptake of PFOA in the same study), thus the lack of PPARα activity observed with PFOS may be due to the limited uptake by the cells.

A difference regarding the effect of PFOS on the expression of PPARα-dependent genes was observed between human and rat liver cells (Bjork and Wallace, 2009). PFOS at a concentration of 25 µM did not induce increased expression of PPARα-dependent genes in human primary hepatocytes or human hepatoma HepG2/C3A cells, whereas rat hepatocytes did respond at this concentration (Bjork and Wallace, 2009).

Other nuclear receptors, i.e., PPARγ, PXR, CAR, and PPARβ/δ

Besides effects on PPARα, several studies show that PFOS can modulate other receptors such as PPARγ, PXR, CAR, and possibly PPARβ/δ, by measuring receptor activation-induced luciferase reporter activity, and increased expression of target gene and/or protein levels (cytochrome P450 isoforms) from translation of downstream regulated genes in rodents (Takacs and Abbott, 2007; Elcombe et al., 2012a; Elcombe et al., 2012b; Rosen et al., 2017; NTP, 2019b). As was seen with PFOA, mice treated with PFOS had increased gene expression of PPARγ, CAR, and ERα transcriptional targets (Rosen et al., 2017).

Male and female Sprague Dawley rats exposed to PFOS by gavage for 28 days exhibited significant increases in expression of *Cyp2b1* and *Cyp2b2* compared to controls, an indication of increased CAR activity (NTP, 2019b). In a study of male Sprague Dawley rats exposed to PFOS in the diet at concentrations of 0, 20 or 100 ppm for 1, 7 or 28 days, increases in liver activity and protein levels of CYP2B and CYP3A, target cytochrome P450 isoforms of CAR and PXR activation, respectively, were observed (Elcombe et al., 2012a). In another study, male Sprague Dawley rats were exposed to PFOS in the diet at concentrations of 0, 20 or 100 ppm for 7 days, and then fed control diet for up to an additional 84 days. Increases in liver activity and protein levels of CYP3A, a marker of PXR activation, persisted throughout the additional 84-day period on control diet in the 100 ppm dose group, whereas liver activity and protein levels of CYP2B, a marker of CAR activation, remained elevated after 1 and 28 days on control diet, were equivalent to levels in untreated animals after 56 days, and were decreased compared to untreated animals after 84 days (Elcombe et al., 2012b). In studies conducted in Cos-1 cells (a monkey kidney fibroblast-like cell line) transfected with plasmids containing various human or murine PPAR genes and a luciferase reporter, PFOS transactivated mouse PPARβ/δ, but not human PPARβ/δ or mouse or human PPARγ (Takacs and Abbott, 2007).

Modulation of endogenous ligands and hormones

Estradiol

The effect of PFOS neonatal or post-weaning exposure (0.1, 1, and 10 mg/kg-day via subcutaneous injection for five days) on serum estradiol levels was investigated in female Sprague Dawley rats (Du et al., 2019). In rats exposed on PND 1-5, PFOS at 0.1, 1 and 10 mg/kg significantly increased serum estradiol, with the estradiol level being the highest at the lowest dose of PFOS. In rats exposed on PND 26-30, only the low and medium doses of PFOS significantly increased serum estradiol levels.

Thyroid Hormones

The effect of PFOS on thyroid hormones has been discussed in detail in Section 5.4 of this document. In the general human population, OEHHA did not find consistent trends across the different studies reviewed by US EPA (2016d) and studies published after this review. In pregnant women, US EPA (2016d) identified three studies that reported positive associations between PFOS and TSH. However, the four studies published since US EPA's review (US EPA, 2016d) (or otherwise not included in that review) reported essentially opposite findings. In animals, the overall body of evidence from the literature, including studies summarized by (US EPA, 2016d) and recent studies newly identified and reviewed in Section 5.4 of this document, suggests that PFOS decreases thyroid hormone levels. Recent mechanistic studies suggest that PFOS may interact with thyroid hormone transporters and receptors in animals, which is

similar to results reported in mechanistic studies with human thyroid hormone transporters and receptors (US EPA, 2016d).

Summary of evidence

Several animal studies have shown that PFOA alters gene expression in the liver, and that these effects are mediated through ERα, PPARα, PPARγ, PXR, and CAR. Evidence that PFOA can bind to or activate ERα, PPARα, and possibly PPARβ/δ comes from in silico modeling studies (human ERα), in vitro studies in human cells or cell lines (ERα, PPARα), and in vitro studies in animal tissue preparations or cell lines (ERα, PPARβ/δ). There is also evidence from studies in animals that PFOA can modulate levels of endogenous hormones, including estradiol, progesterone, testosterone and thyroid hormones, and possibly levels of growth factors in the testis and mammary gland.

Several animal studies have shown that PFOS alters the expression of genes that are regulated by ERα, PPARα, PPARγ, PXR, and CAR, and one reporter gene study shows PFOS activates murine PPARβ/δ in vitro. The evidence for the estrogenic effect of PFOS also comes from increased ER reporter activity in human cell lines, increased proliferation of estrogen-responsive human breast cancer cell lines in several studies, weak binding to ER in fish, and similar gene expression patterns between PFOS and E2 in fish. One reporter gene study indicates PFOS inhibited AR activation by DHT. There is also evidence from animal studies that PFOS can decrease thyroid hormone levels and increase estradiol levels.

KC10: Alters cell proliferation, cell death or nutrient supply

Examples of the types of effects indicative of KC10 have been described by Smith et al. (2016), and include increased cell proliferation, decreased apoptosis, changes in growth factors, energetics and signaling pathways related to cellular replication or cell cycle control, and increased angiogenesis.

This section summarizes data on the effects of PFOA and PFOS on cell proliferation, growth factors, and signaling pathways related to cellular replication and cell cycle control.

PFOA

In Sprague Dawley rats treated with PFOA via gavage for 28 days, hyperplasia of the olfactory and respiratory epithelium were observed (NTP, 2019a). The study authors noted that the pattern of nasal pathology in this study was not suggestive of gavage-related reflux and should be considered treatment-related. Another animal study showed that peripubertal 28-day gavage exposure of female C57BL/6 wild-type and PPARα knockout mice to PFOA (5 mg/kg-day, 5 days/week) stimulated mammary gland development, as evidenced by increased numbers of mammary gland TEBs and duct ends (Zhao et al., 2010). An earlier study from the same laboratory had seen a similar stimulation of mammary gland development and increase of proliferation index at 5 mg/kg-day PFOA in C57BL/6 mice, but inhibition of mammary gland development at 10 mg/kg-day; in Balb/c mice, inhibition of mammary gland development was observed at 5 or 10 mg/kg-day and decreased proliferation index was observed at 5 mg/kg-day (Yang et al., 2009). It is noteworthy that TEBs are sensitive to chemical carcinogen treatment and persistent proliferation of TEBs has been positively associated with development of mammary tumors in rodent bioassays (Russo and Russo, 1996; Fenton, 2006).

After a 72-hour treatment, PFOA increased cell proliferation in a human breast epithelial cell line (MCF-10A) at concentrations of 50 and 100 µM (Pierozan et al., 2018). Furthermore, 100 µM PFOA increased the level of regulatory cell cycle proteins, such as cyclin D1 and cyclin-dependent kinases in MCF-10A cells. Another two studies using the MCF-7 human breast cancer cell line (Maras et al., 2006; Henry and Fair, 2013) demonstrated that PFOA (1 to 100 µM) increased cell proliferation via an E-SCREEN assay. However, in the presence of estradiol (E2), PFOA significantly decreased cell proliferation in the E-SCREEN assay compared with E2 alone (Henry and Fair, 2013). Additionally, PFOA at concentrations ranging from 0.2 ng/ml to 2 µg/ml increased cell proliferation in two human ovarian granulosa cell tumor cell lines, COV434 and KGN, after 72-hour treatments (Gogola et al., 2019). This effect was abolished with pre-treatment of an insulin-like growth factor 1 receptor (IGF1R) antagonist, indicating a possible role of IGF1R in PFOA-induced cell proliferation. Studies have shown that IGF1 signaling regulates cell proliferation, malignant cell invasion, and metastasis in ovarian cancers (Beauchamp et al., 2010).

PFOS

PFOS (20 or 100 ppm in the diet) increased the liver proliferative index and decreased the liver apoptotic index in Sprague Dawley rats. The effects were observed after only 1 day of exposure at 100 ppm and after 7 or 28 days of exposure at both concentrations (Elcombe et al., 2012a). In a follow-up study, in which Sprague Dawley rats were similarly fed PFOS in the diet for 7 days, a sustained decrease in liver apoptotic index was observed 84 days after cessation of exposure (20 or 100 ppm) (Elcombe et al., 2012b).

In studies with a human fetal hepatic cell line (HL-7702), 50 µM PFOS stimulated cell proliferation and altered expression of 27 proteins associated with cell proliferation, including hepatoma-derived growth factor (HdGF), the proliferation biomarker Ki67, cyclin D1, cyclin E2, cyclin A2, cyclin B1, c-Myc, and p53 (Cui et al., 2015).

In human breast epithelial cells (MCF-10A), PFOS increased cell proliferation and cell-cycle progression at concentrations of 1 or 10 µM after a 72-hour treatment (Pierozan and Karlsson, 2018). The ER blocker, ICI 182, 780, partially blocked PFOS-induced cell proliferation, indicating stimulation of proliferation was at least in part driven by ER activation (Pierozan and Karlsson, 2018). Additionally, PFOS at 0.01 µg/ml (~0.025 µM) and 30 µg/ml (~75 µM) was shown to be estrogenic and to induce cell proliferation in the E-SCREEN assay in estrogen-sensitive MCF-7 cells (Henry and Fair, 2013). Similar to PFOA, in the presence of E2, PFOS significantly decreased cell proliferation in the E-SCREEN assay compared with E2 alone. Also similar to PFOA, PFOS increased cell proliferation in the COV434 and KGN human ovarian granulosa cell tumor cell lines after 72-hour treatment with concentrations as low as 0.08 ng/ml, and this effect was abolished by pretreatment with an IGF1 receptor antagonist (Gogola et al., 2019).

Summary of evidence

Several animal studies provide evidence that PFOA increases cell proliferation, based on respiratory tissue hyperplasia observed in rats, and stimulated mammary gland development in mice. In addition, PFOA increased levels of regulatory cell cycle proteins in a human breast epithelial cell line, and increased cell proliferation in multiple studies of human breast and ovarian cell lines.

Two studies in rats provide evidence that PFOS increases cell proliferation and inhibits apoptosis in the liver, with the latter effect being long-lived. In multiple studies of human fetal liver, breast and ovarian cell lines, PFOS increased cell proliferation. In addition, PFOS altered the expression of proteins linked to cell proliferation, including increasing levels of regulatory cell cycle proteins and growth factors in a human fetal liver cell line, and increased cell proliferation in multiple studies of human breast, ovarian, and fetal liver cell lines.

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APPENDIX 9. TOXCAST DATA

OEHHA has organized the ToxCast HTS data for PFOA and PFOS (accessed on May 3, 2021), including:

- Table A9.1. 81 Active ToxCast assays for PFOA
- Table A9.2. 58 Active ToxCast assays for PFOA ammonium salt
- Table A9.3. 260 Active ToxCast assays for PFOS
- Table A9.4. 179 Active ToxCast assays for PFOS potassium salt
- Table A9.5. 26 Active ToxCast assays for PFOS lithium salt

Table A9.1. 81 Active ToxCast assays[1] for PFOA

Assay Name	Gene Symbol	Organism	Cells/Cell Lines	Intended Target Family	AC50 (μM)
LTEA_HepaRG_CAT_up	CAT	human	HepaRG	catalase	28.2
BSK_KF3CT_ICAM1_down	ICAM1	human	keratinocytes and foreskin fibroblasts	cell adhesion molecules	40.0
BSK_LPS_Eselectin_up	SELE	human	umbilical vein endothelium and peripheral blood mononuclear cells	cell adhesion molecules	40.0
LTEA_HepaRG_CDKN1A_dn	CDKN1A	human	HepaRG	cell cycle	0.890
TOX21_HRE_BLA_Agonist_viability	NA	human	cervis ME-180	cell cycle	23.4
TOX21_RT_HEPG2_GLO_00hr_ctrl_viability	NA	human	HepG2	cell cycle	42.0
TOX21_ERb_BLA_Agonist_viability	NA	human	HEK293T	cell cycle	22.7
TOX21_ERb_BLA_Antagonist_viability	NA	human	HEK293T	cell cycle	32.6
APR_HepG2_MicrotubuleCSK_72h_up	NA	human	HepG2	cell morphology	109
APR_HepG2_MitoMembPot_72h_dn	NA	human	HepG2	cell morphology	116
LTEA_HepaRG_CYP1A1_up	CYP1A1	human	HepaRG	cyp	36.1
LTEA_HepaRG_CYP3A7_up	CYP3A7	human	HepaRG	cyp	33.9
LTEA_HepaRG_CYP4A11_up	CYP4A11	human	HepaRG	cyp	12.9
LTEA_HepaRG_CYP4A22_up	CYP4A22	human	HepaRG	cyp	5.58
LTEA_HepaRG_CYP2B6_up	CYP2B6	human	HepaRG	cyp	23.8
CLD_CYP2B6_24hr	CYP2B6	human	primary hepatocyte	cyp	0.499
LTEA_HepaRG_CYP2C8_up	CYP2C8	human	HepaRG	cyp	10.8
LTEA_HepaRG_CYP2C19_up	CYP2C19	human	HepaRG	cyp	22.7
LTEA_HepaRG_CYP2E1_up	CYP2E1	human	HepaRG	cyp	28.5
NVS_ADME_rCYP2A2	Cyp2a2	rat	NA	cyp	1.52
NVS_ADME_rCYP2C11_Activator	Cyp2c11	rat	NA	cyp	0.903

Assay Name	Gene Symbol	Organism	Cells/Cell Lines	Intended Target Family	AC50 (μM)
BSK_3C_uPAR_down	PLAUR	human	umbilical vein endothelium	cytokine	10.0
BSK_LPS_TNFa_down	TNF	human	umbilical vein endothelium and peripheral blood mononuclear cells	cytokine	10.0
LTEA_HepaRG_IL6R_up	IL6R	human	HepaRG	cytokine receptor	168
LTEA_HepaRG_DDIT3_up	DDIT3	human	HepaRG	dna binding	44.7
ATG_AP_1_CIS_up	FOS	human	HepG2	dna binding	81.8
LTEA_HepaRG_FOXO3_up	FOXO3	human	HepaRG	dna binding	71.3
ATG_NRF2_ARE_CIS_up	NFE2L2	human	HepG2	dna binding	47.9
TOX21_ARE_BLA_agonist_ratio	NFE2L2	human	HepG2	dna binding	29.1
LTEA_HepaRG_NFE2L2_up	NFE2L2	human	HepaRG	dna binding	42.0
TOX21_RXR_BLA_Agonist_ratio	RXRA	human	HEK293T	dna binding	17.3
ATG_Sp1_CIS_up	SP1	human	HepG2	dna binding	32.3
LTEA_HepaRG_XBP1_up	XBP1	human	HepaRG	dna binding	29.7
NVS_GPCR_hAdoRA2a	ADORA2A	human	NA	gpcr	16.0
NVS_GPCR_hLTB4_BLT1	LTB4R	human	NA	gpcr	32.9
NVS_GPCR_gLTB4	Ltb4r	guinea pig	NA	gpcr	16.5
LTEA_HepaRG_TGFA_up	TGFA	human	HepaRG	growth factor	82.9
LTEA_HepaRG_THRSP_dn	THRSP	human	HepaRG	growth factor	28.0
NVS_ENZ_hPI3Ka	PIK3CA	human	NA	kinase	14.6
LTEA_HepaRG_PDK4_up	PDK4	human	HepaRG	kinase	24.8
NVS_ENZ_hTie2	TEK	human	NA	kinase	18.2
LTEA_HepaRG_ACLY_up	ACLY	human	HepaRG	lyase	32.8
LTEA_HepaRG_FASN_up	FASN	human	HepaRG	lyase	26.1
LTEA_HepaRG_HMGCS2_up	HMGCS2	human	HepaRG	lyase	5.91
CLD_HMGCS2_48hr	HMGCS2	human	primary hepatocyte	lyase	35.0
LTEA_HepaRG_GADD45B_up	GADD45B	human	HepaRG	mutagenicity response	39.8
LTEA_HepaRG_GADD45G_up	GADD45G	human	HepaRG	mutagenicity response	229
ATG_ERE_CIS_up	ESR1	human	HepG2	nuclear receptor	33.8
ATG_ERa_TRANS_up	ESR1	human	HepG2	nuclear receptor	47.2
TOX21_ERa_BLA_Antagonist_ratio	ESR1	human	HEK293T	nuclear receptor	51.9
TOX21_ERb_BLA_Antagonist_ratio	ESR2	human	HEK293T	nuclear receptor	39.3
TOX21_ERR_Antagonist	ESRRA	human	ERR-HEK293T	nuclear receptor	58.0

Assay Name	Gene Symbol	Organism	Cells/Cell Lines	Intended Target Family	AC50 (μM)
ATG_PXRE_CIS_up	NR1I2	human	HepG2	nuclear receptor	35.3
NVS_NR_hCAR_Antagonist	NR1I3	human	NA	nuclear receptor	18.7
ATG_DR4_LXR_CIS_dn	NR1H3	human	HepG2	nuclear receptor	30.1
ATG_PPRE_CIS_up	PPARA	human	HepG2	nuclear receptor	30.6
ATG_PPARa_TRANS_up	PPARA	human	HepG2	nuclear receptor	21.8
TOX21_PPARd_BLA_agonist_ratio	PPARD	human	HepG2	nuclear receptor	253
TOX21_PPARd_BLA_antagonist_ratio	PPARD	human	HepG2	nuclear receptor	46.7
ATG_PPARg_TRANS_up	PPARG	human	HepG2	nuclear receptor	49.6
NVS_NR_hPPARg	PPARG	human	NA	nuclear receptor	23.3
TOX21_PPARg_BLA_antagonist_ratio	PPARG	human	HepG2	nuclear receptor	5.51
ATG_RORE_CIS_dn	RORA	human	HepG2	nuclear receptor	0.557
ATG_THRa1_TRANS_dn	THRA	human	HepG2	nuclear receptor	22.3
LTEA_HepaRG_ACOX1_up	ACOX1	human	HepaRG	oxidase	15.2
LTEA_HepaRG_FMO3_up	FMO3	human	HepaRG	oxidoreductase	3.24
LTEA_HepaRG_SDHB_up	SDHB	human	HepaRG	oxidoreductase	30.1
NVS_ENZ_hACP1	ACP1	human	NA	phosphatase	0.217
LTEA_HepaRG_ALPP_dn	ALPP	human	HepaRG	phosphatase	116
NVS_ENZ_hPPP1CA	PPP1CA	human	NA	phosphatase	0.474
NVS_ENZ_hPPP2CA	PPP2CA	human	NA	phosphatase	21.4
NVS_ENZ_hBACE	BACE1	human	NA	protease	3.90
BSK_BE3C_MMP1_up	MMP1	human	bronchial epithelial cells	protease	40.0
CLD_GSTA2_48hr	GSTA2	human	primary hepatocyte	transferase	0.266
LTEA_HepaRG_SULT2A1_up	SULT2A1	human	HepaRG	transferase	23.8
LTEA_HepaRG_UGT1A1_up	UGT1A1	human	HepaRG	transferase	2.93
LTEA_HepaRG_ABCC2_up	ABCC2	human	HepaRG	transporter	71.5
LTEA_HepaRG_ABCC3_up	ABCC3	human	HepaRG	transporter	68.6
LTEA_HepaRG_FABP1_up	FABP1	human	HepaRG	transporter	17.2

[1] Assays are alphabetically ordered by “intended target family”, and within each “intended target family” assays are ordered alphabetically by “gene symbol”. This table includes assays with curve-fitting “flags”, although the flags are not shown here. The table does not include assays classified by the US EPA Comptox Chemicals Dashboard as ‘background measurement’ assays (e.g., artifact fluorescence, baseline controls, and internal markers).
AC50: the concentration that induces a half-maximal assay response.

NA, not applicable. This notation is used when no specific target genes are reported by the US EPA Comptox Chemicals Dashboard, and used for cell-free assays such as cell-free systems utilizing enzymes or receptors extracted from tissues or cells of various organisms.

Table A9.2. 58 Active ToxCast assays[1] for PFOA ammonium salt

Assay Name	Gene Symbol	Organism	Cells/Cell Lines	Intended Target Family	AC50 (μM)
LTEA_HepaRG_CAT_up	CAT	human	HepaRG	catalase	9.11
LTEA_HepaRG_BCL2L11_up	BCL2L11	human	HepaRG	cell cycle	5.20
LTEA_HepaRG_CCND1_up	CCND1	human	HepaRG	cell cycle	44.6
ACEA_AR_agonist_AUC_viability	NA	human	22Rv1 prostate cell line	cell cycle	54.4
ACEA_AR_antagonist_AUC_viability	NA	human	22Rv1 prostate cell line	cell cycle	33.7
TOX21_HRE_BLA_Agonist_viability	NA	human	cervis ME-180	cell cycle	48.3
TOX21_p53_BLA_p4_viability	NA	human	HCT116 intestinal cell line	cell cycle	42.4
TOX21_ERb_BLA_Agonist_viability	NA	human	HEK293T	cell cycle	27.0
TOX21_ERb_BLA_Antagonist_viability	NA	human	HEK293T	cell cycle	35.6
APR_HepG2_MitoMembPot_72h_dn	NA	human	HepG2	cell morphology	111
LTEA_HepaRG_CYP1A1_up	CYP1A1	human	HepaRG	cyp	32.6
LTEA_HepaRG_CYP3A4_up	CYP3A4	human	HepaRG	cyp	14.3
LTEA_HepaRG_CYP3A7_up	CYP3A7	human	HepaRG	cyp	17.3
LTEA_HepaRG_CYP4A11_up	CYP4A11	human	HepaRG	cyp	9.87
LTEA_HepaRG_CYP4A22_up	CYP4A22	human	HepaRG	cyp	9.54
LTEA_HepaRG_CYP2B6_up	CYP2B6	human	HepaRG	cyp	5.64
LTEA_HepaRG_CYP2C19_up	CYP2C19	human	HepaRG	cyp	6.44
NVS_ADME_hCYP2C9	CYP2C9	human	NA	cyp	0.202
BSK_LPS_IL8_up	CXCL8	human	Umbilical vein endothelium and peripheral blood mononuclear cells	cytokine	40.0
BSK_BE3C_IP10_down	CXCL10	human	Bronchial epithelial cells	cytokine	40.0
LTEA_HepaRG_IL6_dn	IL6	human	HepaRG	cytokine	21.7
LTEA_HepaRG_DDIT3_up	DDIT3	human	HepaRG	dna binding	86.3
LTEA_HepaRG_EGR1_dn	EGFR1	human	HepaRG	dna binding	19.4
LTEA_HepaRG_FOXO1_up	FOXO1	human	HepaRG	dna binding	48.0
LTEA_HepaRG_HSPA1A_up	HSPA1A	human	HepaRG	dna binding	34.7
LTEA_HepaRG_NFE2L2_up	NFE2L2	human	HepaRG	dna binding	72.6
ATG_NRF2_ARE_CIS_up	NFE2L2	human	HepG2	dna binding	57.2
TOX21_ARE_BLA_agonist_ratio	NFE2L2	human	HepG2	dna binding	43.9

Assay Name	Gene Symbol	Organism	Cells/Cell Lines	Intended Target Family	AC50 (μM)
LTEA_HepaRG_TP53_up	TP53	human	HepaRG	dna binding	40.4
LTEA_HepaRG_LPL_up	LPL	human	HepaRG	esterase	58.1
LTEA_HepaRG_KRT19_up	KRT19	human	HepaRG	filaments	32.9
NVS_GPCR_hTXA2	TBXA2R	human	NA	gpcr	11.2
NVS_GPCR_gLTB4	Ltb4r	guinea pig	NA	gpcr	21.9
LTEA_HepaRG_TGFA_up	TGFA	human	HepaRG	growth factor	18.7
LTEA_HepaRG_TGFB1_up	TGFB1	human	HepaRG	growth factor	26.7
LTEA_HepaRG_THRSP_dn	THRSP	human	HepaRG	growth factor	33.7
LTEA_HepaRG_PDK4_up	PDK4	human	HepaRG	kinase	22.4
LTEA_HepaRG_HMGCS2_up	HMGCS2	human	HepaRG	lyase	10.2
TOX21_AR_BLA_Antagonist_ratio	AR	human	HEK293T	nuclear receptor	50.3
ACEA_AR_antagonist_80hr	AR	human	22Rv1 prostate cell line	nuclear receptor	45.0
NVS_NR_rAR	Ar	rat	NA	nuclear receptor	19.3
ATG_ERE_CIS_up	ESR1	human	HepG2	nuclear receptor	22.7
ATG_ERa_TRANS_up	ESR1	human	HepG2	nuclear receptor	44.7
TOX21_ERa_BLA_Antagonist_ratio	ESR1	human	HEK293T	nuclear receptor	46.9
TOX21_ERb_BLA_Antagonist_ratio	ESR2	human	HEK293T	nuclear receptor	41.5
ATG_PXRE_CIS_up	NR1I2	human	HepG2	nuclear receptor	21.1
ATG_PPARa_TRANS_up	PPARA	human	HepG2	nuclear receptor	6.73
ATG_PPARg_TRANS_up	PPARG	human	HepG2	nuclear receptor	21.8
NVS_NR_hPPARg	PPARG	human	NA	nuclear receptor	26.4
TOX21_PPARd_BLA_antagonist_ratio	PPARD	human	HepG2	nuclear receptor	53.7
ATG_RARa_TRANS_dn	RARA	human	HepG2	nuclear receptor	128
ATG_RXRb_TRANS_up	RXRB	human	HepG2	nuclear receptor	37.7
TOX21_TR_LUC_GH3_Antagonist	Thrb	rat	Pituitary gland cell line	nuclear receptor	77.1
LTEA_HepaRG_ACOX1_up	ACOX1	human	HepaRG	oxidase	8.16
NVS_ENZ_hBACE	BACE1	human	NA	protease	1.25
LTEA_HepaRG_UGT1A1_up	UGT1A1	human	HepaRG	transferase	6.90
LTEA_HepaRG_FABP1_up	FABP1	human	HepaRG	transporter	8.77
LTEA_HepaRG_IGFBP1_up	IGFBP1	human	HepaRG	transporter	42.5

[1] Assays are alphabetically ordered by “intended target family”, and within each “intended target family” assays are ordered alphabetically by “gene symbol”. This table includes assays with curve-fitting “flags”, although the flags are not shown here. The table does not include assays classified by the US EPA Comptox Chemicals Dashboard as ‘background measurement’ assays (e.g., artifact fluorescence, baseline controls, and internal markers).
AC50: the concentration that induces a half-maximal assay response.
NA, not applicable. This notation is used when no specific target genes are reported by the US EPA Comptox Chemicals Dashboard, and used for cell-free assays such as cell-free systems utilizing enzymes or receptors extracted from tissues or cells of various organisms.

Table A9.3. 260 Active ToxCast assays[1] for PFOS

Assay Name	Gene Symbol	Organism	Cells/Cell Lines	Intended Target Family	AC50 (μM)
LTEA_HepaRG_CAT_dn	CAT	human	HepaRG	catalase	82.1
BSK_hDFCGF_CollagenIII_down	COL3A1	human	foreskin fibroblast	cell adhesion molecules	40.0
BSK_BE3C_HLADR_down	HLA-DRA	human	bronchial epithelial cells	cell adhesion molecules	10.0
BSK_hDFCGF_VCAM1_down	VCAM1	human	foreskin fibroblast	cell adhesion molecules	40.0
LTEA_HepaRG_BAX_up	BAX	human	HepaRG	cell cycle	33.4
LTEA_HepaRG_BCL2_up	BCL2	human	HepaRG	cell cycle	34.1
LTEA_HepaRG_CCND1_up	CCND1	human	HepaRG	cell cycle	21.3
APR_HepG2_OxidativeStress_24h_up	γH2AX	human	HepG2	cell cycle	108
APR_HepG2_OxidativeStress_72h_up	γH2AX	human	HepG2	cell cycle	7.98
APR_HepG2_MitoticArrest_24h_up	pH3	human	HepG2	cell cycle	109
APR_HepG2_MitoticArrest_72h_up	pH3	human	HepG2	cell cycle	8.10
APR_HepG2_CellLoss_24h_dn	NA	human	HepG2	cell cycle	111
APR_HepG2_CellLoss_72h_dn	NA	human	HepG2	cell cycle	111
BSK_3C_SRB_down	NA	human	umbilical vein endothelium	cell cycle	40.0
BSK_BE3C_SRB_down	NA	human	bronchial epithelial cells	cell cycle	40.0
BSK_hDFCGF_Proliferation_down	NA	human	foreskin fibroblast	cell cycle	10.0
BSK_hDFCGF_SRB_down	NA	human	foreskin fibroblast	cell cycle	40.0
TOX21_TR_LUC_GH3_Antagonist_viability	NA	rat	pituitary gland GH4	cell cycle	120
TOX21_FXR_BLA_antagonist_viability	NA	human	HEK293T	cell cycle	31.4
TOX21_PPARd_BLA_antagonist_viability	NA	human	HEK293T	cell cycle	71.0
TOX21_DT40	NA	chicken	lymphoblast	cell cycle	115
LTEA_HepaRG_LDH_cytotoxicity	NA	human	HepaRG	cell cycle	80.3
TOX21_ARE_BLA_agonist_viability	NA	human	HepG2	cell cycle	54.2
TOX21_p53_BLA_p1_viability	NA	human	intestinal cells HCT116	cell cycle	78.1
TOX21_FXR_BLA_agonist_viability	NA	human	HEK293T	cell cycle	30.1
TOX21_PPARd_BLA_Agonist_viability	NA	human	HEK293T	cell cycle	57.8
TOX21_p53_BLA_p3_viability	NA	human	intestinal cells HCT116	cell cycle	100

Assay Name	Gene Symbol	Organism	Cells/Cell Lines	Intended Target Family	AC50 (µM)
TOX21_p53_BLA_p4_viability	NA	human	intestinal cells HCT116	cell cycle	81.4
TOX21_VDR_BLA_Agonist_ viability	NA	human	HEK293T	cell cycle	37.3
NCCT_HEK293T_ CellTiterGLO	NA	human	HEK293T	cell cycle	49.9
TOX21_RXR_BLA_Agonist_ viability	NA	human	HEK293T	cell cycle	30.1
ACEA_AR_agonist_AUC_ viability	NA	human	prostate 22Rv1	cell cycle	80.0
ACEA_AR_antagonist_AUC_ viability	NA	human	prostate 22Rv1	cell cycle	33.7
TOX21_HRE_BLA_Agonist_ viability	NA	human	cervix ME-180	cell cycle	41.6
TOX21_RT_HEK293_FLO_ 08hr_viability	NA	human	HEK293T	cell cycle	30.7
TOX21_RT_HEK293_FLO_ 16hr_viability	NA	human	HEK293T	cell cycle	29.8
TOX21_RT_HEK293_FLO_ 24hr_viability	NA	human	HEK293T	cell cycle	28.1
TOX21_RT_HEK293_FLO_ 32hr_viability	NA	human	HEK293T	cell cycle	27.9
TOX21_RT_HEK293_FLO_ 40hr_viability	NA	human	HEK293T	cell cycle	27.7
TOX21_RT_HEK293_GLO_ 08hr_viability	NA	human	HEK293T	cell cycle	71.7
TOX21_RT_HEK293_GLO_ 16hr_viability	NA	human	HEK293T	cell cycle	70.5
TOX21_RT_HEK293_GLO_ 24hr_viability	NA	human	HEK293T	cell cycle	73.4
TOX21_RT_HEK293_GLO_ 32hr_viability	NA	human	HEK293T	cell cycle	66.4
TOX21_RT_HEK293_GLO_ 40hr_viability	NA	human	HEK293T	cell cycle	68.5
NIS_HEK293T_CTG_ Cytotoxicity	NA	human	HEK293T	cell cycle	0.100
TOX21_ERb_BLA_Agonist_ viability	NA	human	HEK293T	cell cycle	25.4
TOX21_ERb_BLA_Antagonist _viability	NA	human	HEK293T	cell cycle	30.3
TOX21_PR_BLA_Agonist_ viability	NA	human	PR-UAS-bla-HEK293T	cell cycle	27.7
TOX21_PR_BLA_Antagonist_ viability	NA	human	PR-UAS-bla-HEK293T	cell cycle	32.8
TOX21_DT40_657	NA	chicken	lymphoblast	cell cycle	67.0
TOX21_PXR_viability	NA	human	PXR-Luc HepG2 cells	cell cycle	99.4
APR_HepG2_MitoMass_24h_ dn	NA	human	HepG2	cell morphology	114
APR_HepG2_MitoMass_72h_ dn	NA	human	HepG2	cell morphology	17.5

Assay Name	Gene Symbol	Organism	Cells/Cell Lines	Intended Target Family	AC50 (µM)
APR_HepG2_NuclearSize_72h_dn	NA	human	HepG2	cell morphology	8.10
BSK_3C_Vis_down	NA	human	umbilical vein endothelium	cell morphology	40.0
TOX21_MMP_ratio_up	NA	human	HepG2	cell morphology	33.2
LTEA_HepaRG_CYP1A2_dn	CYP1A2	human	HepaRG	cyp	20.8
LTEA_HepaRG_CYP3A4_up	CYP3A4	human	HepaRG	cyp	10.1
LTEA_HepaRG_CYP3A7_up	CYP3A7	human	HepaRG	cyp	10.2
LTEA_HepaRG_CYP4A11_dn	CYP4A11	human	HepaRG	cyp	82.5
LTEA_HepaRG_CYP4A11_up	CYP4A11	human	HepaRG	cyp	10.8
LTEA_HepaRG_CYP4A22_dn	CYP4A22	human	HepaRG	cyp	88.2
LTEA_HepaRG_CYP4A22_up	CYP4A22	human	HepaRG	cyp	8.70
LTEA_HepaRG_CYP7A1_dn	CYP7A1	human	HepaRG	cyp	84.1
NVS_ADME_hCYP19A1	CYP19A1	human	NA	cyp	4.14
LTEA_HepaRG_CYP2B6_dn	CYP2B6	human	HepaRG	cyp	90.7
LTEA_HepaRG_CYP2B6_up	CYP2B6	human	HepaRG	cyp	5.52
CLD_CYP2B6_24hr	CYP2B6	human	primary hepatocyte	cyp	34.5
LTEA_HepaRG_CYP2C8_up	CYP2C8	human	HepaRG	cyp	16.7
NVS_ADME_hCYP2C8	CYP2C8	human	NA	cyp	4.70
LTEA_HepaRG_CYP2C9_dn	CYP2C9	human	HepaRG	cyp	85.4
NVS_ADME_hCYP2C9	CYP2C9	human	NA	cyp	2.17e-2
NVS_ADME_rCYP2C11	Cyp2c11	rat	NA	cyp	9.27e-2
NVS_ADME_hCYP2C18	CYP2C18	human	NA	cyp	0.822
LTEA_HepaRG_CYP2C19_up	CYP2C19	human	HepaRG	cyp	11.8
NVS_ADME_hCYP2C19	CYP2C19	human	NA	cyp	4.91
LTEA_HepaRG_CYP2E1_dn	CYP2E1	human	HepaRG	cyp	30.9
NVS_ADME_hCYP4F12	CYP4F12	human	NA	cyp	1.53
BSK_SAg_CD40_down	CD40	human	umbilical vein endothelium and peripheral blood mononuclear cells	cytokine	40.0
BSK_LPS_IL8_up	CXCL8	human	umbilical vein endothelium and peripheral blood mononuclear cells	cytokine	10.0
BSK_BE3C_IP10_down	CXCL10	human	bronchial epithelial cells	cytokine	10.0
BSK_KF3CT_IP10_down	CXCL10	human	keratinocytes and foreskin fibroblasts	cytokine	10.0
BSK_hDFCGF_IP10_down	CXCL10	human	foreskin fibroblast	cytokine	40.0

Assay Name	Gene Symbol	Organism	Cells/Cell Lines	Intended Target Family	AC50 (μM)
BSK_BE3C_IL1a_down	IL1A	human	bronchial epithelial cells	cytokine	40.0
BSK_BE3C_PAI1_down	SERPINE1	human	bronchial epithelial cells	cytokine	40.0
LTEA_HepaRG_FAS_up	FAS	human	HepaRG	cytokine receptor	17.4
LTEA_HepaRG_IL6R_dn	IL6R	human	HepaRG	cytokine receptor	85.3
NHEERL_MED_hDIO2_dn	null	human	NA	deiodinase	93.2
NHEERL_MED_hDIO3_dn	null	human	NA	deiodinase	173
LTEA_HepaRG_DDIT3_up	DDIT3	human	HepaRG	dna binding	33.9
LTEA_HepaRG_EGR1_dn	EGR1	human	HepaRG	dna binding	10.4
ATG_AP_1_CIS_up	FOS	human	HepG2	dna binding	33.7
LTEA_HepaRG_FOXO1_up	FOXO1	human	HepaRG	dna binding	23.8
LTEA_HepaRG_FOXO3_up	FOXO3	human	HepaRG	dna binding	27.3
LTEA_HepaRG_HSPA1A_up	HSPA1A	human	HepaRG	dna binding	19.3
ATG_MRE_CIS_up	MTF1	human	HepG2	dna binding	42.0
LTEA_HepaRG_MYC_up	MYC	human	HepaRG	dna binding	32.3
ATG_NRF2_ARE_CIS_up	NFE2L2	human	HepG2	dna binding	5.82
LTEA_HepaRG_NFE2L2_up	NFE2L2	human	HepaRG	dna binding	14.1
TOX21_ARE_BLA_agonist_ratio	NFE2L2	human	HEK293T	dna binding	25.2
ATG_Pax6_CIS_up	PAX6	human	HepG2	dna binding	84.0
ATG_Sp1_CIS_up	SP1	human	HepG2	dna binding	34.0
APR_HepG2_p53Act_24h_up	TP53	human	HepG2	dna binding	109
APR_HepG2_p53Act_72h_up	TP53	human	HepG2	dna binding	5.43
LTEA_HepaRG_TP53_up	TP53	human	HepaRG	dna binding	302
ATG_p53_CIS_dn	TP53	human	HepG2	dna binding	69.1
TOX21_p53_BLA_p4_ratio	TP53	human	intestinal cells HCT116	dna binding	159
LTEA_HepaRG_LIPC_dn	LIPC	human	HepaRG	esterase	47.2
NVS_ENZ_hPDE4A1	PDE4A	human	NA	esterase	9.62
NVS_ENZ_hPDE5	PDE5A	human	NA	esterase	25.6
LTEA_HepaRG_KRT19_up	KRT19	human	HepaRG	filaments	30.9
NVS_GPCR_hAdoRA2a	ADORA2A	human	NA	gpcr	5.17
NVS_GPCR_hAdra2C	ADRA2C	human	NA	gpcr	16.8
NVS_GPCR_hAdrb1	ADRB1	human	NA	gpcr	31.2
NVS_GPCR_gLTD4	Cysltr1	guinea pig	NA	gpcr	27.2
NVS_GPCR_hDRD4.4	DRD4	human	NA	gpcr	19.3
NVS_GPCR_h5HT5A	HTR5A	human	NA	gpcr	16.7
NVS_GPCR_h5HT6	HTR6	human	NA	gpcr	24.0
NVS_GPCR_h5HT7	HTR7	human	NA	gpcr	7.26
NVS_GPCR_hLTB4_BLT1	LTB4R	human	NA	gpcr	24.3
NVS_GPCR_gLTB4	Ltb4r	guinea pig	NA	gpcr	21.8
NVS_GPCR_hNPY2	NPY2R	human	NA	gpcr	28.0
NVS_GPCR_hOpiate_D1	OPRD1	human	NA	gpcr	12.6
NVS_GPCR_hPY2	P2RY1	human	NA	gpcr	11.8

Assay Name	Gene Symbol	Organism	Cells/Cell Lines	Intended Target Family	AC50 (µM)
NVS_GPCR_hTXA2	TBXA2R	human	NA	gpcr	16.1
LTEA_HepaRG_IGF1_dn	IGF1	human	HepaRG	growth factor	34.7
LTEA_HepaRG_TGFA_up	TGFA	human	HepaRG	growth factor	13.4
ATG_TGFb_CIS_up	TGFB1	human	HepG2	growth factor	70.7
BSK_BE3C_TGFb1_down	TGFB1	human	bronchial epithelial cells	growth factor	40.0
BSK_KF3CT_TGFb1_down	TGFB1	human	keratinocytes and foreskin fibroblasts	growth factor	40.0
LTEA_HepaRG_THRSP_dn	THRSP	human	HepaRG	growth factor	48.2
TOX21_SBE_BLA_Antagonist_ratio	null	human	SBE-bla HEK 293T cell line	growth factor receptor	71.6
NVS_ENZ_hHDAC3	HDAC3	human	NA	hydrolase	9.92
NVS_ENZ_hSIRT2	SIRT2	human	NA	hydrolase	7.58
NVS_ENZ_hSIRT3_Activator	SIRT3	human	NA	hydrolase	7.55
NVS_ENZ_hAKT1	AKT1	human	NA	kinase	38.3
NVS_ENZ_hAKT2	AKT2	human	NA	kinase	26.2
NVS_ENZ_hAurA	AURKA	human	NA	kinase	26.8
NVS_ENZ_hBTK	BTK	human	NA	kinase	21.9
NVS_ENZ_hCDK6	CDK6	human	NA	kinase	20.0
NVS_ENZ_hIKKa	CHUK	human	NA	kinase	30.4
NVS_ENZ_hCSF1R	CSF1R	human	NA	kinase	23.0
NVS_ENZ_hCSF1R_Activator	CSF1R	human	NA	kinase	0.406
NVS_ENZ_hCK1a	CSNK1A1	human	NA	kinase	24.2
NVS_ENZ_hCK1D	CSNK1D	human	NA	kinase	26.4
NVS_ENZ_hDYRK1a	DYRK1A	human	NA	kinase	20.7
NVS_ENZ_hEGFR	EGFR	human	NA	kinase	19.1
NVS_ENZ_hEphA2	EPHA2	human	NA	kinase	20.8
NVS_ENZ_hFGFR1	FGFR1	human	NA	kinase	20.8
NVS_ENZ_hFGFR3	FGFR3	human	NA	kinase	33.3
NVS_ENZ_hVEGFR1	FLT1	human	NA	kinase	8.92
NVS_ENZ_hVEGFR3	FLT4	human	NA	kinase	8.36
NVS_ENZ_hFyn	FYN	human	NA	kinase	41.0
NVS_ENZ_hGSK3b	GSK3B	human	NA	kinase	25.5
NVS_ENZ_hInsR	INSR	human	NA	kinase	12.5
NVS_ENZ_hInsR_Activator	INSR	human	NA	kinase	40.9
NVS_ENZ_hVEGFR2	KDR	human	NA	kinase	32.2
NVS_ENZ_hLck	LCK	human	NA	kinase	40.9
NVS_ENZ_hLynA_Activator	LYN	human	NA	kinase	27.8
NVS_ENZ_hMARK1	MARK1	human	NA	kinase	32.5
NVS_ENZ_hMAPK1	MAPK1	human	NA	kinase	30.4
NVS_ENZ_hMAPK3	MAPK3	human	NA	kinase	31.5
NVS_ENZ_hMAPKAPK5	MAPKAPK5	human	NA	kinase	36.4
NVS_ENZ_hMet	MET	human	NA	kinase	25.7
NVS_ENZ_hTrkA	NTRK1	human	NA	kinase	30.2
NVS_ENZ_hPAK4	PAK4	human	NA	kinase	26.5

Assay Name	Gene Symbol	Organism	Cells/Cell Lines	Intended Target Family	AC50 (µM)
LTEA_HepaRG_PDK4_up	PDK4	human	HepaRG	kinase	24.2
NVS_ENZ_hPI3Ka	PIK3CA	human	NA	kinase	7.42
NVS_ENZ_hAMPKa1	PRKAA1	human	NA	kinase	5.47
NVS_ENZ_hPKA	PRKACA	human	NA	kinase	26.4
NVS_ENZ_hRAF1	RAF1	human	NA	kinase	24.4
NVS_ENZ_hROCK1	ROCK1	human	NA	kinase	33.9
NVS_ENZ_hMsk1	RPS6KA5	human	NA	kinase	29.7
NVS_ENZ_hSGK1	SGK1	human	NA	kinase	29.8
NVS_ENZ_hTie2	TEK	human	NA	kinase	8.69
NVS_ENZ_hZAP70	ZAP70	human	NA	kinase	13.5
LTEA_HepaRG_ACLY_dn	ACLY	human	HepaRG	lyase	32.2
LTEA_HepaRG_FASN_dn	FASN	human	HepaRG	lyase	45.0
LTEA_HepaRG_HMGCS2_dn	HMGCS2	human	HepaRG	lyase	83.6
Tanguay_ZF_120hpf_MORT_up	NA	zebrafish	dechorionated zebrafish embryo	malformation	0.547
Tanguay_ZF_120hpf_YSE_up	NA	zebrafish	dechorionated zebrafish embryo	malformation	8.65
Tanguay_ZF_120hpf_AXIS_up	NA	zebrafish	dechorionated zebrafish embryo	malformation	2.39
Tanguay_ZF_120hpf_SNOU_up	NA	zebrafish	dechorionated zebrafish embryo	malformation	26.0
Tanguay_ZF_120hpf_JAW_up	NA	zebrafish	dechorionated zebrafish embryo	malformation	29.2
Tanguay_ZF_120hpf_PE_up	NA	zebrafish	dechorionated zebrafish embryo	malformation	28.7
Tanguay_ZF_120hpf_TRUN_up	NA	zebrafish	dechorionated zebrafish embryo	malformation	16.8
Tanguay_ZF_120hpf_TR_up	NA	zebrafish	dechorionated zebrafish embryo	malformation	1.92
NHEERL_ZF_144hpf_TERATOSCORE_up	NA	zebrafish	zebrafish embryo	malformation	42.3
Tanguay_ZF_120hpf_ActivityScore	NA	zebrafish	dechorionated zebrafish embryo	malformation	3.45
LTEA_HepaRG_EZR_up	EZR	human	HepaRG	membrane protein	56.5
LTEA_HepaRG_MIR122_dn	MIR122	human	HepaRG	microrna	72.0
ACEA_AR_antagonist_80hr	AR	human	prostate 22Rv1	nuclear receptor	39.0
NVS_NR_hAR	AR	human	NA	nuclear receptor	12.6

Assay Name	Gene Symbol	Organism	Cells/Cell Lines	Intended Target Family	AC50 (µM)
NVS_NR_cAR	AR	chimpanzee	NA	nuclear receptor	7.31
NVS_NR_rAR	AR	rat	NA	nuclear receptor	4.27
ATG_ERE_CIS_up	ESR1	human	HepG2	nuclear receptor	18.1
ATG_ERa_TRANS_up	ESR1	human	HepG2	nuclear receptor	38.9
NVS_NR_hER	ESR1	human	NA	nuclear receptor	27.2
TOX21_ERa_BLA_Antagonist_ratio	ESR1	human	HEK293T	nuclear receptor	86.5
OT_ER_ERaERb_0480	ESR2	human	HEK293T	nuclear receptor	87.4
TOX21_ERb_BLA_Antagonist_ratio	ESR2	human	HEK293T	nuclear receptor	62.2
ATG_PXRE_CIS_up	NR1I2	human	HepG2	nuclear receptor	9.42
ATG_PXR_TRANS_up	NR1I2	human	HepG2	nuclear receptor	19.7
NVS_NR_hPXR	NR1I2	human	NA	nuclear receptor	40.9
NVS_NR_hCAR_Antagonist	NR1I3	human	NA	nuclear receptor	17.6
NVS_NR_hGR	NR3C1	human	NA	nuclear receptor	2.27
ATG_DR4_LXR_CIS_dn	NR1H3	human	HepG2	nuclear receptor	23.8
TOX21_PR_BLA_Antagonist_ratio	PGR	human	PR-UAS-bla-HEK293T	nuclear receptor	35.5
NVS_NR_hPR	PGR	human	NA	nuclear receptor	22.6
NVS_NR_bPR	PGR	bovine	NA	nuclear receptor	22.2
ATG_PPRE_CIS_up	PPARA	human	HepG2	nuclear receptor	33.9
ATG_PPARa_TRANS_up	PPARA	human	HepG2	nuclear receptor	58.9
ATG_PPARg_TRANS_up	PPARG	human	HepG2	nuclear receptor	26.7
NVS_NR_hPPARg	PPARG	human	NA	nuclear receptor	5.94
NVS_NR_hRAR_Antagonist	RARA	human	NA	nuclear receptor	28.4
NVS_NR_hTRa_Antagonist	THRA	human	NA	nuclear receptor	14.6
TOX21_TR_LUC_GH3_Antagonist	Thrb	rat	pituitary gland GH3	nuclear receptor	86.5
LTEA_HepaRG_ACOX1_up	ACOX1	human	HepaRG	oxidase	14.7
LTEA_HepaRG_FMO3_dn	FMO3	human	HepaRG	oxidoreductase	86.3
NVS_ENZ_oCOX2	PTGS2	sheep	NA	oxidoreductase	11.9

Assay Name	Gene Symbol	Organism	Cells/Cell Lines	Intended Target Family	AC50 (µM)
NVS_ENZ_hACP1	ACP1	human	NA	phosphatase	24.1
LTEA_HepaRG_ALPP_up	ALPP	human	HepaRG	phosphatase	40.4
NVS_ENZ_hDUSP3	DUSP3	human	NA	phosphatase	26.0
NVS_ENZ_hPPP1CA	PPP1CA	human	NA	phosphatase	16.1
NVS_ENZ_hPPP2CA	PPP2CA	human	NA	phosphatase	5.39
NVS_ENZ_hPTEN	PTEN	human	NA	phosphatase	7.66
NVS_ENZ_hPTPN1	PTPN1	human	NA	phosphatase	5.33e-2
NVS_ENZ_hPTPN2	PTPN2	human	NA	phosphatase	26.6
NVS_ENZ_hPTPN6	PTPN6	human	NA	phosphatase	23.3
NVS_ENZ_hPTPN11	PTPN11	human	NA	phosphatase	24.3
NVS_ENZ_hPTPN12	PTPN12	human	NA	phosphatase	21.8
NVS_ENZ_hPTPN13	PTPN13	human	NA	phosphatase	10.5
NVS_ENZ_hPTPRB	PTPRB	human	NA	phosphatase	25.4
NVS_ENZ_hPTPRC	PTPRC	human	NA	phosphatase	16.0
NVS_ENZ_hPTPRF	PTPRF	human	NA	phosphatase	11.1
NVS_ENZ_hPTPRM	PTPRM	human	NA	phosphatase	31.0
NVS_ENZ_hBACE	BACE1	human	NA	protease	0.471
LTEA_HepaRG_CASP3_up	CASP3	human	HepaRG	protease	21.1
NVS_ENZ_hCASP5	CASP5	human	NA	protease	13.6
LTEA_HepaRG_CASP8_up	CASP8	human	HepaRG	protease	18.5
NVS_ENZ_hMMP3	MMP3	human	NA	protease	18.8
NVS_ENZ_hMMP7	MMP7	human	NA	protease	1.73
NVS_ENZ_hMMP9	MMP9	human	NA	protease	18.4
BSK_KF3CT_MMP9_down	MMP9	human	keratinocytes and foreskin fibroblasts	protease	10.0
LTEA_HepaRG_MMP10_up	MMP10	human	HepaRG	protease	83.4
NVS_ENZ_hMMP13	MMP13	human	NA	protease	8.08
BSK_BE3C_tPA_down	PLAT	human	bronchial epithelial cells	protease	40.0
BSK_BE3C_uPA_down	PLAU	human	bronchial epithelial cells	protease	40.0
LTEA_HepaRG_GSTA2_dn	GSTA2	human	HepaRG	transferase	41.2
LTEA_HepaRG_UGT1A1_up	UGT1A1	human	HepaRG	transferase	15.3
LTEA_HepaRG_UGT1A6_dn	UGT1A6	human	HepaRG	transferase	56.1
LTEA_HepaRG_ABCB1_up	ABCB1	human	HepaRG	transporter	21.2
LTEA_HepaRG_ABCB11_dn	ABCB11	human	HepaRG	transporter	44.4
LTEA_HepaRG_ABCC2_up	ABCC2	human	HepaRG	transporter	11.6
LTEA_HepaRG_ABCG2_up	ABCG2	human	HepaRG	transporter	6.93
LTEA_HepaRG_FABP1_dn	FABP1	human	HepaRG	transporter	85.9
LTEA_HepaRG_IGFBP1_up	IGFBP1	human	HepaRG	transporter	57.5
NIS_RAIU_inhibition	SLC5A5	human	HEK293T	transporter	11.2
LTEA_HepaRG_SLC22A1_dn	SLC22A1	human	HepaRG	transporter	85.1
LTEA_HepaRG_SLCO1B1_dn	SLCO1B1	human	HepaRG	transporter	82.9

[1] Assays are alphabetically ordered by “intended target family”, and within each “intended target family” assays are ordered alphabetically by “gene symbol”. This table includes assays with curve-fitting “flags”,

although the flags are not shown here. The table does not include assays classified by the US EPA Comptox Chemicals Dashboard as 'background measurement' assays (e.g., artifact fluorescence, baseline controls, and internal markers).
AC50: the concentration that induces a half-maximal assay response.
NA, not applicable. This notation is used when no specific target genes are reported by the US EPA Comptox Chemicals Dashboard, and used for cell-free assays such as cell-free systems utilizing enzymes or receptors extracted from tissues or cells of various organisms.

Table A9.4. 179 Active ToxCast assays[1] for PFOS potassium salt

Assay Name	Gene Symbol	Organism	Cells/Cell Lines	Intended Target Family	AC50 (μM)
LTEA_HepaRG_CAT_dn	CAT	human	HepaRG	catalase	87.0
BSK_BE3C_HLADR_down	HLA-DRA	human	bronchial epithelial cells	cell adhesion molecules	10.0
BSK_LPS_Eselectin_up	SELE	human	umbilical vein endothelium and peripheral blood mononuclear cells	cell adhesion molecules	40.0
BSK_4H_Pselectin_up	SELP	human	umbilical vein endothelium	cell adhesion molecules	40.0
APR_HepG2_CellLoss_24h_dn	NA	human	HepG2	cell cycle	115
APR_HepG2_MitoticArrest_24h_up	pH3	human	HepG2	cell cycle	110
APR_HepG2_OxidativeStress 24h_up	γH2AX	human	HepG2	cell cycle	121
APR_HepG2_CellLoss_72h_dn	NA	human	HepG2	cell cycle	111
APR_HepG2_MitoticArrest_72h_up	pH3	human	HepG2	cell cycle	107
APR_HepG2_OxidativeStress_72h_up	γH2AX	human	HepG2	cell cycle	111
APR_HepG2_StressKinase_72h_up	NA	human	HepG2	cell cycle	112
BSK_KF3CT_SRB_down	NA	human	keratinocytes and foreskin fibroblasts	cell cycle	40.0
BSK_BE3C_SRB_down	NA	human	bronchial epithelial cells	cell cycle	40.0
TOX21_TR_LUC_GH3_Antagonist_viability	NA	rat	pituitary gland GH4	cell cycle	49.4
LTEA_HepaRG_BAX_up	BAX	human	HepaRG	cell cycle	53.6
LTEA_HepaRG_BCL2_up	BCL2	human	HepaRG	cell cycle	43.0
LTEA_HepaRG_CCND1_up	CCND1	human	HepaRG	cell cycle	11.4
TOX21_FXR_BLA_antagonist_viability	NA	human	HEK293T	cell cycle	31.9
TOX21_PPARd_BLA_antagonist_viability	NA	human	HEK293T	cell cycle	43.2
TOX21_DT40	NA	chicken	lymphoblast	cell cycle	44.9

Assay Name	Gene Symbol	Organism	Cells/Cell Lines	Intended Target Family	AC50 (µM)
LTEA_HepaRG_LDH_ cytotoxicity	NA	human	HepaRG	cell cycle	83.4
TOX21_ARE_BLA_agonist_ viability	NA	human	HepG2	cell cycle	76.2
TOX21_FXR_BLA_agonist_ viability	NA	human	HEK293T	cell cycle	29.1
TOX21_PPARd_BLA_Agonist _viability	NA	human	HEK293T	cell cycle	32.4
TOX21_p53_BLA_p3_viability	NA	human	intestinal cells HCT116	cell cycle	54.1
TOX21_p53_BLA_p4_viability	NA	human	intestinal cells HCT116	cell cycle	45.4
TOX21_VDR_BLA_Agonist_ viability	NA	human	HEK293T	cell cycle	18.8
TOX21_AP1_BLA_Agonist_vi ability	NA	human	cervix cell line	cell cycle	50.2
ACEA_AR_agonist_AUC_ viability	NA	human	prostate 22Rv1	cell cycle	69.3
ACEA_AR_antagonist_AUC_ viability	NA	human	prostate 22Rv1	cell cycle	56.0
TOX21_ERR_viability	NA	human	ERR-HEK293T	cell cycle	38.3
TOX21_HRE_BLA_Agonist_ viability	NA	human	cervix ME-180	cell cycle	26.3
TOX21_RT_HEK293_FLO_ 08hr_viability	NA	human	HEK293T	cell cycle	28.5
TOX21_RT_HEK293_FLO_ 16hr_viability	NA	human	HEK293T	cell cycle	24.0
TOX21_RT_HEK293_FLO_ 24hr_viability	NA	human	HEK293T	cell cycle	29.2
TOX21_RT_HEK293_FLO_ 32hr_viability	NA	human	HEK293T	cell cycle	28.8
TOX21_RT_HEK293_FLO_ 40hr_viability	NA	human	HEK293T	cell cycle	29.1
TOX21_RT_HEPG2_FLO_16h r_ctrl_viability	NA	human	HEK293T	cell cycle	14.3
TOX21_RT_HEPG2_FLO_24h r_viability	NA	human	HEK293T	cell cycle	13.4
TOX21_RT_HEPG2_FLO_32h r_ctrl_viability	NA	human	HEK293T	cell cycle	15.7
TOX21_RT_HEPG2_FLO_40h r_ctrl_viability	NA	human	HEK293T	cell cycle	14.8
TOX21_RT_HEPG2_GLO_00 hr_ctrl_viability	NA	human	HEK293T	cell cycle	43.6
NIS_HEK293T_CTG_ Cytotoxicity	NA	human	HEK293T	cell cycle	10.3
TOX21_ERb_BLA_Agonist_ viability	NA	human	HEK293T	cell cycle	42.2
TOX21_ERb_BLA_Antagonist _viability	NA	human	HEK293T	cell cycle	42.5
TOX21_PR_BLA_Agonist_ viability	NA	human	PR-UAS-bla-HEK293T	cell cycle	40.5

Assay Name	Gene Symbol	Organism	Cells/Cell Lines	Intended Target Family	AC50 (µM)
TOX21_PR_BLA_Antagonist_viability	NA	human	PR-UAS-bla-HEK293T	cell cycle	55.9
TOX21_DT40_100	NA	chicken	lymphoblast	cell cycle	43.3
TOX21_DT40_657	NA	chicken	lymphoblast	cell cycle	65.3
TOX21_PXR_viability	NA	human	PXR-Luc HepG2 cells	cell cycle	88.4
APR_HepG2_MitoMass_24h_dn	NA	human	HepG2	cell morphology	120
APR_HepG2_MitoMass_72h_dn	NA	human	HepG2	cell morphology	113
TOX21_MMP_ratio_up	NA	human	HepG2	cell morphology	72.5
LTEA_HepaRG_CYP1A2_dn	CYP1A2	human	HepaRG	cyp	202
LTEA_HepaRG_CYP3A4_up	CYP3A4	human	HepaRG	cyp	8.63
LTEA_HepaRG_CYP3A5_dn	CYP3A5	human	HepaRG	cyp	90.0
LTEA_HepaRG_CYP3A7_up	CYP3A7	human	HepaRG	cyp	9.25
LTEA_HepaRG_CYP4A11_dn	CYP4A11	human	HepaRG	cyp	87.0
LTEA_HepaRG_CYP4A22_dn	CYP4A22	human	HepaRG	cyp	85.8
LTEA_HepaRG_CYP7A1_dn	CYP7A1	human	HepaRG	cyp	82.2
LTEA_HepaRG_CYP2B6_dn	CYP2B6	human	HepaRG	cyp	86.4
LTEA_HepaRG_CYP2B6_up	CYP2B6	human	HepaRG	cyp	3.31
LTEA_HepaRG_CYP2C8_dn	CYP2C8	human	HepaRG	cyp	89.2
LTEA_HepaRG_CYP2C8_up	CYP2C8	human	HepaRG	cyp	21.2
LTEA_HepaRG_CYP2C9_dn	CYP2C9	human	HepaRG	cyp	86.3
NVS_ADME_hCYP2C9	CYP2C9	human	NA	cyp	1.30e-2
NVS_ADME_hCYP2C19	CYP2C19	human	NA	cyp	6.09
LTEA_HepaRG_CYP2C19_dn	CYP2C19	human	HepaRG	cyp	81.9
LTEA_HepaRG_CYP2C19_up	CYP2C19	human	HepaRG	cyp	11.1
LTEA_HepaRG_CYP2E1_dn	CYP2E1	human	HepaRG	cyp	60.4
BSK_BE3C_MIG_down	CXCL9	human	bronchial epithelial cells	cytokine	40.0
BSK_BE3C_IP10_down	CXCL10	human	bronchial epithelial cells	cytokine	10.0
BSK_KF3CT_IP10_down	CXCL10	human	keratinocytes and foreskin fibroblasts	cytokine	40.0
BSK_BE3C_IL1a_down	IL1A	human	bronchial epithelial cells	cytokine	40.0
BSK_KF3CT_IL1a_down	IL1A	human	keratinocytes and foreskin fibroblasts	cytokine	40.0
BSK_BE3C_uPAR_down	PLAUR	human	bronchial epithelial cells	cytokine	40.0
BSK_BE3C_PAI1_down	SERPINE1	human	bronchial epithelial cells	cytokine	40.0
LTEA_HepaRG_IL6R_dn	IL6R	human	HepaRG	cytokine receptor	95.7
NHEERL_MED_hDIO1_dn	DIO1	human	NA	deiodinase	175
NHEERL_MED_hDIO2_dn	NA	human	NA	deiodinase	122

Assay Name	Gene Symbol	Organism	Cells/Cell Lines	Intended Target Family	AC50 (µM)
NHEERL_MED_hDIO3_dn	NA	human	NA	deiodinase	109
LTEA_HepaRG_DDIT3_up	DDIT3	human	HepaRG	dna binding	8.61
LTEA_HepaRG_EGR1_dn	EGR1	human	HepaRG	dna binding	42.6
ATG_AP_1_CIS_up	FOS	human	HepG2	dna binding	37.7
LTEA_HepaRG_JUN_up	JUN	human	HepaRG	dna binding	53.6
ATG_MRE_CIS_up	MTF1	human	HepG2	dna binding	30.7
LTEA_HepaRG_MYC_up	MYC	human	HepaRG	dna binding	12.5
ATG_NRF2_ARE_CIS_up	NFE2L2	human	HepG2	dna binding	30.8
LTEA_HepaRG_NFE2L2_up	NFE2L2	human	HepaRG	dna binding	66.9
ATG_TCF_b_cat_CIS_dn	TCF7	human	HepG2	dna binding	111
APR_HepG2_p53Act_24h_up	TP53	human	HepG2	dna binding	111
APR_HepG2_p53Act_72h_up	TP53	human	HepG2	dna binding	23.7
ATG_p53_CIS_dn	TP53	human	HepG2	dna binding	49.0
LTEA_HepaRG_TP53_up	TP53	human	HepaRG	dna binding	82.6
TOX21_p53_BLA_p4_ratio	TP53	human	intestinal cells HCT116	dna binding	49.1
LTEA_HepaRG_XBP1_dn	XBP1	human	HepaRG	dna binding	73.6
LTEA_HepaRG_LIPC_dn	LIPC	human	HepaRG	esterase	34.2
LTEA_HepaRG_LPL_up	LPL	human	HepaRG	esterase	15.5
LTEA_HepaRG_KRT19_up	KRT19	human	HepaRG	filaments	35.5
NVS_GPCR_hAdoRA2a	ADORA2A	human	NA	gpcr	10.3
NVS_GPCR_hAdra2C	ADRA2C	human	NA	gpcr	17.6
NVS_GPCR_hAdrb1	ADRB1	human	NA	gpcr	31.5
NVS_GPCR_gLTD4	Cysltr1	guinea pig	NA	gpcr	30.9
NVS_GPCR_hDRD4.4	DRD4	human	NA	gpcr	18.8
NVS_GPCR_h5HT5A	HTR5A	human	NA	gpcr	32.7
NVS_GPCR_h5HT7	HTR7	human	NA	gpcr	30.2
NVS_GPCR_hLTB4_BLT1	LTB4R	human	NA	gpcr	25.9
NVS_GPCR_gLTB4	Ltb4r	guinea pig	NA	gpcr	12.1
NVS_GPCR_hTXA2	TBXA2R	human	NA	gpcr	16.4
LTEA_HepaRG_IGF1_dn	IGF1	human	HepaRG	growth factor	90.0
LTEA_HepaRG_TGFA_up	TGFA	human	HepaRG	growth factor	71.6
ATG_TGFb_CIS_up	TGFB1	human	HepG2	growth factor	26.2
LTEA_HepaRG_TGFB1_up	TGFB1	human	HepaRG	growth factor	54.0
BSK_BE3C_TGFb1_down	TGFB1	human	bronchial epithelial cells	growth factor	40.0
BSK_KF3CT_TGFb1_down	TGFB1	human	keratinocytes and foreskin fibroblasts	growth factor	40.0
LTEA_HepaRG_THRSP_dn	THRSP	human	HepaRG	growth factor	57.8
TOX21_SBE_BLA_Antagonist_ratio	null	human	SBE-bla HEK 293T cell line	growth factor receptor	34.6
LTEA_HepaRG_KCNK1_up	KCNK1	human	HepaRG	ion channel	34.0
LTEA_HepaRG_PDK4_up	PDK4	human	HepaRG	kinase	23.0
LTEA_HepaRG_FASN_dn	FASN	human	HepaRG	lyase	65.1
LTEA_HepaRG_HMGCS2_dn	HMGCS2	human	HepaRG	lyase	82.3

Assay Name	Gene Symbol	Organism	Cells/Cell Lines	Intended Target Family	AC50 (µM)
Tanguay_ZF_120hpf_MORT_up	NA	zebrafish	dechorionated zebrafish embryo	malformation	19.8
Tanguay_ZF_120hpf_YSE_up	NA	zebrafish	dechorionated zebrafish embryo	malformation	38.0
Tanguay_ZF_120hpf_AXIS_up	NA	zebrafish	dechorionated zebrafish embryo	malformation	34.6
Tanguay_ZF_120hpf_TR_up	NA	zebrafish	dechorionated zebrafish embryo	malformation	12.5
NHEERL_ZF_144hpf_TERATOSCORE_up	NA	zebrafish	zebrafish embryo	malformation	9.62
Tanguay_ZF_120hpf_ActivityScore	NA	zebrafish	dechorionated zebrafish embryo	malformation	33.7
LTEA_HepaRG_EZR_up	EZR	human	HepaRG	membrane protein	24.7
LTEA_HepaRG_MIR122_dn	MIR122	human	HepaRG	microrna	49.7
LTEA_HepaRG_GADD45B_up	GADD45B	human	HepaRG	mutagenicity response	34.8
LTEA_HepaRG_GADD45G_dn	GADD45G	human	HepaRG	mutagenicity response	36.2
ACEA_AR_antagonist_80hr	AR	human	prostate 22Rv1	nuclear receptor	69.3
NVS_NR_hAR	AR	human	NA	nuclear receptor	20.9
NVS_NR_rAR	AR	rat	NA	nuclear receptor	12.0
ATG_ERE_CIS_up	ESR1	human	HepG2	nuclear receptor	32.6
ATG_ERa_TRANS_up	ESR1	human	HepG2	nuclear receptor	35.9
NVS_NR_bER	ESR1	bovine	NA	nuclear receptor	2.59e-2
ATG_LXRb_TRANS_dn	NR1H2	human	HepG2	nuclear receptor	13.4
ATG_DR4_LXR_CIS_dn	NR1H3	human	HepG2	nuclear receptor	32.2
ATG_PXRE_CIS_up	NR1I2	human	HepG2	nuclear receptor	9.76
ATG_PXR_TRANS_up	NR1I2	human	HepG2	nuclear receptor	14.0
ATG_CAR_TRANS_up	NR1I3	human	HepG2	nuclear receptor	39.5
NVS_NR_hGR	NR3C1	human	NA	nuclear receptor	20.7
TOX21_PR_BLA_Antagonist_ratio	PGR	human	PR-UAS-bla-HEK293T	nuclear receptor	63.5

Assay Name	Gene Symbol	Organism	Cells/Cell Lines	Intended Target Family	AC50 (µM)
NVS_NR_hPR	PGR	human	NA	nuclear receptor	25.4
NVS_NR_bPR	PGR	bovine	NA	nuclear receptor	29.6
ATG_PPARa_TRANS_up	PPARA	human	HepG2	nuclear receptor	17.1
NVS_NR_hPPARa	PPARA	human	NA	nuclear receptor	11.2
ATG_PPARg_TRANS_up	PPARG	human	HepG2	nuclear receptor	22.2
NVS_NR_hPPARg	PPARG	human	NA	nuclear receptor	20.3
NVS_NR_hRARa_Antagonist	RARA	human	NA	nuclear receptor	1.91
TOX21_TR_LUC_GH3_ Antagonist	Thrb	rat	pituitary gland GH3	nuclear receptor	65.1
LTEA_HepaRG_FMO3_dn	FMO3	human	HepaRG	oxidoreductase	90.4
LTEA_HepaRG_ALPP_up	ALPP	human	HepaRG	phosphatase	57.1
NVS_ENZ_hPPP1CA	PPP1CA	human	NA	phosphatase	41.0
LTEA_HepaRG_PPP2R4_up	PPP2R4	human	HepaRG	phosphatase	55.9
NVS_ENZ_hPTPRC	PTPRC	human	NA	phosphatase	18.9
NVS_ENZ_hBACE	BACE1	human	NA	protease	8.72
LTEA_HepaRG_CASP3_up	CASP3	human	HepaRG	protease	22.5
NVS_ENZ_hCASP5	CASP5	human	NA	protease	27.8
NVS_ENZ_hMMP3	MMP3	human	NA	protease	29.4
BSK_KF3CT_MMP9_down	MMP9	human	keratinocytes and foreskin fibroblasts	protease	40.0
LTEA_HepaRG_MMP10_up	MMP10	human	HepaRG	protease	46.6
BSK_BE3C_tPA_down	PLAT	human	bronchial epithelial cells	protease	40.0
BSK_BE3C_uPA_down	PLAU	human	bronchial epithelial cells	protease	40.0
BSK_KF3CT_uPA_down	PLAU	human	keratinocytes and foreskin fibroblasts	protease	40.0
BSK_hDFCGF_TIMP1_up	TIMP1	human	foreskin fibroblast	protease inhibitor	10.0
BSK_KF3CT_TIMP2_down	TIMP2	human	keratinocytes and foreskin fibroblasts	protease inhibitor	40.0
LTEA_HepaRG_GSTA2_dn	GSTA2	human	HepaRG	transferase	71.1
LTEA_HepaRG_SULT2A1_dn	SULT2A1	human	HepaRG	transferase	88.4
LTEA_HepaRG_UGT1A1_dn	UGT1A1	human	HepaRG	transferase	85.5
LTEA_HepaRG_UGT1A1_up	UGT1A1	human	HepaRG	transferase	10.2
LTEA_HepaRG_UGT1A6_dn	UGT1A6	human	HepaRG	transferase	38.5
LTEA_HepaRG_ABCB11_dn	ABCB11	human	HepaRG	transporter	86.8
LTEA_HepaRG_FABP1_dn	FABP1	human	HepaRG	transporter	82.8
LTEA_HepaRG_IGFBP1_up	IGFBP1	human	HepaRG	transporter	73.5

Assay Name	Gene Symbol	Organism	Cells/Cell Lines	Intended Target Family	AC50 (µM)
LTEA_HepaRG_SLC22A1_dn	SLC22A1	human	HepaRG	transporter	78.6
LTEA_HepaRG_SLCO1B1_dn	SLCO1B1	human	HepaRG	transporter	86.2
NIS_RAIU_inhibition	SLC5A5	human	HEK293T	transporter	22.8

[1] Assays are alphabetically ordered by "intended target family", and within each "intended target family" assays are ordered alphabetically by "gene symbol". This table includes assays with curve-fitting "flags", although the flags are not shown here. The table does not include assays classified by the US EPA Comptox Chemicals Dashboard as 'background measurement' assays (e.g., artifact fluorescence, baseline controls, and internal markers).
AC50: the concentration that induces a half-maximal assay response.
NA, not applicable. This notation is used when no specific target genes are reported by the US EPA Comptox Chemicals Dashboard, and used for cell-free assays such as cell-free systems utilizing enzymes or receptors extracted from tissues or cells of various organisms.

Table A9.5. 26 Active ToxCast assays[1] for PFOS lithium salt

Assay Name	Gene Symbol	Organism	Cells/Cell Lines	Intended Target Family	AC50 (µM)
TOX21_MMP_viability	NA	human	HepG2	cell cycle	67.4
TOX21_TR_LUC_GH3_ Antagonist_viability	NA	rat	pituitary gland GH4	cell cycle	52.3
TOX21_ARE_BLA_agonist_ viability	NA	human	HepG2	cell cycle	76.2
TOX21_FXR_BLA_antagonist _viability	NA	human	HEK293T	cell cycle	253
TOX21_PPARd_BLA_ Agonist_viability	NA	human	HEK293T	cell cycle	31.4
TOX21_p53_BLA_p2_viability	NA	human	intestinal cells HCT116	cell cycle	70.0
TOX21_p53_BLA_p3_viability	NA	human	intestinal cells HCT116	cell cycle	49.0
TOX21_p53_BLA_p4_viability	NA	human	intestinal cells HCT116	cell cycle	65.8
TOX21_AP1_BLA_Agonist_vi ability	NA	human	cervix ME-180	cell cycle	38.7
TOX21_H2AX_HTRF_CHO_vi ability	NA	Chinese hamster	CHO-K1	cell cycle	158
TOX21_HRE_BLA_Agonist_ viability	NA	human	cervix ME-180	cell cycle	40.5
TOX21_RT_HEK293_FLO_ 16hr_viability	NA	human	HEK293T	cell cycle	7.40e-2
TOX21_RT_HEK293_FLO_ 24hr_viability	NA	human	HEK293T	cell cycle	8.21e-2
TOX21_RT_HEK293_FLO_ 40hr_viability	NA	human	HEK293T	cell cycle	0.289
TOX21_RT_HEPG2_GLO_00 hr_ctrl_viability	NA	human	HEK293T	cell cycle	45.2
TOX21_ERb_BLA_Agonist_ viability	NA	human	HEK293T	cell cycle	43.4
TOX21_ERb_BLA_Antagonist _viability	NA	human	HEK293T	cell cycle	95.9
TOX21_PR_BLA_Agonist_ viability	NA	human	PR-UAS-bla-HEK293T	cell cycle	50.0
TOX21_PR_BLA_Antagonist_ viability	NA	human	PR-UAS-bla-HEK293T	cell cycle	84.3
TOX21_MMP_ratio_up	NA	human	HepG2	cell morphology	40.5
TOX21_ARE_BLA_agonist_ ratio	NFE2L2	human	HEK293T	dna binding	85.2
TOX21_p53_BLA_p4_ratio	TP53	human	intestinal cells HCT116	dna binding	70.8
TOX21_SBE_BLA_Antagonist _ratio	NA	human	SBE-bla HEK 293T cell line	growth factor receptor	45.3
NVS_NR_bER	ESR1	bovine	NA	nuclear receptor	15.1
TOX21_PR_BLA_Antagonist_ ratio	PGR	human	PR-UAS-bla-HEK293T	nuclear receptor	98.6

Assay Name	Gene Symbol	Organism	Cells/Cell Lines	Intended Target Family	AC50 (µM)
TOX21_TR_LUC_GH3_ Antagonist	Thrb	rat	pituitary gland GH3	nuclear receptor	63.5

[1] Assays are alphabetically ordered by "intended target family", and within each "intended target family" assays are ordered alphabetically by "gene symbol". This table includes assays with curve-fitting "flags", although the flags are not shown here. The table does not include assays classified by the US EPA Comptox Chemicals Dashboard as 'background measurement' assays (e.g., artifact fluorescence, baseline controls, and internal markers).
AC50: the concentration that induces a half-maximal assay response.
NA, not applicable. This notation is used when no specific target genes are reported by the US EPA Comptox Chemicals Dashboard, and used for cell-free assays such as cell-free systems utilizing enzymes or receptors extracted from tissues or cells of various organisms.

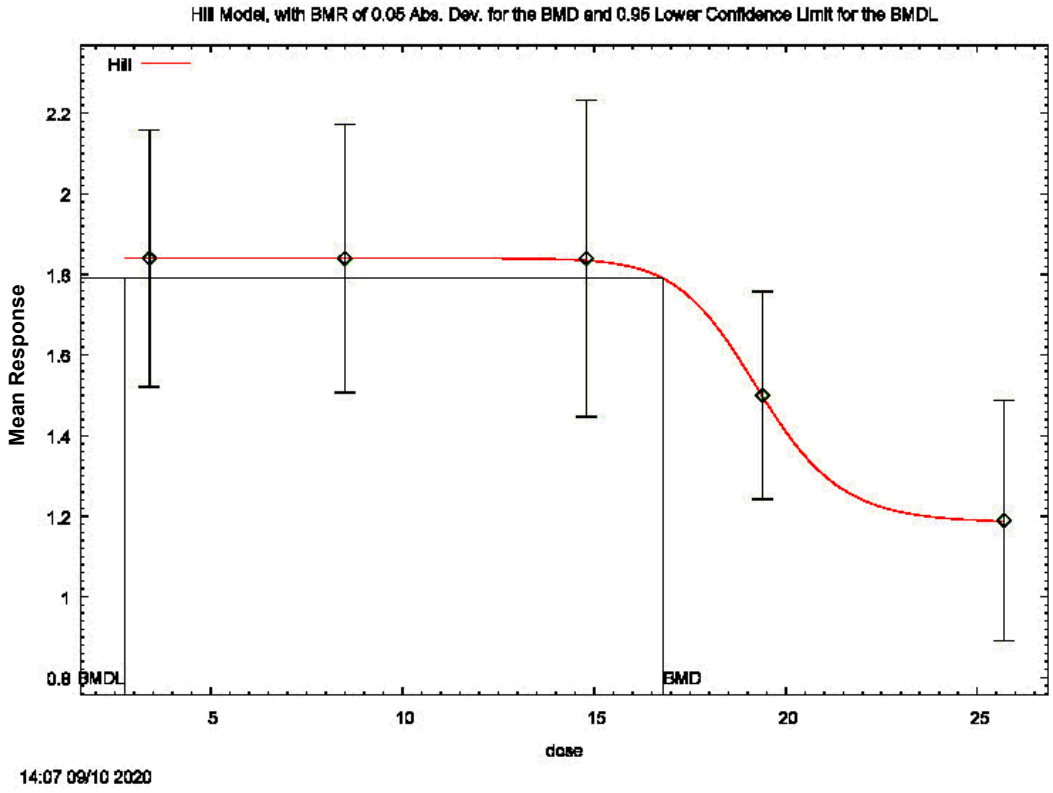
APPENDIX 10. BENCHMARK DOSE MODELING

This appendix provides the BMD modeling outputs for toxicity data that were amenable to dose-response modeling. All models are run with default parameters and a benchmark response of 5% for dichotomous data and one standard deviation from the control mean for continuous data. When appropriate, some models for continuous data are run with modeled variance instead of the default constant variance. Model selection criteria when comparing outputs of different models for the same endpoint/dataset are: scaled residual ≤ the absolute value of two, goodness of fit p-value ≥ 0.05,[28] the Akaike's information criterion (AIC), and visual inspection of the dose-response curve. The lower limit of the 95% confidence interval of the BMD resulting in the benchmark response, the BMDL, is selected as the POD. The model selected for each study to derive a POD is presented below.

Benchmark Dose Analysis Results for Noncancer Endpoints

Perfluorooctanoic Acid

Figure A10.1. BMD modeling of decreased influenza (Hib) antibody levels in humans from Abraham et al. (2020)



[28] US EPA's Benchmark Dose Technical Guidance (2012) suggests using a goodness of fit p-value ≥ 0.1; however, models with less adequate fit (goodness of fit p-value ≥ 0.05) may be used when other criteria are taken into account, such as variability in the endpoint and visual fit.

Model run output for Figure A10.1: Hill Model for decreased influenza (Hib) antibody levels in humans from Abraham et al. (2020).
Note, the benchmark response factor (BMRF, or user defined benchmark response) was derived by OEHHA as follows:

Power(10, at dose Q1) = power(10, 1.84) = 69.2 mg/dl;

10% decrease = 69.2 mg/dl × 0.90 = 62.3 mg/dl;

Log_{10}(62.3 mg/dl) = 1.79.

So, a 10% decrease from Q1 corresponds to a log_{10} value of 1.79, or a difference of 1.84 – 1.79 = 0.05 on the log_{10} scale.

Thus, the BMRF is an absolute deviation of 0.05.

```
================================================================
Hill Model. (Version: 2.18;  Date: 03/14/2017)
Input Data File: C:/Users/csteinmaus/Documents/BMDS/BMDS2704/Data/hil_Abraham PFOA
Hib quintiles_Opt.(d)
Gnuplot Plotting File:  C:/Users/csteinmaus/Documents/BMDS/BMDS2704/Data/hil_Abraham
PFOA Hib quintiles_Opt.plt
Thu Sep 10 14:07:15 2020
================================================================
BMDS Model Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
The form of the response function is:
Y[dose] = intercept + v*dose^n/(k^n + dose^n)

Dependent variable = Mean
Independent variable = Dose
rho is set to 0
Power parameter restricted to be greater than 1
A constant variance model is fit

Total number of dose groups = 5
Total number of records with missing values = 0
Maximum number of iterations = 500
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

Default Initial Parameter Values
alpha =  0.469219
rho =  0 Specified
intercept =  1.84
v =  -0.65
n =  8.78409
k =  19.1971

Asymptotic Correlation Matrix of Parameter Estimates
 ( *** The model parameter(s)  -rho    -n
```

have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	lalpha	intercept	v	k
lalpha	1	-5.7e-008	2.1e-007	-3.9e-007
intercept	-5.7e-008	1	-0.47	-0.25
v	2.1e-007	-0.47	1	-0.31
k	-3.9e-007	-0.25	-0.31	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval	
			Lower Conf. Limit	Upper Conf. Limit
alpha	0.445284	0.0636119	0.320606	0.569961
intercept	1.84178	0.0868517	1.67155	2.012
v	-0.655607	0.181528	-1.0114	-0.299819
n	18	NA		
k	19.3091	1.1682	17.0194	21.5987

NA - Indicates that this parameter has hit a bound
implied by some inequality constraint and thus
has no standard error.

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
3.4	20	1.84	1.84	0.68	0.667	-0.0119
8.5	20	1.84	1.84	0.71	0.667	-0.0119
14.8	20	1.84	1.84	0.84	0.667	0.0244
19.4	20	1.5	1.5	0.55	0.667	-0.000805
25.7	18	1.19	1.19	0.6	0.667	0.000231

Model Descriptions for likelihoods calculated
Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2
Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2
Model A3: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2
Model A3 uses any fixed variance parameters that
were specified by the user

Model R: Yi = Mu + e(i)
Var{e(i)} = Sigma^2

Likelihoods of Interest

Model	Log(likelihood)	# Param's	AIC
A1	-9.356408	6	30.712816
A2	-7.232118	10	34.464235
A3	-9.356408	6	30.712816
fitted	-9.356848	4	26.713697
R	-16.086495	2	36.172990

Explanation of Tests
Test 1: Do responses and/or variances differ among Dose levels?
(A2 vs. R)
Test 2: Are Variances Homogeneous? (A1 vs. A2)
Test 3: Are variances adequately modeled? (A2 vs. A3)
Test 4: Does the Model for the Mean Fit? (A3 vs. fitted)
(Note: When rho=0 the results of Test 3 and Test 2 will be the same.)

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	17.7088	8	0.02352
Test 2	4.24858	4	0.3734
Test 3	4.24858	4	0.3734
Test 4	0.000880611	2	0.9996

The p-value for Test 1 is less than .05. There appears to be a difference between response and/or variances among the dose levels
It seems appropriate to model the data

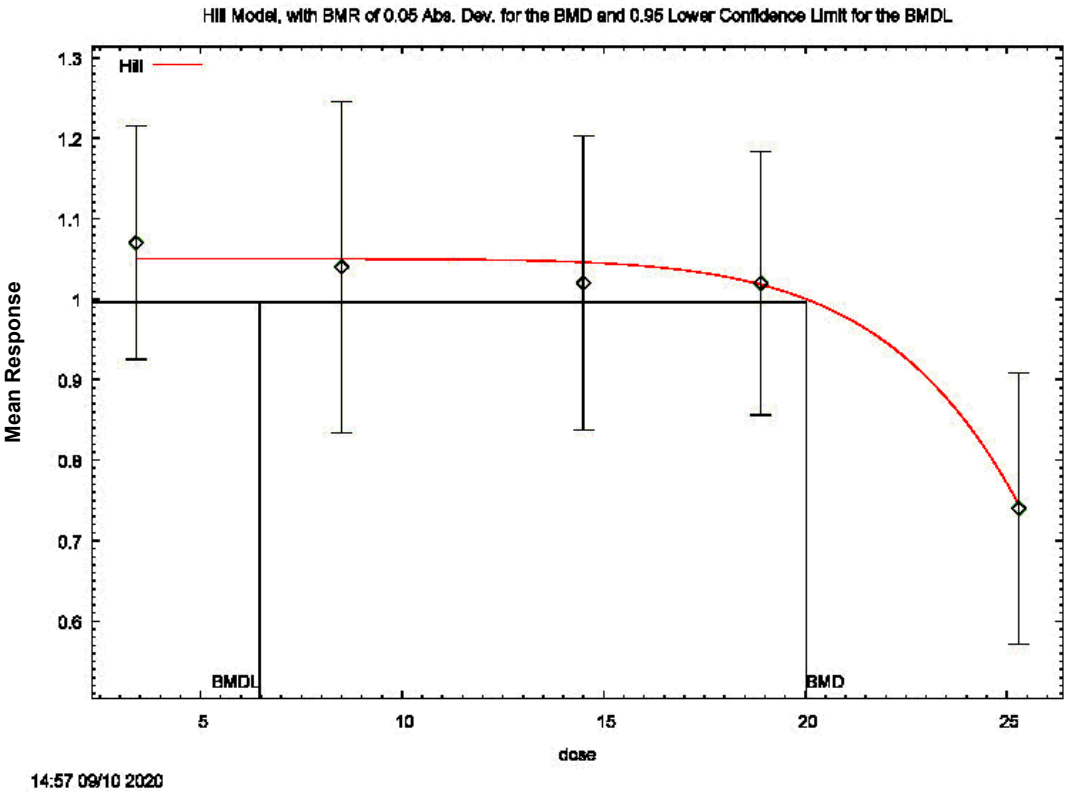
The p-value for Test 2 is greater than .1. A homogeneous variance model appears to be appropriate here

The p-value for Test 3 is greater than .1. The modeled variance appears to be appropriate here

The p-value for Test 4 is greater than .1. The model chosen seems to adequately describe the data

Benchmark Dose Computation
Specified effect = 0.05
Risk Type = Absolute deviation
Confidence level = 0.95
BMD = 16.8106
BMDL = 2.75177
BMDU = 19.2936

Figure A10.2. BMD modeling of decreased tetanus antibody levels in humans from Abraham et al. (2020)



Model run output for Figure A10.2: Hill Model for decreased tetanus antibody levels in humans from Abraham et al. (2020).
Note, the BMRF was derived by OEHHA as follows:

Power(10, at dose Q1) = power(10, 1.07) = 11.75 mg/L (mean antibody cocentration at dose Q1);

10% decrease = 11.75 mg/L × 0.90 = 10.57 mg/L;

Log_{10}(10.57 mg/L) = 1.02.
So, a 10% decrease from Q1 corresponds to a log_{10} value of 1.02, or a difference of 1.07 – 1.02 = 0.05 on the log_{10} scale.
Thus, the BMRF is an absolute deviation of 0.05.
==========
Hill Model. (Version: 2.18; Date: 03/14/2017)
Input Data File: C:/Users/csteinmaus/Documents/BMDS/BMDS2704/Data/hil_Abraham PFOA tetanus quintiles_Opt.(d)
Gnuplot Plotting File: C:/Users/csteinmaus/Documents/BMDS/BMDS2704/Data/hil_Abraham PFOA tetanus quintiles_Opt.plt
Thu Sep 10 14:57:53 2020
==========
BMDS Model Run
~~~~~~~~~~
~~~~~~~~~~

The form of the response function is:
Y[dose] = intercept + v*dose^n/(k^n + dose^n)

Dependent variable = Mean
Independent variable = Dose
rho is set to 0
Power parameter restricted to be greater than 1
A constant variance model is fit

Total number of dose groups = 5
Total number of records with missing values = 0
Maximum number of iterations = 500
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

Default Initial Parameter Values
alpha = 0.13878
rho = 0 Specified
intercept = 1.07
v = -0.33
n = 6.68878
k = 21.5286

Asymptotic Correlation Matrix of Parameter Estimates
(*** The model parameter(s) -rho
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	lalpha	intercept	n	v	k
lalpha	1	-1.6e-005	0.00062	8.6e-005	-0.00062
intercept	-1.6e-005	1	-0.025	-0.62	0.07
v	0.00062	-0.025	1	0.14	-1
n	8.6e-005	-0.62	0.14	1	-0.21
k	-0.00062	0.07	-1	-0.21	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval	
			Lower Conf. Limit	Upper Conf. Limit
alpha	0.132066	0.018677	0.09546	0.168672
intercept	1.04647	0.0533738	0.941856	1.15108
v	-79.6551	8878.19	-17480.6	17321.3
n	7.75749	11.8916	-15.5497	31.0647
k	51.79	756.924	-1431.75	1535.33

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
3.4	20	1.07	1.05	0.31	0.363	0.29
8.5	20	1.04	1.05	0.44	0.363	-0.0788
14.5	20	1.02	1.04	0.39	0.363	-0.275

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
18.9	20	1.02	1.01	0.35	0.363	0.0679
25.3	20	0.74	0.74	0.36	0.363	-0.0034

Model Descriptions for likelihoods calculated
Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2
Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2
Model A3: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2
Model A3 uses any fixed variance parameters that
were specified by the user

Model R: Yi = Mu + e(i)
Var{e(i)} = Sigma^2

Likelihoods of Interest

Model	Log(likelihood)	# Param's	AIC
A1	51.307931	6	-90.615863
A2	52.669574	10	-85.339147
A3	51.307931	6	-90.615863
fitted	51.222612	5	-92.445225
R	46.092155	2	-88.184310

Explanation of Tests
Test 1: Do responses and/or variances differ among Dose levels?
(A2 vs. R)
Test 2: Are Variances Homogeneous? (A1 vs. A2)
Test 3: Are variances adequately modeled? (A2 vs. A3)
Test 4: Does the Model for the Mean Fit? (A3 vs. fitted)
(Note: When rho=0 the results of Test 3 and Test 2 will be the same.)

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	13.1548	8	0.1066
Test 2	2.72328	4	0.6051
Test 3	2.72328	4	0.6051
Test 4	0.170638	1	0.6795

The p-value for Test 1 is greater than .05. There may not be a
diffence between responses and/or variances among the dose levels
Modelling the data with a dose/response curve may not be appropriate

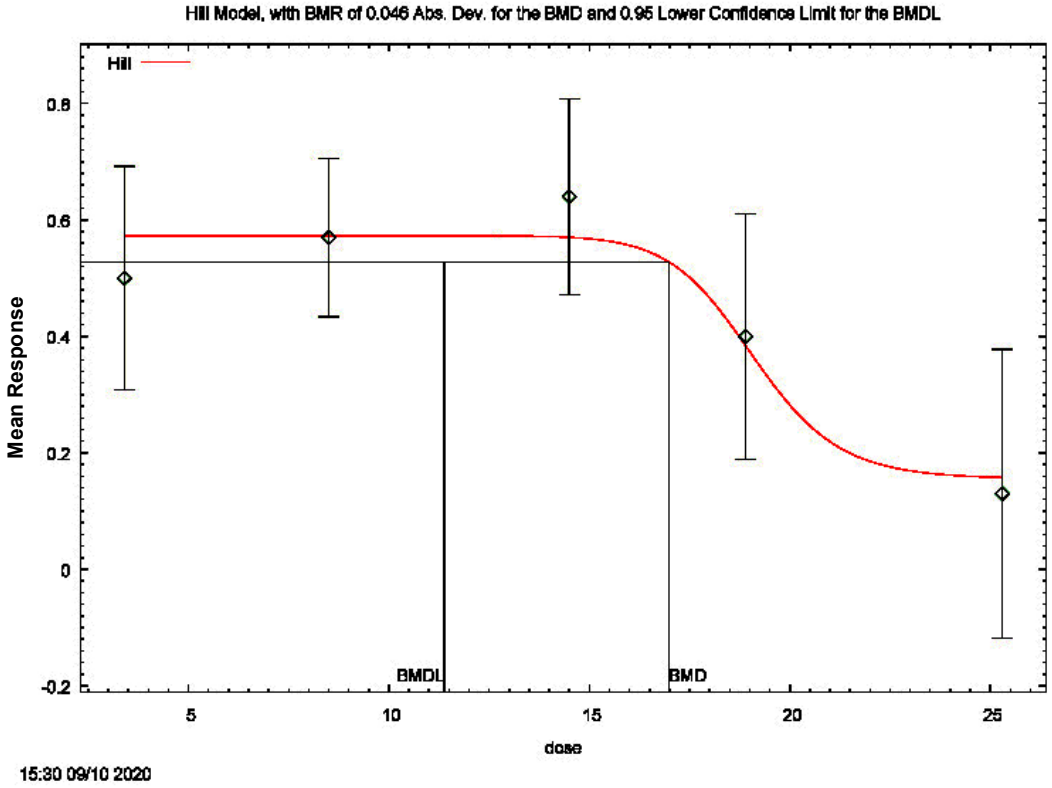
The p-value for Test 2 is greater than .1. A homogeneous variance
model appears to be appropriate here

The p-value for Test 3 is greater than .1. The modeled variance appears
to be appropriate here

The p-value for Test 4 is greater than .1. The model chosen seems to adequately describe the data

Benchmark Dose Computation
Specified effect = 0.05
Risk Type = Absolute deviation
Confidence level = 0.95
BMD = 20.0211
BMDL = 6.4617
BMDU = 23.8054

Figure A10.3. BMD modeling of decreased diphtheria antibody levels in humans from Abraham et al. (2020)



Model run output for Figure A10.3: Hill Model for decreased diphtheria antibody levels in humans from Abraham et al. (2020).
Note, the BMRF was derived by OEHHA as follows:

Power(10, at dose Q1) = power(10, 0.50) = 3.16 IU/ml (mean antibody concentration at dose Q1);

10% decrease = 3.16 IU/ml × 0.90 = 2.846 IU/ml;

Log_{10}(2.846 IU/ml) = 0.454.

So, a 10% decrease from Q1 corresponds to a log_{10} value of 0.454, or a difference of 0.50 –

0.454 = 0.046 on the $\log_{10}$ scale.

Thus, the BMRF is an absolute deviation of 0.046.

```
================================================================
Hill Model. (Version: 2.18;  Date: 03/14/2017)
Input Data File: C:/Users/csteinmaus/Documents/BMDS/BMDS2704/Data/hil_Abraham PFOA
diph quintiles_Opt.(d)
Gnuplot Plotting File:  C:/Users/csteinmaus/Documents/BMDS/BMDS2704/Data/hil_Abraham
PFOA diph quintiles_Opt.plt
Thu Sep 10 15:30:13 2020
================================================================
BMDS Model Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
The form of the response function is:
Y[dose] = intercept + v*dose^n/(k^n + dose^n)

Dependent variable = Mean
Independent variable = Dose
Power parameter restricted to be greater than 1
The variance is to be modeled as Var(i) = exp(lalpha  + rho * ln(mean(i)))
Total number of dose groups = 5
Total number of records with missing values = 0
Maximum number of iterations = 500
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

Default Initial Parameter Values
lalpha = -1.75423
rho =  0
intercept = 0.5
v = -0.37
n = 9.80546
k = 20.9148

Asymptotic Correlation Matrix of Parameter Estimates
( *** The model parameter(s)  -n
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix )
```

	lalpha	rho	intercept	v	k
lalpha	1	0.92	0.17	-0.65	0.38
rho	0.92	1	0.093	-0.77	0.41
intercept	0.17	0.093	1	-0.39	-0.2
v	-0.65	-0.77	-0.39	1	-0.39
k	0.38	0.41	-0.2	-0.39	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval	
			Lower Conf. Limit	Upper Conf. Limit
lalpha	-2.3853	0.398083	-3.16553	-1.60507

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval	
			Lower Conf. Limit	Upper Conf. Limit
rho	-0.606513	0.480212	-1.54771	0.334686
intercept	0.572972	0.0462347	0.482353	0.66359
v	-0.417998	0.12579	-0.664542	-0.171455
n	18	NA		
k	19.0701	1.05478	17.0027	21.1374

NA - Indicates that this parameter has hit a bound
implied by some inequality constraint and thus
has no standard error.

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
3.4	20	0.5	0.573	0.41	0.359	-0.908
8.5	20	0.57	0.573	0.29	0.359	-0.037
14.5	20	0.64	0.57	0.36	0.36	0.87
18.9	20	0.4	0.381	0.45	0.407	0.211
25.3	20	0.13	0.158	0.53	0.531	-0.232

Model Descriptions for likelihoods calculated
Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2
Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2
Model A3: Yij = Mu(i) + e(ij)
Var{e(ij)} = exp(lalpha + rho*ln(Mu(i)))
Model A3 uses any fixed variance parameters that
were specified by the user

Model R: Yi = Mu + e(i)
Var{e(i)} = Sigma^2

Likelihoods of Interest

Model	Log(likelihood)	# Param's	AIC
A1	40.276290	6	-68.552579
A2	44.254859	10	-68.509717
A3	43.092126	7	-72.184252
fitted	42.034964	5	-74.069927
R	31.491216	2	-58.982431

Explanation of Tests
Test 1: Do responses and/or variances differ among Dose levels?
(A2 vs. R)
Test 2: Are Variances Homogeneous? (A1 vs. A2)
Test 3: Are variances adequately modeled? (A2 vs. A3)
Test 4: Does the Model for the Mean Fit? (A3 vs. fitted)
(Note: When rho=0 the results of Test 3 and Test 2 will be the same.)

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	25.5273	8	0.001265
Test 2	7.95714	4	0.09316
Test 3	2.32547	3	0.5077
Test 4	2.11432	2	0.3474

The p-value for Test 1 is less than .05. There appears to be a difference between response and/or variances among the dose levels It seems appropriate to model the data

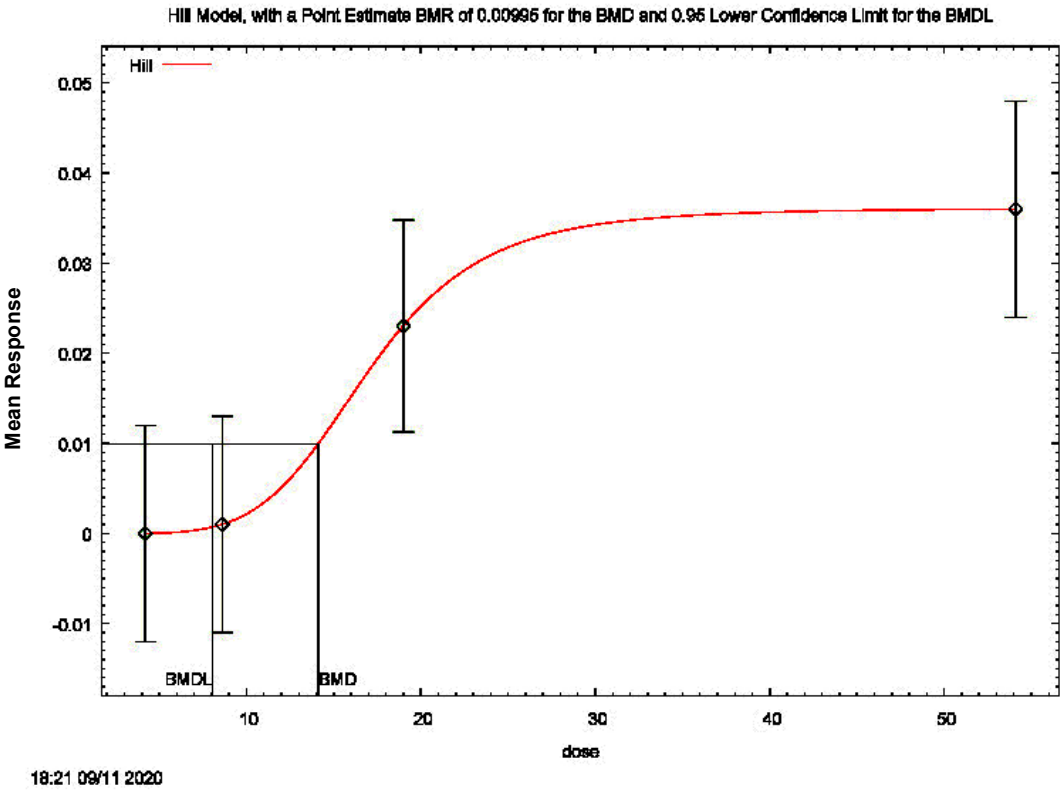
The p-value for Test 2 is less than .1. A non-homogeneous variance model appears to be appropriate

The p-value for Test 3 is greater than .1. The modeled variance appears to be appropriate here

The p-value for Test 4 is greater than .1. The model chosen seems to adequately describe the data

Benchmark Dose Computation
Specified effect = 0.046
Risk Type = Absolute deviation
Confidence level = 0.95
BMD = 16.9793
BMDL = 11.3762
BMDU = 19.2778

Figure A10.4. BMD modeling of increased mean ALT concentrations in humans from Darrow et al. (2016)



Model run output for Figure A10.4: Hill Model for increased mean ALT levels in humans from Darrow et al. (2016).
Note, the BMRF was derived by OEHHA as follows:

A BMR of 1% would give a b of:

b = ln(1 + 0.01) = 0.00995. Thus, 0.00995 was used as the BMR of 1%.
Furthermore, the model did not converge with all quintiles, thus, quintile 5 was excluded.
==========
Hill Model. (Version: 2.18; Date: 03/14/2017)
Input Data File: C:/Users/csteinmaus/Documents/BMDS/BMDS2704/Data/hil_Darrow PFOA ALT means b 4 levels_Opt.(d)
Gnuplot Plotting File: C:/Users/csteinmaus/Documents/BMDS/BMDS2704/Data/hil_Darrow PFOA ALT means b 4 levels_Opt.plt
Fri Sep 11 18:21:52 2020
==========
BMDS Model Run
~~~~~~~~~~
The form of the response function is:

Y[dose] = intercept + v*dose^n/(k^n + dose^n)

Dependent variable = Mean
~~~~~~~~~~

Independent variable = Dose
rho is set to 0
Power parameter restricted to be greater than 1
A constant variance model is fit

Total number of dose groups = 4
Total number of records with missing values = 0
Maximum number of iterations = 500
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

Default Initial Parameter Values
alpha = 0.228025
rho = 0 Specified
intercept = 0
v = 0.036
n = 18
k = 21.3636

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -rho
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	lalpha	intercept	n	v	k
lalpha	1	-2.1e-008	4.9e-009	-1.1e-008	-4.1e-008
intercept	-2.1e-008	1	-0.75	0.71	0.7
v	4.9e-009	-0.75	1	-0.62	-0.38
n	-1.1e-008	0.71	-0.62	1	0.81
k	-4.1e-008	0.7	-0.38	0.81	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval	
			Lower Conf. Limit	Upper Conf. Limit
alpha	0.227988	0.00205653	0.223957	0.232019
intercept	-2.59953e-005	0.00640626	-0.012582	0.01253
v	0.0361177	0.00950303	0.0174921	0.0547433
n	5.1685	11.1492	-16.6836	27.0206
k	17.0337	5.10574	7.02659	27.0407

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
4.2	6145	0	-1.41e-008	0.48	0.477	2.32e-006
8.6	6145	0.001	0.001	0.48	0.477	-2.46e-007
19	6145	0.023	0.023	0.47	0.477	-5.79e-006
54.1	6145	0.036	0.036	0.48	0.477	1.72e-006

Degrees of freedom for Test A3 vs. fitted <= 0

Model Descriptions for likelihoods calculated
Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2
Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2
Model A3: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2
Model A3 uses any fixed variance parameters that
were specified by the user

Model R: Yi = Mu + e(i)
Var{e(i)} = Sigma^2

Likelihoods of Interest

Model	Log(likelihood)	# Param's	AIC
A1	5880.307248	5	-11750.614495
A2	5882.335686	8	-11748.671371
A3	5880.307248	5	-11750.614495
fitted	5880.307248	5	-11750.614495
R	5867.834254	2	-11731.668509

Explanation of Tests
Test 1: Do responses and/or variances differ among Dose levels?
(A2 vs. R)
Test 2: Are Variances Homogeneous? (A1 vs. A2)
Test 3: Are variances adequately modeled? (A2 vs. A3)
Test 4: Does the Model for the Mean Fit? (A3 vs. fitted)
(Note: When rho=0 the results of Test 3 and Test 2 will be the same.)

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	29.0029	6	<.0001
Test 2	4.05688	3	0.2554
Test 3	4.05688	3	0.2554
Test 4	4.36557e-011	0	NA

The p-value for Test 1 is less than .05. There appears to be a
difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is greater than .1. A homogeneous variance
model appears to be appropriate here

The p-value for Test 3 is greater than .1. The modeled variance appears
to be appropriate here

NA - Degrees of freedom for Test 4 are less than or equal to 0. The Chi-Square
test for fit is not valid

Benchmark Dose Computation

Specified effect = 0.00995
Risk Type = Point estimate
Confidence level = 0.95
BMD = 14.1371
BMDL = 8.04229
BMDU = 18.5908

BMD modeling of increased ORs for high ALT in humans from Gallo et al. (2012)

Note: When all ten deciles are used, logprobit was the best fitting model (p=0.18; Figure A10.5A). Although p >0.10, this model resulted in a very steep dose-response curve at the lower doses (almost vertical) and an unusually low BMD (0.43 ng/ml), which did not correlate well with the observed data. Because of this, and because the fit was much improved with the logistic when the higher doses were removed, only deciles 1-5 were used in these BMD calculations (Figure A10.5B).

Figure A10.5A: BMD modeling of increased ORs for high ALT in humans from Gallo et al. (2012) (all ten deciles plotted)

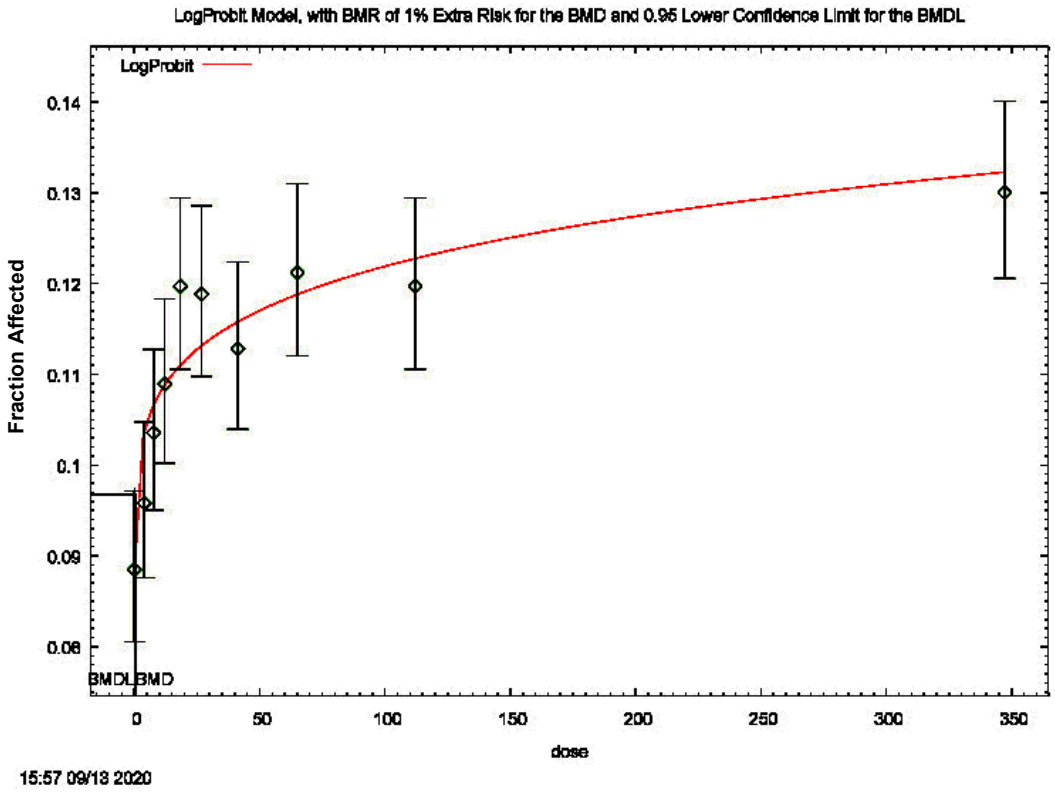
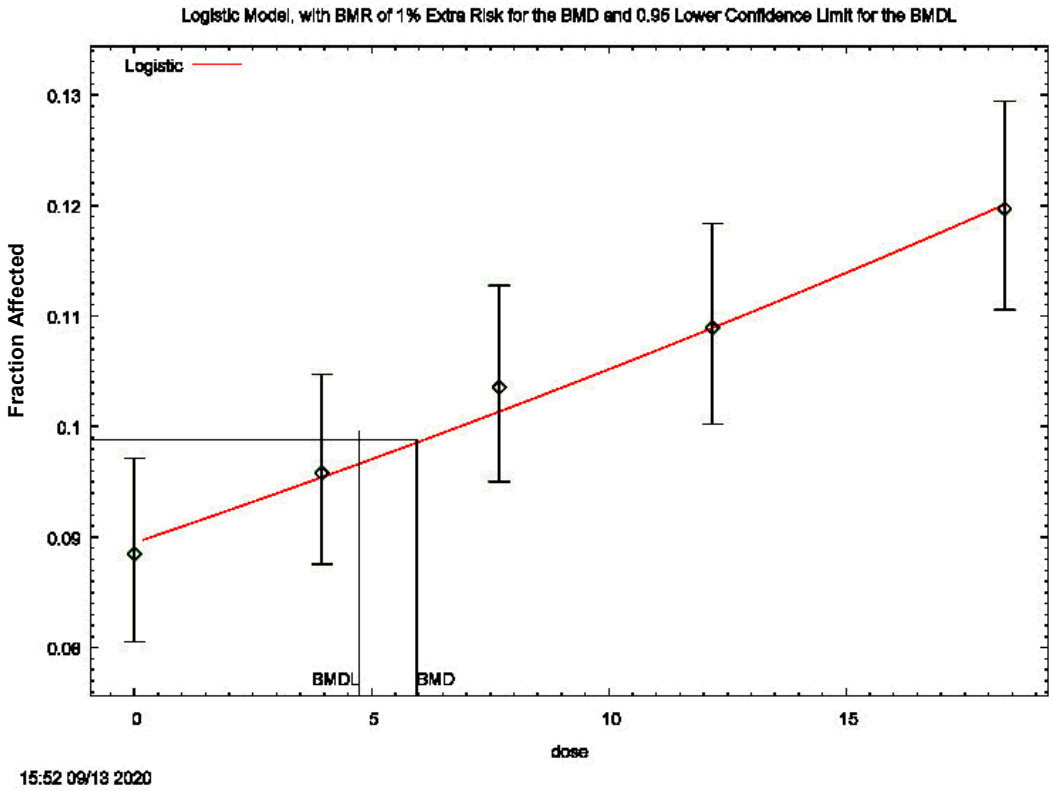


Figure A10.5B: BMD modeling of increased ORs for high ALT in humans from Gallo et al. (2012) (deciles 1-5 plotted)



Model run output for Figure A10.5B: Logistic Model for increased ORs for high ALT levels in humans from Gallo et al. (2012).

```
==============================================================================
Logistic Model. (Version: 2.15; Date: 3/20/2017)
Input Data File: C:/Users/csteinmaus/Documents/BMDS/BMDS2704/Data/log_Gallo PFOA ALT ORs 5_Opt.(d)
Gnuplot Plotting File:  C:/Users/csteinmaus/Documents/BMDS/BMDS2704/Data/log_Gallo PFOA ALT ORs 5_Opt.plt
Sun Sep 13 15:52:04 2020
==============================================================================
 BMDS_Model_Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
The form of the probability function is:
P[response] = 1/[1+EXP(-intercept-slope*dose)]

Dependent variable = case
Independent variable = dose0
Slope parameter is not restricted

Total number of observations = 5
Total number of records with missing values = 0
Maximum number of iterations = 500
Relative Function Convergence has been set to: 1e-008
```

Parameter Convergence has been set to: 1e-008

Default Initial Parameter Values
background = 0 Specified
intercept = -2.31763
slope = 0.018037

Asymptotic Correlation Matrix of Parameter Estimates
(*** The model parameter(s) -background
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	intercept	slope
intercept	1	-0.81
slope	-0.81	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval	
			Lower Conf. Limit	Upper Conf. Limit
intercept	-2.3176	0.0371119	-2.39034	-2.24487
slope	0.0179348	0.0033493	0.0113703	0.0244993

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-7702.32	5			
Fitted model	-7702.47	2	0.302117	3	0.9596
Reduced model	-7716.75	1	28.8656	4	<.0001

AIC: 15408.9

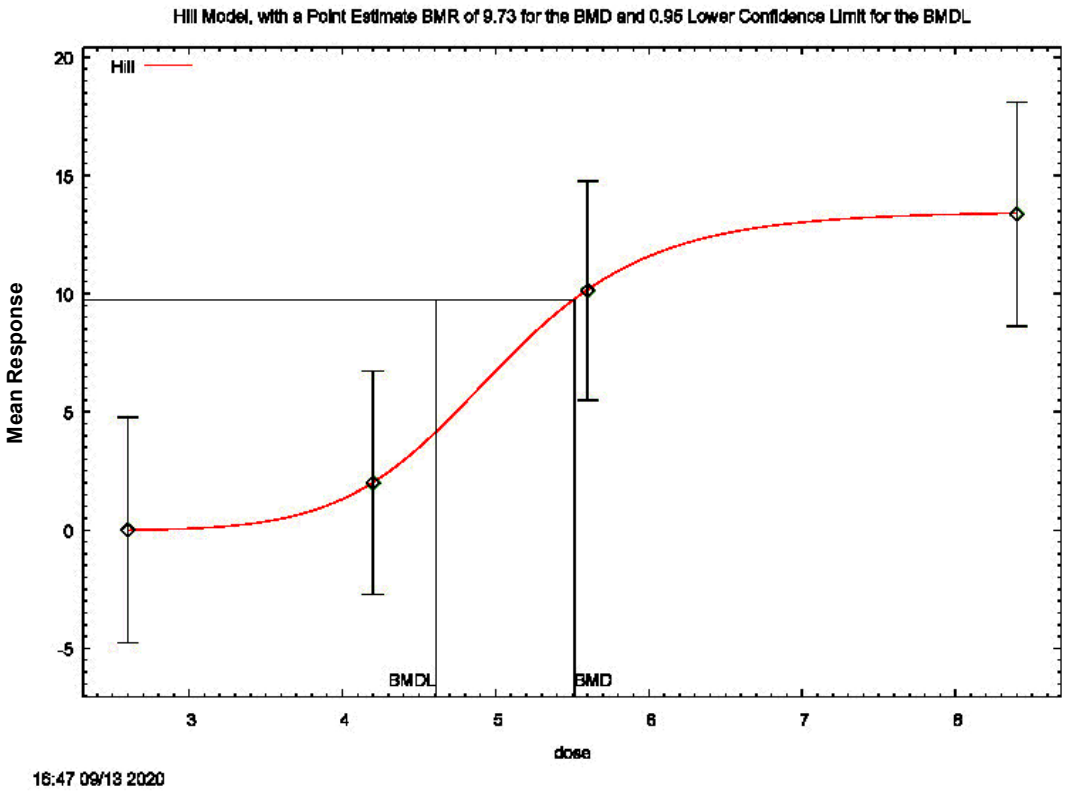
Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0897	416.543	411.000	4645.000	-0.285
3.9500	0.0956	444.198	445.000	4645.000	0.040
7.6900	0.1016	471.884	481.000	4645.000	0.443
12.1800	0.1092	507.135	506.000	4645.000	-0.053
18.3400	0.1204	559.242	556.000	4645.000	-0.146

Chi^2 = 0.30 d.f. = 3 P-value = 0.9595

Benchmark Dose Computation
Specified effect = 0.01
Risk Type = Extra risk
Confidence level = 0.95
BMD = 5.9513
BMDL = 4.74341
BMDU = 8.24403

Figure A10.6. BMD modeling of increased mean serum total cholesterol in humans from Lin et al. (2019)



Model run output for Figure A10.6: Hill Model for increased mean serum total cholesterol levels in humans from Lin et al. (2019).

Note, the BMRF was derived by OEHHA as follows. The mean TC values in each quartile were not provided. However, the average TC in all subjects combined was provided. This overall average could be used, in combination with the mean differences, to estimate the mean TC in each quartile. These calculations are given in the "lipid reference level calculations" excel spreadsheet. Based on these calculations, the estimated mean TC in quartile 1 is 194.6. A 5% increase in this value is a mean difference of 194.6 × 5% = 9.73. Thus, 9.73 was used as the BMR of 5%.

```
================================================================
Hill Model. (Version: 2.18;  Date: 03/14/2017)
Input Data File: C:/Users/csteinmaus/Documents/BMDS/BMDS2704/Data/hil_Lin PFOA
TC_Opt.(d)
Gnuplot Plotting File:  C:/Users/csteinmaus/Documents/BMDS/BMDS2704/Data/hil_Lin PFOA
TC_Opt.plt
Sun Sep 13 16:47:22 2020
================================================================
BMDS Model Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
The form of the response function is:
Y[dose] = intercept + v*dose^n/(k^n + dose^n)

Dependent variable = Mean
```

Independent variable = Dose
rho is set to 0
Power parameter restricted to be greater than 1
A constant variance model is fit

Total number of dose groups = 4
Total number of records with missing values = 0
Maximum number of iterations = 500
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

Default Initial Parameter Values
alpha = 1286.34
rho = 0 Specified
intercept = 0
v = 13.36
n = 18
k = 6.1941

Asymptotic Correlation Matrix of Parameter Estimates
(*** The model parameter(s) -rho
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	lalpha	intercept	n	v	k
lalpha	1	-9.6e-009	3.6e-009	1.9e-009	3.1e-009
intercept	-9.6e-009	1	-0.72	0.5	0.53
v	3.6e-009	-0.72	1	-0.59	-0.045
n	1.9e-009	0.5	-0.59	1	0.2
k	3.1e-009	0.53	-0.045	0.2	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval	
			Lower Conf. Limit	Upper Conf. Limit
alpha	1280.61	60.4356	1162.16	1399.06
intercept	-0.0203507	2.47126	-4.86394	4.82324
v	13.4584	3.62057	6.36216	20.5545
n	9.92358	7.34258	-4.46761	24.3148
k	5.00174	0.577992	3.8689	6.13459

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
2.6	221	0	-5.08e-007	35.9	35.8	2.11e-007
4.2	222	2	2	35.7	35.8	2.84e-007
5.6	227	10.1	10.1	35.5	35.8	1.69e-007
8.4	228	13.4	13.4	36.4	35.8	9.4e-008

Degrees of freedom for Test A3 vs. fitted <= 0
Model Descriptions for likelihoods calculated
Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2

Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2
Model A3: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2
Model A3 uses any fixed variance parameters that
were specified by the user

Model R: Yi = Mu + e(i)
Var{e(i)} = Sigma^2

Likelihoods of Interest

Model	Log(likelihood)	# Param's	AIC
A1	-3661.635617	5	7333.271234
A2	-3661.551213	8	7339.102426
A3	-3661.635617	5	7333.271234
fitted	-3661.635617	5	7333.271234
R	-3672.265777	2	7348.531554

Explanation of Tests
Test 1: Do responses and/or variances differ among Dose levels?
(A2 vs. R)
Test 2: Are Variances Homogeneous? (A1 vs. A2)
Test 3: Are variances adequately modeled? (A2 vs. A3)
Test 4: Does the Model for the Mean Fit? (A3 vs. fitted)
(Note: When rho=0 the results of Test 3 and Test 2 will be the same.)

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	21.4291	6	0.001536
Test 2	0.168809	3	0.9825
Test 3	0.168809	3	0.9825
Test 4	9.09495e-013	0	NA

The p-value for Test 1 is less than .05. There appears to be a
difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is greater than .1. A homogeneous variance
model appears to be appropriate here

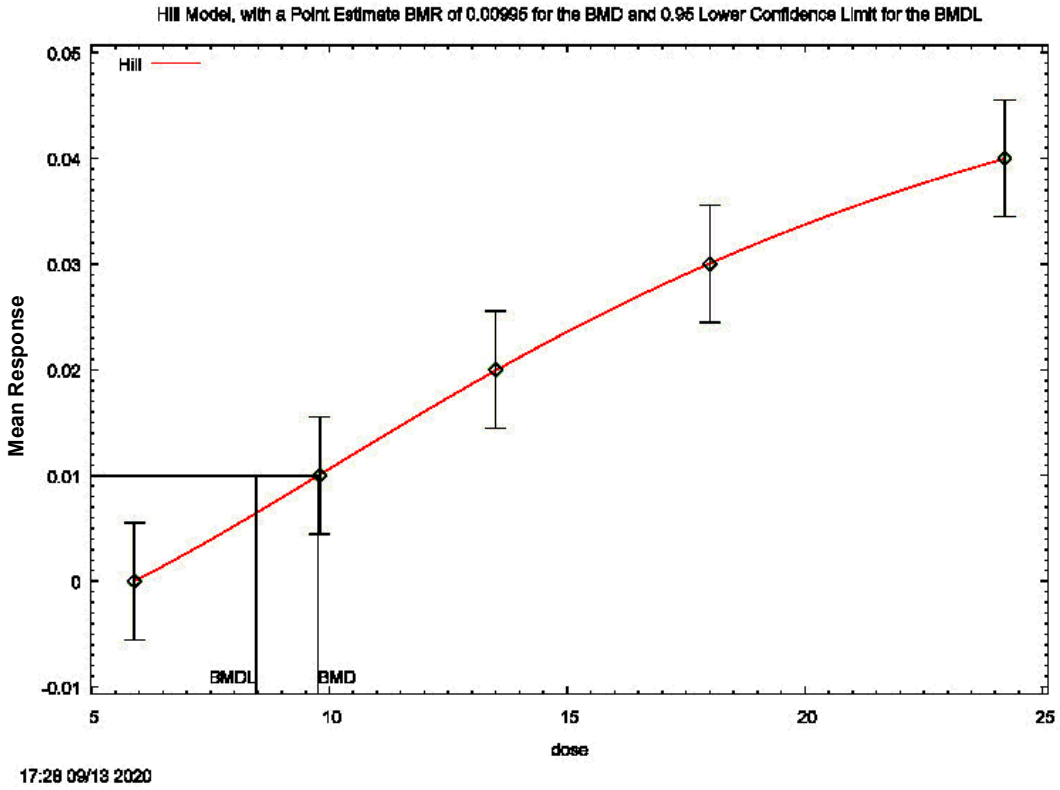
The p-value for Test 3 is greater than .1. The modeled variance appears
to be appropriate here

NA - Degrees of freedom for Test 4 are less than or equal to 0. The Chi-Square
test for fit is not valid

Benchmark Dose Computation
Specified effect = 9.73
Risk Type = Point estimate
Confidence level = 0.95

BMD = 5.51357
BMDL = 4.61048
BMDU = 7.05482

Figure A10.7. BMD modeling of increased mean serum total cholesterol in humans from Steenland et al. (2009)



Model run output for Figure A10.7: Hill Model for increased mean serum total cholesterol levels from Steenland et al. (2009).

Note, the BMRF was derived by OEHHA as follows. A 5% increase was within the range of the observed effects but close to the upper limit (the percent increase in the highest decile was 5.1%). Because of this, a BMR of 1% was used.
Percent change in TC = [exp(b) - 1] x 100%
or, exp(b) = percent change + 1
or, exp(b) = 0.01 + 1 = 1.01
or, b = ln(1.01) = 0.00995.
Thus, b = 0.00995 was used as the BMR of 1%.

==========

Hill Model. (Version: 2.18; Date: 03/14/2017)
Input Data File: C:/Users/csteinmaus/Documents/BMDS/BMDS2704/Data/hil_Steenland PFOA TC means 5_Opt.(d)
Gnuplot Plotting File: C:/Users/csteinmaus/Documents/BMDS/BMDS2704/Data/hil_Steenland PFOA TC means 5_Opt.plt
Sun Sep 13 17:28:05 2020

==========

BMDS Model Run
~~~~~~

The form of the response function is:
Y[dose] = intercept + v*dose^n/(k^n + dose^n)

Dependent variable = Mean
Independent variable = Dose
rho is set to 0
Power parameter restricted to be greater than 1
A constant variance model is fit

Total number of dose groups = 5
Total number of records with missing values = 0
Maximum number of iterations = 500
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

Default Initial Parameter Values
alpha = 0.0370352
rho = 0 Specified
intercept = 0
v = 0.04
n = 18
k = 13.5

Asymptotic Correlation Matrix of Parameter Estimates
(*** The model parameter(s) -rho
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	lalpha	intercept	n	v	k
lalpha	1	2.1e-008	-1.3e-008	2.1e-008	-6.5e-009
intercept	2.1e-008	1	-0.93	0.95	-0.75
v	-1.3e-008	-0.93	1	-0.99	0.94
n	2.1e-008	0.95	-0.99	1	-0.9
k	-6.5e-009	-0.75	0.94	-0.9	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval	
			Lower Conf. Limit	Upper Conf. Limit
alpha	0.0370272	0.000344197	0.0363526	0.0377018
intercept	-0.00722434	0.0160511	-0.038684	0.0242353
v	0.0688417	0.0656045	-0.0597409	0.197424
n	2.07285	2.18476	-2.2092	6.3549
k	16.6084	9.65775	-2.32041	35.5373

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
5.9	4629	0	-1.18e-005	0.192	0.192	0.00416
9.8	4629	0.01	0.0101	0.192	0.192	-0.0185
13.5	4629	0.02	0.0199	0.192	0.192	0.0298
~~~~~~

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
18	4629	0.03	0.0301	0.192	0.192	-0.0213
24.2	4629	0.04	0.04	0.192	0.192	0.00581

Model Descriptions for likelihoods calculated
Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2
Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2
Model A3: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2
Model A3 uses any fixed variance parameters that
were specified by the user

Model R: Yi = Mu + e(i)
Var{e(i)} = Sigma^2

Likelihoods of Interest

Model	Log(likelihood)	# Param's	AIC
A1	26571.646401	6	-53131.292802
A2	26571.646401	10	-53123.292802
A3	26571.646401	6	-53123.292802
fitted	26571.645532	5	-53133.291064
R	26509.306509	2	-53014.613018

Explanation of Tests
Test 1: Do responses and/or variances differ among Dose levels?
(A2 vs. R)
Test 2: Are Variances Homogeneous? (A1 vs. A2)
Test 3: Are variances adequately modeled? (A2 vs. A3)
Test 4: Does the Model for the Mean Fit? (A3 vs. fitted)
(Note: When rho=0 the results of Test 3 and Test 2 will be the same.)

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	124.68	8	<.0001
Test 2	0	4	1
Test 3	0	4	1
Test 4	0.00173865	1	0.9667

The p-value for Test 1 is less than .05. There appears to be a
difference between response and/or variances among the dose levels
It seems appropriate to model the data

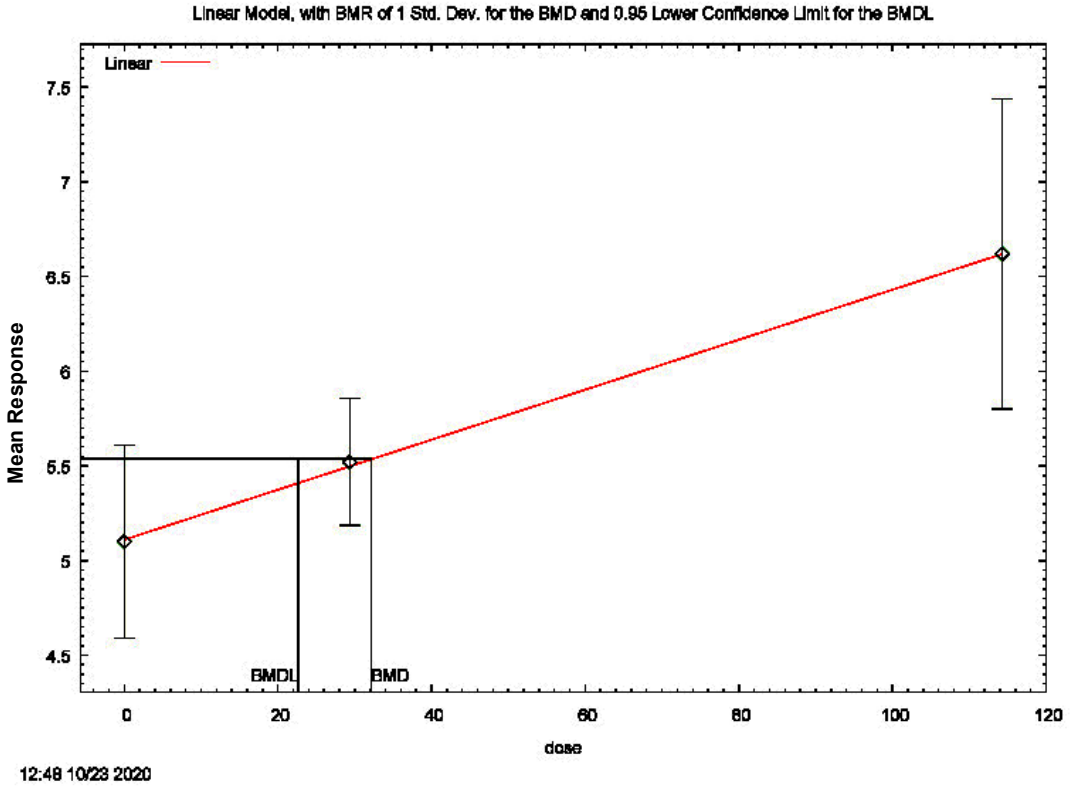
The p-value for Test 2 is greater than .1. A homogeneous variance
model appears to be appropriate here

The p-value for Test 3 is greater than .1. The modeled variance appears
to be appropriate here

The p-value for Test 4 is greater than .1. The model chosen seems
to adequately describe the data

Benchmark Dose Computation
Specified effect = 0.00995
Risk Type = Point estimate
Confidence level = 0.95
BMD = 9.76261
BMDL = 8.46747
BMDU = 11.1839

Figure A10.8. BMD modeling of increased relative liver weight weight in male BALB/c mice from Yu et al. (2016)



Model run output for Figure A10.8: BMD modeling of increased relative liver weight in male BALB/c mice from Yu et al. (2016).

```
================================================================
Polynomial Model. (Version: 2.21;  Date: 03/14/2017)
Input Data File: K:/BMD saved files/Chemicals/PFOA/lin_Yu 2016 rel liv weight_Opt.(d)
Gnuplot Plotting File:  K:/BMD saved files/Chemicals/PFOA/lin_Yu 2016 rel liv weight_Opt.plt
Fri Oct 23 12:48:38 2020
 ================================================================
 BMDS Model Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
The form of the response function is:
Y[dose] = beta_0 + beta_1*dose + beta_2*dose^2 + ...
```

Dependent variable = Mean
Independent variable = Dose
rho is set to 0
Signs of the polynomial coefficients are not restricted
A constant variance model is fit
Total number of dose groups = 3
Total number of records with missing values = 0
Maximum number of iterations = 500
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008
Default Initial Parameter Values
alpha = 0.225533
rho = 0 Specified
beta_0 = 5.1136
beta_1 = 0.0132208
Asymptotic Correlation Matrix of Parameter Estimates
(*** The model parameter(s) -rho
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	alpha	beta_0	beta_1
alpha	1	1.3e-009	1.1e-009
beta_0	1.3e-009	1	-0.7
beta_1	1.1e-009	-0.7	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval	
			Lower Conf. Limit	Upper Conf. Limit
alpha	0.180611	0.0659499	0.0513518	0.309871
beta_0	5.1136	0.154253	4.81127	5.41593
beta_1	0.0132208	0.00226408	0.00878333	0.0176584

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
0.011	5	5.1	5.11	0.41	0.425	-0.0723
29.34	5	5.52	5.5	0.27	0.425	0.0973
114.3	5	6.62	6.62	0.66	0.425	-0.025

Model Descriptions for likelihoods calculated
Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2
Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2
Model A3: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2
Model A3 uses any fixed variance parameters that were specified by the user
Model R: Yi = Mu + e(i)
Var{e(i)} = Sigma^2

Likelihoods of Interest

Model	Log(likelihood)	# Param's	AIC
A1	5.343231	4	-2.686463
A2	7.255811	6	-2.511622
A3	5.343231	4	-2.686463
fitted	5.335563	3	-4.671127
R	-3.557768	2	11.115535

Explanation of Tests
Test 1: Do responses and/or variances differ among Dose levels?
(A2 vs. R)
Test 2: Are Variances Homogeneous? (A1 vs. A2)
Test 3: Are variances adequately modeled? (A2 vs. A3)
Test 4: Does the Model for the Mean Fit? (A3 vs. fitted)
(Note: When rho=0 the results of Test 3 and Test 2 will be the same.)

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	21.6272	4	0.0002377
Test 2	3.82516	2	0.1477
Test 3	3.82516	2	0.1477
Test 4	0.0153362	1	0.9014

The p-value for Test 1 is less than .05. There appears to be a difference between response and/or variances among the dose levels
It seems appropriate to model the data

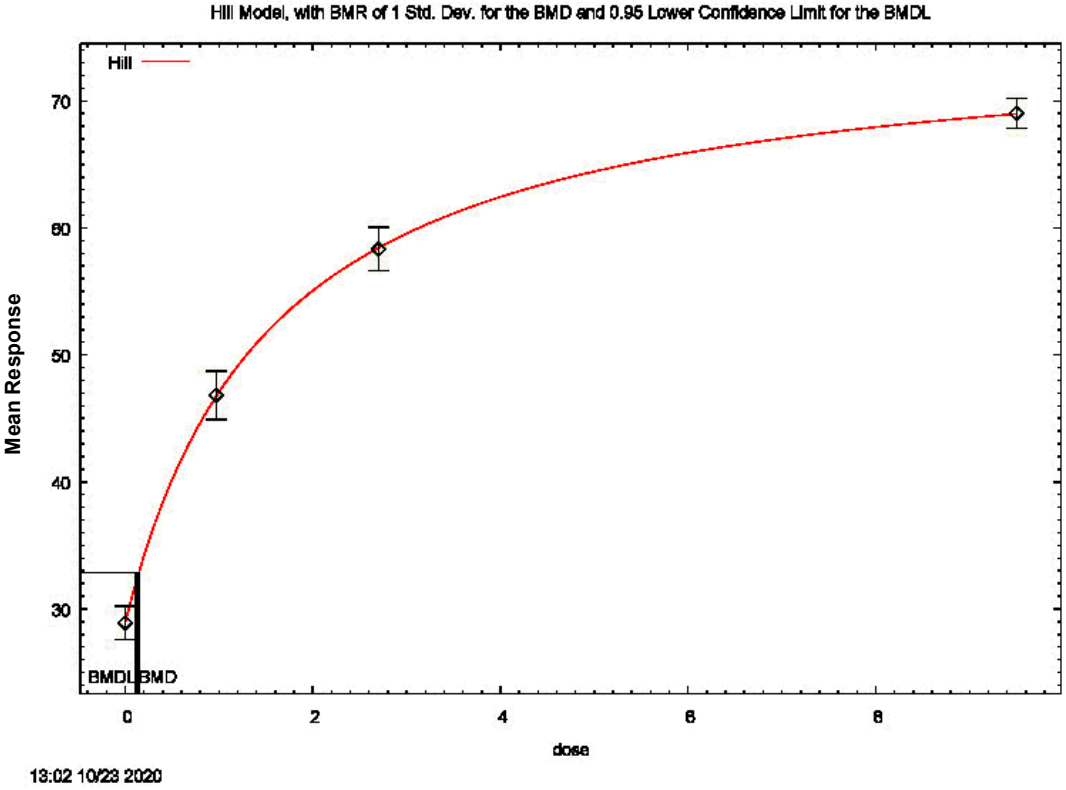
The p-value for Test 2 is greater than .1. A homogeneous variance model appears to be appropriate here

The p-value for Test 3 is greater than .1. The modeled variance appears to be appropriate here

The p-value for Test 4 is greater than .1. The model chosen seems to adequately describe the data

Benchmark Dose Computation
Specified effect = 1
Risk Type = Estimated standard deviations from the control mean
Confidence level = 0.95
BMD = 32.145
BMDL = 22.6469
BMDU = 53.8869

Figure A10.9. BMD modeling of increased p53 levels in male BALB/c mice Li et al. (2017)



Model run output for Figure A10.9: BMD modeling of increased p53 levels in male BALB/c mice Li et al. (2017).

```
================================================================================
Hill Model. (Version: 2.18;  Date: 03/14/2017)
Input Data File: K:/BMD saved files/Chemicals/PFOA/hil_Li female p53_Opt.(d)
Gnuplot Plotting File:  K:/BMD saved files/Chemicals/PFOA/hil_Li female p53_Opt.plt
Fri Oct 23 13:02:53 2020
================================================================================
BMDS Model Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
The form of the response function is:
Y[dose] = intercept + v*dose^n/(k^n + dose^n)
Dependent variable = Mean
Independent variable = Dose
Power parameter restricted to be greater than 1
The variance is to be modeled as Var(i) = exp(lalpha  + rho * ln(mean(i)))
Total number of dose groups = 4
Total number of records with missing values = 0
Maximum number of iterations = 500
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

Default Initial Parameter Values
lalpha = 2.84693
```

rho = 0
intercept = 28.8963
v = 40.1338
n = 0.0741803
k = 4.10814

Asymptotic Correlation Matrix of Parameter Estimates
(*** The model parameter(s) -n
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	lalpha	rho	intercept	v	k
lalpha	1	-1	0.041	0.045	0.088
rho	-1	1	-0.042	-0.045	-0.088
intercept	0.041	-0.042	1	-0.38	0.44
v	0.045	-0.045	-0.38	1	0.55
k	0.088	-0.088	0.44	0.55	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval	
			Lower Conf. Limit	Upper Conf. Limit
lalpha	2.41404	1.80205	-1.11792	5.946
rho	0.102867	0.463534	-0.805643	1.01138
intercept	28.9066	0.721242	27.493	30.3203
v	46.6915	1.39224	43.9628	49.4203
n	1	NA		
k	1.56858	0.153601	1.26753	1.86963

NA - Indicates that this parameter has hit a bound
implied by some inequality constraint and thus
has no standard error.

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
0	30	28.9	28.9	3.48	3.98	-0.0142
0.97	30	46.8	46.7	5.08	4.07	0.101
2.7	30	58.3	58.4	4.55	4.12	-0.15
9.5	30	69	69	3.21	4.16	0.0643

Model Descriptions for likelihoods calculated
Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2
Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2
Model A3: Yij = Mu(i) + e(ij)
Var{e(ij)} = exp(lalpha + rho*ln(Mu(i)))
Model A3 uses any fixed variance parameters that were specified by the user
Model R: Yi = Mu + e(i)
Var{e(i)} = Sigma^2

Likelihoods of Interest

Model	Log(likelihood)	# Param's	AIC
A1	-228.781417	5	467.562835
A2	-224.581124	8	465.162248
A3	-228.756484	6	469.512968
fitted	228.775516	-5	467.551033
R	-388.287724	2	780.575449

Explanation of Tests
Test 1: Do responses and/or variances differ among Dose levels? (A2 vs. R)
Test 2: Are Variances Homogeneous? (A1 vs. A2)
Test 3: Are variances adequately modeled? (A2 vs. A3)
Test 4: Does the Model for the Mean Fit? (A3 vs. fitted)
(Note: When rho=0 the results of Test 3 and Test 2 will be the same.)

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	327.413	6	<.0001
Test 2	8.40059	3	0.03842
Test 3	8.35072	2	0.01537
Test 4	0.038065	1	0.8453

The p-value for Test 1 is less than .05. There appears to be a difference between response and/or variances among the dose levels It seems appropriate to model the data

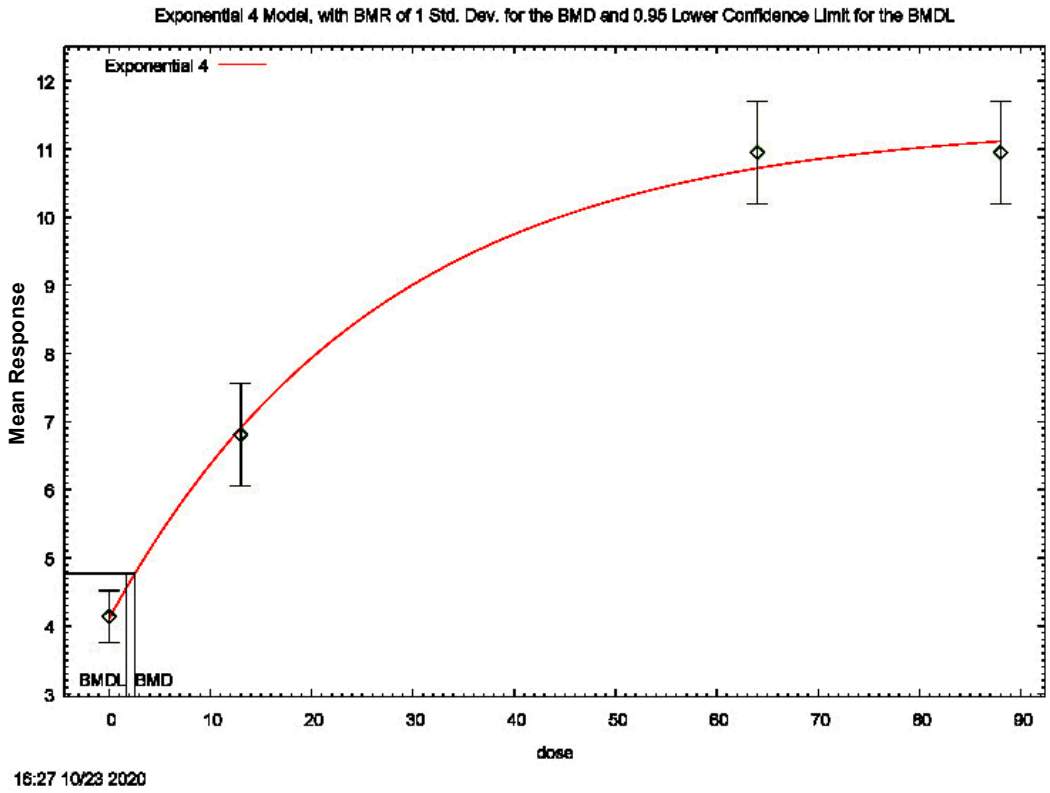
The p-value for Test 2 is less than .1. A non-homogeneous variance model appears to be appropriate

The p-value for Test 3 is less than .1. You may want to consider a different variance model

The p-value for Test 4 is greater than .1. The model chosen seems to adequately describe the data

Benchmark Dose Computation
Specified effect = 1
Risk Type = Estimated standard deviations from the control mean
Confidence level = 0.95
BMD = 0.145968
BMDL = 0.112334
BMDU = 0.2355

Figure A10.10. BMD modeling of increased relative liver weight in male BALB/c mice from Guo et al. (2019)



Model run output for Figure A10.10: BMD modeling of increased relative liver weight in male BALB/c mice from Guo et al. (2019).

```
==========================================================================
Exponential Model. (Version: 1.11;  Date: 03/14/2017)
Input Data File: K:/BMD saved files/Chemicals/PFOA/exp_Guo 2019 rel liv weight_Opt.(d)
Gnuplot Plotting File:
Fri Oct 23 16:27:42 2020
==========================================================================
 BMDS Model Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
The form of the response function by Model:
Model 2:     Y[dose] = a * exp{sign * b * dose}
Model 3:     Y[dose] = a * exp{sign * (b * dose)^d}
Model 4:     Y[dose] = a * [c-(c-1) * exp{-b * dose}]
Model 5:     Y[dose] = a * [c-(c-1) * exp{-(b * dose)^d}]
Note: Y[dose] is the median response for exposure = dose;
sign = +1 for increasing trend in data;
sign = -1 for decreasing trend.

Model 2 is nested within Models 3 and 4.
Model 3 is nested within Model 5.
Model 4 is nested within Model 5.
```

Dependent variable = Mean
Independent variable = Dose
Data are assumed to be distributed: normally
Variance Model: exp(lnalpha +rho *ln(Y[dose]))
The variance is to be modeled as Var(i) = exp(lalpha + log(mean(i)) * rho)

Total number of dose groups = 4
Total number of records with missing values = 0
Maximum number of iterations = 500
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

MLE solution provided: Exact

Initial Parameter Values

Variable	Model 2	Model 3	Model 4	Model 5
lnalpha	-2.6178	-2.6178	-2.6178	-2.6178
rho	1.29136	1.29136	1.29136	1.29136
a	4.98718	4.98718	3.933	3.933
b	0.0102914	0.0102914	0.0337541	0.0337541
c	0 *	0 *	2.92334	2.92334
d	1 *	1	1 *	1

* Indicates that this parameter has been specified

Parameter Estimates by Model

Variable	Model 2	Model 3	Model 4	Model 5
lnalpha	-0.178462	-0.178462	-2.65372	-2.57698
rho	0.483039	0.483039	1.29459	1.25001
a	5.35374	5.35374	4.10528	4.12238
b	0.00909555	0.00909555	0.0373018	0.0547297
c	--	--	2.77402	2.65328
d	--	1	--	1.96892

-- Indicates that this parameter does not appear in model

Std. Err. Estimates by Model

Variable	Model 2	Model 3	Model 4	Model 5
lnalpha	1.5504	1.5504	1.17786	1.18104
rho	0.750434	0.750434	0.570423	0.572084
a	0.316622	0.316622	0.189448	0.193895
b	0.000907091	0.000907091	0.00650846	3.96733
c	NA	NA	0.149678	0.153129
d	NA	NA	NA	419.199

NA - Indicates that this parameter was specified (by the user or because of the model form) or has hit a bound implied by some inequality constraint and thus has no standard error.

Table of Stats From Input Data

Dose	N	Obs Mean	Obs Std Dev
0.02	12	4.14	0.6
13	12	6.81	1.19

Dose	N	Obs Mean	Obs Std Dev
64	12	10.95	1.19
88	12	10.95	1.19

Estimated Values of Interest

Model	Dose	Est Mean	Est Std Dev	Scaled Res.
2	0.02	5.355	1.372	-3.068
	13	6.026	1.411	1.925
	64	9.582	1.579	3.001
	88	11.92	1.664	-2.019
3	0.02	5.355	1.372	-3.068
	13	6.026	1.411	1.925
	64	9.582	1.579	3.001
	88	11.92	1.664	-2.019
4	0.02	4.111	0.6624	0.1531
	13	6.904	0.9266	-0.3506
	64	10.72	1.232	0.6495
	88	11.11	1.261	-0.4527
5	0.02	4.122	0.6682	0.09129
	13	6.852	0.9179	-0.1574
	64	10.94	1.23	0.03446
	88	10.94	1.23	0.03432

Other models for which likelihoods are calculated:
Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2
Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2
Model A3: Yij = Mu(i) + e(ij)
Var{e(ij)} = exp(lalpha + log(mean(i)) * rho)
Model R: Yij = Mu + e(i)
Var{e(ij)} = Sigma^2

Likelihoods of Interest

Model	Log(likelihood)	DF	AIC
A1	-25.30928	5	60.61856
A2	-22.04414	8	60.08828
A3	-23.09363	6	58.18726
R	-77.88171	2	159.7634
2	-43.5176	4	95.0352
3	-43.5176	4	95.0352
4	-23.42929	5	56.85859
5	-23.09363	6	58.18726

Additive constant for all log-likelihoods = -44.11. This constant added to the above values gives the log-likelihood including the term that does not depend on the model parameters.
Explanation of Tests
Test 1: Does response and/or variances differ among Dose levels? (A2 vs. R)
Test 2: Are Variances Homogeneous? (A2 vs. A1)

Test 3: Are variances adequately modeled? (A2 vs. A3)
Test 4: Does Model 2 fit the data? (A3 vs. 2)
Test 5a: Does Model 3 fit the data? (A3 vs. 3)
Test 5b: Is Model 3 better than Model 2? (3 vs. 2)

Test 6a: Does Model 4 fit the data? (A3 vs. 4)
Test 6b: Is Model 4 better than Model 2? (4 vs. 2)
Test 7a: Does Model 5 fit the data? (A3 vs. 5)
Test 7b: Is Model 5 better than Model 3? (5 vs. 3)
Test 7c: Is Model 5 better than Model 4? (5 vs. 4)

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	111.7	6	< 0.0001
Test 2	6.53	3	0.08848
Test 3	2.099	2	0.3501
Test 4	40.85	2	< 0.0001
Test 5a	40.85	2	< 0.0001
Test 5b	-1.421e-014	0	N/A
Test 6a	0.6713	1	0.4126
Test 6b	40.18	1	< 0.0001
Test 7a	1.048e-008	0	N/A
Test 7b	40.85	2	< 0.0001
Test 7c	0.6713	1	0.4126

The p-value for Test 1 is less than .05. There appears to be a difference between response and/or variances among the dose levels, it seems appropriate to model the data.

The p-value for Test 2 is less than .1. A non-homogeneous variance model appears to be appropriate.

The p-value for Test 3 is greater than .1. The modeled variance appears to be appropriate here.

The p-value for Test 4 is less than .1. Model 2 may not adequately describe the data; you may want to consider another model.

The p-value for Test 5a is less than .1. Model 3 may not adequately describe the data; you may want to consider another model.

Degrees of freedom for Test 5b are less than or equal to 0. The Chi-Square test for fit is not valid.

The p-value for Test 6a is greater than .1. Model 4 seems to adequately describe the data.

The p-value for Test 6b is less than .05. Model 4 appears to fit the data better than Model 2.

Degrees of freedom for Test 7a are less than or equal to 0.
The Chi-Square test for fit is not valid.

The p-value for Test 7b is less than .05. Model 5 appears to fit the data better than Model 3.

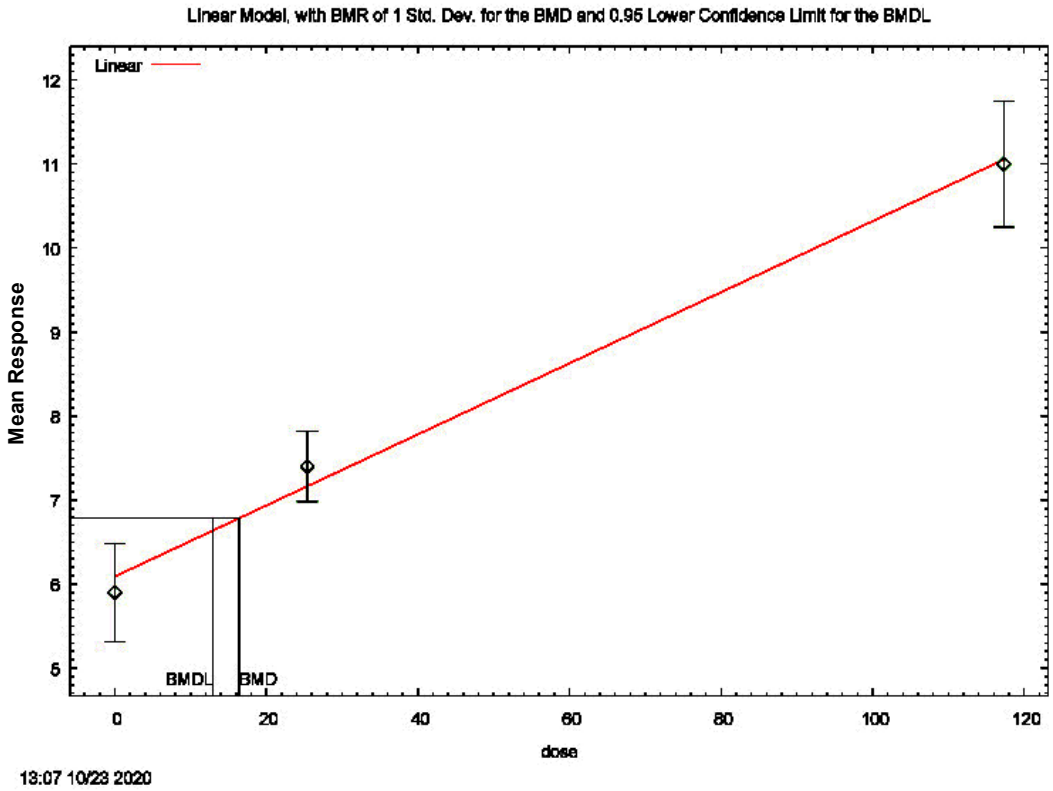
The p-value for Test 7c is greater than .05. Model 5 does not seem to fit the data better than Model 4.

Benchmark Dose Computations:
Specified Effect = 1.000000
Risk Type = Estimated standard deviations from control
Confidence Level = 0.950000

BMD and BMDL by Model

Model	BMD	BMDL	BMDU
2	25.077	18.7265	35.2831
3	25.077	18.7265	35.2831
4	2.55421	1.70954	4.13933
5	5.76493	1.79716	12.2396

Figure A10.11. BMD modeling of increased relative liver weight in pregnant CD-1 mice at ED 11.5 from Blake et al. (2020)



Model run output for Figure A10.11: BMD modeling of increased relative liver weight in pregnant CD-1 mice at ED 11.5 from Blake et al. (2020).

```
================================================================
Polynomial Model. (Version: 2.21;  Date: 03/14/2017)
Input Data File: K:/BMD saved files/Chemicals/PFOA/lin_Blake 2020 rel liv ED11_Opt.(d)
Gnuplot Plotting File:  K:/BMD saved files/Chemicals/PFOA/lin_Blake 2020 rel liv ED11_Opt.plt
Fri Oct 23 13:07:29 2020
================================================================
BMDS Model Run
~~~
```

Parameter Convergence has been set to: 1e-008
Default Initial Parameter Values
alpha = 0.516667
rho = 0 Specified
beta_0 = 6.08666
beta_1 = 0.0423268

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -rho
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	alpha	beta_0	beta_1
alpha	1	3.1e-009	4e-008
beta_0	3.1e-009	1	-0.69
beta_1	4e-008	-0.69	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval	
			Lower Conf. Limit	Upper Conf. Limit
alpha	0.483504	0.139576	0.209941	0.757067
beta_0	6.08666	0.195191	5.70409	6.46922
beta_1	0.0423268	0.0028169	0.0368058	0.0478478

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
0	8	5.9	6.09	0.7	0.695	-0.759
25.4	8	7.4	7.16	0.5	0.695	0.969
117.3	8	11	11.1	0.9	0.695	-0.21

Model Descriptions for likelihoods calculated
Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2
Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2
Model A3: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2
Model A3 uses any fixed variance parameters that
were specified by the user
Model R: Yi = Mu + e(i)
Var{e(i)} = Sigma^2

Likelihoods of Interest

Model	Log(likelihood)	# Param's	AIC
A1	-2.473335	4	12.946670
A2	-1.156162	6	14.312324
A3	-2.473335	4	12.946670
fitted	-3.279650	3	12.559300
R	-31.390009	2	66.780018

Explanation of Tests
Test 1: Do responses and/or variances differ among Dose levels?
(A2 vs. R)
Test 2: Are Variances Homogeneous? (A1 vs. A2)
Test 3: Are variances adequately modeled? (A2 vs. A3)
Test 4: Does the Model for the Mean Fit? (A3 vs. fitted)
(Note: When rho=0 the results of Test 3 and Test 2 will be the same.)

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	60.4677	4	<.0001
Test 2	2.63435	2	0.2679
Test 3	2.63435	2	0.2679
Test 4	1.61263	1	0.2041

The p-value for Test 1 is less than .05. There appears to be a difference between response and/or variances among the dose levels
It seems appropriate to model the data

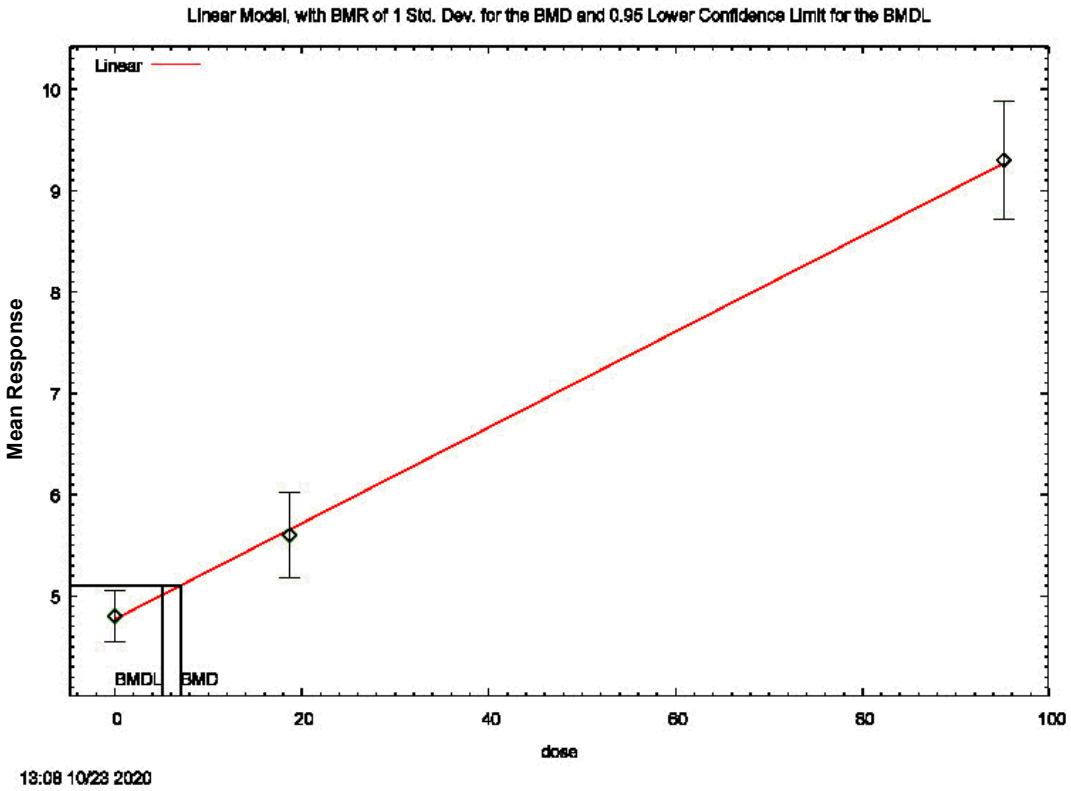
The p-value for Test 2 is greater than .1. A homogeneous variance model appears to be appropriate here

The p-value for Test 3 is greater than .1. The modeled variance appears to be appropriate here

The p-value for Test 4 is greater than .1. The model chosen seems to adequately describe the data

Benchmark Dose Computation
Specified effect = 1
Risk Type = Estimated standard deviations from the control mean
Confidence level = 0.95
BMD = 16.428
BMDL = 12.9484
BMDU = 21.9988

Figure A10.12. BMD modeling of increased relative liver weight in pregnant CD-1 mice at ED 17.5 from Blake et al. (2020)



Model run output for Figure A10.12: BMD modeling of increased relative liver weight in pregnant CD-1 mice at ED 17.5 from Blake et al. (2020).

```
================================================================
Polynomial Model. (Version: 2.21;  Date: 03/14/2017)
Input Data File: K:/BMD saved files/Chemicals/PFOA/lin_Blake 2020 rel liv ED17_Opt.(d)
Gnuplot Plotting File:  K:/BMD saved files/Chemicals/PFOA/lin_Blake 2020 rel liv ED17_Opt.plt
Fri Oct 23 13:08:54 2020
================================================================
BMDS Model Run
~~~
```

Default Initial Parameter Values
lalpha = -1.28494
rho = 0
beta_0 = 4.75952
beta_1 = 0.0476401

Asymptotic Correlation Matrix of Parameter Estimates

	lalpha	rho	beta_0	beta_1
lalpha	1	-0.99	-0.063	0.095
rho	-0.99	1	0.063	-0.096
beta_0	-0.063	0.063	1	-0.52
beta_1	0.095	-0.096	-0.52	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval	
			Lower Conf. Limit	Upper Conf. Limit
lalpha	-5.62452	2.05483	-9.65192	-1.59712
rho	2.19217	1.10534	0.0257544	4.35859
beta_0	4.76933	0.0996775	4.57397	4.96469
beta_1	0.0473241	0.00285874	0.0417211	0.0529271

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
0	8	4.8	4.77	0.3	0.333	0.261
18.7	8	5.6	5.65	0.5	0.401	-0.383
95.1	8	9.3	9.27	0.7	0.69	0.124

Model Descriptions for likelihoods calculated
Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2
Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2
Model A3: Yij = Mu(i) + e(ij)
Var{e(ij)} = exp(lalpha + rho*ln(Mu(i)))
Model A3 uses any fixed variance parameters that
were specified by the user
Model R: Yi = Mu + e(i)
Var{e(i)} = Sigma^2

Likelihoods of Interest

Model	Log(likelihood)	# Param's	AIC
A1	5.021679	4	-2.043358
A2	7.632736	6	-3.265472
A3	7.148940	5	-4.297880
fitted	7.078860	4	-6.157719
R	-28.885821	2	61.771641

Explanation of Tests

Test 1: Do responses and/or variances differ among Dose levels?

(A2 vs. R)
Test 2: Are Variances Homogeneous? (A1 vs. A2)
Test 3: Are variances adequately modeled? (A2 vs. A3)
Test 4: Does the Model for the Mean Fit? (A3 vs. fitted)
(Note: When rho=0 the results of Test 3 and Test 2 will be the same.)

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	73.0371	4	<.0001
Test 2	5.22211	2	0.07346
Test 3	0.967592	1	0.3253
Test 4	0.140161	1	0.7081

The p-value for Test 1 is less than .05. There appears to be a difference between response and/or variances among the dose levels It seems appropriate to model the data

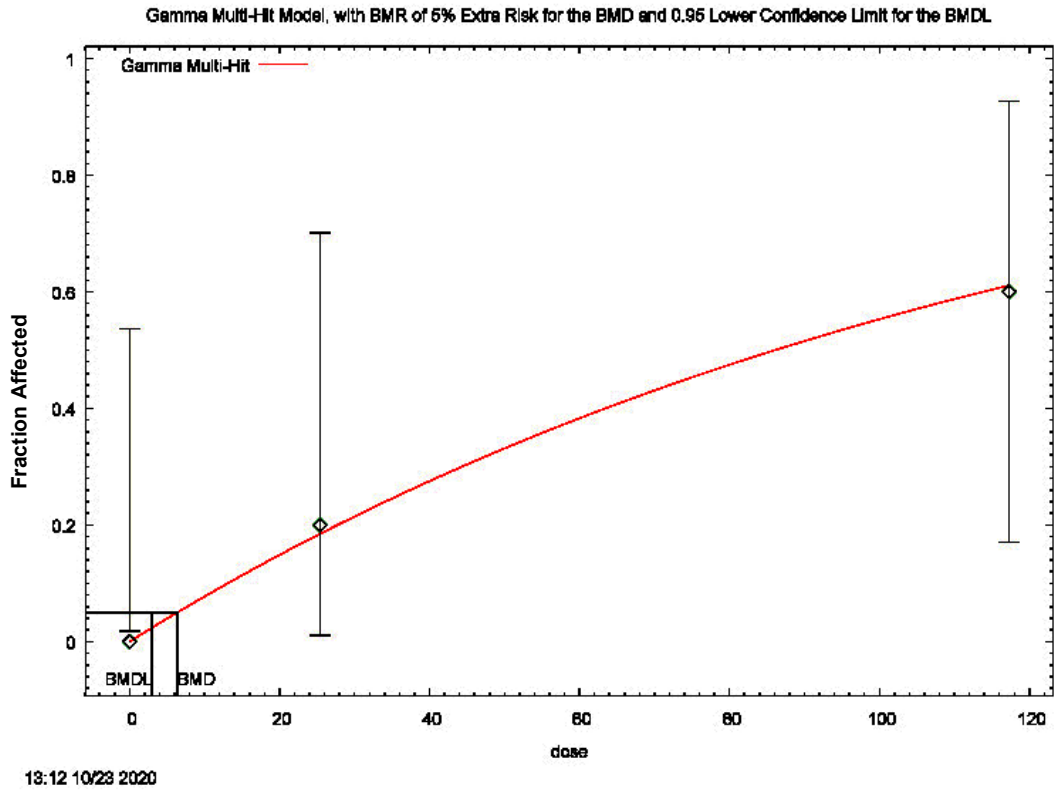
The p-value for Test 2 is less than .1. A non-homogeneous variance model appears to be appropriate

The p-value for Test 3 is greater than .1. The modeled variance appears to be appropriate here

The p-value for Test 4 is greater than .1. The model chosen seems to adequately describe the data

Benchmark Dose Computation
Specified effect = 1
Risk Type = Estimated standard deviations from the control mean
Confidence level = 0.95
BMD = 7.03424
BMDL = 5.06239
BMDU = 10.3874

Figure A10.13. BMD modeling of increased liver cell death in pregnant CD-1 mice at ED 11.5 from Blake et al. (2020)



Model run output for Figure A10.13: BMD modeling of increased liver cell death in pregnant CD-1 mice at ED 11.5 from Blake et al. (2020).

```
================================================================
Gamma Model. (Version: 2.17;  Date: 6/22/2017)
Input Data File: K:/BMD saved files/Chemicals/PFOA/gam_Blake 2020 liv cell death
ed11_Opt.(d)
Gnuplot Plotting File:  K:/BMD saved files/Chemicals/PFOA/gam_Blake 2020 liv cell death
ed11_Opt.plt
Fri Oct 23 13:12:01 2020
================================================================
BMDS_Model_Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
The form of the probability function is:
P[response]= background+(1-background)*CumGamma[slope*dose,power],
where CumGamma(.) is the cummulative Gamma distribution function
Dependent variable = Effect
Independent variable = Dose
Power parameter is restricted as power >=1
Total number of observations = 3
Total number of records with missing values = 0
Maximum number of iterations = 500
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008
```

Default Initial (and Specified) Parameter Values
Background = 0.142857
Slope = 0.00977874
Power = 1.3

Asymptotic Correlation Matrix of Parameter Estimates
(*** The model parameter(s) -Background -Power
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	Slope
Slope	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval	
			Lower Conf. Limit	Upper Conf. Limit
Background	0	NA		
Slope	0.00804597	0.00413652	-6.14598e-005	0.0161534
Power	1	NA		

NA - Indicates that this parameter has hit a bound
implied by some inequality constraint and thus
has no standard error.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-5.86707	3			
Fitted model	-5.87204	1	0.00994311	2	0.995
Reduced model	-8.69873	1	5.66331	2	0.05892

AIC: 13.7441

Goodness of Fit

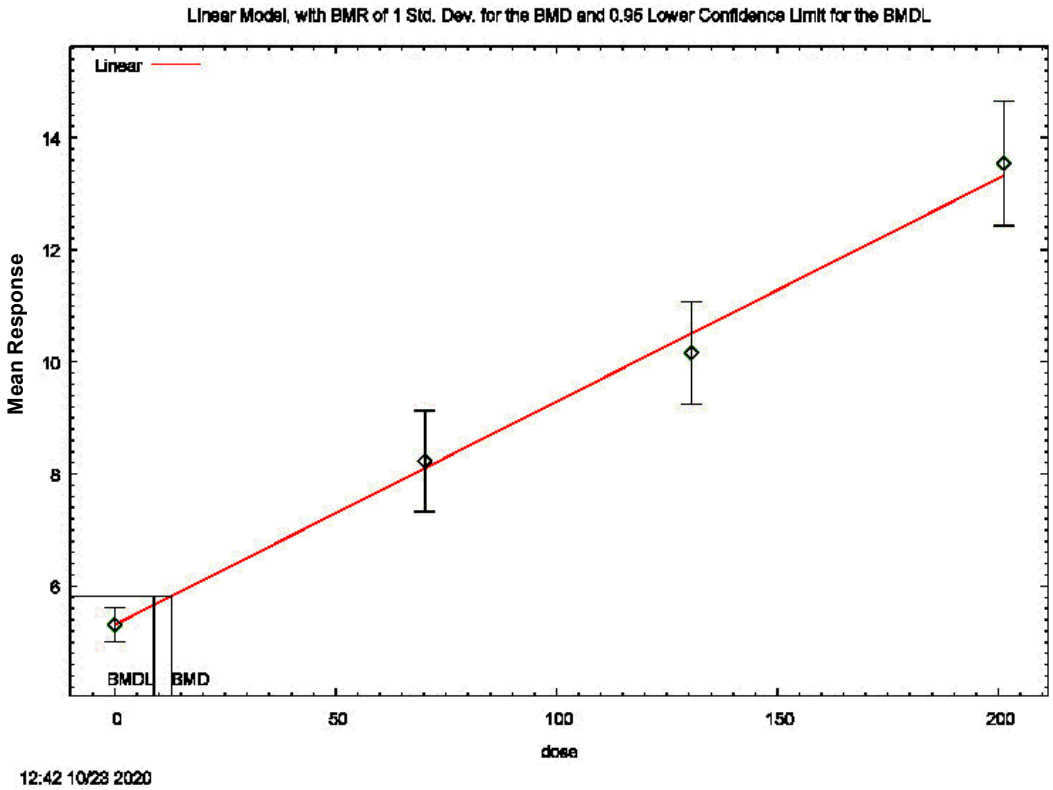
Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0000	0.000	0.000	5.000	0.000
25.4000	0.1848	0.924	1.000	5.000	0.087
117.3000	0.6109	3.054	3.000	5.000	-0.050

Chi^2 = 0.01 d.f. = 2 P-value = 0.9950

Benchmark Dose Computation
Specified effect = 0.05
Risk Type = Extra risk
Confidence level = 0.95
BMD = 6.37503
BMDL = 3.00033
BMDU = 89.7605

Perfluorooctane Sulfonic Acid

Figure A10.14. BMD modeling of increased relative liver weight in male C57BL/6 mice from Xing et al. (2016)



Model run output for Figure A10.14: BMD modeling of increased relative liver weight in male C57BL/6 mice from Xing et al. (2016).

```
================================================================
Polynomial Model. (Version: 2.21;  Date: 03/14/2017)
Input Data File: K:/BMD saved files/Chemicals/PFOS/lin_Xing 2016 liv weight_Opt.(d)
Gnuplot Plotting File:  K:/BMD saved files/Chemicals/PFOS/lin_Xing 2016 liv weight_Opt.plt
Fri Oct 23 12:42:38 2020
================================================================
BMDS Model Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
The form of the response function is:
Y[dose] = beta_0 + beta_1*dose + beta_2*dose^2 + ...
Dependent variable = Mean
Independent variable = Dose
Signs of the polynomial coefficients are not restricted
The variance is to be modeled as Var(i) = exp(lalpha + log(mean(i)) * rho)

Total number of dose groups = 4
Total number of records with missing values = 0
Maximum number of iterations = 500
Relative Function Convergence has been set to: 1e-008
```

Parameter Convergence has been set to: 1e-008

Default Initial Parameter Values
lalpha = 0.377751
rho = 0
beta_0 = 5.27261
beta_1 = 0.040168

Asymptotic Correlation Matrix of Parameter Estimates

	lalpha	rho	beta_0	beta_1
lalpha	1	-0.99	-0.078	0.083
rho	-0.99	1	0.076	-0.081
beta_0	-0.078	0.076	1	-0.54
beta_1	0.083	-0.081	-0.54	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval	
			Lower Conf. Limit	Upper Conf. Limit
lalpha	-5.91848	1.75177	-9.35188	-2.48508
rho	2.73789	0.798373	1.17311	4.30267
beta_0	5.31108	0.154427	5.00841	5.61375
beta_1	0.0397629	0.00222688	0.0353983	0.0441275

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
0.02	10	5.31	5.31	0.42	0.51	-0.0116
70.23	10	8.23	8.1	1.26	0.909	0.439
130.6	10	10.2	10.5	1.28	1.3	-0.835
201.2	10	13.5	13.3	1.56	1.79	0.4

Model Descriptions for likelihoods calculated
Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2
Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2
Model A3: Yij = Mu(i) + e(ij)
Var{e(ij)} = exp(lalpha + rho*ln(Mu(i)))
Model A3 uses any fixed variance parameters that
were specified by the user
Model R: Yi = Mu + e(i)
Var{e(i)} = Sigma^2

Likelihoods of Interest

Model	Log(likelihood)	# Param's	AIC
A1	-25.447815	5	60.895630
A2	-18.444360	8	52.888720
A3	-20.036279	6	52.072559
fitted	-20.764402	4	49.528805
R	-66.562230	2	137.124460

Explanation of Tests
Test 1: Do responses and/or variances differ among Dose levels?
(A2 vs. R)
Test 2: Are Variances Homogeneous? (A1 vs. A2)
Test 3: Are variances adequately modeled? (A2 vs. A3)
Test 4: Does the Model for the Mean Fit? (A3 vs. fitted)
(Note: When rho=0 the results of Test 3 and Test 2 will be the same.)

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	96.2357	6	<.0001
Test 2	14.0069	3	0.002896
Test 3	3.18384	2	0.2035
Test 4	1.45625	2	0.4828

The p-value for Test 1 is less than .05. There appears to be a difference between response and/or variances among the dose levels
It seems appropriate to model the data

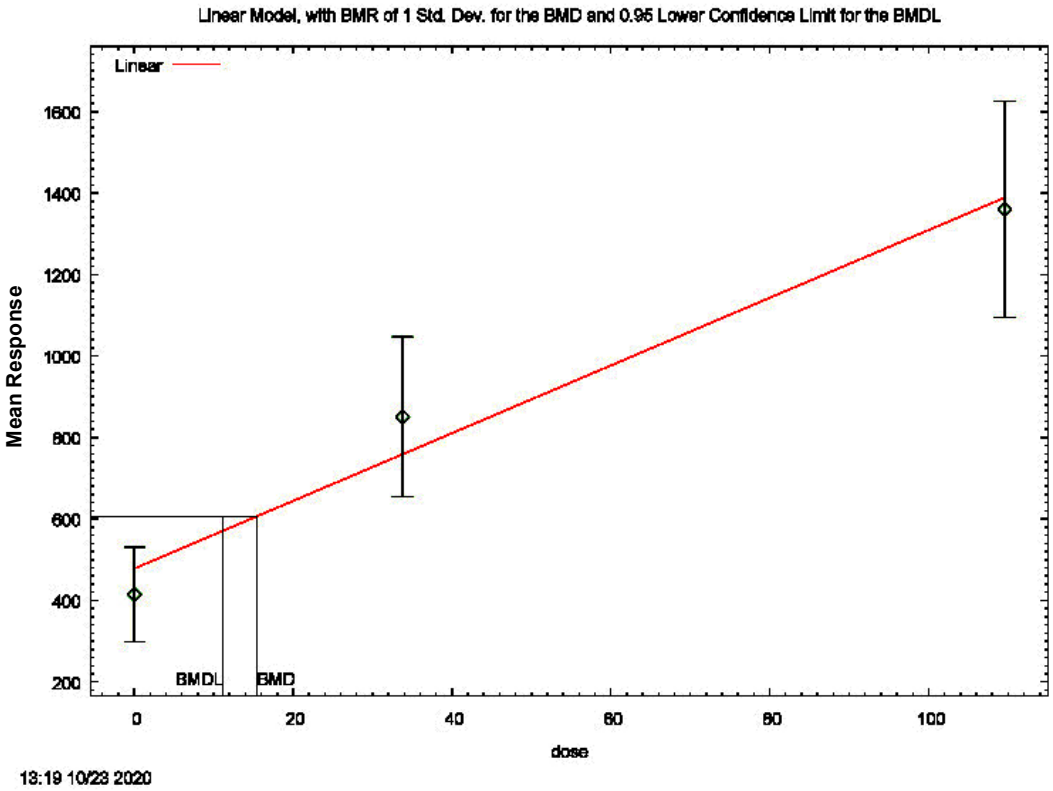
The p-value for Test 2 is less than .1. A non-homogeneous variance model appears to be appropriate

The p-value for Test 3 is greater than .1. The modeled variance appears to be appropriate here

The p-value for Test 4 is greater than .1. The model chosen seems to adequately describe the data

Benchmark Dose Computation
Specified effect = 1
Risk Type = Estimated standard deviations from the control mean
Confidence level = 0.95
BMD = 12.8255
BMDL = 8.93086
BMDU = 19.588

Figure A10.15. BMD modeling of increased liver triglycerides in female CD-1 mice from Lai et al. (2018)



Model run output for Figure A10.15: BMD modeling of increased liver triglycerides in female CD-1 mice from Lai et al. (2018).

```
================================================================
Polynomial Model. (Version: 2.21;  Date: 03/14/2017)
Input Data File: K:/BMD saved files/Chemicals/PFOS/lin_Lai 2018 liv tg_Opt.(d)
Gnuplot Plotting File:  K:/BMD saved files/Chemicals/PFOS/lin_Lai 2018 liv tg_Opt.plt
Fri Oct 23 13:19:17 2020
================================================================
BMDS Model Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
The form of the response function is:
Y[dose] = beta_0 + beta_1*dose + beta_2*dose^2 + ...
Dependent variable = Mean
Independent variable = Dose
rho is set to 0
Signs of the polynomial coefficients are not restricted
A constant variance model is fit

Total number of dose groups = 3
Total number of records with missing values = 0
Maximum number of iterations = 500
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008
```

Default Initial Parameter Values
alpha = 1
rho = 0 Specified
beta_0 = 477.882
beta_1 = 8.30982

Asymptotic Correlation Matrix of Parameter Estimates
(*** The model parameter(s) -rho
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	lalpha	beta_0	beta_1
lalpha	1	6.1e-010	1.9e-010
beta_0	6.1e-010	1	-0.72
beta_1	1.9e-010	-0.72	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval	
			Lower Conf. Limit	Upper Conf. Limit
alpha	16567.9	6763.71	3311.3	29824.6
beta_0	477.882	53.6998	372.633	583.132
beta_1	8.30982	0.811248	6.7198	9.89983

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
0.0239	4	415	478	72.7	129	-0.988
33.78	4	851	759	124	129	1.43
109.6	4	1.36e+003	1.39e+003	167	129	-0.44

Model Descriptions for likelihoods calculated
Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2
Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2

Model A3: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2
Model A3 uses any fixed variance parameters that
were specified by the user

Model R: Yi = Mu + e(i)
Var{e(i)} = Sigma^2

Likelihoods of Interest

Model	Log(likelihood)	# Param's	AIC
A1	-62.424521	4	132.849042
A2	-61.166630	6	134.333259
A3	-62.424521	4	132.849042
fitted	-64.291449	3	134.582898
R	-77.951075	2	159.902150

Explanation of Tests

Test 1: Do responses and/or variances differ among Dose levels?
(A2 vs. R)
Test 2: Are Variances Homogeneous? (A1 vs. A2)
Test 3: Are variances adequately modeled? (A2 vs. A3)
Test 4: Does the Model for the Mean Fit? (A3 vs. fitted)
(Note: When rho=0 the results of Test 3 and Test 2 will be the same.)

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	33.5689	4	<.0001
Test 2	2.51578	2	0.2843
Test 3	2.51578	2	0.2843
Test 4	3.73386	1	0.05332

The p-value for Test 1 is less than .05. There appears to be a difference between response and/or variances among the dose levels
It seems appropriate to model the data

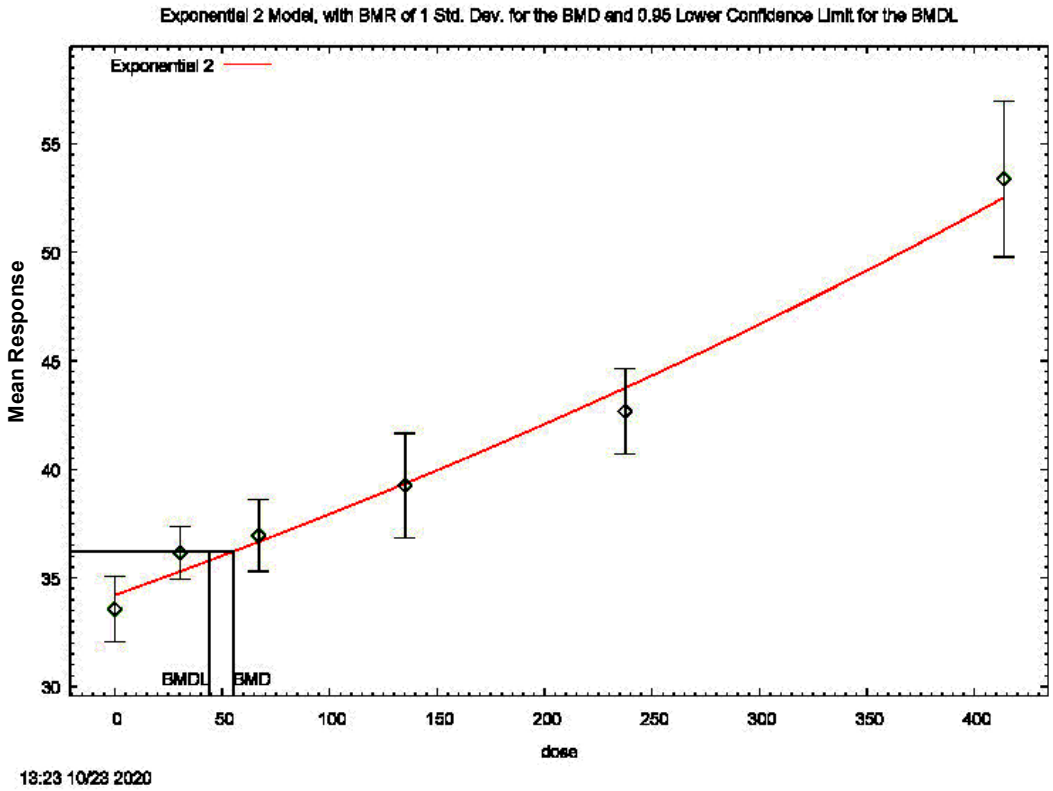
The p-value for Test 2 is greater than .1. A homogeneous variance model appears to be appropriate here

The p-value for Test 3 is greater than .1. The modeled variance appears to be appropriate here

The p-value for Test 4 is less than .1. You may want to try a different model

Benchmark Dose Computation
Specified effect = 1
Risk Type = Estimated standard deviations from the control mean
Confidence level = 0.95
BMD = 15.4897
BMDL = 11.1741
BMDU = 24.04

Figure A10.16. BMD modeling of increased relative liver weight in female Sprague Dawley rats from NTP (2019b)



Model run output for Figure A10.16: BMD modeling of increased relative liver weight in female Sprague Dawley rats from NTP (2019b).

```
==============================================================
Exponential Model. (Version: 1.11;  Date: 03/14/2017)
Input Data File: K:/BMD saved files/Chemicals/PFOS/exp_NTP 2019 female liv weight_Opt.(d)
Gnuplot Plotting File:
Fri Oct 23 13:24:29 2020
==============================================================
BMDS Model Run
~~~
```

Dependent variable = Mean
Independent variable = Dose
Data are assumed to be distributed: normally
Variance Model: exp(lnalpha +rho *ln(Y[dose]))
The variance is to be modeled as Var(i) = exp(lalpha + log(mean(i)) * rho)

Total number of dose groups = 6
Total number of records with missing values = 0
Maximum number of iterations = 500
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

MLE solution provided: Exact

Initial Parameter Values

Variable	Model 2	Model 3	Model 4	Model 5
lnalpha	-12.1843	-12.1843	-12.1843	-12.1843
rho	3.83765	3.83765	3.83765	3.83765
a	34.1558	34.1558	31.882	31.882
b	0.00104589	0.00104589	0.000785948	0.000785948
c	0 *	0 *	3.34797	3.34797
d	1 *	1	1 *	1

* Indicates that this parameter has been specified

Parameter Estimates by Model

Variable	Model 2	Model 3	Model 4	Model 5
lnalpha	-11.8604	-11.8604	-13.7212	-12.3537
rho	3.75336	3.75336	4.26605	3.89281
a	34.2029	34.2029	33.9708	34.2256
b	0.0010363	0.0010363	1.71187e-007	7.36298e-007
c	--	--	7316.82	3879.51
d	--	1	--	1.10059

-- Indicates that this parameter does not appear in model

Std. Err. Estimates by Model

Variable	Model 2	Model 3	Model 4	Model 5
lnalpha	4.9863	4.9863	5.26548	5.64019
rho	1.35453	1.35453	1.43135	1.53272
a	0.399311	0.399311	0.419397	0.649752
b	7.07734e-005	7.07734e-005	9.41746e-006	3.75474e-005
c	NA	NA	402450	217472
d	NA	NA	NA	0.202644

NA - Indicates that this parameter was specified (by the user or because of the model form) or has hit a bound implied by some inequality constraint and thus has no standard error.

Table of Stats From Input Data

Dose	N	Obs Mean	Obs Std Dev
0.054	10	33.56	2.09
30.5	10	36.15	1.71

Dose	N	Obs Mean	Obs Std Dev
67	10	36.95	2.31
135.1	10	39.25	3.35
237.5	10	42.67	2.75
413.6	9	53.37	4.68

Estimated Values of Interest

Model	Dose	Est Mean	Est Std Dev	Scaled Res.
2	0.054	34.2	2.012	-1.014
	30.5	35.3	2.134	1.258
	67	36.66	2.291	0.3974
	135.1	39.34	2.616	-0.1122
	237.5	43.75	3.192	-1.067
	413.6	52.51	4.496	0.5766
3	0.054	34.2	2.012	-1.014
	30.5	35.3	2.134	1.258
	67	36.66	2.291	0.3974
	135.1	39.34	2.616	-0.1122
	237.5	43.75	3.192	-1.067
	413.6	52.51	4.496	0.5766
4	0.054	33.97	1.934	-0.6755
	30.5	35.27	2.095	1.331
	67	36.82	2.296	0.1773
	135.1	39.72	2.699	-0.5489
	237.5	44.07	3.37	-1.318
	413.6	51.57	4.71	1.149
5	0.054	34.23	2.013	-1.047
	30.5	35.24	2.131	1.348
	67	36.64	2.299	0.4255
	135.1	39.45	2.655	-0.2396
	237.5	43.95	3.275	-1.234
	413.6	52.13	4.566	0.816

Other models for which likelihoods are calculated:
Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2
Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2
Model A3: Yij = Mu(i) + e(ij)
Var{e(ij)} = exp(lalpha + log(mean(i)) * rho)
Model R: Yij = Mu + e(i)
Var{e(ij)} = Sigma^2

Likelihoods of Interest

Model	Log(likelihood)	DF	AIC
A1	-89.95135	7	193.9027
A2	-83.54031	12	191.0806
A3	-84.99057	8	185.9811
R	-143.3548	2	290.7096
2	-87.11333	4	182.2267

Model	Log(likelihood)	DF	AIC
3	-87.11333	4	182.2267
4	-87.82322	5	185.6464
5	-87.68515	6	187.3703

Additive constant for all log-likelihoods = -54.22. This constant added to the above values gives the log-likelihood including the term that does not depend on the model parameters.

Explanation of Tests
Test 1: Does response and/or variances differ among Dose levels? (A2 vs. R)
Test 2: Are Variances Homogeneous? (A2 vs. A1)
Test 3: Are variances adequately modeled? (A2 vs. A3)
Test 4: Does Model 2 fit the data? (A3 vs. 2)

Test 5a: Does Model 3 fit the data? (A3 vs. 3)
Test 5b: Is Model 3 better than Model 2? (3 vs. 2)

Test 6a: Does Model 4 fit the data? (A3 vs. 4)
Test 6b: Is Model 4 better than Model 2? (4 vs. 2)

Test 7a: Does Model 5 fit the data? (A3 vs. 5)
Test 7b: Is Model 5 better than Model 3? (5 vs. 3)
Test 7c: Is Model 5 better than Model 4? (5 vs. 4)

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	119.6	10	< 0.0001
Test 2	12.82	5	0.0251
Test 3	2.901	4	0.5746
Test 4	4.246	4	0.3738
Test 5a	4.246	4	0.3738
Test 5b	2.842e-014	0	N/A
Test 6a	5.665	3	0.1291
Test 6b	-1.42	1	N/A
Test 7a	5.389	2	0.06757
Test 7b	-1.144	2	N/A
Test 7c	0.2762	1	0.5992

The p-value for Test 1 is less than .05. There appears to be a difference between response and/or variances among the dose levels, it seems appropriate to model the data.

The p-value for Test 2 is less than .1. A non-homogeneous variance model appears to be appropriate.

The p-value for Test 3 is greater than .1. The modeled variance appears to be appropriate here.

The p-value for Test 4 is greater than .1. Model 2 seems to adequately describe the data.

The p-value for Test 5a is greater than .1. Model 3 seems to adequately describe the data.

Degrees of freedom for Test 5b are less than or equal to 0. The Chi-Square test for fit is not valid.

The p-value for Test 6a is greater than .1. Model 4 seems to adequately describe the data.

The p-value for Test 6b is less than .05. Model 4 appears to fit the data better than Model 2.

The p-value for Test 7a is less than .1. Model 5 may not adequately describe the data; you may want to consider another model.

The p-value for Test 7b is less than .05. Model 5 appears to fit the data better than Model 3.

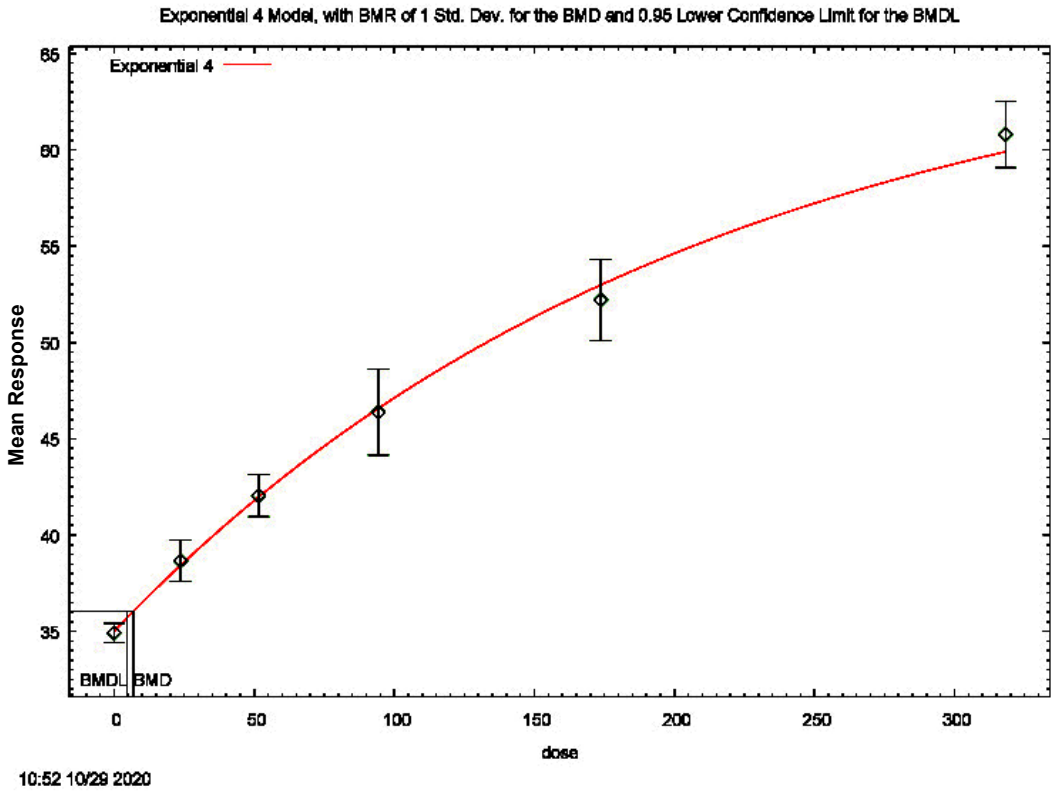
The p-value for Test 7c is greater than .05. Model 5 does not seem to fit the data better than Model 4.

Benchmark Dose Computations:
Specified Effect = 1.000000
Risk Type = Estimated standard deviations from control
Confidence Level = 0.950000

BMD and BMDL by Model

Model	BMD	BMDL	BMDU
2	55.1412	44.119	70.0943
3	55.1412	44.119	96.7343
4	45.4488	35.7996	58.8881
5	56.7917	36.2354	104.724

Figure A10.17. BMD modeling of increased relative liver weight in male Sprague Dawley rats from NTP (2019b)



Model run output for Figure A10.17: BMD modeling of increased relative liver weight in male Sprague Dawley rats from NTP (2019b).

```
================================================================
Exponential Model. (Version: 1.11;  Date: 03/14/2017)
Input Data File: K:/BMD saved files/Chemicals/PFOS/exp_NTP 2019 male liv weight_Opt.(d)
Gnuplot Plotting File:
Thu Oct 29 10:53:03 2020
================================================================
BMDS Model Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

The form of the response function by Model:
Model 2: Y[dose] = a * exp{sign * b * dose}
Model 3: Y[dose] = a * exp{sign * (b * dose)^d}
Model 4: Y[dose] = a * [c-(c-1) * exp{-b * dose}]
Model 5: Y[dose] = a * [c-(c-1) * exp{-(b * dose)^d}]

Note: Y[dose] is the median response for exposure = dose;
sign = +1 for increasing trend in data;
sign = -1 for decreasing trend.

Model 2 is nested within Models 3 and 4.
Model 3 is nested within Model 5.
Model 4 is nested within Model 5.
```

Dependent variable = Mean
Independent variable = Dose
Data are assumed to be distributed: normally
Variance Model: exp(lnalpha +rho *ln(Y[dose]))
The variance is to be modeled as Var(i) = exp(lalpha + log(mean(i)) * rho)

Total number of dose groups = 6
Total number of records with missing values = 0
Maximum number of iterations = 500
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

MLE solution provided: Exact
Initial Parameter Values

Variable	Model 2	Model 3	Model 4	Model 5
lnalpha	-15.8946	-15.8946	-15.8946	-15.8946
rho	4.48618	4.48618	4.48618	4.48618
a	37.5477	37.5477	33.174	33.174
b	0.00165161	0.00165161	0.00682224	0.00682224
c	0 *	0 *	1.9244	1.9244
d	1 *	1	1 *	1

* Indicates that this parameter has been specified

Parameter Estimates by Model

Variable	Model 2	Model 3	Model 4	Model 5
lnalpha	-6.32147	-6.32147	-17.333	-17.333
rho	2.2163	2.2163	4.89051	4.89051
a	37.5374	37.5374	35.0088	35.0088
b	0.00166409	0.00166409	0.00478913	0.00478913
c	--	--	1.90908	1.90908
d	--	1	--	1

-- Indicates that this parameter does not appear in model

Std. Err. Estimates by Model

Variable	Model 2	Model 3	Model 4	Model 5
lnalpha	7.76758	7.76759	5.26047	5.26047
rho	2.0385	2.0385	1.38161	1.38161
a	0.681746	0.681747	0.300973	0.300973
b	0.000152463	0.000152463	0.000865858	0.000865859
c	NA	NA	0.0999593	0.0999594
d	NA	NA	NA	NA

NA - Indicates that this parameter was specified (by the user or because of the model form) or has hit a bound implied by some inequality constraint and thus has no standard error.

Table of Stats From Input Data

Dose	N	Obs Mean	Obs Std Dev
0	10	34.92	0.696
23.7	10	38.66	1.49

Dose	N	Obs Mean	Obs Std Dev
51.6	10	42.04	1.52
94.3	10	46.38	3.13
173.7	10	52.21	2.94
318.2	10	60.8	2.43

Estimated Values of Interest

Model	Dose	Est Mean	Est Std Dev	Scaled Res.
2	0	37.54	2.355	-3.514
	23.7	39.05	2.461	-0.4979
	51.6	40.9	2.59	1.388
	94.3	43.92	2.803	2.781
	173.7	50.12	3.245	2.038
	318.2	63.74	4.235	-2.197
3	0	37.54	2.355	-3.514
	23.7	39.05	2.461	-0.4979
	51.6	40.9	2.59	1.388
	94.3	43.92	2.803	2.781
	173.7	50.12	3.245	2.038
	318.2	63.74	4.235	-2.197
4	0	35.01	1.028	-0.273
	23.7	38.42	1.291	0.5791
	51.6	41.98	1.603	0.1242
	94.3	46.57	2.066	-0.2973
	173.7	52.98	2.832	-0.8629
	318.2	59.9	3.823	0.7436
5	0	35.01	1.028	-0.273
	23.7	38.42	1.291	0.5791
	51.6	41.98	1.603	0.1242
	94.3	46.57	2.066	-0.2973
	173.7	52.98	2.832	-0.8629
	318.2	59.9	3.823	0.7436

Other models for which likelihoods are calculated:
Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2
Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2
Model A3: Yij = Mu(i) + e(ij)
Var{e(ij)} = exp(lalpha + log(mean(i)) * rho)
Model R: Yij = Mu + e(i)
Var{e(ij)} = Sigma^2

Likelihoods of Interest

Model	Log(likelihood)	DF	AIC
A1	-74.47572	7	162.9514
A2	-62.46333	12	148.9267
A3	-67.87277	8	151.7455
R	-161.2307	2	326.4615
2	-93.59988	4	195.1998

Model	Log(likelihood)	DF	AIC
3	-93.59988	4	195.1998
4	-68.62996	5	147.2599
5	-68.62996	5	147.2599

Additive constant for all log-likelihoods = -55.14. This constant added to the above values gives the log-likelihood including the term that does not depend on the model parameters.

Explanation of Tests
Test 1: Does response and/or variances differ among Dose levels? (A2 vs. R)
Test 2: Are Variances Homogeneous? (A2 vs. A1)
Test 3: Are variances adequately modeled? (A2 vs. A3)
Test 4: Does Model 2 fit the data? (A3 vs. 2)

Test 5a: Does Model 3 fit the data? (A3 vs. 3)
Test 5b: Is Model 3 better than Model 2? (3 vs. 2)

Test 6a: Does Model 4 fit the data? (A3 vs. 4)
Test 6b: Is Model 4 better than Model 2? (4 vs. 2)

Test 7a: Does Model 5 fit the data? (A3 vs. 5)
Test 7b: Is Model 5 better than Model 3? (5 vs. 3)
Test 7c: Is Model 5 better than Model 4? (5 vs. 4)

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	197.5	10	< 0.0001
Test 2	24.02	5	0.0002147
Test 3	10.82	4	0.02868
Test 4	51.45	4	< 0.0001
Test 5a	51.45	4	< 0.0001
Test 5b	-5.116e-013	0	N/A
Test 6a	1.514	3	0.679
Test 6b	49.94	1	< 0.0001
Test 7a	1.514	3	0.679
Test 7b	49.94	1	< 0.0001
Test 7c	-5.684e-013	0	N/A

The p-value for Test 1 is less than .05. There appears to be a difference between response and/or variances among the dose levels, it seems appropriate to model the data.

The p-value for Test 2 is less than .1. A non-homogeneous variance model appears to be appropriate.

The p-value for Test 3 is less than .1. You may want to consider a different variance model.

The p-value for Test 4 is less than .1. Model 2 may not adequately

describe the data; you may want to consider another model.

The p-value for Test 5a is less than .1. Model 3 may not adequately describe the data; you may want to consider another model.

Degrees of freedom for Test 5b are less than or equal to 0.
The Chi-Square test for fit is not valid.

The p-value for Test 6a is greater than .1. Model 4 seems to adequately describe the data.

The p-value for Test 6b is less than .05. Model 4 appears to fit the data better than Model 2.

The p-value for Test 7a is greater than .1. Model 5 seems to adequately describe the data.

The p-value for Test 7b is less than .05. Model 5 appears to fit the data better than Model 3.

Degrees of freedom for Test 7c are less than or equal to 0.
The Chi-Square test for fit is not valid.

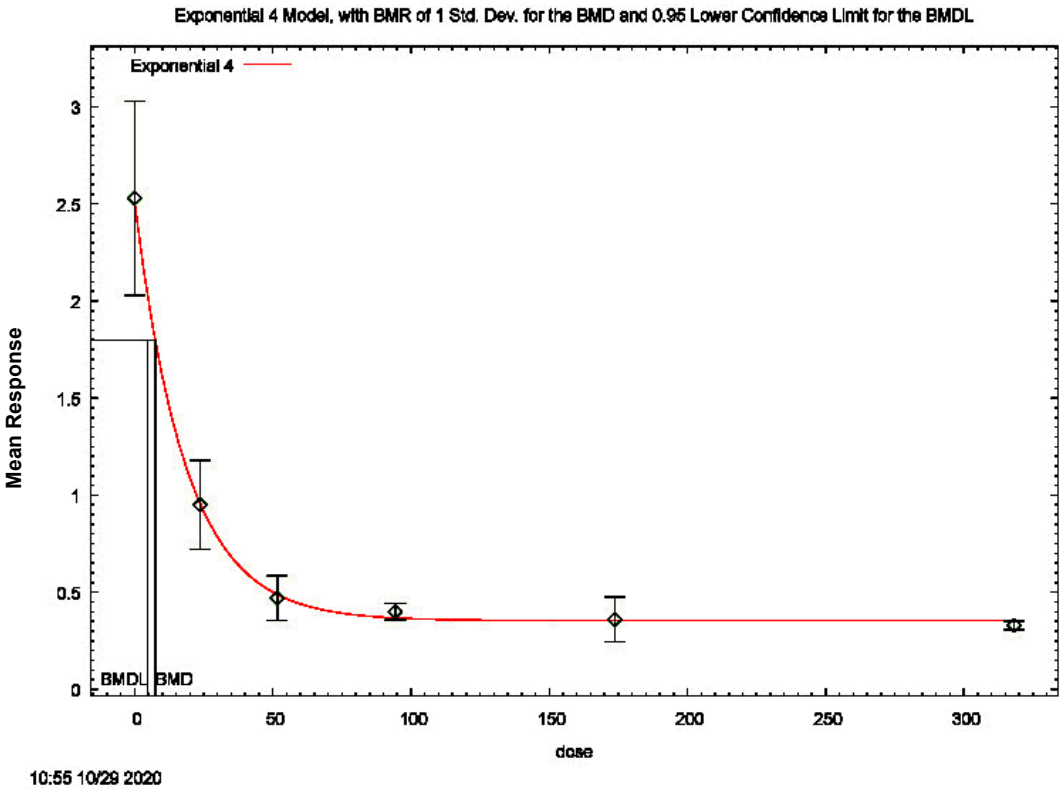
Benchmark Dose Computations:

Specified Effect = 1.000000
Risk Type = Estimated standard deviations from control
Confidence Level = 0.950000

BMD and BMDL by Model

Model	BMD	BMDL	BMDU
2	36.5705	20.6092	54.6849
3	36.5705	20.6092	54.6849
4	6.85744	4.70931	10.4181
5	6.85744	4.70931	10.5731

Figure A10.18. BMD modeling of decreased free T4 in male Sprague Dawley rats from NTP (2019b)



Model run output for Figure A10.18: BMD modeling of decreased free T4 in male Sprague Dawley rats from NTP (2019b).

```
========================================================================
Exponential Model. (Version: 1.11;  Date: 03/14/2017)
Input Data File: K:/BMD saved files/Chemicals/PFOS/exp_NTP 2019 male freeT4_Opt.(d)
Gnuplot Plotting File:
Thu Oct 29 10:55:42 2020
 ========================================================================
BMDS Model Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
The form of the response function by Model:
Model 2: Y[dose] = a * exp{sign * b * dose}
Model 3: Y[dose] = a * exp{sign * (b * dose)^d}
Model 4: Y[dose] = a * [c-(c-1) * exp{-b * dose}]
Model 5: Y[dose] = a * [c-(c-1) * exp{-(b * dose)^d}]

Note: Y[dose] is the median response for exposure = dose;
sign = +1 for increasing trend in data;
sign = -1 for decreasing trend.

Model 2 is nested within Models 3 and 4.
Model 3 is nested within Model 5.
Model 4 is nested within Model 5.
```

Dependent variable = Mean
Independent variable = Dose
Data are assumed to be distributed: normally
Variance Model: exp(lnalpha +rho *ln(Y[dose]))
The variance is to be modeled as Var(i) = exp(lalpha + log(mean(i)) * rho)

Total number of dose groups = 6
Total number of records with missing values = 0
Maximum number of iterations = 500
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

MLE solution provided: Exact

Initial Parameter Values

Variable	Model 2	Model 3	Model 4	Model 5
lnalpha	-2.62382	-2.62382	-2.62382	-2.62382
rho	2.46849	2.46849	2.46849	2.46849
a	0.364387	0.662731	2.6565	2.6565
b	0.0047355	1.3676e-005	0.01922	0.01922
c	0 *	0 *	0.118308	0.118308
d	1 *	2	1 *	1

* Indicates that this parameter has been specified

Parameter Estimates by Model

Variable	Model 2	Model 3	Model 4	Model 5
lnalpha	-1.69663	-6.02037	-2.50112	-2.50102
rho	0.0740169	-32.5821	1.98513	1.98559
a	2.46523	0.84	2.51089	2.5072
b	0.0329466	1.56641e-005	0.0538167	0.0533173
c	--	--	0.14101	0.141483
d	--	4.21197	--	1.01627

NC = No Convergence
-- Indicates that this parameter does not appear in model

Std. Err. Estimates by Model

Variable	Model 2	Model 3	Model 4	Model 5
lnalpha	0.220405	NA	0.241422	0.241551
rho	0.0465958	NA	0.288022	0.288401
a	0.133022	NA	0.217577	0.223448
b	0.0032016	NA	0.00557817	0.00880357
c	NA	NA	0.0139465	0.0154707
d	NA	NA	NA	0.228883

NA - Indicates that this parameter was specified (by the user or because of the model form) or has hit a bound implied by some inequality constraint and thus has no standard error.

Table of Stats From Input Data

Dose	N	Obs Mean	Obs Std Dev
0	10	2.53	0.7
23.7	10	0.95	0.32
51.6	10	0.47	0.16
94.3	10	0.4	0.06
173.7	10	0.36	0.16
318.2	10	0.33	0.03

Estimated Values of Interest

Model	Dose	Est Mean	Est Std Dev	Scaled Res.
2	0	2.465	0.4427	0.4627
	23.7	1.129	0.4301	-1.317
	51.6	0.4503	0.4157	0.1496
	94.3	0.1103	0.3946	2.322
	173.7	0.008062	0.3582	3.107
	318.2	6.901e-005	0.3003	3.474
3	0	0.84	0.8438	6.333
	23.7	0.84	0.8438	0.4122
	51.6	0.84	0.8438	-1.387
	94.3	0.84	0.8438	-1.649
	173.7	0.84	0.8438	-1.799
	318.2	0.84	0.8438	-1.911
4	0	2.511	0.7141	0.08462
	23.7	0.9565	0.274	-0.07469
	51.6	0.4883	0.1406	-0.4112
	94.3	0.3675	0.106	0.968
	173.7	0.3542	0.1022	0.1779
	318.2	0.3541	0.1022	-0.7447
5	0	2.507	0.7132	0.1011
	23.7	0.9602	0.275	-0.1168
	51.6	0.486	0.1399	-0.3624
	94.3	0.3671	0.1059	0.9837
	173.7	0.3549	0.1024	0.1584
	318.2	0.3547	0.1023	-0.7641

Other models for which likelihoods are calculated:
Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2
Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2
Model A3: Yij = Mu(i) + e(ij)
Var{e(ij)} = exp(lalpha + log(mean(i)) * rho)
Model R: Yij = Mu + e(i)
Var{e(ij)} = Sigma^2

Likelihoods of Interest

Model	Log(likelihood)	DF	AIC
A1	39.92491	7	-65.84982
A2	87.97322	12	-151.9464
A3	76.18026	8	-136.3605

Model	Log(likelihood)	DF	AIC
R	-19.81312	2	43.62624
2	26.96114	4	-45.92229
3	-19.81312	5	49.62624
4	73.99365	5	-137.9873
5	73.9962	6	-135.9924

Additive constant for all log-likelihoods = -55.14. This constant added to the above values gives the log-likelihood including the term that does not depend on the model parameters.

Explanation of Tests

Test 1: Does response and/or variances differ among Dose levels? (A2 vs. R)
Test 2: Are Variances Homogeneous? (A2 vs. A1)
Test 3: Are variances adequately modeled? (A2 vs. A3)
Test 4: Does Model 2 fit the data? (A3 vs. 2)

Test 5a: Does Model 3 fit the data? (A3 vs. 3)
Test 5b: Is Model 3 better than Model 2? (3 vs. 2)

Test 6a: Does Model 4 fit the data? (A3 vs. 4)
Test 6b: Is Model 4 better than Model 2? (4 vs. 2)

Test 7a: Does Model 5 fit the data? (A3 vs. 5)
Test 7b: Is Model 5 better than Model 3? (5 vs. 3)
Test 7c: Is Model 5 better than Model 4? (5 vs. 4)

Tests of Interest

Test	-2*log(Likelihood Ratio)	D. F.	p-value
Test 1	215.6	10	< 0.0001
Test 2	96.1	5	< 0.0001
Test 3	23.59	4	< 0.0001
Test 4	98.44	4	< 0.0001
Test 5a	192	3	< 0.0001
Test 5b	-93.55	1	N/A
Test 6a	4.373	3	0.2239
Test 6b	94.07	1	< 0.0001
Test 7a	4.368	2	0.1126
Test 7b	187.6	1	< 0.0001
Test 7c	0.005103	1	0.943

The p-value for Test 1 is less than .05. There appears to be a difference between response and/or variances among the dose levels, it seems appropriate to model the data.

The p-value for Test 2 is less than .1. A non-homogeneous variance model appears to be appropriate.

The p-value for Test 3 is less than .1. You may want to

consider a different variance model.

The p-value for Test 4 is less than .1. Model 2 may not adequately describe the data; you may want to consider another model.

The p-value for Test 5a is less than .1. Model 3 may not adequately describe the data; you may want to consider another model.

The p-value for Test 5b is less than .05. Model 3 appears to fit the data better than Model 2.

The p-value for Test 6a is greater than .1. Model 4 seems to adequately describe the data.

The p-value for Test 6b is less than .05. Model 4 appears to fit the data better than Model 2.

The p-value for Test 7a is greater than .1. Model 5 seems to adequately describe the data.

The p-value for Test 7b is less than .05. Model 5 appears to fit the data better than Model 3.

The p-value for Test 7c is greater than .05. Model 5 does not seem to fit the data better than Model 4.

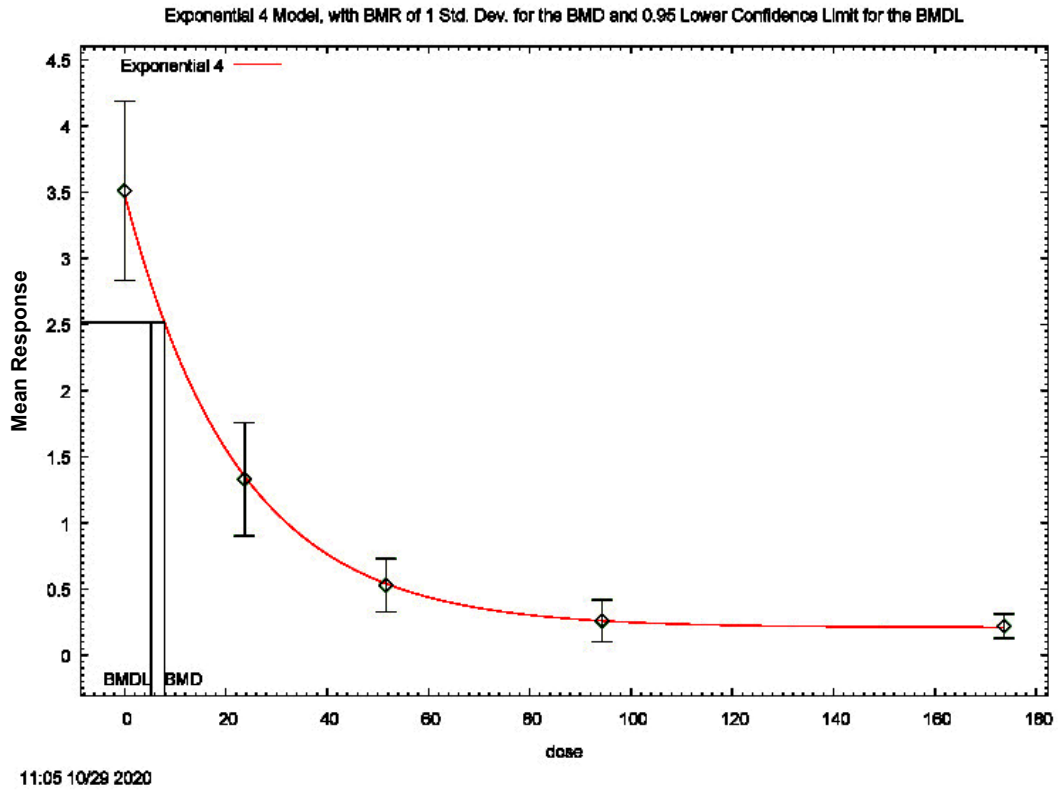
Benchmark Dose Computations:
Specified Effect = 1.000000
Risk Type = Estimated standard deviations from control

Confidence Level = 0.950000

BMD and BMDL by Model

Model	BMD	BMDL	BMDU
2	6.00736	4.19822	9.4973
3	-0	-0	Not computed
4	7.47141	4.78086	14.083
5	7.65985	4.78261	15.7676

Figure A10.19. BMD modeling of decreased total T4 in male Sprague Dawley rats from NTP (2019b) (high dose excluded)



Model run output for Figure A10.19: BMD modeling of decreased total T4 in male Sprague Dawley rats from NTP (2019b) (high dose excluded).

```
================================================================
Exponential Model. (Version: 1.11;  Date: 03/14/2017)
Input Data File: K:/BMD saved files/Chemicals/PFOS/exp_NTP 2019 male totT4 high
dose_Opt.(d)
Gnuplot Plotting File:
Thu Oct 29 11:05:50 2020
================================================================
BMDS Model Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
The form of the response function by Model:
Model 2: Y[dose] = a * exp{sign * b * dose}
Model 3: Y[dose] = a * exp{sign * (b * dose)^d}
Model 4: Y[dose] = a * [c-(c-1) * exp{-b * dose}]
Model 5: Y[dose] = a * [c-(c-1) * exp{-(b * dose)^d}]

Note: Y[dose] is the median response for exposure = dose;
sign = +1 for increasing trend in data;
sign = -1 for decreasing trend.

Model 2 is nested within Models 3 and 4.
Model 3 is nested within Model 5.
```

Model 4 is nested within Model 5.

Dependent variable = Mean
Independent variable = Dose
Data are assumed to be distributed: normally
Variance Model: exp(lnalpha +rho *ln(Y[dose]))
The variance is to be modeled as Var(i) = exp(lalpha + log(mean(i)) * rho)

Total number of dose groups = 5
Total number of records with missing values = 0
Maximum number of iterations = 500
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

MLE solution provided: Exact

Initial Parameter Values

Variable	Model 2	Model 3	Model 4	Model 5
lnalpha	-1.63191	-1.63191	-1.63191	-1.63191
rho	1.33961	1.33961	1.33961	1.33961
a	0.240579	0.608535	3.6855	3.6855
b	0.0150546	7.02825e-005	0.0368227	0.0368227
c	0 *	0 *	0.0568509	0.0568509
d	1 *	2	1 *	1

* Indicates that this parameter has been specified

Parameter Estimates by Model

Variable	Model 2	Model 3	Model 4	Model 5
lnalpha	-1.22909	2.95289	-1.70796	-1.70796
rho	0.40255	-15.1302	1.3087	1.3087
a	3.42992	1.17	3.48038	3.48038
b	0.0352566	0.00159613	0.0443829	0.0443829
c	--	--	0.0603702	0.0603702
d	--	17.7628	--	1

NC = No Convergence
-- Indicates that this parameter does not appear in model
Std. Err. Estimates by Model

Variable	Model 2	Model 3	Model 4	Model 5
lnalpha	0.222157	NA	0.230198	0.230198
rho	0.0896499	NA	0.218597	0.218597
a	0.20411	NA	0.29379	0.29379
b	0.00295564	NA	0.00502326	0.00502326
c	NA	NA	0.0119747	0.0119747
d	NA	NA	NA	NA

NA - Indicates that this parameter was specified (by the user or because of the model form) or has hit a bound implied by some inequality constraint and thus has no standard error.

Table of Stats From Input Data

Dose	N	Obs Mean	Obs Std Dev
0	10	3.51	0.95
23.7	10	1.33	0.6
51.6	10	0.53	0.28
94.3	10	0.26	0.22
173.7	10	0.22	0.13

Estimated Values of Interest

Model	Dose	Est Mean	Est Std Dev	Scaled Res.
2	0	3.43	0.6932	0.3653
	23.7	1.487	0.5859	-0.849
	51.6	0.5562	0.4806	-0.1721
	94.3	0.1234	0.355	1.217
	173.7	0.00751	0.2021	3.325
3	0	1.17	1.335	5.544
	23.7	1.17	1.335	0.3791
	51.6	1.17	1.335	-1.516
	94.3	1.17	1.335	-2.156
	173.7	1.17	1.335	-2.251
4	0	3.48	0.9628	0.09728
	23.7	1.352	0.5187	-0.1363
	51.6	0.5412	0.2849	-0.1246
	94.3	0.2599	0.1763	0.002205
	173.7	0.2116	0.1541	0.1728
5	0	3.48	0.9628	0.09728
	23.7	1.352	0.5187	-0.1363
	51.6	0.5412	0.2849	-0.1246
	94.3	0.2599	0.1763	0.002205
	173.7	0.2116	0.1541	0.1728

Other models for which likelihoods are calculated:
Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2
Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2
Model A3: Yij = Mu(i) + e(ij)
Var{e(ij)} = exp(lalpha + log(mean(i)) * rho)
Model R: Yij = Mu + e(i)
Var{e(ij)} = Sigma^2

Likelihoods of Interest

Model	Log(likelihood)	DF	AIC
A1	9.347685	6	-6.69537
A2	31.52834	10	-43.05669
A3	30.74894	7	-47.49788
R	-39.43493	2	82.86987
2	17.68492	4	-27.36985
3	-39.43493	5	88.86987
4	30.5611	5	-51.1222
5	30.5611	5	-51.1222

Additive constant for all log-likelihoods = -45.95. This constant added to the above values gives the log-likelihood including the term that does not depend on the model parameters.

Explanation of Tests
Test 1: Does response and/or variances differ among Dose levels? (A2 vs. R)
Test 2: Are Variances Homogeneous? (A2 vs. A1)
Test 3: Are variances adequately modeled? (A2 vs. A3)
Test 4: Does Model 2 fit the data? (A3 vs. 2)

Test 5a: Does Model 3 fit the data? (A3 vs. 3)
Test 5b: Is Model 3 better than Model 2? (3 vs. 2)

Test 6a: Does Model 4 fit the data? (A3 vs. 4)
Test 6b: Is Model 4 better than Model 2? (4 vs. 2)

Test 7a: Does Model 5 fit the data? (A3 vs. 5)
Test 7b: Is Model 5 better than Model 3? (5 vs. 3)
Test 7c: Is Model 5 better than Model 4? (5 vs. 4)

Tests of Interest

Test	-2*log(Likelihood Ratio)	D. F.	p-value
Test 1	141.9	8	< 0.0001
Test 2	44.36	4	< 0.0001
Test 3	1.559	3	0.6688
Test 4	26.13	3	< 0.0001
Test 5a	140.4	2	< 0.0001
Test 5b	-114.2	1	N/A
Test 6a	0.3757	2	0.8287
Test 6b	25.75	1	< 0.0001
Test 7a	0.3757	2	0.8287
Test 7b	140	0	N/A
Test 7c	-4.619e-013	0	N/A

The p-value for Test 1 is less than .05. There appears to be a difference between response and/or variances among the dose levels, it seems appropriate to model the data.

The p-value for Test 2 is less than .1. A non-homogeneous variance model appears to be appropriate.

The p-value for Test 3 is greater than .1. The modeled variance appears to be appropriate here.

The p-value for Test 4 is less than .1. Model 2 may not adequately describe the data; you may want to consider another model.

The p-value for Test 5a is less than .1. Model 3 may not adequately describe the data; you may want to consider another model.

The p-value for Test 5b is less than .05. Model 3 appears to fit the data better than Model 2.

The p-value for Test 6a is greater than .1. Model 4 seems to adequately describe the data.

The p-value for Test 6b is less than .05. Model 4 appears to fit the data better than Model 2.

The p-value for Test 7a is greater than .1. Model 5 seems to adequately describe the data.

Degrees of freedom for Test 7b are less than or equal to 0. The Chi-Square test for fit is not valid.

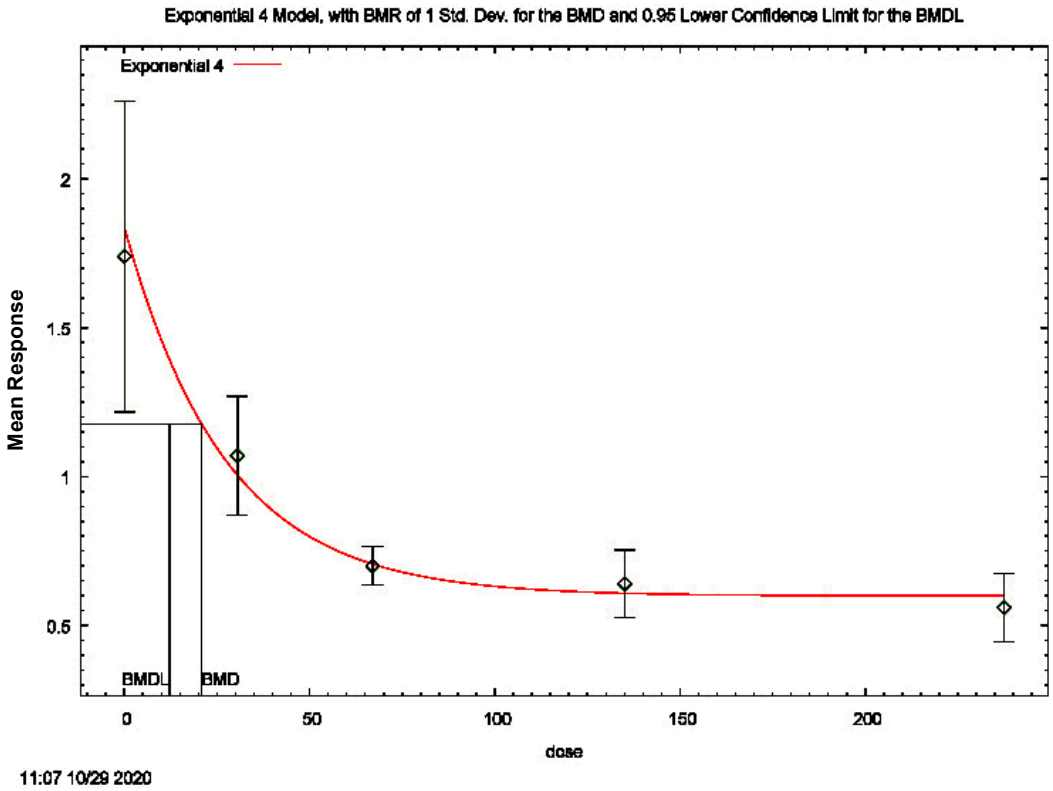
Degrees of freedom for Test 7c are less than or equal to 0. The Chi-Square test for fit is not valid.

Benchmark Dose Computations:
Specified Effect = 1.000000
Risk Type = Estimated standard deviations from control
Confidence Level = 0.950000

BMD and BMDL by Model

Model	BMD	BMDL	BMDU
2	6.40359	4.41377	10.5654
3	-0	-0	Not computed
4	7.8571	5.19419	13.6877
5	7.8571	5.19419	14.725

Figure A10.20. BMD modeling of decreased free T4 in female Sprague Dawley rats from NTP (2019b) (high dose excluded)



Model run output for Figure A10.20: BMD modeling of decreased free T4 in female Sprague Dawley rats from NTP (2019b) (high dose excluded).

==========

Exponential Model. (Version: 1.11; Date: 03/14/2017)
Input Data File: K:/BMD saved files/Chemicals/PFOS/exp_NTP 2019 female freeT4 high dose_Opt.(d)
Gnuplot Plotting File:
Thu Oct 29 11:07:17 2020

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BMDS Model Run

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The form of the response function by Model:
Model 2: Y[dose] = a * exp{sign * b * dose}
Model 3: Y[dose] = a * exp{sign * (b * dose)^d}
Model 4: Y[dose] = a * [c-(c-1) * exp{-b * dose}]
Model 5: Y[dose] = a * [c-(c-1) * exp{-(b * dose)^d}]

Note: Y[dose] is the median response for exposure = dose;
sign = +1 for increasing trend in data;
sign = -1 for decreasing trend.

Model 2 is nested within Models 3 and 4.
Model 3 is nested within Model 5.
~~~~~~~~~~

Model 4 is nested within Model 5.

Dependent variable = Mean
Independent variable = Dose
Data are assumed to be distributed: normally
Variance Model: exp(lnalpha +rho *ln(Y[dose]))
The variance is to be modeled as Var(i) = exp(lalpha + log(mean(i)) * rho)

Total number of dose groups = 5
Total number of records with missing values = 0
Maximum number of iterations = 500
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

MLE solution provided: Exact

Initial Parameter Values

Variable	Model 2	Model 3	Model 4	Model 5
lnalpha	-2.59855	-2.59855	-2.59855	-2.59855
rho	3.05927	3.05927	3.05927	3.05927
a	0.582269	0.826107	1.827	1.827
b	0.00413243	1.41578e-005	0.017773	0.017773
c	0 *	0 *	0.291918	0.291918
d	1 *	2	1 *	1

* Indicates that this parameter has been specified

Parameter Estimates by Model

Variable	Model 2	Model 3	Model 4	Model 5
lnalpha	-1.744	-1.7883	-2.56371	-2.56769
rho	3.60746	-10.3545	2.84981	2.92667
a	1.21261	0.942	1.83708	1.79019
b	0.00355905	1.18396e-005	0.0365556	0.0328328
c	--	--	0.326198	0.343342
d	--	4.33687	--	1.43342

-- Indicates that this parameter does not appear in model

Std. Err. Estimates by Model

Variable	Model 2	Model 3	Model 4	Model 5
lnalpha	0.337133	NA	0.244905	0.249298
rho	0.919305	NA	0.505485	0.534869
a	0.10609	NA	0.195972	0.196265
b	0.00051845	NA	0.00643411	0.005464
c	NA	NA	0.0374588	0.0424576
d	NA	NA	NA	0.479716

NA - Indicates that this parameter was specified (by the user or because of the model form) or has hit a bound implied by some inequality constraint and thus has no standard error.

Table of Stats From Input Data

Dose	N	Obs Mean	Obs Std Dev
0.054	10	1.74	0.73
30.5	10	1.07	0.28
67	10	0.7	0.09
135.1	10	0.64	0.16
237.5	10	0.56	0.16

Estimated Values of Interest

Model	Dose	Est Mean	Est Std Dev	Scaled Res.
2	0.054	1.212	0.5918	2.82
	30.5	1.088	0.4867	-0.1161
	67	0.9553	0.385	-2.097
	135.1	0.7497	0.2487	-1.395
	237.5	0.5207	0.1289	0.9633
3	0.054	0.942	0.5572	4.529
	30.5	0.942	0.5572	0.7264
	67	0.942	0.5572	-1.373
	135.1	0.942	0.5572	-1.714
	237.5	0.942	0.5572	-2.168
4	0.054	1.835	0.6589	-0.4542
	30.5	1.005	0.2796	0.7332
	67	0.7062	0.169	-0.1151
	135.1	0.6081	0.1366	0.7379
	237.5	0.5995	0.1339	-0.9323
5	0.054	1.79	0.6493	-0.2438
	30.5	1.046	0.2959	0.2539
	67	0.6678	0.1534	0.6632
	135.1	0.6149	0.136	0.5839
	237.5	0.6146	0.1359	-1.272

Other models for which likelihoods are calculated:
Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2
Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2
Model A3: Yij = Mu(i) + e(ij)
Var{e(ij)} = exp(lalpha + log(mean(i)) * rho)
Model R: Yij = Mu + e(i)
Var{e(ij)} = Sigma^2

Likelihoods of Interest

Model	Log(likelihood)	DF	AIC
A1	27.85952	6	-43.71904
A2	54.24186	10	-88.48372
A3	50.81643	7	-87.63287
R	4.240573	2	-4.481145
2	31.39641	4	-54.79282
3	4.240573	5	1.518855
4	49.70844	5	-89.41688
5	50.15681	6	-88.31362

Additive constant for all log-likelihoods = -45.95. This constant added to the above values gives the log-likelihood including the term that does not depend on the model parameters.

Explanation of Tests
Test 1: Does response and/or variances differ among Dose levels? (A2 vs. R)
Test 2: Are Variances Homogeneous? (A2 vs. A1)
Test 3: Are variances adequately modeled? (A2 vs. A3)
Test 4: Does Model 2 fit the data? (A3 vs. 2)

Test 5a: Does Model 3 fit the data? (A3 vs. 3)
Test 5b: Is Model 3 better than Model 2? (3 vs. 2)

Test 6a: Does Model 4 fit the data? (A3 vs. 4)
Test 6b: Is Model 4 better than Model 2? (4 vs. 2)

Test 7a: Does Model 5 fit the data? (A3 vs. 5)
Test 7b: Is Model 5 better than Model 3? (5 vs. 3)
Test 7c: Is Model 5 better than Model 4? (5 vs. 4)

Tests of Interest

Test	-2*log(Likelihood Ratio)	D. F.	p-value
Test 1	100	8	< 0.0001
Test 2	52.76	4	< 0.0001
Test 3	6.851	3	0.07681
Test 4	38.84	3	< 0.0001
Test 5a	93.15	2	< 0.0001
Test 5b	-54.31	1	N/A
Test 6a	2.216	2	0.3302
Test 6b	36.62	1	< 0.0001
Test 7a	1.319	1	0.2507
Test 7b	91.83	1	< 0.0001
Test 7c	0.8967	1	0.3437

The p-value for Test 1 is less than .05. There appears to be a difference between response and/or variances among the dose levels, it seems appropriate to model the data.

The p-value for Test 2 is less than .1. A non-homogeneous variance model appears to be appropriate.

The p-value for Test 3 is less than .1. You may want to consider a different variance model.

The p-value for Test 4 is less than .1. Model 2 may not adequately describe the data; you may want to consider another model.

The p-value for Test 5a is less than .1. Model 3 may not adequately describe the data; you may want to consider another model.

The p-value for Test 5b is less than .05. Model 3 appears to fit the data better than Model 2.

The p-value for Test 6a is greater than .1. Model 4 seems to adequately describe the data.

The p-value for Test 6b is less than .05. Model 4 appears to fit the data better than Model 2.

The p-value for Test 7a is greater than .1. Model 5 seems to adequately describe the data.

The p-value for Test 7b is less than .05. Model 5 appears to fit the data better than Model 3.

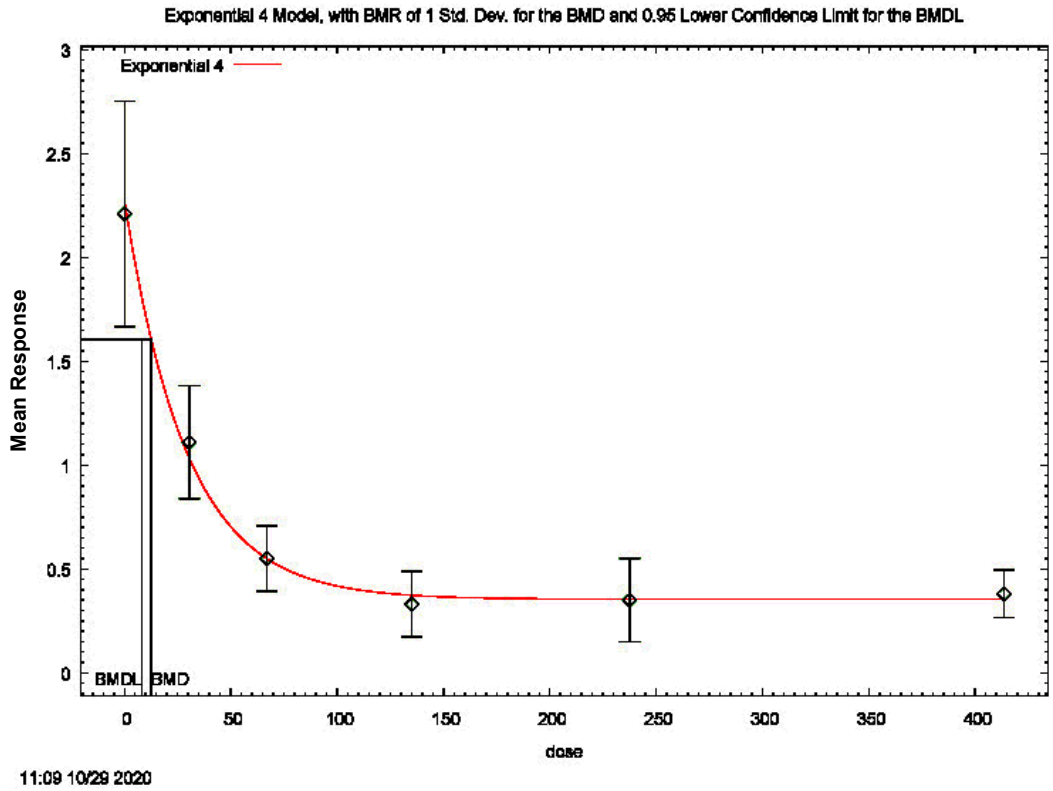
The p-value for Test 7c is greater than .05. Model 5 does not seem to fit the data better than Model 4.

Benchmark Dose Computations:
Specified Effect = 1.000000
Risk Type = Estimated standard deviations from control
Confidence Level = 0.950000

BMD and BMDL by Model

Model	BMD	BMDL	BMDU
2	188.193	100.614	445.578
3	82335.7	185.908	2.375e+006
4	20.8485	12.0925	52.8862
5	26.1553	13.1887	53.4664

Figure A10.21. BMD modeling of decreased total T4 in female Sprague Dawley rats from NTP (2019b)



Model run output for Figure A10.21: BMD modeling of decreased total T4 in female Sprague Dawley rats from NTP (2019b).

```
==========================================================================
Exponential Model. (Version: 1.11;  Date: 03/14/2017)
Input Data File: K:/BMD saved files/Chemicals/PFOS/exp_NTP 2019 female totT4_Opt.(d)
Gnuplot Plotting File:
Thu Oct 29 11:09:42 2020
==========================================================================
BMDS Model Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

The form of the response function by Model:
Model 2: Y[dose] = a * exp{sign * b * dose}
Model 3: Y[dose] = a * exp{sign * (b * dose)^d}
Model 4: Y[dose] = a * [c-(c-1) * exp{-b * dose}]
Model 5: Y[dose] = a * [c-(c-1) * exp{-(b * dose)^d}]

Note: Y[dose] is the median response for exposure = dose;
sign = +1 for increasing trend in data;
sign = -1 for decreasing trend.

Model 2 is nested within Models 3 and 4.
Model 3 is nested within Model 5.
Model 4 is nested within Model 5.
```

Dependent variable = Mean
Independent variable = Dose
Data are assumed to be distributed: normally
Variance Model: exp(lnalpha +rho *ln(Y[dose]))
The variance is to be modeled as Var(i) = exp(lalpha + log(mean(i)) * rho)

Total number of dose groups = 6
Total number of records with missing values = 0
Maximum number of iterations = 500
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

MLE solution provided: Exact

Initial Parameter Values

Variable	Model 2	Model 3	Model 4	Model 5
lnalpha	-1.85945	-1.85945	-1.85945	-1.85945
rho	1.31752	1.31752	1.31752	1.31752
a	0.378064	0.677915	2.3205	2.3205
b	0.00357207	7.7376e-006	0.0127321	0.0127321
c	0 *	0 *	0.135439	0.135439
d	1 *	2	1 *	1

* Indicates that this parameter has been specified

Parameter Estimates by Model

Variable	Model 2	Model 3	Model 4	Model 5
lnalpha	-1.55384	-3.5949	-1.86964	-1.87846
rho	0.0787933	-16.4755	1.29137	1.29137
a	2.14448	0.829153	2.27293	2.23463
b	0.0184725	6.02767e-006	0.034085	0.0312502
c	--	--	0.155619	0.161448
d	--	4.26099	--	1.23067

-- Indicates that this parameter does not appear in model

Std. Err. Estimates by Model

Variable	Model 2	Model 3	Model 4	Model 5
lnalpha	0.217422	NA	0.229921	0.229373
rho	0.0635918	NA	0.269636	0.268228
a	0.137424	NA	0.203019	0.205509
b	0.00215864	NA	0.00504129	0.00517153
c	NA	NA	0.0212704	0.0228504
d	NA	NA	NA	0.327459

NA - Indicates that this parameter was specified (by the user or because of the model form) or has hit a bound implied by some inequality constraint and thus has no standard error.

Table of Stats From Input Data

Dose	N	Obs Mean	Obs Std Dev
0.054	10	2.21	0.76

Dose	N	Obs Mean	Obs Std Dev
30.5	10	1.11	0.38
67	10	0.55	0.22
135.1	10	0.33	0.22
237.5	10	0.35	0.28
413.6	9	0.38	0.15

Estimated Values of Interest

Model	Dose	Est Mean	Est Std Dev	Scaled Res.
2	0.054	2.142	0.4738	0.4516
	30.5	1.221	0.4634	-0.7558
	67	0.622	0.4513	-0.5047
	135.1	0.1768	0.4295	1.128
	237.5	0.02667	0.3986	2.565
	413.6	0.001031	0.3507	3.242
3	0.054	0.8292	0.7756	5.63
	30.5	0.8292	0.7756	1.145
	67	0.8292	0.7756	-1.138
	135.1	0.8292	0.7756	-2.035
	237.5	0.8292	0.7756	-1.954
	413.6	0.8292	0.7756	-1.737
4	0.054	2.269	0.6665	-0.2818
	30.5	1.032	0.4008	0.6127
	67	0.5493	0.2667	0.008358
	135.1	0.3729	0.2077	-0.6534
	237.5	0.3543	0.2009	-0.06764
	413.6	0.3537	0.2007	0.3929
5	0.054	2.234	0.6569	-0.1151
	30.5	1.091	0.4135	0.1466
	67	0.5172	0.2554	0.4055
	135.1	0.366	0.2043	-0.557
	237.5	0.3608	0.2024	-0.1686
	413.6			0.2849

Other models for which likelihoods are calculated:
Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2
Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2
Model A3: Yij = Mu(i) + e(ij)
Var{e(ij)} = exp(lalpha + log(mean(i)) * rho)
Model R: Yij = Mu + e(i)
Var{e(ij)} = Sigma^2

Likelihoods of Interest

Model	Log(likelihood)	DF	AIC
A1	28.51891	12	-43.03781
A2	46.17054	12	-68.34107
A3	43.71268	8	-71.42537
R	-14.50826	2	33.01651

Model	Log(likelihood)	DF	AIC
2	20.69547	4	-33.39095
3	-14.50826	5	39.01651
4	43.13407	5	-76.26814
5	43.4172	6	-76.26814

Additive constant for all log-likelihoods = -54.22. This constant added to the above values gives the log-likelihood including the term that does not depend on the model parameters.

Explanation of Tests
Test 1: Does response and/or variances differ among Dose levels? (A2 vs. R)
Test 2: Are Variances Homogeneous? (A2 vs. A1)
Test 3: Are variances adequately modeled? (A2 vs. A3)
Test 4: Does Model 2 fit the data? (A3 vs. 2)

Test 5a: Does Model 3 fit the data? (A3 vs. 3)
Test 5b: Is Model 3 better than Model 2? (3 vs. 2)

Test 6a: Does Model 4 fit the data? (A3 vs. 4)
Test 6b: Is Model 4 better than Model 2? (4 vs. 2)

Test 7a: Does Model 5 fit the data? (A3 vs. 5)
Test 7b: Is Model 5 better than Model 3? (5 vs. 3)
Test 7c: Is Model 5 better than Model 4? (5 vs. 4)

Tests of Interest

Test	-2*log(Likelihood Ratio)	D. F.	p-value
Test 1	121.4	10	< 0.0001
Test 2	35.3	5	< 0.0001
Test 3	4.916	4	0.2961
Test 4	46.03	4	< 0.0001
Test 5a	116.4	3	< 0.0001
Test 5b	-70.41	1	N/A
Test 6a	1.157	3	0.7633
Test 6b	44.88	1	< 0.0001
Test 7a	0.591	2	0.7442
Test 7b	115.9	1	< 0.0001
Test 7c	0.5663	1	0.4517

The p-value for Test 1 is less than .05. There appears to be a difference between response and/or variances among the dose levels, it seems appropriate to model the data.

The p-value for Test 2 is less than .1. A non-homogeneous variance model appears to be appropriate.

The p-value for Test 3 is greater than .1. The modeled variance appears to be appropriate here.

The p-value for Test 4 is less than .1. Model 2 may not adequately describe the data; you may want to consider another model.

The p-value for Test 5a is less than .1. Model 3 may not adequately describe the data; you may want to consider another model.

The p-value for Test 5b is less than .05. Model 3 appears to fit the data better than Model 2.

The p-value for Test 6a is greater than .1. Model 4 seems to adequately describe the data.

The p-value for Test 6b is less than .05. Model 4 appears to fit the data better than Model 2.

The p-value for Test 7a is greater than .1. Model 5 seems to adequately describe the data.

The p-value for Test 7b is less than .05. Model 5 appears to fit the data better than Model 3.

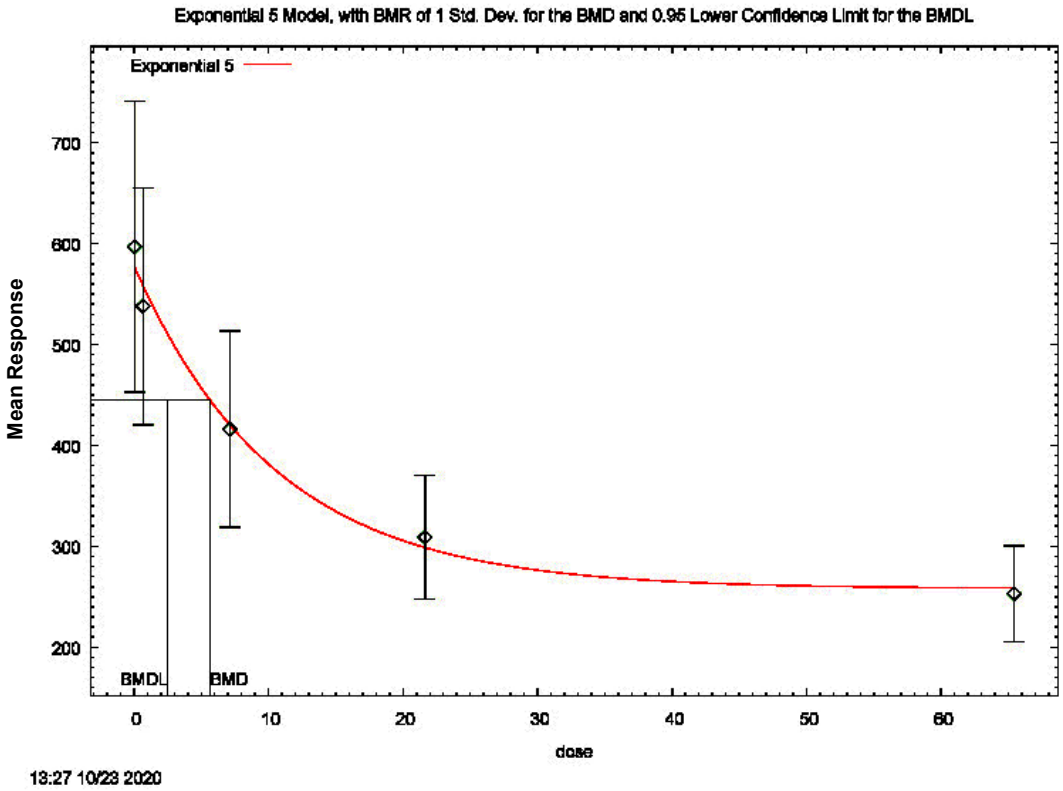
The p-value for Test 7c is greater than .05. Model 5 does not seem to fit the data better than Model 4.

Benchmark Dose Computations:
Specified Effect = 1.000000
Risk Type = Estimated standard deviations from control
Confidence Level = 0.950000

BMD and BMDL by Model

Model	BMD	BMDL	BMDU
2	13.5173	8.90055	23.9262
3	210178	308.041	4.136e+006
4	12.5322	8.19881	22.3109
5	16.1711	8.55386	29.0349

Figure A10.22. BMD modeling of decreased plaque forming cell response in male c57/BL6 mice from Dong et al. (2009) (BMR = 1 standard deviation)



Model run output for Figure A10.22: BMD modeling of decreased plaque forming cell response in male c57/BL6 mice from Dong et al. (2009).

```
================================================================
Exponential Model. (Version: 1.11;  Date: 03/14/2017)
Input Data File: K:/BMD saved files/Chemicals/PFOS/exp_Dong 2009 pfc no high dose_Opt.(d)
Gnuplot Plotting File:
Fri Oct 23 13:28:02 2020
================================================================
BMDS Model Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

The form of the response function by Model:
Model 2: Y[dose] = a * exp{sign * b * dose}
Model 3: Y[dose] = a * exp{sign * (b * dose)^d}
Model 4: Y[dose] = a * [c-(c-1) * exp{-b * dose}]
Model 5: Y[dose] = a * [c-(c-1) * exp{-(b * dose)^d}]

Note: Y[dose] is the median response for exposure = dose;
sign = +1 for increasing trend in data;
sign = -1 for decreasing trend.

Model 2 is nested within Models 3 and 4.
Model 3 is nested within Model 5.
Model 4 is nested within Model 5.
```

Dependent variable = Mean
Independent variable = Dose
Data are assumed to be distributed: normally
Variance Model: exp(lnalpha +rho *ln(Y[dose]))
The variance is to be modeled as Var(i) = exp(lalpha + log(mean(i)) * rho)

Total number of dose groups = 5
Total number of records with missing values = 0
Maximum number of iterations = 500
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

MLE solution provided: Exact

Initial Parameter Values

Variable	Model 2	Model 3	Model 4	Model 5
lnalpha	-5.5662	-5.5662	-5.5662	-5.5662
rho	2.52721	2.52721	2.52721	2.52721
a	320.885	320.885	626.85	626.85
b	0.0118183	0.0118183	0.0562866	0.0562866
c	0 *	0 *	0.384386	0.384386
d	1 *	1	1 *	1

* Indicates that this parameter has been specified

Parameter Estimates by Model

Variable	Model 2	Model 3	Model 4	Model 5
lnalpha	-7.40745	-7.40747	-5.92711	-5.92711
rho	2.857	2.85701	2.57226	2.57226
a	501.266	501.266	572.107	572.107
b	0.0112284	0.0112284	0.084283	0.084283
c	--	--	0.441593	0.441593
d	--	1	--	1

-- Indicates that this parameter does not appear in model

Std. Err. Estimates by Model

Variable	Model 2	Model 3	Model 4	Model 5
lnalpha	5.64667	5.85396	4.26621	4.26621
rho	0.943073	0.978063	0.712088	0.712088
a	30.8634	32.0567	39.9535	39.9535
b	0.00164939	0.00178318	0.0245768	0.0245768
c	NA	NA	0.0454404	0.0454405
d	NA	NA	NA	NA

NA - Indicates that this parameter was specified (by the user or because of the model form) or has hit a bound implied by some inequality constraint and thus has no standard error.

Table of Stats From Input Data

Dose	N	Obs Mean	Obs Std Dev
0.048	10	597	202

Dose	N	Obs Mean	Obs Std Dev
0.674	10	538	164
7.132	10	416	136
21.64	10	309	85.4
65.43	10	253	66.4

Estimated Values of Interest

Model	Dose	Est Mean	Est Std Dev	Scaled Res.
2	0.048	501	177.1	1.714
	0.674	497.5	175.3	0.7307
	7.132	462.7	158.1	-0.934
	21.64	393.1	125.3	-2.124
	65.43	240.4	62.06	0.6396
3	0.048	501	177.1	1.714
	0.674	497.5	175.3	0.7307
	7.132	462.7	158.1	-0.934
	21.64	393.1	125.3	-2.124
	65.43	240.4	62.06	0.6396
4	0.048	570.8	181.2	0.4569
	0.674	554.5	174.6	-0.2983
	7.132	427.8	125	-0.2977
	21.64	304.2	80.65	0.1879
	65.43	253.9	63.93	-0.04578
5	0.048	570.8	181.2	0.4569
	0.674	554.5	174.6	-0.2983
	7.132	427.8	125	-0.2977
	21.64	304.2	80.65	0.1879
	65.43	253.9	63.93	-0.04578

Other models for which likelihoods are calculated:
Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2
Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2
Model A3: Yij = Mu(i) + e(ij)
Var{e(ij)} = exp(lalpha + log(mean(i)) * rho)
Model R: Yij = Mu + e(i)
Var{e(ij)} = Sigma^2

Likelihoods of Interest

Model	Log(likelihood)	DF	AIC
A1	-269.4221	6	550.8442
A2	-262.0043	10	544.0086
A3	-262.0841	7	538.1682
R	-286.3701	2	576.7402
2	-268.6484	4	545.2969
3	-268.6484	4	545.2969
4	-262.3839	5	534.7679
5	-262.3839	5	534.7679

Additive constant for all log-likelihoods = -45.95. This constant added to the

above values gives the log-likelihood including the term that does not depend on the model parameters.

Explanation of Tests
Test 1: Does response and/or variances differ among Dose levels? (A2 vs. R)
Test 2: Are Variances Homogeneous? (A2 vs. A1)
Test 3: Are variances adequately modeled? (A2 vs. A3)
Test 4: Does Model 2 fit the data? (A3 vs. 2)

Test 5a: Does Model 3 fit the data? (A3 vs. 3)
Test 5b: Is Model 3 better than Model 2? (3 vs. 2)

Test 6a: Does Model 4 fit the data? (A3 vs. 4)
Test 6b: Is Model 4 better than Model 2? (4 vs. 2)

Test 7a: Does Model 5 fit the data? (A3 vs. 5)
Test 7b: Is Model 5 better than Model 3? (5 vs. 3)
Test 7c: Is Model 5 better than Model 4? (5 vs. 4)

Tests of Interest

Test	-2*log(Likelihood Ratio)	D. F.	p-value
Test 1	48.73	8	< 0.0001
Test 2	14.84	4	0.005055
Test 3	0.1596	3	0.9838
Test 4	13.13	3	0.004367
Test 5a	13.13	3	0.004367
Test 5b	-6.071e-011	0	N/A
Test 6a	0.5996	2	0.741
Test 6b	12.53	1	0.0004007
Test 7a	0.5996	2	0.741
Test 7b	12.53	1	0.0004007
Test 7c	0	0	N/A

The p-value for Test 1 is less than .05. There appears to be a difference between response and/or variances among the dose levels, it seems appropriate to model the data.

The p-value for Test 2 is less than .1. A non-homogeneous variance model appears to be appropriate.

The p-value for Test 3 is greater than .1. The modeled variance appears to be appropriate here.

The p-value for Test 4 is less than .1. Model 2 may not adequately describe the data; you may want to consider another model.

The p-value for Test 5a is less than .1. Model 3 may not adequately describe the data; you may want to consider another model.

Degrees of freedom for Test 5b are less than or equal to 0.

The Chi-Square test for fit is not valid.

The p-value for Test 6a is greater than .1. Model 4 seems to adequately describe the data.

The p-value for Test 6b is less than .05. Model 4 appears to fit the data better than Model 2.

The p-value for Test 7a is greater than .1. Model 5 seems to adequately describe the data.

The p-value for Test 7b is less than .05. Model 5 appears to fit the data better than Model 3.

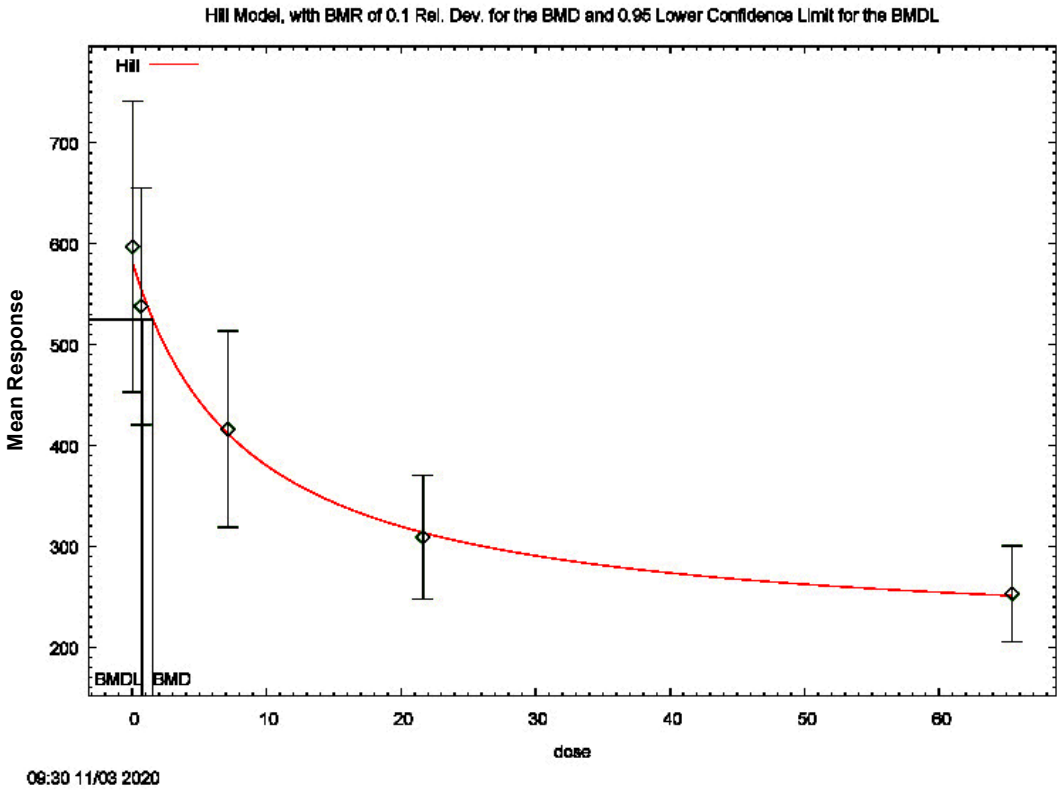
Degrees of freedom for Test 7c are less than or equal to 0.
The Chi-Square test for fit is not valid.

Benchmark Dose Computations:
Specified Effect = 1.000000
Risk Type = Estimated standard deviations from control
Confidence Level = 0.950000

BMD and BMDL by Model

Model	BMD	BMDL	BMDU
2	38.856	24.4058	67.3771
3	38.856	24.4058	67.3771
4	9.98166	5.08531	23.9077
5	9.98166	5.08531	23.9077

Figure A10.23. BMD modeling of decreased plaque forming cell response in male c57/BL6 mice from Dong et al. (2009) (BMR = 0.1 relative deviation)



Model run output for Figure A10.23: BMD modeling of decreased plaque forming cell response in male c57/BL6 mice from Dong et al. (2009).

```
================================================================
Hill Model. (Version: 2.18;  Date: 03/14/2017)
Input Data File: K:/BMD saved files/Chemicals/PFOS/hil_Dong 2009 pfc no high dose_Opt.(d)
Gnuplot Plotting File:  K:/BMD saved files/Chemicals/PFOS/hil_Dong 2009 pfc no high
dose_Opt.plt
Tue Nov 03 09:30:20 2020
================================================================
BMDS Model Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
The form of the response function is:
Y[dose] = intercept + v*dose^n/(k^n + dose^n)

Independent variable = Dose
Power parameter restricted to be greater than 1
The variance is to be modeled as Var(i) = exp(lalpha  + rho * ln(mean(i)))

Total number of dose groups = 5
Total number of records with missing values = 0
Maximum number of iterations = 500
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008
```

Default Initial Parameter Values
lalpha = 9.88224
rho = 0
intercept = 597
v = -344
n = 1.19729
k = 6.65559

Asymptotic Correlation Matrix of Parameter Estimates
(*** The model parameter(s) -n
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	lalpha	rho	intercept	v	k
lalpha	1	-1	0.34	-0.41	-0.12
rho	-1	1	-0.35	0.42	0.12
intercept	0.34	-0.35	1	-0.76	-0.54
v	-0.41	0.42	-0.76	1	-0.028
k	-0.12	0.12	-0.54	-0.028	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval	
			Lower Conf. Limit	Upper Conf. Limit
lalpha	-5.75113	4.23737	-14.0562	2.55397
rho	2.54262	0.707137	1.15666	3.92858
intercept	582.785	43.8176	496.904	668.666
v	-374.609	50.0391	-472.684	-276.534
n	1	NA		
k	8.47933	4.00913	0.621581	16.3371

NA - Indicates that this parameter has hit a bound
implied by some inequality constraint and thus
has no standard error.

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
0.048	10	597	581	202	184	0.28
0.674	10	538	555	164	174	-0.313
7.132	10	416	412	136	119	0.116
21.64	10	309	314	85.4	84.1	-0.175
65.43	10	253	251	66.4	63.4	0.092

Model Descriptions for likelihoods calculated
Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2
Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2
Model A3: Yij = Mu(i) + e(ij)
Var{e(ij)} = exp(lalpha + rho*ln(Mu(i)))

Model A3 uses any fixed variance parameters that
were specified by the user
Model R: Yi = Mu + e(i)
Var{e(i)} = Sigma^2

Likelihoods of Interest

Model	Log(likelihood)	# Param's	AIC
A1	-269.422105	6	550.844210
A2	-262.004309	10	544.008618
A3	-262.084125	7	538.168249
fitted	-262.335552	5	534.671105
R	-286.370083	2	576.740166

Explanation of Tests
Test 1: Do responses and/or variances differ among Dose levels?
(A2 vs. R)
Test 2: Are Variances Homogeneous? (A1 vs. A2)
Test 3: Are variances adequately modeled? (A2 vs. A3)
Test 4: Does the Model for the Mean Fit? (A3 vs. fitted)
(Note: When rho=0 the results of Test 3 and Test 2 will be the same.)

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	48.7315	8	<.0001
Test 2	14.8356	4	0.005055
Test 3	0.159631	3	0.9838
Test 4	0.502856	2	0.7777

The p-value for Test 1 is less than .05. There appears to be a
difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is less than .1. A non-homogeneous variance
model appears to be appropriate

The p-value for Test 3 is greater than .1. The modeled variance appears
to be appropriate here

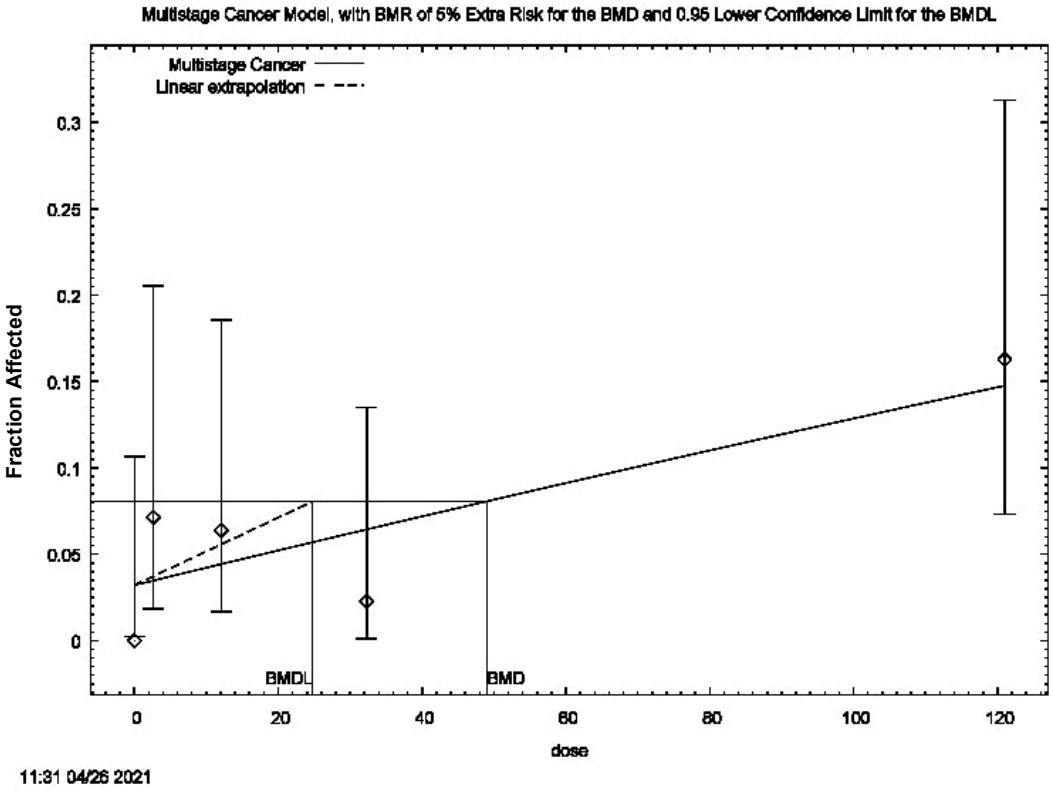
The p-value for Test 4 is greater than .1. The model chosen seems
to adequately describe the data

Benchmark Dose Computation
Specified effect = 0.1
Risk Type = Relative deviation
Confidence level = 0.95
BMD = 1.56217
BMDL = 0.746895
BMDU = 6.44306

Benchmark Dose Analysis Results for Cancer Endpoints

Perfluorooctane Sulfonic Acid

Figure A10.24. BMD modeling of hepatocellular tumor incidence in male Sprague Dawley rats from Butenhoff et al. (2012b)



Model Run Output for Figure A10.24: BMD modeling of hepatocellular tumor incidence in male Sprague Dawley rats from Butenhoff et al. (2012b).

```
 ========================================================================
Multistage Model. (Version: 3.4;  Date: 05/02/2014)
Input Data File: K:/BMD saved files/Chemicals/PFOS/msc_Butenhoff pfos male serum
hep_Opt.(d)
Gnuplot Plotting File:  K:/BMD saved files/Chemicals/PFOS/msc_Butenhoff pfos male serum
hep_Opt.plt
Mon Apr 26 11:30:59 2021
========================================================================
BMDS_Model_Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
The form of the probability function is:
P[response] = background + (1-background)*[1-EXP(
-beta1*dose^1)]

The parameter betas are restricted to be positive
```

Dependent variable = Effect
Independent variable = Dose

Total number of observations = 5
Total number of records with missing values = 0
Total number of parameters in model = 2
Total number of specified parameters = 0
Degree of polynomial = 1

Maximum number of iterations = 500
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

Default Initial Parameter Values
Background = 0.0288539
Beta(1) = 0.00115644

Asymptotic Correlation Matrix of Parameter Estimates

	Background	Beta(1)
Background	1	-0.46
Beta(1)	-0.46	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval	
			Lower Conf. Limit	Upper Conf. Limit
Background	0.0321432	0.0165415	-0.000277513	0.0645638
Beta(1)	0.00104566	0.000531353	4.23e-006	0.00208709

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-45.8404	5			
Fitted model	-48.8849	2	6.08895	3	0.1074
Reduced model	-51.9101	1	12.1394	4	0.01634

AIC: 101.77

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0140	0.0322	1.318	0.000	41.000	-1.167
2.6400	0.0348	1.462	3.000	42.000	1.295
12.1000	0.0443	2.083	3.000	47.000	0.650
32.3000	0.0643	2.829	1.000	44.000	-1.124
121.0000	0.1472	6.328	7.000	43.000	0.289

Chi^2 = 4.81 d.f. = 3 P-value = 0.1864

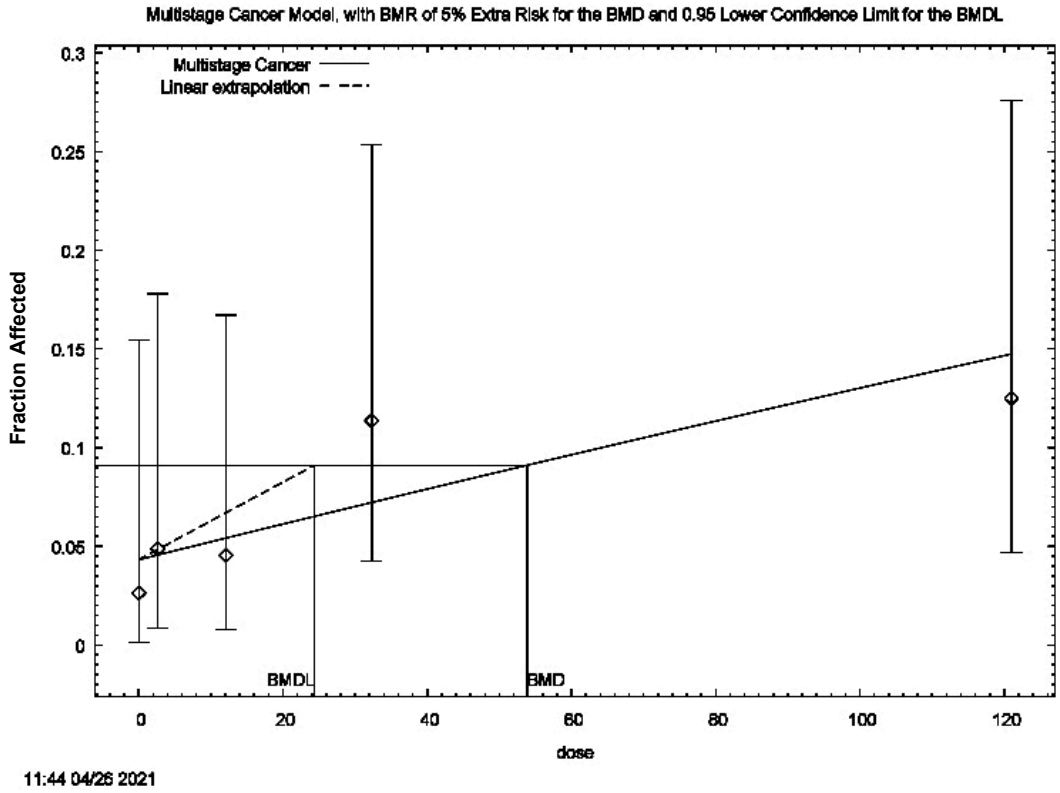
Benchmark Dose Computation
Specified effect = 0.05
Risk Type = Extra risk
Confidence level = 0.95
BMD = 49.0534
BMDL = 24.7835

BMDU = 171.534

Taken together, (24.7835, 171.534) is a 90 % two-sided confidence interval for the BMD

Cancer Slope Factor = 0.00201747

Figure A10.25. BMD modeling of pancreatic islet cell carcinoma incidence in male Sprague Dawley rats from Butenhoff et al. (2012b)



Model Run Output for Figure A10.25: BMD modeling of pancreatic islet cell carcinoma incidence in male Sprague Dawley rats from Butenhoff et al. (2012b).

```
================================================================
Multistage Model. (Version: 3.4;  Date: 05/02/2014)
Input Data File: K:/BMD saved files/Chemicals/PFOS/msc_Butenhoff pfos male serum
panc_Opt.(d)
Gnuplot Plotting File:  K:/BMD saved files/Chemicals/PFOS/msc_Butenhoff pfos male serum
panc_Opt.plt
Mon Apr 26 11:44:10 2021
================================================================
BMDS_Model_Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

The form of the probability function is:
P[response] = background + (1-background)*[1-EXP(
```

-beta1*dose^1)]

The parameter betas are restricted to be positive
Dependent variable = Effect
Independent variable = Dose

Total number of observations = 5
Total number of records with missing values = 0
Total number of parameters in model = 2
Total number of specified parameters = 0
Degree of polynomial = 1

Maximum number of iterations = 500
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

Default Initial Parameter Values
Background = 0.0476913
Beta(1) = 0.000791577

Asymptotic Correlation Matrix of Parameter Estimates

	Background	Beta(1)
Background	1	-0.56
Beta(1)	-0.56	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval	
			Lower Conf. Limit	Upper Conf. Limit
Background	0.0432278	0.0206316	0.00279061	0.083665
Beta(1)	0.000952916	0.000613586	-0.00024969	0.00215552

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-51.4005	5			
Fitted model	-52.1608	2	1.52043	3	0.6776
Reduced model	-53.8129	1	4.82475	4	0.3058

AIC: 108.322

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0140	0.0432	1.643	1.000	38.000	-0.513
2.6400	0.0456	1.871	2.000	41.000	0.097
12.1000	0.0542	2.385	2.000	44.000	-0.256
32.3000	0.0722	3.178	5.000	44.000	1.061
121.0000	0.1474	5.897	5.000	40.000	-0.400

Chi^2 = 1.62 d.f. = 3 P-value = 0.6540

Benchmark Dose Computation

Specified effect = 0.05
Risk Type = Extra risk
Confidence level = 0.95
BMD = 53.8277
BMDL = 24.3624
BMDU = 657.141

Taken together, (24.3624, 657.141) is a 90% two-sided confidence interval for the BMD

Cancer Slope Factor = 0.00205235

Model Run Output for BMD modeling of multisite tumors: hepatocellular adenoma and pancreatic islet cell carcinoma in male Sprague Dawley rats from Butenhoff et al. (2012b).

```
================================================================
MS_COMBO. (Version: 1.10;  Date: 01/29/2017)
Input Data File: K:\BMD saved files\Chemicals\PFOS\butenhoff 2012 pfos multi.(d)
Gnuplot Plotting File:  K:\BMD saved files\Chemicals\PFOS\butenhoff 2012 pfos multi.plt
Mon Apr 26 11:48:44 2021
================================================================
BMDS_Model_Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
The form of the probability function is:
P[response] = background + (1-background)*[1-EXP(
-beta1*dose^1)]

The parameter betas are restricted to be positive

Dependent variable = Effect
Independent variable = Dose
Data file name = Butenhoffpfosmaleserumhep.dax

Total number of observations = 5
Total number of records with missing values = 0
Total number of parameters in model = 2
Total number of specified parameters = 0
Degree of polynomial = 1

Maximum number of iterations = 500
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

Default Initial Parameter Values
Background = 0.0288539
Beta(1) = 0.00115644

Asymptotic Correlation Matrix of Parameter Estimates
                Background   Beta(1)
 Background     1            -0.57
```

	Background	Beta(1)
Beta(1)	-0.57	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval	
			Lower Conf. Limit	Upper Conf. Limit
Background	0.0321432	*	*	*
Beta(1)	0.00104566	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-45.8404	5			
Fitted model	-48.8849	2	6.08895	3	0.1074
Reduced model	-51.9101	1	12.1394	4	0.01634

AIC: 101.77

Log-likelihood Constant 40.11446893476365

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0140	0.0322	1.318	0.000	41.000	-1.167
2.6400	0.0348	1.462	3.000	42.000	1.295
12.1000	0.0443	2.083	3.000	47.000	0.650
32.3000	0.0643	2.829	1.000	44.000	-1.124
121.0000	0.1472	6.328	7.000	43.000	0.289

Chi^2 = 4.81 d.f. = 3 P-value = 0.1864

Benchmark Dose Computation
Specified effect = 0.05
Risk Type = Extra risk
Confidence level = 0.95
BMD = 49.0534
BMDL = 24.7835
BMDU = 171.534

Taken together, (24.7835, 171.534) is a 90 % two-sided confidence interval for the BMD

Multistage Cancer Slope Factor = 0.00201747

==========

MS_COMBO. (Version: 1.10; Date: 01/29/2017)
Input Data File: K:\BMD saved files\Chemicals\PFOS\butenhoff 2012 pfos multi.(d)
Gnuplot Plotting File: K:\BMD saved files\Chemicals\PFOS\butenhoff 2012 pfos multi.plt
Mon Apr 26 11:48:44 2021

==========

BMDS_Model_Run

~~~~~~~~~~

The form of the probability function is:
~~~~~~~~~~

P[response] = background + (1-background)*[1-EXP(
-beta1*dose^1)]

The parameter betas are restricted to be positive

Dependent variable = Effect
Independent variable = Dose
Data file name = Butenhoffpfosmaleserumpanc.dax

Total number of observations = 5
Total number of records with missing values = 0
Total number of parameters in model = 2
Total number of specified parameters = 0
Degree of polynomial = 1

Maximum number of iterations = 500
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

Default Initial Parameter Values
Background = 0.0476913
Beta(1) = 0.000791577

Asymptotic Correlation Matrix of Parameter Estimates

	Background	Beta(1)
Background	1	-0.63
Beta(1)	-0.63	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval	
			Lower Conf. Limit	Upper Conf. Limit
Background	0.0432278	*	*	*
Beta(1)	0.000952916	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-51.4005	5			
Fitted model	-52.1608	2	1.52043	3	0.6776
Reduced model	-53.8129	1	4.82475	4	0.3058

AIC: 108.322

Log-likelihood Constant 44.494124583256585

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0140	0.0432	1.643	1.000	38.000	-0.513

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
2.6400	0.0456	1.871	2.000	41.000	0.097
12.1000	0.0542	2.385	2.000	44.000	-0.256
32.3000	0.0722	3.178	5.000	44.000	1.061
121.0000	0.1474	5.897	5.000	40.000	-0.400

Chi^2 = 1.62 d.f. = 3 P-value = 0.6540

Benchmark Dose Computation
Specified effect = 0.05
Risk Type = Extra risk
Confidence level = 0.95
BMD = 53.8277
BMDL = 24.3624
BMDU = 3.61147e+276

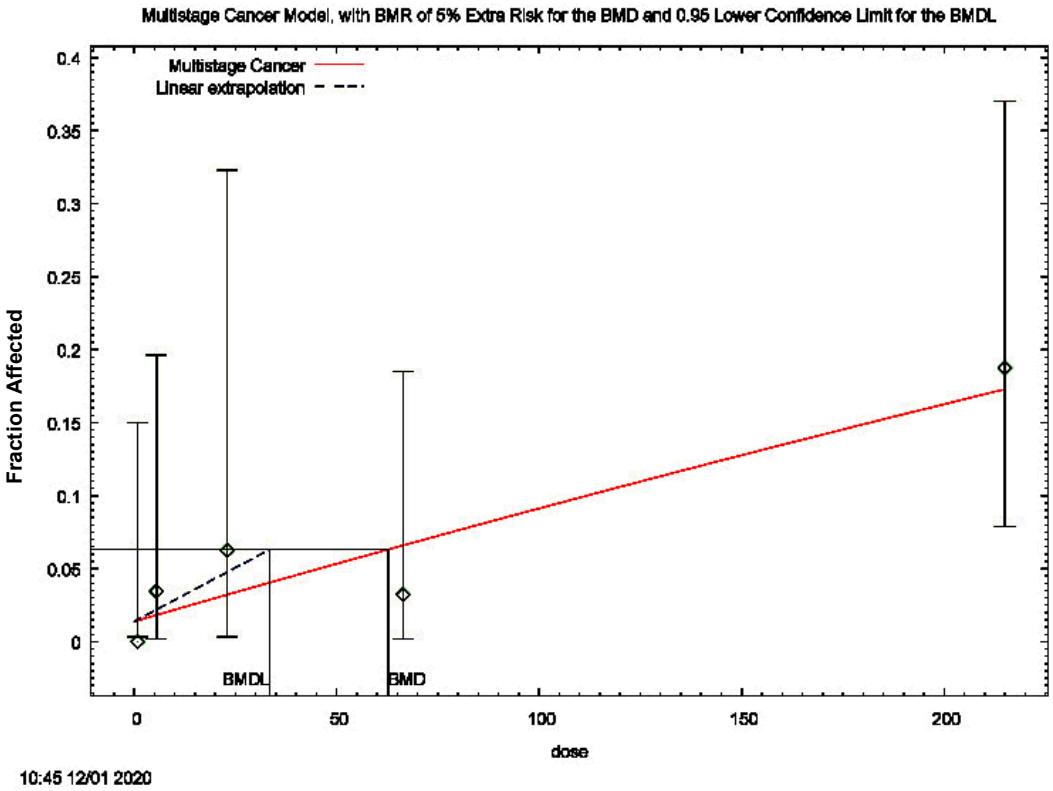
Taken together, (24.3624, 3.61147e+276) is a 90% two-sided confidence interval for the BMD

Multistage Cancer Slope Factor = 0.00205235
**** Start of combined BMD and BMDL Calculations.****
Combined Log-Likelihood 101.04566952341376
Combined Log-likelihood Constant 84.608593518020228

Benchmark Dose Computation
Specified effect = 0.05
Risk Type = Extra risk
Confidence level = 0.95
BMD = 25.6649
BMDL = 14.7461
BMDU = 64.2098

Multistage Cancer Slope Factor = 0.00339073

Figure A10.26. BMD modeling of hepatocellular tumor incidence in female Sprague Dawley rats from Butenhoff et al. (2012b)



Model Run Output for Figure A10.26: BMD modeling of hepatocellular tumor incidence in female Sprague Dawley rats from Butenhoff et al. (2012b).

```
================================================================
Multistage Model. (Version: 3.4;  Date: 05/02/2014)
Input Data File: K:/BMD saved files/Chemicals/PFOS/msc_Butenhoff pfos female serum
hep_Opt.(d)
Gnuplot Plotting File:  K:/BMD saved files/Chemicals/PFOS/msc_Butenhoff pfos female serum
hep_Opt.plt
Tue Dec 01 10:45:30 2020
================================================================
BMDS_Model_Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
The form of the probability function is:
P[response] = background + (1-background)*[1-EXP(
-beta1*dose^1)]
The parameter betas are restricted to be positive
Dependent variable = Effect
Independent variable = Dose
Total number of observations = 5
Total number of records with missing values = 0
Total number of parameters in model = 2
Total number of specified parameters = 0
Degree of polynomial = 1
```

Maximum number of iterations = 500
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

Default Initial Parameter Values
Background = 0.0145684
Beta(1) = 0.000858241

Asymptotic Correlation Matrix of Parameter Estimates

	Background	Beta(1)
Background	1	-0.44
Beta(1)	-0.44	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval	
			Lower Conf. Limit	Upper Conf. Limit
Background	0.0137622	0.0167901	-0.0191458	0.0466703
Beta(1)	0.000818058	0.000367259	9.82424e-005	0.00153787

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-27.9507	5			
Fitted model	-29.0869	2	2.27239	3	0.5178
Reduced model	-33.1343	1	10.3672	4	0.03468

AIC: 62.1738

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.8400	0.0144	0.404	0.000	28.000	-0.640
5.4900	0.0182	0.527	1.000	29.000	0.657
23.0000	0.0321	0.514	1.000	16.000	0.688
66.4000	0.0659	2.043	1.000	31.000	-0.755
215.0000	0.1728	5.530	6.000	32.000	0.220

Chi^2 = 1.93 d.f. = 3 P-value = 0.5862

Benchmark Dose Computation
Specified effect = 0.05
Risk Type = Extra risk
Confidence level = 0.95
BMD = 62.7013
BMDL = 33.4769
BMDU = 168.724

Taken together, (33.4769, 168.724) is a 90% two-sided confidence interval for the BMD

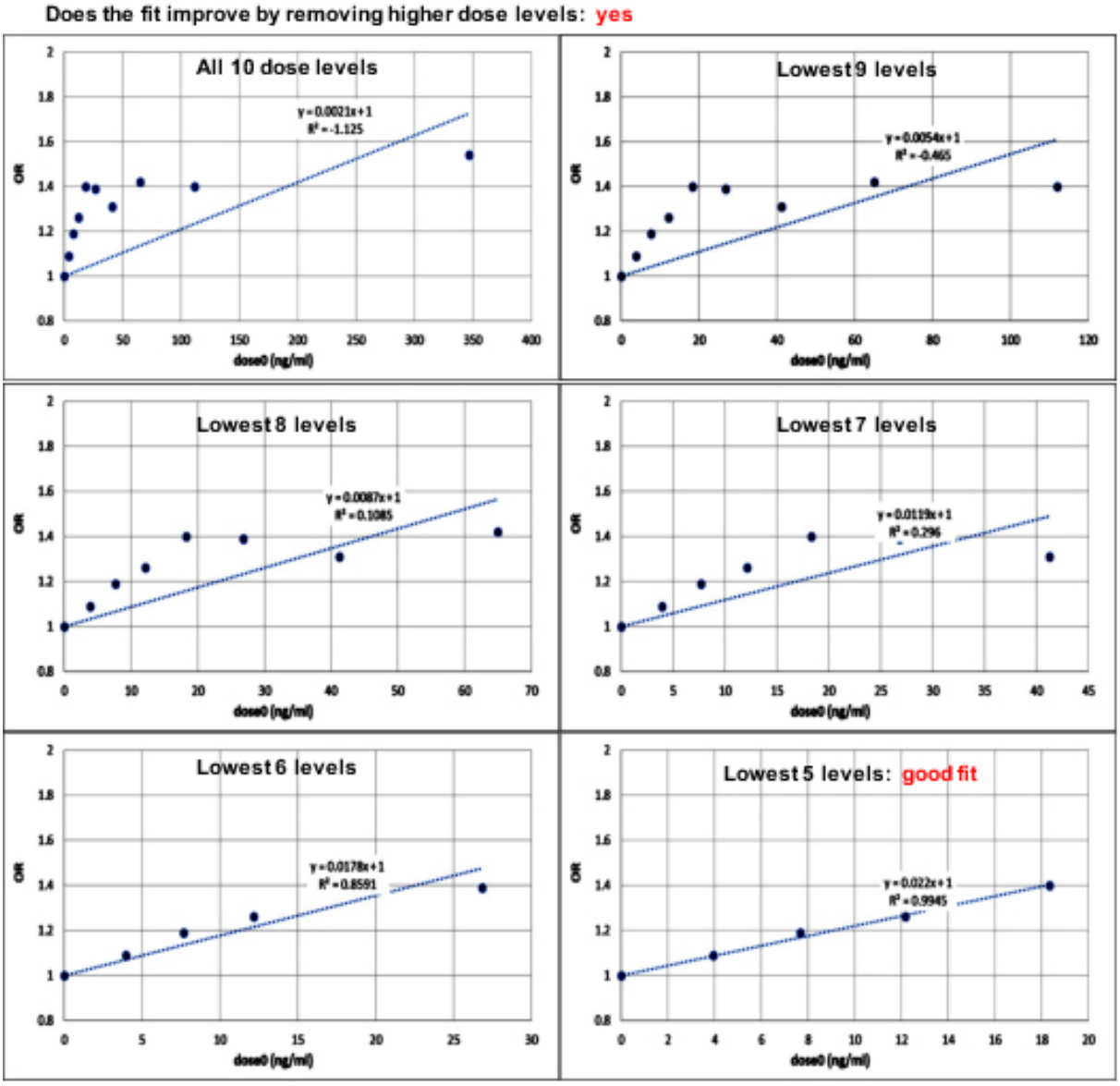
Cancer Slope Factor = 0.00149357

APPENDIX 11. GENERALIZED LEAST SQUARES FOR TREND IN STATA

Stata output of ORs for high ALT from Gallo et al. (2012).

"No intercept model" used: lowest dose subtracted out
Poor model fit when all ten dose levels are used. Fit improved substantially when only the lowest five doses were used.

Figure A11.1. Dose-response graphs plotting all ten dose levels in comparison with plotting only the lowest nine, eight, seven, six or five doses.



Results:
The regression model between PFOA and lnOR using GLST in Stata

Generalized least- squares regression Number of obs = 4
Goodness-of-fit chi2(3) = 0.29 Model ch2(1) = 25.71
Prob > chi2 = 0.9621 Prob >chi2 = 0.0000

logrr	Coef.	Std. Err.	z	P>|z|	[95% Conf.	Interval]
dose0	.017	0.00354	5.07	0.000	.0110127	0.248891

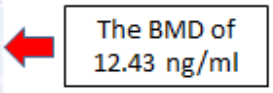
This regression coefficient was then used to calculate the BMD01 and BMDL01 using the following equations:

$OR = \exp^{(dose \times slope)}$ (eq 1)
lnOR = dose x slope (eq 2)
dose = lnOR / slope (eq 3)
BMD01* = 0.1181 / 0.01795 = 6.58 ng/ml
BMDL01* = 0.1181 / 0.024889 = 4.74 ng/ml

*Since the lowest dose (5.85 ng/ml) was subtracted out (i.e. no intercept model), this dose was added back in:

BMD = 5.85 + 6.58 = 12.43 ng/ml
BMDL = 5.85 + 4.74 = 10.59 ng/ml

Decile	Dose (ng/ml)	Log10 dose	N	OR	CIL	CIU	Logrr	Percent with high ALT	Percent over baseline
1	5.853917	0.767447	4645	1			0	8.85%	0.0%
2	9.807348	0.991552	4645	1.09	0.94	1.26	0.086178	9.6%	0.7%
3	13.53996	1.131617	4645	1.19	1.03	1.37	0.173953	10.4%	1.5%
4	18.03516	1.25612	4645	1.26	1.09	1.45	0.231112	10.9%	2.1%
5	24.19555	1.383735	4645	1.4	1.22	1.62	0.336472	12.0%	3.1%
6	32.69365	1.514463	4645	1.39	1.21	1.6	0.329304	11.9%	3.0%
7	47.11991	1.673204	4645	1.31	1.14	1.52	0.270027	11.3%	2.4%
8	70.89587	1.850621	4645	1.42	1.23	1.64	0.350657	12.1%	3.3%
9	117.927	2.071613	4645	1.4	1.21	1.62	0.336472	12.0%	3.1%
10	353.0504	2.547837	4645	1.54	1.33	1.78	0.431782	13.0%	4.2%



APPENDIX 12. ALTERNATIVE METHODS FOR CALCULATING THE CANCER SLOPE FACTOR AND CANCER HEALTH-PROTECTIVE CONCENTRATION

Generalized least-squares for trend (glst): A potential disadvantage of the method described in the main PHG document to calculate the CSFs is that it assumes that the relative risk (RR) estimates for the different exposure categories are independent. However, they are not independent because for each study they rely on a common reference group. This lack of independence can lead to an underestimate of the variance of the dose-response slope derived from these data (Greenland and Longnecker, 1992). That is, it can lead to an artificially narrow CI around b. It could also lead to a less efficient estimator of b. The glst method is a way of correcting for this lack of independence. However, a potential weakness of the glst model is that it assumes a linear relationship between the exposure and the logarithm of the RR, a relationship that may or may not fit the data.

The effect of this lack of independence was evaluated by running dose-response analyses of the Shearer et al. (2021) and Vieira et al. (2013) data with and without the glst correction. The glst command in Stata/IC (version 15.1) was used to provide dose-response and variance estimates corrected for the lack of independence, and the variance-weighted least squares (vwls) command (also in Stata/IC) was used to provide uncorrected estimates of these variables. The vwls method is similar to the glst method in that it also assumes a linear-log relationship between exposure and RR, however it does not correct for lack of independence in the data. Printouts from these analyses are shown in Figure A12.1. As seen, the correction for lack of independence had only a small effect on the dose-response slopes (labeled "Coef." in the printouts) or their upper 95% CI. As such, these findings suggest that the lack of independence in the Shearer et al. (2021) and Vieira et al. (2013) results likely had only small effects on the CSF calculations.

Figure A12.1. Regression coefficients and their 95% CIs between the log of the RCC or kidney cancer ORs and serum PFOA concentrations using data from Shearer et al. (2021) and Vieira et al. (2013): adjusted (glst) and unadjusted (vwls) for OR dependence

Shearer et al. (2021):

Adjusted:

```
. glst logrr d0, se(se) cov(n case) cc

Generalized least-squares regression                Number of obs   =       3
Goodness-of-fit chi2(2)     =    0.84               Model chi2(1)   =    8.39
Prob > chi2                 =  0.6568               Prob > chi2     =  0.0038
------------------------------------------------------------------------------
       logrr |      Coef.   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
          d0 |   .0582026   .0200985     2.90   0.004     .0188103    .0975949
------------------------------------------------------------------------------
```

Unadjusted:

```
. vwls logrr d0 if logrr != 0, sd(se) nocons

Variance-weighted least-squares regression      Number of obs     =          3
Goodness-of-fit chi2(2)     =    0.44           Model chi2(1)     =       9.06
Prob > chi2                 =  0.8018           Prob > chi2       =     0.0026
------------------------------------------------------------------------------
       logrr |      Coef.   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
          d0 |   .0646938   .0214912     3.01   0.003     .0225718    .1068157
------------------------------------------------------------------------------
```

Vieira et al. (2013):

Adjusted:

```
. glst logrr dose0, se(se) cov(n case) cc

Generalized least-squares regression                Number of obs   =       3
Goodness-of-fit chi2(2)     =    0.58               Model chi2(1)   =    9.53
Prob > chi2                 =  0.7478               Prob > chi2     =  0.0020
------------------------------------------------------------------------------
       logrr |      Coef.   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
       dose0 |   .0108576   .0035177     3.09   0.002     .0039631    .0177521
------------------------------------------------------------------------------
```

Unadjusted:

```
. vwls logrr dose0 if logrr != 0, sd(se) nocons

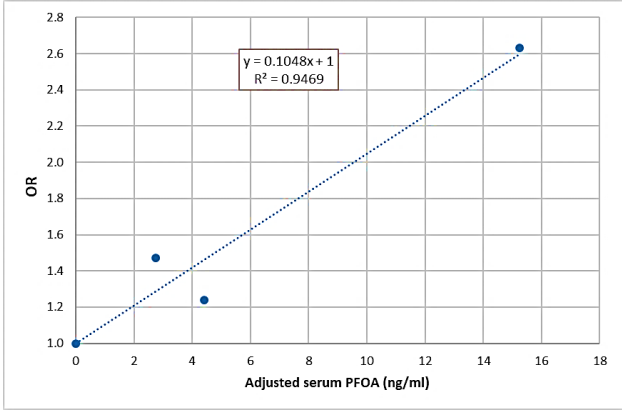
Variance-weighted least-squares regression      Number of obs     =          3
Goodness-of-fit chi2(2)     =    0.58           Model chi2(1)     =       9.42
Prob > chi2                 =  0.7500           Prob > chi2       =     0.0021
------------------------------------------------------------------------------
       logrr |      Coef.   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
       dose0 |   .0106432   .0034669     3.07   0.002     .0038482    .0174382
------------------------------------------------------------------------------
```

As noted above, another difference between the glst model and the linear model used for the final CSF calculations is that the former assumes a linear relationship between dose and the log of the RR estimates. Plotting both the linear-linear model (used in Equation 1) and the linear-log model (used in the glst method) shows that both provide a reasonably good fit (both visually and based on R^2 values), but with the linear model being slightly better for both studies (Figure A12.2). This suggests that the linear-linear model was a good choice for the CSF calculations.

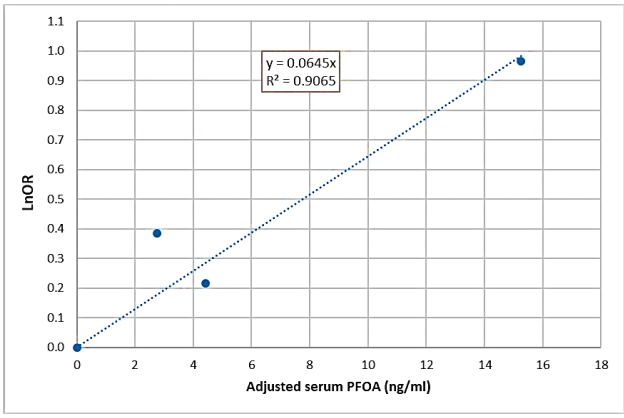
Figure A12.2. Plots, unweighted regression coefficients, and coefficients of determination (R^2) of RCC or kidney cancer ORs or natural log ORs (lnOR) and serum PFOA concentrations using data from Shearer et al. (2021) and Vieira et al. (2013)

Shearer et al. (2021):

OR (linear model, Equation 1)

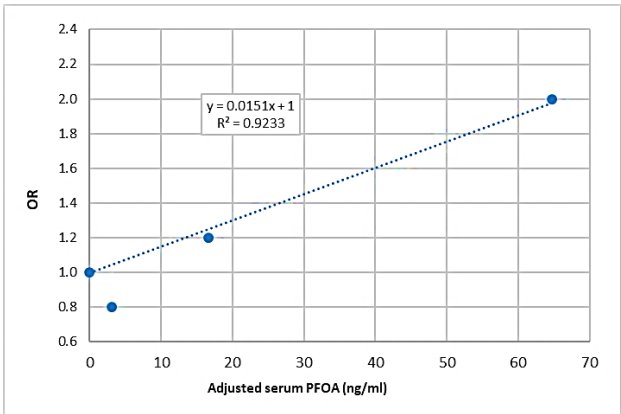


LnOR (glst model)

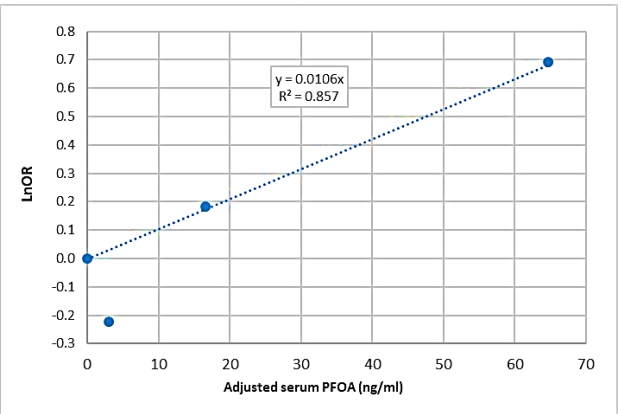


Vieira et al. (2013):

OR (linear model, Equation 1)



LnOR (glst model)



Lifetime, cumulative, or peak exposure: Exposure assessment in the Shearer et al. (2021) study was based on a single serum measurement collected in adulthood, while exposure assessment in the Vieira et al. (2013) study was based on modeled estimates of a single year of exposure, also in adulthood. Because of the long half-life of PFOA in serum, these measurements are likely a good indication of several years of exposure. Regardless, the use of a single measurement at a single point in time or for a relatively short period of time raises concerns about the importance of even longer-term cumulative exposure, and concerns about whether important time windows of exposure (e.g., childhood exposures) might have been missed.

Currently, little information is available on the relative cancer risks from longer-term lower PFOA exposures (e.g., cumulative exposure) versus shorter-term higher PFOA exposures (e.g., peak exposure). In addition, information from human studies on the most important time window (e.g., latency period) or life stage of exposure are not currently available. Despite this, several factors suggest that the exposure measurements in the Shearer et al. (2021) and Vieira et al. (2013) studies are good metrics for assessing the true cancer risks of PFOA. The first factor is that in animal studies, cancer risks from PFOA were not increased when exposure occurred perinatally compared to when it occurred later in life (NTP, 2020). This suggests that the Shearer et al. (2021) and Vieira et al. (2013) studies did not underestimate cancer risks by not including earlier life exposures. The second factor is that the Barry et al. (2013) study of PFOA and kidney cancer examined various exposure lag periods and found that the model using unlagged exposures provided a better fit than the models using lag periods of 10 to 20 years. This finding suggests that the cancer latency of PFOA is not several decades long, and that important latency effects were not missed in either study.

The third factor is that RR estimates for PFOA and kidney cancer in the two population-based studies in the C8 area, Barry et al. (2013) and Vieira et al. (2013), were similar regardless of whether the exposure metric was cumulative exposure or an indicator of lifetime peak exposure. Table A.X.1 shows the RR estimates for kidney cancer in these two studies. As shown, the RR estimates in the higher exposure categories of the C8 studies were all near 2.0, regardless of whether the exposure metric was cumulative exposure over a number of years or an estimate of the lifetime peak. The fact that the RRs for cumulative exposure are not markedly higher suggests that the peak exposure metrics OEHHA used in its CSF calculations are a good way to assess the true cancer risks of PFOA.

Table A12.1. Relative risk estimates for modeled serum PFOA and kidney cancer in the C8 population-based studies

Barry et al. (2013)			**Vieira et al. (2013)**					
Cumulative exposure Exposure window: 1952-2004			Cumulative exposure Exposure window: 1986-2005			Single year average Exposure window: 1986-1995		
Category	%	RR (95% CI)	Category	%	RR (95% CI)	Category	%	RR (95% CI)
Quartile 1	25%	1.00	Tertile 1	33%	0.8 (0.4-1.5)	Tertile 1	33%	0.8 (0.4-1.5)
Quartile 2	25%	1.34 (0.71-2.52)	Tertile 2	33%	1.2 (0.7-2.0)	Tertile 2	33%	1.2 (0.7-2.0)
Quartile 3	25%	1.95 (1.03-3.70)	Tertile 3*	23%	2.0 (1.3-3.2)	Tertile 3*	23%	2.0 (1.3-3.2)
Quartile 4	25%	2.04 (1.07-3.88)	>90th pct	10%	2.1 (1.1-4.2)	>90th pct	10%	2.0 (1.0-3.9)

Abbreviations: %, percentage of participants in each exposure category; pct, percentile; RR, relative risk estimate
* Tertile 3 excluding the upper 10th percentile

US EPA Benchmark Dose Software (BMDS): Categorical data from case-control studies cannot be used in the US EPA BMDS since these models are based on cancer risk, and the information needed to calculate risks is not available from the Shearer et al. (2021) or Vieira et al. (2013) publications. More specifically, although the number of RCC or kidney cancer cases in each exposure category is known, the exact number of people that generated these cases in each exposure category is not. These denominators could be estimated using the total number of participants in the underlying cohorts (e.g., the total number of people in the screening arm of the PLCO trial) and percentages of controls in each exposure category. However, entering

these estimates into the BMDS would lead to an artificially narrow 95% CI around the regression slope, and thus an artificially elevated BMDL.

One advantage of the BMDS is that the fit of a number of different dose-response models can be assessed (e.g., logistic, multi-stage, Weibull), allowing selection of the best fitting model. However, as discussed above, the linear model used in the above calculations provides a very good fit to the Shearer et al. (2021) and Vieira et al. (2013) data and it is unlikely that a different model will provide a fit that is substantially better.

APPENDIX 13. CANCER HEALTH-PROTECTIVE CONCENTRATIONS USING DIFFERENT VALUES FOR BASELINE RISK

Table A13.1. The effect on cancer health protective concentrations of using lower values of baseline risk (R_O)

Reduction in R_O	0%	5%	10%	15%	20%	25%
R_O Vieira	0.0202	0.0192	0.0182	0.0172	0.0162	0.0152
R_O Shearer	0.0182	0.0173	0.0164	0.0155	0.0146	0.0137
b Vieira	0.0146	0.0146	0.0146	0.0146	0.0146	0.0146
b Shearer	0.0980	0.0980	0.0980	0.0980	0.0980	0.0980
CSF_{serum} Vieira	0.00029	0.00028	0.00027	0.00025	0.00024	0.00022
CSF_{serum} Shearer	0.00178	0.00169	0.00160	0.00152	0.00143	0.00134
CF (ml/kg-day)	0.28	0.28	0.28	0.28	0.28	0.28
CSF_{intake} Vieira	0.00105	0.00100	0.00095	0.00090	0.00084	0.00079
CSF_{intake} Shearer	0.00637	0.00605	0.00573	0.00541	0.00509	0.00478
CSF_{intake} geometric mean	0.00259	0.00246	0.00233	0.00220	0.00207	0.00194
10^{-6} risk	0.000001	0.000001	0.000001	0.000001	0.000001	0.000001
DWI (L/kg-day)	0.053	0.053	0.053	0.053	0.053	0.053
C (ng/L)	0.00729	0.00767	0.00810	0.00857	0.00911	0.00972
$C_{rounded}$ (ng/L)	0.007	0.008	0.008	0.009	0.009	0.010

Abbreviations: b, regression slope between serum PFOA (ng/ml) and RCC relative risk; C, cancer health protective concentration; CSF_{intake} cancer slope factor or excess RCC risk per ng/kg-day intake of PFOA; CSF_{serum}, cancer slope factor or excess RCC risk per ng/ml serum PFOA; DWI, drinking water intake; R_O, baseline risk (i.e., RCC risk in the "unexposed" group)

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PFAS reduction during biosolids drying correlates to initial moisture content and is accompanied by detection of PFAS in dryer condensate

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Land application of biosolids has come under scrutiny for its potential to convey PFAS. Some water resource recovery facilities (WRRFs) have been forced to choose between implementing more extensive biosolids treatment technologies to achieve regulatory limits or altering their biosolids handling approach. Many novel biosolids treatments for PFAS removal include advanced thermal processes such as pyrolysis or gasification. Such processes require drying of biosolids as a preparatory step for treatment. However, little is known about the isolated impact of biosolids drying on the removal of PFAS from biosolids, or what fate PFAS may undergo during the drying process. The objective of this research was to elucidate how biosolids' PFAS profiles change due to drying, and to understand if PFAS can be transported with the gaseous vapor phase into liquid condensate. Biosolids were collected from five WRRFs and dried in a lab-scale oven. Additionally, biosolids from one of the WRRFs were dried in a system that captured and condensed vapor in a methanol impinger. Analysis of the PFAS profiles of the biosolids before and after drying indicated an average of 58% reduction in detectable PFAS across all sites, with the range being 43–74%. A positive correlation was established between the initial moisture content of the biosolids and reduction in detectable PFAS (R^2 = 0.61). The condensate contained 12–22% of the initial PFAS from the wet biosolids, indicating that PFAS can leave with the gas-phase vapor generated during drying. This research supports the conclusion that biosolids drying can reduce detectable concentrations of PFAS, and operational parameters could be adjusted to optimize dryers for this purpose. Treatment technologies could then be applied to the condensate to destroy the PFAS contained therein.

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Water impact

Reduction of PFAS concentrations in biosolids may be necessary in some regions to meet land application regulations. While the implementation of novel treatment technologies may be prohibitive in terms of scale and energy costs, biosolids drying is a conventional technology that can reduce the concentration of PFAS in biosolids. After drying, some PFAS were detected in dryer condensate, indicating that treatment of condensate could disrupt PFAS cycles within WRRFs.

1.0 Introduction

Per- and polyfluoroalkyl substances (PFAS) are a recalcitrant group of chemicals that are ubiquitous throughout society and have been associated with several human health concerns, including decreased antibody response, decreased fetal growth, increased risk of kidney cancer, and neurotoxicity.[1–4] Due to their presence in consumer goods and industrial discharges, many PFAS end up in wastewater and biosolids.[5–7] Land application of biosolids has been scrutinized recently for its capacity to convey PFAS into the environment.[7,8] As a result, some states have restricted land application based on the PFAS concentration in biosolids, and other states have banned it entirely.[8,9] Now, water resource recovery facilities (WRRFs) must account for PFAS when designing their biosolids management plans.

Land application has long been regarded as one of the most cost effective biosolids handling methods, as well as a sustainable method for recovering nutrients.[10–12] Employing

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land application to manage biosolids is also beneficial for reducing the amount of material sent to landfills.[10] Landfills emit volatile PFAS to the atmosphere and have potential to leach PFAS into nearby groundwater.[13,14] Moreover, sending biosolids to landfills may become costly or impracticable as space becomes limited and moisture content becomes a concern. Alternatively, incinerators, though potentially capable of defluorinating PFAS, may generate toxic gases like dioxin, furan, and fluorocarbons, including short-chain PFAS.[6,15,16] Furthermore, new incinerators are difficult to install if not part of an existing incinerator upgrade or expansion. Thermal processes have garnered recent interest as possible solids handling processes that could help mitigate PFAS in biosolids. Pyrolysis has been shown to reduce the detectable concentrations of PFAS in biochar, and gasification also likely reduces PFAS in the solid-phase.[17–20] Drawbacks to these emerging thermal processes include that they alter the inherent value of biosolids because biochar does not contain the same available carbon and nutrient content, and the long-term operational feasibility of these processes is still unknown.[21–23] Implementation of these thermal processes would require drying of the feed biosolids.[20,24,25]

Interestingly, drying may provide an avenue to help manage PFAS in biosolids. Previous research found that detectable PFAS concentrations were lower in biosolids after drying, in both a lab-scale oven and a full-scale dryer, and that reduction of detectable PFAS concentration was positively correlated to the initial moisture content of the biosolids sample.[26] Other research investigating the phenomenon of PFAS reduction during biosolids drying is limited, and comparison of data is complicated by varying analytical techniques. For example, other studies found PFAS concentrations increased during biosolids drying but analyzed fewer species.[27–29] The study which analyzed the most distinct PFAS species saw both increases and decreases to the PFAS profile of biosolids during drying across multiple sites.[30]

While multiple studies have reported decreases in detectable PFAS concentration, little information is available that elaborates on the mechanism by which this occurs. Only one study has captured and analyzed the vapor from the drying process, and in this full-scale study the mass of PFAS entering the regenerative thermal oxidizer (RTO) from a rotary biosolids dryer was orders of magnitude lower than the mass of PFAS removed from biosolids during drying.[31] More research is needed to determine the removal mechanism and phase distribution of PFAS during and after biosolids drying. Reductions in PFAS concentration have been observed at temperatures of 105 °C, which excludes mineralization as a removal mechanism; therefore, it is plausible that PFAS are being transferred to the vapor generated during drying.[63] Understanding the transport and fate of PFAS during biosolids drying will reveal avenues for appropriate management, including possible treatment, of PFAS after drying.

The objective of this research was to determine the reduction of detectable PFAS during biosolids drying across a range of moisture contents in biosolids and to establish if biosolids drying yielded PFAS in the gas-phase that could be detected in downstream condensate. It was hypothesized that higher moisture content would yield greater reductions in detectable PFAS concentration and that PFAS would be removed from biosolids during drying *via* aerosolization and appear in the captured condensate.[26,32,33] To test these hypotheses, triplicate samples of biosolids from five full-scale WRRFs were collected and dried in a lab-scale oven. The vapor from one set of drying experiments was captured with a methanol impinger to measure PFAS in condensate. This work sought to elucidate the feasibility of PFAS removal *via* transport with water during biosolids drying and determine the fraction of detectable PFAS reduction that could be explained by transfer to condensate.

2.0 Methods

2.1 Experimental setup

Biosolids samples were collected from five full-scale WRRFs with average flowrates ranging from 30 to 200 million gallons per day (MGD). Four of the five WRRFs employ secondary treatment systems with biological nutrient removal (BNR), while one facility (WRRF no. 1) utilizes secondary treatment for biochemical oxygen demand (BOD) removal only. All WRRFs use gravity thickening followed by anaerobic digestion and dewatering with centrifuges. The biosolids from these five WRRFs were dried in triplicate in a lab-scale oven overnight (24 hours) at 105 °C to determine the reduction of detectable PFAS achieved during biosolids drying. Biosolids from five different WRRFs were used for experiments to better understand how varied initial moisture contents impact PFAS removal. For these drying experiments, the wet and dry biosolids samples (before and after drying, respectively) were analyzed for PFAS as described below.

In addition to the oven-drying experiments, one set of biosoilds was dried in triplicate in a lab-scale heating system that allowed for vapor collection. The vapor collection system was customized by modifying a high-temperature conversion system (SI Fig. S1).[34] The customized system included a stainless-steel reactor vessel, a ceramic radiative heater, an inert gas purge system, and a gas collection system consisting of one impinger (20 mL of methanol, HPLC grade, >99.8%, Alfa Aesar™) in an ice bath. A pressure gauge and a thermocouple were used to monitor pressure and temperature inside of the reactor, respectively. The temperature of the system was raised at a rate of 8–15 °C min^{-1} to 105 °C, and the residence time of drying the biosolids in the reactor was 12 hours. To capture the system's off-gas, vapor from one drying experiment passed through PFAS-free tubing to the methanol impinger, where it was condensed (and henceforth referred to as condensate). The mass of the dried biosolids was determined gravimetrically, and the mass of the PFAS in the condensate was determined

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as a function of their concentration in the methanol impinger and the methanol + water volume; the volume of liquid in the impinger increased during the experiments from the water that transferred from the wet biosolids to the impinger during drying. The initial wet biosolids and the liquid from the impinger were stored in a freezer until shipment to Eurofins. The dry biosolids were stored at room temperature until shipment to Eurofins.

Negative controls were run to check for background PFAS contamination in this gas-phase collection system. Baked Ottawa sand (20–30 mesh, Spectrum™ Chemical) was loaded into the reactor, and 20 mL of methanol were loaded into the impinger. No detects were reported in the sand. The only species that was detected in the methanol negative controls was perfluoropropionic acid (PFPrA), at 1.4 μg L^{-1} in the methanol impinger. PFPrA was excluded from analysis for all gas-phase methanol samples because no samples had PFPrA concentrations greater than 10× the blank concentration. Pure methanol did not contain any reportable PFAS. The biosolids and liquid samples were put into containers provided by Eurofins for PFAS analysis. Samples were shipped in a cooler with ice packs to Eurofins for PFAS analysis.

2.2 PFAS analysis

The PFAS analysis employed in this experiment has been outlined previously.[26] A list of the sixty targeted PFAS analytes is included in the SI, section S1. Isotopically labeled analogs, referred to as extracted internal standards (EIS), were used for quantition where available. The list of EISs and their corresponding target analytes are listed in the SI Table S1. PFAS were analyzed with a SCIEX 5500+ triple quad LC/MS/MS system operated in multiple reaction monitoring (MRM) mode. Biosolids samples were manually homogenized in their collection containers before being subsampled (nominally 1 g for pre-drying and post-drying samples). The EISs of the native analytes were spiked into each subsample, then were sonicated with basic methanol for an hour. Water was added to the extracts to re-constitute them, and then they were cleaned up *via* solid-phase extraction (SPE) using a WAX SPE cartridge (Strata X-AW or equivalent) to retain the desired PFAS. Then, basic methanol was used to elute the IDAs and target analytes from the SPE cartridge and $^{13}C_2$-PFOA was added as the internal standard (ISTD) to fortify the extracts. A solution of 80 : 20 methanol/water was added to obtain a final extract volume of 10 mL. The same extraction procedure was used for pre-drying and post-drying biosolids samples.

To ensure the accuracy of the measurements of the 60 target analytes, initial and continual verification of accuracy and precision are achieved *via* laboratory control sample aliquots, laboratory control sample duplicates, laboratory method blanks, method detection limit (MDL) studies, MDL verification aliquots, and performance testing studies. The descriptions of these processes can be found in the corresponding laboratory standard operating procedures (SOPs). These procedures and performance test results are audited *via* 3rd party laboratory accreditation bodies for the relevant regulatory programs, including the National Environmental Laboratory Accreditation Program and the United States Department of Defense Environmental Laboratory Accreditation Program.

Additional measures of method performance monitored within each sample include EIS recovery, ISTD response, analyte retention times, analyte ion ratios, and analyte signal-to-noise ratios. The criteria for these sample-specific quality controls and identification elements are specified in the corresponding laboratory SOP. PFAS concentrations are reported on the basis of a sample's dry weight, which is determined after moisture has been removed at 105 °C and the sample has cooled to room temperature in a desiccator.

2.3 Statistical analysis

Concentrations of PFAS were normalized to μmol of F per kg of dry solids, where F represents the fluorine content of a given species of PFAS. Reported data were in units of μg PFAS per kg dried biosolids, so each measurement was divided by the molecular weight of the species and then multiplied by its F content per molecule. All data are available in the SI in units of μmol of F per kg of dry solids and also as the more commonly reported values of μg PFAS per kg of dry solids. For the condensate capture and mass balance experiment, the concentrations of PFAS in the biosolids and methanol were multiplied by the sample mass or volume, respectively. As such, the amount of total PFAS was normalized to moles of F in each targeted PFAS. Biosolids from source 5 were chosen by the anonymous utility partner for this phase of the experiment.

Effective statistical analysis required complete triplicate data, therefore, non-detects in the data were filled in with the reporting limits of the analytical method where triplicate data were incomplete. By contrast, if a species was not detected across any triplicates for either wet or dry biosolids, reporting limits were not used to fill in the data. Though this approach compensates for the potential trace presence of PFAS that evades detection, it slightly reduced the calculated percent removals of total PFAS compared to not using reporting limits (values of zero). Figure captions indicate when reporting limits are included in a given figure.

Additionally, significance was set to an alpha value of 0.05 or less for paired Student's *t*-tests. All statistical analyses were conducted in GraphPad Prism v10.4.2 (GraphPad Software, La Jolla, CA).

3.0 Results & discussion

3.1 Detectable PFAS removal during drying

Drying substantially reduced the presence of detectable PFAS in biosolids. In all five biosolids sample sets the total sum of detectable PFAS concentrations was significantly lower after drying (*p*-values of 0.0021, 0.0091, 0.0012, 0.0034, 0.0023, respectively) (Fig. 1). When categorized by speciation, nearly

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all classes of PFAS exhibited a measured concentration reduction during biosolids drying across all sites, including terminal species such as perfluorocarboxylic acids (PFCAs) and perfluorosulfonic acids (PFSAs). The average reduction in total detectable PFAS concentration across all samples was approximately 60% (57.6%), though reported reductions in concentration ranged from 42.3% to nearly 74.2% (Fig. 1). This range of percentages suggests that characteristic differences in biosolids samples impact PFAS concentration reduction during biosolids drying.

The presence of water affects the reduction in detectable PFAS during biosolids drying. The percent reduction was positively correlated to initial moisture content (Fig. 2). The R^2 value was 0.61 for a sample size of 18, which implies that 61% of variation in PFAS concentration reduction was explained by moisture content. The initial moisture contents of all samples were within a narrow range, from 72.4% (27.6% total solids) to 78.5% (21.5% total solids). This strong trendline justifies future research into a wider range of initial moisture (and total solids) contents. Also, while these data imply a mechanism of PFAS loss *via* transport with water, the exact removal mechanism cannot be determined from these data alone. The temperature of the lab-scale oven was slightly above the boiling point of water (105 °C). Previous research on the volatilization of pure chemical PFAS revealed that, at temperatures around 100 °C, thermogravimetrically analyzed weight loss of long-chain PFCAs such as PFOA, PFNA, and PFDA was less than 10%, and for PFSAs such as PFBS, PFHxS, and PFOS, weight loss was negligible.[36] The lack of removal

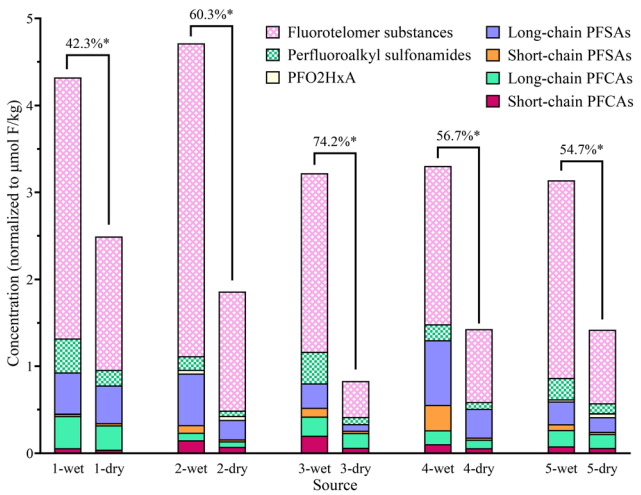


Fig. 1 Average concentration of categorized PFAS (normalized to µmol F kg^{-1}) in pre-drying ("wet") and post-drying ("dry") solids at five sites, labeled 1–5 (n = 3). Fluorotelomer substances include FTSes and FTCAs. Perfluoroalkyl sulfonamides include NMeFOSAA, NEtFOSAA, NMeFOSE, and NEtFOSE. According to the EPA, long-chain PFSAs have 6+ carbons, short-chain PFSAs have <6 carbons, long-chain PFCAs have 8+ carbons, and short-chain PFCAs have <8 carbons.[35] Reporting limits were substituted in for non-detects. Total PFAS percent removals were calculated for each triplicate and averaged (shown above each sample). Asterisk denotes statistical significance. Reprinted with permission. © The Water Research Foundation.

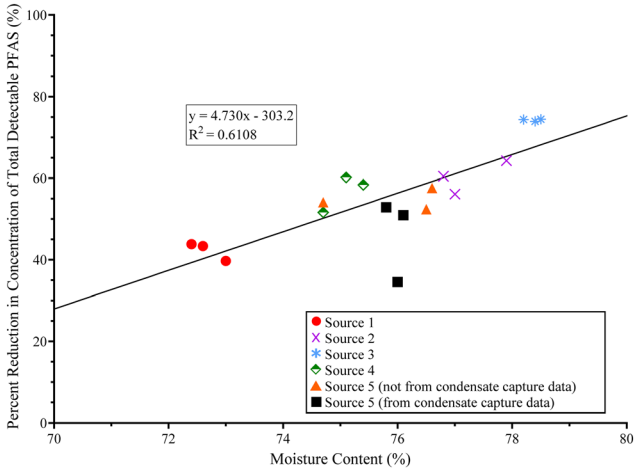


Fig. 2 Initial moisture content of biosolids is positively correlated to concentration reduction of total detectable PFAS (n = 18). Reporting limits were substituted in for non-detects. Reprinted with permission. © The Water Research Foundation.

of pure chemical PFAS at 100 °C indicates that volatilization alone is not a substantial removal mechanism for PFAS at this temperature. The percent reductions of PFAS in these biosolids drying experiments indicate that a removal mechanism other than (or in addition to) volatilization occurred.[36]

The removal of both volatile and nonvolatile PFAS *via* attachment to aerosols has been demonstrated for aeration tanks.[32,33] By extension, the formation of aerosols during boiling may occur *via* the bubble microtome effect.[37] Due to the tendency of PFAS to partition to the air–water interface (AWI), both small air bubbles underwater (*e.g.*, boiling bubbles) and small water droplets in the air (*e.g.*, aerosols) may contain significant amounts of PFAS, as both have high surface area to volume ratios; the surface area of an aerosol is effectively composed of the AWI.[37,38] Therefore, drying biosolids may incite the boiling of the water within and around the biosolids, thus leading to the generation of PFAS-laden aerosols.[26] An increase in the moisture content of biosolids may result in greater formation of aerosols, thereby giving rise to the observed positive correlation between moisture content and percent reduction of total detectable PFAS shown in Fig. 2. This trend supports data from previous research, which found a similar positive correlation between these variables.[26]

3.2 Incomplete mass balance on PFAS removal to the lab-scale dryer condensate

A fraction of the PFAS entered the lab-scale dryer condensate, yet the PFAS mass balance was not complete. On average, 16% of total PFAS from the wet biosolids were detected in the condensate. Additionally, the biosolids' detectable PFAS mass was reduced by an average of 46%. The "unaccounted" fraction of PFAS (represented by the white bars in Fig. 3 and 4a–c) corresponds to the difference between the initial PFAS

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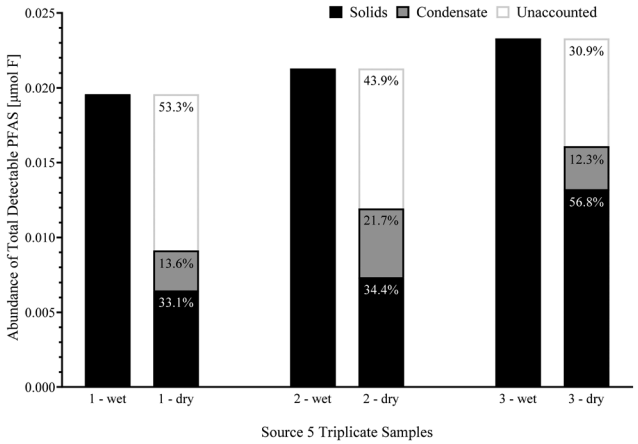


Fig. 3 Phase distribution of total detectable PFAS after drying (from source 5 triplicate samples). Abundance of PFAS was determined by normalizing reported concentrations to µmol F kg^{-1} solids or nmol F L^{-1} liquid, then multiplying that value by either the mass of solids or the volume of liquid in the impinger (Table S2). Only detected species were used for this figure (*i.e.*, reporting limits were not substituted in for non-detects). Reprinted with permission. © The Water Research Foundation.

mass in the wet biosolids and the combined mass detected in the dry solids and impinger methanol; the fate of this mass of unaccounted PFAS is unknown. On average, 43% of total PFAS as F remained unaccounted for across all triplicates, with a range from 31% to 53%.

Several reasons could contribute to explaining the unaccounted fraction. It is possible that PFAS were lost *via* sorption to part of the experimental apparatus since the entire surface area was not extracted for PFAS, *e.g.*, the tubing of the impinger system was not rinsed with methanol. If PFAS sorbed to the tubing, they did so by way of entering the gas-phase, which suggests that they should have been detected in the captured condensate but did not make it to the impinger. Additionally, a portion of unaccounted PFAS may stem from detectable PFAS transforming to undetectable PFAS, such as trifluoroacetic acid.[39] Approximately 10% weight loss of PFOA has been reported at temperatures of 100 °C, accompanied by the generation of volatile, fluorinated compounds.[36] OTM-50 was not employed in this research, and it is possible that non-polar volatile PFAS were generated as products of incomplete destruction but were not detected in the condensate.[40] Experimental data would be needed to validate this removal pathway. It is also possible there is an artifact arising from the heterogenous distribution of PFAS in biosolids samples, or that chemical extraction of PFAS from the dried solids samples was less efficient than extraction from wet solids samples.[41] Within the third triplicate specifically, the volume of methanol in the impinger was approximately eight mL less than the other triplicates by the end of the experiment. Although the reason for this difference in methanol volumes was not identified, it is possibly due to sample loss, thus reducing the mass of PFAS recovered in the condensate of the third triplicate in

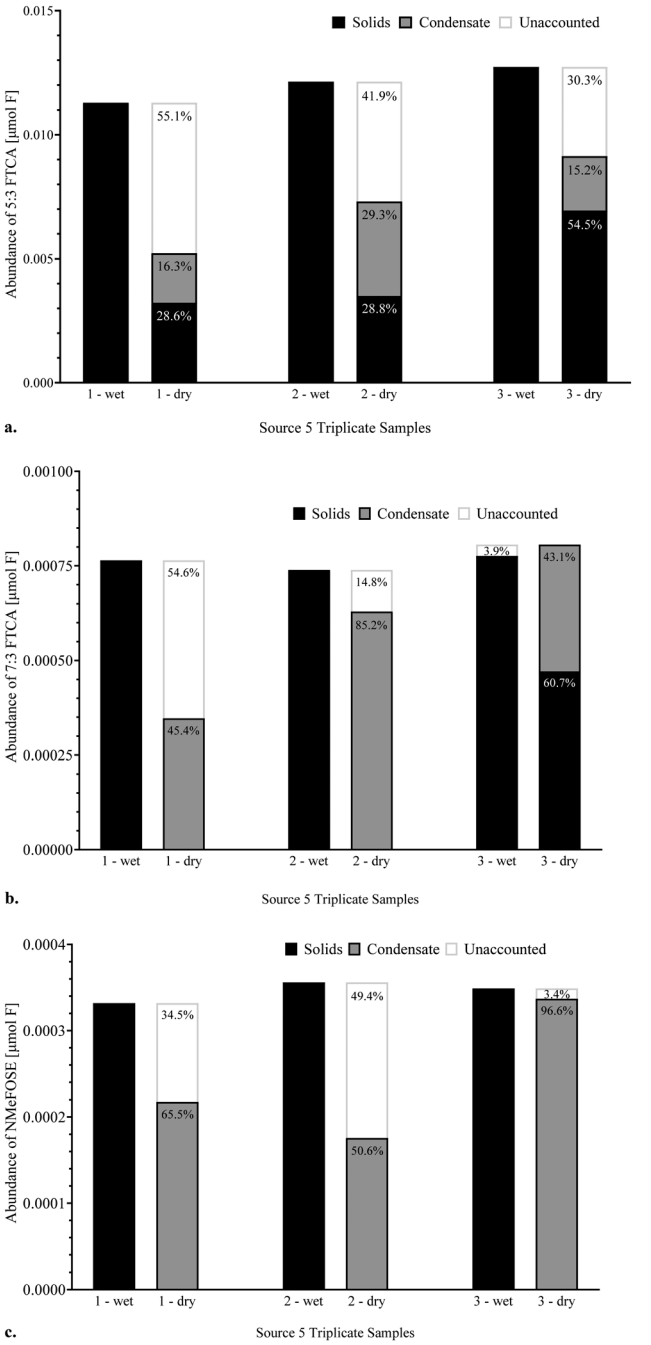


Fig. 4 a. Phase distribution of 5 : 3 FTCA after drying (from source 5 triplicate samples). b. Phase distribution of 7 : 3 FTCA after drying (from source 5 triplicate samples). c. Phase distribution of NMeFOSE after drying (from source 5 triplicate samples). Only detected species were used for this figure (*i.e.*, reporting limits were not substituted in for non-detects). Reprinted with permission. © The Water Research Foundation.

Fig. 3 and 4a–c. Despite the unclosed F mass balance, these results demonstrate a proof-of-concept that PFAS can be captured in dryer condensate.

Precursor species, including fluorotelomer carboxylic acids (FTCAs), were removed from biosolids during drying and captured in the condensate. Three species were

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detected across all three triplicates of the captured condensate: 5:3 FTCA (Fig. 4a), 7:3 FTCA (Fig. 4b), and NMeFOSE (Fig. 4c); one species was detected only in one triplicate: NEtFOSE. More than three-quarters of the total PFAS in the condensate was attributable to 5:3 FTCA, across all triplicates. Though NMeFOSE and 7:3 FTCA comprised a smaller fraction of total PFAS across all phases than 5:3 FTCA, they exhibited substantial removal to the condensate.

Not only are PFAS leaving with the gas-phase and appearing in the condensate, but in the case of 7:3 FTCA and NMeFOSE, some PFAS may not be detected at all in the dried solids. None of the dried biosolids triplicates had detectable levels of NMeFOSE, and only one had 7:3 FTCA, despite all three condensate triplicates revealing detectable amounts of both, as shown in Fig. 4b and c. In the sample with the lowest condensate fraction of 7:3 FTCA and NMeFOSE, nearly half of the amount present in the pre-dried biosolids was captured in the condensate. Since the temperature of the oven was approximately 105 °C, it is possible that this removal mechanism was physical (*i.e.*, removal *via* attachment to aerosols) rather than a direct phase transition (*i.e.*, volatilization); the relatively low volatility and high water solubility of FTCAs is consistent with the tendency to partition to aerosols.[33]

3.3 Species specific concentration reduction rates

On average, the concentration of total detectable PFAS in the pre-drying biosolids decreased by 57.6% after drying. The reduction in concentration of precursor species, on average, accounted for over 80% of this observed concentration reduction of detectable PFAS. Specifically, 5:3 FTCA contributed 60% to the reduction in concentration of total PFAS, and the top three precursors (5:3 FTCA, 6:2 FTCA, and 7:3 FTCA) contributed more than 71% (Fig. 5). Of the terminal species, the average reduction in concentration of PFOS was the greatest, at more than 10% of the total PFAS-as-F (Fig. 5). All of the ten species that contributed to more than 1% of the concentration reduction of total detectable PFAS are shown in Fig. 5 below. These data demonstrate that the majority of detectable PFAS concentration reduction observed during drying is attributable to a limited number of species. It should be noted that the capacity for a species to contribute to the reduction in concentration of total detectable PFAS is heavily dependent on the initial concentration of the species. For example, 5:3 FTCA constituted the largest proportion of the PFAS profile for all biosolids samples across all sites. Therefore, reducing its concentration will contribute significantly to the reduction in concentration of total detectable PFAS.

3.4 Role of FTCAs and quantification methods

The analytical technique used for this experiment detected 60 distinct species of PFAS, including all 40 species

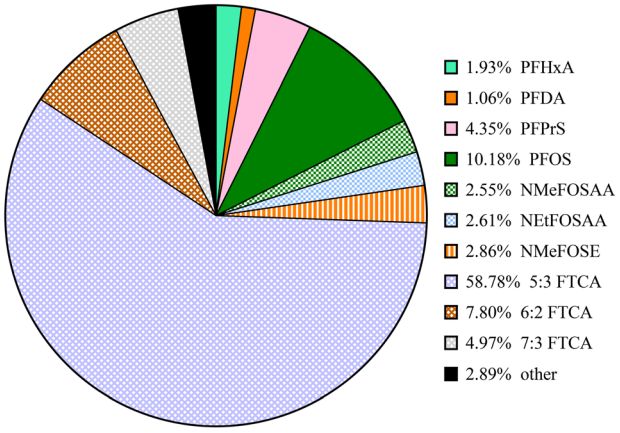


Fig. 5 Species that contributed at least 1% to the reduction in concentration of total detectable PFAS from biosolids. Reporting limits were substituted in for non-detects. Each species' contribution to the percent concentration reduction of total detectable PFAS from the biosolids was averaged for each site (n = 3), then the averages of each species across all sites were averaged (n = 5). Reprinted with permission. © The Water Research Foundation.

detected by EPA method 1633.[42] Five species that would have been excluded by method 1633 were detected in the experimental samples, making up, on average, 10.5% of the pre-dried biosolids' PFAS profiles and 1.6% of the post-dried biosolids' PFAS profiles. However, this experiment did not include detection of polyfluoroalkyl phosphoric acid diesters (diPAPs), which have been substantial PFAS species in other biosolids studies (54% and 56% of the total PFAS mass in biosolids, on average).[30,43]

Differing analytical techniques will detect different species of PFAS, which may influence concentration reduction calculations.[43] Some techniques exclude FTCAs in their analysis.[29] 5:3 FTCA was the most prevalent species of PFAS across all triplicates for all samples, including pre-dried solids, dried solids, and condensate. Had FTCAs not been detected in this set of experiments, the calculated average percent removal of total PFAS would have been 48% across all sites (instead of 58%). Furthermore, the exclusion of FTCAs from analysis dramatically reduces the detectable concentration of total PFAS to approximately 33% of the original values, as shown in comparing Fig. 6a and b. The detection (or lack of detection) of specific PFAS has a large influence on the results and conclusions drawn from an experiment.

Despite being the most prevalent PFAS species detected in biosolids from all sources, 5:3 FTCA is not a contaminant of direct industrial output; it is a metabolite from the transformation of other precursors, including 6:2 diPAP, 6:2 FTOH, and 6:2 FTSA, which may have extensive industrial applications.[44–50] 5:3 FTCA has been identified as the most highly concentrated PFAS present in landfill leachate.[46] Leachate being sent to the head of a WRRF may, therefore,

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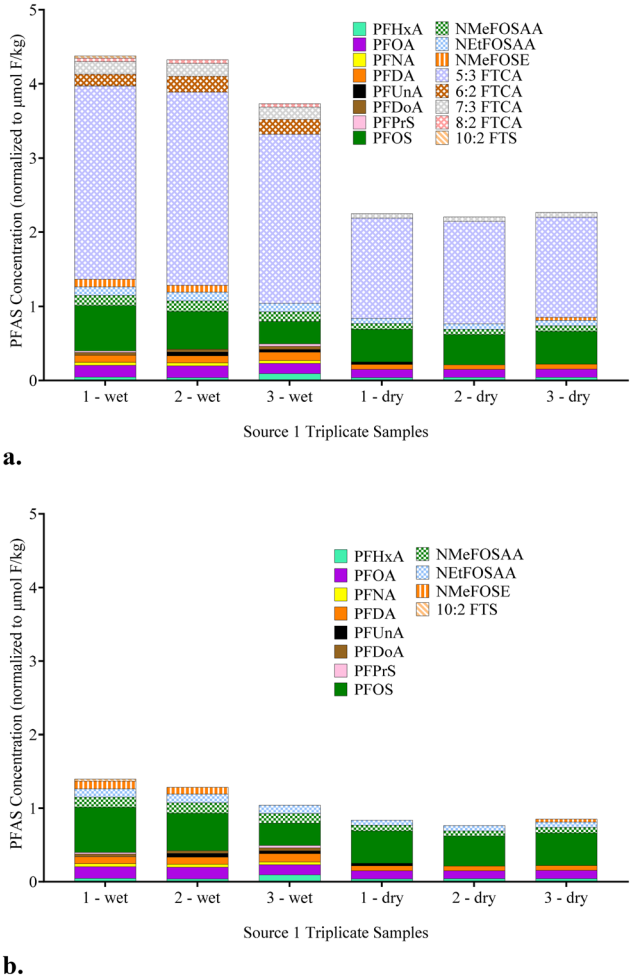


Fig. 6 a. Original PFAS profile of triplicates from the first site. b. PFAS profile of triplicate data from the first site excluding FTCA compounds (approximately 1/3 of the total PFAS from each sample is shown). Only detected species were used for this figure (*i.e.*, reporting limits were not substituted in for non-detects). Reprinted with permission. © The Water Research Foundation.

be a substantial source of 5 : 3 FTCA in wastewater treatment streams.

The FTCAs in land-applied biosolids may undergo transformation in food crops (*e.g.*, pumpkin and wheat) to terminal PFAS such as PFHxA, PFPrA, and PFOA.[51,52] Though FTCAs may not be regulated stringently, their presence is indicative of potential future contamination as they degrade and transform into perfluoroalkyl acid (PFAA) species.[51–55] This insight is especially crucial when paired with the condensate PFAS profile data that indicates 5 : 3 FTCA and 7 : 3 FTCA both left with the gas-phase during drying (Fig. 4a and b). The escape of these compounds, and their transformation potential, indicate that drying requires effective air pollution controls to reduce potential emission of not only these precursors, but their transformation products as well. Further research on the efficacy of current air pollution control technologies and possibly condensate treatment on PFAS-containing dryer emissions would be useful.[31]

3.5 PFAS treatment in condensate with PFAS from dryers

While drying could be a viable technology to reduce detectable PFAS levels in biosolids, treatment requirements for the resulting condensate from the drying process need to be further investigated. Condensate volumes from sludge dryers can vary significantly based on factors such as sludge type, dryer type, and operating conditions. Nonetheless, sludge dryers can produce substantial condensate volumes that may require further treatment and can be recycled to biological treatment portions of the plant.[56] In addition to conventional nutrients (*e.g.*, phosphorus and nitrogen), the condensate can also contain PFAS.[29] The mobilization of PFAS from the biosolids into the liquid phase (*i.e.*, condensate) represents an opportunity to disrupt the PFAS contamination cycle at WRRFs.[26]

To break this cycle, a separation step followed by destruction could be an efficient method to drastically reduce PFAS concentrations.[13] One possible separation technique is foam fractionation, where air bubbles are utilized to separate surface-active molecules (*i.e.*, PFAS). Through this process, PFAS are concentrated in a foamate solution that can be directed for subsequent destruction. This step offers a dual advantage as the PFAS concentrations in the foam can be enriched by a factor ranging from 100 to 100 000, and the volume requiring treatment is significantly smaller compared to not using foam fractionation.[57]

To date, various destruction techniques have been utilized to eliminate PFAS, each with its own distinct advantages and disadvantages, indicating a need for a customized approach for each WRRF.[58] Among other technologies, field installations of supercritical water oxidation (SCWO) and electrochemical oxidation (EO) have recently garnered significant attention. In SCWO, water is heated above its critical point to create a supercritical state where it exhibits properties of both a liquid and a gas. This high-temperature and high-pressure environment allows for the complete oxidation of organic contaminants, like PFAS, breaking them down into byproducts such as carbon dioxide, water, and mineral acids.[59] During EO, an electrical current is applied to the contaminated water or solution, causing oxidation reactions to occur at the electrode surfaces. These oxidation reactions break down the PFAS compounds into less harmful byproducts through electrochemical processes.[60] Combining foam fractionation with either SCWO or EO could offer a promising solution to prevent the re-mobilization of PFAS back into the biological treatment section of a WRRF after removal of the PFAS from the biosolids stream. The net life cycle cost of this flow diagram (drying with PFAS treatment of the condensate) may be advantageous when compared to PFAS destruction in the full biosolids stream.

3.6 Future perspectives

The current study has revealed that PFAS transport to dryer condensate during biosolids drying is feasible. Due to the heterogeneity of biosolids samples, it is necessary to analyze

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the PFAS profiles of dryer condensate from different biosolids samples to establish the reproducibility of these experiments. Additionally, a more robust experimental design is required to further elucidate PFAS fate, including their potential for transformation and removal during biosolids drying. Effective detection of precursor compounds, especially diPAPs, is key to developing a more comprehensive understanding of PFAS fate.[61] Future work should include the quantification of diPAPs in sample analysis and the extraction of PFAS from laboratory equipment with a methanol rinse. Augmenting targeted detection methods with analytical tools such as the total oxidizable precursor (TOP) Assay may also improve the understanding of PFAS fate.

4.0 Conclusions

This research confirms that concentrations of detectable PFAS in biosolids are reduced during drying. Furthermore, the majority of concentration reduction is attributable to a reduction in precursor species, particularly 5:3 FTCA. Therefore, reduction in concentration of PFAS in biosolids during drying may provide an avenue by which PFAS can be captured and destroyed, thus mitigating contamination cycles. However, should biosolids drying not be paired with some form of air pollution control and/or condensate treatment, dryer air emissions or condensate may contain precursors such as 5:3 FTCA, 7:3 FTCA, and NMeFOSE that can then transform into PFAAs.[62] While the vapor capture technique utilized herein revealed removal to condensate of some PFAS, more research is needed to more fully close the F mass balance from drying of biosolids, by either confirming transformation products or detecting previously undetectable PFAS. Measurements of PFAS concentration reduction are constrained by the chosen detection method.

This research aimed to help fill the gap in the literature on the effect of drying on biosolids' PFAS profiles, including concentration reduction percentages and phase distribution, and to correlate PFAS concentration reduction with initial biosolids moisture content. Moisture content of the initial biosolids sample correlated to greater reduction of total detectable PFAS concentration, indicating that aerosolization during biosolids drying is a potential avenue by which PFAS are being removed.[26,37] This process circumvents the requirement for high temperatures associated with volatilization and mineralization, and may be a substantial removal mechanism for PFAS in biosolids dryers. By developing a robust understanding of how PFAS concentrations are reduced during biosolids drying and where exactly they end up, full-scale facilities can be optimized to control and increase the removal of PFAS from biosolids during drying and to capture and treat the removed PFAS. Overall, drying could be used to increase the accessibility of biosolids land application.

Conflicts of interest

There are no conflicts to declare.

Data availability

The data supporting this article have been included as part of the supplementary information (SI).

Supplementary information is available and describes the reactor setup, PFAS analytes quantified, and PFAS profiles of wet and dry biosolids samples. See DOI: https://doi.org/10.1039/d5ew01120e.

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PFAS Destruction by a Hazardous Waste Incinerator: Testing Results



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PFAS Destruction by a Hazard Waste Incinerator: Testing Results

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Abbreviations

°C	degree(s) Celsius
°F	degree(s) Fahrenheit
µg/m^3	microgram(s) per cubic meter
µg/sample	microgram(s) per sample
afterburner	afterburner chamber
AFFF	aqueous film-forming foam
ASTM	ASTM International
ATA	Additional Target Analyte
ATG	Alliance Technical Group
BT	breakthrough
CAS No.	Chemical Abstracts Service registry number
CF_4	tetrafluoromethane
C_2F_6	hexafluoroethane
CFR	Code of Federal Regulations
CHES	Clean Harbors Environmental Services
CO_2	carbon dioxide
DE	destruction efficiency
DI	deionized
DOD	U.S. Department of Defense
DQI	data quality indicator
DRE	destruction and removal efficiency
EA	EA Engineering, Science, and Technology, Inc., PBC
EPA	U.S. Environmental Protection Agency
EPA ORD	U.S. EPA Office of Research and Development
Eurofins	Eurofins Environment Testing
FRC	Fluorinated Replacement Chemical
FSMB	field sample media blank
FTIR	Fourier transform infrared
HDPE	high-density polyethylene
HWI	hazardous waste incinerator
lb/hr	pound(s) per hour
LC/MS/MS	liquid chromatography/tandem mass spectrometry
LCS	laboratory control sample
LCSD	laboratory control sample duplicate
MDC	minimum detectable concentration
MDL	method detection limit
Method 0010	EPA SW-846 Method 0010
MeOH	methanol
mL	milliliter(s)

MS	matrix spike
MSD	matrix spike duplicate
Na_2CO_3	sodium carbonate
ng/dscm	nanogram(s) per dry standard cubic meter
ng/g	nanogram(s) per gram
ng/L	nanogram(s) per liter
ng/m^3	nanogram(s) per cubic meter
ng/sample	nanogram(s) per sample
NH_4OH	ammonium hydroxide
No.	number
O_2	oxygen
OTM-45	EPA Other Test Method 45
OTM-50	EPA Other Test Method 50
PFAS	per- and polyfluoroalkyl substances
PID	product of incomplete destruction
POHC	principal organic hazardous constituent
ppb	part(s) per billion
ppbv	part(s) per billion by volume
QA	quality assurance
QC	quality control
RPD	relative percent difference
SOP	Standard Operating Procedure
Spectrum	Spectrum Environmental Solutions, Inc.
SSI	Superior Spiking Industries
STPB	sampling train proof blank
TMT-15	15 percent aqueous solution of trimercapto-s-triazine trisodium salt
UMO	used motor oil
VFC	volatile fluorinated compound
XAD-2	Tradename for hydrophobic copolymer of styrene-divinylbenzene resin

Given the length and complexity of the definitions for the PFAS abbreviations, this report deviates from generally accepted rules of abbreviation handling for PFAS abbreviations only. To make the descriptions within the text of this report less cumbersome, PFAS target analytes and PFAS classes are not defined at first use in the text and the abbreviations only are used. The table below provides the group, abbreviation, and full name of the PFAS target analytes referenced in this report.

The remainder of the abbreviations used in the report are compiled in the Abbreviations list on page xi and are defined at first use in the body of the report.

PFAS Target Analyte Abbreviations

Class	Abbreviation	Compound
Perfluorinated Carboxylic Acids (PFCAs)	PFBA	perfluorobutanoic acid
PFCAs	PFPeA	perfluoropentanoic acid
PFCAs	PFHxA	perfluorohexanoic acid
PFCAs	PFHpA	perfluoroheptanoic acid
PFCAs	PFOA	perfluorooctanoic acid
PFCAs	PFNA	perfluorononanoic acid
PFCAs	PFDA	perfluorodecanoic acid
PFCAs	PFUnA	perfluoroundecanoic acid
PFCAs	PFDoA	perfluorododecanoic acid
PFCAs	PFTriA	perfluorotridecanoic acid
PFCAs	PFTeA	perfluorotetradecanoic acid
PFCAs	PFHxDA	perfluorohexadecanoic acid
PFCAs	PFODA	perfluorooctadecanoic acid
Perfluorinated Sulfonic Acids (PFSAs)	PFBS	perfluorobutanesulfonic acid
PFSAs	PFPeS	perfluoropentanesulfonic acid
PFSAs	PFHxS	perfluorohexanesulfonic acid
PFSAs	PFHpS	perfluoroheptanesulfonic acid
PFSAs	PFOS	perfluorooctanesulfonic acid
PFSAs	PFNS	perfluorononanesulfonic acid
PFSAs	PFDS	perfluorodecanesulfonic acid
PFSAs	PFDoS	perfluorododecanesulfonic acid
Perfluorinated Sulfonamides (FOSAs)	FOSA	perfluorooctanesulfonamide

Class	Abbreviation	Compound
FOSAs	NMeFOSA	N-methyl-perfluorooctane sulfonamide
FOSAs	NEtFOSA	N-ethylperfluoro-1-octanesulfonamide
Perfluorinated Sulfonamidoacetic Acids (FOSAAs)	NMeFOSAA	N-methylperfluorooctanesulfonamidoacetic acid
FOSAAs	NEtFOSAA	N-ethylperfluorooctanesulfonamidoacetic acid
Perfluorinated Sulfonamide Ethanols (FOSEs)	N-MeFOSE	2-(N-methylperfluoro-1-octanesulfonamido) ethanol
FOSEs	N-EtFOSE	2-(N-ethylperfluoro-1-octanesulfonamido) ethanol
Fluorotelomer Sulfonates (FTSs)	4:2 FTS	1H, 1H, 1H, 2H, 2H-perfluorohexanesulfonic acid
FTSs	6:2 FTS	1H, 1H, 1H, 2H, 2H-perfluorooctanesulfonic acid
FTSs	8:2 FTS	1H, 1H, 1H, 2H, 2H-perfluorodecanesulfonic acid
FTSs	10:2 FTS	1H, 1H, 1H, 2H, 2H-perfluoroundecanesulfonic acid
Fluorinated Replacement Chemicals (FRCs)	DONA	4,8-dioxa-3H-perfluorononanoic acid
FRCs	HFPO-DA	hexafluoropropylene oxide dimer acid
FRCs	9Cl-PF3ONS	9-chlorohexadecafluoro-3-oxanonane-1-sulfonic acid
FRCs	11Cl-PF3OUdS	11-chloroeicosafluoro-3-oxaundecane-1-sulfonic acid
Additional Target Analytes (ATAs)	PFECA F	perfluoro-3-methoxypropanoic acid
ATAs	PFECA A	perfluoro-4-methoxybutanoic acid
ATAs	PFECA B	nonafluoro-3,6-dioxaheptanoic acid
ATAs	PES	perfluoro (2-ethoxyethane) sulfonic acid
ATAs	3:3 FTCA	3-perfluoropropylpropanoic acid
ATAs	5:3 FTCA	5:3 fluorotelomer carboxylic acid
ATAs	7:3 FTCA	7:3 fluorotelomer carboxylic acid
ATAs	8:2 FTUCA	8:2 fluorotelomer unsaturated acid
ATAs	10:2 FTCA	10:2 fluorotelomer carboxylic acid
ATAs	8:2 FTCA	8:2 fluorotelomer carboxylic acid
ATAs	6:2 FTCA	6:2 fluorotelomer carboxylic acid
ATAs	PFECHS	perfluoro(perfluoroethyl)cyclohexanesulfonic acid
ATAs	6:2 FTUCA	6:2 fluorotelomer unsaturated acid

Executive Summary

Per- and polyfluoroalkyl substances (PFAS) are a large group of synthetic chemicals. The wide use of both polymeric and non-polymeric PFAS in manufactured products is based on their unique heat resistance, chemical resistance, and surface-active properties, which are associated with the multiple carbon-fluorine bonds and various functional groups characteristic of these chemicals. Unfortunately, the stability that results from the multiple carbon-fluorine bonds and contributes to the utility of PFAS also makes these chemicals difficult to degrade naturally. This, combined with the broad use of PFAS, has led to significant persistence of some of these chemicals in the environment. This persistence, combined with emerging evidence of human and ecological toxicity associated with certain PFAS, have raised concerns regarding the potential risk of these "forever chemicals" to human health and the environment.

Thes concerns have led to a comprehensive search for technologies and methods that can effectively destroy PFAS, either when co-mingled with environmental media or in more concentrated forms in industrial wastes. Given the wide range of individual PFAS and their inherent chemical stability, Resource Conservation and Recovery Act (RCRA)-permitted hazardous waste incineration, which operate at conditions to destroy other persistent organic compounds, may be a candidate technology for ensuring complete, non-selective destruction of PFAS.

Full-scale experience with incineration of PFAS in commercial hazardous waste incinerators has been limited to date. Two previous comprehensive test programs were conducted in 2021 and 2022 at a RCRA-permitted hazardous waste incinerator in Utah, which is owned and operated by Clean Harbors Environmental Services (CHES). The CHES test programs demonstrated high (>99.9999 percent) destruction and removal efficiency (DRE) for PFAS present in aqueous film-forming foam (AFFF) concentrate and dynamically spiked into the incinerator for DRE demonstration. PFAS effectively destroyed in these tests included legacy materials (no longer manufactured due to health and environmental concerns) and replacement PFAS, including GenX. The uncertainty about products of incomplete destruction (PIDs), even after demonstration of high PFAS DREs, led to additional testing at the same unit in November 2024.

The November 2024 testing incorporated most components of the 2021 and 2022 test campaigns, including sampling of liquid waste feed streams, input chemicals and utilities, process residuals, and exhaust emissions for PFAS target analytes, to support an overall PFAS mass balance. This test program included feeding AFFF concentrate and dynamic spiking of nine PFAS compounds for DRE determination. For each test condition, triplicate runs were conducted under normal incinerator operating conditions with mixed solid and liquid waste feeds used to maintain a temperature in the rotary kiln between 950 and 1196 °C and over 1100 °C in the afterburner. As during the two previous test programs, EPA's Other Test Method-45 (OTM-45) was used to sample exhaust emissions for 49 semi-volatile, polar PFAS target analytes. Other Test Method-50 (OTM-50), released by EPA in January 2024, was used in this test for sampling exhaust emissions for 30 volatile fluorinated compounds (VFCs), which could include potential PFAS PIDs such as tetrafluoromethane (CF_4) and hexafluoroethane (C_2F_6). EPA's SW-846 Method 0010 (Method 0010) was used for sampling exhaust emissions for semi-volatile, non-polar PFAS. In addition, exhaust emissions were sampled and analyzed by Fourier transform infrared (FTIR) spectroscopy using ASTM method D6348, as an alternate means of quantifying CF_4 and C_2F_6.

Nine semi-volatile PFAS, for which EPA has established human health toxicity assessments, were spiked as to determine the DRE and destruction efficiency (DE). C_2F_6 was also spiked to enable determination of DRE for this difficult-to-destroy VFC.

OTM-45 results were similar to those from the two previous tests for semi-volatile polar PFAS with most of OTM-45's target list not detected or near the detection limit. Some PFAS were also found as contaminates in the blanks, such as hexafluoropropylene oxide dimer acid. Method 0010 results indicated no semi-volatile non-polar PFAS were identified in the exhaust emissions. OTM-50 sample analysis was conducted by both Eurofins and EPA. Concentrations of all 30 VFCs in the samples analyzed by Eurofins were below detection level for these analytes in all nine runs. EPA analytical results indicated low level detections for six OTM-50 VFC analytes near the detection limit. For two of these compounds, concentrations in the blanks were in the same range as the concentrations in the actual samples.

For the nine spiked PFAS, calculated DREs ranged from 99.95 percent to 99.9999 percent. Based on the results of OTM-50 and FTIR sampling and analysis, DRE for C_2F_6 ranged from 99.99 percent to 99.9999 percent depending on the detection limits of the analytical method.

DREs relate the amount of a compound in the stack emissions to the mass fed into the system without regard of the amount of the destruction of the compound but the removal from the emissions. DE calculations were performed to determine total PFAS destruction by the entire treatment system. DE was based, to the extent practicable, on the comprehensive overall PFAS mass balance, using total mass of PFAS entering the process in all streams and the total mass of PFAS leaving the system in process residuals and exhaust emissions. For the nine spiked PFAS, calculated DEs ranged from 99.97 percent to 99.9999 percent.

An EPA dispersion model, AERSCREEN, was used to predict ground-level ambient air concentrations for twelve PFAS measured in exhaust emissions. All modeled values were between two and eight orders of magnitude below levels outlined for the regulated PFAS concentrations in several state ambient air quality regulations and guidelines.

The results of the November 2024 PFAS destruction testing demonstrated for the third time the Aragonite RCRA-permitted hazardous waste incineration facility can produce DREs as high as 99.9999 percent for a variety of PFAS. The testing also demonstrated that incineration of PFAS-containing wastes can achieve low to non-detectable concentration of VFCs, below 1.00 parts per billion by volume, that could be considered PFAS PIDs in exhaust emissions. DRE values from 99.99 to 99.9999 percent were demonstrated for C_2F_6, supporting the potential use of C_2F_6 as an indicator for PFAS destruction. Implementation of this approach may help to reduce the complexity and cost of future tests at this and similar facilities by relying primarily on verification that C_2F_6 can be destroyed at high efficiencies, but more data is needed.

This is the most comprehensive PFAS incineration test to date and provides valuable data about PFAS incineration but is not intended to be a general recommendation of incineration for the treatment of PFAS. There are some uncertainties and limitations to the data. The results here may only apply to the incinerator that was tested and not all hazardous waste incinerators. Each incinerator may need to be tested to determine its performance. As the methods improve, greater certainty will occur and target lists may grow, making future results even more comprehensive. Here, the method to identify semi-volatile fluorinated compounds in the emissions, modified Method 0010, is still under development and

no PIDs were observed. This is promising, but as the method is improved greater sensitivity may help detect fluorinated compounds in the emissions. The presence of polar volatile fluorinated compounds was not investigated, since there is no standardized method for polar volatile fluorinated compounds, such as trifluoroacetic acid, and may be an area for future development. The results from the OTM-45 trains had HFPO-DA in the trains and in the proof blank, likely from contamination. This may show that great cleaning or other modifications may be needed to OTM-45 to reduce contamination.

1 Introduction

1.1 Background

Widespread detection of per- and polyfluoroalkyl substances (PFAS) in environmental media, along with mounting evidence of their harmful effects on human health and ecosystems, is driving the need to identify effective PFAS remediation and destruction technologies (U.S. Environmental Protection Agency [EPA] 2024a). Due to the strength of multiple carbon-fluorine chemical bonds in these compounds, many PFAS are difficult to completely degrade. There are insufficient data on the performance of commercially available destruction technologies with capacities to treat large amounts of PFAS-contaminated wastes. Evaluation of PFAS destruction technologies is difficult due to the wide array of different physical and chemical properties of the large number of chemicals classified as PFAS. Recent development of new methods to sample and measure PFAS and potential products of incomplete destruction (PIDs) makes more comprehensive evaluations of various remediation and destruction technologies possible.

Incineration is an established destruction technology for a wide range of materials, including difficult-to-destroy hazardous organic compounds. The high temperatures and residence times in hazardous waste incinerators (HWIs) may provide adequate conditions to break the carbon-fluorine bonds, and other bonds, in PFAS (Blotevogel 2023). Many wastes currently being processed at commercial Resource Conservation and Recovery Act-permitted HWIs contain PFAS, sometimes at significant concentrations, as in the case of aqueous film-forming foam (AFFF) concentrate wastes.

PFAS destruction studies on the scale described in this report and the reports from two previous Clean Harbors Environmental Services (CHES) tests have been conducted at only a few other HWI facilities. Therefore, publicly available data on large-scale testing of PFAS destruction in HWIs are very limited.

1.2 Test Program Overview

CHES has previously conducted two full-scale performance tests of PFAS destruction at its HWI in Aragonite, Utah (Crisenbery et al. 2024). The test programs were conducted in 2021 and 2022, and both successfully demonstrated greater than 99.9999 percent destruction and removal efficiency (DRE) for a target list of semi-volatile, polar PFAS. At the time those test programs were conducted, there was no sampling and analytical method for assessing the identities and concentrations of volatile fluorinated compounds (VFCs) that may be produced as PIDs. In January 2024, EPA released Other Test Method 50 (OTM-50) for the sampling and analysis of selected VFCs. This method was used in this study to characterize whether PFAS PIDs are present in thermal treatment exhaust emissions.

This report summarizes the third PFAS destruction study conducted at CHES. The third test was conducted from November 12 to 14, 2024 in collaboration with the U.S. EPA Office of Research and Development (EPA ORD) and U.S. Department of Defense (DOD) Strategic Environmental Research and Development Program (SERDP). The test team consisted of contributors from CHES; EPA ORD; DOD; Focus Environmental, Inc.; EA Engineering, Science, and Technology, Inc., PBC (EA); Alliance Technical Group (ATG); Eurofins Environment Testing (Eurofins); Spectrum Environmental Solutions, Inc. (Spectrum); Superior Spiking Industries (SSI); Sanborn Head & Associates; and Teri Bales (an independent data validator). The test team developed the test plan, executed the field testing,

conducted the laboratory analysis, performed data validation, and worked together to prepare this project report.

The report was reviewed internally by EPA scientists and externally by independent technical reviewers. The two independent reviewers are knowledgeable in the incineration field and in PFAS incineration and provided feedback on the technical merit and structure of the report. Their reviews were implemented into this final version.

1.3 Test Objectives

The key study objectives for the sampling and analysis campaign were:

- Determine the efficacy of an HWI for the treatment of PFAS as indicated by DRE and destruction efficiency (DE) results
- Perform a PFAS mass balance around the entire incineration system
- Characterize PIDs that are observed in exhaust emissions
- Investigate the incinerator's performance by determining the DRE of hexafluoroethane (C_2F_6) and correlating the DRE of C_2F_6 to the presence of PIDs and PFAS in the exhaust emissions

1.4 Technical Approach

A detailed work plan was developed before conducting the tests to facilitate systematic execution of the field activities, including collecting data on process operations, process sampling and analysis, exhaust emissions sampling and analysis, Fourier transform infrared (FTIR) monitoring, PFAS spiking, C_2F_6 spiking, and quality control (QC) testing. The testing program was conducted under three conditions, with triplicate runs performed for each, resulting in a total of nine runs over three days. The incineration system was operated with typical waste feeds and at normal process operating conditions during each run. During Runs 1A, 2A, and 3A, AFFF was fed to the rotary kiln at rates similar to those used during normal operations, and the rotary kiln was spiked with nine selected PFAS spiking compounds. Runs 1B, 2B, and 3B served as baseline runs, during which no AFFF was fed and no PFAS spiking was conducted. During Runs 1C, 2C, and 3C, C_2F_6 spiking was conducted. No AFFF was fed and no PFAS spiking was conducted during Runs 1C, 2C, or 3C. The waste feed, spiking materials, and exhaust emissions sampling methods used during each run are presented in Table 1-1.

The following conventions are used in this document for reporting analytical results and performing mass balance calculation:

- The term "method detection limit" (MDL) is a laboratory reporting convention which defines the lowest level for detection for which false positive results are assumed to be absent with a 99.9% confidence. MDLs are used in this report to define performance and perform all emission, DRE, DE, and mass balance calculations. Analytical values reported as non-detect are below the MDL.
- The term "reporting limit" is a laboratory reporting convention which defines the floor of the quantifiable range of the analytical method. The given certainty of the measured concentrations

is applicable above this limit. The reporting limits for the methods are not used in this report to calculate performance or perform any calculations.

- The term "PFAS" is used in this report to discuss the targeted PFAS measured by the employed methods. The lack of standards and methods to measure all PFAS, or those not on targeted lists limits these evaluations to the PFAS in the methods used.

To achieve the objectives, a targeted PFAS mass balance was assessed by sampling and analyzing the waste feeds into the incinerator, chemicals fed to the emission control system, process water inputs to the incinerator and emission control system, residuals out of the incinerator and the emission control system, and exhaust emissions. The sampling and analysis of process streams included incinerator liquid waste feed streams, treatment chemicals, process water, and solid and liquid process residual streams. The samples from these process locations were analyzed for 40 PFAS target analytes using EPA Method 1633 (liquid chromatography/tandem mass spectrometry [LC/MS/MS] with isotope dilution) (EPA 2024b). The exhaust emissions were characterized using EPA's Other Test Method 45 (OTM-45) (EPA 2021) for 49 PFAS target analytes and OTM-50 (EPA 2025a) for 30 target VFC analytes. Semi-volatile nonpolar fluorinated compounds were sampled using Method 0010 (EPA 1986) and analyzed using EPA's SW-846 Method 8270C (EPA 1996). Tetrafluoromethane (CF_4) and C_2F_6 were monitored using FTIR by ASTM D-6348-12 (ASTM 2020). The solid and liquid process residuals were sampled and analyzed using EPA Method 1633.

To evaluate the efficacy of this HWI for destroying PFAS, AFFF was fed into the rotary kiln along with nine semi-volatile polar PFAS that have EPA published human health toxicity assessments (EPA 2024c, 2025b, 2025c) that were spiked as principal organic hazardous constituents (POHCs) for the purpose of measuring DRE and DE.

To attempt to help evaluate the performance of the HWI, C_2F_6 was also spiked into the rotary kiln to determine DRE for a difficult-to-destroy VFC. Since nontargeted analyses are not quantifiable due to lack of standards and calibrations, every PFAS could not be evaluated. The injection of C_2F_6 as an indicator compound provides data for a hard to destroy PFAS that can provide insight into the performance of the system with an easily measured compound. This study provides more data to determine if the destruction of C_2F_6 corelates with high destruction of known PFAS and the absences of PIDs. Further research is necessary to determine if the indicator gas may be used to aid in the evaluation incinerators' destruction of PFAS in the future.

Table 1-1. Feed Conditions and PFAS Emissions Sampling and Monitoring Methods

Run No.	Normal Waste Feed Materials	AFFF Feed Materials	PFAS Spiking Feed Materials	C_2F_6 Spiking Feed Materials	OTM-45 Emissions Sampling	Method 0010 Emissions Sampling	OTM-50 Emissions Sampling	ASTM D-6348-12 Emissions Monitoring
1A	X	X	X	---	X	X	X	X
1B	X	---	---	---	---	---	X	X
1C	X	---	---	X	---	---	X	X
2A	X	X	X	---	X	X	X	X
2B	X	---	---	---	---	---	X	X
2C	X	---	---	X	---	---	X	X
3A	X	X	X	---	X	X	X	X
3B	X	---	---	---	---	---	X	X
3C	X	---	---	X	---	---	X	X

Notes:
No. = Number
OTM-45 = EPA Other Test Method 45
Method 0010 = EPA SW-846 Method 0010
OTM-50 = EPA Other Test Method 50
ASTM = ASTM International
X = feed condition or sampling/monitoring method was applicable
--- = feed condition or sampling/monitoring method was not applicable

2 Incineration Facility Description

2.1 Overview

CHES is a provider of hazardous waste treatment and disposal services in North America and operates ten HWIs at five locations. In the past, as shown in compliance test reports, these units have consistently demonstrated compliance with high DREs through test-burn studies using Class 1 POHCs.

The CHES Aragonite Incineration facility is located in northern Utah at 11600 North Aptus Road, Grantsville, Utah. The facility processes approximately 60,000 tons of hazardous waste each year. The incinerator has a thermal input limit of approximately 156 million Btu/hr (British thermal units per hour) and consists of a slagging rotary kiln with a vertical afterburner chamber (afterburner). The emission control system consists of a spray dryer, powdered activated carbon injection system, baghouse, saturator, packed bed scrubber, induced draft fan, and stack.

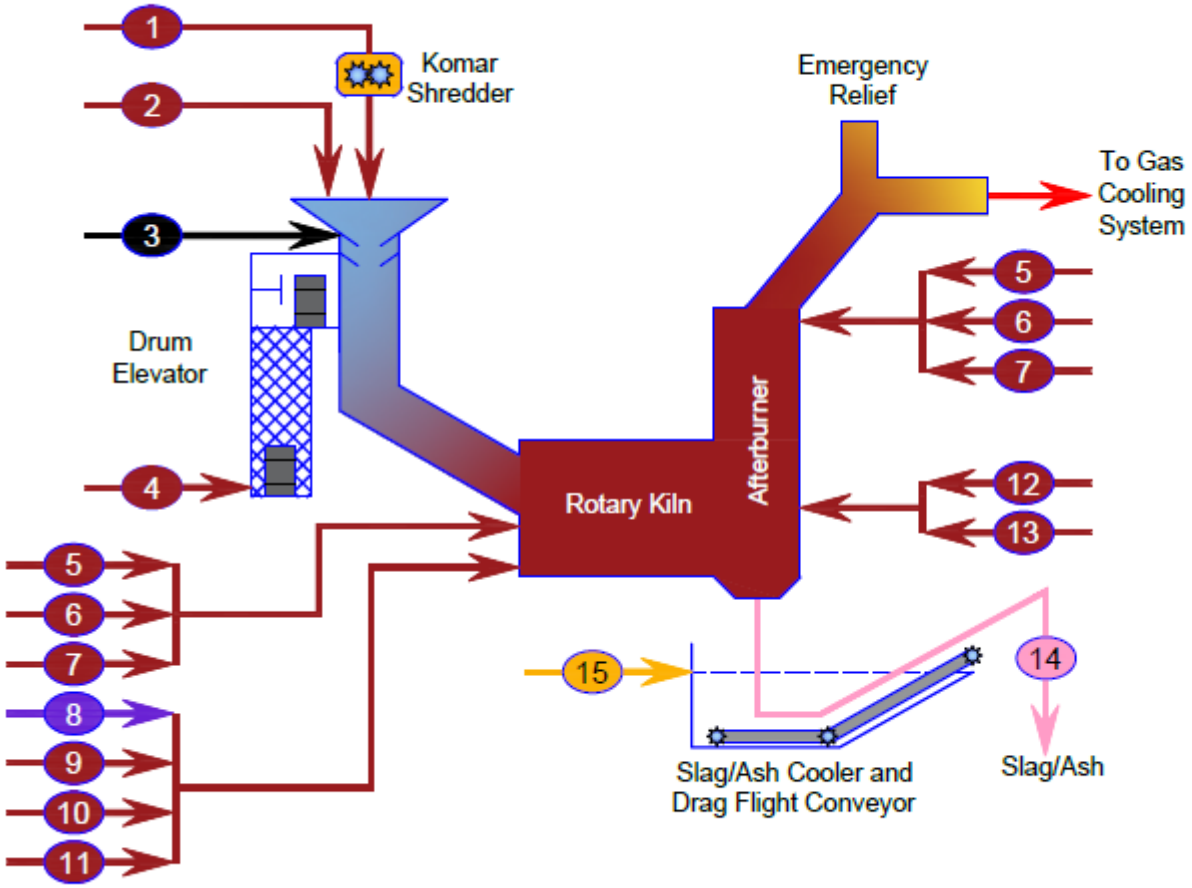
A process flow diagram of the waste feed systems, rotary kiln, and afterburner is presented on Figure 2-1. This figure also shows sampling points for waste feed, PFAS spiking compound, slag/ash, and process water. A process flow diagram of the emission control system showing sampling points for chemical reagents, process water, process residuals, and exhaust emissions is presented on Figure 2-2.

2.2 Waste Feed Systems

The solid waste feed system is capable of feeding bulk solids (including soil), solids that are shredded and fed by a Komar shredder, and containerized solids. The Komar shredder is used for size reduction of bulk and drummed solids, liquids and sludges, lab packs, and other containerized wastes. Bulk solids and output from the Komar shredder are fed to the rotary kiln through a solids waste feed chute in the front wall. Containerized waste is raised by the waste feed elevator and discharged into the solid waste feed chute. Because all the solid wastes are heterogeneous, obtaining representative samples was not possible and solid waste feed samples were not collected. Therefore, any PFAS that may have been in the solid materials are not included in either the PFAS material balance or DRE calculations, potentially biasing the DRE estimates low.

Liquids or sludges may be stored and fed from tanks, tanker trailers, drums, or totes. Waste blend liquids and used motor oil (UMO) are fed to the rotary kiln through lances located inside the burner can. Direct burn liquids from drums and totes, sludge, and direct burn liquids from a tanker are fed to the rotary kiln through individual lances in the front wall. For Runs 1A, 2A, and 3A, the direct burn liquids from a tanker consisted of a commercial AFFF product. This stream was not used during the other runs (Runs 1B, 1C, 2B, 2C, 3B, and 3C).

Waste blend liquids and UMO are fed to the north afterburner and south afterburner wall lances located inside their respective burner cans. Aqueous liquids are fed to the afterburner through lances in both the north and south walls and corrosive waste is fed to the afterburner through a lance in the south wall.



Sampling Point	Stream
1	Komar Solids
2	Bulk Solids (including Soil)
3	PFAS Spike
4	Containerized Solids
5	Waste Blend Liquid
6	Fuel Oil (Not Used)
7	Used Motor Oil
8	C_2F_6 Spike
9	Direct Burn - Drums
10	Direct Burn - Tote
11	Direct Burn - Tank Truck (AFFF)
12	Aqueous Liquid
13	Corrosive Waste
14	Slag/Ash
15	Process Water

Figure 2-1. Combustion Systems Process Stream Sampling Points

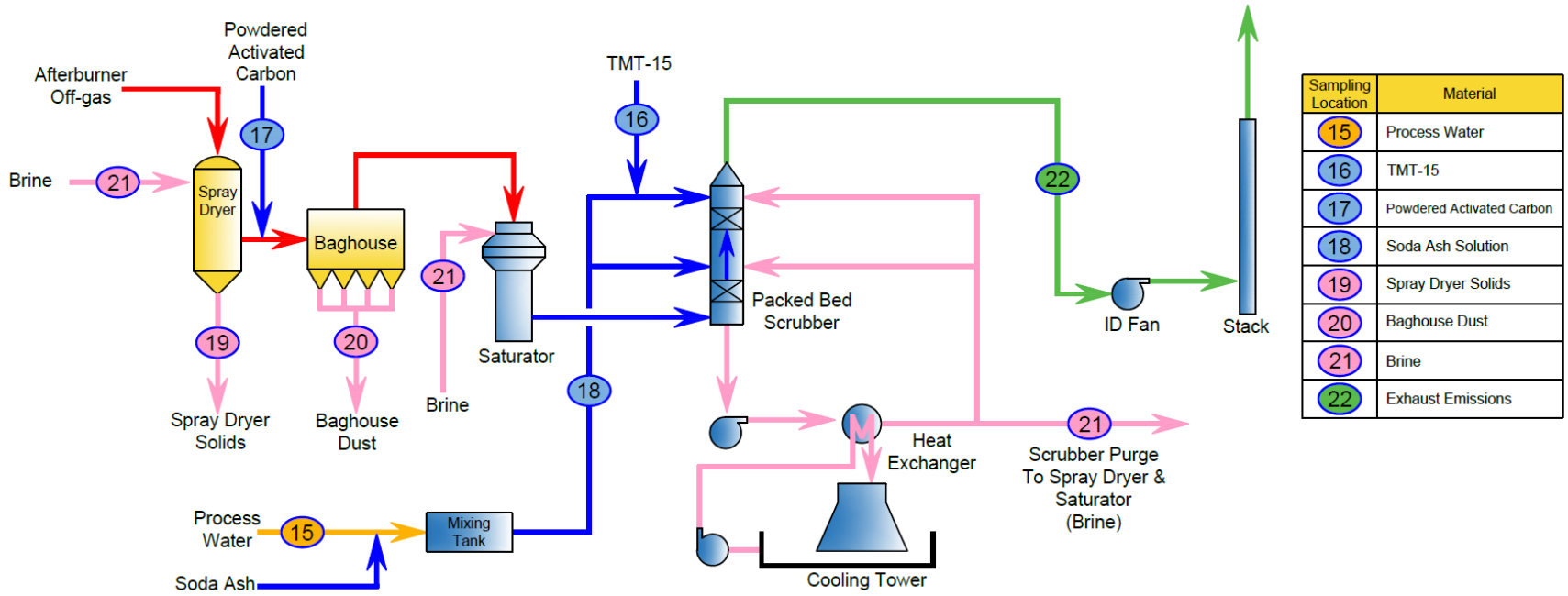


Figure 2-2. Emission Control System Process Stream Sampling Points

2.3 Combustion Systems

The thermal treatment process includes a high-temperature slagging rotary kiln incinerator with an afterburner. Combustion air is delivered to the front wall of the rotary kiln by a forced draft fan. Combustion air is also delivered to the burners on the north and south walls of the afterburner by two forced draft fans, one for each burner. The oxygen (O_2) concentration in the combustion gas at the afterburner exit ranges from approximately 9 to 11 percent. During the testing, the exit gas temperature of the rotary kiln ranged from 2,006 to 2,233 degrees Fahrenheit (°F) (1,097 to 1,223 degrees Celsius [°C]) and the exit gas temperature of the afterburner ranged from 2,048 to 2,096°F (1,120 to 1,147°C). Non-combustible solid material residence time in the rotary kilns was estimated by CHES personnel to be approximately 60 minutes.

Slag/ash is discharged from the rotary kiln into a water-filled slag cooler, where it is cooled and moisturized before being removed by a drag flight conveyor and deposited into a roll-off container. Process water is added to the slag cooler to cool the slag/ash.

2.4 Emission Control System

The emission control system consists of spray dryer, powdered activated carbon injection system, baghouse, Na_2CO_3 solution supply system, saturator, packed bed scrubber, decommissioned electrostatic precipitator, induced draft fan, and stack. Exhaust emissions samples were collected from the outlet duct of the packed bed scrubber because this location is more accessible and safer than collecting samples from sampling ports on the stack platform.

The purpose of the spray dryer is to cool the gas before entering the baghouse. The emission control system is a zero-blowdown process and the purge water (brine) from the packed bed scrubber is evaporated in the spray dryer. Some solids collect on the walls of the spray dryer and in the duct that connects the spray dryer to the baghouse. Solids that slough off the spray dryer walls, and dust collected in the outlet duct, are collectively defined as “spray dryer solids” and are discharged through an airlock into a roll-off bin.

Powdered activated carbon is injected upstream of the baghouse for control of polychlorinated dibenzo-p-dioxins, polychlorinated dibenzofurans, and mercury. The baghouse also removes particulate matter from the flue gas. “Baghouse dust” is discharged from the baghouse through airlocks and is conveyed by screw conveyors to a roll-off container.

The baghouse is followed by a saturator. A brine solution is sprayed into the gas to cool and precondition it in the saturator before the gas enters the packed scrubber.

The gas then flows to the packed scrubber where a Na_2CO_3 solution is used to react with and neutralize acid gases including hydrogen chloride, chlorine, sulfur dioxide, and hydrogen fluoride. Na_2CO_3 is stored as a dry powder in a silo and metered into a mixing tank where it is mixed with process water to form a Na_2CO_3 solution. The flow rate of the Na_2CO_3 solution into the packed scrubber is controlled by pH measurements of the scrubber recirculation water. In the packed scrubber, acid gases are absorbed by the recirculating water stream and are neutralized by reaction with the Na_2CO_3. Since this reaction produces sodium chloride, sodium sulfite, and sodium fluoride salts, some of the water (brine) must be purged from the packed scrubber to control the concentration of total dissolved solids in the scrubber

water. Part of this brine is sent to the saturator and the rest of it is sent to the spray dryer. The packed scrubber discharge water is routed through a heat exchanger to lower its temperature before it is recycled into the packed scrubber.

TMT-15 is also injected into the packed scrubber recirculating water for additional mercury control. TMT-15 reacts with mercury and other heavy metals to form a stable, virtually insoluble complex of heavy metal and TMT-15 that is easy to separate from the water.

The gas leaving the packed scrubber goes through a decommissioned electrostatic precipitator, which is no longer needed to meet emission standards. The electrostatic precipitator shell is in place, but its internal parts have been removed. The gas then flows through an induced draft fan and is discharged through the stack.

3 Process Parameter Data Collection

3.1 Continuous Process Monitoring

During runs, CHES plant personnel were responsible for operating the incineration process. Personnel confirmed the system was operated within permitted operating limits and maintained operating parameters within typical operating ranges. This was to ensure that the PFAS destruction testing process conditions were representative of normal operations.

Key process parameters were monitored and their values recorded electronically at 1-minute intervals on the CHES distributed control system. The project team reduced the 1-minute data records to calculate the minimum, average, and maximum rates from the start time to the end time of each run. The standard deviation of the data within a run was also calculated. The types of parameters that were monitored and recorded include solid waste feed rates to the rotary kiln; liquid waste feed rates to the rotary kiln; liquid waste feed rates to the afterburner; chemical reagent feed rates to the emission control system; temperatures; liquid flow rates to the scrubber system; differential pressures; broken bag indicators; pH of the recycle water going into the packed scrubber; total fluorine and total chlorine waste feed rates; exhaust emissions concentrations of sulfur dioxide, nitric oxide, nitrogen dioxide, carbon dioxide (CO_2), carbon monoxide, O_2, total hydrocarbons, and exhaust emissions flow rates. Detailed records of process parameters are presented in Appendix B for each day of testing. Summaries of waste feed rates to the rotary kiln and the afterburner are presented in Table 3-1 and Table 3-2, respectively. A summary of key process parameters that may affect the DRE of PFAS is presented in Table 3-3.

Table 3-1. Waste Feed Rates to Rotary Kiln

Date	Run No.	Komar Feed Drop to Rotary Kiln (lb/hr)	Containerized Solids Feed Drop to Rotary Kiln (lb/hr)	Bulk Solids Bin Weight to Rotary Kiln (lb/hr)	Waste Blend Liquid Flow to Rotary Kiln (lb/hr)	UMO Flow to Rotary Kiln (lb/hr)	Drum Direct Burn Educt 1 to Rotary Kiln (lb/hr)	Tote and Drum Direct Burn Educt 2 to Rotary Kiln (lb/hr)	Tanker Direct Burn Flow to Rotary Kiln (lb/hr)
11/13/24	Run 1A	5,116	1,169	5,838	216	106	569	610	878
11/12/24	Run 1B-1	4,058	1,460	6,279	680	0	652	647	0
11/12/24	Run 1B-2	4,403	2,697	7,785	905	587	554	497	0
11/12/24	Run 1C	2,755	1,947	5,498	903	302	531	806	0
11/13/24	Run 2A	3,192	749	6,126	636	95	690	671	888
11/14/24	Run 2B	8,571	831	5,635	0	0	366	173	0
11/14/24	Run 2C	7,063	710	5,570	0	0	366	173	0
11/14/24	Run 3A	6,052	649	5,737	348	212	343	564	752
11/14/24	Run 3B	6,430	722	6,280	0	0	287	430	0
11/14/24	Run 3C	7,765	1,191	6,025	247	0	341	467	0
NA	Minimum	2,755	649	5,498	0	0	287	173	0
NA	Average	5,540	1,212	6,077	394	130	470	504	252
NA	Maximum	8,571	2,697	7,785	905	587	690	806	888

Notes:
lb/hr = pound(s) per hour
UMO = used motor oil
NA = not applicable

Table 3-2. Waste Feed Rates to Afterburner Chambers

Date	Run No.	Blend Liquid Flow to North ABC (lb/hr)	Blend Liquid Flow to South ABC (lb/hr)	UMO Flow to North ABC (lb/hr)	UMO Flow to South ABC (lb/hr)	Corrosive Waste Feed to South ABC (lb/hr)	Aqueous Liquid Flow to North ABC (lb/hr)	Aqueous Liquid Flow to South ABC (lb/hr)
11/13/24	Run 1A	733	767	401	462	669	1,640	1,385
11/12/24	Run 1B-1	724	1,177	172	390	368	1,126	2,285
11/12/24	Run 1B-2	1,002	1,276	358	643	703	1,677	841
11/12/24	Run 1C	1,006	1,453	0	45	669	2,763	1,417
11/13/24	Run 2A	911	814	540	296	765	2,109	1,677
11/14/24	Run 2B	610	489	364	519	454	1,817	2,096
11/14/24	Run 2C	782	646	1	85	528	1,912	1,126
11/14/24	Run 3A	741	731	402	420	403	1,976	1,476
11/14/24	Run 3B	610	489	364	519	454	1,817	2,096
11/14/24	Run 3C	389	1,043	338	428	569	1,974	2,072
NA	Minimum	389	489	0	45	368	1,126	841
NA	Average	751	888	294	381	558	1,881	1,647
NA	Maximum	1,006	1,453	540	643	765	2,763	2,285

Notes:
ABC = afterburner chamber
NA = not applicable

Table 3-3. Process Monitoring Data Summary for Key Parameters

Date	Run No.	Rotary Kiln Exit Gas Temperature (°F)	ABC Exit Gas Temperature (°F)	Spray Dryer Outlet Temperature (°F)	Saturator Temperature (°F)	Stack Temperature (°F)	1st Stage Scrubber Brine Flow (gpm)	2nd Stage Scrubber Brine Flow (gpm)	Saturator Brine Flow (gpm)	Baghouse Differential Pressure (in. w.c.)	Scrubber Differential Pressure (in. w.c.)	1st stage Neutralization Feed pH (Std Units)	2nd stage Neutralization Feed pH (Std Units)
11/13/24	Run 1A	2,060	2,073	369	171	148	2,030	2,118	344	5.8	5.1	6.7	6.9
11/12/24	Run 1B-1	2,116	2,048	369	171	148	1,976	2,144	338	5.8	5.0	6.7	6.9
11/12/24	Run 1B-2	2,006	2,096	370	170	146	1,977	2,135	337	5.9	5.0	6.7	6.9
11/12/24	Run 1C	2,167	2,082	369	172	149	1,979	2,135	337	5.9	4.9	6.7	6.9
11/13/24	Run 2A	2,025	2,054	369	171	148	2,067	2,104	342	5.8	5.2	6.7	6.9
11/14/24	Run 2B	2,211	2,054	372	171	151	2,006	2,155	368	5.6	5.0	6.7	6.7
11/14/24	Run 2C	2,189	2,051	368	170	149	2,010	2,150	368	5.8	4.9	6.7	6.8
11/14/24	Run 3A	2,044	2,052	371	171	150	2,017	2,123	368	5.9	4.9	6.7	6.9
11/14/24	Run 3B	2,233	2,060	369	171	150	2,012	2,140	367	5.9	5.0	6.7	6.8
11/14/24	Run 3C	2,137	2,053	369	172	150	2,010	2,118	368	5.9	5.0	6.7	6.8
NA	Minimum	2,006	2,048	368	170	146	1,976	2,104	337	5.6	4.9	6.7	6.7
NA	Average	2,119	2,062	370	171	149	2,009	2,132	354	5.8	5.0	6.7	6.9
NA	Maximum	2,233	2,096	372	172	151	2,067	2,155	368	5.9	5.2	6.7	6.9

Notes:
ABC = afterburner chamber
gpm = gallon(s) per minute
in. w.c. = inch(es) of water column
NA = not applicable
Std Units = standard units

3.2 Parameters Derived by Mass Balance Calculations

Three process parameters that were not continuously monitored but were calculated by mass balance or mass and energy balance calculations include (1) Na_2CO_3 solution flow rate, (2) process water flow rate, and (3) brine flow rate. The Na_2CO_3 solution flow rate and process water flow rate are used in the overall plant PFAS mass balance, DRE, and DE calculations. The brine flow rate is an internal process stream that does not affect the overall plant PFAS mass balance, DRE, or DE calculations. Descriptions of the methods used to perform these calculations are presented below.

The Na_2CO_3 solution flow rate was computed using mass balance calculations. Na_2CO_3 is stored as a dry powder in a silo, and the Na_2CO_3 level in the silo is monitored continuously. The mass flow rate of Na_2CO_3 powder was calculated at 1-minute intervals based on the depth of the Na_2CO_3 powder in the silo, the diameter of the silo, and the bulk density of the dry Na_2CO_3. The Na_2CO_3 powder is mixed with process water to feed Na_2CO_3 to the scrubber as a solution; this solution was sampled and analyzed to determine its PFAS content. The flow rate of the Na_2CO_3 solution was calculated based on the mass flow rate of Na_2CO_3 powder and the concentration of Na_2CO_3 in solution. CHES provided the Na_2CO_3 solution density measurements. A chart showing the correlation between Na_2CO_3 solution density and Na_2CO_3 concentration was then used to calculate the flow rate of the process water used to create the solution. An example of these calculations is presented in Appendix B, Table B-3.

The flow rate of process water to the system is not monitored. Process water is introduced into the system to make Na_2CO_3 solution, and the flow rate of process water for this purpose was calculated as described above. Process water is also introduced into the system in the slag/ash cooler. The flow rate of this stream was determined using mass and energy balance calculations based on the flow rate of the slag/ash, the temperature of the slag/ash as it exits the rotary kiln (assumed to be the same as the off-gas temperature), the temperature of the slag/ash exiting the slag/ash cooler, and the residual moisture in the slag/ash. These calculations are presented in Appendix B, Table B-4.

The flow rate of brine is also not monitored, but the flow rate of this stream does not affect any DRE, DE, or mass balance calculations since it is an internal process stream. However, it may be useful in interpreting the PFAS mass balance between the spray dryer solids, baghouse dust, and scrubber blowdown streams. It can be calculated by a mass and energy balance around the entire plant. These calculations were performed as part of the 2022 PFAS test program (Crisenbery et al. 2024), and the brine flow rate from those calculations — 128 gallons per minute — was applied to this study.

3.3 Process Residual Measurements

Solid process residual streams include slag/ash, spray dryer solids, and baghouse dust. Each bin is weighed empty when it is put into service and weighed again when it is full and taken out of service. Therefore, it is not possible to get a weight of material in each bin at the beginning and end of each run. For these three streams, the average fill rate for a bin was calculated from the time it was put in service before a run started until it was taken out of service after a run ended. Detailed calculations of residual stream flow rates are presented in Appendix C. A summary of residual stream flow rates is presented in Table 3-4.

Table 3-4. Residual Stream Flow Rates

Run No.	Date	Slag/Ash (lb/hr)	Spray Dryer Solids (lb/hr)	Baghouse Dust (lb/hr)	Total (lb/hr)
Run 1A	11/13/2024	5,302	118	1,107	6,526
Run 1B-1	11/12/2024	2,155	118	977	3,249
Run 1B-2	11/12/2024	2,236	118	977	3,330
Run 1C	11/12/2024	2,236	118	977	3,330
Run 2A	11/13/2024	5,109	118	1,107	6,333
Run 2B	11/14/2024	5,410	118	1,073	6,601
Run 2C	11/14/2024	5,410	118	1,073	6,601
Run 3A	11/14/2024	4,787	118	1,073	5,978
Run 3B	11/14/2024	5,410	118	1,073	6,601
Run 3C	11/14/2024	5,974	118	1,073	7,165

4 Process Stream Sampling and Analysis

4.1 Overview

The Test Plan is based on a comprehensive approach to sampling process streams that includes as many process inputs and outputs as practical. The purpose of collecting and analyzing the process samples is to develop as complete a PFAS mass balance as possible to help evaluate PFAS destruction. This approach results in identifying potential sources of PFAS in process streams entering the process, and PFAS sinks for process streams leaving the process. Process sampling includes liquid waste feed streams, treatment chemicals, process water, and process residues. Containerized, shredded, and bulk solids waste feed streams were not sampled. Due to their complexity and heterogeneity, it was not practical to sample and perform PFAS analyses on the solid waste feed materials. Therefore, PFAS that may be contained in the solid waste feed streams were not included in the mass balance, DRE, or DE calculations.

4.2 Scope of Sample Collection

During the test program, representative process stream samples were only collected during Runs 1A, 2A, and 3A. No process stream samples were collected during Runs 1B, 1C, 2B, 2C, 3B, or 3C. The types of process streams that were sampled and analyzed include liquid waste feed streams, treatment chemicals, utilities, and process residues. The types of streams within each of these categories are listed below.

All liquid waste feed streams were sampled and analyzed, including the following:

Liquids waste feed streams:

- Waste blend liquids (fed to rotary kiln, north afterburner, south afterburner)
- UMO (fed to rotary kiln, north afterburner, south afterburner)
- Drum direct burn educt (fed to rotary kiln)
- Tote direct burn educt (fed to rotary kiln)
- Tanker direct burn (AFFF fed to rotary kiln)
- Aqueous liquid (fed to north afterburner, south afterburner)
- Corrosive waste (fed to south afterburner)

For liquid waste streams that are fed to the system in more than one location, samples were collected at one location, analyzed, and the analytical results applied to multiple streams.

Treatment chemicals:

- Powdered activated carbon

- Na_2CO_3 solution
- TMT-15

Utilities:

- Process water

Process residues:

- Slag/ash
- Spray dryer solids
- Baghouse dust

Schematic diagrams of the combustion and emission control processes showing sampling location for each process stream were previously presented on Figure 2-1 and Figure 2-2, respectively.

4.3 General Process Sampling Approach

Given the ubiquity of PFAS in the environment, particular care was taken with all sampling activities to avoid inadvertent PFAS contamination of samples. Process sampling activities were conducted following best practices as avoiding use of the following materials in proximity to sampling activities:

- Teflon® or low-density polyethylene containing materials
- Water-proofed materials
- Water-proof markers
- Water-proof clothing and footwear
- Fire-retardant clothing
- Coated Tyvek®
- Personal care products and synthetic sunscreens
- Food and drink containers

In addition to observing general best practices during sample collection activities, individuals collecting samples for PFAS analysis wore new, powder-free nitrile gloves at all times and placed all samples in high density polyethylene (HDPE) sample jars. All hand-held sampling equipment was disposable and was used at only one sampling location and for only one sampling event.

4.4 Sampling Responsibilities

CHES personnel were responsible for collecting all process samples under the direction of EA. EA personnel were responsible for overall coordination of process sampling activities, compositing grab samples provided by CHES plant personnel, on-site storage of process samples, and delivery of process samples packaged for shipment with completed chain-of-custody forms to the Eurofins courier.

The EA SOP used for obtaining process samples was provided in an approved Work Plan as an attachment to the EPA quality assurance project plan (QAPP). Process sampling logs showing the time of collection of each grab sample are presented in Appendix D. Grab samples were collected for each sample type, and the grab samples were then combined into a composite sample for each run. The composite samples were homogenized, sealed, and stored in a secure area of the CHES on-site laboratory to minimize the potential for cross-contamination. At the end of the test program, the process samples were packaged and preserved in coolers, along with Request for Analysis/Chain-of-Custody forms and transferred to the Eurofins courier.

4.5 Sample Transport and Analysis

The Eurofins courier delivered the project's properly preserved process samples under chain of custody to the Eurofins laboratory in West Sacramento, California. The Eurofins West Sacramento laboratory was responsible for the analyses of all process samples and reporting final analytical results. Samples were analyzed by LC/MS/MS by EPA Method 1633. The process sample analytical data reports were prepared to comply with DOD Data Validation Guidelines Module 6. This reporting process provides the necessary quality assurance (QA)/QC results and supporting documentation enabling independent data validation and quality assessment of the analytical results.

For liquid waste feed streams, a duplicate and a matrix spike (MS)/matrix spike duplicate (MSD) analysis were performed on one sample of each waste type. For process residual streams, MS/MSD analyses were performed on one sample of each residual type. For every extraction batch (less than or equal to 20 samples), method blank and MS analyses were performed. Isotope dilution internal standards were spiked into the samples to assess extraction efficiencies, and internal standards were spiked into final extracts to assess instrument drift. The LC/MS/MS instrument was calibrated using a minimum of five concentration levels. All assays were bracketed by passing continuing calibration verification evaluations. A maximum of ten samples were assayed between continuing calibration verifications. All laboratory QC procedures and results are documented in the Eurofins Analytical Report in Appendix J-1.

4.6 Analytical Results

Select summary tables representing process sample analytical results for Run 1A and containing data for target analytes of EPA Method 1633 are presented in Tables 4-1 through 4-5. A complete set of tables for Runs 1A, 2A, and 3A are included in Appendix A-1, Table A-1-14, Table A-1-15, and Table A-1-16, respectively. These tables include the analytical results, MDL, data qualifier flags applied by the laboratory, and the data qualified flags applied by the data validator. Complete process sample analytical results are presented in Eurofins Analytical Report, which is included as Appendix J-1.

Spray dryer solids consisted of materials with a range of particle sizes. These samples were sieved in the laboratory into three size fractions as part of their preparation for analysis: less than 250 micrometers, 250 micrometers to 2 millimeters, and greater than 2 millimeters. Each size fraction was weighed and analyzed separately. The concentration values reported for Spray dryer solids are the mass weighted average concentrations of the three size fractions. Concentration data for the individual size fractions are presented in Appendix A-1, Table A-1-16.

Analytical results for process stream samples were generally consistent between runs in terms of the types of PFAS and order of magnitude concentrations observed for all but two waste streams. The waste blend liquid stream was determined after the analyses were conducted to be a two-phase liquid with a dark-brown top phase and a dark-green bottom phase. Analytical results for this stream were significantly different between Runs 1A, 1B, and 1C. It appears that the samples that were analyzed came from different phases for Runs 1A and 1B, and that the sample analyzed for Run 1C may have been a mixture of materials from the two phases. The variability of the analytical results for this stream would have had a very minor effect on the overall PFAS mass balance because the PFAS in this stream made up less than 0.0033% of the total PFAS fed to the system.

The analytical results for the Run 1A process water had detectable concentrations for 11 PFAS, with PFOS (58 nanograms per liter [ng/L]) and 6:2 FTS (27 ng/L) having the two highest concentrations. These results were unexpected and likely due to an unidentified external source of contamination. The analytical results for Run 2A and Run 3A for process water were all non-detect, except for 6:2 FTS, which was detected at concentrations of 2.49 ng/L and 1.42 ng/L respectively.

4.7 Quality Assurance/Quality Control Results

Throughout the project, various measures were implemented to ensure the overall quality and usability of the collected data. The field investigation activities included collection of additional QC samples (e.g., duplicates, MS/MSD, etc.). Duplicate samples were submitted, and field and laboratory QC requirements were completed. A detailed discussion of the process sampling and analytical QA/QC results is presented in Section 8.0.

Table 4-1. PFAS in Liquid Waste Feeds to Rotary Kiln — Run 1A

CAS No.	Acronym	Waste Blend Liquid (ng/L)	Drum Direct Burn Educt (ng/L)	Tote Direct Burn Educt (ng/L)	UMO (ng/L)	Tanker Direct Burn[(a)] (ng/L)
375-22-4	PFBA	44,500.00	<50.00	3,080.00	4,710.00	23,500,000
2706-90-3	PFPeA	969.00	<25.00	92.80	<50.00	29,500,000
307-24-4	PFHxA	42,500.00	<25.00	108.00	<50.00	87,000,000
375-85-9	PFHpA	<500.00	<25.00	36.00	<500.00	32,600,000
335-67-1	PFOA	919.00	<25.00	55.70	121.00	103,000,000
375-95-1	PFNA	84.10	<25.00	9.34	<50.00	<1,250,000
335-76-2	PFDA	<50.00	<25.00	1.42	<50.00	<1,250,000
2058-94-8	PFUnA	<50.00	<25.00	1.01	<50.00	<1,250,000
307-55-1	PFDoA	<55.00	<27.50	<1.10	<55.00	<1,380,000
72629-94-8	PFTriA	<58.00	<29.00	<1.16	<58.00	<1,450,000

CAS No.	Acronym	Waste Blend Liquid (ng/L)	Drum Direct Burn Educt (ng/L)	Tote Direct Burn Educt (ng/L)	UMO (ng/L)	Tanker Direct Burn[(a)] (ng/L)
376-06-7	PFTeA	<81.00	<40.50	<1.62	<81.00	<2,030,000
375-73-5	PFBS	3,760.00	<25.00	1,350.00	152.00	150,000,000
2706-91-4	PFPeS	628.00	<25.00	46.40	<50.00	194,000,000
355-46-4	PFHxS	1,770.00	<25.00	185.00	65.60	1,150,000,000
375-92-8	PFHpS	<50.00	<25.00	22.00	<50.00	160,000,000
1763-23-1	PFOS	14,800.00	<25.00	927.00	<50.00	6,520,000,000
68259-12-1	PFNS	<50.00	<25.00	<1.00	<50.00	4,880,000
335-77-3	PFDS	457.00	<25.00	<1.00	<50.00	3,650,000
79780-39-5	PFDoS	<53.00	<26.50	<1.06	<53.00	2,130,000
754-91-6	FOSA	<50.00	<25.00	2.03	<50.00	<1,250,000
31506-32-8	NMeFOSA	<50.00	<25.00	<1.00	<50.00	<1,250,000
4151-50-2	NEtFOSA	<50.00	<25.00	<1.00	<50.00	<1,250,000
2355-31-9	NMeFOSAA	<50.00	<25.00	<1.00	<50.00	<1,250,000
2991-50-6	NEtFOSAA	808.00	<25.00	<1.00	<50.00	<1,250,000
24448-09-7	N-MeFOSE	4,190.00	<125.00	<5.00	<250.00	<6,250,000
1691-99-2	N-EtFOSE	490.00	<125.00	<5.00	<250.00	<6,250,000
757124-72-4	4:2 FTS	<100.00	<50.00	<2.00	<100.00	<2,500,000
27619-97-2	6:2 FTS	<1,000.00	<50.00	451.00	794.00	<2,500,000
39108-34-4	8:2 FTS	<100.00	<50.00	26.50	<100.00	<2,500,000
13252-13-6	HFPO-DA	<390.00	<19.50	320.00	<390.00	<975,000
919005-14-4	DONA	<50.00	<25.00	<1.00	<50.00	<1,250,000
756426-58-1	9Cl-PF3ONS	<58.00	<29.00	<1.16	<58.00	<1,450,000
763051-92-9	11Cl-PF3OUdS	<50.00	<25.00	<1.00	<50.00	<1,250,000
377-73-1	PFECA F	<500.00	<25.00	105.00	374.00	<1,250,000
863090-89-5	PFECA A	<500.00	<25.00	3.15	<50.00	<1,250,000
151772-58-6	PFECA B	<72.00	<36.00	<1.44	<72.00	<1,800,000
113507-82-7	PES	<50.00	<25.00	<1.00	<50.00	<1,250,000
356-02-5	3:3 FTCA	<1,000.00	<50.00	<24.00	<100.00	<2,500,000
914637-49-3	5:3 FTCA	505.00	<125.00	<5.00	<250.00	<6,250,000
812-70-4	7:3 FTCA	<250.00	<125.00	<5.00	<250.00	<6,250,000
NA	**Subtotal: Non-detect**	**5,267.00**	**1,558.00**	**62.54**	**3,517.00**	**60,335,000**
NA	**Subtotal: Detected**	**116,380.10**	**0.00**	**6,822.35**	**6,216.60**	**8,460,260,000**
NA	**Grand Total**	**121,647.10**	**1,558.00**	**6,884.89**	**9,733.60**	**8,520,595,000**

Notes:
(a) AFFF concentrate
Yellow shading = compound spiked during Runs 1A, 2A, 3A
Pink shading = compound detected
CAS No. = Chemical Abstracts Survey registry number

NA = not applicable

Table 4-2. PFAS in Liquid Waste Feeds to North Afterburner Chamber— Run 1A

CAS No.	Acronym	Blend Liquid (ng/L)	Aqueous Liquid (ng/L)	UMO (ng/L)
375-22-4	PFBA	44,500.00	3,740.00	4,710.00
2706-90-3	PFPeA	969.00	<50.00	<50.00
307-24-4	PFHxA	42,500.00	632.00	<50.00
375-85-9	PFHpA	<500.00	122.00	<500.00
335-67-1	PFOA	919.00	281.00	121.00
375-95-1	PFNA	84.10	63.60	<50.00
335-76-2	PFDA	<50.00	<50.00	<50.00
2058-94-8	PFUnA	<50.00	<50.00	<50.00
307-55-1	PFDoA	<55.00	<55.00	<55.00
72629-94-8	PFTriA	<58.00	<58.00	<58.00
376-06-7	PFTeA	<81.00	<81.00	<81.00
375-73-5	PFBS	3,760.00	2,960.00	152.00
2706-91-4	PFPeS	628.00	249.00	<50.00
355-46-4	PFHxS	1,770.00	327.00	65.60
375-92-8	PFHpS	<50.00	<50.00	<50.00
1763-23-1	PFOS	14,800.00	508.00	<50.00
68259-12-1	PFNS	<50.00	<50.00	<50.00
335-77-3	PFDS	457.00	<50.00	<50.00
79780-39-5	PFDoS	<53.00	<53.00	<53.00
754-91-6	FOSA	<50.00	<50.00	<50.00
31506-32-8	NMeFOSA	<50.00	<50.00	<50.00
4151-50-2	NEtFOSA	<50.00	<50.00	<50.00
2355-31-9	NMeFOSAA	<50.00	<50.00	<50.00
2991-50-6	NEtFOSAA	808.00	<50.00	<50.00
24448-09-7	N-MeFOSE	4,190.00	<250.00	<250.00
1691-99-2	N-EtFOSE	490.00	<250.00	<250.00
757124-72-4	4:2 FTS	<100.00	<100.00	<100.00
27619-97-2	6:2 FTS	<1,000.00	484.00	794.00
39108-34-4	8:2 FTS	<100.00	<100.00	<100.00
13252-13-6	HFPO-DA	<390.00	<39.00	<390.00
919005-14-4	DONA	<50.00	<50.00	<50.00
756426-58-1	9Cl-PF3ONS	<58.00	<58.00	<58.00
763051-92-9	11Cl-PF3OUdS	<50.00	<50.00	<50.00
377-73-1	PFECA F	<500.00	<50.00	374.00
863090-89-5	PFECA A	<500.00	<50.00	<50.00

CAS No.	Acronym	Blend Liquid (ng/L)	Aqueous Liquid (ng/L)	UMO (ng/L)
151772-58-6	PFECA B	<72.00	<72.00	<72.00
113507-82-7	PES	<50.00	<50.00	<50.00
356-02-5	3:3 FTCA	<1,000.00	<100.00	<100.00
914637-49-3	5:3 FTCA	505.00	1,430.00	<250.00
812-70-4	7:3 FTCA	<250.00	<250.00	<250.00
NA	**Subtotal: Non-detect**	**5,267.00**	**2,266.00**	**3,517.00**
NA	**Subtotal: Detected**	**116,380.10**	**10,796.60**	**6,216.60**
NA	**Grand Total**	**121,647.10**	**13,062.60**	**9,733.60**

Notes:
Yellow shading = compound spiked during Runs 1A, 2A, 3A
Pink shading = compound detected
ABC = afterburner chamber
NA = not applicable

Table 4-3. PFAS in Liquid Waste Feeds to South Afterburner Chamber— Run 1A

CAS No.	Acronym	Blend Liquid (ng/L)	Aqueous Liquid (ng/L)	UMO (ng/L)	Corrosive Waste Feed (ng/L)
375-22-4	PFBA	44,500.00	3,740.00	4,710.00	195.00
2706-90-3	PFPeA	969.00	<50.00	<50.00	69.50
307-24-4	PFHxA	42,500.00	632.00	<50.00	57.80
375-85-9	PFHpA	<500.00	122.00	<500.00	19.50
335-67-1	PFOA	919.00	281.00	121.00	109.00
375-95-1	PFNA	84.10	63.60	<50.00	680.00
335-76-2	PFDA	<50.00	<50.00	<50.00	<5.00
2058-94-8	PFUnA	<50.00	<50.00	<50.00	8.13
307-55-1	PFDoA	<55.00	<55.00	<55.00	<5.50
72629-94-8	PFTriA	<58.00	<58.00	<58.00	<5.80
376-06-7	PFTeA	<81.00	<81.00	<81.00	<8.10
375-73-5	PFBS	3,760.00	2,960.00	152.00	7.73
2706-91-4	PFPeS	628.00	249.00	<50.00	<5.00
355-46-4	PFHxS	1,770.00	327.00	65.60	14.30
375-92-8	PFHpS	<50.00	<50.00	<50.00	<5.00
1763-23-1	PFOS	14,800.00	508.00	<50.00	45.20
68259-12-1	PFNS	<50.00	<50.00	<50.00	<5.00
335-77-3	PFDS	457.00	<50.00	<50.00	<5.00
79780-39-5	PFDoS	<53.00	<53.00	<53.00	<5.30
754-91-6	FOSA	<50.00	<50.00	<50.00	<5.00
31506-32-8	NMeFOSA	<50.00	<50.00	<50.00	<5.00

CAS No.	Acronym	Blend Liquid (ng/L)	Aqueous Liquid (ng/L)	UMO (ng/L)	Corrosive Waste Feed (ng/L)
4151-50-2	NEtFOSA	<50.00	<50.00	<50.00	<5.00
2355-31-9	NMeFOSAA	<50.00	<50.00	<50.00	<5.00
2991-50-6	NEtFOSAA	808.00	<50.00	<50.00	<5.00
24448-09-7	N-MeFOSE	4,190.00	<250.00	<250.00	<25.00
1691-99-2	N-EtFOSE	490.00	<250.00	<250.00	<25.00
757124-72-4	4:2 FTS	<100.00	<100.00	<100.00	<10.00
27619-97-2	6:2 FTS	<1,000.00	484.00	794.00	324.00
39108-34-4	8:2 FTS	<100.00	<100.00	<100.00	122.00
13252-13-6	HFPO-DA	<390.00	<39.00	<390.00	<3.90
919005-14-4	DONA	<50.00	<50.00	<50.00	<5.00
756426-58-1	9Cl-PF3ONS	<58.00	<58.00	<58.00	<5.80
763051-92-9	11Cl-PF3OUdS	<50.00	<50.00	<50.00	<5.00
377-73-1	PFECA F	<500.00	<50.00	374.00	37.50
863090-89-5	PFECA A	<500.00	<50.00	<50.00	<5.00
151772-58-6	PFECA B	<72.00	<72.00	<72.00	<7.20
113507-82-7	PES	<50.00	<50.00	<50.00	<5.00
356-02-5	3:3 FTCA	<1,000.00	<100.00	<100.00	<10.00
914637-49-3	5:3 FTCA	505.00	1,430.00	<250.00	<25.00
812-70-4	7:3 FTCA	<250.00	<250.00	<250.00	<25.00
NA	**Subtotal: Non-detect**	**5,267.00**	**2,266.00**	**3,517.00**	**231.60**
NA	**Subtotal: Detected**	**116,380.10**	**10,796.60**	**6,216.60**	**1,689.66**
NA	**Grand Total**	**121,647.10**	**13,062.60**	**9,733.60**	**1,921.26**

Notes:
Yellow shading = compound spiked during Runs 1A, 2A, 3A
Pink shading = compound detected
ABC = afterburner chamber
NA = not applicable

Table 4-4. PFAS in Chemicals and Process Water to Emission Control System — Run 1A

CAS No.	Acronym	Powdered Activated Carbon (ng/g)	Soda Ash (ng/L)	TMT-15 (ng/g)	Process Water (ng/L)
375-22-4	PFBA	6.34	<10.00	<10.00	<0.94
2706-90-3	PFPeA	<0.20	<5.00	<5.00	<0.47
307-24-4	PFHxA	<0.20	<5.00	<5.00	2.95
375-85-9	PFHpA	<0.20	<5.00	<5.00	0.79
335-67-1	PFOA	1.79	<5.00	<5.00	3.27
375-95-1	PFNA	<0.20	<5.00	<5.00	0.56
335-76-2	PFDA	<0.20	<5.00	<5.00	<0.47
2058-94-8	PFUnA	<0.20	<5.00	<5.00	<0.47
307-55-1	PFDoA	<0.20	<5.50	<5.50	<0.52
72629-94-8	PFTriA	<0.20	<5.80	<5.80	<0.55
376-06-7	PFTeA	<0.23	<8.10	<8.10	<0.76
375-73-5	PFBS	0.43	<5.00	8.45	0.76
2706-91-4	PFPeS	<0.20	<5.00	<5.00	0.66
355-46-4	PFHxS	<0.20	<5.00	<5.00	7.00
375-92-8	PFHpS	<0.20	<5.00	<5.00	0.96
1763-23-1	PFOS	<0.20	5.79	11.00	58.50
68259-12-1	PFNS	<0.20	<5.00	<5.00	<0.47
335-77-3	PFDS	<0.20	<5.00	<5.00	<0.47
79780-39-5	PFDoS	<0.20	<5.30	<5.30	<0.50
754-91-6	FOSA	0.31	<5.00	12.70	<0.47
31506-32-8	NMeFOSA	<0.20	<5.00	<5.00	<0.47
4151-50-2	NEtFOSA	<0.20	<5.00	<5.00	<0.47
2355-31-9	NMeFOSAA	<0.20	<5.00	<5.00	<0.47
2991-50-6	NEtFOSAA	<0.20	<5.00	<5.00	<0.47
24448-09-7	N-MeFOSE	<1.00	<25.00	<25.00	<2.36
1691-99-2	N-EtFOSE	<1.00	<25.00	<25.00	<2.36
757124-72-4	4:2 FTS	<0.40	<10.00	<10.00	<0.94
27619-97-2	6:2 FTS	<0.40	<10.00	16.70	27.00
39108-34-4	8:2 FTS	<0.40	<10.00	<10.00	5.84
13252-13-6	HFPO-DA	<0.20	<3.90	<3.90	<0.37
919005-14-4	DONA	<0.20	<5.00	<5.00	<0.47
756426-58-1	9Cl-PF3ONS	<0.20	<5.80	<5.80	<0.55
763051-92-9	11Cl-PF3OUdS	<0.30	<5.00	<5.00	<0.47
377-73-1	PFECA F	<0.20	<5.00	<5.00	<0.47
863090-89-5	PFECA A	<0.20	<5.00	<5.00	<0.47
151772-58-6	PFECA B	<0.25	<7.20	<7.20	<0.68
113507-82-7	PES	<0.20	<5.00	<5.00	<0.47
356-02-5	3:3 FTCA	<0.40	<10.00	<10.00	<0.94

CAS No.	Acronym	Powdered Activated Carbon (ng/g)	Soda Ash (ng/L)	TMT-15 (ng/g)	Process Water (ng/L)
914637-49-3	5:3 FTCA	<1.00	<25.00	<25.00	<2.36
812-70-4	7:3 FTCA	<1.00	<25.00	<25.00	<2.36
NA	**Subtotal: Non-detect**	**11.38**	**306.60**	**286.60**	**23.27**
NA	**Subtotal: Detected**	**8.87**	**5.79**	**48.85**	**108.29**
NA	**Grand Total**	**20.25**	**312.39**	**335.45**	**131.57**

Notes:
Yellow shading = compound spiked during Runs 1A, 2A, 3A
Pink shading = compound detected
NA = not applicable
ng/g = nanogram(s) per gram

Table 4-5. PFAS in Residuals Streams — Run 1A

CAS No.	Acronym	Slag/Ash (ng/g)	Spray Dryer[(a)] Solids (ng/g)	Baghouse Dust (ng/g)	Brine (ng/L)
375-22-4	PFBA	24.10	<0.39	1.82	<0.94
2706-90-3	PFPeA	<0.14	<0.19	<0.20	2.57
307-24-4	PFHxA	0.51	<0.19	<0.20	1.86
375-85-9	PFHpA	<0.14	<0.19	<0.20	<0.47
335-67-1	PFOA	0.80	<0.24	<0.25	<0.47
375-95-1	PFNA	0.41	<0.19	<0.20	<0.47
335-76-2	PFDA	0.69	<0.19	<0.20	<0.47
2058-94-8	PFUnA	<0.14	<0.19	<0.20	<0.47
307-55-1	PFDoA	<0.14	<0.19	<0.20	<0.52
72629-94-8	PFTriA	<0.14	<0.19	<0.20	<0.55
376-06-7	PFTeA	<0.17	<0.22	<0.23	<0.76
375-73-5	PFBS	4.42	0.27	<0.20	<0.47
2706-91-4	PFPeS	1.00	<0.19	<0.20	<0.47
355-46-4	PFHxS	15.40	<0.19	<0.20	<0.47
375-92-8	PFHpS	0.57	<0.19	<0.20	<0.47
1763-23-1	PFOS	43.70	<0.20	<0.20	0.85
68259-12-1	PFNS	<0.14	<0.19	<0.20	<0.47
335-77-3	PFDS	0.23	<0.19	<0.20	<0.47
79780-39-5	PFDoS	<0.14	<0.19	<0.20	<0.50
754-91-6	FOSA	<0.18	<0.24	<0.25	<0.47
31506-32-8	NMeFOSA	<0.14	<0.19	<0.20	<0.47
4151-50-2	NEtFOSA	<0.14	<0.19	<0.20	<0.47
2355-31-9	NMeFOSAA	<0.14	<0.19	<0.20	<0.47

CAS No.	Acronym	Slag/Ash (ng/g)	Spray Dryer[(a)] Solids (ng/g)	Baghouse Dust (ng/g)	Brine (ng/L)
2991-50-6	NEtFOSAA	<0.14	<0.19	<0.20	<0.47
24448-09-7	N-MeFOSE	<0.71	<0.97	<1.00	<2.35
1691-99-2	N-EtFOSE	<0.71	<0.97	<1.00	<2.35
757124-72-4	4:2 FTS	<0.29	<0.39	<0.40	<0.94
27619-97-2	6:2 FTS	0.38	<0.39	<0.40	1.74
39108-34-4	8:2 FTS	<0.29	<0.39	<0.40	<0.94
13252-13-6	HFPO-DA	2.06	<0.19	<0.20	<0.37
919005-14-4	DONA	<0.14	<0.19	<0.20	<0.47
756426-58-1	9Cl-PF3ONS	<0.14	<0.19	<0.20	<0.55
763051-92-9	11Cl-PF3OUdS	<0.21	<0.29	<0.30	<0.47
377-73-1	PFECA F	<0.14	<0.19	<0.20	<0.47
863090-89-5	PFECA A	<0.14	<0.19	<0.20	<0.47
151772-58-6	PFECA B	<0.18	<0.24	<0.25	<0.68
113507-82-7	PES	<0.14	<0.19	<0.20	<0.47
356-02-5	3:3 FTCA	<0.29	<0.39	<0.40	<0.94
914637-49-3	5:3 FTCA	<0.71	<0.97	<1.00	<2.35
812-70-4	7:3 FTCA	<0.71	<0.97	<1.00	<2.35
NA	**Subtotal: Non-detect**	**6.74**	**11.69**	**12.08**	**26.95**
NA	**Subtotal: Detected**	**94.27**	**0.47**	**1.82**	**7.02**
NA	**Grand Total**	**101.01**	**12.15**	**13.90**	**33.96**

Notes:
Yellow shading = compound spiked during Runs 1A, 2A, 3A
Pink shading = compound detected
NA = not applicable

5 PFAS Spiking

5.1 PFAS Spiking Objective

The incinerator PFAS spiking was coordinated and executed by EA. One of the overall testing objectives was to be able to demonstrate up to 99.9999 percent DRE for select target analytes. To demonstrate DRE performance at this level, the mass of any PFAS target analyte in the waste feed was planned to be at least 1,000,000 times the mass that can be detected in the exhaust emissions at the analytical MDL. Since the native mass of the PFAS target analytes in the waste feed materials prior to the test was unknown, spiking to fortify their concentration levels was used to ensure that sufficient masses of target analytes were fed during the test for selected compounds to achieve the DRE performance demonstration objective.

5.2 PFAS Spiking Target Analytes

Nine PFAS were selected to be spiked into the rotary kiln to facilitate determining DRE and DE values. These chemicals were selected from the compounds that the EPA had published human health toxicity assessments (EPA 2025c). Three of the perfluoroalkyl acids were not readily available in the acid (H^+) form required for the test program. Therefore, their potassium (K^+) salt forms were purchased for spiking in place of the acid (H^+) forms.

The liquid chromatography methodology (LC/MS/MS) used for the analysis of the individual PFAS compounds quantifies the anionic form (A^-) of the PFAS target analyte. Therefore, the initial form of the spiked PFAS compound, whether acid or the potassium salt, does not affect the analytical quantification of the target analytes. The PFAS spiking compounds include:

- PFHxA CAS No. 307-24-4 $C_6HF_{11}O_2$ MW 314.05
- PFOA CAS No. 335-67-1 $C_8HF_{15}O_2$ MW 414.07
- PFNA CAS No. 375-95-1 $C_9HF_{17}O_2$ MW 464.08
- PFDA CAS No. 335-76-2 $C_{10}HF_{19}O_2$ MW 514.08
- PFBA CAS No. 375-22-4 $C_4HF_7O_2$ MW 214.04
- K-PFBS CAS No. 29420-49-3 $C_4F_9O_3SK$ MW 338.19
- K-PFHxS CAS No. 3871-99-6 $C_6F_{13}O_3SK$ MW 438.20
- K-PFOS CAS No. 2795-39-3 $C_8F_{17}O_3SK$ MW 538.22
- HFPO-DA CAS No. 13252-13-6 $C_6HF_{11}O_3$ MW 330.05

5.3 Calculation of PFAS Spiking Rates

The required spiking mass feed rates were calculated using the estimated exhaust emissions flow rate, the OTM-45 sample volume, and the MDL for each specific analyte in the exhaust emissions. The calculated feed amounts for all nine PFAS were adjusted to account for the purity of the purchased chemicals. For PFBS, PFHxS, and PFOS, which were supplied as potassium salts, the PFAS spiking compound amounts were further adjusted to reflect the molecular weights of the salt forms, ensuring the calculations were based on equivalent molar quantities of the PFAS target analytes. These adjustments were necessary to support consistent interpretation of DRE results across analytes with varying physical and chemical properties. The spiking rate calculations for each of the nine PFAS are provided in Attachment 1 of Appendix E.

5.4 PFAS Spiking Material Preparation

Reagent grade PFAS spiking compounds were purchased from the following vendors:

- Matrix Scientific (Columbia, SC)
- Smolecule (San Antonio, TX)
- Indofine Chemical Company (Hillsborough Township, NJ)

Safety data sheets and Certificate of Analysis for the PFAS spiking compounds are presented in Appendix E, Attachment 5 and 6 respectively.

The PFAS spiking compound mass amounts were precisely weighed to two decimal places (plus or minus 5 milligrams) using a calibrated analytical scale. The weights were recorded, and the weighed portions were placed in labeled PFAS-free HDPE containers and sealed. Each of the sealed containers held the measured mass of an individual PFAS. The containers were then bundled into groups of the nine spiking compounds, each in their own HDPE container. Multiple containers were used for some compounds, as needed. The individual bundles were then packaged in PFAS-free, disposable cardboard boxes, sealed, and labeled with sequential identification numbers by EA personnel. The sequential identification number and corresponding spiking material weights were recorded on the PFAS Spiking Bundle Tracking Spreadsheet. Each box was labeled with a sequential identification number matching its assigned bundle. Photographs of the spike material packaging system are shown in Appendix E, Attachment 2.

5.5 PFAS Spiking Execution

PFAS spiking was conducted during Run 1A, 2A, and 3A. PFAS spiking was not conducted during Runs 1B, 1C, 2B, 2C, 3B, or 3C. During each day when PFAS spiking was conducted, the required number of spiking material boxes were removed from storage and staged at the rotary kiln feed chute. The first container of PFAS spiking compounds was fed into the rotary kiln at least 60 minutes before any process stream sampling or exhaust emissions sampling began. This lead time allowed the PFAS concentration in the rotary kiln slag/ash to reach equilibrium, as the residence time of the solids in the rotary kiln was approximately 1 hour. The residence time estimate was based on historical observations by CHES of the

elapsed time between the start of material feeding and its appearance in the slag/ash discharge during a cold startup.

The CHES operator performed the spiking by placing the individual boxes sequentially into the rotary kiln feed chute (as shown on Appendix E, Figure E-1) at 15-minute intervals. Once initiated, spiking continued without interruption through the preconditioning period and until the run sampling was completed (Run 1A, 2A, or 3A).

The CHES operator recorded the identification number printed on each box and the time each box was dropped was documented on the PFAS Spiking Bundle Feed Log, as well as at the conclusion of each run. The recorded times from the PFAS Spiking Bundle Feed Log Sheet were transferred to the PFAS Spiking Bundle Tracking Spreadsheet (Appendix E, Attachment 3). The total mass of PFAS spiking compound added during the run was calculated accordingly and is presented in the tables in Appendix E, Attachment 4.

Table 5-1 presents the total amount of each PFAS spiking compound in the form that it was spiked during each run. Table 5-1 also presents the total amount of each target analyte in the acid form, which includes adjusting the form spiked for chemical purity. For the three compounds that were spiked as potassium salts, molecular weights were used to calculate the mass of the acid form for that chemical. The mass of the PFAS acid form was used in all mass and energy balance, DRE, and DE calculations.

Table 5-1. PFAS Spiking Compound Amounts During Sampling Runs

Bottle Number	Analyte	Purity (%)	MW of K Salt Form	MW of Acid Form	Run 1A Weight in Form Spiked[(a)] (g)	Run 2A Weight in Form Spiked[(a)] (g)	Run 3A Weight in Form Spiked[(a)] (g)	Run 1A Weight of Acid Form[(b,c)] (g)	Run 2A Weight of Acid Form[(b,c)] (g)	Run 3A Weight of Acid Form[(b,c)] (g)
1	PFOA	96	NA	414.07	172.32	140.01	183.09	165.43	134.41	175.77
2	K-PFOS	95	538.22	500.13	146.40	118.95	155.55	129.24	105.01	137.31
3	HFPO-DA	97	NA	330.05	1,128.16	916.63	1,198.67	1094.32	889.13	1162.71
4	K-PFBS	98	338.19	300.1	194.56	158.08	206.72	169.19	137.47	179.77
5	K-PFHxS	95	438.2	400.12	71.20	57.85	75.65	61.76	50.18	65.62
6	PFHxA	98	NA	314.05	186.40	151.45	198.05	182.67	148.42	194.09
7	PFBA	98	NA	214.04	898.08	729.69	954.21	880.12	715.10	935.13
8	PFNA	97	NA	464.08	65.76	53.43	69.87	63.79	51.83	67.77
9	PFDA	98	NA	514.08	92.16	74.88	97.92	90.32	73.38	95.96
1–9	**NA**	**NA**	**NA**	**NA**	**2,955.04**	**2,400.97**	**3,139.73**	**2,836.83**	**2,304.92**	**3,014.13**

Notes:
(a) As purchased form, not adjusted for purity of analytes. Includes mass of K in K-PFOS, K-PFBS, and K-PFHxS.
(b) Adjusted to account for chemical purity of material.
(c) Includes molecular weight conversion from K salt form for K-PFOS, K-PFBS, and K-PFHxS to acid form.
% = percent
g = gram(s)
K = potassium
MW = molecular weight
NA = not applicable

6 C_2F_6 Spiking

6.1 Overview

The C_2F_6 spiking services were provided by SSI. A complete description of the C_2F_6 spiking procedures and results is presented in Appendix F. This section describes the process for and results of spiking C_2F_6 into the rotary kiln during Runs 1C, 2C, and 3C. C_2F_6 has been proposed as a potential indicator compound POHC for conducting DRE testing for a wide range of PFAS. The purpose for spiking C_2F_6 is to evaluate the performance of the incinerator by determining the DRE for a stable compound. The evaluation of C_2F_6 as a potential indicator compound will be performed by correlating the DRE values achieved for C_2F_6 with the DREs of other PFAS and the presence of PIDs in the exhaust emissions.

The C_2F_6 spiking rate required to achieve a target DRE value is based on the exhaust emissions flow rate and the analytical detection limit of C_2F_6 in the exhaust emissions. For planning purposes, the maximum exhaust emissions flow rate that had been measured during previous testing in 2021 and 2022 (Crisenbery et al. 2024) was used in the C_2F_6 spiking calculations. The exhaust emissions flow rate from Run 8 of the June 2021 test program is 38,565 dry standard cubic feet per minute.

Both OTM-50 sampling and analysis as well as FTIR monitoring were used to assess C_2F_6 emission concentrations. Prior to conducting the PFAS test program, the estimated analytical detection limit for C_2F_6 analyzed by OTM-50 was estimated by Eurofins to be 0.031 parts per billion (ppb) (0.175 micrograms per cubic meter [µg/m^3]). The MDC was determined by Method #3, determination of analytical algorithm errors per ASTM D6348-12 Section A2.3. The MDC for C_2F_6 analyzed by FTIR was estimated by Spectrum to be in the range of 10 to 25 ppb (56 to 141 µg/m^3). Therefore, the lower MDL value from the OTM-50 analysis was used to perform C_2F_6 spiking calculations. Spiking C_2F_6 at a feed rate (12.43 lb/hr) was deemed sufficient to achieve a concentration in the exhaust emissions of 15 parts per million by volume if there was no C_2F_6 destruction during thermal treatment. This spiking rate was estimated to allow demonstration of a C_2F_6 DRE value of 99.99979 percent at the Eurofins OTM-50 estimated pre-test MDL. After the first run, spiking C_2F_6 at a rate of 12.43 lb/hr, the plan for the remaining testing, Runs 2C and 3C, was changed to increase the feed rate to 45 lb/hr. This allowed for a higher DRE to be calculated and for a greater possibility of detectable measurement of C_2F_6 in exhaust emissions.

6.2 Spiking Equipment

The spiking system was comprised of a cylinder of C_2F_6 gas placed on a calibrated weigh scale, pressure gauges, pressure regulators, a weight data logger, a mass flow meter, a mass flow rate data logger, and a set of control and shutoff valves. A schematic of this setup is displayed on Figure 6-1. Nitrogen gas was used to purge the lines before each run.

Prior to the test, CHES ran a ¼-inch stainless steel tube from the sludge port on the rotary kiln to the spiking equipment. The spiking equipment was set up on a working platform on the north side of the afterburner. The stainless steel tubing was provided with a ¼-inch female Swagelok connector and a ¼-inch ball valve type shutoff. SSI set up their equipment and connected it to the tubing provided by CHES.

6.3 Spiking Procedures

The C_2F_6 spiking gas was injected through the sludge port on the rotary kiln. This port fires below the burner. The project team purposely chose to inject the C_2F_6 gas through this port to avoid feeding it through a burner flame. This feed location exposes the spiked gas material to conservatively lower temperature conditions than if the gas were fed through a burner port. No other waste materials were fed at this location during the spiking process to avoid the occurrence of back pressure in the lines.

Before each run, the spiking system was primed with the C_2F_6 gas and the flow rate was set and stabilized at the target flow rate. The target C_2F_6 feed rate was then maintained for the duration of the run. During the run, weigh scale recordings of the cylinder weight loss were manually recorded at 5-minute time intervals. Records were kept both electronically and manually as shown in Appendix F.

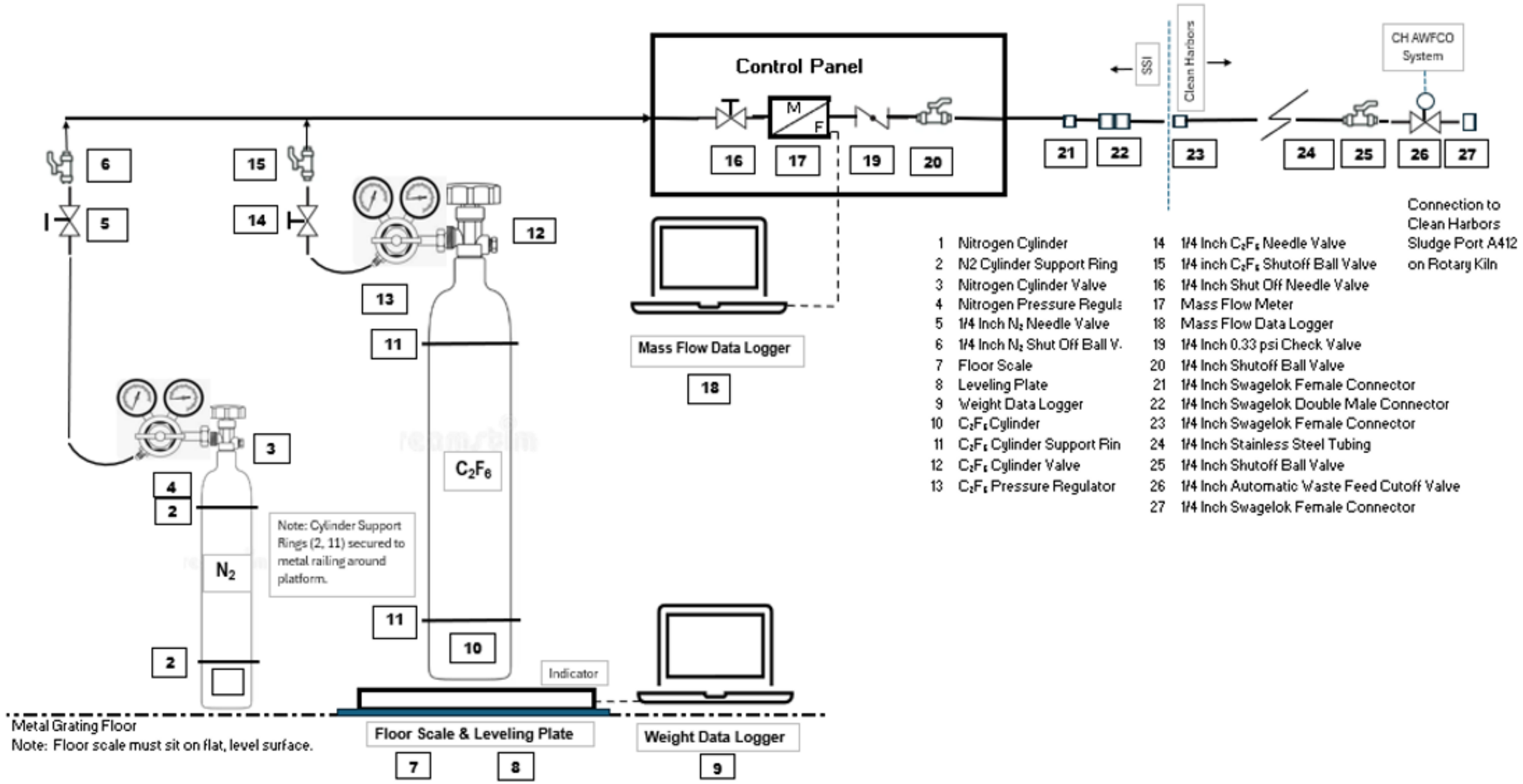
Two independent spiking rate measurement methods were used:

- The Weight Loss versus Time Method
- The Mass Flow Meter Method

For the Weight Loss versus Time Method, the spiking material was released out of the cylinder into the feed line. Consequently, the mass remaining in the cylinder and on the weight scale decreases. The rate at which the mass changes with time defines the spiking material injection rate. A computer-based data logger was used to record the cylinder weight at 1-minute time intervals. The instantaneous and cumulative average spiking rates were subsequently calculated from these data. This method provided a continuous record of the spiking rate. The accuracy of the scale system was demonstrated both pre-test and post-test by placing a weight standard on the scale. The calibration certified check weights are traceable to the National Institute of Standards and Technology.

Weight measurements were also manually recorded on a paper log sheet at 5-minute time intervals. The 5-minute average feed rate and the cumulative feed rate are also recorded on the log. This record provided a backup spiking record if the data logger failed during a run.

The mass flow meter provided a continuous flow rate measurement. The data from the mass flow meter were used during the runs to adjust the flow rate of the spiked gas. These data were not used in the mass balance calculations. The data from the mass flow meter were recorded on a data logger at 1-minute intervals. SSI could not recover the data from the disk at the end of the test program. However, the inability to recover data had no effect on meeting the test objectives because the data from the Weight Loss versus Time method were utilized in all C_2F_6 spiking rate calculations.



1	Nitrogen Cylinder	14	1/4 Inch C_2F_6 Needle Valve
2	N2 Cylinder Support Ring	15	1/4 inch C_2F_6 Shutoff Ball Valve
3	Nitrogen Cylinder Valve	16	1/4 Inch Shut Off Needle Valve
4	Nitrogen Pressure Regula	17	Mass Flow Meter
5	1/4 Inch N_2 Needle Valve	18	Mass Flow Data Logger
6	1/4 Inch N_2 Shut Off Ball V.	19	1/4 Inch 0.33 psi Check Valve
7	Floor Scale	20	1/4 Inch Shutoff Ball Valve
8	Leveling Plate	21	1/4 Inch Swagelok Female Connector
9	Weight Data Logger	22	1/4 Inch Swagelok Double Male Connector
10	C_2F_6 Cylinder	23	1/4 Inch Swagelok Female Connector
11	C_2F_6 Cylinder Support Rin	24	1/4 Inch Stainless Steel Tubing
12	C_2F_6 Cylinder Valve	25	1/4 Inch Shutoff Ball Valve
13	C_2F_6 Pressure Regulator	26	1/4 Inch Automatic Waste Feed Cutoff Valve
		27	1/4 Inch Swagelok Female Connector

Figure 6-1. Hexafluoroethane (C_2F_6) Spiking Equipment Diagram

6.4 Spiking Results

The planned and measured amounts of gas spiked to the rotary kiln during each run are summarized in Table 6-1. The C_2F_6 feed rate was slightly below the actual gas flow rate because the purity of C_2F_6 in the gas was 99.9 percent. Detailed spiking results are presented in Appendix F. Log sheets are provided that document the start and stop time for each run. A summary of spiking results recorded on the Weight Loss versus Time data logger is presented in Appendix F, Attachment A-1, Table A-2. This data was used for all mass balance and DRE calculations. The measured spiking accuracy values ranged from 98.02 to 99.84 percent. Detailed spiking data are presented in Appendix F, Attachment A-1, Table A-3.

Spiking results that were recorded manually are presented in Appendix F, Attachment A-2 in Tables A-4, A-5, and A-6 for Runs 1C, 2C, and 3 C, respectively. Spiking results are graphed in Appendix F, Attachment A-2 on Figures A-1, A-2, and A-3 for Runs 1C, 2C, and 3C, respectively. Field log sheets for these data are presented in Appendix F, Attachment A-3 on Figures A-4, A-5, and A-6 for Runs 1C, 2C, and 3C, respectively.

Table 6-1. C_2F_6 Spiking Results Summary

Run No.	Gas Feed Rate[(a)] (lb/hr)	Target Gas Feed Rate (lb/hr)	Gas Feed Rate Accuracy[(b)] (%)	C_2F_6 Feed Rate[(c)] (lb/hr)
Run 1C	12.41	12.43	99.84	12.40
Run 2C	44.11	45.00	98.02	44.07
Run 3C	44.68	45.00	99.29	44.64

Notes:
(a) Data from manual data log.
(b) Gas Feed Rate Accuracy = (Gas Feed Rate [lb/hr]/ Target Gas Feed Rate [lb/hr]) * 100%
(c) Calculated at a C_2F_6 material purity shown on Certificate of Analysis of >99.9%
% = percent

6.5 Quality Assurance/Quality Control Procedures

QA checks included spiking material composition documentation and test equipment calibrations, performance of leak checks, and redundancy of system measurements of the C_2F_6 spiking rate. The QA checks included the following elements:

- The composition of the C_2F_6 spiking gas was analyzed by the industrial gas supplier (Linde) prior to the test. A certificate of analysis for C_2F_6 spiking material is provided in Appendix F, Attachment A-4, Figure A-7.

- The General Electronic platform scale was calibrated prior to the test by Carlton Scale in Knoxville, Tennessee, using certified check weights. The calibration range is 0 to 400 pounds. Carlton Scale performed a post-test calibration check when the scales were returned after the completion of the test. The post-test calibration was performed on January 14, 2025; however, the Carlton Scale representative confirmed that the scale had not been used since it was returned following the field test on November 20, 2024. Certificates of calibration for the pre-

test and post-test calibrations are included in Appendix F, Attachment A-4, Figure A-8, and Figure A-9.

- The Emerson mass flow meter was calibrated by Coastal Flow Liquid Measurement, Inc. in Houston, Texas, before the test. The calibration range is set for 0 to 20 lb/hr. A certificate of calibration is included in Appendix F, Attachment A-4, Figure A-10.

- SSI performed leak checks on the spiking system and all valves prior to use during the test program. Upon completion of successful leak checks, the system was purged for 2 minutes with nitrogen gas before each run.

- Redundant electronic and manual C_2F_6 flow rate recording systems were used. These systems are described in Section 3.0. (*FTIR Experimental Parameters*) of Appendix G.

Based on the above procedures, it was determined that the data presented for this spiking program is sufficiently accurate and representative and suitable for its intended use to demonstrate the DRE of C_2F_6.

7 FTIR Monitoring

7.1 Overview

The FTIR monitoring was conducted by Spectrum. Long-path extractive FTIR spectroscopy by Method ASTM D6348-12 was used to continuously profile incinerator exhaust emissions for PIDs. This testing methodology was chosen for its ability to speciate and quantify PFAS analytes present within the relatively complex mixture of gases present in the incinerator exhaust on a continuous, (near) real-time basis. The complete FTIR Monitoring Report is presented in Appendix G.

The Spectrum FTIR WaveRunIR™ system was pre-configured and optimized to detect and accurately quantify C_2F_6 and CF_4 at sensitivities below single-digit ppbv in near real-time (4- to 5-minute spectral signal averaging periods). The hardware/software configurations needed to achieve these requirements are described in detail within Section 3 (*FTIR Experimental Parameters*) of Appendix G, while the analytical figures-of-merit achieved in the field, per ASTM D6348-12 definitions, are rigorously presented in Section 5 (*FTIR Data Quality Assurance/Quality Control [QA/QC]*) of Appendix G.

Analytical parameters-directly pertinent to the exhaust emissions test results, including those related to FTIR system detection limits, also known as MDCs, are included in Appendix G. Sample measurement accuracies as derived from certified analyte gas spiking recovery percentages (percent recoveries) within the representative gas sample matrices, are also included in Appendix G. Table 7-1 summarizes the field detection capabilities demonstrated for the FTIR hardware/software assembly deployed on this project.

Table 7-2 summarizes the field recovery (i.e., sampling system + analytical measurement accuracies) capabilities demonstrated for the FTIR hardware/software assembly deployed on this project. An acceptable criterion, per ASTM D6348-12, is nominally plus or minus 30 percent (i.e., 70 to 130 percent recovery).

The summary of FTIR field test results, listed for each run, is shown in Table 7-3. The graphical real-time series data plots are presented in Section 4 (*FTIR Stack Emission Test Results*) of Appendix G. The values in Table 7-3 represent average concentration amounts and pertinent minimum/maximum values. In this case, zero values are expressed more appropriately as the measurement system's MDC.

Table 7-1. FTIR Empirically Determined Field Detection Limits for C_2F_6 and CF_4

Species	Quant Region NEA_{RMS} (abs units)	$A_{refpeak}$ (abs units)	Instrumental detection limit (ppbv)	Analytical Method MDC[(a)] (ppbv)
C_2F_6	1.46×10^{-5}	0.0034	0.22	0.62
CF_4	8.07×10^{-5}	0.0245	0.17	0.60

Notes:
(a) This minimum detectable concentration (MDC) evaluation of dataset 1B corresponds to the MDC #3 determination of analytical algorithm errors per ASTM D6348-12 Section A2.3.
NEA_{RMS} = root mean square value of the noise equivalent absorbance
$A_{refpeak}$ = absorbance of the reference peak

Table 7-2. FTIR Analyte Spiking Parameters and Recoveries

Analyte Run Date and Gas Standard #	DF of Spike into Sample	Gas Standard $[C_2F_6/CF_4]_{direct}$ CS (ppbv)	Expected FTIR $[C_2F_6/CF_4]$ (ppbv)	Measured FTIR $[C_2F_6/CF_4]$ (ppbv)	C_2F_6/CF_4 Recoveries (%)
11/13/24, #2	0.026	9910/9930	257.7/258.2	240.7/257.6	***93.4/99.8***
11/13/24, #2	0.0135	9910/9930	133.8/134.0	126.5/141.1	***94.6/105.2***
11/13/24, #2	0.0054	9910/9930	53.5/53.6	49.0/59.2	***91.6/110.4***
11/13/24, #2	0.0025	9910/9930	24.8/24.8	22.9/33.9	***92.4/136.5***
11/13/24, #1	0.0255	1010/1006	25.7/25.6	25.0/32.2	***97.1/125.5***
11/13/24, #1	0.0131	1010/1006	13.2/13.2	12.7/16.1	***96.0/122.2***
11/13/24, #1	0.0064	1010/1006	6.5/6.4	5.2/8.3	***80.4/128.9***
11/13/24, #1	0.0033	1010/1006	3.3/3.3	2.5/4.9	***75.0/145.0***
11/14/24, #1	0.0244	1010/1006	24.6/24.5	25.4/26.4	***103.1/107.5***
11/14/24, #1	0.0127	1010/1006	12.8/12.8	14.2/15.4	***110.7/120.5***
11/14/24, #1	0.0057	1010/1006	5.7/5.7	6.8/7.8	***118.1/136.0***
11/14/24, #1	0.003	1010/1006	3.0/3.0	4.1/3.5	***129.5/116.0***

Notes:
% = percent
CS = calibration spike concentration
DF = dilution factor, the percentage (divided by 100) of analyte spike gas within sample gas
ppbv = parts per billion by volume

Table 7-3. Summary of FTIR Results

Parameter	PFAS Spiking and AFFF Feed Run 1A (ppb)	PFAS Spiking and AFFF Feed Run 2A (ppb)	PFAS Spiking and AFFF Feed Run 3A (ppb)	Baseline Run 1B (ppb)	Baseline Run 2B (ppb)	Baseline Run 3B (ppb)	C_2F_6 Spiking Run 1C (ppb)	C_2F_6 Spiking Run 2C (ppb)	C_2F_6 Spiking Run 3C (ppb)
C_2F_6 MDC	0.62	0.62	0.62	0.62	0.62	0.62	0.62	0.62	0.62
C_2F_6 Minimum	---	---	---	---	---	---	---	0.62	---
C_2F_6 Average	0.62	0.62	0.62	0.62	0.62	0.62	0.62	1.21	0.62
C_2F_6 Maximum	---	---	---	---	---	---	---	1.39	---
CF_4 MDC	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6
CF_4 Minimum	2.2	0.6	---	0.6	---	---	---	---	---
CF_4 Average	4.4	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6
CF_4 Maximum	13.4	4.2	---	14.3	---	---	---	---	---
CHF_3 MDC	0.57	0.57	0.57	0.57	0.57	0.57	0.57	0.57	0.57
CHF_3 Minimum	---	---	---	---	---	---	---	---	---
CHF_3 Average	0.57	0.57	0.57	0.57	0.57	0.57	0.57	0.57	0.57
CHF_3 Maximum	---	---	---	---	---	---	---	---	---

Notes:

--- = value not observed

Non-detect values are reported at the MDC; therefore, no minimum nor maximum was observed.

8 Exhaust Emissions Sampling

8.1 Overview

The exhaust emissions sampling was conducted by ATG. The ATG Source Testing Report is presented in H. Samples were collected from a vertical duct on the discharge side of the packed scrubber. During Runs 1A, 2A, and 3A, the exhaust emissions sampling was conducted using the following methods:

- OTM-45
  Semi-volatile polar PFAS (49 target analytes)

- Method 0010
  Semi-volatile non-polar compounds (106 non-PFAS target analytes, plus a characterization of unknowns)

- OTM-50
  Volatile nonpolar PFAS (30 target analytes) plus characterization of unknowns

During Runs 1B, 1C, 2B, 2C, 3B, and 3C, the exhaust emissions sampling was conducted using OTM-50.

8.2 Stack Sampling Methods — EPA Methods 1, 2, 3A, and 4

Each run also included the use of EPA Method 1 (Sample and Velocity Traverses for Stationary Sources), EPA Method 2 (Determination of Exhaust Emissions Velocity and Volumetric Flow Rate), EPA Method 3A (Determination of Oxygen and Carbon Dioxide Concentrations), and EPA Method 4 (Determination of Moisture Content in Exhaust Emissions). Descriptions of EPA Methods 1, 2, 3A, and 4 are presented below.

8.2.1 EPA Method 1 — Sample and Velocity Traverses for Stationary Sources

EPA Method 1 was used to assure that representative measurements of volumetric flow rates were obtained by dividing the cross-section of the stack into perpendicular transects and then locating a traverse sampling location point at intervals along each transect. Acceptable sample location points were located at least two stack equivalent diameters downstream from a flow disturbance and one-half equivalent diameter upstream from a flow disturbance. Acceptable cyclonic flow conditions were confirmed prior to testing using EPA Method 1, Section 11.4.

8.2.2 EPA Method 2 — Determination of Exhaust Emissions Velocity and Volumetric Flow Rate

EPA Method 2 was used to measure the exhaust emissions velocity with an S-type pitot tube connected to a differential pressure measurement device, and to measure the exhaust emissions temperature using a calibrated thermocouple connected to a thermocouple readout indicator. Type-S pitot tubes conforming to the geometric specifications in the test method were used, along with an inclined manometer. The measurements were made at the traverse points specified by EPA Method 1. The molecular weight of the gas stream was determined from independent measurements of O_2, CO_2, and moisture content. The exhaust emissions volumetric flow rate was calculated at the measurement plane using the average square root of the velocity head, the area of the stack at the measurement plane, the

average temperature, the stack static pressure, the molecular weight of the gas stream, and the moisture content.

8.2.3 EPA Method 3A — Determination of Oxygen and Carbon Dioxide Concentrations

EPA Method 3A is an instrumental method used to measure the concentration of O_2 and CO_2 in the exhaust emissions, and nitrogen was then calculated by difference. Data was collected online with a continuous emissions monitoring system and reported in 1-minute averages. The sampling system consisted of a stainless-steel probe, Teflon sample line, gas conditioning system, and gas analyzer. The gas conditioning system was a non-contact condenser used to remove moisture from the exhaust emissions. A portable non-contact condenser was placed in the system directly after the probe. The performance requirements of the method were met to validate data.

8.2.4 EPA Method 4 — Determination of Moisture Content in Exhaust Emissions

EPA Method 4 is a manual, non-isokinetic method used to measure the moisture content of gas streams. Gas was sampled at a constant sampling rate through a probe and impinger train. Moisture was removed using a series of pre-weighed impingers containing methodology-specific liquids and silica gel immersed in an ice water bath. The impingers were weighed after each run to determine the percent moisture. Independent Method 4 samples were collected in conjunction with each sampling train (OTM-45, OTM-50, Method 0010).

8.3 EPA OTM-45 Sampling and Analysis

The OTM-45 sampling train was operated to collect samples during Runs 1A, 2A, and 3A. PFAS spiking was being conducted and AFFF was being fed during these runs. The process data and stack testing data during these runs were used to calculate exhaust emissions rates and to perform a PFAS mass balance for the entire incineration system. The data were also used to calculate DRE and DE for EPA Method 1633 target analytes.

8.3.1 OTM-45 Sampling Method Description

EPA OTM-45 uses an extractive isokinetic sampling method to collect semi-volatile polar PFAS in the exhaust emissions. Semi-volatile polar PFAS are defined as compounds with boiling points greater than 100°C. Gaseous and particulate pollutants were withdrawn from the emission source at an isokinetic sampling rate and collected in a multicomponent sampling train.

The principal components of the train included a high-efficiency, quartz-fiber filter to collect organic-laden particulate, an XAD-2 sorbent trap, a set of chilled impingers, and a breakthrough (BT) XAD-2 sorbent trap located after the impingers and before the last impinger containing silica gel desiccant. XAD-2 is the tradename for hydrophobic copolymers of styrene-divinylbenzene resin. The purpose of the BT XAD-2 sorbent trap is to collect PFAS that pass through the other components of the sampling train. Specific components of the exhaust emissions sampling train included:

- Glass nozzle
- Heated, glass-lined probe
- Heated quartz filter in filter holder with a silicon O-ring seal

- Ice water cooled condenser
- XAD-2 sorbent trap with cooling jacket
- Short-stem knockout impinger in ice water bath
- Three impingers with 100 milliliters (mL) deionized (DI) water in an ice water bath
- BT XAD-2 sorbent
- Trap with cooling jacket
- Impinger containing 200 g of silica gel desiccant
- Ground glass connections for impingers
- O-rings to seal connections for all ball joints
- Vacuum sampling pump
- Calibrated dry gas meter to measure the collected sample volume

A schematic of the OTM-45 sampling train as implemented by ATG is shown on Figure 8-1.

After a sampling run was completed, the probe was removed from the sample train and the open outlet was capped. The filter inlet, the filter-holder outlet, and inlet to the XAD-2 sorbent trap were capped with certified PFAS free aluminum foil (foil). The XAD-2 sorbent trap was separated from the condensate knockout trap and the openings were capped with foil or parafilm. The impingers and both XAD-2 sorbent traps were weighed to determine moisture. The particulate filter was removed from the filter holder, folded or rolled with the particulate on the inside, and placed in an HDPE sample container. Any residual particulate matter on the glass nozzle, probe liner, or front half of the filter holder was recovered by brushing and rinsing these sample train components three times with a 95 percent methanol (MeOH)/5 percent ammonium hydroxide (NH_4OH) solution and collecting the rinse solution into an HDPE bottle. The XAD-2 sorbent traps were capped, and all recovered samples were immediately placed in coolers and kept on ice. The back-half of the filter holder, coil condenser and connecting glassware were rinsed three times with 95 percent MeOH/5 percent NH_4OH solution and collecting the rinse solution into an HDPE bottle. The condensate and impinger water were combined in a 1-liter HDPE bottle.

The ATG SOP for the PFAS OTM-45 exhaust emissions sampling includes detailed protocols for the following procedures:

- Stack sampling equipment and glassware cleaning procedures (in laboratory prior to field deployment)
- Laboratory preparation of sampling media (i.e., XAD-2 sorbent traps, particulate filters, MeOH/5 percent NH_4OH impinger solution, and DI water)

- Prevention of cross-contamination from equipment that previously may have been used to sample PFAS or sources (i.e., isolation of sampling equipment, use of new storage coolers, new containers, proper selection of consumables, etc.)

- Avoiding materials of construction that may contain fluoropolymers (i.e., Viton O-rings, Teflon filter frits, or other fluoropolymers items)

- Packaging procedures to ensure the cleanliness of equipment was maintained during shipping and transport to the field.

8.3.2 OTM-45 Sample Analysis & Results

At the end of the field testing program, the OTM-45 samples were packaged and preserved in coolers. Request for Analysis/Chain of Custody forms were placed in the cooler and the cooler was transferred to the Eurofins courier. The Eurofins courier delivered the OTM-45 samples under strict chain of custody to the Eurofins laboratory in Knoxville, Tennessee. The Eurofins Knoxville laboratory was responsible for analyses of all OTM-45 samples and for reporting final analytical results. Samples were analyzed by LC/MS/MS. The analytical results for this project, including the laboratory QC procedures, are provided in the Eurofins Analytical Report in Appendix J-2.

Detailed OTM-45 analytical results are presented in tables in Appendix A-1. These tables include the analytical results, MDL, data qualifier flags applied by the laboratory, and data qualifier flags applied by the data validator. A summary of emission concentrations for Runs 1A, 2A, and 3A is presented in Table 8-1.

8.3.3 OTM-45 QA/QC Samples

OTM-45 field sampling and analysis data quality was assessed using a variety of QA/QC procedures, including collection and analysis of the following types of field QA/QC samples:

- Sampling train proof blank (STPB)

- Glassware proof blank

- Field sample media blanks (FSMB)

The following types of laboratory QA samples were also analyzed:

- Laboratory media checks

- Laboratory method blanks

- Laboratory control samples (LCSs)

- Laboratory control sample duplicates (LCSDs)

Summaries of OTM-45 field sample QA/QC data and OTM-45 laboratory sample QA/QC data are presented in Section 9. Section 9 also provides a detailed discussion of the purpose of each type of QA/QC sample, along with the corresponding analytical results and related sample data.

8.3.4 Results Summary

Nineteen PFAS were detected in one or more exhaust emissions samples; concentrations were all non-detect for the remaining 30 compounds. Exhaust emissions concentrations were calculated for 147 data points (49 compounds times three runs). Figure 8-2 presents a frequency distribution of measured stack emission concentrations. Approximately 69 percent of targeted PFAS results were non-detect and approximately 96 percent had concentrations less than 10 nanograms per cubic meter (ng/m^3) for individual analytes.

One compound, HFPO-DA, had a stack emission concentration ranging from 107 to 2,223 nanograms per dry standard cubic meter (ng/dscm). However, the total contamination in the sampling train proof blank was 31.52 nanograms. Therefore, it is plausible that this analyte is contamination from an external source and not derived from the incinerator off-gas.

HFPO-DA emission results from HWI have historically been inconsistent. Another HWI study also reported HFPO-DA in emissions under different equipment design and process operating conditions (Hofman et al. 2025). However, two previous test programs in June of 2021 and February of 2022 at the CHES Aragonite incinerator produced HFPO-DA exhaust emissions concentrations of <0.23 ng/dscm at 7 percent O_2 and <7.21 ng/dscm, respectively (Crisenbery et al. 2024). Recent tests conducted by Veolia North America at an HWI in Port Arthur Texas also reported non-detect levels of HFPO-DA in stack emissions (Veolia 2025). The inconsistency in measured HFPO-DA emissions suggests that the presence or absence of HFPO-DA in emissions can be system dependent and/or the result of background contamination from sampling or analysis activities. The historical data lends credence to the conclusion that the emissions measured during the most recent CHES test were derived from an external source of contamination.

Appendix K, HFPO-DA Data Analysis, describes the analytical results and data evaluation procedures that are used to come to this conclusion. In addition to the test results described above, there are several other lines of evidence to support this conclusion:

- HFPO-DA is thermally unstable and decarboxylates at 194°F (Ryan 2019)
- Pilot scale tests in the EPA Rainbow Furnace indicate that HFPO-DA is destroyed at >99.99 percent efficiency at 1,382°F, the lowest temperature that was tested. (Weber, 2025). The operating temperatures in both the rotary kiln and afterburner during the Clean Harbors tests were in excess of 2,000°F.
- Chemical kinetic models indicate that HFPO-DA is much less thermally stable than PFOA and that other shorter chain PFCAs decompose at roughly the same temperature as PFOA (Blotevogel et al. 2023).
- If the HFPO-DA were not destroyed in the incinerator, other thermally stable compounds would also have been expected in the exhaust emissions at similar or higher concentrations. The other eight spiked PFAS compounds (with one exception for PFBA on Run 1A) had exhaust emissions concentrations of <5 ng/dscm 7 percent O_2 as shown in Table 8-1.
- HFPO-DA was not detected in any emission control system process residual stream (spray dryer solids, baghouse dust, or brine).

- HPFO-DA is highly soluble in water (739 grams per liter) (EPA 2022). If it were present in the process gas entering the emission control system, it would be efficiently removed in the packed scrubber. No HFPO-DA was detected in the packed scrubber brine.

Appendix K presents a detailed discussion of the steps that were taken to investigate the magnitude of the HFPO-DA emissions. Several approaches were taken to attempt to identify potential sources of HFPO-DA contamination:

- Analysis of OTM-45 sample data and QA data
- Analysis of rinses from graduated glass cylinders used in OTM-45 sample preparation
- OTM-45 sample extracts confirmation analysis by a second laboratory
- Post test analysis of samples of the wet scrubber packing materials
- Post test analysis of wipe samples collected on scrubber internal walls
- Post test analysis of new baghouse bag materials
- Post test analysis of OTM-45 sampling train O-rings and filter holders

Discussions of the results of each of these analyses are presented in Appendix K.

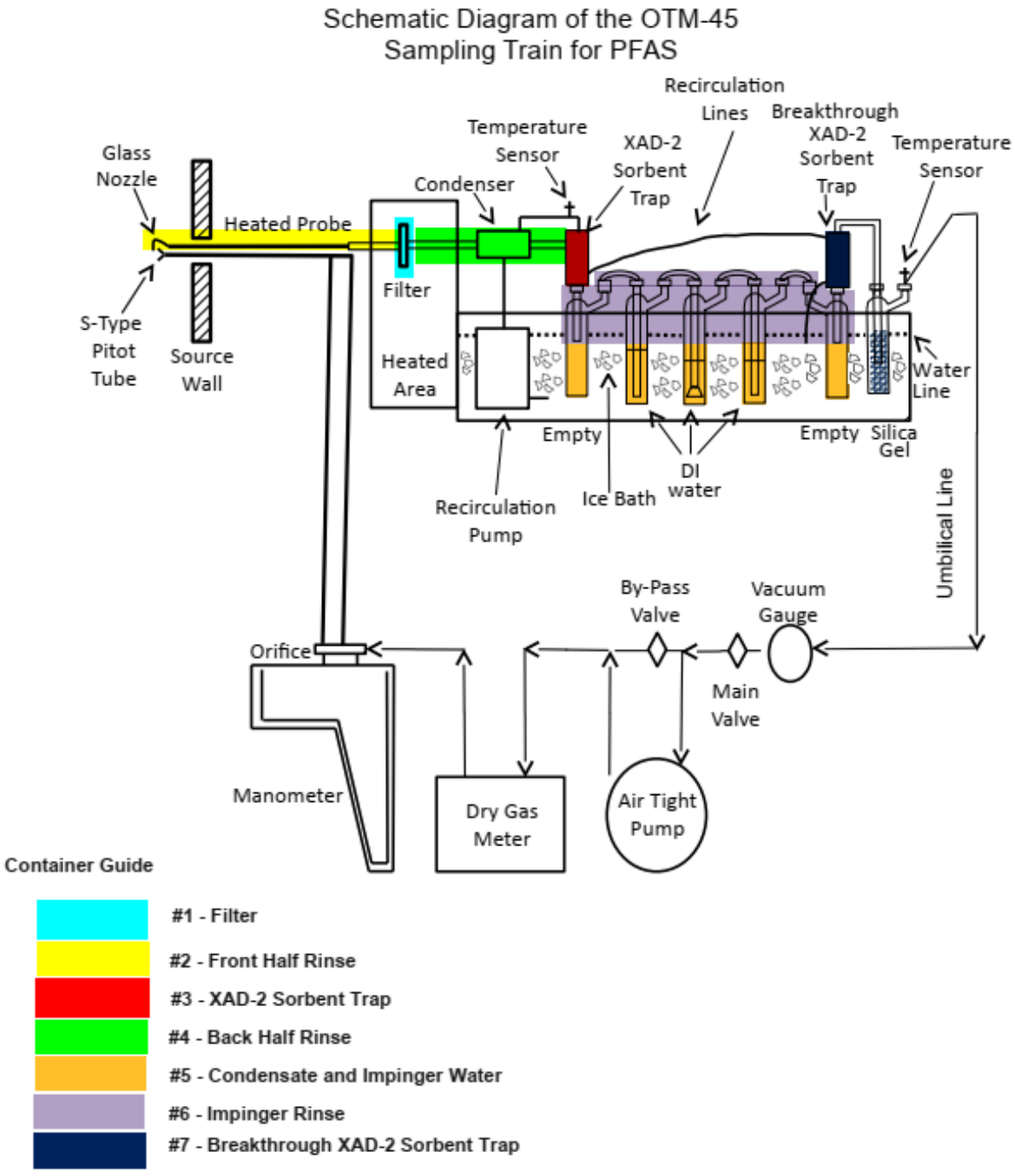


Figure 8-1. Other Test Method (OTM) 45 Sampling Train

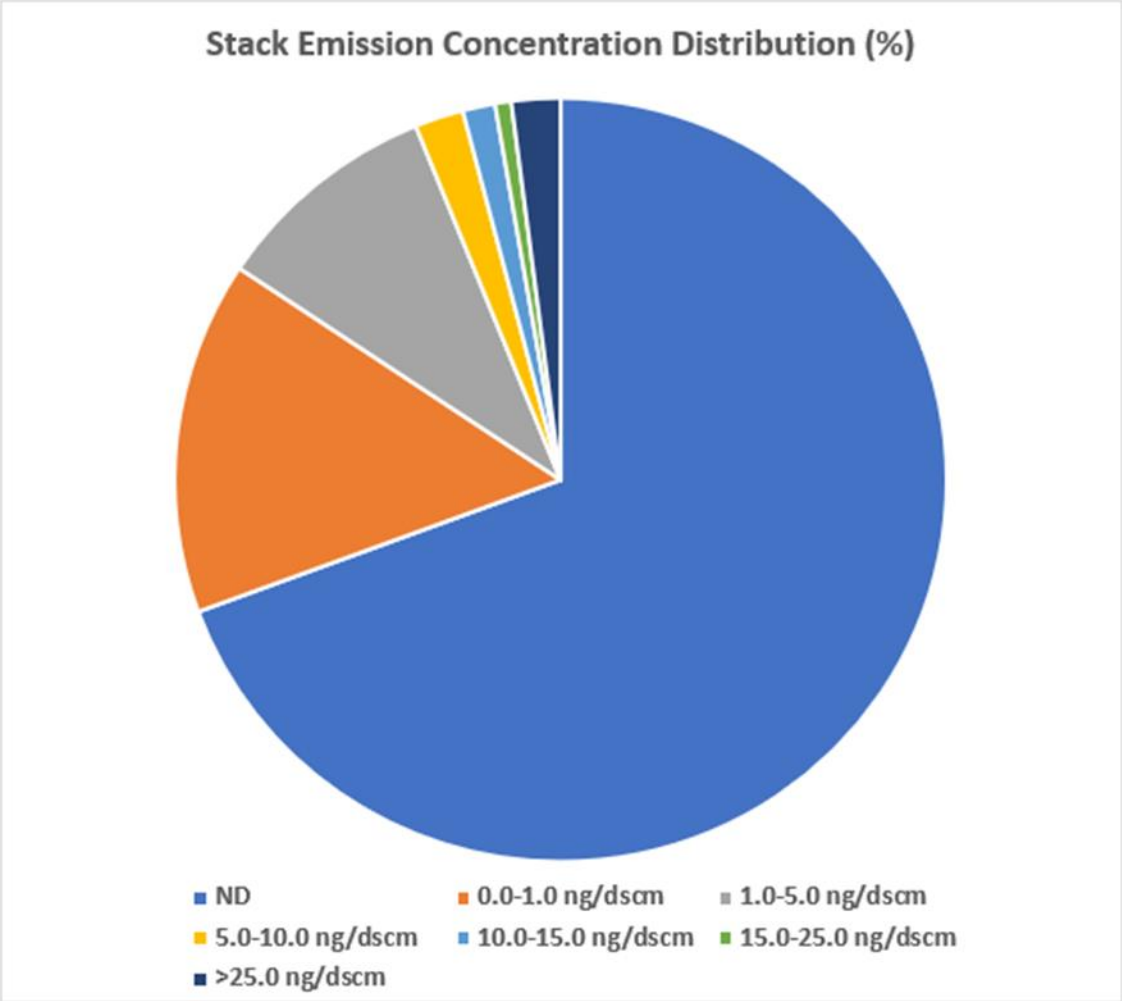
Table 8-1. Summary of Emission Concentrations of Other Test Method 45 Exhaust Emissions

PFAS Class	CAS No.	Acronym	Run 1A Exhaust Emissions Concentration (ng/dscm @ 7% O_2)	Run 2A Exhaust Emissions Concentration (ng/dscm @ 7% O_2)	Run 3A Exhaust Emissions Concentration (ng/dscm @ 7% O_2)	Average Concentration (ng/dscm @ 7% O_2)
PFCAs	375-22-4	PFBA	<5.68	<4.70	<4.26	<4.88
PFCAs	2706-90-3	PFPeA	<12.78	<8.30	<2.44	<7.84
PFCAs	307-24-4	PFHxA	<2.21	<0.66	<0.76	<1.21
PFCAs	375-85-9	PFHpA	<4.05	<4.49	<1.67	<3.40
PFCAs	335-67-1	PFOA	<1.00	<1.50	<0.79	<1.09
PFCAs	375-95-1	PFNA	<0.37	<7.47	<0.24	<2.69
PFCAs	335-76-2	PFDA	<0.36	<0.35	<0.35 ND	<0.35
PFCAs	2058-94-8	PFUnA	<0.28	13.86	<0.24 ND	<4.79
PFCAs	307-55-1	PFDoA	<0.19	<0.46	<0.20	<0.28
PFCAs	72629-94-8	PFTriA	<0.24	<19.33	<0.23 ND	<6.60
PFCAs	376-06-7	PFTeA	<0.29 ND	<0.59	<0.28 ND	<0.39
PFCAs	67905-19-5	PFHxDA	<0.33 ND	<0.44	<0.35 ND	<0.37
PFCAs	16517-11-6	PFODA	<0.28 ND	<0.21	<0.28 ND	<0.26
PFSAs	375-73-5	PFBS	<2.11	<1.81	<2.33	<2.08
PFSAs	2706-91-4	PFPeS	<0.13 ND	<0.10 ND	<0.14 ND	<0.12 ND
PFSAs	355-46-4	PFHxS	<0.15 ND	<0.11 ND	<0.15 ND	<0.14 ND
PFSAs	375-92-8	PFHpS	<0.32 ND	<0.25 ND	<0.30 ND	<0.29 ND
PFSAs	1763-23-1	PFOS	<0.44	<0.47	<0.58	<0.50
PFSAs	68259-12-1	PFNS	<0.13 ND	<0.11 ND	<0.15 ND	<0.13 ND
PFSAs	335-77-3	PFDS	<0.24 ND	<0.19 ND	<0.23 ND	<0.22 ND
PFSAs	79780-39-5	PFDoS	<0.32 ND	<0.27 ND	<0.31 ND	<0.30 ND
FOSAs	754-91-6	FOSA	<0.18 ND	<0.15 ND	<0.18 ND	<0.17 ND
FOSAs	31506-32-8	NMeFOSA	<0.27 ND	<0.21 ND	<0.27 ND	<0.25 ND
FOSAs	4151-50-2	NEtFOSA	<0.33 ND	<0.24 ND	<0.30 ND	<0.29 ND
FOSAAs	2355-31-9	NMeFOSAA	<0.24 ND	<0.17 ND	<0.22 ND	<0.21 ND

PFAS Class	CAS No.	Acronym	Run 1A Exhaust Emissions Concentration (ng/dscm @ 7% O_2)	Run 2A Exhaust Emissions Concentration (ng/dscm @ 7% O_2)	Run 3A Exhaust Emissions Concentration (ng/dscm @ 7% O_2)	Average Concentration (ng/dscm @ 7% O_2)
FOSAAs	2991-50-6	NEtFOSAA	<0.20 ND	<0.15 ND	<0.21 ND	<0.19 ND
FOSEs	24448-09-7	N-MeFOSE	<1.85	<1.80	<3.43 ND	<2.36
FOSEs	1691-99-2	N-EtFOSE	<0.26 ND	<0.21 ND	<0.26 ND	<0.24 ND
FTSs	757124-72-4	4:2 FTS	<0.13 ND	<0.10 ND	<0.13 ND	<0.12 ND
FTSs	27619-97-2	6:2 FTS	<1.71 ND	<1.53 ND	<2.93 ND	<2.06 ND
FTSs	39108-34-4	8:2 FTS	<0.26 ND	<0.17 ND	<0.23 ND	<0.22 ND
FTSs	120226-60-0	10:2 FTS	<0.31 ND	<0.24 ND	<0.36 ND	<0.31 ND
FRCs	13252-13-6	HFPO-DA	<161.24	<2645.40	133.83	<980.16
FRCs	919005-14-4	DONA	<0.57 ND	<0.49 ND	<0.55 ND	<0.54 ND
FRCs	756426-58-1	9Cl-PF3ONS	<0.15 ND	<0.11 ND	<0.15 ND	<0.14 ND
FRCs	763051-92-9	11Cl-PF3OUdS	<0.27 ND	<0.22 ND	<0.30 ND	<0.26 ND
ATAs	377-73-1	PFECA F	<0.41 ND	<0.32 ND	<0.39 ND	<0.37 ND
ATAs	863090-89-5	PFECA A	<0.34 ND	<0.22 ND	<0.29 ND	<0.28 ND
ATAs	151772-58-6	PFECA B	<1.21	<0.20 ND	<0.26 ND	<0.56
ATAs	113507-82-7	PES	<0.24 ND	<0.15 ND	<0.21 ND	<0.20 ND
ATAs	356-02-5	3:3 FTCA	<0.46 ND	<0.32 ND	<0.43 ND	<0.41 ND
ATAs	914637-49-3	5:3 FTCA	<0.66	<0.59	<0.68	<0.64
ATAs	812-70-4	7:3 FTCA	<0.49 ND	<0.36 ND	<0.49 ND	<0.45 ND
ATAs	70887-84-2	8:2 FTUCA	<0.37 ND	<0.24 ND	<0.32 ND	<0.31 ND
ATAs	53826-13-4	10:2 FTCA	<0.81 ND	<0.62 ND	<0.79 ND	<0.74 ND
ATAs	27854-31-5	8:2 FTCA	<0.71 ND	<0.51 ND	<0.65 ND	<0.62 ND
ATAs	53826-12-3	6:2FTCA	<0.75 ND	<0.56 ND	<0.73 ND	<0.68 ND
ATAs	133201-07-7	PFECHS	<0.34 ND	<0.24 ND	<0.32 ND	<0.30 ND
ATAs	70887-88-6	6:2 FTUCA	<0.19 ND	<0.13 ND	<0.19 ND	<0.17 ND

PFAS Class	CAS No.	Acronym	Run 1A Exhaust Emissions Concentration (ng/dscm @ 7% O_2)	Run 2A Exhaust Emissions Concentration (ng/dscm @ 7% O_2)	Run 3A Exhaust Emissions Concentration (ng/dscm @ 7% O_2)	Average Concentration (ng/dscm @ 7% O_2)
NA	NA	**Subtotal Non-detect**	**12.20**	**8.92**	**17.61**	**10.73**
NA	NA	**Subtotal Detected**	**194.66**	**2,712.39**	**147.78**	**1,020.45**
NA	NA	**Grand Total**	**206.86**	**2,721.31**	**165.39**	**1,031.18**

Notes:
Yellow shading = compound spiked during Runs 1A, 2A, 3A
Pink shading = detected analytical result in one or more exhaust emissions sample fractions (FH, BH, Condensate, BT XAD-2 Sorbent Trap)
ND = Analytical results non-detect in all four exhaust emissions sample fractions (FH, BH, Condensate, BT XAD-2 Sorbent Trap)
% = percent
Conc. = concentration
NA = not applicable
Concentrations calculated using MDL values for non-detects.



Concentration Range	Number of Data Points	Distribution (%)	Cumulative (%)
ND	102	69.4	69.4
0.0-1.0 ng/dscm	22	15.0	84.4
1.0-5.0 ng/dscm	14	9.5	93.9
5.0-10.0 ng/dscm	3	2.0	95.9
10.0-15.0 ng/dscm	2	1.4	97.3
15.0-25.0 ng/dscm	1	0.7	98.0
>25.0 ng/dscm	3	2.0	100.0
Total	147	100.0	

Figure 8-2. Summary of Other Test Method (OTM) 45 Exhaust Emissions Emission Concentrations

8.4 EPA OTM-50 Sampling and Analysis

OTM-50 samples were collected under all three test conditions. During Runs 1A, 2A, and 3A, OTM-50 sample results were available to identify and quantify PIDs produced when significant amounts of PFAS and precursor compounds were being fed to the incinerator, including the nine spiked PFAS and AFFF.

Runs 1B, 2B, and 3B were conducted with normal waste feed conditions, with no PFAS spiking or AFFF waste feed. These B-labeled runs afforded baseline C_2F_6 emission rates to be subtracted from measured C_2F_6 exhaust emissions during Runs 1C, 2C, and 3C when C_2F_6 was being spiked into the unit. This baseline adjustment is necessary to determine the inherent emission rates for C_2F_6 from the spiked C_2F_6.

Runs 1C, 2C, and 3C were conducted with normal waste feed conditions, plus C_2F_6 spiking to provide data to determine DRE for C_2F_6.

8.4.1 OTM-50 Sampling Method Description

EPA OTM-50 uses an extractive non-isokinetic sampling train to collect volatile PFAS exhaust emissions. A gas sample is withdrawn from the emission source, passed through a series of four chilled midget impingers to condense moisture and remove acid gases, and then collected in two evacuated ceramic-coated (Silonite™) canisters, each equipped with a critical orifice. After sample collection, any condensate collected in the impingers is discarded. One canister was analyzed by Eurofins and the second canister was analyzed by EPA ORD. The specific components of the sampling train included:

- Inert coated stainless steel or glass-lined heated probe
- Hot box and particulate matter filter
- Ice water bath with three impingers with 15 mL DI water and one empty impinger
- Critical orifices (two)
- Ceramic-coated (Silonite™) canisters (two)
- Bypass vacuum pump

A schematic of the OTM-50 sampling train with two canisters is shown in Figure 8-3.

The two canister samples for each run are normally collected simultaneously during approximately a 1-hour sampling period. The initial plan was to use flow controllers for the Eurofins canisters and critical orifices for the EPA canisters. However, during Run 1B, the Eurofins canister did not collect a significant amount of sample while the EPA canister received sufficient exhaust emissions for the sampling run. Therefore, this run was labeled Run 1B-1 and the EPA canister was retained but the Eurofins canister was voided. A second Eurofins canister was then fitted with a critical orifice to collect the canister sample for analysis by Eurofins. The run was repeated and labeled Run 1B-2. Because the two canisters were not collected concurrently, they are not considered duplicates. Critical orifices were used to collect all other OTM-50 samples.

8.4.2 OTM-50 Sample Analysis and Results

At the end of the field test program, the Eurofins canisters from all runs were transferred to the Eurofins courier, along with Request for Analysis/Chain-of-Custody forms. The Eurofins courier delivered OTM-50 under strict chain of custody to the Eurofins laboratory in Knoxville, Tennessee. The Eurofins Knoxville laboratory was responsible for analyses of these OTM-50 sample canisters and reporting final analytical results. Samples were analyzed by gas chromatography/mass spectroscopy by the OTM-50 canister method. All analytical results and laboratory QC procedures are documented in the Eurofins Analytical Report in Appendix J-3. The Eurofins sample canisters were diluted at the dilution factors shown in Appendix J-3 prior to analysis.

The second canister from each run was retained by EPA personnel and shipped by FedEx to the EPA laboratory in Research Triangle Park, North Carolina, for analysis.

A comparison of emission concentrations of the Eurofins and EPA analytical data sets for Runs 1A, 1B, and 1C are presented in Table 8-2. A comparison of emission concentrations based on the Eurofins and EPA analytical data sets for Runs 2A, 2B, and 2C is presented in Table 8-3. A comparison of emission concentrations based on the Eurofins and EPA analytical data sets for Runs 3A, 3B, and 3C is presented in Table 8-4.

Eurofins results for all analytes were below the MDL for both the pre-test and post-test blank samples. EPA results for all but two analytes were below the MDL for both the pre-test and post-test blank samples. Chlorodifluoromethane and trichlorofluoromethane were reported at concentrations slightly above the detection limit. The pre-test and post-test blank data are presented in Section 9.

Detailed OTM-50 sample analytical results from the Eurofins laboratory are summarized in Appendix A-2, Tables A-2-5. The results of the Eurofins analyses are non-detect for all thirty target analytes for all nine runs. Eurofins' OTM-50 unknowns analysis did not identify any other fluorinated compounds. These tables include MDLs reported on both an undiluted and diluted sample basis. Table A-2-6 presents the analytical results, MDLs, data qualifier flags applied by the laboratory, data qualifier flags applied by the data validator, and the exhaust emissions rates.

Detailed OTM-50 sample analytical results from the EPA laboratory are summarized in Appendix A-2 and Table A-2-7. Table A-2-8 presents the analytical results, the MDLs, and stack emission rates. No data qualifier flags were applied by the EPA laboratory and the data were not independently validated. EPA analyses produced MDLs that were slightly lower than the MDLs reported by Eurofins for all but three target compounds. As shown in Tables 8-2 through 8-4, EPA analyses detected six compounds in one or more runs above the detection limit but below the reporting limit. Two of the compounds, chlorodifluoromethane (R-22) and trichlorofluoromethane (R-11), are common refrigerants in the atmosphere, were in the pre-test and post-test blanks at similar concentrations as the actual samples and were likely in the gas used to pressurize the canisters. The EPA's OTM-50 unknowns analysis did not identify any other fluorinated compounds.

CF_4, which has high thermal stability, was not detected in two of the EPA samples but was detected in the other seven EPA samples at low concentrations ranging from 1.64 to 6.31 µg/m^3. The highest CF_4 concentrations (3.35 to 6.31 µg/m^3) were measured during Runs 1A, 2A and 3A when PFAS were being spiked and AFFF was being fed. The second highest concentrations (1.64 to 3.83 µg/m^3) were measured during Runs 1C, 2C and 3C when C_2F_6 was being spiked. The lowest concentrations (<0.93 to 3.70 µg/m^3)

were measured during Runs 1B, 2B and 3B when no AFFF was being fed, no PFAS was being spiked, and no C_2F_6 was being spiked.

8.4.3 OTM-50 QA/QC Samples

OTM-50 field sampling and analysis data quality was assessed by collecting both pre-test and post-test blank samples. The pre-test blank is collected with a laboratory cleaned set of glassware, filter housing, and probe. The post-test blank is collected with a set of glassware, filter housing and probe that has been used during the project and field cleaned. These results are presented in Section 9. Concentrations of all analytes were below the MDL except for chlorodifluoromethane and trichlorofluoromethane in the EPA pre-test sample.

Eurofins also reported results for laboratory method blanks which are presented in Section 9. The concentrations of all analytes in the method blank were below the MDL.

8.4.4 Results Summary

Eurofins analytical results of the 30 target analytes from all nine runs were non-detect. Values for the non-detect results are reported using the MDL. MDL values reported by EPA are, on average, about four times lower than those reported by Eurofins. EPA reported hits near the detection limits and below the reporting limits for several compounds. Several of the compounds are common refrigerants that may have been in the compressed air cylinders used to pressurize the canisters, as they were also in blanks. The presence of CF_4 near the detection limit provides some evidence of the production of a PID from the high concentrations of PFAS added to the kiln. The analysis of unknowns did not detect any fluorinated compounds.

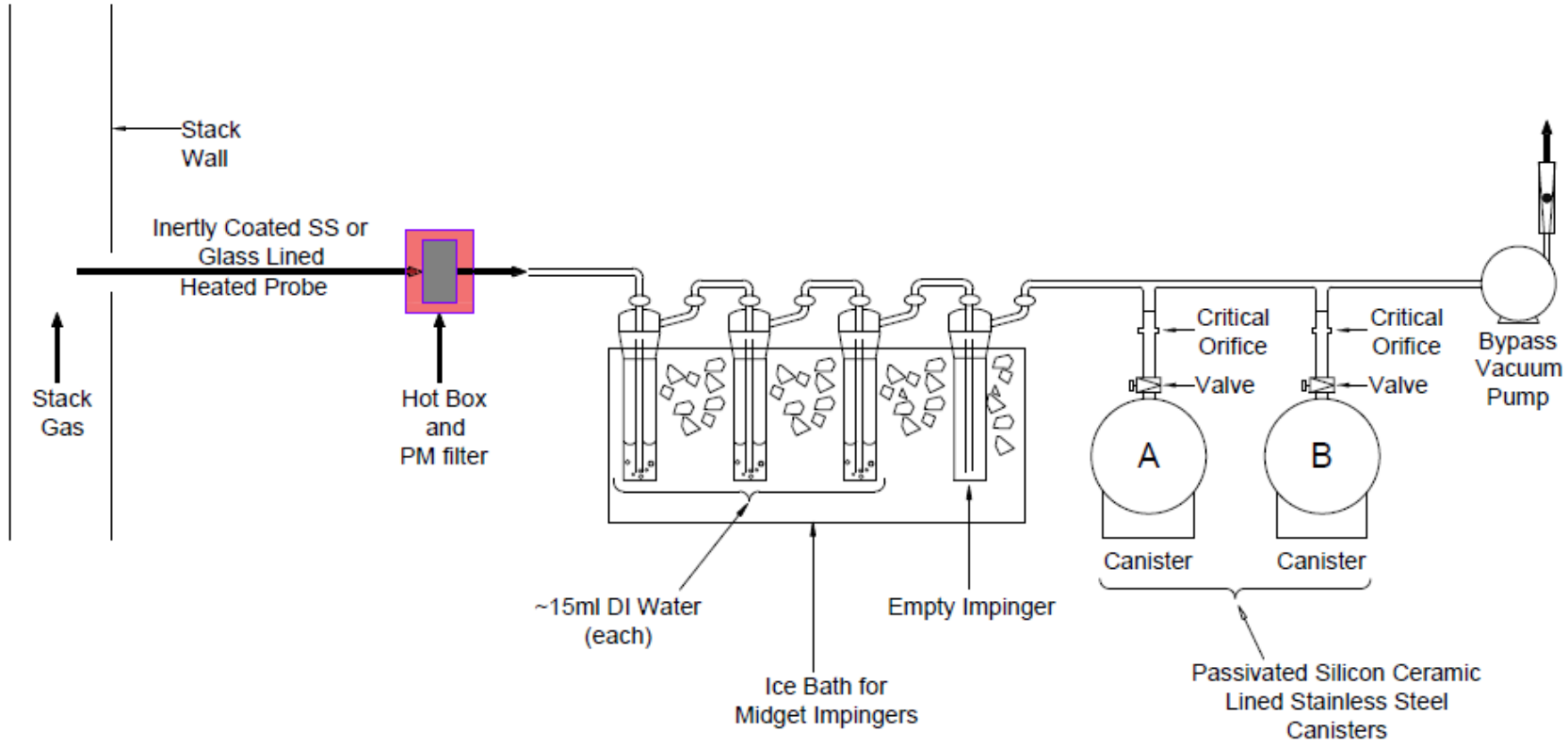


Figure 8-3. Other Test Method (OTM) 50 Sampling Train with Multiple Canisters. PM = particulate matter

Table 8-2. Other Test Method (OTM) 50 Emissions — Runs 1A, 1B, and 1C

CAS No.	Analyte	Eurofins Run 1A Result (µg/m³)	EPA Run 1A Result (µg/m³)	Eurofins Run 1B-2 Result (µg/m³)	EPA Run 1B-1 Result (µg/m³)	Eurofins Run 1C Result (µg/m³)	EPA Run 1C Result (µg/m³)
116-14-3	Tetrafluoroethene	<0.456	<0.220	<0.475	<0.220	<0.459	<0.220
75-46-7	Trifluoromethane	<1.29	<0.290	<1.35	<0.290	<1.3	<0.290
76-19-7	Octafluoropropane	<1.11	<0.350	<1.16	<0.350	<1.12	<0.350
75-10-5	Difluoromethane (HFC-32)	<1.34	<0.050	<1.4	<0.050	<1.35	<0.050
593-53-3	Fluoromethane	<2.18	<1.330	<2.27	<1.330	<2.19	<1.330
354-33-6	1,1,1,2,2-Pentafluoroethane (HFC-125)	<0.99	0.79	<1.03	<0.200	<0.997	<0.200
116-15-4	Hexafluoropropene	<0.517	<0.150	<0.539	<0.150	<0.521	<0.150
355-25-9	Decafluorobutane	<0.879	<0.650	<0.917	<0.650	<0.885	<0.650
678-26-2	Dodecafluoropentane	<1.99	<0.370	<2.07	<0.370	<2	<0.370
355-42-0	Tetradecafluorohexane	<2.08	<1.810	<2.17	<1.810	<2.09	<1.810
375-61-1	1H-Perfluoropentane	<1.46	<0.810	<1.53	<0.810	<1.47	<0.810
335-57-9	Hexadecafluoroheptane	<0.955	<0.320	<0.997	<0.320	<0.962	<0.320
3330-15-2	Heptafluoropropyl 1,2,2,2-tetrafluoroethyl ether	<1.02	<0.320	<1.07	<0.320	<1.03	<0.320
355-37-3	1H-Perfluorohexane	<0.827	<0.310	<0.863	<0.310	<0.833	<0.310
375-83-7	1H-Perfluoroheptane	<1.55	<0.460	<1.62	<0.460	<1.56	<0.460
3330-14-1	2H-Perfluoro-5-methyl-3,6-dioxanonane (E2)	<1.22	<1.980	<1.28	<1.980	<1.23	<1.980
335-65-9	1H-Perfluorooctane	<1.09	<1.410	<1.13	<1.410	<1.09	<1.410
307-34-6	Octadecafluorooctane	<1.24	<1.430	<1.29	<1.430	<1.25	<1.430
375-17-7	1H-Nonafluorobutane	<1	<0.320	<1.05	<0.320	<1.01	<0.320
2252-84-8	1H-Heptafluoropropane	<0.481	<0.320	<0.502	<0.320	<0.485	<0.320
811-97-2	1,1,1,2-Tetrafluoroethane	<1.13	<0.340	<1.18	<0.340	<1.14	<0.340
420-46-2	1,1,1-Trifluoroethane	<2.28	<0.340	<2.37	<0.340	<2.29	<0.340
75-45-6	Chlorodifluoromethane	<1.49	0.48	<1.55	0.26	<1.5	<0.240
75-72-9	Chlorotrifluoromethane	<1.07	<0.370	<1.11	<0.370	<1.07	<0.370
115-25-3	Octafluorocyclobutane (FC-C318)	<0.493	<0.320	<0.514	<0.320	<0.496	<0.320
559-40-0	Octafluorocyclopentene (FC-C1418)	<1.15	<0.130	<1.2	<0.130	<1.16	<0.130
75-69-4	Trichlorofluoromethane	<1.56	0.66	<1.62	0.51	<1.57	<0.450
75-02-5	Vinyl Fluoride[(a)]	<0.737	---	<0.769	---	<0.742	---
79-38-9	Chlorotrifluoroethene[(a)]	<0.444	---	<0.464	---	<0.447	---
76-13-1	1,1,2-Trichloro-1,2,2-trifluoroethane[(a)]	<0.577	---	<0.602	---	<0.581	---

CAS No.	Analyte	Eurofins Run 1A Result (µg/m³)	EPA Run 1A Result (µg/m³)	Eurofins Run 1B-2 Result (µg/m³)	EPA Run 1B-1 Result (µg/m³)	Eurofins Run 1C Result (µg/m³)	EPA Run 1C Result (µg/m³)
76-16-4	Hexafluoroethane (C_2F_6)	<0.527	<0.140	<0.549	<0.140	<0.53	<0.140
428-59-1	Hexafluoropropylene oxide	<10.4	<6.630	<10.9	<6.630	<10.5	<6.630
75-73-0	Tetrafluoromethane	<5.31	3.35	<5.54	<0.930	<5.34	1.88
NA	**Subtotal: Non-detect**	**<50.84**	**<22.10**	**<53.08**	**<22.30**	**<51.20**	**<22.990**
NA	**Subtotal: Detected**	**0.00**	**1.93**	**0.00**	**0.78**	**0.00**	**0.00**
NA	**Grand Total**	**<50.84**	**<24.029**	**<53.08**	**<23.077**	**<51.20**	**<22.99**

Notes:
(a) Compound is not a target analyte listed in OTM-50. It was reported by Eurofins but not reported by EPA.
Pink shading = detected analytical result
--- = not analyzed
NA = not applicable

Table 8-3. Other Test Method (OTM) 50 Emissions — Runs 2A, 2B, and 2C

CAS No.	Analyte	Eurofins Run 2A Result (µg/m³)	EPA Run 2A Result (µg/m³)	Eurofins Run 2B Result (µg/m³)	EPA Run 2B Result (µg/m³)	Eurofins Run 2C Result (µg/m³)	EPA Run 2C Result (µg/m³)
116-14-3	Tetrafluoroethene	<0.481	<0.220	<0.469	<0.220	<0.453	<0.220
75-46-7	Trifluoromethane	<1.37	<0.290	<1.33	<0.290	<1.28	<0.290
76-19-7	Octafluoropropane	<1.17	<0.350	<1.14	<0.350	<1.1	<0.350
75-10-5	Difluoromethane (HFC-32)	<1.42	<0.050	<1.39	<0.050	<1.34	<0.050
593-53-3	Fluoromethane	<2.3	<1.330	<2.24	<1.330	<2.16	<1.330
354-33-6	1,1,1,2,2-Pentafluoroethane (HFC-125)	<1.05	<0.200	<1.02	<0.200	<0.983	<0.200
116-15-4	Hexafluoropropene	<0.546	<0.150	<0.533	<0.150	<0.514	<0.150
355-25-9	Decafluorobutane	<0.929	<0.650	<0.905	<0.650	<0.873	<0.650
678-26-2	Dodecafluoropentane	<2.1	<0.370	<2.05	<0.370	<1.97	<0.370
355-42-0	Tetradecafluorohexane	<2.2	<1.810	<2.14	<1.810	<2.07	<1.810
375-61-1	1H-Perfluoropentane	<1.55	<0.810	<1.51	<0.810	<1.45	<0.810
335-57-9	Hexadecafluoroheptane	<1.01	<0.320	<0.984	<0.320	<0.949	<0.320
3330-15-2	Heptafluoropropyl 1,2,2,2-tetrafluoroethyl ether	<1.08	<0.320	<1.05	<0.320	<1.01	<0.320
355-37-3	1H-Perfluorohexane	<0.874	<0.310	<0.852	<0.310	<0.822	<0.310
375-83-7	1H-Perfluoroheptane	<1.64	<0.460	<1.6	<0.460	<1.54	<0.460
3330-14-1	2H-Perfluoro-5-methyl-3,6-dioxanonane (E2)	<1.29	<1.980	<1.26	<1.980	<1.22	<1.980
335-65-9	1H-Perfluorooctane	<1.15	<1.410	<1.12	<1.410	<1.08	<1.410
307-34-6	Octadecafluorooctane	<1.31	<1.430	<1.28	<1.430	<1.23	<1.430

CAS No.	Analyte	Eurofins Run 2A Result (µg/m³)	EPA Run 2A Result (µg/m³)	Eurofins Run 2B Result (µg/m³)	EPA Run 2B Result (µg/m³)	Eurofins Run 2C Result (µg/m³)	EPA Run 2C Result (µg/m³)
375-17-7	1H-Nonafluorobutane	<1.06	<0.320	<1.03	1.644	<0.996	<0.320
2252-84-8	1H-Heptafluoropropane	<0.509	<0.320	<0.496	0.489	<0.478	<0.320
811-97-2	1,1,1,2-Tetrafluoroethane	<1.19	<0.340	<1.16	<0.340	<1.12	<0.340
420-46-2	1,1,1-Trifluoroethane	<2.4	<0.340	<2.34	<0.340	<2.26	<0.340
75-45-6	Chlorodifluoromethane	<1.57	0.376	<1.53	0.477	<1.48	<0.240
75-72-9	Chlorotrifluoromethane	<1.13	<0.370	<1.1	<0.370	<1.06	<0.370
115-25-3	Octafluorocyclobutane (FC-C318)	<0.52	<0.320	<0.507	<0.320	<0.489	<0.320
559-40-0	Octafluorocyclopentene (FC-C1418)	<1.21	<0.130	<1.18	<0.130	<1.14	<0.130
75-69-4	Trichlorofluoromethane	<1.64	<0.450	<1.6	0.711	<1.55	<0.450
75-02-5	Vinyl Fluoride[(a)]	<0.778	---	<0.759	---	<0.732	---
79-38-9	Chlorotrifluoroethene[(a)]	<0.47	---	<0.458	---	<0.442	---
76-13-1	1,1,2-Trichloro-1,2,2-trifluoroethane[(a)]	<0.609	---	<0.594	---	<0.573	---
76-16-4	Hexafluoroethane (C_2F_6)	<0.556	<0.140	<0.542	<0.140	<0.523	<0.140
428-59-1	Hexafluoropropylene oxide	<11	<6.630	<10.7	<6.630	<10.4	<6.630
75-73-0	Tetrafluoromethane	<5.61	6.31	<5.47	<0.930	<5.27	1.64
NA	**Subtotal: Non-detect**	**<53.72**	**<22.75**	**<52.34**	**<21.66**	**<50.56**	**<22.99**
NA	**Subtotal: Detected**	**0.00**	**0.38**	**0.00**	**3.32**	**0.00**	**0.00**
NA	**Grand Total**	**<53.72**	**<23.126**	**<52.34**	**<24.981**	**<50.56**	**<22.990**

Notes:
(a) Compound is not a target analyte listed in OTM-50. It was reported by Eurofins but not reported by EPA.
Pink shading = detected analytical result
--- = not analyzed
NA = not applicable

Table 8-4. Other Test Method (OTM) 50 Emissions — Runs 3A, 3B, and 3C

CAS No.	Analyte	Eurofins Run 3A Result (µg/m³)	EPA Run 3A Result (µg/m³)	Eurofins Run 3B Result (µg/m³)	EPA Run 3B Result (µg/m³)	Eurofins Run 3C Result (µg/m³)	EPA Run 3C Result (µg/m³)
116-14-3	Tetrafluoroethene	<0.518	<0.220	<0.466	<0.220	<0.459	<0.220
75-46-7	Trifluoromethane	<1.47	<0.290	<1.32	<0.290	<1.3	<0.290
76-19-7	Octafluoropropane	<1.26	<0.350	<1.14	<0.350	<1.12	<0.350
75-10-5	Difluoromethane (HFC-32)	<1.53	<0.050	<1.38	<0.050	<1.35	<0.050
593-53-3	Fluoromethane	<2.48	<1.330	<2.23	<1.330	<2.19	<1.330
354-33-6	1,1,1,2,2-Pentafluoroethane (HFC-125)	<1.12	<0.200	<1.01	<0.200	<0.997	<0.200
116-15-4	Hexafluoropropene	<0.588	<0.150	<0.529	<0.150	<0.521	<0.150

CAS No.	Analyte	Eurofins Run 3A Result (µg/m³)	EPA Run 3A Result (µg/m³)	Eurofins Run 3B Result (µg/m³)	EPA Run 3B Result (µg/m³)	Eurofins Run 3C Result (µg/m³)	EPA Run 3C Result (µg/m³)
355-25-9	Decafluorobutane	<0.999	<0.650	<0.9	<0.650	<0.885	<0.650
678-26-2	Dodecafluoropentane	<2.26	<0.370	<2.03	<0.370	<2	<0.370
355-42-0	Tetradecafluorohexane	<2.36	<1.810	<2.13	<1.810	<2.09	<1.810
375-61-1	1H-Perfluoropentane	<1.66	<0.810	<1.5	<0.810	<1.47	<0.810
335-57-9	Hexadecafluoroheptane	<1.09	<0.320	<0.978	<0.320	<0.962	<0.320
3330-15-2	Heptafluoropropyl 1,2,2,2-tetrafluoroethyl ether	<1.16	<0.320	<1.04	<0.320	<1.03	<0.320
355-37-3	1H-Perfluorohexane	<0.94	<0.310	<0.847	<0.310	<0.833	<0.310
375-83-7	1H-Perfluoroheptane	<1.76	<0.460	<1.58	<0.460	<1.56	<0.460
3330-14-1	2H-Perfluoro-5-methyl-3,6-dioxanonane (E2)	<1.39	<1.980	<1.25	<1.980	<1.23	<1.980
335-65-9	1H-Perfluorooctane	<1.23	<1.410	<1.11	<1.410	<1.09	<1.410
307-34-6	Octadecafluorooctane	<1.41	<1.430	<1.27	<1.430	<1.25	<1.430
375-17-7	1H-Nonafluorobutane	<1.14	0.917	<1.03	<0.320	<1.01	<0.320
2252-84-8	1H-Heptafluoropropane	<0.547	<0.320	<0.493	0.586	<0.485	<0.320
811-97-2	1,1,1,2-Tetrafluoroethane	<1.28	<0.340	<1.16	<0.340	<1.14	<0.340
420-46-2	1,1,1-Trifluoroethane	<2.59	<0.340	<2.33	<0.340	<2.29	<0.340
75-45-6	Chlorodifluoromethane	<1.69	0.469	<1.52	0.348	<1.5	0.248
75-72-9	Chlorotrifluoromethane	<1.21	<0.370	<1.09	<0.370	<1.07	<0.370
115-25-3	Octafluorocyclobutane (FC-C318)	<0.56	<0.320	<0.504	<0.320	<0.496	<0.320
559-40-0	Octafluorocyclopentene (FC-C1418)	<1.31	<0.130	<1.18	<0.130	<1.16	<0.130
75-69-4	Trichlorofluoromethane	<1.77	0.607	<1.59	0.47	<1.57	0.470
75-02-5	Vinyl Fluoride[(a)]	<0.837	---	<0.754	---	<0.742	---
79-38-9	Chlorotrifluoroethene[(a)]	<0.505	---	<0.455	---	<0.447	---
76-13-1	1,1,2-Trichloro-1,2,2-trifluoroethane[(a)]	<0.655	---	<0.59	---	<0.581	---
76-16-4	Hexafluoroethane (C_2F_6)	<0.598	<0.140	<0.539	<0.140	<0.53	<0.140
428-59-1	Hexafluoropropylene oxide	<11.8	<6.630	<10.7	<6.630	<10.5	<6.630
75-73-0	Tetrafluoromethane	<6.03	5.74	<5.43	3.70	<5.34	3.83
NA	**Subtotal: Non-detect**	**<57.75**	**<21.98**	**<52.08**	**<21.98**	**<51.20**	**<22.30**
NA	**Subtotal: Detected**	**0.00**	**1.99**	**0.00**	**1.40**	**0.00**	**0.72**
NA	**Grand Total**	**<57.75**	**<23.973**	**<52.08**	**<23.379**	**<51.20**	**<23.018**

Notes:
(a) Compound is not a target analyte listed in OTM-50. It was reported by Eurofins but not reported by EPA.
Pink shading = detected analytical result
--- = not analyzed
NA = not applicable

8.5 Method 0010 Sampling and Analysis

A Method 0010 (EPA 1986) sampling train was used to collect semi-volatile non-polar compounds which could potentially have been produced as PIDs.

8.5.1 Method 0010 Sampling Method Description

Method 0010 uses an extractive isokinetic sampling train to collect semi-volatile non-polar compounds in the exhaust emissions. Semi-volatile compounds are defined as those with boiling points greater than 100°C. Gaseous and particulate compounds are withdrawn from the emission source at an isokinetic sampling rate and collected in a multicomponent sampling train.

The principal components of the train included a high-efficiency, quartz-fiber particulate filter to collect organic-laden particulate, an XAD-2 sorbent trap, and a set of chilled impingers charged with 100 mL of DI water. Specific components of the exhaust emissions sampling train include:

- Glass nozzle
- Heated, glass-lined probe
- Temperature sensor
- Pitot tube
- Manometer
- Heated quartz filter in filter holder with silicon O-ring seal
- Ice water cooled condenser
- XAD-2 sorbent trap, with cooling jacket
- Short-stem knockout impinger in ice water bath
- Three impingers with 100 mL DI water in an ice water bath
- Impinger containing 200 g of silica gel desiccant
- Ground glass connections for impingers
- O-rings to seal connections for all ball joints
- Vacuum sampling pump
- Calibrated dry gas meter to measure the collected sample volume

A schematic of the Method 0010 sampling train is shown on Figure 8-4.

After a sampling run was completed, the probe was removed from the sample train and the open outlet was capped. The filter inlet, the filter-holder outlet, and inlet to the XAD-2 sorbent trap were capped

with aluminum foil. The XAD-2 sorbent trap was separated from the condensate knockout trap and the openings were capped with aluminum foil. The impingers and XAD-2 sorbent trap were weighed to determine moisture. The particulate filter was removed from the filter holder, folded or rolled with the particulate on the inside, and placed in a glass petri dish. Any residual particulate matter on the glass nozzle, probe liner, or front half of the filter holder was recovered by brushing and rinsing these sample train components three times with acetone, followed by brushing and rinsing three times with methylene chloride. The rinse solutions were combined into an amber glass bottle. The back-half of the filter holder, coil condenser and connecting glassware were rinsed three times with acetone and three times with methylene chloride. The rinses were combined and placed in an amber glass bottle. The condensate and impinger water were combined in a 1-liter amber glass bottle. All impingers and connecting glassware prior to the silica gel impinger are rinsed three times with ultra filtered DI water and added to the same bottle. The impingers and glassware are rinsed three times with acetone and three times with methylene chloride and the rinses were placed in a separate amber glass bottle. The silica gel impinger was reweighed to determine moisture. All recovered samples were immediately placed in coolers and kept cold on ice.

The ATG SOP for the SW-846 Method 0010 exhaust emissions sampling includes detailed protocols for the following procedures:

- Stack sampling equipment and glassware and ancillary tools cleaning procedures (in laboratory prior to field deployment)
- Prevention of cross-contamination from equipment that previously may have been used to sample other sources (i.e., isolation of sampling equipment, field cleaning of sampling equipment, use of new coolers and consumables, proper selection of consumables, etc.)
- Avoiding, to the extent possible, materials of construction that may contain fluoropolymers (i.e., Viton O-rings, Teflon filter frits, or other fluoropolymers items)
- Packaging procedures to ensure the cleanliness of equipment was maintained during shipping and transport to the field.

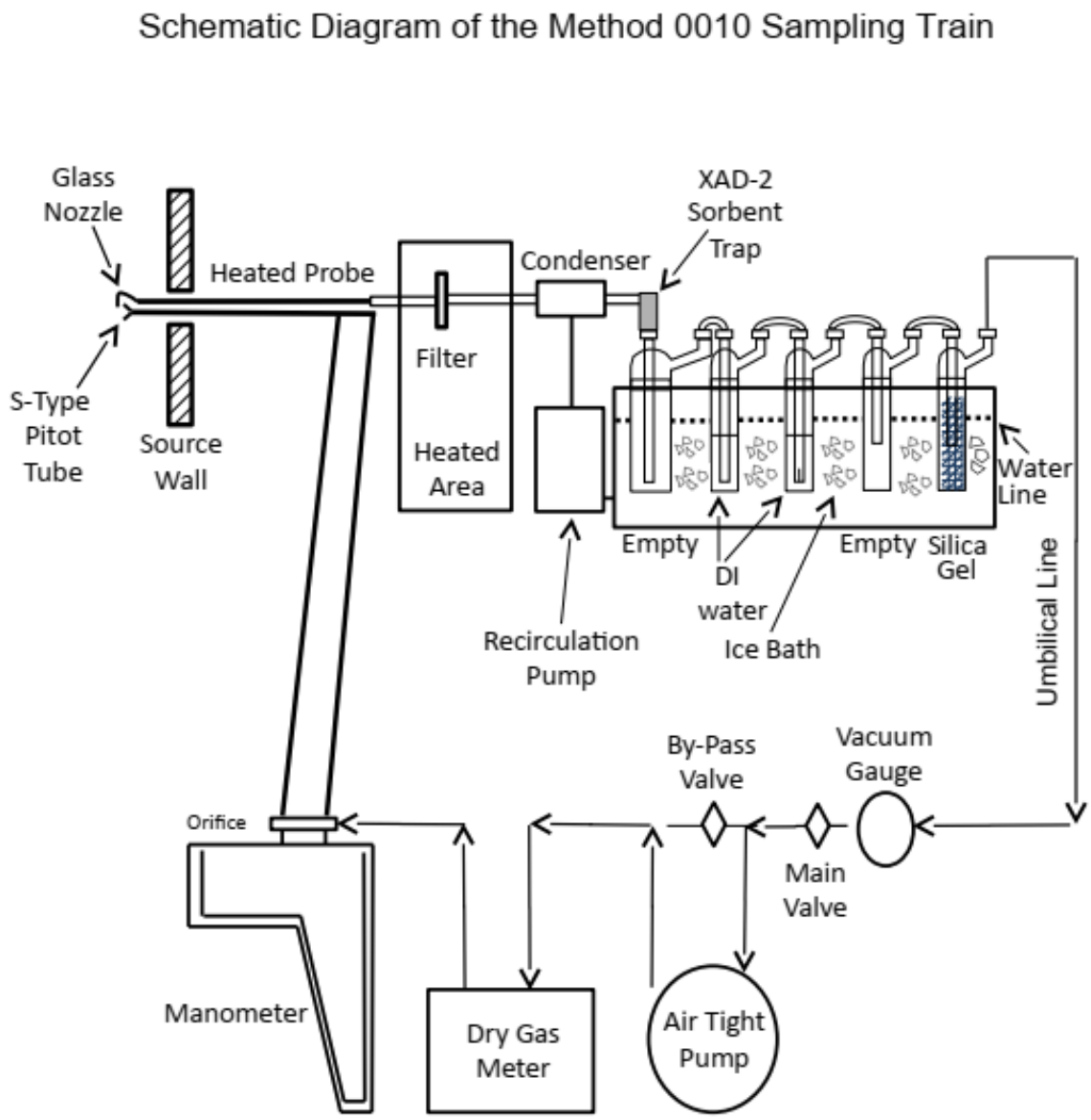


Figure 8-4. SW-846 Method 0010 Sampling Train

8.5.2 Method 0010 Sample Analysis and Results

At the end of the test program, the Method 0010 samples were packaged and preserved in coolers. Request for Analysis/Chain-of-Custody forms were placed in the cooler and the cooler was transferred to the Eurofins courier. The Eurofins courier delivered Method 0010 samples under strict chain of custody to the Eurofins laboratory in Knoxville, Tennessee. The Eurofins Knoxville laboratory was responsible for the analyses of all Method 0010 samples and reporting final analytical results. Samples were analyzed by gas chromatography/mass spectroscopy by EPA Method 8270C.

All analytical results and laboratory QC procedures and results are documented in the Eurofins Analytical Report in Appendix J-4. Reduced data for the semi-volatile compounds are included in Appendix A-3, Table A-3-1 through Table A-3-4. The 8270 analysis only measured N-Nitrosodimethylamine above the reporting limit. Of the handful of compounds detected above the detection limit, phenol was the only compound observed in all three runs. No fluorinated unknown compounds were observed.

8.5.3 Method 0010 QA/QC Samples

Method field sampling and analysis data quality was assessed using a variety of QA/QC procedures, including collection and analysis of the following types of field QA/QC samples:

- Proof blank train
- Reagent blanks (filter, XAD-2 sorbent trap, methylene chloride, acetone, DI water)

The analytical results for the proof blank train are discussed in Section 9.2.2.2.3.

The following types of laboratory QA samples were also analyzed:

- Laboratory media checks
- Laboratory method blanks
- LCSs (not summarized)
- LCSDs (not summarized)

Analytical results for all analytes in laboratory media checks and method blanks were non-detect. All Method 0010 QA data are summarized in the Eurofins analytical reports in Appendix J-4.

8.5.4 Results Summary

No fluorinated PIDs were identified by the Method 0010 analysis.

9 Data Quality and Usability

9.1 Overview

This section of the report is an evaluation at the conclusion of data collection activities, which uses the results from data validation with context of the overall project decisions and objectives. Using both quantitative and qualitative methods, the assessment determines whether project execution and the resulting data have met project QA objectives. Both sampling and analytical activities are considered to assess whether the collected data are of the right type, quality, and quantity to meet project objectives.

Data quality indicators (DQIs) (representativeness, comparability, precision, accuracy, completeness) are important components in assessing data usability. These DQIs are evaluated in the subsequent sections. The results of the evaluation demonstrate that the data presented in this report are of high quality overall. A small subset of data was qualified to indicate uncertainty due to analytical quality issues, as described below. The Data Validation Reports in Appendix L present explanations for all qualified data in greater detail. QA/QC measures for each sampling and analytical method are discussed separately in the following sections, with dedicated subsections addressing process and exhaust emission samples for each DQI.

9.2 Sampling Data Quality Review

This section presents an evaluation of sampling data quality as part of the overall data usability assessment. This evaluation incorporates results from field QA/QC procedures, considering both process and stack sampling activities.

9.2.1 Bias

Bias is a measure of confidence in a measurement. The smaller the difference between the measurement of a parameter and its true or expected value, the more accurate the measurement and the less bias present. The more precise or reproducible the result, the more reliable the result.

9.2.1.1 Process Samples

Accuracy of field sampling for process samples was evaluated through the analysis of one equipment blank associated with spray dryer solids and disposable funnels for liquid process samples for Runs 1A, 2A, and 3A.

As shown in Table 9-1, five PFAS were detected in the equipment blank made by rinsing the sampling equipment (sieve) with 134.2 mL of DI water: HFPO-DA (1.9 ng/L), PFOS (2.16 ng/L), PFHxA (0.931 ng/L), PFOA (0.786 ng/L), and PFBA (1.77 ng/L). Among these, only PFOS was detected in the spray dryer solids sample, at a concentration of 0.35 ng/g. Assuming that the entire PFOS mass from the equipment blank transferred to the 4,594 g spray dryer solids sample, the contribution would equate to a negligible fraction relative to the measured value. None of the other PFAS detected in the blank were observed in the spray dryer solids sample. As such, the data indicated no significant impact from equipment blank contamination, and the field sample results for process samples are considered accurate and reliable. While not shown here in detail, similar results were observed for equipment blanks from Runs 2A and 3A, supporting consistent and reliable sampling performance across all events.

For liquid process samples, disposable funnels were used, with a new funnel dedicated to each individual sample to prevent cross-contamination. One funnel was selected and rinsed as a representative equipment blank to assess potential background PFAS contributions. Two PFAS—PFNA (3.01 ng/L) and PFUnA (0.814 ng/L)—were detected in the funnel rinse blank (Table 9-2). PFNA was subsequently detected in waste blend liquid, aqueous liquid, tote (educt station #2), and corrosive waste, while PFUnA was detected in tote (educt station #2) and corrosive waste.

Table 9-1. EPA 1633 Sampling Equipment (Sieve) Blank QA/QC Data Summary

PFAS Class	CAS No.	Acronym	Equipment Blank (ng/L)
PFCAs	375-22-4	PFBA	1.770
PFCAs	2706-90-3	PFPeA	<0.466
PFCAs	307-24-4	PFHxA	0.931
PFCAs	375-85-9	PFHpA	<0.466
PFCAs	335-67-1	PFOA	0.786
PFCAs	375-95-1	PFNA	<0.466
PFCAs	335-76-2	PFDA	<0.466
PFCAs	2058-94-8	PFUnA	<0.466
PFCAs	307-55-1	PFDoA	<0.512
PFCAs	72629-94-8	PFTriA	<0.540
PFCAs	376-06-7	PFTeA	<0.754
PFSAs	375-73-5	PFBS	<0.466
PFSAs	2706-91-4	PFPeS	<0.466
PFSAs	355-46-4	PFHxS	<0.466
PFSAs	375-92-8	PFHpS	<0.466
PFSAs	1763-23-1	PFOS	2.160
PFSAs	68259-12-1	PFNS	<0.466
PFSAs	335-77-3	PFDS	<0.466
PFSAs	79780-39-5	PFDoS	<0.494
FOSAs	754-91-6	FOSA	<0.466
FOSAs	31506-32-8	NMeFOSA	<0.466
FOSAs	4151-50-2	NEtFOSA	<0.466
FOSAAs	2355-31-9	NMeFOSAA	<0.466
FOSAAs	2991-50-6	NEtFOSAA	<0.466
FOSEs	24448-09-7	N-MeFOSE	<2.33
FOSEs	1691-99-2	N-EtFOSE	<2.33
FTSs	757124-72-4	4:2 FTS	<0.931
FTSs	27619-97-2	6:2 FTS	<0.931
FTSs	39108-34-4	8:2 FTS	<0.931
FRCs	13252-13-6	HFPO-DA	1.900
FRCs	919005-14-4	DONA	<0.466
FRCs	756426-58-1	9Cl-PF3ONS	<0.540
FRCs	763051-92-9	11Cl-PF3OUdS	<0.466
ATAs	377-73-1	PFECA F	<0.466
ATAs	863090-89-5	PFECA A	<0.466

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PFAS Class	CAS No.	Acronym	Equipment Blank (ng/L)
ATAs	151772-58-6	PFECA B	<0.671
ATAs	113507-82-7	PES	<0.466
ATAs	356-02-5	3:3 FTCA	<0.931
ATAs	914637-49-3	5:3 FTCA	<2.33
ATAs	812-70-4	7:3 FTCA	<2.33

Notes:
Yellow shading = compound spiked during Runs 1A, 2A, 3A
Pink shading = detected analytical result
--- = not analyzed

Table 9-2. EPA 1633 Sampling Equipment (Funnel) Blank QA/QC Data Summary

PFAS Class	CAS No.	Acronym	Funnel RB P-1051 (ng/L)
PFCAs	375-22-4	PFBA	<0.943
PFCAs	2706-90-3	PFPeA	<0.472
PFCAs	307-24-4	PFHxA	<0.472
PFCAs	375-85-9	PFHpA	<0.472
PFCAs	335-67-1	PFOA	<0.472
PFCAs	375-95-1	PFNA	3.01
PFCAs	335-76-2	PFDA	<0.472
PFCAs	2058-94-8	PFUnA	0.814
PFCAs	307-55-1	PFDoA	<0.519
PFCAs	72629-94-8	PFTriA	<0.547
PFCAs	376-06-7	PFTeA	<0.764
PFSAs	375-73-5	PFBS	<0.472
PFSAs	2706-91-4	PFPeS	<0.472
PFSAs	355-46-4	PFHxS	<0.472
PFSAs	375-92-8	PFHpS	<0.472
PFSAs	1763-23-1	PFOS	<0.472
PFSAs	68259-12-1	PFNS	<0.472
PFSAs	335-77-3	PFDS	<0.472
PFSAs	79780-39-5	PFDoS	<0.5
FOSAs	754-91-6	FOSA	<0.472
FOSAs	31506-32-8	NMeFOSA	<0.472
FOSAs	4151-50-2	NEtFOSA	<0.472
FOSAAs	2355-31-9	NMeFOSAA	<0.472
FOSAAs	2991-50-6	NEtFOSAA	<0.472
FOSEs	24448-09-7	N-MeFOSE	<2.36
FOSEs	1691-99-2	N-EtFOSE	<2.36
FTSs	757124-72-4	4:2 FTS	<0.943
FTSs	27619-97-2	6:2 FTS	<0.943
FTSs	39108-34-4	8:2 FTS	<0.943
FRCs	13252-13-6	HFPO-DA	<0.368

PFAS Class	CAS No.	Acronym	Funnel RB P-1051 (ng/L)
FRCs	919005-14-4	DONA	<0.472
FRCs	756426-58-1	9Cl-PF3ONS	<0.547
FRCs	763051-92-9	11Cl-PF3OUdS	<0.472
ATAs	377-73-1	PFECA F	<0.472
ATAs	863090-89-5	PFECA A	<0.472
ATAs	151772-58-6	PFECA B	<0.679
ATAs	113507-82-7	PES	<0.472
ATAs	356-02-5	3:3 FTCA	<0.943
ATAs	914637-49-3	5:3 FTCA	<2.36
ATAs	812-70-4	7:3 FTCA	<2.36

Notes:
Yellow shading = compound spiked during Runs 1A, 2A, 3A
Pink shading = detected analytical result
--- = not analyzed

9.2.1.2 Exhaust Emission Samples

Exhaust emissions samples were collected and analyzed to evaluate the presence of PFAS in emissions and assess potential sampling-related contamination.

9.2.1.2.1 OTM-45

Multiple QC blanks were included in accordance with OTM-45 protocols to verify the accuracy and integrity of the field sampling procedures during Runs 1A, 2A, and 3A. Accuracy of field sampling for exhaust emissions samples was evaluated through the collection and analysis of multiple QC blanks, including STPBs, Glassware Proof Blanks, and FSMBs, during Runs 1A, 2A, and 3A. These blanks were used to assess potential contamination from sampling equipment, glassware, reagents, and field procedures, in accordance with OTM-45 method protocols.

9.2.1.2.1.1 Sample Train Proof Blank

The definition of a STPB from OTM-45 Section 3.32 is as follows:

> "Sampling Train Proof Blank. The complete field assembly and recovery of a clean OTM-45 sampling train without actual sampling, including bringing the train to sampling location, heating, and leak checks. The STFB is conducted using clean glassware that has not been previously used for exhaust emissions sampling as part of the current field test. The purpose of the STPB is to determine if method analytes or other interferences are introduced into the sample from the clean, unused sample train glassware, train assembly, preparation, and recovery, including the field environment."

As shown in Table 9-3, 10 compounds were detected in the STPB. PFHpA, PFOA, PFBS, and HFPO-DA were the only PFAS with concentrations measured over the reporting limit, as presented in the Eurofins analytical report in Appendix J-2. Details of the impacted samples and the full range of PFAS concentration ratios—calculated as detected levels in the samples divided by the corresponding detected STPB values—is provided in Table 9-4.

Table 9-3. Other Test Method (OTM) 45 Field Sample QA/QC Data Summary

PFAS Class	CAS No.	Acronym	STPB Front Half (ng/sample)	STPB Back Half (ng/sample)	STPB Impinger Components (ng/sample)	STPB BT XAD-2 (ng/sample)	Glassware Proof Blank Rinse (ng/sample)	FSMB Filter (ng/sample)	FSMB XAD-2 (ng/sample)	FSMB MeOH/5% NH_4OH (ng/sample)	FSMB DI Water (ng/sample)
PFCAs	375-22-4	PFBA	<1.300	<7.100	0.931	<7.100	<1.780	<1.300	<7.100	<1.780	<0.550
PFCAs	2706-90-3	PFPeA	<0.180	<0.830	<0.130	<0.830	<0.208	<0.180	<0.830	<0.208	<0.130
PFCAs	307-24-4	PFHxA	0.597	<0.740	0.770	<0.740	<0.185	<0.210	<0.740	<0.185	<0.380
PFCAs	375-85-9	PFHpA	1.650	<1.600	3.390	<1.600	<0.400	<0.620	<1.600	<0.400	<0.130
PFCAs	335-67-1	PFOA	1.130	0.570	0.353	<0.390	<0.098	<0.650	<0.390	<0.098	<0.190
PFCAs	375-95-1	PFNA	0.085	<0.170	<0.105	<0.170	<0.043	<0.085	<0.170	<0.043	<0.105
PFCAs	335-76-2	PFDA	0.329	<0.420	0.198	<0.420	<0.105	<0.250	<0.420	<0.105	<0.130
PFCAs	2058-94-8	PFUnA	<0.170	<0.220	<0.150	<0.220	<0.055	<0.170	<0.220	<0.055	<0.150
PFCAs	307-55-1	PFDoA	0.167	0.321	<0.105	0.260	<0.033	<0.100	0.281	<0.033	<0.105
PFCAs	72629-94-8	PFTriA	<0.140	<0.240	<0.160	<0.240	<0.060	<0.140	<0.240	<0.060	<0.160
PFCAs	376-06-7	PFTeA	<0.170	<0.290	<0.224	<0.290	<0.073	<0.170	<0.290	<0.073	<0.225
PFCAs	67905-19-5	PFHxDA	<0.290	<0.180	<0.284	<0.180	<0.045	<0.290	<0.180	<0.045	<0.285
PFCAs	16517-11-6	PFODA	<0.220	<0.160	<0.254	<0.160	<0.040	<0.220	<0.160	<0.040	<0.255
PFSAs	375-73-5	PFBS	<0.890	2.110	<0.090	2.060	<0.125	<0.890	2.010	<0.125	<0.090
PFSAs	2706-91-4	PFPeS	<0.120	<0.058	<0.120	<0.058	<0.015	<0.120	<0.058	<0.015	<0.120
PFSAs	355-46-4	PFHxS	<0.110	<0.130	<0.115	<0.130	<0.033	<0.110	<0.130	<0.033	<0.115
PFSAs	375-92-8	PFHpS	<0.110	<0.440	<0.229	<0.440	<0.110	<0.110	<0.440	<0.110	<0.230
PFSAs	1763-23-1	PFOS	<0.450	0.587	0.158	0.488	<0.083	<0.450	0.555	<0.083	<0.130
PFSAs	68259-12-1	PFNS	<0.120	<0.140	<0.075	<0.140	<0.035	<0.120	<0.140	<0.035	<0.075
PFSAs	335-77-3	PFDS	<0.110	<0.320	<0.165	<0.320	<0.080	<0.110	<0.320	<0.080	<0.165
PFSAs	79780-39-5	PFDoS	<0.095	<0.590	<0.160	<0.590	<0.148	<0.095	<0.590	<0.148	<0.160
FOSAs	754-91-6	FOSA	<0.088	<0.260	<0.115	<0.260	<0.065	<0.088	<0.260	<0.065	<0.115
FOSAs	31506-32-8	NMeFOSA	<0.150	<0.350	<0.170	<0.350	<0.088	<0.150	<0.350	<0.088	<0.170
FOSAs	4151-50-2	NEtFOSA	<0.160	<0.320	<0.269	<0.320	<0.080	<0.160	<0.320	<0.080	<0.270
FOSAAs	2355-31-9	NMeFOSAA	<0.120	<0.210	<0.204	<0.210	<0.053	<0.120	<0.210	<0.053	<0.205
FOSAAs	2991-50-6	NEtFOSAA	<0.140	<0.190	<0.150	<0.190	<0.048	<0.140	<0.190	<0.048	<0.150
FOSEs	24448-09-7	N-MeFOSE	<4.900	<0.300	<0.204	<0.300	<0.075	<4.900	<0.300	<0.075	<0.205
FOSEs	1691-99-2	N-EtFOSE	<0.120	<0.390	<0.145	<0.390	<0.098	<0.120	<0.390	<0.098	<0.145
FTSs	757124-72-4	4:2 FTS	<0.091	<0.095	<0.115	<0.095	<0.024	<0.091	<0.095	<0.024	<0.115
FTSs	27619-97-2	6:2 FTS	<4.000	<0.250	<0.549	<0.250	<0.063	<4.000	<0.250	<0.063	<0.550
FTSs	39108-34-4	8:2 FTS	<0.140	<0.140	<0.259	<0.140	<0.035	<0.140	<0.140	<0.035	<0.260
FTSs	120226-60-0	10:2 FTS	<0.320	<0.230	<0.214	<0.230	<0.058	<0.320	<0.230	<0.058	<0.215
FRCs	13252-13-6	HFPO-DA	29.000	2.520	<0.469	<2.400	<0.600	<4.700	<2.400	<0.600	0.842
FRCs	919005-14-4	DONA	<0.140	<1.100	<0.279	<1.100	<0.275	<0.140	<1.100	<0.275	<0.280
FRCs	756426-58-1	9Cl-PF3ONS	<0.098	<0.120	<0.130	<0.120	<0.030	<0.098	<0.120	<0.030	<0.130
FRCs	763051-92-9	11Cl-PF3OUdS	<0.200	<0.360	<0.140	<0.360	<0.090	<0.200	<0.360	<0.090	<0.140
ATAs	377-73-1	PFECA F	<0.150	<0.570	<0.289	<0.570	<0.143	<0.150	<0.570	<0.143	<0.290

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PFAS Class	CAS No.	Acronym	STPB Front Half (ng/sample)	STPB Back Half (ng/sample)	STPB Impinger Components (ng/sample)	STPB BT XAD-2 (ng/sample)	Glassware Proof Blank Rinse (ng/sample)	FSMB Filter (ng/sample)	FSMB XAD-2 (ng/sample)	FSMB MeOH/5% NH_4OH (ng/sample)	FSMB DI Water (ng/sample)
ATAs	863090-89-5	PFECA A	<0.200	<0.086	<0.394	<0.086	<0.022	<0.200	<0.086	<0.022	<0.395
ATAs	151772-58-6	PFECA B	<0.150	<0.190	<0.289	<0.190	<0.048	<0.150	<0.190	<0.048	<0.290
ATAs	113507-82-7	PES	<0.140	<0.088	<0.254	<0.088	<0.022	<0.140	<0.088	<0.022	<0.255
ATAs	356-02-5	3:3 FTCA	<0.290	<0.280	<0.439	<0.280	<0.070	<0.290	<0.280	<0.070	<0.440
ATAs	914637-49-3	5:3 FTCA	<0.480	<0.340	<0.599	<0.340	<0.085	<0.480	<0.340	<0.085	<0.600
ATAs	812-70-4	7:3 FTCA	<0.350	<0.380	<0.394	<0.380	<0.095	<0.350	<0.380	<0.095	<0.395
ATAs	70887-84-2	8:2 FTUCA	<0.220	<0.130	<0.399	<0.130	<0.033	<0.220	<0.130	<0.033	<0.400
ATAs	53826-13-4	10:2 FTCA	<0.420	<0.930	<0.599	<0.930	<0.233	<0.420	<0.930	<0.233	<0.600
ATAs	27854-31-5	8:2 FTCA	<0.350	<0.590	<0.648	<0.590	<0.148	<0.350	<0.590	<0.148	<0.650
ATAs	53826-12-3	6:2FTCA	<0.430	<0.720	<0.599	<0.720	<0.180	<0.430	<0.720	<0.180	<0.600
ATAs	133201-07-7	PFECHS	<0.220	<0.210	<0.319	<0.210	<0.053	<0.220	<0.210	<0.053	<0.320
ATAs	70887-88-6	6:2 FTUCA	<0.140	<0.100	<0.180	<0.100	<0.025	<0.140	<0.100	<0.025	<0.180

Notes:
Yellow shading = compound spiked during Runs 1A, 2A, 3A
Pink shading = detected analytical result in one or more exhaust emissions sample fractions (FH, BH, Condensate, BT XAD-2 Sorbent Trap)
% = percent
ng/sample = nanogram(s) per sample

Table 9-4. Sample Train Proof Blank Detects and Associated Sample Versus Blank Ratios

PFAS	STPB Mass (ng/sample)	Run 1A Mass (ng/sample)	Run 2A Mass (ng/sample)	Run 3A Mass (ng/sample)	Run 1A/ STPB Ratio	Run 2A/ STPB Ratio	Run 3A/ STPB Ratio
PFBA	0.931	9.4	6.3	3.1	10	7	3
PFHxA	1.367	6.94	2.049	1.12	5	1	1
PFHpA	5.04	12.68	10.93	2.17	3	2	0
PFOA	2.053	2.74	4.036	1.064	1	2	1
PFNA	0.0852	1.16	23.36	0.384	14	274	5
PFDA	0.527	0.841	0.841	---	2	2	---
PFBS	4.17	5.71	4.76	5.106	1	1	1
PFOS	1.233	1.404	1.954	1.272	1	2	1
HFPO-DA	31.52	505.3	8290.6	401.9	16	263	13

Notes:
--- = <MDL

9.2.1.2.1.2 Glassware Proof Blank

A glassware proof blank consists of a solvent rinse of a set of sampling train glassware prior to its use in the field. All analytes in the glassware proof blank were non-detect.

9.2.1.2.1.3 Field Sample Media Blanks

The FSMB includes the sampling media (i.e., particulate filter, XAD-2 sorbent trap and reagents (95 percent MeOH/5 percent NH_4OH rinsing solvents and DI water) associated with the field sample collection and recovery. These samples are treated as actual samples in all respects, including shipment to the sampling site, exposure to sampling site conditions, sample storage, and processing by all of the same analytical procedures. The purpose of the FSMB is to determine if target analytes or other interferences are introduced into the sample media from shipping, storage, and the field environment and procedures. As shown in Table 9-3, for the filter media and 95 percent MeOH/5 percent NH_4OH media blanks, all analytes are reported as non-detect. There were three target compounds (PFDoA [0.281 ng/sample], PFBS [2.010 ng/sample], and PFOS [0.555 ng/sample]) reported in the XAD-2 sorbent trap. One target analyte (HFPO-DA) was reported at 0.842 ng/sample for the DI water.

PFDoA concentrations in Runs 1A and 3A were approximately twice those observed in the blanks, while the levels in Run 2A were about six times higher than the sum of the fractions in the FSMB. PFBS concentrations were up to three times higher for Runs 1A, 2A, and 3A than the sum of the fractions in the FSMB. PFOS levels were generally comparable to the blanks, ranging from 30 to 90 percent higher than the sum of the fractions in the FSMB.

9.2.1.2.1.4 Stack Sampling QA/QC

A number of stack sampling QA/QC procedures were used, and are described in Appendices B, C, D and E of the ATG Test Report in Appendix F. These procedures were applicable to measuring exhaust emissions flow rates for OTM-45, OTM-50, and Method 0010. They include performing leak and temperature checks as required by the sampling method, using certified calibration gases for O_2 and CO_2

continuous emissions monitors, verifying CEM system response times, and performing bias/drift determinations.

9.2.1.2.2 OTM-50

Accuracy of OTM-50 field sampling was assessed through background determinations, system purging, and field and laboratory sample integrity checks conducted in accordance with the method. Accuracy was measured by analyzing pre-test and post-test blanks. As shown in Table 9-5, chlorodifluoromethane and trichlorofluoromethane were both detected in the EPA pre-test blank at concentrations similar to those observed in actual samples. Results from analysis of all target analytes were not detected in the Eurofins pre-test and post-test blanks or the method blank.

9.2.1.2.3 Method 0010

Multiple QC blanks were included in accordance with Method 0010 protocols to verify the accuracy and integrity of the field sampling procedures during Runs 1A, 2A, and 3A. Accuracy of field sampling for exhaust emissions samples was evaluated through the collection and analysis of multiple QC blanks, including proof blanks and reagent blanks. These blanks were used to assess potential contamination from sampling equipment, glassware, reagents, and field procedures in accordance with Method 0010 protocols.

9.2.1.2.3.1 Proof Blank

A Method 0010 proof blank was collected by the complete field assembly and recovery of a clean Method 0010 sampling train without actual sampling, including bringing the train to sampling location, heating, and leak checks. The proof blank was conducted using clean glassware that had not been previously used for exhaust emissions sampling as part of the current field test. The purpose of the STPB is to determine if method analytes or other interferences are introduced into the sample from the clean, unused sample train glassware, train assembly, preparation, and recovery, including the field environment. Proof blank samples included the filter, front half solvent rinse, XAD-2 sorbent trap, back half solvent rinse, and impinger solvent rinse. Three detections were recorded on the blank train: di-n-butyl phthalate at 3.59 micrograms per sample (µg/sample) in the filter and front half solvent rinse, acetophenone at 10.8 µg/sample in the XAD-2 sorbent trap and solvent rinse, and phenol at 3.71 µg/sample in the XAD-2 sorbent trap and solvent rinse. All other analytes in blank train sample fractions were non-detect.

9.2.1.2.3.2 Reagent Blanks

Reagent blanks were analyzed for the filter, methylene chloride, acetone, DI water, and XAD-2 sorbent trap. Analytical results for all target analytes for all reagent blank samples were non-detect.

9.2.2 Precision

Precision is the degree of agreement among repeated measurements of the same characteristic on the same sample or on separate samples collected as close as possible in time and place. Field sampling precision is measured with the field duplicate relative percent differences (RPDs), and laboratory precision is measured with RPDs for laboratory duplicates, such as MS and MSD pairs.

9.2.2.1 Process Samples

Field duplicate process samples were collected for Runs 1A, 2A, and 3A to assess the overall sampling and measurement precision for this sampling effort. While most of the field duplicate process samples were within the precision limits of RPD less than or equal to 30 percent (for results greater than five times the reporting limit) or differences less than the value of the reporting limit (Appendix J-1), there were seven occasions where RPDs exceeded the limit up to 50 percent for some analytes.

9.2.2.2 Exhaust Emission Samples

No field duplicate samples were collected for exhaust emissions sampling; therefore, field sampling precision for exhaust emission samples could not be directly evaluated using RPDs.

9.3 Laboratory Analytical Data Quality Review

This section presents a detailed evaluation of the laboratory analytical data quality to determine whether the PFAS results generated during the study meet project data quality objectives. The assessment integrates findings from laboratory QA/QC measures including method blanks, LCS/LCSD, MS/MSD, surrogate recoveries, and isotope dilution standards to evaluate accuracy, precision, completeness, representativeness, and comparability of the data. Results were reviewed against method-specific acceptance criteria, with qualifiers applied to indicate any deviations. Subsections below address each data quality indicator individually, with separate evaluations for process and exhaust emission samples across all laboratory methods used in this study.

9.3.1 Accuracy

Accuracy of laboratory analytical data is measured through percent recoveries in calibration verification samples, laboratory control samples including method blanks, LCSs, LCSDs, MS/MSDs, and through isotope dilution standards.

9.3.1.1 Process Samples

For process samples, eight method blanks were prepared and analyzed. Method blanks were non-detects.

LCSs were analyzed for each analytical batch and demonstrated that the analytical system was in control during sample preparation and analysis, with the following exceptions. There was one LCS and two LCSDs which failed with qualifiers. The qualifier for outside acceptance limits low biased is " *- " for LCS and/or LCSD. The qualifier for outside acceptance limits high biased is " *+ " for LCS and/or LCSD. Analytes PFDS, PFNS, 9 Cl-PF3ONS, 11Cl-PF3OUdS, and PFODA had recoveries below acceptance criteria in multiple LCSs and LCSDs associated with process samples. The recoveries for 3:3 FTCA, PFHpS, 5:3 FTCA, ADONA, PFTrDA, PFDoS, and PFHpS were above acceptance limits in the LCS.

MS/MSDs for PFAS analysis were performed on Run 1A process samples. Of the thirteen process sample collection locations, eight locations had multiple analytes with recoveries outside the acceptable limits. A summary of the recoveries is provided in Appendix J-1. The low recoveries indicate that the complex sample matrix may have introduced some uncertainty into the analytical results.

Prior to sample preparation and analysis, isotope dilution standards were added by the laboratory to measure relative responses of target analytes. For a subset of analytes, the recoveries of one or more of these standards were outside the acceptance limits. Though sample cleanup procedures were routinely used, residual interferences due to the complexity of the matrices were the attributed cause. Recoveries outside acceptance limits may be the result of matrix interferences. For analytes with isotope-dilution recoveries of less than 25 percent or over 150 percent, the results for associated target analytes were qualified as estimated and flagged as “J” or “UJ”.

9.3.1.2 Exhaust Emission Samples

This section presents a comprehensive review of the laboratory analytical data quality for exhaust emission samples, evaluated according to the specific analytical methods employed during the study. Each method is assessed for compliance with QA/QC standards, with attention given to any observed deviations and their potential impact on data usability. The subsections include method-specific discussions such as OTM-45, including observations related to calibration checks, ion ratio variances, and qualifier usage. Associated laboratory controls, including media checks and method blanks, are also summarized to assess potential background contamination and confirm the integrity of the analytical process. The goal of this evaluation is to ensure that all reported results meet the required data quality objectives and are suitable for interpretation and reporting.

9.3.1.2.1 OTM-45

Several continuing calibration verifications displayed recoveries outside of the QA criteria for one or more target analytes for the OTM-45 and the process samples. The samples associated with the continuing calibration verification were non-detected for the affected analytes; therefore, the data has been reported. The transition ion mass ratios for PFOS, PFPeA, PFHpS, and/or HFPO-DA in some of the parent samples were outside established limits. The results for these analytes in affected samples were flagged with the qualifier “I”.

9.3.1.2.1.1 Laboratory Media Checks

Laboratory media blanks are samples of sampling media (particulate filter, MeOH/5 percent NH_4OH, DI water, and XAD-2 sorbent trap) that are prepared and archived at the laboratory during the field sample collection campaign. The samples are collected from the same lots of materials that are used for the actual field samples, but they are not taken to the field. The purpose of the laboratory media checks is to identify potential contamination sources in the specific media used to collect field samples.

As shown in Table 9-6, the OTM-45 particulate filter had one detection (PFBS) near the MDL and the XAD-2 sorbent trap had two detections (PFHxA and PFHpA) with concentrations near the MDL. This is in line with previous studies reporting detections in the media blanks for new, clean XAD-2 sorbent traps (Barranco et al. 2020, Gehrmann et al. 2024).

Table 9-5. Other Test Method (OTM) 50 Eurofins and EPA QA/QC Results

CAS No.	Analyte	Eurofins Pre-test Blank Result (µg/m³)	EPA Pre-test Blank Result (µg/m³)	Eurofins Post-test Blank Result (µg/m³)	EPA Post-test Blank Result (µg/m³)	Eurofins MB 140-93657/4 Result (µg/m³)
116-14-3	Tetrafluoroethene	<0.622	<0.229	<0.453	<0.229	<0.151
75-46-7	Trifluoromethane	<1.77	<0.298	<1.28	<0.298	<0.43
76-19-7	Octafluoropropane	<1.52	<0.356	<1.1	<0.356	<0.369
75-10-5	Difluoromethane (HFC-32)	<1.84	<0.046	<1.34	<0.046	<0.447
593-53-3	Fluoromethane	<2.97	<1.357	<2.16	<1.357	<0.724
354-33-6	1,1,1,2,2-Pentafluoroethane (HFC-125)	<1.35	<0.199	<0.983	<0.199	<0.329
116-15-4	Hexafluoropropene	<0.706	<0.156	<0.514	<0.156	<0.172
355-25-9	Decafluorobutane	<1.2	<0.660	<0.873	<0.660	<0.292
678-26-2	Dodecafluoropentane	<2.71	<0.377	<1.97	<0.377	<0.66
355-42-0	Tetradecafluorohexane	<2.84	<1.837	<2.07	<1.837	<0.691
375-61-1	1H-Perfluoropentane	<2	<0.829	<1.45	<0.829	<0.486
335-57-9	Hexadecafluoroheptane	<1.3	<0.330	<0.949	<0.330	<0.317
3330-15-2	Heptafluoropropyl 1,2,2,2-tetrafluoroethyl ether	<1.39	<0.322	<1.01	<0.322	<0.339
355-37-3	1H-Perfluorohexane	<1.13	<0.319	<0.822	<0.319	<0.275
375-83-7	1H-Perfluoroheptane	<2.12	<0.472	<1.54	<0.472	<0.515
3330-14-1	2H-Perfluoro-5-methyl-3,6-dioxanonane (E2)	<1.67	<2.010	<1.22	<2.010	<0.407
335-65-9	1H-Perfluorooctane	<1.48	<1.438	<1.08	<1.438	<0.361
307-34-6	Octadecafluorooctane	<1.69	<1.453	<1.23	<1.453	<0.412
375-17-7	1H-Nonafluorobutane	<1.37	<0.322	<0.996	<0.322	<0.333
2252-84-8	1H-Heptafluoropropane	<0.657	<0.328	<0.478	<0.328	<0.16
811-97-2	Ethane, 1,1,1,2-tetrafluoro-	<1.54	<0.344	<1.12	<0.344	<0.376
420-46-2	1,1,1-Trifluoroethane	<3.11	<0.345	<2.26	<0.345	<0.756
75-45-6	Chlorodifluoromethane	<2.03	0.387	<1.48	<0.086	<0.495
75-72-9	Chlorotrifluoromethane	<1.46	<0.374	<1.06	<0.374	<0.355
115-25-3	Octafluorocyclobutane (FC-C318)	<0.672	<0.325	<0.489	<0.325	<0.164
559-40-0	Octafluorocyclopentene (FC-C1418)	<1.57	<0.132	<1.14	<0.132	<0.382
75-69-4	Trichlorofluoromethane	<2.12	0.57	<1.55	<0.16	<0.517

KD1794

CAS No.	Analyte	Eurofins Pre-test Blank Result (µg/m³)	EPA Pre-test Blank Result (µg/m³)	Eurofins Post-test Blank Result (µg/m³)	EPA Post-test Blank Result (µg/m³)	Eurofins MB 140-93657/4 Result (µg/m³)
75-02-5	Vinyl Fluoride[(a)]	<1.01	---	---	---	<0.245
79-38-9	Chlorotrifluoroethene[(a)]	<0.607	---	---	---	<0.148
76-13-1	1,1,2-Trichloro-1,2,2-trifluoroethane[(a)]	<0.787	---	---	---	<0.192
76-16-4	Hexafluoroethane (C_2F_6)	<0.719	<0.140	<0.523	<0.140	<0.175
428-59-1	Hexafluoropropylene oxide	<14.2	<6.744	<10.4	<6.744	<3.46
75-73-0	Tetrafluoromethane	<7.25	<0.945	<5.27	<0.945	<1.76
NA	**Subtotal: Non-detect**	**<69.41**	**<7.88**	**<50.56**	**<8.12**	**<16.90**
NA	**Subtotal: Detected**	**0.00**	**0.95**	**0.00**	**0.00**	**0.00**
NA	**Grand Total**	**<69.41**	**<8.831**	**<50.56**	**<8.123**	**<16.90**

Notes:

(a) Compound is not a target analyte listed in OTM-50. It was reported by Eurofins but not reported by EPA.

Pink shading = detected analytical result

--- = not analyzed

NA = not applicable

9.3.1.3 Laboratory Method Blanks

Laboratory method blanks for OTM-45 are DI water that is run through the analytical process alongside a batch of analytical samples. The purpose of the laboratory method blanks is to identify any potential contamination sources within analytical equipment in the processing of samples. Three method blanks were run in the same analytical batch as parent samples and the results are reported in Table 9-6.

In the first method blank, PFBA was detected at 1.449 ng/sample. The second method blank showed detections for PFHxA and PFHpA at 0.411 and 2.689 ng/sample, respectively. No PFAS were detected in the third method blank. The discussion below addresses how these detections may have impacted the sample results, or if any impacts were observed. The results are reported and no blank subtraction was performed.

PFBA was detected in the impinger portions for Runs 1A, 2A and 3A, at levels ranging from 3.1 ng/sample to 9.4 ng/sample. Additionally, one QA sample (the impinger rinse) contained PFBA at 0.931 ng/sample.

PFHxA was detected in seven samples: 0.397 ng/sample for FH R2A, 0.779 ng/sample in BH R2A, 0.873 ng/sample in impinger R2A, 1.12 ng/sample in impinger R3A, 1.12 ng/sample in BH R1A, 1.66 ng/sample in FH R1A, and 4.16 ng/sample in impinger R1A.

PFHpA was detected in eight samples: 0.837 ng/sample in FH R2A, 2.17 ng/sample in impinger R3A, 2.55 ng/sample in BH R1A, 3.97 ng/sample in BH R2A, 4.06 ng/sample impinger R1A, 6.07 ng/sample in FH R1A, and 6.96 ng/sample in impinger R2A. Of these detections, three sample results — FH R2A, impinger R3A, and BH R1A — were below the method blank level. The remaining five exceeded the method blank, with concentrations ranging from 1.5 times (BH R2A) to approximately 2.6 times (impinger R2A) higher than the method blank.

Three QA samples were also impacted by PFHpA. The media check filter (0.823 ng/sample) and FH proof blank (1.65 ng/sample) were both below the method blank level, while the impinger in proof blank (3.39 ng/sample) exceeded the method blank, with a concentration approximately 1.3 times higher.

9.3.1.3.1 OTM-50

OTM-50 method blanks were performed by analyzing the same nitrogen gas that is used to pressurize the canisters. Analytical results of all method blanks were non-detects.

Table 9-6. Other Test Method (OTM) 45 Eurofins and EPA QA/QC Results

PFAS Class	CAS No.	Acronym	Filter[a] A-2499 Media Check (ng/sample)	XAD-2[b] A-2501 Media Check (ng/sample)	MB 140-93798/1-B Method Blank (ng/sample)	MB 140-93945/1-B Method Blank (ng/sample)	MB 140-93949/1-B Method Blank (ng/sample)
PFCAs	375-22-4	PFBA	<7.100	<1.280	1.449	<1.280	<7.100
PFCAs	2706-90-3	PFPeA	<0.830	<0.177	<0.260	<0.177	<0.830
PFCAs	307-24-4	PFHxA	<0.740	0.230	<0.760	0.411	<0.740
PFCAs	375-85-9	PFHpA	<1.600	0.823	<0.260	2.689	<1.600
PFCAs	335-67-1	PFOA	<0.390	<0.638	<0.380	<0.638	<0.390
PFCAs	375-95-1	PFNA	<0.170	<0.083	<0.210	<0.084	<0.170
PFCAs	335-76-2	PFDA	<0.420	<0.245	<0.260	<0.246	<0.420
PFCAs	2058-94-8	PFUnA	<0.220	<0.167	<0.300	<0.167	<0.220
PFCAs	307-55-1	PFDoA	<0.130	<0.098	<0.210	<0.098	<0.130
PFCAs	72629-94-8	PFTriA	<0.240	<0.137	<0.320	<0.138	<0.240
PFCAs	376-06-7	PFTeA	<0.290	<0.167	<0.450	<0.167	<0.290
PFCAs	67905-19-5	PFHxDA	<0.180	<0.285	<0.570	<0.285	<0.180
PFCAs	16517-11-6	PFODA	<0.160	<0.216	<0.510	<0.216	<0.160
PFSAs	375-73-5	PFBS	0.752	<0.874	<0.180	<0.874	<0.500
PFSAs	2706-91-4	PFPeS	<0.058	<0.118	<0.240	<0.118	<0.058
PFSAs	355-46-4	PFHxS	<0.130	<0.108	<0.230	<0.108	<0.130
PFSAs	375-92-8	PFHpS	<0.440	<0.108	<0.460	<0.108	<0.440
PFSAs	1763-23-1	PFOS	<0.330	<0.442	<0.260	<0.442	<0.330
PFSAs	68259-12-1	PFNS	<0.140	<0.118	<0.150	<0.118	<0.140
PFSAs	335-77-3	PFDS	<0.320	<0.108	<0.330	<0.108	<0.320
PFSAs	79780-39-5	PFDoS	<0.590	<0.093	<0.320	<0.093	<0.590
FOSAs	754-91-6	FOSA	<0.260	<0.086	<0.230	<0.086	<0.260
FOSAs	31506-32-8	NMeFOSA	<0.350	<0.147	<0.340	<0.147	<0.350
FOSAs	4151-50-2	NEtFOSA	<0.320	<0.157	<0.540	<0.157	<0.320
FOSAAs	2355-31-9	NMeFOSAA	<0.210	<0.118	<0.410	<0.118	<0.210

PFAS Class	CAS No.	Acronym	Filter[a] A-2499 Media Check (ng/sample)	XAD-2[b] A-2501 Media Check (ng/sample)	MB 140-93798/1-B Method Blank (ng/sample)	MB 140-93945/1-B Method Blank (ng/sample)	MB 140-93949/1-B Method Blank (ng/sample)
FOSAAs	2991-50-6	NEtFOSAA	<0.190	<0.137	<0.300	<0.138	<0.190
FOSEs	24448-09-7	N-MeFOSE	<0.300	<4.810	<0.410	<4.810	<0.300
FOSEs	1691-99-2	N-EtFOSE	<0.390	<0.118	<0.290	<0.118	<0.390
FTSs	757124-72-4	4:2 FTS	<0.095	<0.089	<0.230	<0.089	<0.095
FTSs	27619-97-2	6:2 FTS	<0.250	<3.930	<1.100	<3.930	<0.250
FTSs	39108-34-4	8:2 FTS	<0.140	<0.137	<0.520	<0.138	<0.140
FTSs	120226-60-0	10:2 FTS	<0.230	<0.314	<0.430	<0.314	<0.230
FRCs	13252-13-6	HFPO-DA	<2.400	<4.610	<0.940	<4.620	<2.400
FRCs	919005-14-4	DONA	<1.100	<0.137	<0.560	<0.138	<1.100
FRCs	756426-58-1	9Cl-PF3ONS	<0.120	<0.096	<0.260	<0.096	<0.120
FRCs	763051-92-9	11Cl-PF3OUdS	<0.360	<0.196	<0.280	<0.196	<0.360
ATAs	377-73-1	PFECA F	<0.570	<0.147	<0.580	<0.147	<0.570
ATAs	863090-89-5	PFECA A	<0.086	<0.196	<0.790	<0.196	<0.086
ATAs	151772-58-6	PFECA B	<0.190	<0.147	<0.580	<0.147	<0.190
ATAs	113507-82-7	PES	<0.088	<0.137	<0.510	<0.138	<0.088
ATAs	356-02-5	3:3 FTCA	<0.280	<0.285	<0.880	<0.285	<0.280
ATAs	914637-49-3	5:3 FTCA	<0.340	<0.471	<1.200	<0.471	<0.340
ATAs	812-70-4	7:3 FTCA	<0.380	<0.344	<0.790	<0.344	<0.380
ATAs	70887-84-2	8:2 FTUCA	<0.130	<0.216	<0.800	<0.216	<0.130
ATAs	53826-13-4	10:2 FTCA	<0.930	<0.412	<1.200	<0.413	<0.930
ATAs	27854-31-5	8:2 FTCA	<0.590	<0.344	<1.300	<0.344	<0.590
ATAs	53826-12-3	6:2FTCA	<0.720	<0.422	<1.200	<0.422	<0.720
ATAs	133201-07-7	PFECHS	<0.210	<0.216	<0.640	<0.216	<0.210
ATAs	70887-88-6	6:2 FTUCA	<0.100	<0.137	<0.360	<0.138	<0.100

Notes:

(a) MDL for PFBS is 0.5 ng/sample.

(b) MDL for PFHxA is 0.609 ng/sample for PFHxA and 0.206 ng/sample for PFHpA.

Yellow shading = compound spiked during Runs 1A, 2A, 3A
Pink shading = detected analytical result

9.3.1.3.2 Method 0010

9.3.1.3.2.1 Laboratory Media Checks

Laboratory media blanks are samples of sampling media (particulate filter and XAD-2 sorbent trap) that are prepared and archived at the laboratory during the field sample collection campaign. The samples are collected from the same lots of materials that are used for the actual field samples, but they are not taken to the field. The purpose of the laboratory media checks is to identify potential contamination sources in the specific media used to collect field samples.

All target analytes in both media samples were non-detect.

9.3.1.3.2.2 Laboratory Method Blanks

Each matrix (filter, XAD-2 sorbent trap, and condensate) has its own method blank. The filter method blank is created using filter material from the same batch that was sent to the field. The condensate method blank is created using laboratory DI water. Analytical results for all method blanks were non-detects.

9.3.2 Precision

Precision was evaluated to assess the reproducibility of PFAS measurements in both process and exhaust emission samples. Precision results were compared against the established acceptance criterion of an RPD less than or equal to 30 percent, as detailed below.

9.3.2.1 Process Samples

MS/MSD pairs for the process samples were prepared, analyzed, and reported with the process sample results for PFAS. All MS/MSD recoveries are presented in Appendix J-1. MS/MSD precision met the acceptance criterion of RPD less than or equal to 30 percent for most of the process samples, with some sample locations having RPD of 30 percent for one or more analytes.

Seven LCS and LCSD pairs were analyzed with analytical batches. The corresponding recoveries are presented in Appendix J-2. LCS/LCSD precision met the acceptance criterion of RPD less than or equal to 30 percent for all of the process samples.

9.3.2.2 Exhaust Emission Samples

9.3.2.2.1 OTM-45

Analytical laboratory precision for OTM-45 exhaust emission samples was assessed using method-defined internal QC checks, including surrogate recoveries and LCS performance, as summarized in Appendix J-2. Overall, the consistency of PFAS detections and concentrations across the three runs suggests acceptable precision for the stack sampling and analysis effort.

Three LCS and LCSD pairs were analyzed with analytical batches. The corresponding recoveries are presented in Appendix J-2. For the first pair, precision met the acceptance criteria of RPD less than or equal to 30 percent for all analytes. In the second pair, four analytes exceeded 30 percent, with three around 36 percent and one analyte, PES, at 48 percent. For the third pair, eleven analytes exceeded 30 percent, with the highest exceedance observed for 5:3 FTCA at 75 percent.

9.3.2.2.2 OTM-50

One duplicate OTM-50 sample was analyzed to assess precision. All analytes were non-detect, which is the same as the original sample.

9.3.2.2.3 Method 0010

Analytical laboratory precision for Method 0010 exhaust emission samples was assessed using method-defined internal QC checks, including surrogate recoveries and LCS performance, as summarized in Appendix J-4. Overall, the consistency of PFAS results (all non-detect) across the three runs suggests acceptable precision for the stack sampling and analysis effort.

Three LCS and LCSD pairs were analyzed with analytical batches. The corresponding recoveries are presented in Appendix J-4. For the first pair, precision met the acceptance criteria of RPD less than or equal to 30 percent for all but one of the analytes (2-chloropheono). In the second and third pairs, precision met the acceptance criteria of RPD less than or equal to 30 percent for all analytes.

9.3.3 Completeness

Completeness is a measure of the amount of valid data obtained from a measurement system compared to the amount of data expected under normal conditions. The validity of the sample results is determined through the data validation process. Any rejected (R) sample results are considered to be incomplete. The data that are qualified as estimated or estimated non-detected (UJ) are considered to be valid and usable. The completeness is calculated and reported for each method and analyte combination. The number of valid results divided by the number of possible individual analyte results expressed as a percentage determines the completeness of the data set. The laboratory provided data meeting system QC acceptance criteria for all samples tested. In several instances the laboratory reported both an initial and re-analysis set of results for each process sample for the PFAS analysis. The data validator reviewed each set of results and selected the appropriate result for reporting. A total of 3,230 process sample analytical results with EPA Method 1633, 1,484 results with OTM-45, 2,332 with Method 0010, and 396 with OTM-50 were generated, and 0 were rejected, for a completeness of 100 percent. The completeness goals were met.

9.3.4 Representativeness

This sub-section qualitatively expresses the degree to which data accurately reflects samples collected at the site. Factors that affect the representativeness of analytical data include proper sample collection and preservation techniques, analytical holding times, and use of standard analytical methods.

9.3.4.1 Process Samples

The process samples associated with this study include materials like waste feed materials, chemicals, residual streams, process waters, and other materials consisting of complex matrices. The analytical method chosen for the PFAS characterization of these samples is EPA Method 1633 which is expressly appropriate for aqueous, solids, biosolids, and tissue samples. Therefore, its application is appropriate with some necessary qualifications noted.

The original analyses for all process samples were completed within the most restrictive 28 day holding time requirements of Method 1633. During the data review period certain isotope dilution internal standards were observed to be outside of the target recovery limits for some analytes. Under these

conditions, re-analyses were conducted, albeit not within the original holding time of 28 days. The Method 1633 recommended holding time for samples other than aqueous samples is 90 days when stored with proper refrigeration. Therefore, all of the original analyses and re-analyses of the process samples were conducted within 90 days of sample collection.

The case narratives of the analytical reports (Appendix J-1) provide additional details about the re-extraction handling. Where improved results were observed in the reanalysis, these results were reported and the data was qualified using "estimated" data flags.

9.3.4.2 Exhaust Emission Samples

Exhaust emission sampling activities in this project integrated protocols from EPA's OTM-45 for targeted semi-volatile polar compounds and Modified Method 0010 for targeted and non-targeted semi-volatile nonpolar compounds. OTM-50 was employed for volatile fluorinated analytes. The OTM-45 and OTM-50 methods were applied under controlled, documented procedures consistent with Quality Assurance Project Plan protocols.

Where deviations occurred (e.g., re-extractions past method-specified holding times due to isotope standard recovery issues), initial analyses were conducted within holding times. Some samples were originally reported at a dilution due to concentrations of native analytes. Per client request, samples were re-analyzed undiluted. The re-analysis of these samples was performed outside of the analytical holding time. OTM-45 requires a 28-day holding time from extraction to analysis. These samples were reanalyzed on day 42. OTM-45 also states that extracts may be archived for up to 1 year. Both sets of data are presented in the analytical report (Appendix J-2).

9.3.5 Comparability

Comparability is the extent to which data from one study can be compared directly to either past data from the current project or data from another study. Using standardized sampling and analytical methods, units of reporting, and sampling location selection procedures helps ensure comparability.

To ensure that data derived from this field effort are comparable from run to run, all like media samples were submitted for analysis by the same analytical method (with like analytical parameters and similar detection/reporting limits); units of measure (e.g., microgram per liter, microgram per kilogram) are the same for all reporting; and like media were sampled, handled, and prepared in the same manner according to field collection SOPs. Every effort was made to maintain consistent operations during the testing. The data variability observed reflects the complex and heterogeneous nature of commercial HWI feeds and the effects of the spiked PFAS.

9.3.6 Data Validation

Data validation consists of evaluating the completeness, correctness, and conformance of a data set against the method, SOP or contract requirements in the approved Quality Assurance Project Plan. A third party data validation was conducted on all samples by Terri Bales. The validation was conducted as the protocols listed in DOD Data Validation Guidelines Module 6. The data was validated for compliance with the following primary benchmarks:

- Sample preservation and holding times

- Analytical method requirements
- Laboratory specific requirements (i.e., compliance with laboratory SOPs)
- Project specific requirements, if applicable
- Completeness of the data package submittals

9.3.6.1 Process Samples

Overall, the data quality objectives for accuracy, precision, and completeness were met for the process sample data analyzed by EPA Method 1633. Data quality qualifiers were applied to the data where HFPO-DA was reported as exceeding the calibration range for the Dilution Factor 1. Detailed information on the data quality assessment is included in the Data Validation Report (Appendix L-1) for the process samples (EPA Method 1633) data. Data without qualifiers and data qualified as estimated are usable for the purpose of supporting project objectives. All validated data were also found to be representative and comparable for all samples collected and analyzed for EPA Method 1633. The data quality for the EPA Method 1633 analysis is deemed acceptable for its intended use.

9.3.6.2 Exhaust Emission Samples

This section provides a summary of data quality assessments for exhaust emission samples analyzed using EPA Methods OTM-45, OTM-50, and Method 0010. Accuracy, precision, and completeness objectives were met across all methods. Data qualifiers were applied where applicable, and full validation details are provided in Appendices L-2 through L-4. Overall, the data quality is acceptable for its intended use.

9.3.6.2.1 OTM-45

Overall, the data quality objectives for accuracy, precision, and completeness were met for EPA OTM-45 data. Qualifiers for data quality were applied to the data where HFPO-DA was reported as exceeding the calibration range for the Dilution Factor 1. Detailed information on the data quality assessment is included in the Data Validation Report (Appendix L-2) for EPA OTM-45 data. Data without qualifiers and data qualified as estimated are usable for the purpose of supporting project objectives. All validated data were also found to be representative and comparable for all samples collected and analyzed for EPA Method OTM-45. The data quality for the OTM-45 analysis is deemed acceptable for its intended use.

9.3.6.2.2 OTM-50

Overall, the data quality objectives for accuracy, precision, and completeness were met for EPA OTM-50 data. Qualifiers for data quality were applied to the data related to information provided by the laboratory concerning the independent CO_2 bias test and continuous calibration verification percent differences exceeding the 20 percent criteria. Detailed information on the data quality assessment is included in the Data Validation Report (Appendix L-3) for EPA OTM-50 data. Data without qualifiers and data qualified as estimated are usable for the purpose of supporting project objectives. All validated data were also found to be representative and comparable for all samples collected and analyzed for EPA Method OTM-50. The data quality for the OTM-50 analysis is deemed acceptable for its intended use.

9.3.6.2.3 Method 0010

Overall, the data quality objectives for accuracy, precision, and completeness were met for EPA Method 0010 data. Qualifiers for data quality were applied to the impinger fraction sample data for low surrogate recoveries of an acid extractable surrogate and low surrogate recoveries for specified analytes. Qualifiers were also applied to phenol results for two back-half fraction samples associated with low level phenol results reported in the blank train sample. Detailed information on the data quality assessment is included in the Data Validation Report (Appendix L-4) for EPA Method 0010 data. Data without qualifiers and data qualified as estimated are usable for the purpose of supporting project objectives. All validated data were also found to be representative and comparable for all samples collected and analyzed for EPA Method 0010. The data quality for the M0010 analysis is deemed acceptable for its intended use.

9.3.7 Data Usability Summary

Overall, the data are viable and usable for addressing the stated objectives of this test. Data without qualifiers and data qualified as estimated are usable for purposes supporting project objectives. Validated data were also found to be representative and comparable for all samples.

10 Mass Balance, DRE, and DE Calculations

10.1 PFAS Mass Balance Calculations

A PFAS mass balance around the incineration system is presented in Appendix A-1, Table A-1-2. A mass flow rate for each analyte in each stream was calculated as the measured concentration of the analyte times the mass flow rate of the stream in lb/hr. For liquid streams, analytical results were reported in units of ng/L. Therefore, measured flow rates in lb/hr were converted to flows in liters/hour using density factors for the liquid streams as reported by the CHES Aragonite Laboratory. The density factors for each liquid stream are presented in Appendix I. Mass balance calculations for process streams are presented in Appendix A-1, Table A-1-13 through Table A-1-16. Mass balance calculations for exhaust emissions are presented in Appendix A-1, Table A-1-17 through Table A-1-19.

The relative amounts of total PFAS in each process stream and the exhaust emissions are shown for Run 1A on Figure 10-1. Similar graphs for Runs 2A and Run 3A are almost identical to Figure 10-1 and are presented in Appendix A-1, Figure A-1-2 and Figure A-1-3. Figure 10-1 should be interpreted with the following qualifiers and observations:

- The streams included in the mass balance are waste liquid feeds, treatment chemicals, process water, process residues, and exhaust emissions.
- All process and exhaust emissions streams except waste feed solids were accounted for in developing the PFAS mass balances. Komar solids, containerized solids, and bulk solids were fed during the test program but were not sampled and analyzed for PFAS due to waste non-homogeneity. Therefore, PFAS feed rates in these solids streams could not be calculated and these streams are not shown on Figure 10-1. As previously discussed, excluding the potential PFAS contributions from the solid waste streams likely biases the PFAS mass feed rate low which in turn biases the DRE results low.
- Process water flow rate was not measured directly because there is no flow meter; however, the flow rate was calculated by a mass and energy balance as described in Section 3.2.
- Brine is an internally recycled stream and does not contribute to PFAS mass flow into or out of the battery limits of the incineration process. Therefore, this stream is not shown on Figures 10-1.
- The total PFAS mass balance for all process streams includes the forty PFAS target analytes reported by EPA Method 1633. An additional nine analytes are reported by OTM-45 for exhaust emissions samples and are included in the mass balance. Therefore, the mass balances for the process streams and the exhaust emissions streams are not on the same basis in terms of the specific analytes included. However, these nine additional analytes comprised only 1.99 percent, 0.12 percent, and 2.41 percent of the total PFAS in the exhaust emissions during Run 1A, 2A, and 3A, respectively.
- The tanker direct burn stream was comprised of AFFF concentrate and had high concentrations of many PFAS, particularly PFOS and PFHxS. This stream contributed approximately 78 to 82 percent of the total PFAS mass fed to the incineration system during the test program.

- The PFAS spiking compounds comprised approximately 18 to 22 percent of the total PFAS mass fed to the system during the test program.
- All other waste feed streams (excluding the AFFF and PFAS spikes) that were sampled and analyzed accounted for from 0.001 to 0.003 percent of the total PFAS input.
- Based on the reported HFPO-DA analytical results, 78, 97, and 81 percent of the total PFAS exhaust emissions are comprised of HFPO-DA for Run 1A, 2A, and 3A, respectively. As previously noted in Section 8.3.4 and discussed in detail in Appendix K, most of the exhaust emissions analytical results for HFPO-DA are plausibly due to contamination from an external source and not actual emissions from the incinerator.

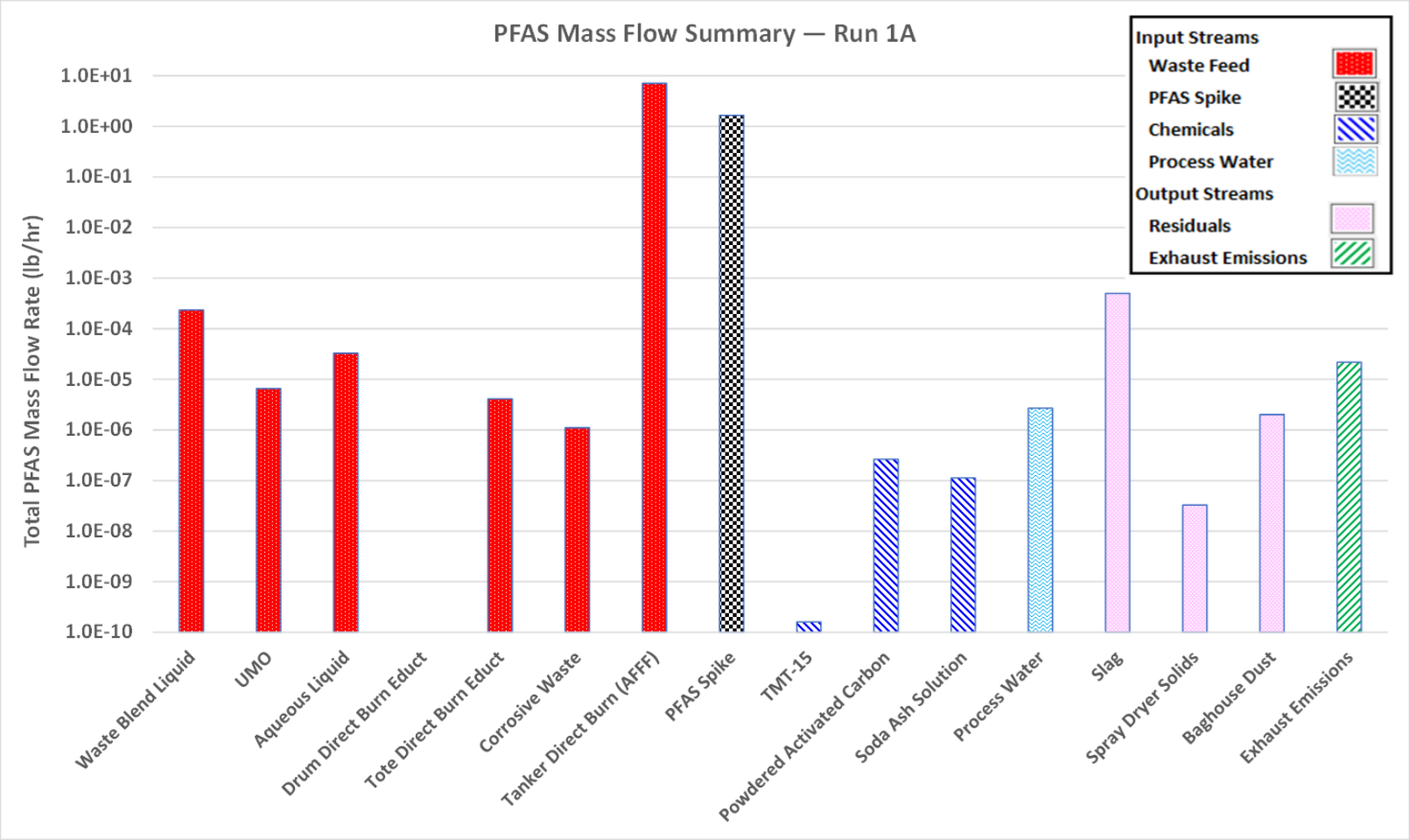


Figure 10-1. Total PFAS Mass Balance – Run 1A

10.2 PFAS Destruction and Removal Efficiency Calculations

One of the strategic objectives for the test program was to spike sufficient masses of the nine PFAS to be able to demonstrate up to 99.9999 percent DRE. To demonstrate DRE at this level, the mass of a PFAS target analyte in the waste feed is required to be at least 1,000,000 times the mass that can be detected in the exhaust emissions at the analytical MDL. This section describes the formulas that were used to calculate DRE values and summarizes the DRE results.

DRE was calculated using Equation 1 and definitions specified in 40 CFR §264.343:

$$DRE = \{1 - (w_{out} / w_{in})\} \times 100 \text{ percent} \qquad \text{(Equation 1)}$$

where:

- W_{in} = mass feed rate of one POHC in the waste streams feeding the incinerator
- W_{out} = mass emission rate of the same POHC present in exhaust emissions prior to release to the atmosphere.

For this report, a POHC is an individual PFAS analyte. Note that PFAS present in treatment chemicals or process water are not included in the W_{in} component of the DRE calculation, since they are not waste feeds and are introduced to the process downstream of the combustion systems. Likewise, PFAS in process residuals are not included in the W_{out} component of the DRE calculation since they are not released to the atmosphere. However, these other mass flows are included in a separate DE calculation that is presented in Section 10.3.

In addition to the spiked PFAS, DRE values were also calculated for the other EPA Method 1633 target analytes for which there was sufficient PFAS mass in the waste feed materials to demonstrate a minimum DRE value of 99.99 percent at the exhaust emissions analytical detection limit. As described in EPA/625/6-89/019, Guidance on Setting Permit Conditions and Reporting Trial Burn Results (January 1989), Section 4.2.1, one of the requirements for planning a DRE test is to ensure that sufficient levels of POHCs are fed so that 99.99 percent DRE can be detected. DRE values were calculated for any analyte for which there was sufficient mass in the waste feed materials to demonstrate a minimum DRE value of 99.99 percent based on the analytical detection limit in the exhaust emissions. A feed sufficiency test was implemented to determine if sufficient mass of each EPA 1633 target analyte was present in the waste feed materials to demonstrate the following levels of DRE:

- 99.99 percent (mass in feed 10,000 times the mass in the exhaust emissions at the analytical detection limit)
- 99.999 percent (mass in feed 100,000 times the mass in the exhaust emissions at the analytical detection limit)
- 99.9999 (mass in feed 1,000,000 times the mass in the exhaust emissions at the analytical detection limit)

The detailed results of the feed sufficiency test are presented in Table 10-1 and the results are summarized below:

- 99.99 percent DRE 16 target analytes
- 99.999 percent DRE 15 target analytes
- 99.9999 percent DRE 11 target analytes

All nine spiked compounds passed the feed sufficiency test at the 99.9999 percent level.

Sixteen target analytes passed the feed sufficiency test at a minimum DRE value of 99.99 percent. This included all PFCAs with 10 carbon atoms or less, all PFSAs, and HFPO-DA. The other 24 target analytes from EPA 1633 did not pass the feed sufficiency test at the 99.99 percent DRE level. In addition, there were nine additional PFAS that are target analytes for OTM-45 but are not target analytes for EPA 1633 for which DRE values could not be calculated because there is no analytical data available for the feed materials.

DRE results for these 16 target analytes are presented in Table 10-2 as Case 1. DRE values range from 99.95 percent up to greater than 99.9999 percent. There are 48 DRE values reported (16 target analytes times 3 runs). 34 of the DRE results are the highest value that can theoretically be demonstrated based on the feed sufficiency test results presented in Table 10-1. Only one DRE value less than 99.99 percent was calculated (HFPO-DA). As discussed in Section 8.3.4, it is plausible that HFPO-DA measured in the emissions is contamination from an external source and not derived from the incinerator off-gas. Therefore, the DRE result for this compound is likely biased low.

In all cases, DRE values were calculated using the most conservative possible assumptions. Analytes that were not detected in the feed materials are included as zero values in the waste feed material mass balance. Analytes that were not detected in the exhaust emissions samples are assumed to be present at the MDL in the mass balance. Exhaust emissions concentrations were not blank corrected, even though detectable amounts of 10 analytes were reported in one or more analytical fractions of the STPB. In addition, PFAS inputs from the bulk and containerized solid waste feed streams to the incinerator could not be measured due to waste heterogeneity and containerization. Therefore, any PFAS that were in the solid waste streams are not included in the DRE calculations. As a result of this group of conservative assumptions, it is likely that the actual DRE values are greater than the calculated values presented in this report. This set of assumptions was used to perform all calculations reported in this document, with the one exception of the sensitivity study discussed in the next paragraph.

A DRE sensitivity analysis was conducted using the MDL in DRE calculations for process samples which had non-detect analytical results. The results of this analysis are shown in Table 10-3 as Case 2. This analysis shows that all but three DRE values exceed 99.99 percent. For analytes which met the 99.99 percent feed sufficiency test shown in Table 10-1, there is virtually no difference in DRE values between Case 1 (Table 10-2) and Case 2 (Table 10-3). This is because these compounds have significant amounts of PFAS detected in the waste feed streams and the added mass from including non-detects in the exhaust emissions is insignificant. Conversely, for those analytes that did not meet the 99.99 percent feed sufficiency test, the mass contributing by using the MDL instead of zero for non-detect is significant and greatly increases the number of compounds meeting the 99.99 percent feed sufficiency test and increases DRE values compared to Case 1.

Table 10-1. Destruction and Removal Efficiency PFAS Feed Sufficiency Test Results

PFAS Class	CAS No.	Acronym	>99.9999% DRE?[a] Run 1A	>99.9999% DRE?[a] Run 2A	>99.9999% DRE?[a] Run 3A	>99.999% DRE?[b] Run 1A	>99.999% DRE?[b] Run 2A	>99.999% DRE?[b] Run 3A	>99.99% DRE?[c] Run 1A	>99.99% DRE?[c] Run 2A	>99.99% DRE?[c] Run 3A
PFCAs	375-22-4	PFBA	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
PFCAs	2706-90-3	PFPeA	No	No	No	Yes	Yes	Yes	Yes	Yes	Yes
PFCAs	307-24-4	PFHxA	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
PFCAs	375-85-9	PFHpA	No	No	No	Yes	Yes	Yes	Yes	Yes	Yes
PFCAs	335-67-1	PFOA	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
PFCAs	375-95-1	PFNA	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
PFCAs	335-76-2	PFDA	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
PFCAs	2058-94-8	PFUnA	No	No	No	No	No	No	No	No	No
PFCAs	307-55-1	PFDoA	No	No	No	No	No	No	No	No	No
PFCAs	72629-94-8	PFTriA	No	No	No	No	No	No	No	No	No
PFCAs	376-06-7	PFTeA	No	No	No	No	No	No	No	No	No
PFCAs	67905-19-5	PFHxDA	No	No	No	No	No	No	No	No	No
PFCAs	16517-11-6	PFODA	No	No	No	No	No	No	No	No	No
PFSAs	375-73-5	PFBS	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
PFSAs	2706-91-4	PFPeS	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
PFSAs	355-46-4	PFHxS	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
PFSAs	375-92-8	PFHpS	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
PFSAs	1763-23-1	PFOS	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
PFSAs	68259-12-1	PFNS	No	No	No	Yes	Yes	Yes	Yes	Yes	Yes
PFSAs	335-77-3	PFDS	No	No	No	Yes	Yes	Yes	Yes	Yes	Yes
PFSAs	79780-39-5	PFDoS	No	No	No	No	No	No	Yes	Yes	Yes
FOSAs	754-91-6	FOSA	No	No	No	No	No	No	No	No	No
FOSAs	31506-32-8	NMeFOSA	No	No	No	No	No	No	No	No	No
FOSAs	4151-50-2	NEtFOSA	No	No	No	No	No	No	No	No	No
FOSAAs	2355-31-9	NMeFOSAA	No	No	No	No	No	No	No	No	No
FOSAAs	2991-50-6	NEtFOSAA	No	No	No	No	No	No	No	No	No
FOSEs	24448-09-7	N-MeFOSE	No	No	No	No	No	No	No	No	No
FOSEs	1691-99-2	N-EtFOSE	No	No	No	No	No	No	No	No	No
FTSs	757124-72-4	4:2 FTS	No	No	No	No	No	No	No	No	No
FTSs	27619-97-2	6:2 FTS	No	No	No	No	No	No	No	No	No
FTSs	39108-34-4	8:2 FTS	No	No	No	No	No	No	No	No	No
FTSs	120226-60-0	10:2 FTS	No	No	No	No	No	No	No	No	No
FRCs	13252-13-6	HFPO-DA	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes
FRCs	919005-14-4	DONA	No	No	No	No	No	No	No	No	No
FRCs	756426-58-1	9Cl-PF3ONS	No	No	No	No	No	No	No	No	No
FRCs	763051-92-9	11Cl-PF3OUdS	No	No	No	No	No	No	No	No	No
ATAs	377-73-1	PFECA F	No	No	No	No	No	No	No	No	No
ATAs	863090-89-5	PFECA A	No	No	No	No	No	No	No	No	No
ATAs	151772-58-6	PFECA B	No	No	No	No	No	No	No	No	No
ATAs	113507-82-7	PES	No	No	No	No	No	No	No	No	No
ATAs	356-02-5	3:3 FTCA	No	No	No	No	No	No	No	No	No
ATAs	914637-49-3	5:3 FTCA	No	No	No	No	No	No	No	No	No
ATAs	812-70-4	7:3 FTCA	No	No	No	No	No	No	No	No	No
ATAs	70887-84-2	8:2 FTUCA	No	No	No	No	No	No	No	No	No
ATAs	53826-13-4	10:2 FTCA	No	No	No	No	No	No	No	No	No
ATAs	27854-31-5	8:2 FTCA	No	No	No	No	No	No	No	No	No
ATAs	53826-12-3	6:2FTCA	No	No	No	No	No	No	No	No	No
ATAs	133201-07-7	PFECHS	No	No	No	No	No	No	No	No	No
ATAs	70887-88-6	6:2 FTUCA	No	No	No	No	No	No	No	No	No
NA	NA	Sum of "No" Values	38	39	38	34	34	34	33	33	33
NA	NA	Sum of "Yes" Values	11	10	11	15	15	15	16	16	16

Notes:
(a) Was the Total Waste Feed and Spike PFAS Input Rate Adequate to Demonstrate >99.9999% DRE at the Exhaust Emissions Analytical Detection Limit?
(b) Was the Total Waste Feed and Spike PFAS Input Rate Adequate to Demonstrate >99.999% DRE at the Exhaust Emissions Analytical Detection Limit?
(c) Was the Total Waste Feed and Spike PFAS Input Rate Adequate to Demonstrate >99.99% DRE at the Exhaust Emissions Analytical Detection Limit?
Yellow shading = compound spiked during Runs 1A, 2A, 3A
Blue shading = not target analytes for EPA Method 1633; DRE cannot be calculated.
Green shading = cell contents equal “Yes”
% = percent
NA = not applicable

Table 10-2. Destruction and Removal Efficiency Summary — Case 1

PFAS Class	CAS No.	Acronym	Run 1A DRE[a,b,c] (%)	Run 2A DRE[a,b,c] (%)	Run 3A DRE[a,b,c] (%)
PFCAs	375-22-4	PFBA	99.999887	99.999898	99.999922
PFCAs	2706-90-3	PFPeA	99.994552	99.996657	99.998918
PFCAs	307-24-4	PFHxA	99.999869	99.999957	99.999958
PFCAs	375-85-9	PFHpA	99.998439	99.998268	99.999230
PFCAs	335-67-1	PFOA	99.999942	99.999913	99.999954
PFCAs	375-95-1	PFNA	99.999893	99.997660	99.999937
PFCAs	335-76-2	PFDA	99.999927	99.999923	99.999936
PFCAs	2058-94-8	PFUnA	---	---	---
PFCAs	307-55-1	PFDoA	---	---	---
PFCAs	72629-94-8	PFTriA	---	---	---
PFCAs	376-06-7	PFTeA	---	---	---
PFSAs	375-73-5	PFBS	99.999901	99.999910	99.999883
PFSAs	2706-91-4	PFPeS	99.999991	99.999994	99.999990
PFSAs	355-46-4	PFHxS	99.999998	99.999999	99.999998
PFSAs	375-92-8	PFHpS	99.999975	99.999979	99.999974
PFSAs	1763-23-1	PFOS	99.999999	99.999999	99.999999
PFSAs	68259-12-1	PFNS	99.999663	99.999782	99.999621
PFSAs	335-77-3	PFDS	99.999165	99.999373	99.999150
PFSAs	79780-39-5	PFDoS	99.998107	99.997866	99.997751
FOSAs	754-91-6	FOSA	---	---	---
FOSAs	31506-32-8	NMeFOSA	---	---	---
FOSAs	4151-50-2	NEtFOSA	---	---	---
FOSAAs	2355-31-9	NMeFOSAA	---	---	---
FOSAAs	2991-50-6	NEtFOSAA	---	---	---
FOSEs	24448-09-7	N-MeFOSE	---	---	---
FOSEs	1691-99-2	N-EtFOSE	---	---	---
FTSs	757124-72-4	4:2 FTS	---	---	---
FTSs	27619-97-2	6:2 FTS	---	---	---
FTSs	39108-34-4	8:2 FTS	---	---	---
FRCs	13252-13-6	HFPO-DA	99.997319	99.951677	99.997966
FRCs	919005-14-4	DONA	---	---	---
FRCs	756426-58-1	9Cl-PF3ONS	---	---	---
FRCs	763051-92-9	11Cl-PF3OUdS	---	---	---
ATAs	377-73-1	PFECA F	---	---	---
ATAs	863090-89-5	PFECA A	---	---	---
ATAs	151772-58-6	PFECA B	---	---	---
ATAs	113507-82-7	PES	---	---	---
ATAs	356-02-5	3:3 FTCA	---	---	---
ATAs	914637-49-3	5:3 FTCA	---	---	---
ATAs	812-70-4	7:3 FTCA	---	---	---

Notes:
(a) Case 1: ND = 0 for Process Streams, ND = MDL for Exhaust Emissions
(b) Conditional Formatting Legend for DRE Values:

99.99995	DE > 99.9999%
99.99950	99.999% < DE < 99.9999%
99.99500	99.99% < DE < 99.999%
99.95000	DE < 99.99%

(c) DRE reported for compounds for which the mass of PFAS in the feed was sufficient to demonstrate >99.99% DRE as shown in Table 10-1.
Yellow shading = compound spiked during Runs 1A, 2A, 3A
--- = not analyzed
% = percent

Table 10-3. Destruction and Removal Efficiency Summary — Case 2

PFAS Class	CAS No.	Acronym	Run 1A DRE[(a,b,c)] (%)	Run 2A DRE[(a,b,c)] (%)	Run 3A DRE[(a,b,c)] (%)
PFCAs	375-22-4	PFBA	99.999887	99.999898	99.999922
PFCAs	2706-90-3	PFPeA	99.994552	99.996657	99.998918
PFCAs	307-24-4	PFHxA	99.999869	99.999957	99.999958
PFCAs	375-85-9	PFHpA	99.998440	99.998268	99.999230
PFCAs	335-67-1	PFOA	99.999942	99.999913	99.999954
PFCAs	375-95-1	PFNA	99.999896	99.997734	99.999939
PFCAs	335-76-2	PFDA	99.999929	99.999925	99.999937
PFCAs	2058-94-8	PFUnA	99.997147	99.867824	99.997392
PFCAs	307-55-1	PFDoA	99.998306	99.996058	99.997957
PFCAs	72629-94-8	PFTriA	99.997898	99.841050	99.997847
PFCAs	376-06-7	PFTeA	99.998202	99.996563	99.998066
PFSAs	375-73-5	PFBS	99.999901	99.999910	99.999883
PFSAs	2706-91-4	PFPeS	99.999991	99.999994	99.999990
PFSAs	355-46-4	PFHxS	99.999998	99.999999	99.999998
PFSAs	375-92-8	PFHpS	99.999975	99.999979	99.999974
PFSAs	1763-23-1	PFOS	99.999999	99.999999	99.999999
PFSAs	68259-12-1	PFNS	99.999663	99.999782	99.999621
PFSAs	335-77-3	PFDS	99.999165	99.999373	99.999150
PFSAs	79780-39-5	PFDoS	99.998107	99.997866	99.997752
FOSAs	754-91-6	FOSA	99.998145	99.998588	99.997976
FOSAs	31506-32-8	NMeFOSA	99.997305	99.997956	99.996988
FOSAs	4151-50-2	NEtFOSA	99.996727	99.997712	99.996657
FOSAAs	2355-31-9	NMeFOSAA	99.997626	99.998368	99.997594
FOSAAs	2991-50-6	NEtFOSAA	99.997981	99.998536	99.997727
FOSEs	24448-09-7	N-MeFOSE	99.996290	99.996577	99.992429
FOSEs	1691-99-2	N-EtFOSE	99.999487	99.999600	99.999431
FTSs	757124-72-4	4:2 FTS	99.999333	99.999541	99.999280
FTSs	27619-97-2	6:2 FTS	99.991434	99.992688	---

PFAS Class	CAS No.	Acronym	Run 1A DRE[a,b,c] (%)	Run 2A DRE[a,b,c] (%)	Run 3A DRE[a,b,c] (%)
FTSs	39108-34-4	8:2 FTS	99.998717	99.999176	99.998751
FRCs	13252-13-6	HFPO-DA	99.997322	99.951749	99.997968
FRCs	919005-14-4	DONA	99.994224	99.995364	99.993909
FRCs	756426-58-1	9Cl-PF3ONS	99.998678	99.999085	99.998589
FRCs	763051-92-9	11Cl-PF3OUdS	99.997305	99.997865	99.996697
ATAs	377-73-1	PFECA F	99.995834	99.996920	99.995746
ATAs	863090-89-5	PFECA A	99.996550	99.997923	99.996767
ATAs	151772-58-6	PFECA B	99.991584	99.998666	99.998012
ATAs	113507-82-7	PES	99.997632	99.998527	99.997714
ATAs	356-02-5	3:3 FTCA	99.997676	99.998460	99.997615
ATAs	914637-49-3	5:3 FTCA	99.998675	99.998883	99.998504
ATAs	812-70-4	7:3 FTCA	99.999025	99.999314	99.998918

Notes:

(a) Case 2: ND = MDL for Process Streams, ND = MDL for Exhaust Emissions

(b) Conditional Formatting Legend for DRE Values:

99.99995	DE > 99.9999%
99.99950	99.999% < DE < 99.9999%
99.99500	99.99% < DE < 99.999%
99.95000	DE < 99.99%

(c) DRE reported for compounds for which the mass of PFAS in the feed was sufficient to demonstrate >99.99% DRE as shown in Table 10-1.

Yellow shading = compound spiked during Runs 1A, 2A, 3A

--- = not analyzed

% = percent

10.3 PFAS Destruction Efficiency Calculations

DE is not a term that is defined in the U.S. hazardous waste regulations. For this report, DE calculations have been developed to indicate the degree of destruction of each PFAS based on its total mass entering the system in all waste feeds, process chemicals, and process water, and the total out of the system in process residual streams and exhaust emissions. The intent of the DE calculation is to assess how much PFAS is destroyed, not just transferred from one media to another media. DE was calculated using Equation 2.

$$DE = \{1 - [(W_{out} + R_{out}) / (W_{in} + C_{in} + P_{in})]\} \times 100 \qquad \text{(Equation 2)}$$

where:

- W_{in} = mass feed rate of one POHC in the waste streams feeding the incinerator
- C_{in} = mass feed rate of the same POHC in treatment chemical feeds (powdered activated carbon, Na_2CO_3 solution, TMT-15
- P_{in} = mass flow rate of POHC in process water feed
- R_{out} = mass flow rate of POHC out in residual streams (slag/ash, spray dryer solids, baghouse dust)
- W_{out} = mass emission rate of the same POHC present in exhaust emissions prior to release to the atmosphere

A summary of the DE Results is shown in Table 10-4 for Case 1. Detailed DE calculations for all EPA Method 1633 target analytes are presented in Appendix A-1, Table A-1-8 through Table A-1-12. DE values ranged from large negative values to greater than 99.9999 percent. Analytes which have low or negative DE values also had very low concentrations of the analyte in the waste feed materials. The low or negative DE values are primarily a result of how non-detects were handled in calculations, with non-detects for process steams using a zero-concentration value in calculations and using the MDLs for non-detects for exhaust emissions samples.

The DE calculations were repeated, using MDLs for non-detects for both process samples and exhaust emissions samples (Case 2). These results are presented in Table 10-5 and show that the DE values ranged from greater than 99.85 percent up to greater than 99.99 percent. The wide variation in the DE results presented in Table 10-4 and Table 10-5 indicate that DE results are only meaningful if there is a significant mass of PFAS in the waste feed materials to the incinerator.

10.4 C_2F_6 Destruction Removal Efficiency Calculations

During Runs 1B, 2B, and 3B, the incinerator was run with normal waste feeds but without either AFFF waste feed or PFAS spiking. The intent was to create low C_2F_6 background samples to compare to concentrations measured during Runs 1C, 2C, and 3C when C_2F_6 was spiked to the rotary kiln to determine DRE for the spiked C_2F_6. However, the concentration of C_2F_6 was non-detect in all sample canisters from Runs 1B, 2B, 3B, 1C, 2C, and 3C. Non-detect C_2F_6 concentrations were obtained from both

the Eurofins and EPA laboratory analyses of OTM-50 samples. Therefore, no background correction was required to be made for the calculation of DRE values for the spiked C_2F_6.

Three different sets of DRE values were calculated for C_2F_6. All three sets of DRE values used the amount of spiked C_2F_6 as the POHC value. DRE values were calculated using C_2F_6 stack emission rates using OTM-50 data from EPA and Eurofins and the concentrations measured by Spectrum with an FTIR. The average MDL reported by EPA was 0.024 ppb, the average MDL reported by Eurofins was 0.094 ppb, and the MDC # 3 (determination of analytical algorithm errors per ASTM D6348-12 Section A2.3) for C_2F_6 measured by FTIR was 0.62 ppb. Based on these MDL and MDC values, C_2F_6 DRE values of greater than 99.9999 percent, greater than 99.999 percent, and greater than 99.99 percent were calculated using the EPA, Eurofins, and Spectrum data, respectively. These DRE calculations are presented in Table 10-6. These data indicate that C_2F_6 can be destroyed at a very high efficiency at the normal incinerator operating conditions.

Table 10-4. Destruction Efficiency Summary — Case 1

PFAS Class	CAS No.	Acronym	Run 1A DE[(a,b)] (%)	Run 2A DE[(a,b)] (%)	Run 3A DE[(a,b)] (%)
PFCAs	375-22-4	PFBA	99.9753	99.9693	99.9875
PFCAs	2706-90-3	PFPeA	99.9946	99.9893	99.9989
PFCAs	307-24-4	PFHxA	99.9983	99.9977	99.9991
PFCAs	375-85-9	PFHpA	99.9984	99.9983	99.9992
PFCAs	335-67-1	PFOA	99.9976	99.9968	99.9993
PFCAs	375-95-1	PFNA	99.9940	99.9882	99.9999
PFCAs	335-76-2	PFDA	99.9929	99.9943	99.9999
PFCAs	2058-94-8	PFUnA	-401.5992	---	---
PFSAs	375-73-5	PFBS	99.9894	99.9803	99.9965
PFSAs	2706-91-4	PFPeS	99.9967	99.9947	99.9985
PFSAs	355-46-4	PFHxS	99.9918	99.9934	99.9984
PFSAs	375-92-8	PFHpS	99.9977	99.9946	99.9988
PFSAs	1763-23-1	PFOS	99.9958	99.9931	99.9982
PFSAs	68259-12-1	PFNS	99.9997	99.9998	99.9996
PFSAs	335-77-3	PFDS	99.9592	99.9994	99.9992
PFSAs	79780-39-5	PFDoS	99.9981	99.9979	99.9978
PFSAs	754-91-6	FOSA	-85.9725	-13994.9209	-6993.6646
FOSAs	31506-32-8	NMeFOSA	---	---	-1.7382
FOSAs	4151-50-2	NEtFOSA	---	---	---
FOSAs	2355-31-9	NMeFOSAA	---	---	81.7836
FOSAAs	2991-50-6	NEtFOSAA	98.7049	---	98.9529
FOSAAs	24448-09-7	N-MeFOSE	97.7046	96.1175	10.8533
FOSEs	1691-99-2	N-EtFOSE	97.2881	97.2630	91.3209
FOSEs	757124-72-4	4:2 FTS	---	---	---
FTSs	27619-97-2	6:2 FTS	37.3318	31.4358	69.3331
FTSs	39108-34-4	8:2 FTS	88.8651	-24.8873	92.5904
FTSs	13252-13-6	HFPO-DA	99.9956	99.9452	99.9978
FRCs	919005-14-4	DONA	---	---	---
FRCs	756426-58-1	9Cl-PF3ONS	---	---	---
FRCs	763051-92-9	11Cl-PF3OUdS	---	---	---
FRCs	377-73-1	PFECA F	90.9523	92.8522	94.0378
ATAs	863090-89-5	PFECA A	-1797.4660	---	---
ATAs	151772-58-6	PFECA B	---	---	---
ATAs	113507-82-7	PES	---	---	---
ATAs	356-02-5	3:3 FTCA	---	95.1570	---
ATAs	914637-49-3	5:3 FTCA	98.7141	98.7430	92.3704
ATAs	812-70-4	7:3 FTCA	---	---	---

Notes:

(a) Case 1: ND = 0 for Process Streams, ND = MDL for Exhaust Emissions

(b) Conditional Formatting Legend:

99.995 DE > 99.99%

99.95 99.9% < DE < 99.99%

99.5 99% < DE < 99.9%
99 DE < 99%
Yellow shading = compound spiked during Runs 1A, 2A, 3A
--- = not analyzed
% = percent

Table 10-5. Destruction Efficiency Summary — Case 2

PFAS Class	CAS No.	Acronym	Run 1A DE[(a,b)] (%)	Run 2A DE[(a,b)] (%)	Run 3A DE[(a,b)] (%)
PFCAs	375-22-4	PFBA	99.9753	99.9692	99.9874
PFCAs	2706-90-3	PFPeA	99.9905	99.9883	99.9943
PFCAs	307-24-4	PFHxA	99.9982	99.9976	99.9990
PFCAs	375-85-9	PFHpA	99.9948	99.9945	99.9944
PFCAs	335-67-1	PFOA	99.9974	99.9966	99.9992
PFCAs	375-95-1	PFNA	99.9935	99.9879	99.9973
PFCAs	335-76-2	PFDA	99.9926	99.9939	99.9980
PFCAs	2058-94-8	PFUnA	99.9012	99.7759	99.8819
PFSA	375-73-5	PFBS	99.9893	99.9802	99.9965
PFSAs	2706-91-4	PFPeS	99.9966	99.9945	99.9983
PFSAs	355-46-4	PFHxS	99.9918	99.9934	99.9983
PFSAs	375-92-8	PFHpS	99.9976	99.9944	99.9986
PFSAs	1763-23-1	PFOS	99.9958	99.9931	99.9982
PFSAs	68259-12-1	PFNS	99.9751	99.9802	99.9735
PFSAs	335-77-3	PFDS	99.9512	99.9676	99.9613
PFSAs	79780-39-5	PFDoS	99.9418	99.9217	99.9225
PFSAs	754-91-6	FOSA	99.8773	99.8678	99.8248
FOSAs	31506-32-8	NMeFOSA	99.9013	99.9060	99.8815
FOSAs	4151-50-2	NEtFOSA	99.9008	99.9058	99.8812
FOSAs	2355-31-9	NMeFOSAA	99.9017	99.9064	99.8821
FOSAAs	2991-50-6	NEtFOSAA	99.9022	99.9066	99.8825
FOSAAs	24448-09-7	N-MeFOSE	99.9006	99.9048	99.8771
FOSEs	1691-99-2	N-EtFOSE	99.9036	99.9077	99.8841
FOSEs	757124-72-4	4:2 FTS	99.9034	99.9076	99.8841
FTSs	27619-97-2	6:2 FTS	99.8729	99.8810	99.8475
FTSs	39108-34-4	8:2 FTS	99.9028	99.9072	99.8835
FTSs	13252-13-6	HFPO-DA	99.9956	99.9452	99.9977
FRCs	919005-14-4	DONA	99.8983	99.9034	99.8785
FRCs	756426-58-1	9Cl-PF3ONS	99.9160	99.9198	99.8991
FRCs	763051-92-9	11Cl-PF3OUdS	99.8536	99.8602	99.8239
FRCs	377-73-1	PFECA F	99.9000	99.9050	99.8804
ATAs	863090-89-5	PFECA A	99.9007	99.9060	99.8814
ATAs	151772-58-6	PFECA B	99.9091	99.9196	99.8987
ATAs	113507-82-7	PES	99.9017	99.9066	99.8823
ATAs	356-02-5	3:3 FTCA	99.9018	99.9065	99.8825
ATAs	914637-49-3	5:3 FTCA	99.9029	99.9071	99.8833

PFAS Class	CAS No.	Acronym	Run 1A DE[(a,b)] (%)	Run 2A DE[(a,b)] (%)	Run 3A DE[(a,b)] (%)
ATAs	812-70-4	7:3 FTCA	99.9032	99.9074	99.8838

Notes:
(a) Case 2: ND = MDL for Process Streams, ND = MDL for Exhaust Emissions
(b) Conditional Formatting Legend:
99.995 DE > 99.99%
99.95 99.9% < DE < 99.99%
99.5 99% < DE < 99.9%
99 DE < 99%
Yellow shading = compound spiked during Runs 1A, 2A, 3A
--- = not analyzed
% = percent

Table 10-6. Hexafluoroethane (C_2F_6) DRE Calculations

Data Source	Parameter	Units	Run 1C	Run 2C	Run 3C	Notes and Data Sources
NA	Exhaust Emissions Flow Rate	scfm	34,095	32,101	33,040	Appendix H, Table 2-1
NA	Exhaust Emissions Flow Rate	lb-mol/hr	5,308	4,998	5,144	Calculation
NA	C_2F_6 Spiking Rate	lb/hr	12.40	44.07	44.64	Appendix F, Table 1
Eurofins (OTM-50)	C_2F_6 Emission Concentration	ppb	<0.0939	<0.0927	<0.0939	Eurofins EDD 140-39565-1[a]
Eurofins (OTM-50	C_2F_6 Emission Concentration	µg/m^3	<0.530	<0.523	<0.530	Eurofins EDD 140-39565-1[a]
Eurofins (OTM-50)	C_2F_6 Emission Rate	lb/hr	<6.63 E-05	<6.39 E-05	<6.49 E-05	Table A-2-4
Eurofins (OTM-50)	C_2F_6 DRE	%	>99.99947	>99.99985	>99.99985	Calculation
EPA (OTM-50)	C_2F_6 Emission Concentration	ppb	<0.024	<0.024	<0.024	EPA Spreadsheet[a]
EPA (OTM-50)	C_2F_6 Emission Concentration	µg/m^3	<0.14	<0.14	<0.14	Table A-2-8
EPA (OTM-50)	C_2F_6 Emission Rate	lb/hr	<1.75 E-05	<1.71 E-05	<1.71 E-05	Table A-2-8
EPA (OTM-50)	C_2F_6 DRE	%	>99.99986	>99.99996	>99.99996	Calculation
Spectrum (FTIR)	C_2F_6 Emission Concentration	ppb	<0.62	1.21	<0.62	Appendix G, Tables 1-1 and 1-3
Spectrum (FTIR)	C_2F_6 Emission Concentration	µg/m^3	<3.50	6.83	<3.50	Calculation
Spectrum (FTIR)	C_2F_6 Emission Rate	lb/hr	<4.54 E-04	8.34 E-04	<4.40 E-04	Calculation

Data Source	Parameter	Units	Run 1C	Run 2C	Run 3C	Notes and Data Sources
Spectrum (FTIR)	C_2F_6 DRE	%	>99.99634	99.99811	>99.99901	Calculation

Notes:
a. found in Appendix A-2
% = percent
EDD = Electronic Data Deliverable
lb-mol/hr = pound-mole(s) per hour
NA = not applicable

11 Dispersion Modeling Results

The EPA screening air dispersion model AERSCREEN was used to estimate the ambient air impacts from the exhaust emissions of 12 PFAS emitted from the CHES Aragonite incinerator stack during the testing. The complete dispersion modeling report is presented in Appendix M.

The 12 PFAS for which ambient impacts were modeled include PFBA, PFHxA, PFOA, PFNA, PFDA, PFDoA, PFBS, PFHxS, PFOS, FOSA, HPFO-DA and 6:2 FTS. These compounds are target analytes for OTM-45 and have ambient concentration guidelines or standards in one or more states. Regulatory standards or guidance values were identified for Michigan, Minnesota, New Jersey, New York, and Texas (Bryan 2025). It should be noted that establishing ambient air standards is an emerging area of PFAS regulation with some States, and there are currently no federal standards.

The dispersion modeling analysis was based on the average PFAS emission rates measured during Run 1A, 1B, and 1C. Safety factors are defined as the ambient regulatory standard or guideline divided by the modeled maximum ambient concentration. The dispersion modeling analysis indicates the safety factors for the 12 PFAS range from two to eight orders of magnitude.

12 Conclusions and Lessons Learned

12.1 Conclusions

The key conclusions from this test program include:

- HWIs can destroy PFAS, resulting in low concentrations in exhaust emissions, mostly below 25.0 ng/m^3, and process residues, below 101 ng/g.
    - 19 PFAS were detected in one or more exhaust emissions samples; concentrations were all below MDL for the remaining 30 compounds. Approximately 69 percent of PFAS exhaust emissions results for individual analytes were non-detect.
    - DRE values ranging from of 99.999 to greater than 99.9999 percent were achieved for all spiked PFAS except HFPO-DA, which ranged from 99.95 to 99.998 percent. The exhaust emissions samples for HFPO-DA had concentrations above background level and the resulting DRE values are likely biased low as a result of this. As described in Appendix K, the exhaust emission levels are likely due to external contamination.
    - DE values ranging from 99.94 percent to greater than 99.9999 percent were achieved for the nine PFAS spiking compounds. For PFAS that were not spiked, DE values ranged from large negative values to greater than 99.99 percent. The large negative DE values were calculated for PFAS that had concentrations below MDLs in several waste feed streams and for which zero values were used for the concentrations in the calculations. A sensitivity study was performed using the MDL instead of zero for non-detects, and these PFAS then had DE values ranging from 99.77 percent to greater than 99.99 percent.
- Eurofins' analyses had no VFCs above the MDL.
- Samples analyzed by EPA ORD had six compounds above the MDL, but below the reporting limit, with two were also in the blanks and are likely from the air used to pressurize the canisters.
- No fluorinated semi-volatile, nonpolar compounds were identified by EPA Method 0010 sampling and analysis.
- The DRE values for C_2F_6 ranged from greater than 99.99 to 99.9999 percent depending on the feed rate of C_2F_6 and the measurement method used.
    - OTM-50 analysis by EPA ORD for C_2F_6 DREs = >99.99986 to >99.99996
    - OTM-50 analysis by Eurofins for C_2F_6 DREs = >99.99947 to >99.99985
    - FTIR by Spectrum for C_2F_6 DREs = >99.99811 to >99.99901.
- Modeled ambient air concentrations were compared to regulatory standards or guidelines from five state regulatory agencies (Michigan, Minnesota, New Jersey, New York, and Texas). The analysis shows that the maximum ambient concentrations for the 12 PFAS that were modeled

range from two to eight orders of magnitude below known state ambient regulatory standards or guidelines.

12.1.1 Destruction and Removal Efficiency Demonstration

This test program and two previous test programs (Crisenbery et al. 2024) at the CHES Aragonite incinerator demonstrate that DRE values in the range of 99.999 percent to greater than 99.9999 percent are readily achievable if sufficient mass of POHC is available in the waste feed material. This generally requires spiking with a POHC, but for some analytes, native concentrations were sufficient to achieve DRE values in this range. The amount of PFAS spiking compound required to achieve the desired DRE can be calculated based on the MDL of the analyte in the exhaust emissions, the exhaust emissions flow rate, and the exhaust emissions sample volume.

12.1.2 Destruction Efficiency Demonstration

DE values in the range of 99 percent to greater than 99.999 percent were readily demonstrated when a sufficient mass of an analyte was available in the waste feed material, either by spiking or as a native concentration. However, for analytes that had low concentrations in the waste feed inputs, calculated DE values varied widely from large negative values if zero values were used for non-detects in calculations up to greater than 99.9 percent if MDLs were used for non-detects in DE calculations.

12.1.3 C_2F_6 as a Spiking Indicator Compound

DRE values in the range of 99.99 percent to greater than 99.9999 percent were demonstrated for C_2F_6. The level of DRE that could be demonstrated depended partially on the C_2F_6 spiking rate but more importantly on the MDL that was achieved by OTM-50 or the MDC achieved by FTIR. OTM-50 was able to achieve MDLs one to two orders of magnitude lower than the MDC determined by FTIR.

The DRE values achieved for C_2F_6 were similar (99.999 percent to greater than 99.9999 percent) to those achieved for spiked PFAS. The high DREs of PFAS, the absence of PIDs, and the high DRE of C_2F_6 indicate that the Aragonite incinerator and emission control system has a high efficacy for PFAS destruction. This result is another data point that demonstrates correlation between high C_2F_6 DREs and high incinerator performance. More data is needed to determine if a there is credence to the potential use of C_2F_6 as a POHC indicator for other PFAS. Implementation of this procedure may mitigate the complexity and cost of future tests at this facility to mainly include another verification that C_2F_6 can still be destroyed at these same levels.

12.1.4 CF_4 as a Potential PID

CF_4 was detected in seven of the nine EPA OTM-50 samples at concentrations near the detection limit and was not present in the blanks. CF_4 was also detected by FTIR during Runs 1A, 2A, and 1B. The presence of CF_4 near the detection limit provides some evidence of the production of a PID from the high concentrations of PFAS added to the kiln.

12.2 Lessons Learned

12.2.1 Problematic Destruction Efficiency Calculation — How to Avoid Negative Values

DE tests are best planned so that there is sufficient PFAS mass in the feed material to demonstrate the desired DE values. To demonstrate 99 percent DE, the mass of PFAS in the inlet streams must be at least

100 times the mass in the outlet streams. If DE tests are conducted without spiking PFAS, the native concentrations of PFAS in feed materials may be very low and result in low DE values. This issue is clearly illustrated in Table 10-5 which shows that DE values of greater than 99.9 percent were achieved for the nine compounds that were spiked and DE values of less than 99 percent were commonly reported for compounds that were not spiked. For the calculations in Table 10-5, a value of zero was used in DE calculations for analytes that were not detected in the input materials.

The DE calculations were repeated using the MDL value for those compounds that were below detection levels. These results are presented in Table 10-6 and show that all DE values exceed 99 percent. One conclusion from these analyses is that DE values are only valid if there is sufficient mass of the PFAS analyte in the feed material to demonstrate the desired level of DE. A second conclusion is that DE values are highly dependent on what values (zero or MDL) are used in calculations for analytical results that are below the detection limit.

12.2.2 Feed Characterization and Variability

Hazardous wastes fed to a large, commercial HWI vary widely in chemical and physical form, as well as instantaneous feed rates. Wastes include bulk solids, packaged solids, and bulk and packaged organic and inorganic liquid wastes. Daily operations at a commercial HWI are based on a “burn plan”, which factors in waste attributes such as heating value, halogen content, mineral ash levels, and material inventory, to ensure that the HWI can be operated consistently and predictably within permit limits, to attain the objective of complete destruction of the hazardous waste. Although each waste accepted for treatment is characterized consistent with regulatory requirements, many bulk and packaged solid waste have an inherent variability, creating short term fluctuations in the chemical and physical properties, particularly with respect to emerging contaminants such as PFAS. This, combined with the operational requirements of the burn plan, can result in fluctuations in feed properties. Hence, despite careful planning of destruction testing, including conservative assumptions and allowances, test results will typically reflect this inherent waste feed variability. Additionally, limitations of analytical methods, particularly for PFAS, can further constrain comprehensive waste characterization efforts. To enhance both waste characterization and fluorine mass balance assessments, incorporating complementary analytical approaches, such as total organic fluorine measurements, can be beneficial.

12.2.3 Stack Sampling Process and Glassware QA/QC

The stack sampling and analytical methods met the quality metrics in the methods and provided data of known and acceptable quality. However, the proof blank had HFPO-DA and some other PFAS in the extracts. The presence of PFAS in the proof blank does cast doubt on the presence of some PFAS in the exhaust emissions, such as HFPO-DA and in compliance tests would invalidate the tests. The search to try to find the cause of the contamination re-emphasized the cautions in OTM-45. The glassware, seals, O-rings, filter support materials, and other train and recovery components can be constructed of materials not mentioned in the method, however, they must be demonstrated to be free of the targeted PFAS. OTM-45 Rev 1 specifically prohibits the use of glass fritted filter supports for this reason. Appendix K provides data for the analyses of various parts of the train to help identify potential contamination. It was found that silicone O-rings, fritted glass filter supports, and other materials did have PFAS on them. This reiterates the importance of ensuring materials and recovery supplies do not contain PFAS, cleaning techniques ensure that potential PFAS contamination is removed, and proof and/or field sample train blanks and other equipment blanks should be collected for components that could be potential sources of contamination.

Additional blanks (e.g., proof blanks and field blanks) would be beneficial to determine whether contamination has occurred and to help ensure the trains are effectively recovered. Although additional blanks add to the time and costs of the testing, the additional data can be extremely valuable in confirming potential sample train contamination.

12.2.4 Data Validation Process Standards

The results of the analyses for this testing campaigns were reviewed for a data quality assessment based on the laboratory's (Eurofins) analytical method procedures, and the requirements outlined by DOD Data Validation Guidelines Module 6: Data Validation Procedure for PFAS Analysis by Quality Systems Manual for Environmental Laboratories Table B-24. With the complex nature of the developing methods for PFAS and the use of draft and OTM methods, the National Functional Guidelines for Organic Superfund Methods Data Review may better apply to the data validation than the DoD procedures. The Superfund guidelines allow for more flexibility with complex analytical methods and would help simplify the data validation.

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Appendices

The Appendices can be found at https://doi.org/10.23719/1532481 through data.gov. The files are too large to include in the body of this report and still make it readily accessible and transferable. Note: Some Appendix materials may not be fully Section 508-compliant.

Appendix A - Mass Balance, DRE, and Emission Calculations

Appendix B - Process Monitoring Data

Appendix C - Residual Streams Mass Flow Rate Data

Appendix D - Process Sampling Logs

Appendix E - PFAS Spiking Report

Appendix F - C_2F_6 Spiking Report

Appendix G - FTIR Monitoring Report

Appendix H - Source Testing Report

Appendix I - Clean Harbors Analytical Data Report

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Appendix L - Data Validation Reports

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Appendix N - Deviations from the Work Plan



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Comprehensive analysis of PFAS presence from environment to plate

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Per- and polyfluoroalkyl substances (PFAS) pose an emerging environmental risk impacting food products and ecosystems. This study analyzes over 150,000 entries from food safety authorities and scientific publications from 2017 onwards. Our findings show that fish & seafood, and biota have the highest PFAS concentrations due to environmental contamination and bioaccumulation. Surface water samples also frequently contain PFAS, raising concerns about long-term ecological and human health effects. Comprehensive strategies are essential to mitigate these risks.

PFAS (per- and polyfluoroalkyl substances) constitute a group of synthetic chemicals that find extensive application in both industrial and consumer products. They are commonly used in firefighting foam, non-stick cookware, waterproof fabrics, and food packaging, among others. One of the concerning characteristics of PFAS is their persistence and bioaccumulative nature. These chemicals do not readily degrade in the environment, leading to their long-lasting presence, and they can accumulate in the food chain. The persistence of PFAS means that they do not break down easily over time, even in the face of environmental degradation processes. As a result, PFAS can persist in soil, water, and air for extended periods, leading to widespread environmental contamination. The bioaccumulative nature of PFAS raises concerns about potential health risks for both humans and wildlife. Studies have linked exposure to certain PFAS compounds with adverse health effects, including reproductive issues, developmental problems, liver and kidney damage, and an increased risk of certain cancers[1–5].

The available information on PFAS (Per- and Polyfluoroalkyl Substances) health impacts is currently incomplete and fragmented, leading to difficulties in prioritizing which PFAS should be of greater environmental and health concern. There is an ongoing debate about whether to assess PFAS as a collective group from a health perspective or to evaluate individual PFAS based on their specific potential health impacts. PFAS can be categorized into two primary families: polymer and non-polymer PFAS[5]. Health concerns have predominantly been associated with non-polymer PFAS, which includes perfluorinated carboxylic acids (PFCAs) and perfluorinated sulfonic acids (PFSAs)[6].

The determination of PFAS in food products and the environment involves a series of analytical techniques, including sampling, extraction, and analysis. Conventional methods, such as LC-MS/MS, are effective in PFAS analysis but are often expensive and not widely accessible. On the other hand, emerging low-cost methods offer the advantage of affordability but lack sensitivity compared to conventional techniques, sometimes falling short of meeting regulatory detection limits. The development of low-cost sensors has the potential to be a significant change in PFAS analysis, as they can aid in identifying and prioritizing samples for further investigation and swift intervention[7].

Given the widespread occurrence of PFAS in various environmental media and their presence in a wide array of products, it is essential to address this issue comprehensively. Continuous monitoring, research, and regulatory efforts are necessary to minimize human exposure to PFAS and mitigate their potential health and environmental impact. Therefore, the objective of this review is to compile existing data on the occurrence of PFAS, including measured max and mean values, geographical origin, sampling strategies, contamination sources, and recovery values of the applied analytical methods. By gathering this comprehensive information, we aim to gain insights into the prevalence and distribution of PFAS compounds in various regions. This review aims to enhance our understanding of the worldwide prevalence of PFAS and their potential sources of contamination, explore the presence of PFAS compounds in food and environmental samples, and gain insights into the processes of bioaccumulation from the environment to the food chain through an in-depth analysis of publicly available data from public authorities and peer-reviewed literature.

Literature search

Search criteria and search strings

The search protocol used for identification of the relevant scientific publication and reports in Pubmed and ScienceDirect on PFAS in food, limited to the papers published in the last 5 years, contained the following strings:

("polyfluoroalkyl" OR "PFAS" OR "perfluoroalkyl") AND "sample" AND ("food" OR "feed" OR "supplements" OR "drinking water").

On the other hand, the search protocol used for identification of the relevant scientific publication on PFAS in environmental sources, limited to the studies published in the last 5 years, was:

("polyfluoroalkyl" OR "PFAS" OR "perfluoroalkyl") AND "sample" AND ("soil" OR "water" OR "aquatic environment" OR "land") AND

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Table 1 | List of data sources

Environmental samples
Water quality: https://www.eea.europa.eu/data-and-maps/data/waterbase-water-quality-icm
Water Appendix: http://chm.pops.int/portals/0/download.aspx?d=UNEP-POPS-GMP-RMR-WEOG-ANNEX-WaterAppendix2-2021.English.xlsx
Australian PFAS chemicals map: https://pfas.australianmap.net/2019-kilmore-wastewater-management-facility-victoria-pfas/
Occurrence and distribution of per-and polyfluoroalkyl substances (PFAS) in surface and groundwaters in an urbanized and agricultural area, Southern Brazil: https://link.springer.com/article/10.1007/s11356-022-22603-x
Measurement of per- and polyfluoroalkyl substances (PFAS) in Hematophagous Arthropods (Ticks) as a Novel Strategy to Monitor Environmental Contamination Levels: https://www.rsc.org/suppdata/d1/em/d1em00209k/d1em00209k1.pdf
Target and suspect screening of 4777 per- and polyfluoroalkyl substances (PFAS) in river water, wastewater, groundwater, and biota samples in the Danube River: Basin https://doi.org/10.1016/j.jhazmat.2022.129276
Comprehensive screening of polar emerging organic contaminants including PFASs and evaluation of the trophic transfer behavior in a freshwater food web https://pubmed.ncbi.nlm.nih.gov/35545009/
Per- and Polyfluoroalkyl-Contaminated Freshwater Impacts Adjacent Riparian Food Webs https://pubmed.ncbi.nlm.nih.gov/32870664/
Food samples
Results from the monitoring of veterinary medicinal product residues and other substances in live animals and animal products—Denmark https://zenodo.org/records/6275567#.Y2T9qdJByV4
Results from the monitoring of veterinary medicinal product residues and other substances in live animals and animal products—Cyprus https://zenodo.org/record/6275444#.Y2T9lNJByV4
Results from the monitoring of veterinary medicinal product residues and other substances in live animals and animal products—Germany https://zenodo.org/record/6275816#.Y2UCN9JByV5
Results from the monitoring of veterinary medicinal product residues and other substances in live animals and animal products—Norway https://zenodo.org/records/6276534#.Y2T9ktJByV4
Perfluoroalkyl acids in drinking water of China in 2017: distribution characteristics, influencing factors, and potential risks https://www.sciencedirect.com/science/article/pii/S0160412018316131
WHO GEMS food contaminants database: https://extranet.who.int/gemsfood/Search.aspx
Dietary exposure of Chinese adults to perfluoroalkyl acids via animal-origin foods: Chinese total diet study (2005–2007 and 2011–2013) https://pubmed.ncbi.nlm.nih.gov/31070369/
Levels of persistent organic pollutants (POPs) in foods from the first regional Sub-Saharan Africa total diet study: https://pubmed.ncbi.nlm.nih.gov/31881431/
Locally caught freshwater fish across the United States are likely a significant source of exposure to PFOS and other perfluorinated compounds: https://www.sciencedirect.com/science/article/pii/S0013935122024926?via%3Dihub
Per- and polyfluoroalkyl-contaminated freshwater impacts adjacent Riparian food web: https://pubmed.ncbi.nlm.nih.gov/32870664

("concentration" OR "occurrence" OR "exposure") NOT ("thyroid"[Title]) NOT ("allergy"[Title]) NOT ("cancer"[Title]) NOT ("biomarker"[Title]) NOT ("clinical"[Title/Abstract]) NOT ("breast milk"[Title]).

Publications that do not include data in any format (table, supplementary material, links, data sources references) were not considered as relevant for this study. Publications that include a meta-analysis of other studies were not considered as relevant, but they were used to identify references to data sources. The lists of publications were organized in two sheets of the tool, namely 'PFAS food' and 'PFAS environment', along with appropriate metadata fields related to:

- Time period covered by the samples.
- Geographical coverage
- Data is available only in aggregated form in the paper or in a more granular format in a supplementary file
- Foods and environmental media covered by the conducted research.

Exclusion criteria:

- focused on medical issues.
- focused on the detection of PFAS in the population.
- new treatment and removal techniques
- development of a risk assessment strategy
- new technique for the enrichment of food packaging material
- no data available

Search process

The process of selecting studies for inclusion in the review was rigorously evaluated by three independent reviewers (M.P., M.K., and M.D.). Mendeley was instrumental in identifying and eliminating duplicate publications, ensuring that each article was considered only once. The initial screening involved assessing titles and abstracts, guided by predefined inclusion and exclusion criteria. Articles deemed potentially relevant progressed to the subsequent stage for more comprehensive evaluation. In this phase, a second round of selection took place based on a thorough examination of the full text of the publications included. Each author independently reviewed the potentially relevant studies, adhering to predefined eligibility criteria, to determine the final set of studies to be incorporated into the review.

Selected data sources

Table 1 presents the data sources selected for PFAS after applying the prioritization methodology. The total number of records collected for PFAS from external sources is 152.019. Each record represents the analysis of a sample for a specific PFAS compound. Additionally, more records were collected for PFAS from scientific publications[6–44]. Regarding the food samples, we included publicly available data from sources such as locally caught freshwater fish across the U.S. and a contaminated pond in Sweden, as well. These additional sources were selected to capture a range of potential contamination scenarios, including both commonly consumed food products and more localized environmental exposures.

Data collection process

The information extracted from the selected full-text articles and relevant databases for inclusion in the study covered various key parameters. These included details on the analytical method employed, the percent recovery, the sampling strategy, the samples' geographical origin, the matrix under

examination, the maximum resulting concentration of PFAS, the mean value of PFAS, and the identified source of contamination.

Data for PFAS occurrence in foods have been extracted from the existing data platform of Agroknow, literature review, and external data sources. Data from external data sources were identified for PFAS and extracted datasets were selected as the ones that fit best with this study's objectives. Collected data covers high-level food categories, environmental media, 125 PFAS, and a time of more than 20 years from 2000 to 2021.

Temporal gap analysis

In terms of temporal coverage, the data collected and processed from external sources and literature cover the years as illustrated in Table 2. The total records of food samples collected were 85,998, while environmental samples totaled 66,021. Table 2 shows the total records collected per year. Interestingly, after 2005, there was a notable rise in publicly available data regarding PFAS in both food and the environment. However, it is important to highlight that 1866 records within the dataset lack information regarding the sampling year, which underscores the importance of ensuring comprehensive data collection practices for accurate scientific analysis.

Distribution of PFAS in food products and environment

All samples were subjected to analysis using advanced liquid chromatography techniques (high-performance liquid chromatography–tandem mass spectrometry (HPLC–MS/MS), ultra-performance liquid chromatography–tandem mass spectrometry (UPLC–MS/MS)). The recovery rate for these analyses ranged from 70.6% to 99.6%. Out of the total samples analyzed, a substantial proportion were collected using spatial sampling strategies, while the remaining samples were obtained through selective, suspect, and random approaches.

Table 2 | Temporal coverage of the collected data for PFAS analysis in food products and environment

Year	Records
2000	38
2001	9
2002	10
2003	96
2004	169
2005	860
2006	415
2007	697
2008	1416
2009	1105
2010	675
2011	1210
2012	942
2013	1163
2014	1626
2015	2276
2016	7234
2017	14,631
2018	8984
2019	33,096
2020	33,125
2021	40,376

Table 3 presents the results of PFAS presence in various environmental matrices, including surface, wastewater, biota, particulate matter, groundwater, and soil. The "Total samples" column represents the number of samples reported for each environmental matrix. The ">LOQ" column shows the number of samples in each matrix where PFAS concentrations were found to be above the limit of quantification (LOQ). In parallel, the 'Between LOD and LOQ' column specifies the number of samples where concentrations were observed to fall between the limit of detection (LOD) and the limit of quantification (LOQ). For example, among the 17,212 surface samples, PFAS concentrations exceeded the LOQ in 14,906 samples, with an additional 2107 samples registering concentrations between LOD and LOQ. This suggests a significant presence of PFAS in surface environments.

Table 4 presents data on PFAS presence in various food and beverage samples, as well as drinking water. It highlights the potential for PFAS contamination in different food products. Notably, drinking water shows a noteworthy presence of PFAS, with 723 out of 2973 samples having concentrations above the LOQ. This suggests a significant concern for public health, given the direct human exposure to PFAS through water consumption. In contrast, certain food categories display varying levels of PFAS presence. For example, fish and seafood samples show a particularly high incidence of PFAS, with 1323 out of 9015 samples exceeding the LOQ. This is concerning given the common dietary consumption of seafood, as elevated PFAS levels could pose health risks to consumers. Conversely, fruits and vegetables exhibit relatively lower contamination rates, indicating a lower risk of PFAS exposure through consumption. Contaminated soil or water sources used for irrigation could be potential routes of PFAS contamination in these foods. Similarly, among the 5524 dairy product samples tested, 100 samples had PFAS concentrations above the LOQ, while 2760 samples had concentrations between the LOD and LOQ.

Additionally, in this study, a comprehensive statistical analysis was conducted to assess the presence of PFAS in both food and the environment, with a focus on concentrations measured. Tables 5 and 6 display the average and maximum concentrations of PFAS observed from samples detected >LOQ within each category. The maximum PFAS values detected in food samples within the seafood category, exceeded 0,2 mg/kg (ww), while the highest PFAS concentrations found in environmental samples, particularly in ticks categorized as biota, exceeded 2 mg/kg. The detection of PFAS concentrations exceeding 0.2 mg/kg within the seafood category indicates substantial contamination within certain areas of the marine ecosystem. The identification of PFAS concentrations exceeding 2 mg/kg (ww) in ticks, which are part of the biota category, highlights the contamination of certain terrestrial ecosystems.

Presence of PFAS in environment (per analytical compound)

The most prevalent PFAS compounds found in environmental samples include PFOS, PFOA, PFHxs, PFUnA, PFDA, PFHpA, PFNA, PFPea, PFHxA, and PFTrDA. Out of these, six belong to the category

Table 3 | Presence of PFAS in different environmental samples

	Surface water	Rainwater	Wastewater	Biota	Particulate matter	Groundwater	Soil
Total samples	17,212	33,500	186	2559	65	12,216	283
>LOQ	**14,906**	8782	73	771	23	2035	134
Between LOD and LOQ	2107	24718	6	404	10	9982	18
<LOD	199	0	107	1384	32	199	131

Bold indicates the highest number of records >LOQ.

of long-chain PFAS, while four fall into the short-chain category. Table 7. provides comprehensive information on PFAS compounds detected above the LOQ within the two categories exhibiting the highest number of records (surface water) or the highest concentration (biota). The highest number of records >LOQ observed in the category of surface waters is primarily attributed to the PFOS, PFOA, and PFHxS compounds. One significant source can be industrial discharges, as certain industries have historically released PFOS, PFOA, and other PFAS chemicals into the environment as part of their manufacturing processes. Another notable source is aqueous film-forming foam (AFFF), which contains PFOS and can contribute to soil contamination when used during firefighting training exercises or in emergency response situations. Additionally, the disposal of products containing PFOS and PFOA in landfills poses a risk, as these chemicals can leach into the surrounding soil over time. Lastly, PFOS and PFOA can be transported through the air and deposited onto soil surfaces through atmospheric deposition.

Table 4 | Presence of PFAS in different food samples

	Total samples	>LOQ	Between LOD and LOQ	<LOD
Drinking water	2973	**723**	**906**	1344
Poultry & products	4285	105	2237	1943
Oils/Fats	2582	182	1440	960
Cereals & Grains	6028	12	2882	3134
Dairy Products	5524	100	2760	2664
Fish & Seafood	9015	**1323**	**4258**	3434
Fruits	11,548	2	5762	5784
Herbs & Spices	1440	0	960	480
Honey	480	0	240	240
Seeds & Nuts	2004	69	965	970
Meat & products	9103	176	4826	4101
Pasta	240	0	240	0
Vegetables	21,896	19	11,047	10,830
Beverages	2400	0	1200	1200
Sucrose	720	0	240	480
Chocolate/ Coffee/Tea	1680	0	1200	480
Ready to eat	1920	0	720	1200
Infant	720	0	480	240
Other	1440	0	0	1440

Bold indicates the hignest number of records >LOD.

Presence of PFAS in food products per analytical compound

The most prevalent PFAS compounds detected in food samples consist of PFOS, PFOA, PFHxS, PFUnA, PFDA, PFOSA, PFNA, PFBA, PFHpA, and PFPeA.) Table 8 presents a comprehensive overview of PFAS presence per individual analytical compounds in drinking water and Fish & Seafood categories. These categories were selected based on the abundance of publicly available data resulted >LOQ (drinking water) and the observed higher concentration levels within them (Fish & seafood). Among these compounds, perfluorooctane sulfonate (PFOS) emerged as the predominant contaminant in these categories. PFOS enters water supplies through various pathways, including industrial discharge and firefighting foam runoff. Its persistence and resistance to degradation make it particularly troublesome for water treatment facilities, as conventional treatment methods may not effectively remove it. Similarly, seafood, poultry and meat products have garnered attention due to their propensity to contain higher concentrations of PFAS compared to some other food types. This phenomenon can be attributed to a combination of factors. Firstly, PFAS compounds possess a remarkable ability to bioaccumulate in organisms, meaning that as they move up the food chain, they become more concentrated. This bioaccumulation process is particularly pronounced in ecosystems. Moreover, some PFAS, like perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA), exhibit exceptional environmental persistence, lingering in water bodies and allowing for their gradual accumulation. Especially, seafood's proximity to contamination sources, its filter-feeding behavior in the case of mollusks, and the global distribution of PFAS all contribute to the higher concentrations found in seafood. In 2020, the European Food Safety Authority (EFSA) proposed a draft opinion setting a tolerable intake of 8 ng/kg/week for the combined sum of these substances, equivalent to an annual dose of 0.42 μg/kg[45]. Diet serves as the primary exposure pathway, with PFOS intake strongly linked to fish and seafood consumption.

Geographical distribution of PFAS in food products and environment

Table 9 provides information about the geographical origin of the total samples analyzed. It is important to note that this data is not representative of the contamination level in each country, but rather reflects the monitoring programs for PFAS in those regions. Records from Australia have been excluded due to Australia's extensive mapping efforts on PFAS contamination, accessible at the following link: https://pfas.australianmap.net/. Increased awareness and monitoring efforts in France and in Italy might contribute to a higher number of reported samples compared to other countries. The high presence of PFAS in the United States, compared to some other countries, can be attributed to a combination of historical factors and proactive monitoring efforts. The US has a legacy of substantial PFAS usage across various industries and manufacturing facilities, dating back decades. Moreover, the use of PFAS-containing firefighting foams at military installations has been a notable source of contamination. A robust regulatory framework and an extensive system for monitoring environmental contaminants have led to increased awareness and testing in the United States, potentially contributing to higher detection rates.

Table 5 | Mean values and max values of PFAS concentration in environmental samples (mg/kg) (ww)

Resulted concentration	Surface water	Rainwater	Soil	Biota	Groundwater	Wastewater	Particulate matter
Max value	0.51	0.182	0.111	**2.32**	0.106	0.057	6.00E−04
Mean value	1.00E−03	1.00E−04	3.00E−03	0.02	1.00E−03	6.00E−03	6.00E−06

Bold indicates the highest resulted concentration.

Table 6 | Mean and max values of PFAS concentration in food samples (mg/kg) (ww)

Resulted concentration	Drinking water	Fish & seafood	Meat & products	Poultry & products	Fruits	Vegetables
Max value	1.00E−04	**0.28**	0.035	0.012	2.00E−06	2.00E−05
Mean value	3.28E−06	0.014	3.00E−04	2.00E−04	2.00E−06	6.07E−06

Bold indicates the highest resulted concentration.

Table 7 | Presence of PFAS per analytical compound in environmental samples (surface water and biota)

CAS number	PFAS	Surface water			Biota		
		<LOD	Between LOD and LOQ	>LOQ	<LOD	Between LOD and LOQ	> LOQ
1763-23-1	PFOS	3	2061	**5858**	38	26	34
335-67-1	PFOA	19	2	**5085**	141	31	85
2058-94-8	PFUnA	8	11	6	18	19	119
335-76-2	PFDA	5	8	83	49	35	73
375-95-1	PFNA	6	3	57	32	47	78
335-46-4	PFHxS	**7**	1	**2929**	35	3	12
375-85-9	PFHpA	3	8	113	88	18	49
2706-90-3	PFPeA	12	1	127	104	47	1
307-24-4	PFHxA	6	3	177	102	17	37
72629- 94-8	PFTrDA	13	1	14	52	12	88
375-22-4	PFBA	11	1	125	148	3	0
335-77-3	PFDS	0	1	30	45	1	2
307-55-1	PFCA	10	1	21	50	25	79
67905-19-5	PFHxDA	13	0	2	60	45	46

Bold indicates the highest number of records >LOQ.

Table 8 | Presence of PFAS per analytical compound in food samples (Drinking water + Fish & seafood)

CAS Number	PFAS	Drinking water			Fish & seafood		
		<LOD	Between LOD and LOQ	>LOQ	<LOD	Between LOD and LOQ	>LOQ
1763-23-1	PFOS	30	25	11	107	225	**536**
335-67-1	PFOA	48	38	**138**	121	226	26
2058-94-8	PFUnA	63	31	34	119	223	27
335-76-2	PDFA	40	48	40	119	223	27
375-95-1	PFNA	34	33	61	121	223	25
335-46-4	PFHxS	54	32	59	116	112	22
375-85-9	PFHpA	75	35	34	119	223	22
2706-90-3	PFPeA	84	31	30	114	134	18
307-24-4	PFHxA	80	38	27	125	223	14
72629- 94-8	PFTrDA	63	28	37	112	112	18
754-91-6	PFOSA	36	24	5	112	204	11
375-22-4	PFBA	43	24	78	112	112	9
335-77-3	PFDS	27	30	71	112	112	9
307-55-1	PFCA	63	27	38	112	112	16

Bold indicates the highest number of records >LOQ.

Nevertheless, it is essential to recognize that PFAS contamination is a global concern, and many nations worldwide are grappling with similar challenges that require coordinated efforts to address effectively. Italy's National Research Council (CNR) initiated comprehensive monitoring of PFAS presence within the country in 2013. This proactive approach likely contributes to the robust detection of PFAS contamination in Italy. Norway, despite its renowned pristine landscapes, is not exempt from the presence of PFAS compounds. These contaminants find their way into the environment through various sources, including industrial activities, firefighting foams, and the use of PFAS-containing products. Elevated PFAS levels have been detected in some drinking water supplies, prompting regulatory measures to limit exposure. Norwegian authorities are actively engaged in monitoring, research, and remediation efforts to comprehend the extent and impact of PFAS contamination in soil, water, and wildlife[46–48]. Germany's rigorous approach to monitoring PFAS contamination in food is driven by its status as the largest food retail sector in the European Union[49–51]. PFAS can enter the food chain from a variety of sources, including contaminated soil, water, and packaging materials. As a response, German authorities conduct systematic testing and monitoring of a wide range of food items, including fish, meat, dairy, and vegetables, to assess PFAS levels and potential health risks to consumers. Germany adheres to European Union (EU) regulations, which establish maximum residue levels (MRLs) for PFAS in certain food categories, ensuring that food products available in the market comply with established safety standards. Additionally, attention is given to sites with historical PFAS contamination, as they can represent potential sources of PFAS in the food chain, emphasizing the importance of comprehensive monitoring and safety measures to safeguard food quality and public health.

Contamination sources of PFAS

Table 10 provides a comprehensive overview of PFAS occurrence across different contamination source categories. Notably, fire stations are associated with the highest number of records (65), emphasizing the frequent

Table 9 | Top 10 countries with the highest number of records

Country	Records > LOQ	Records < LOQ
France	4938	23,280
Italy	4949	6756
USA	3147	951
Germany	2622	285
Netherlands	1722	1118
Denmark	81	2175
Sweden	1141	820
China	879	867
Canada	1004	0
Norway	54	560

Table 10 | Number of records per contamination sources

Contamination source category	Number of records
Fire station	65
Industry	49
Military	52
Landfill	39

presence of PFAS contamination in these locations, due to the historical use of PFAS-containing firefighting foams. Industry-related sites, represented by 49 records, are another significant source of PFAS contamination, stemming from industrial processes and materials. Military installations, with 52 records, are notable for their association with PFAS contamination, often linked to the use of PFAS-containing firefighting agents. Landfills, accounting for 39 records, also stand out as contributors to PFAS environmental contamination, with PFAS-containing products disposed of in these sites leaching into the surrounding environment.

Per- and polyfluoroalkyl substances (PFAS) represent a group of water-soluble chemical compounds widely employed for various applications over the past six decades. Notably, perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) are among the most extensively studied within this group[52–55]. Human exposure to PFAS primarily occurs through dietary intake, although in specific instances, drinking water can also be a significant source, along with minor contributions from dust and indoor air exposure[56]. These chemicals have attracted significant attention due to their widespread use across various industrial and consumer applications, raising concerns about their environmental persistence and potential health risks. One of the key areas of focus has been on understanding the environmental behavior of PFAS, particularly their persistence and tendency to biomagnify in food chains. PFAS have been found to resist degradation processes, leading to their accumulation in ecosystems over time. Thus, the collective efforts of researchers worldwide have resulted in a substantial body of literature on PFAS, covering a wide range of topics, including their environmental fate, exposure pathways, toxicity mechanisms, and potential mitigation measures[57–59]. While a majority of the available data originates from studies conducted in the European Union, the United States, Australia, and China, there are still considerable information gaps for many regions worldwide[60].

Analyzing the geographical distribution of PFAS from existing available data, an interesting pattern emerges. The results demonstrate that within the European Union, France, Italy, Germany, the Netherlands, Denmark, Sweden, and Norway exhibit a significant number of samples exceeding the LOQ for PFAS contamination. This observation is consistent with prior studies examining contamination sites across Europe and within scientific literature[61,62]. This geographic disparity offers intriguing insights into the sources and pathways of PFAS contamination. However, a monitoring campaign for chemical contaminants was conducted in France in 2021, while other countries, such as Sweden, Germany, Italy, the Netherlands, and Norway, have established National Monitoring Programs for chemical contaminants. Therefore, the number of records exceeding the LOQ in these specific countries can be attributed to these programs. This underscores the presence of PFAS in both the environment and food, highlighting the necessity for all European countries to implement a comprehensive approach to address this issue[16–64]. The presence of PFAS in the United States, when compared to certain other nations, can be attributed to a combination of historical factors and proactive monitoring endeavors. The United States has a historical legacy of extensive PFAS usage across a range of industries and manufacturing facilities, for many decades. Additionally, the utilization of PFAS-containing firefighting foams at military installations has significantly contributed to environmental contamination[65]. Moreover, in more industrialized nations, fluorochemical production plants (FPPs) have played a significant role in the direct discharge of long-chain legacy PFAS into surface water and the atmosphere[66–68]. Notably, the United States' commitment to comprehensive monitoring and research initiatives has fostered a deeper understanding of PFAS contamination, shedding light on the scope of their presence in various environmental and food matrices. The United States (through the National Health and Nutrition Examination Survey or NHANES), Canada (via the Canadian Health Measures Survey), Germany (with the Human Biomonitoring Commission of the Federal Environmental Agency), and France (under the French Ministries of Health and Environment), have instituted national biomonitoring programs[69]. Thus, besides environmental pollution, also EU's proactive approach to investigation and monitoring plays a significant role in detecting and addressing PFAS contamination in food[49–62,64–75].

The presence of PFAS in surface water and drinking water samples analyzed, underscores the potential contamination of water sources, emphasizing the pervasiveness of these compounds in the environment[76,77]. However, it's notable that PFAS were typically found at low concentrations in these samples. This indicates that while contamination is prevalent, the levels detected are generally minimal. Despite their low concentrations, the presence of PFAS in numerous samples remains concerning due to their enduring presence in the environment, prompting worries about potential long-term effects on ecosystems and human health. Additionally, the high mean values of PFAS detected in food samples indicate a significant concern within this study. PFAS compounds, which have entered water sources, tend to accumulate in the tissues of organisms over time, creating a reservoir of these chemicals. As organisms consume contaminated water and prey on one another, PFAS concentrations increase up the food chain. Consequently, top-level consumers, such as fish and seafood, exhibit much higher PFAS concentrations in their tissues than in the surrounding environment. Apart from the biomagnification of existing PFAS compounds, there were also indications of precursor substances accumulating in the food chain, indicating the possibility of their transformation into more persistent PFAS compounds in higher trophic levels[78,79].

Therefore, the detection of the highest maximum concentrations in Fish & seafood and biota categories demonstrates the bioaccumulation potential, pointing out the need for vigilance in monitoring PFAS in the food chain[80,81]. Thus, the high PFAS levels in the Fish & seafood category indicate widespread contamination in specific areas of the aquatic environment, originating from industrial discharges, urban runoff, or atmospheric deposition. In contrast, ticks are ectoparasites that feed on the blood of various host animals, including mammals and birds. According to literature, PFAS compounds have permeated the terrestrial food web, with ticks acting as bioindicators[82,83]. Elevated PFAS levels in biota are indicative of widespread environmental contamination, which can have cascading effects on wildlife health, ecosystem dynamics, and even potential risks to humans. Besides, our findings align with other studies, collectively highlighting the widespread presence and concerning levels of PFAS contamination across various environmental and food matrices, underscoring the urgent need for comprehensive mitigation strategies[84–89]. Moreover, it emphasizes the crucial role of establishing stringent regulations by regulatory bodies to curb PFAS contamination and safeguard public health and the environment.

In general, PFAS exhibits remarkable stability because all the hydrogen atoms in their carbon–fluorine (C–F) bonds are replaced by fluorine atoms, enhancing their overall molecular energy. The unique structure of PFAS imparts them with hydrophobic and lipophobic characteristics, as well as exceptional chemical and thermal stability, which collectively render them resistant to hydrolysis, photolysis, metabolism, and biodegradation[90]. Our study revealed the prevalence of PFOS and PFOA in most environmental samples, while PFHxS were found in surface water samples. Notably, these compounds were also detected in fish and seafood products, suggesting their transmission to aquatic life through contaminated surface water[91–93].

Moreover, the significant detection of PFAS in drinking water samples indicates potential routes of exposure, with PFAS potentially making its way into poultry and meat products through drinking water or even into crops via irrigation practices. PFOS, PFOA, and PFOSA contain eight carbon atoms, which contribute to their enhanced stability, and this can enlighten their frequent detection in seafood, meat, and poultry products[94]. Additionally, in marine environments, research has shown that organisms at higher trophic levels exhibit a significantly greater ability to convert PFOSA into PFOS, as evidenced by the proportion of these compounds in their PFAS profiles and body burdens[78].

Analyzing the data, it becomes clear that most PFAS compounds detected in food samples mostly belong to the long-chain PFAS category. In contrast, the environmental samples contain both long-chain and short-chain PFAS. Short-chain PFAS with hydrophilic properties exerts more significant effects on surface waters, while long-chain PFAS with hydrophobic characteristics can accumulate in sediments, posing risks to aquatic organisms[95]. This disparity indicates a notable difference in the distribution and prevalence of PFAS compounds in these two types of matrixes. The presence of long-chain PFAS in food samples is significant as they tend to bioaccumulate in living organisms. This is especially concerning as long-chain PFAS are typically associated with a higher potential for health and environmental risks due to their persistence and bioaccumulative properties. In environmental samples, the detection of both long-chain and short-chain PFAS compounds indicates that various sources may contribute to environmental contamination. This reflects the diverse applications and sources of PFAS in the environment, ranging from historical uses to ongoing emissions. The presence of short-chain PFAS in the environment is noteworthy as they are often considered less bioaccumulative and less persistent, but they are still of concern due to their potential to leach into water sources and impact aquatic ecosystems.

Addressing these concerning findings necessitates a multifaceted approach. First and foremost, there is a critical need to identify and investigate potential sources of PFAS contamination in marine and terrestrial ecosystems and the environment, with the aim of preventing further pollution[96,97]. Additionally, comprehensive health risk assessments are imperative to evaluate the potential health implications for consumers of contaminated foodstuff[98,99]. Furthermore, it is essential to assess the ecological impact of PFAS contamination on these ecosystems, considering potential effects on biodiversity and food chains. In parallel, regulatory measures should be contemplated, including the establishment or revision of standards for PFAS levels in food products, to safeguard both the environment and human health. Finally, the development and implementation of mitigation strategies, remediation efforts, and enhanced waste management practices are essential steps in reducing PFAS contamination in affected ecosystems.

In conclusion, given the interconnected nature of environmental challenges, understanding how factors such as PFAS presence intersect with climate change is vital for safeguarding public health and ecosystems. This necessitates a holistic approach involving research, policy, and action to mitigate their combined implications. Moreover, ensuring the availability of public data for laboratory testing of PFAS in environmental and food samples, as well as promoting data sharing, is essential for fostering transparency, collaboration, and informed decision-making in addressing PFAS contamination and its impacts on climate and public health.

The distribution of PFAS in food and the environment, as explored in our study, is subject to notable limitations that warrant acknowledgment. Firstly, while our data sources, drawn from publications and reports, provide a diverse array of information, they inherently carry biases and may overlook certain regions and food categories. Notably, our analysis revealed significant data gaps in regions like Africa and South America, limiting the broader applicability of our findings. Additionally, temporal coverage presents a challenge, with sparse data available from recent years and a scarcity of historical records prior to 2005, impacting both the comprehensiveness and temporal relevance of our analysis. Furthermore, the high contamination prevalence observed in our sample set may be influenced by targeted sampling efforts in areas known for elevated contamination risks, potentially skewing the overall distribution patterns. Methodologically, while we prioritized the inclusion of available public data and categorized samples based on their utility, it's crucial to note that concentrations below the limit of detection in certain categories do not necessarily imply the absence of contamination but rather reflect limitations in data sources. Consequently, our study should be viewed as an in-depth exploration of publicly available data rather than an authoritative account of PFAS distribution and impact. Moving forward, addressing these limitations through refined methodologies is essential for a more comprehensive understanding of PFAS distribution dynamics and informed decision-making in environmental and food safety policies.

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Competing interests

The authors declare no competing interests.

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Technical BRIEF
INNOVATIVE RESEARCH FOR A SUSTAINABLE FUTURE

Per- and Polyfluoroalkyl Substances (PFAS): Incineration to Manage PFAS Waste Streams

Background

Per- and polyfluoroalkyl substances (PFAS) are a very large class of man-made chemicals that include PFOA, PFOS and GenX chemicals. Since the 1940s, PFAS have been manufactured and used in a variety of industries in the United States and around the globe. PFAS are found in everyday items such as food packaging, non-stick stain repellent, and waterproof products, including clothes and other products used by outdoor enthusiasts. PFAS are also widely used in industrial applications and for firefighting. PFAS can enter the environment through production or waste streams and can be very persistent in the environment and the human body. PFAS have many and varied pathways into waste streams, presenting challenges for ultimate disposal. Determining the appropriate method for ultimate disposal of PFAS wastes is a complex issue due to their volatility, solubility, and environmental mobility and persistence. EPA is currently considering multiple disposal techniques, including incineration, to effectively treat and dispose of PFAS waste.

Options and Considerations for the Disposal of PFAS Waste via Incineration

One potential disposal method for PFAS waste is through high temperature chemical breakdown, or incineration. Incineration has been used as a method of destroying related halogenated organic chemicals such as polychlorinated biphenyls (PCBs) and ozone-depleting substances (ODSs), where sufficiently high temperatures and long residence times break the carbon-halogen bond, after which the halogen can be scrubbed from the flue gas, typically as an alkali-halogen. PFAS compounds are difficult to break down due to fluorine's electronegativity and the chemical stability of fluorinated compounds. Incomplete destruction of PFAS compounds can result in the formation of smaller PFAS products, or products of incomplete combustion (PICs), which may not have been researched and thus could be a potential chemical of concern.



Incineration of halogenated organic compounds occurs via unimolecular decomposition and radical reaction. For unimolecular decomposition, fluorinated organic compounds likely require higher temperatures to achieve 99.99% destruction in 1 second residence time than do their chlorinated counterparts. Unimolecular decomposition of highly fluorinated organics most likely occurs through breakage of C-C or C-F bonds (Tsang et al., 1998). The most difficult fluorinated organic compound to decompose is CF_4, requiring temperatures over 1,400°C, but is easily monitored, making it a potential candidate for destructibility trials.

Fluorinated organic compounds can also be degraded via incineration by free radical initiation, propagation, and branching mechanisms. Although hydroxyl radical reaction with hydrocarbons is a common combustion flame-propagating mechanism, the strength of the C-F bond makes this pathway unlikely and would instead leave atomic hydrogen, formed at high temperatures, as the likely radical reacting with the carbon-bonded fluorine.

Radical reactions are more likely for chloroalkanes than fluoroalkanes, due to the lower bond energy of C-Cl (Tsang et al., 1998). If formed, the extremely high electronegativity of fluorine radicals results in their quick combination with other radical species, preventing flame-sustaining free radical propagation and branching processes. This propensity to terminate free radical chemistry make PFAS effective fire suppressants.

The stability of perfluorinated radicals leads to higher concentrations and correspondingly increased propensity to recombine, creating larger molecules that are products of incomplete combustion (PIC) and distinctive from the original fluorinated organics. These reactions are promoted by partial organic combustion resulting from insufficient temperatures, time, and mixing. In addition, the presence of catalytic surfaces, often metals, promotes further reaction and PIC formation in post-combustion regions. This scenario has been most studied related to the formation of polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDDs/PCDFs) in the cool-down regions of waste incinerators.

The effectiveness of incineration to destroy PFAS compounds and the tendency for formation of fluorinated or mixed halogenated organic byproducts is not well understood. Few experiments have been conducted under oxidative and temperature conditions representative of field-scale incineration. Limited studies on the thermal destructibility of fluorotelomer-based polymers found no detectable levels of perfluorooctanoic acid after 2 second residence time and 1,000°C (Yamada et al., 2005; Taylor et al., 2014). Emission studies, particularly for PICs, have been incomplete due to lack of necessary measurement methods suitable for the comprehensive characterization of fluorinated and mixed halogenated organic compounds.

Addressing Gaps in Research for PFAS Waste

The extent to which PFAS-containing waste material in the United States is incinerated is not fully documented or understood. PFAS compounds are not listed as hazardous wastes under the Resource Conservation and Recovery Act (RCRA) nor as hazardous air pollutants under Clean Air Act regulations, so they are not subject to the tracking systems associated with these regulations.

EPA is currently considering multiple disposal techniques, including incineration, to effectively treat and dispose of PFAS wastes. EPA researchers are currently studying PFAS incineration, sampling and analytical methods development, and industrial field sampling. Research on thermal stability of PFAS compounds, the ability to fully capture and identify PFAS compounds and their thermal decomposition byproducts, and the efficacy of emission control technologies are areas of targeted research. These efforts, in cooperation with states and industries, is aimed at proper disposal of PFAS-laden wastes without media-to-media transfer or environmental release.

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Note: This technical brief is a summary of the science and does not necessarily reflect EPA policy.

Interim Guidance on the Destruction and Disposal of Perfluoroalkyl and Polyfluoroalkyl Substances and Materials Containing Perfluoroalkyl and Polyfluoroalkyl Substances—Version 2 (2024)

INTERIM GUIDANCE FOR PUBLIC COMMENT
APRIL 8, 2024

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KD1841

Addendum

Addendum
03/12/2025

Note that some of the tools listed on page 102 in Section 4.c.i “Identifying potentially vulnerable populations” are no longer available and they are not accessible through the web page hyperlinks in this guidance. Additionally, note that Executive Order (EO) 14096, “Revitalizing Our Nation’s Commitment to Environmental Justice for All,” referenced on page 101 in Section 4.c “Considering vulnerability” and page 106 in Section 4.c.iii “Considerations for community engagement” was rescinded on January 20, 2025, via EO 14148, “Initial Rescissions of Harmful Executive Orders and Actions.”

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Abbreviations

AFFF	aqueous film-forming foam
APCD	air pollution control device
ARFF	aircraft rescue firefighting
BDL	below detection limit
BMP	best management practice
C	Celsius
CAA	Clean Air Act
CaF_2	calcium fluoride
CaO	calcium oxide
$Ca(OH)_2$	calcium hydroxide
C&D	construction and demolition
CDC	Centers for Disease Control and Prevention
CDR	Chemical Data Reporting
CEJST	Climate and Economic Justice Screening Tool
CERCLA	Comprehensive Environmental Response, Compensation, and Liability Act
CF_4	carbon tetrafluoride
C_2F_6	hexafluoroethane
C_3F_8	octafluoropropane
CFR	Code of Federal Regulations
CHES	Clean Harbors Environmental Services
CHF_3	fluoroform
CIC	combustion–ion chromatography
CI/MS	chemical ionization mass spectrometry
CKD	cement kiln dust
DE	destruction efficiency
DoD	Department of Defense
DOE	Department of Energy
DRE	destruction and removal efficiency
ECHO	Enforcement Compliance and History Online
EJ	environmental justice
EPA	United States Environmental Protection Agency
ESP	electrostatic precipitator
ESTCP	Environmental Security Technology Certification Program
F	Fahrenheit
FAA	Federal Aviation Administration
FBC	fluidized bed combustor
FF	fabric filter
FML	flexible membrane liner
FTIR	Fourier transform infrared spectrometry
FTOH	fluorotelomer alcohol
FTS	fluorotelomer sulfonate
FY 2020 NDAA	National Defense Authorization Act for Fiscal Year 2020
GAC	granular activated carbon
GCCS	gas collection and control system
HAP	hazardous air pollutant

HF	hydrogen fluoride
HFPO-DA	hexafluoropropylene oxide-dimer acid, 2,3,3,3-Tetrafluoro-2-(1,1,2,2,3,3,3-heptafluoropropoxy) propanoic acid, ($C_6F_{11}O_3H_2$)
H_2O_2	hydrogen peroxide
HWC	hazardous waste combustor
HWI	hazardous waste incinerator
LC/MS/MS	liquid chromatography/tandem mass spectrometry
LFG	landfill gas
LWAK	lightweight aggregate kiln
MDL	method detection limit
MF	microfiltration
MnO_2	manganese(IV) oxide
MSW	municipal solid waste
MWC	municipal waste combustor
NADP	National Atmospheric Deposition Program
NASA	National Aeronautics and Space Administration
NESHAP	National Emission Standards for Hazardous Air Pollutants
NF	nanofiltration
ng/L	nanograms per liter
NIOSH	National Institute for Occupational Safety and Health
NMOC	nonmethane organic compound
NPDES	National Pollutant Discharge Elimination System
NPDWR	National Primary Drinking Water Regulation
NSPS	New Source Performance Standards
NTA	non-targeted analysis
OECD	Organization for Economic Cooperation and Development
ORD	Office of Research and Development
OSTP	Office of Science and Technology Policy
OTM	Other Test Method
PAC	powdered activated carbon
PBPK	physiologically based pharmacokinetic
PCB	polychlorinated biphenyl
PFAA	perfluoroalkyl acid
PFAS	perfluoroalkyl and polyfluoroalkyl substances
PFASTT	PFAS Thermal Treatment Database
PFBS	perfluorobutanesulfonic acid ($C_4F_9SO_3H$)
PFC	perfluorocarbon
PFHxS	perfluorohexanesulfonic acid ($C_6F_{13}SO_3H$)
PFOA	perfluorooctanoic acid ($C_8F_{15}O_2H$)
PFOS	perfluorooctanesulfonic acid ($C_8F_{17}SO_3H$)
PIC	product of incomplete combustion
PID	product of incomplete destruction
PIGE	particle-induced gamma emission spectrometry
PITT	PFAS Innovative Treatment Team
POHC	principal organic hazardous constituent
POTW	publicly owned treatment works
ppb	parts per billion
ppmv	parts per million by volume

PTFE	polytetrafluoroethylene
PVDF	polyvinylidene fluoride or polyvinylidene difluoride
RCRA	Resource Conservation and Recovery Act
RDF	refuse-derived fuel
RO	reverse osmosis
RSEI	Risk-Screening Environmental Indicators
SBIR	Small Business Innovation Research
SDA	spray dryer absorber
SDWA	Safe Drinking Water Act
SERDP	Strategic Environmental Research and Development Program
SSI	sewage sludge incinerator
TOF	total organic fluorine
TRI	Toxics Release Inventory
TRL	technology readiness level
TSCA	Toxic Substances Control Act
TWTDS	treatment works treating domestic sewage
UF	ultrafiltration
UIC	underground injection control
U.S.C.	United States Code
USDW	underground source of drinking water
UST	underground storage tank
VOC	volatile organic compound
WWTP	wastewater treatment plant
XPS	X-ray photo-electron spectroscopy

Executive Summary

This 2024 update of the U.S. Environmental Protection Agency (EPA)'s 2020 interim guidance presents currently available information on the destruction and disposal of perfluoroalkyl and polyfluoroalkyl substances (PFAS) and PFAS-containing materials. It focuses on the current state of science and associated uncertainties for three large-scale capacity technologies that can destroy PFAS or control PFAS release into the environment: thermal destruction, landfills, and underground injection. It also includes a framework for evaluating emerging technologies for PFAS destruction or disposal.

EPA published the first edition of this guidance as required by the National Defense Authorization Act (NDAA) of 2020. The NDAA directs EPA to address the destruction and disposal of PFAS and specific PFAS-containing materials, including aqueous film-forming foam, contaminated media, textiles (other than consumer goods), and various wastes from water treatment. The NDAA also requires EPA to review and update the guidance as appropriate, but no less frequently than every three years.

The primary audience of this guidance is decision-makers who need to identify the most effective means for destroying or disposing of PFAS-containing materials and wastes. The audience may also include regulators, waste managers, and the public, including affected communities. EPA is recommending that managers of PFAS wastes consider the nature of the waste, location, potential for environmental release, and other factors to determine the most appropriate destruction, disposal, or storage method. As a general approach, EPA recommends that decision-makers prioritize the use of destruction and disposal technologies that have a lower potential for PFAS release to the environment, over destruction and disposal technology options with a greater potential for environmental release of PFAS. EPA acknowledges that each destruction and disposal technology has limitations. However, managing PFAS to minimize environmental releases during destruction and disposal activities supports protection of human health and the environment.

While the purpose of an effective destruction and disposal technology is to prevent or minimize environmental releases, it is also important to distinguish between a potential environmental release and a direct human exposure. A PFAS release does not inherently imply direct and immediate human exposure and a release does not necessarily present an unacceptable risk to specific populations.

EPA has developed a fact sheet for the public, including potentially affected communities, that provides a more concise and less technical presentation of key materials.

Key Findings and Updates

EPA describes destruction and disposal options based on each technology's potential to destroy PFAS or control PFAS release into the environment. This approach is consistent with EPA's mission to protect human health and the environment. There are several different types of technologies that have been used for PFAS treatment and disposal, and their potential to destroy PFAS or control PFAS releases is described herein (see Section 1). In general, the following technologies (in no particular order) have a lower potential for environmental release of PFAS compared to other technologies in the same category and are viewed as the more protective technologies:

- **Underground Injection - Permitted Class I non-hazardous industrial or hazardous waste injection wells** are the waste management approach with a lower potential for environmental release when

compared to other destruction and disposal options. These wells may help ensure that injected fluids are confined and cannot enter underground sources of drinking water. However, the limited number of wells currently receiving off-site PFAS and waste transportation logistics may significantly limit the type and quantity of PFAS-containing fluids appropriate for underground injection.

- **Landfills - Permitted hazardous waste landfills** have the most protective landfill engineering controls and practices for the containment of PFAS waste and would be more effective at minimizing PFAS release into the environment than other landfill types. EPA recommends Subtitle C landfills when PFAS levels of the waste are relatively high and landfill disposal is the selected option. However, for all landfill types, new information demonstrates landfilling could have higher PFAS releases to the environment than previously thought in 2020.
- **Thermal Treatment - Permitted hazardous waste combustors** such as commercial incinerators, cement kilns, and lightweight aggregate kilns and granular activated carbon (GAC) reactivation units with thermal oxidizers may operate under conditions more conducive to destroying PFAS and controlling related products of incomplete combustion. Research suggests that the use of higher temperatures, well mixed combustion environments, and longer residence times may be more conducive to destroying PFAS and controlling related products of incomplete combustion. Although limited data have been obtained since the 2020 version of the interim guidance (including data suggestive of adequate temperature ranges to break down PFAS), uncertainties remain about the effectiveness of thermal treatment. EPA encourages additional testing with EPA-approved or EPA-evaluated methods by waste managers of thermal treatment operations, including for products of incomplete combustion and the presence of PFAS in all associated waste streams, to evaluate whether thermal treatment technologies are minimizing potential environmental releases. EPA's new analytical method, OTM-50, will allow better characterization of products of incomplete combustion (PICs) that will help to address some of these uncertainties when additional data are collected as recommended in this guidance (see Appendix A).

This list includes the three technology categories described in Section 3 and does not preclude new or emerging technologies. When considering the bullets above, it is important to note that real-world performance and testing data are generally limited. Additional performance and testing data—including data on destruction and removal of PFAS in thermal treatment devices, ongoing research activities, and long-term performance data for landfills and underground injection—may change EPA's understanding of each technology's ability to control PFAS. Data needs and priorities are summarized in this update.

EPA continues to seek collaboration with thermal treatment facilities to conduct air emission testing during thermal treatment of PFAS and PFAS-containing materials. EPA provides guidance for this and for independent analysis in Appendix A. In addition to performance and testing data, the 2024 guidance identifies other, high-priority data gaps that need to be addressed by researchers across government, academia, and industry for EPA to provide meaningful updates on effective destruction and disposal practices.

EPA partnered with industry and academia to collect data on four emerging technologies for PFAS destruction: mechanochemical degradation, electrochemical oxidation, gasification and pyrolysis, and supercritical water oxidation. While the results from these studies show promise for PFAS destruction, further work using newly available methods is needed to more fully characterize the outputs of these processes and to evaluate their performance for a wider range of PFAS-containing materials. Managers of PFAS and PFAS-containing materials may consider these or other emerging technologies for PFAS disposal or destruction. To assist PFAS material managers with evaluating whether an emerging destruction (or disposal) technology is suitable for a particular PFAS-containing material, EPA developed

a technology evaluation framework. The framework provides a transparent, consistent approach for evaluating destruction and disposal technologies for PFAS and PFAS-containing material and includes considerations for communities. EPA recommends that managers of PFAS-containing materials use the technology evaluation framework to evaluate emerging destruction and disposal technologies and to inform their decisions about managing PFAS-containing materials. EPA also encourages technology developers to generate and publicly release data that can be used to complete the framework (see Section 6).

EPA updated tools, methods, and approaches for considering the impacts of potential releases and exposure on communities located near destruction and disposal facilities, including identifying vulnerable populations overburdened by cumulative impacts, incorporating vulnerability into decision-making, and engaging the community (see Section 4).

What's Next?

EPA and other government, academic, and private institutions will continue to conduct research to better understand PFAS destruction and disposal. EPA will consider public comments it receives on this version of the guidance as well as additional advancements in PFAS research and science to revise this interim guidance within the next three years.[1] For information about the Agency's plans and actions related to PFAS, visit EPA's website pages *PFAS Strategic Roadmap: EPA's Commitments to Action 2021-2024* and *Key Actions to Address PFAS*.

[1] EPA may explore opportunities to provide more frequent technical updates as information becomes available.

1. Introduction

This second version interim guidance provides information on technologies that may be feasible and appropriate for the destruction or disposal of perfluoroalkyl and polyfluoroalkyl substances (PFAS) and PFAS-containing materials. It represents the current state of science and the associated uncertainties for large-capacity technologies that can destroy PFAS or control PFAS release into the environment. This is an update to the interim guidance the U.S. Environmental Protection Agency (EPA) issued in 2020.

EPA published the first version of this interim guidance and made it available for public comment.[2] EPA received 77 unique public comments (out of 5,066 total comments) on the interim guidance from states, Tribes, industries, trade organizations, environmental organizations, and members of the public. This updated version incorporates this public feedback.

The 2024 interim guidance contains the following new information:

Section 1: Introduction

- Updated review of PFAS destruction and disposal technologies.

Section 2: Description of PFAS-Containing Materials Identified in the FY 2020 NDAA

- Additional details on spent water treatment materials.
- Updated information on biosolids generation and management.

Section 3: Technologies for the Destruction and Disposal of PFAS and PFAS-Containing Materials

- Additional information on thermal treatment and landfills.
- Additional information on testing and monitoring at thermal treatment facilities.

Section 4: Considerations for Potentially Vulnerable Populations Living Near Likely Destruction or Disposal Sites

- Updated list of tools for vulnerability screening.

Section 5: Research Needs and Data Gaps for Destruction and Disposal Technologies

- Increased focus on research needs to inform future updates to this interim guidance.
- Updated information on ongoing research and development activities.

Section 6: Emerging Technologies for PFAS Destruction and Disposal (NEW)

- EPA efforts to identify, review, and test emerging technologies for PFAS destruction.
- Technology evaluation framework that utilizes a multiple-lines-of-evidence approach to evaluate a technology/PFAS material combination.

Appendix A: EPA Guidance to Conduct PFAS Emissions Field Testing at Commercial Thermal Destruction Sources (NEW)

- EPA request to access commercial sources to conduct air emissions testing during PFAS thermal destruction.
- Guidance for facilities to conduct source testing prior to accepting/processing PFAS materials at full-scale thermal destruction facilities.

Appendix B: Summary of the Clean Harbors Test Data (NEW)

- Two source test campaigns conducted at a hazardous waste combustor.

Appendix C: Summary of the Chemours Thermal Oxidizer Test Data (NEW)

- Source test campaign conducted at a chemical manufacturer.

[2] Interim PFAS Destruction and Disposal Guidance; Notice of Availability for Public Comment. U.S. EPA. 85 Federal Register 83554. December 22, 2020.

Appendix D: Summary of Costs and Considerations (NEW)

- Summary of costs and factors that should be considered when estimating costs to destroy or dispose of PFAS and PFAS-containing materials.

1.a Purpose and audience of this interim guidance

The purpose of this interim guidance is to describe technologies for managing the destruction and disposal of PFAS-containing materials and to recommend practices associated with these technologies that minimize PFAS releases to the environment. This purpose is consistent with EPA's mission to protect human health and the environment and the National Defense Authorization Act (NDAA) for Fiscal Year 2020, Public Law No: 116-92 (hereafter, "FY 2020 NDAA"), signed into law on December 19, 2019.

Section 7361 of the FY 2020 NDAA (see Figure 1-1) directs EPA to publish interim guidance on the destruction and disposal of PFAS and PFAS-containing materials no later than one year from the date of enactment of the FY 2020 NDAA. EPA published the initial interim guidance on December 18, 2020. The FY 2020 NDAA also requires EPA to review and revise the interim guidance at least every three years, if appropriate. This 2024 update fulfills that requirement.

The primary audience of this guidance is decision-makers who need to identify the most effective means for destroying or disposing of PFAS-containing materials and wastes. The audience may also include regulators, waste managers, and technology developers who can use the technology evaluation framework in Section 6 to evaluate emerging technology applicability to PFAS destruction or disposal. This guidance may also interest the general public; particularly communities near PFAS destruction and disposal sites who may have an opportunity to offer input or provide consultation to decision-makers regarding destruction and disposal decisions based on relevant decision-makers determination. EPA has developed a fact sheet for the public, including potentially affected communities, that provides a more concise and less technical presentation of this guidance.

This interim guidance meets one of many commitments EPA made under the Agency's PFAS Strategic Roadmap. The PFAS Strategic Roadmap, released in 2021, describes EPA's whole-of-Agency approach to address PFAS and identifies concrete actions EPA is taking to safeguard public health, protect the environment, and hold polluters accountable. For more information on EPA's actions to address PFAS, visit *PFAS Strategic Roadmap: EPA's Commitments to Action 2021-2024* and *Key Actions to Address PFAS*.

SEC. 7361. PFAS DESTRUCTION AND DISPOSAL GUIDANCE.

(a) IN GENERAL.—Not later than 1 year after the date of enactment of this Act, the Administrator shall publish interim guidance on the destruction and disposal of perfluoroalkyl and polyfluoroalkyl substances and materials containing perfluoroalkyl and polyfluoroalkyl substances, including—

(1) aqueous film-forming foam;
(2) soil and biosolids;
(3) textiles, other than consumer goods, treated with perfluoroalkyl and polyfluoroalkyl substances;
(4) spent filters, membranes, resins, granular carbon, and other waste from water treatment;
(5) landfill leachate containing perfluoroalkyl and polyfluoroalkyl substances; and
(6) solid, liquid, or gas waste streams containing perfluoroalkyl and polyfluoroalkyl substances from facilities manufacturing or using perfluoroalkyl and polyfluoroalkyl substances.

(b) CONSIDERATIONS; INCLUSIONS.—The interim guidance under subsection (a) shall—

(1) take into consideration—
(A) the potential for releases of perfluoroalkyl and polyfluoroalkyl substances during destruction or disposal, including through volatilization, air dispersion, or leachate; and
(B) potentially vulnerable populations living near likely destruction or disposal sites; and
(2) provide guidance on testing and monitoring air, effluent, and soil near potential destruction or disposal sites for releases described in paragraph (1)(A).

(c) REVISIONS.—The Administrator shall publish revisions to the interim guidance under subsection (a) as the Administrator determines to be appropriate, but not less frequently than once every 3 years.

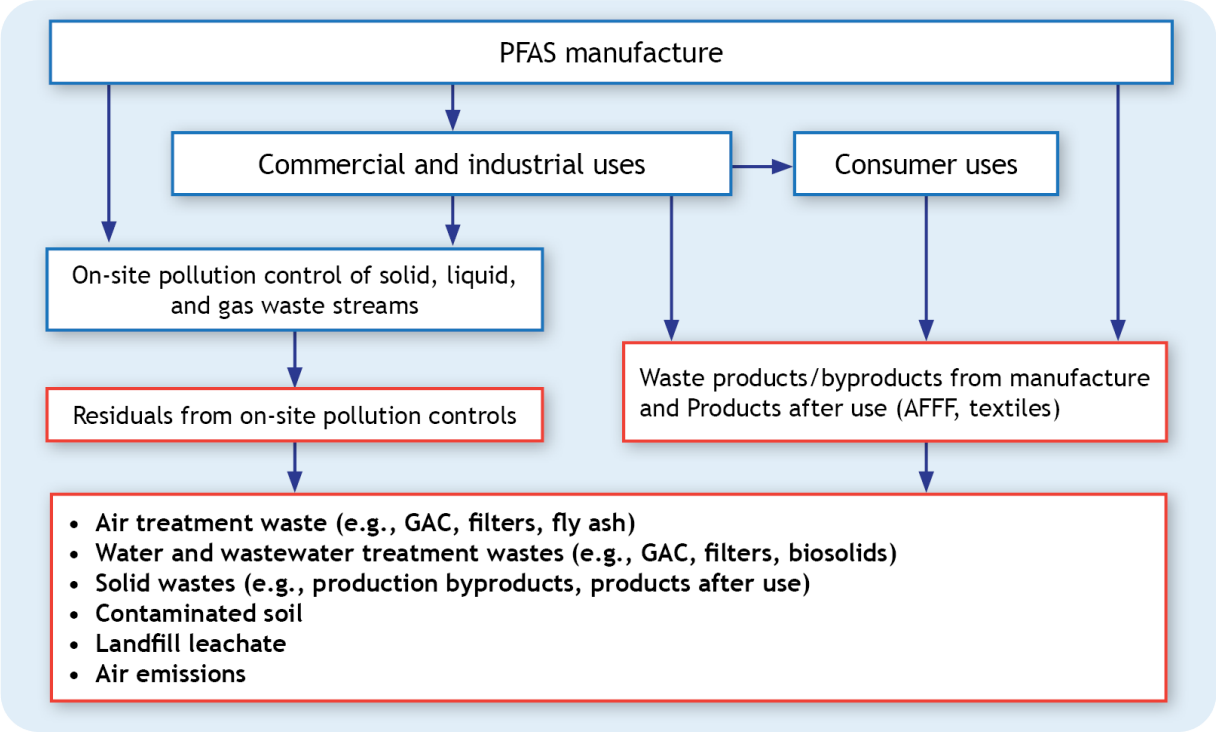
Figure 1-1. FY 2020 NDAA Section 7361.

1.b Scope and use of interim guidance

1.b.i PFAS and PFAS-containing materials in this interim guidance

Section 7361 of the FY 2020 NDAA (see Figure 1-1) lists six types of PFAS-containing materials and wastes. Although the information included in this interim guidance is likely to be suitable for other types of PFAS and PFAS-containing materials, this interim guidance addresses destruction and disposal centering on these six material types, which are described in more detail in Section 2. In this document, the PFAS-containing materials covered by the FY 2020 NDAA that will be destroyed or disposed of are referred to as "waste."

Figure 1-2 shows conceptually how manufacturing activities could result in material streams that are within the intended scope of this interim guidance, including GAC and aqueous film-forming foam (AFFF).



Note: The red-outlined portions show where the FY 2020 NDAA material types occur in the course of manufacture, use, and disposal of PFAS and PFAS-containing materials that are within the scope of this interim guidance.

Figure 1-2. Generation of PFAS materials identified in the FY 2020 NDAA.

1.b.ii Destruction and disposal technologies addressed in this interim guidance

This interim guidance presents three widely-used, commercially available destruction and disposal technologies that may be effective for managing PFAS and PFAS-containing materials. Advances in scientific understanding allow EPA to make some recommendations, however, filling information gaps and addressing uncertainties will improve the scientific understanding and could lead to additional guidance in the future. EPA updates this guidance at least every three years.

This interim guidance describes technologies with the potential to destroy PFAS or control releases of PFAS into the environment. Other waste management options are not discussed, because they are not within the intended scope of this interim guidance. For example, land application of biosolids containing PFAS does not control PFAS releases into the environment.

Current technologies. In developing this second version of the interim guidance, EPA focused on the three widely-used, commercially available destruction and disposal technologies that may be effective for managing PFAS wastes—thermal treatment, landfills, and underground injection. EPA also focused on reviewing data and information about the ability of each of these technologies to manage PFAS-containing materials through containment or destruction (i.e., breaking carbon–fluorine bonds and mineralization) while limiting releases of PFAS to the environment. In doing so, EPA prioritized addressing the scientific issues and uncertainties highlighted in the 2020 interim guidance and, when possible, relied on publicly available, peer-reviewed data and information (see Section 3).

Emerging technologies. Concerns about the effectiveness of current, widely-used technologies for managing PFAS and PFAS-containing materials have motivated efforts to develop new technologies and apply existing technologies to PFAS materials. EPA is aware of and, in some cases, has participated in efforts to identify, develop, and test non-thermal destruction technologies for PFAS-containing materials (see Section 6).

In some cases, managers of PFAS and PFAS-containing materials may consider emerging technologies for PFAS destruction. When considering the use of emerging technologies, managers of PFAS waste should work with state, territorial, or Tribal government agencies. To assist PFAS material managers with evaluating whether an emerging destruction (or disposal) technology is suitable for a particular PFAS-containing material, EPA developed a technology evaluation framework (see Section 6). The framework provides a transparent, consistent approach for evaluating destruction and disposal technologies for PFAS materials, including considerations for communities, including vulnerable populations.

In 2024, EPA is neither recommending nor discouraging the use of any emerging technology for managing the PFAS-containing materials specified in the FY 2020 NDAA. Instead, the Agency recommends that managers of PFAS-containing materials use the technology evaluation framework to evaluate emerging destruction and disposal technologies and to inform their decisions about managing PFAS-containing materials. EPA also encourages technology developers to generate and publicly release data that can be used to complete the framework (see Section 6).

Generally, destruction and disposal of PFAS-containing materials are currently not federally regulated. PFAS-containing materials may be managed in non-hazardous and hazardous waste treatment and disposal systems. Some exceptions exist, such as risk management requirements in cases where permits for production of new chemicals specify methods to be used for destruction or disposal following the review of a Toxic Substances Control Act (TSCA) new chemicals notice. Unless governing regulations require specific actions to the contrary, this guidance recommends selecting an approach with a higher potential to control PFAS releases into the environment, as possible.

Table 1-1. Destruction and Disposal Technologies Discussed in This Interim Guidance, with Examples of PFAS-Containing Materials

Destruction and Disposal Technology, by Physical Phase of Materials	Examples of PFAS-Containing Materials (within the Scope of the FY 2020 NDAA) That Could Be Managed Using These Technologies

Solid phase:
Landfill disposal
Thermal treatment
	• Drinking water, groundwater, and wastewater treatment residuals
	  ○ Biosolids/sewage sludge
	  ○ Spent GAC
	  ○ Ion exchange resins
	• Air waste stream treatment residuals
	  ○ Spent GAC
	  ○ Fly ash
	• Contaminated soil
	• End-of-life products (e.g., textiles)
	• Solidified liquid wastes

Liquid phase: Underground injection Thermal treatment	• Sewage sludge (liquid) • Landfill leachate • AFFF (spent or concentrate)

Destruction and Disposal Technology, by Physical Phase of Materials	Examples of PFAS-Containing Materials (within the Scope of the FY 2020 NDAA) That Could Be Managed Using These Technologies
	• End-of-life products (e.g., spent cleaning solvents) • Pollution control residuals (e.g., concentrates) from PFAS production and use
Gas phase: Thermal treatment	• Landfill gas (LFG) • Emissions from manufacture, use, or destruction of PFAS

1.b.iii Use of this interim guidance

This interim guidance provides information and suggested considerations for evaluating destruction and disposal options. This second version of the interim guidance is based on currently available research and information in 2023 and is responsive to the scope of the FY 2020 NDAA. It presents background information on the manufacture and uses of PFAS, as well as solid, liquid, and gas waste streams containing PFAS, including those materials identified in the FY 2020 NDAA: AFFF, soils and biosolids, textiles, spent water treatment materials, landfill leachate, and PFAS waste streams from facilities manufacturing or using PFAS (see Section 2). Most significantly, it provides up-to-date information on potential releases during the destruction and disposal of PFAS and PFAS-containing materials (see Section 3) and identifies data gaps to be filled that can inform future EPA guidance (see Section 5). This version also incorporates information received during the 2020 Interim Guidance's public comment period.

This version of the interim guidance takes the following into account:

- It is based on currently available information on technology performance and capabilities for destruction and disposal of the PFAS and PFAS-containing materials specified in the FY 2020 NDAA (see Section 1.b.i).
- It focuses on three widely-used, commercially available destruction and disposal technologies that may be effective for managing PFAS wastes (i.e., thermal treatment, landfills, underground injection) (see Section 3).
- It advises how to assess and consider the impacts of potential releases and exposure on communities, including identifying vulnerable populations, incorporating vulnerability into decision-making, and performing community engagement (see Section 4). The FY 2020 NDAA states that the interim guidance should take into consideration potentially vulnerable populations living near destruction or disposal sites.
- It identifies research needs to inform future updates to this interim guidance and provides updated information on ongoing research and development activities (see Section 5).
- It discusses the development of emerging technologies and includes a framework for evaluating technology/PFAS material combinations (see Section 6).
- It does *not* establish what concentrations of PFAS in wastes, spent products, or other materials or media would necessitate destruction or disposal. Regulatory mechanisms are more appropriate for establishing such concentrations.
- It provides references to destruction and disposal facility locators that decision-makers can use to request cost estimates. EPA recommends obtaining cost estimates from applicable destruction and disposal facilities as described in Section 1.c.ii. In addition, Appendix D provides some cost

information that PFAS material managers may want to consider when evaluating different techniques to destroy or dispose of PFAS and PFAS-containing materials. Note that cost information presented in this appendix has a relatively high level of uncertainty associated with it. Moreover, the cost of PFAS destruction and disposal may change based on demand and evolution of available technologies. Additionally, overall costs may vary widely based on the type of materials or the distance to a facility.

This interim guidance is not a regulation. Any discussion of EPA's regulatory authorities is for the purpose of describing controls relevant to the destruction or disposal of PFAS and should not be considered a description of the applicability of those authorities or affect EPA's or any other agency's ability to enforce under these authorities in the future. For information about EPA's other plans and actions related to PFAS, visit EPA's *PFAS Strategic Roadmap: EPA's Commitments to Action 2021-2024* and *Key Actions to Address PFAS*.

1.c Considerations for managing PFAS and PFAS-containing materials

The information presented in this interim guidance can help managers of PFAS or PFAS-containing materials make informed decisions regarding destruction and disposal. To assist decision-makers, EPA reviews destruction and disposal options for thermal treatment, landfills, and underground injection based on the relative potential for releasing PFAS to the environment. This approach highlights destruction and disposal options with lower potential for PFAS releases to the environment compared to other options. This approach is consistent with EPA's mission to protect human health and the environment and is designed to facilitate decisions that protect human health and the environment as much as possible.

EPA recognizes that the potential to control PFAS releases to the environment is one of several factors to consider when selecting among options for destroying or disposing of PFAS and PFAS-containing materials in a way that is consistent with maximizing protection to public health and the environment. Other factors include whether it is imperative to destroy or dispose of the waste immediately, availability of interim storage or destruction and disposal options, the type and volume of waste materials, the concentrations of PFAS in the waste, and the presence of and potential impacts to communities, including vulnerable communities. In the following sections, EPA provides guidance for these decisions in terms of technology considerations and infrastructure considerations.

1.c.i Technology Considerations - Relative potential for PFAS releases to the environment

Using the information presented in Section 3, and considering the significant uncertainties that remain, EPA listed destruction and disposal options in terms of relative potential for releasing PFAS to the environment. When considering the data and information presented in Section 3, EPA considered the type of data available (e.g., laboratory-, pilot-, or field-scale studies), the amount of data available (e.g., data from different operating conditions), and the quality of the data.

When considering the order, it is important to note that available performance and testing data are generally limited. Additional performance and testing data—including data on destruction and removal of PFAS in thermal treatment devices, and long-term performance data for landfills and underground injection—are needed to support more specific guidance. When these data and information become available, EPA may reconsider and revise the order presented below.

As a general approach, EPA encourages managers of PFAS and PFAS-containing materials to prioritize destruction and disposal options with a lower potential for releasing PFAS to the environment over the use of destruction and disposal technology options with a higher potential for environmental release of PFAS. EPA also encourages testing with EPA-approved methods of thermal treatment operations, including products of incomplete combustion and the presence of PFAS in all associated waste streams (see Appendix A), to evaluate whether site-specific conditions are minimizing potential environmental releases.

While the purpose of an effective destruction and disposal technology is to prevent or minimize environmental releases, it is also important to distinguish between a potential environmental release and a direct human exposure. A PFAS release does not inherently imply direct and immediate human exposure and a release does not necessarily present an unacceptable risk to specific populations.

EPA encourages managers of PFAS and PFAS-containing materials to make decisions in consideration of factors discussed in this guidance to select current or future disposal or destruction technologies that protect human health and the environment as much as possible. Based on currently available information cited in this guidance, EPA has listed the following disposal and destruction options from lowest to highest based on their relative potential to release PFAS to the environment:

- **Interim storage with controls.** While not a destruction or disposal method, interim storage may be a short-term option if the destruction or disposal of PFAS and PFAS-containing materials is not imperative, on-site storage capacity is readily available, and interim storage has proper controls in place to reduce releases into the environment. Storage may be more appropriate for some PFAS materials than others. For example, EPA recommends interim storage of low volumes of containerized or high PFAS-concentration materials. In contrast, some materials may be less appropriate for storage because they are continuously generated or are typically high-volume. With proper engineering controls in place, interim storage can control PFAS releases in the short-term.
- **Underground injection (Class I non-hazardous industrial and hazardous waste wells).** EPA has determined the use of Class I non-hazardous industrial waste and hazardous waste wells for high concentration liquid PFAS waste has a lower potential for environmental release when compared to other PFAS destruction and disposal options, and there is relatively low uncertainty in this determination. The standards associated with the construction, operation, and monitoring of these Class I wells are designed to isolate liquid wastes deep below the land surface and ensure protection of underground sources of drinking water (USDWs). Class I hazardous waste wells operators must conduct mechanical integrity testing more often than Class I non-hazardous industrial waste operators. They also must establish and follow additional procedures for reporting and correcting mechanical integrity problems. Class I hazardous waste well operators must also develop and follow a waste analysis plan and conduct annual tests of cement at the base of the well. While the standards associated with Class I hazardous wells are more stringent than for non-hazardous industrial wells, EPA has determined that the probability of failures for both has been demonstrated to be low. While Class I wells are an option for managing PFAS-containing fluids, this technology may not be appropriate everywhere. The limited number of wells currently receiving PFAS, well location, and waste transportation logistics may significantly limit the type and quantity of PFAS-containing fluids appropriate for underground injection. Additional Class I wells may need to be constructed where geologically suitable, and existing well permits may need to be modified to meet the capacity needs for PFAS disposal (see Section 3.c.v).

- **Hazardous waste landfills (Resource Conservation and Recovery Act (RCRA) Subtitle C).** This class of landfills has the most stringent environmental controls in place for minimizing environmental releases and migration of some PFAS from disposed waste. EPA recommends Subtitle C landfills when PFAS levels of the waste are relatively high and landfill disposal is the selected option. Permitted hazardous waste landfills have relatively low quantities of landfill leachate[3], which lessens the probability of mobilizing PFAS that is reversibly bound to the waste. These landfills are required to handle and treat leachate as listed hazardous waste (hazardous waste number F039 per 40 CFR 261.31), lessening the likelihood of environmental release. Because hazardous waste landfills generally do not receive organic wastes that decompose, PFAS migration via LFG is anticipated to be relatively low but LFG collection and destruction equipment could be installed if anaerobic decomposition is shown (see Section 3.b). While recent studies have improved understanding of PFAS migration, additional research is needed to resolve uncertainties (see Section 3.b.vi).
- **Landfill disposal in all landfill types: Landfill disposal of stable polymeric PFAS**. Stable polymeric PFAS, such as polytetrafluoroethylene (Teflon™) and fluorinated ethylene propylene (FEP), are large molecules that are not susceptible to hydrolysis or oxidation. They are neither volatile nor water soluble and, as a result, they are expected to remain within the waste mass for long periods of time. This PFAS category tends not to migrate with the leachate and is unlikely to volatilize with the LFG; therefore, permitted hazardous and municipal solid waste (MSW) landfills (MSWLFs) may provide a good disposal option. Some data are available to support this assessment, but more data are needed to resolve uncertainties (see Sections 3.b.i and 3.b.vi).
- **GAC reactivation units with thermal oxidizers operating under certain conditions**. Carbon reactivation systems, with the associated use of off-gas incineration (i.e., afterburners operating at temperatures >1,100°C[4]) and gas scrubbing units, can potentially destroy PFAS without significant environmental releases, or without PFAS remaining on the reactivated carbon. However, as discussed in Section 3.a.i, more data are needed for confirmation, particularly regarding reactor conditions, differing carbons, and products of incomplete combustion (PICs). There is uncertainty associated with this option because it is based on limited research. While recent studies have improved understanding, additional research is needed to resolve uncertainties on PFAS emissions from full-scale reactivation facilities (see Section 3.a.v).
- **Thermal treatment units operating under certain conditions.** Hazardous waste combustors (HWCs), including commercial incinerators, cement kilns, lightweight aggregate kilns (LWAKs), and thermal oxidizers, that operate under certain conditions (i.e., feeding liquid PFAS-containing materials, higher temperatures >1,100°C[4], well mixed, and adequate residence time) may be more effective at adequately destroying (mineralizing) PFAS and minimizing PICs. There is uncertainty associated with this option because it is based on limited research and observations, there are limited data for the treatment of solid materials or containerized wastes, and there are limited emissions data (e.g., no data for PIC formation and the presence of PFAS in air pollution control device residuals) at full-scale facilities (see Section 3.a.v) due to previous methodology limitations.
- **Landfill disposal in MSWLFs that have composite liners and leachate and gas collection and treatment systems: Landfill disposal of volatile or water soluble PFAS.** MSWLFs receive non-hazardous waste and most have composite liners and gas collection systems. Soluble PFAS in these

[3] EPA defines landfill leachate as the liquid that is formed when rain water filters through wastes placed in a landfill. When this liquid comes in contact with buried wastes, it leaches, or draws out, chemicals or constituents from those wastes (*https://www.epa.gov/landfills/municipal-solid-waste-landfills*).

[4] Maximum PIC destruction was observed at and above 1,090°C as described by Shields et al., 2023.

landfills are likely to be released over time in landfill leachate if they are not adequately bound or sequestered in immobile solids. Further research is needed on the ability of various solidification techniques to retain soluble PFAS for extended periods under conditions found in MSWLFs. Although MSWLFs generally have leachate collection and management processes in place, the typical practices employed are not expected to prevent further PFAS migration. In addition, MSWLFs generally receive significant quantities of organic wastes (e.g., food scraps) that decompose, and thereby can cause PFAS migration via LFG. Multiple studies have reported elevated levels of PFAS in leachate and LFG. Studies estimate that up to 15 percent of mobile PFAS disposed in MSW landfills is emitted to the environment over time. EPA does not recommend MSWLF disposal of media containing relatively high concentrations of soluble or volatile PFAS. While recent studies have improved understanding of PFAS migration in MSWLFs, additional research is needed to resolve uncertainties (see Section 3.b).

- **Thermal treatment at lower temperatures** including municipal waste combustors (MWCs), sewage sludge incinerators (SSIs), or HWCs operating at lower temperatures or feeding solid or containerized PFAS-containing materials. Some HWCs operate at temperatures near or below 1,100°C, MWCs typically operate between 850 and 1,000°C, and SSIs typically operate at even lower temperatures. There is uncertainty associated with this option because it is based on very limited research. While limited, existing datapoints suggest the potential for air emissions of PFAS and PICs orders of magnitude above detection limits at temperatures below 1,000°C (Shields et al., 2023). There are no data on the treatment of solid materials or containerized wastes at lower temperatures. There are currently insufficient data and information to conclude anything about PIC formation or PFAS air emissions from full-scale combustion units operating at these lower temperatures or when feeding solid materials or containerized wastes. Because there are insufficient data available, there is low confidence in the reliability of this technology to control PFAS releases (see Section 3.a).
- **Construction and Demolition (C&D) landfills: Landfill disposal of volatile, water soluble, or oxidizable PFAS.** This class of landfills may not have a composite liner or leachate collection system. Because of the potential for environmental releases, EPA does not recommend that PFAS waste, other than wastes containing stable polymeric PFAS, be disposed of in this type of landfill. While few studies have documented emissions of PFAS from C&D landfills, the known mobility of PFAS in landfills and lack of engineered controls at C&D landfills make them unsuitable for PFAS disposal. For these reasons, EPA does not expect that C&D landfills will minimize PFAS releases to the environment (see Section 3.b).

1.c.ii Infrastructure considerations – communities, costs, and capacity

Waste management infrastructure in the United States is a complex and varied system, crucial for managing the vast amounts of waste generated, including PFAS-containing waste. Therefore, in addition to considering potential for environmental release, decision-makers should consider site- or project-specific factors that may influence decisions about managing PFAS-contaminated wastes. Specific factors include, but are not limited to, the composition of the waste, the types and concentrations of PFAS, transportation costs, destruction and disposal costs, and impacts to communities.

EPA emphasizes the importance for decision-makers (managers of PFAS-containing materials making decisions about destruction and disposal) to consider potential impacts to nearby communities, including vulnerable populations, when evaluating destruction, disposal, and storage options. Those concerns are amplified because the uncertainties in the effectiveness of these options to limit or control environmental releases can contribute to adverse impacts, including potentially disproportionate and

cumulative impacts, for vulnerable communities. Therefore, EPA recommends decision-makers screen communities located in the vicinity of potential releases from the destruction, disposal, and storage options (considering fate and transport) in order to consider the potential for community impacts, including adverse and disproportionate impacts (see Section 4), and to consider potential measures to address such impacts. Depending on site-specific circumstances (e.g., PFAS concentrations, impacted media, and potential exposure pathways), the size and shape of this area (vicinity) will vary. In deciding how to address PFAS waste, site-specific considerations and waste characteristics should factor into the decision. For example, destruction or disposal of waste with a high concentration of PFAS using a method that has a higher potential for environmental release may not be well suited for a site where there is greater likelihood of potential exposure to vulnerable populations.

The waste management infrastructure's design and operation are heavily influenced by regional needs, local regulations, and technological advancements. As a result, waste management options vary by the type of waste and by location due to factors like availability, feasibility, and transportation requirements. For instance, urban areas may incur higher disposal costs due to limited space while rural areas may face higher transportation costs to distant disposal facilities. Furthermore, technological advances specifically related to the management of PFAS-containing materials can potentially reshape the cost and operational landscape of PFAS waste management. EPA recommends that decision-makers request cost estimates directly from potentially applicable destruction and disposal facilities. In addition, decision-makers can refer to Appendix D, which summarizes costs and factors to consider when estimating costs to destroy or dispose of PFAS and PFAS-containing materials.

Decision-makers seeking lists of waste management sites in the United States can access various resources depending on the type of waste and location. It is important to note that often the most direct source is local government websites, such as county or city environmental services or public works departments. These websites typically provide comprehensive lists and maps of local facilities, along with their types, hours of operation, and accepted waste materials. For specialized waste like hazardous or medical waste, state environmental protection agencies or EPA are valuable resources. EPA's website, in particular, offers databases and tools such as *RCRAInfo*, which provides detailed information about facilities managing hazardous waste.

A general yet incomplete list of potential treatment and disposal facilities may also be available through the EPA Incident Waste Decision Support Tool (*I-WASTE DST*). This list includes landfills (hazardous waste, MSW, industrial, and C&D), HWCs, and nonhazardous combustors (sewage sludge and municipal waste) but does not include all technologies available for PFAS destruction and disposal (e.g., cement kilns, carbon reactivation facilities, and underground injection facilities). I-WASTE DST is updated periodically and is limited to information available online.

Similar to cost estimation, management capacity estimates can also be challenging. Facilities that can potentially manage PFAS-containing waste are not evenly distributed. Their number and capacity vary by region. Some areas across the United States face challenges due to limited available landfill space or capacity limits. Incineration and underground injection capacities can also be greatly influenced by technological capabilities and local regulations.

2. Description of PFAS-Containing Materials Identified in the FY 2020 NDAA

The FY 2020 NDAA identifies six types of materials that commonly contain PFAS (see Figure 1-1 and the Executive Summary). This section discusses each FY 2020 NDAA material type, its origin, potential sources of PFAS, current disposal and treatment methods, and potential releases to the environment.

Data on FY 2020 NDAA-relevant material types come from a variety of sources, and more PFAS data will be available from EPA-managed datasets in the near future. For example, EPA's Chemical Data Reporting (CDR) dataset includes production volumes for manufactured and imported amounts and conveys certain industrial processing and use activities. One data element distinguishes amounts that are recycled instead of discharged or released to a waste stream. In the 2020 CDR reporting cycle, submitters began using Organization for Economic Cooperation and Development (OECD)-based industrial processing and use codes to better harmonize those data (U.S. EPA, 2020a).

In 2022, EPA issued two memoranda recommending EPA and state National Pollutant Discharge Elimination System (NPDES) permitting authorities and publicly owned treatment works (POTW) pretreatment program authorities conduct certain activities to align wastewater and stormwater NPDES permits, pretreatment program implementation activities, and biosolids monitoring with the goals in EPA's *PFAS Strategic Roadmap* (U.S. EPA, 2022a, 2022d). The memoranda recommend that EPA and state permitting authorities use the most current sampling and analysis methods in their NPDES programs to identify known or suspected sources of PFAS and to take actions using their pretreatment and permitting authorities, such as imposing technology-based limits on sources of PFAS discharges. The memoranda will also help the Agency obtain comprehensive information through monitoring regarding the sources and quantities of PFAS discharges, informing other EPA efforts to address PFAS.

Additionally, EPA's Toxics Release Inventory (TRI) program collects data related to industrial releases and waste management of certain chemicals. The TRI dataset includes, among other information, quantities of environmental releases to all media above certain thresholds (including on-site disposals and land application), as well as quantities transferred to off-site waste management facilities, including POTW. Under Section 7321 of the FY 2020 NDAA, certain PFAS were added to the TRI list beginning with Reporting Year 2020 (U.S. EPA, 2020b). As of Reporting Year 2023 (reporting forms due by July 1, 2024), a total of 189 PFAS are on the TRI list. TRI reporting data can be accessed at *https://www.epa.gov/toxics-release-inventory-tri-program/tri-data-and-tools*.

In October 2023, EPA finalized the PFAS 8(a)(7) reporting rule under TSCA (88 FR 70516, October 11, 2023). In accordance with obligations under TSCA, as amended by the FY 2020 NDAA, the PFAS 8(a)(7) reporting rule requires any person that manufactures (including import) or has manufactured (including imported) PFAS or PFAS-containing articles in any year since January 1, 2011, to electronically report information regarding PFAS uses, production volumes, disposal, exposures, and hazards. The PFAS 8(a)(7) rule will help EPA better understand the sources and quantities of PFAS manufactured in the United States and provide EPA with the most comprehensive dataset of PFAS manufactured in the United States. EPA will begin receiving reports under the PFAS 8(a)(7) rule in 2025.

2.a Solid, liquid, or gas waste streams containing PFAS from facilities manufacturing or using PFAS

PFAS do not occur naturally. They are synthesized for use in a diverse array of industrial and commercial applications. Industrial waste streams containing PFAS stem from two main sources: (1) primary manufacturing facilities of PFAS and (2) secondary industries that use PFAS or manufacture finished products that contain PFAS. A 2009 survey by OECD identified 27 primary manufacturers and processors of PFAS globally (OECD, 2011). At the time of that survey, more than 90 percent of the global annual production of PFAS was generated by eight manufacturers (Posner et al., 2009), all of which participated in EPA's 2010/2015 Perfluorooctanoic Acid (PFOA) Stewardship Program (U.S. EPA, 2006).

The goal of the PFOA Stewardship Program was to reduce PFOA facility emissions and their use in products by 95 percent by 2010, compared with 2006 baseline levels, and to eliminate PFOA from all facility emissions and products by 2015 (U.S. EPA, 2006). Companies that did not participate in the PFOA Stewardship Program likely continue to use these chemical substances. Long-chain PFAS and their precursors may still be produced as unintentional byproducts and may persist in facility emissions and as product impurities in small quantities (3M Company, 1999; Boucher et al., 2019; Lehmler, 2009; Kissa, 2001). Perfluoroalkyl sulfonic acids containing six or more carbons and perfluoroalkyl carboxylic acids with seven or more carbons are generally considered to be long-chain PFAS. PFOA and PFOS are examples of long-chain PFAS, and PFBS and PFHxA are examples of short-chain PFAS. Furthermore, information on the toxicity and environmental fate and transport of alternative PFAS chemistries is limited (Sun et al., 2016; Wang et al., 2014).

For the 2020 CDR reporting cycle, 52 sites reported a total domestic manufacture and import production volume of 339,000 tons for PFAS in 2019. This represents the aggregate production volume for PFAS produced and imported into the United States across all industries. Although the aggregate production volume might not include all PFAS sources (for instance, a specific chemical or site may not meet reporting obligations), it provides a proxy quantity of all PFAS domestically produced and imported.

PFAS might be released into the environment at every step in the production process, including during synthesis, polymerization, application, transport, usage, waste stream management, and disposal (3M Company, 2000b).

Table 2-1 lists examples of PFAS uses and the resulting solid, liquid, and gas waste streams for primary and secondary manufacturers of PFAS-containing materials and certain service sectors as indicated by industry, national and global inventories, and research. However, this list is not exhaustive or representative of all current uses, applications, recovery and recycling practices, or treatment technologies that could affect the volume and characteristics of the resulting waste streams. EPA recognizes the need for continued research to better characterize the multi-media PFAS-containing materials targeted for destruction or disposal, as discussed in Section 5.a.

2.a.i Solid phase wastes

Primary manufacturing and secondary industrial use of PFAS can generate solid waste streams with PFAS-containing materials (OECD, 2011, 2015). For example, some PFAS synthesis processes can produce tars and polymers consisting of high-molecular-weight byproducts that are either fully or partially fluorinated. These byproducts can be recycled back into the process or treated as waste (3M Company, 2000a, 2000b). Solid wastes such as fly ash (e.g., thermal treatment) or spent GAC (e.g., drinking water treatment) can also be produced from PFAS incineration and other treatment processes.

Other important solid-phase materials include sludges and biosolids (see Section 2.c) that result from stabilizing or treating process waters and wastewaters, either on site or at a municipal wastewater treatment plant (WWTP)[5] that receives influent from industrial sources (Venkatesan & Halden, 2013). In addition to solids produced via treatment, spent water treatment media (such as activated carbon and ion exchange resins) are part of this waste stream (see Section 2.e). Other direct industrial sources of solid wastes containing PFAS include intentional residuals, such as cuttings and fibers from textile manufacturing (see Section 2.d), and materials unintentionally produced outside of product specification. The concentrations and composition of PFAS in solid wastes generated from primary and secondary industrial sources vary by facility and depend on factors such as facility- or industry-specific production processes and the types and quantities of PFAS produced or used (ITRC, 2020).

The characteristics of these solid phase PFAS-containing wastes vary significantly, depending on several factors. As discussed in Section 3.b, the type of PFAS (e.g., polymeric, soluble, or volatile PFAS) as well as the type of waste with which the PFAS is comingled (e.g., biodegradable or non-biodegradable) will dictate the potential and pathways for environmental release. The type of environmental release will, in turn, inform the most appropriate disposal option(s) for each type of PFAS-containing solid waste.

2.a.ii Liquid phase wastes

Primary manufacturers and secondary industrial users of PFAS can generate liquid phase wastes in the form of: (1) liquid byproducts of PFAS synthesis to be recycled or disposed of (e.g., to a landfill) following stabilization, solidification, or another management method; (2) process wastewater resulting from activities using PFAS as a processing aid (e.g., surfactant, emulsifier, mist suppressant, sizing agent) and production of finished products containing PFAS; (3) spills or unintentional releases of liquid wastes and products containing PFAS; and (4) AFFF/water/foam mixtures from the use of fire-extinguishing agents (see Section 2.b) for emergency response activities and emergency response trainings at industrial facilities.

Table 2-1 provides examples of liquid wastes containing PFAS generated by industrial sources and their uses. This is not an all-inclusive list of industries nor waste streams.

Another liquid phase waste stream is wastewater effluent discharged directly from a primary manufacturer or secondary industrial PFAS user. Effluent from wastewater treatment facilities that receive wastewater from industrial PFAS sources may also contain PFAS. According to several studies, conventional wastewater treatment technologies are generally ineffective at destroying or controlling PFAS (Schultz et al., 2006) and may result in higher measurable PFAAs (e.g., PFOA, perfluorooctane sulfonate [PFOS], and their homologues) when precursor compounds (e.g., fluorotelomers) are degraded during the treatment process (Buck et al., 2011; Dauchy et al., 2017a; Schultz et al., 2006; Sinclair & Kannan, 2006). Less often, primary industrial PFAS manufacturers have opted to transport liquid wastes off site for incineration (North Carolina Department of Environmental Quality, 2017).

2.a.iii Gas phase wastes

Studies suggest that PFAS in air emissions from manufacturing facilities are a source of both localized (i.e., within a short radius of the facility) and long-distance (i.e., global) transport of PFAS within the environment (Davis et al., 2007; Dreyer et al., 2009). Non-volatile forms of PFAS, such as the anionic

[5] Regulations at 40 CFR part 122.2 and part 403.3 identify these “municipal wastewater treatment plants" as treatment works treating domestic sewage (TWTDS) or publicly owned treatment works (POTW), dependent on their ownership.

PFAAs (e.g., PFOA, PFOS, their homologues), are associated with airborne particulates when emitted as aerosols from stack emissions at primary manufacturing facilities (Barton et al., 2006; Dreyer et al., 2015). Gas phase emissions of volatile and semivolatile PFAS, and the subsequent transformation of precursor compounds into persistent PFAAs, are a potential mechanism for the atmospheric transport of PFAS. For example, volatile fluorotelomer alcohols (FTOHs) and perfluoroalkyl sulfonamides can transform into perfluoroalkyl carboxylic acids (e.g., PFOA and homologues) and perfluoroalkyl sulfonates (e.g., PFOS and homologues); these can be deposited at significant distances from their origin, which may result in soil and groundwater contamination (Dreyer et al., 2009; Ellis et al., 2004; Martin et al., 2006; Schenker et al., 2008).

Table 2-1. Examples of PFAS Waste Streams by Industry Type.
This is not an all-inclusive list of industries or waste streams.

Industry Type	Uses	Examples of Waste Streams[a]			Source
		Solid	Liquid	Gas	
Primary chemical manufacturing	PFAS synthesis, feedstocks for primary products, feedstocks for secondary users, processing aids (fluoropolymers)	Process byproducts (tars), sludges/residuals, off-spec materials[b], treatment residuals (GAC/anion exchange resins), spill residues (replacement and legacy), *particulate emissions*	Degraded/stabilized process wastes, wastewater effluent, *stack emissions condensate*	Stack emissions, fugitive volatiles	3M Company (1999, 2000b)
Secondary Manufacturing (Industry Users of PFAS-Containing Materials)[c]					
Adhesives manufacturing	Component of solvent- and water-based adhesives, rubber to allow bonding to steel, and urea-formaldehyde adhesive resins for wood particleboard bonding	*Used filter media and filter residues, residues of cured adhesives, empty containers, used shop rags (from cleaning), contaminated soil (from spill cleanup residues)*	*Residues of liquid adhesives, off-spec products[b], contaminated wastewater (from spill cleanup residues)* *For cleaning: equipment startup, cleaning, and flushing wastes; spent cleaning solvents; and contaminated wastewater*	Stack emissions, fugitive volatiles	ASC (n.d.), RadTech International North America (2010)
Cleaning product manufacturing	Component of household cleaners; car wash products; automobile waxes; wiper fluid; strongly acidic or basic cleaners for concrete, masonry, airplanes		*Off-spec products[b], liquid residues from empty containers, and spills*	Stack emissions, fugitive volatiles	3M Company (1999)

Industry Type	Uses	Examples of Waste Streams[a]			Source
		Solid	Liquid	Gas	
	Used for removal of adhesives, dry cleaning of textiles or metal surfaces, machine parts cleaning after nickel plating, and removal of calcium sulfate from reverse osmosis membranes				
Computers/ electronics manufacturing	Component of sealant for electric circuits, zinc battery electrolyte, wetting agents in solders, polar solvents used before welding Used for removal of cured epoxy resins from integrated circuit modules, treatment of insulated wire, alkaline manganese battery MnO_2 cathode treatment, production of polymer electrolyte membrane for fuel cells, cleaning of electronic components, and coating of the surface of magnetic recording devices	*Collected airborne particulates for cleaning/surface preparation*	*Spent acid solution for cleaning/surface preparation, liquid residues from empty containers, and spills*	Stack emissions, fugitive volatiles	U.S. EPA (1990)
Film/ lithography manufacturing	Used in coatings for surface tension, static discharge, and adhesion control for films, papers, and printing plates, and as a surfactant in mixtures used to process imaging films	Rags and wipes discarded by applicator; solids coated with PFAS from processing, sampling, quality assurance; off-spec products[b]	Solvent waste, liquid residues from empty containers, spills, and unused application mix	Coating application exhaust	3M Company (1999), Bowden et al. (2002)
Metal plating/ fabrication	Used as a surfactant, wetting agent, and mist suppressing agent; as a wetting agent fume suppressant for chromium plating and chromium anodizing; as a dispersion product used to coat metals; as a	*Off-spec products*[b]	Spent plating or etching baths, rinse water effluent, liquid residues from empty containers, and spills	Stack emissions, fugitive volatiles	3M Company (1999), U.S. EPA (2009b)

Industry Type	Uses	Examples of Waste Streams[a]			Source
		Solid	Liquid	Gas	
	blocking agent for aluminum foil; in plating baths; and to treat metal surfaces				
Oil and gas drilling/ extraction/ refinery/ support	Component of chemical barrier used for containing oil spills Used as a surfactant for recovery in oil/gas recovery wells, a jet fuel/hydrocarbon solvent, and in hydraulic oils Used as a gasoline/petroleum product evaporation inhibitor in storage tanks in the following forms: a floating layer of cereal grains treated with PFAS, an aqueous layer containing PFAS		Applied product[d] (oil spills, oil and gas recovery wells), *liquid residues from empty containers, and spills*		UNEP (2011), Kissa (2001)
Paint/coating manufacturing	Component of coatings, paints, varnishes, dyes, ink jet printer inks, and ski waxes	*Pigment dust*	*Unused paint products, off-spec products[b], liquid residues from empty containers, and spills*	*Fugitive volatiles, atomized paint*	Waste Management and Research Center (1992)
Paper products/ packaging manufacturing	Waterproofing and grease proofing for products including food contact paper (e.g., plates, popcorn bags, pizza box liners, food containers, wraps), non-food contact applications (e.g., folding cartons, carbonless forms, masking papers)	Dusts; *solids coated with PFAS from processing, sampling, quality assurance; off-spec products[b]*	Spillage, cleanup, and releases during opening, rinsing, and cleaning of PFAS totes	Fugitive volatiles	U.S. EPA (2009a)
Pesticide/ fertilizer/ other agriculture chemical manufacturing	Pesticide and herbicide ingredients and packaging	*Particulate emissions*	*Liquid residues from empty containers, spills, off-spec products[b], cleaning of equipment, and process wastewaters*	*Fugitive volatiles*	U.S. EPA (2023b)

Industry Type	Uses	Examples of Waste Streams[a]			Source
		Solid	Liquid	Gas	
Plastic materials/ resins/ rubber product manufacturing	Used to make membranes used in fuel cells; chlor-alkali cells; water, caustic soda, and caustic potash electrolyzers; silicone rubber sealants; composite resins; polytetrafluoroethylene (PTFE, or Teflon); and polyvinylidene fluoride or polyvinylidene difluoride (PVDF) Processing aid for PVDF manufacture, mold-release agent in foam molding, and antiblocking agent for rubbers	Dusts, spillage (micropowders and resins), cuttings, scrap, debris, and off-spec products[b]; particulate emissions	*Liquid residues from empty containers, spills, and unused application mix*	Fumes from PTFE heating (volatile)	Ebnesajjad (2015)
Textiles/ apparel/ leather/ carpets/ fiber manufacturing	Dispersion products that coat fabrics: jackets, shoes, umbrellas, carpets, upholstery, leather, tents, sails	Solids coated with PFAS from cutting, shearing, packaging, lab and color sampling, quality assurance; flakes or dust containing PFAS; *off-spec products*[b]	Spills, wastewater effluent from product adhering to inside of drum, and unused application mix	Releases of vapors and aerosols during application of surface treatment and mechanical finishing	U.S. EPA (2009a)
Aerospace component manufacturing	Mechanical components such as tubing, hoses, and seals; brake and hydraulic fluid additive; wire and cable insulation; used in coating and paint		*Wastewater effluent*		FluoroCouncil (2019)
Automotive component manufacturing	Mechanical components such as tubing, hoses, and seals; brake and hydraulic fluid additive; anti-mist film on windshields; used in coating/paint; used in coatings or surface treatments of textiles and upholstery		*Wastewater effluent*		FluoroCouncil (2019), ITRC (2020)

Industry Type	Uses	Examples of Waste Streams[a]			Source
		Solid	Liquid	Gas	
Semiconductor manufacturing	Etching solutions for photolithography, glass etching, plastics etching, fused silica, aluminum; liquid etchant in photo mask rendering		Spent plating or etching baths, PFOA residues from photoresist developers associated with semiconductor liquid waste streams, liquid residues from empty containers, and spills Photoresists and antireflective coatings stripped off from semiconductor devices before shipment are present in waste solvent streams	Photoresists and antireflective coatings stripped off from semiconductor devices before shipment are present in waste gas streams	Bowden et al. (2002), Tremblay (2015)
Building and construction materials manufacturing	Component of cement and primers used to coat cement mortar; used in wire and cable insulation and coatings for wood particleboards	*Cuttings and debris, off-spec materials*[b]	*Wastewater effluent*		Buck et al. (2012), FluoroCouncil (2019), U.S. EPA (2009a)
Mining industry	Surfactant for recovery of metals from ores; used in ore flotation to separate metal salts from soil, electrowinning of metals, and nitrogen flotation to recover uranium	Contaminated rock from applied product[d]	Applied product[d]		ITRC (2020)
Medical uses	Video endoscopes; catheters; saline solutions for in vitro diagnostics; treatment/coatings for textiles such as hospital gowns, curtains, drapes; dialysis machines	*Laboratory/medical solid wastes (e.g., tubing, filters, films)*			FluoroCouncil (2019), Posner (2012)
Cosmetics and personal care product manufacturing	Used in cosmetics, hair conditioning formulations, hair creams, and toothpaste	*Off-spec materials*[b]	*Wastewater effluent*		Danish EPA (2018), FluoroCouncil (2019), Schultes et al. (2018)

Industry Type	Uses	Examples of Waste Streams[a]			Source
		Solid	Liquid	Gas	
Fire suppression systems[e]	AFFF and certain dry fire-extinguishing agents	Contaminated soil and debris from applied product[d]	Applied product[d]		See Section 2.b for more information. Gaines (2023)

[a] The italicized waste streams may contain PFAS, given what wastes the relevant industry sectors are known to generate and given applications of PFAS. The presence and concentration of PFAS have not been quantified.

[b] "Off-spec materials" or "off-spec products" are materials or products that do not meet specified standards or requirements and are discarded rather than sold or used.

[c] Some industries listed under "Secondary Manufacturing" may also include primary manufacturing of PFAS. The waste streams resulting from manufacture of PFAS in these industry sectors are addressed in the first row, "Primary chemical manufacturing."

[d] "Applied product" refers to the intentional application of a PFAS-containing product to the environment.

[e] Fire suppression systems, which include AFFF, are commonly found in manufacturing, storage, extraction and refining, and national defense facilities, as well as airports, fire departments, and other federal facilities (e.g., facilities operated by the National Aeronautics and Space Administration [NASA] and the U.S. Department of Energy [DOE]). Although this is an industrial use, not an industrial sector, the prevalence of AFFF in the above primary and secondary PFAS manufacturing industries warrants highlighting these waste streams.

2.b Aqueous film-forming foam

AFFFs are a group of PFAS-containing fire extinguishing agents for low-flashpoint hydrocarbon fuel fires (Tuve et al., 1964). AFFFs are intended for use where a significant flammable liquid fire hazard exists (FFFC, 2016).

AFFFs are based on synthetic fluorosurfactants that provide unique low surface tension and positive spreading coefficient characteristics. When mixed with water and applied, AFFFs form an aqueous film and a foam solution to coat the liquid fuel, seal fuel vapor, and reduce oxygen availability, extinguishing the fire and preventing burnback (FFFC, 2016; SERDP, 2020; Sheinson et al., 2002).

Until application, AFFF is managed as a concentrated product containing less than 2 percent PFAS fluorosurfactants by weight for a typical 3 percent AFFF concentrate (ITRC, 2020). AFFF is stored in either fixed, structural dispensing systems, such as those in hangars and aboard vessels, or in mobile, vehicle-based systems (i.e., aircraft rescue firefighting [ARFF] vehicles) (Field et al., 2017). Reserve AFFF concentrate inventory may be stored in hangars or warehouses. The amount of AFFF concentrate in the finished foam varies by manufacturer and application circumstances, but is usually between 1 and 6 percent, meaning the fluorosurfactants are diluted to less than a fraction of a percent (FFFC, 2016; ITRC, 2020).

A 2004 inventory estimated that there were 4.6 million gallons of legacy PFOS-containing AFFF in the United States (Darwin, 2011). AFFF inventory depletion is determined by frequency of use for firefighting, training, or testing; transfers between locations; and other factors. However, AFFF's characteristically long shelf life means there should be little disposal due to expiration (FFFC, 2016), increasing the possibility that legacy PFOS-containing AFFF concentrate remains in service or reserve inventories. Fluorotelomer-based AFFF surfactant products became available in the 1970s (Prevedouros, 2006). In 2017, the U.S. Department of Defense (DoD) edited its military specification for AFFF to include

no more than 800 parts per billion (ppb) of PFOA and PFOS in the concentrate, the limit set by DoD Quality Systems Manual 5.1. However, this specification does not mention any other PFAS standards.

In the United States, AFFF and associated systems are or have been in service at federal facilities, civil airports, and oil refineries. Civilian fire departments also use or have used AFFF. The federal government is working to identify areas of current and former federal properties where PFOS- or PFOA-containing AFFFs have been used (Darwin, 2011; DoD, 2020). Through September 30, 2023, DoD has determined that 715 active military installations, base realignment and closure (BRAC) locations, National Guard facilities, and formerly used defense sites (FUDS) properties require an assessment of PFAS use or potential release. DoD is performing preliminary assessments/site inspections (PA/SIs) at these installations, which is the first phase of the cleanup process and may take two to three years to complete. As of September 30, 2023, DoD has completed the PA/SI phase at 570 installations. The Department has determined that no further action is required at 118 of these installations, while 452 are proceeding to the next step in the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) process (DoD, 2024).

The FY 2020 NDAA prohibits AFFF use at any military installation, effective October 1, 2024 or up to two years later if the Secretary of Defense deems a waiver necessary. DoD issued a policy in January 2016 to discontinue land-based AFFF training and testing activities, unless the AFFF could be captured and properly disposed. Since then, DoD has managed any mission-critical AFFF use in response to an emergency event as a spill response to mitigate impacts to the environment (DoD, 2019, 2020). DoD, among other entities, is also investing in research and development for fluorine-free AFFF alternatives (SERDP, 2020). In 2023, DoD issued a new military specification for fluorine-free foam (MIL-PRF-32725) (DoD, 2023).

Examples of AFFF users and locations in the United States are listed in Table 2-2. Note that the list of sources in the table is non-exhaustive.

Table 2-2. Examples of AFFF Users and Locations in the United States

AFFF User	Locations	Comments
DoD	• Hundreds of military installations (active and former) with AFFF use • In-service systems installed at an estimated 1,350 locations in aircraft hangars and on an estimated 3,000 ARFF vehicles	• In 2016, DoD discontinued AFFF use for military installation training and testing unless the AFFF could be captured and properly disposed and is investing in fluorine-free alternatives • The FY 2020 NDAA requires DoD to end any land-based AFFF by October 1, 2024 (with extensions possible until 2026) • Sources: DoD (2017, 2019, 2020); CBO (2019); SERDP (2020)
NASA	• Multiple properties with ARFF apparatus • Five properties with fixed systems	• In late 2018, NASA issued order to cease training with AFFF • Source: SERDP (2020)

AFFF User	Locations	Comments
DOE	• Lawrence Berkeley National Laboratory • Brookhaven National Laboratory • Los Alamos National Laboratory • Strategic Petroleum Reserve facilities (Gulf of Mexico)	• Sources: DOE (2021 *DOE Initial Assessment of PFAS at DOE Sites*); Darwin (2011)
Airports and supporting facilities	• 523 Federal Aviation Administration (FAA)-certified civilian airports in 2018 • Since 2006, an annual average of nearly 600 certified airports • Supporting facilities include firefighting training sites, such as the FAA Technical Center's Fire Training Area	• Starting in 1972, FAA regulations allowed for the use of AFFF among a number of different extinguishing agents at civilian airports; since 2006, FAA guidance (FAA, 2006) provides that AFFF must conform to the applicable military specification (AC 150/5210-6 and MIL-F-23485F) at most civilian airports [a] • 80 percent of respondents to a 2017 survey of U.S. and Canadian airports reported training-related discharge directly to the ground; two-thirds reported testing-related discharge directly to the ground • FAA has taken steps to reduce AFFF discharges during testing since 2019 • Sources: DOT (2019); FAA (2006, 2019); Thalheimer et al., (2017); U.S. EPA (2020c)
Fire departments	• At civilian fire departments throughout the United States	• AFFF may be present at fire departments • Some states have begun inventorying and reporting having AFFF (e.g., Michigan, New Hampshire) • Some states have take-back programs to help local fire departments identify AFFF in inventory and assist with removal and disposal (e.g., Vermont) • Sources: Michigan PFAS Action Response Team (n.d.); New Hampshire Department of Environmental Services (2020); Vermont Agency of Natural Resources (n.d.)
Oil refineries and processing facilities	• Oil refineries and related facilities (e.g., storage facilities)	• Little information is available about AFFF in this sector, though published industry guidelines recommend AFFF for pipeline emergencies • Survey-based data suggest this sector is the second largest consumer of AFFF after federal agencies • Sources: Darwin (2011); Noll & Hildebrand (2016)

AFFF User	Locations	Comments
Ships and other vessels	• Ships and other marine vessels, including the U.S. Coast Guard	• Little information is available about AFFF quantities on ships • There has been a shift toward non-fluorinated AFFF for some uses including testing and training, though the Coast Guard has indicated that certain uses (e.g., required inspections) must continue to use fluorinated AFFF • Source: U.S. EPA (2020e)

[a] An exception exists for airports with low departure traffic and serving aircraft less than 90 feet in length. See 14 Code of Federal Regulations (CFR) 139.317 for more information (FAA, 2006).

Fate and transport of PFAS in AFFF after use depends on the release circumstances and chemical-specific properties. Though sometimes classified as incidental releases (Thalheimer et al., 2017), equipment failure, accidental releases, or operator error can result in substantial leaks (Anderson et al., 2016; Resolution Consultants, 2016; Leidos, 2016).

Engineering controls (such as dikes, barriers, or basins) may be installed at facilities with significant flammable liquid hazards to contain foam solution and runoff for later disposal (FFFC, 2016). Where such hazards do not significantly exist, or installed engineering controls are otherwise not practicable, firefighting personnel may block sewer drains or deploy portable dikes as containment measures (FFFC, 2016). Runoff can then be pumped out, and the affected environmental media can be removed for disposal (ITRC, 2020). C&D debris originating from facilities where AFFF was historically released may also be a source of PFAS in landfills and groundwater (Solo-Gabriele et al., 2020).

Though subject to site-specific characteristics and conditions, studies demonstrate AFFF use at airports is a source of PFAS in soil and groundwater (Ahrens et al., 2015; Dauchy et al., 2017b; Høisæter et al., 2019). Further, PFAA precursors from original AFFF concentrate products may transform in the environment to more mobile products over time (Houtz et al., 2013), expanding plumes long after AFFF use is discontinued.

In the 2022 EPA-issued memoranda that recommended NPDES and POTW pretreatment program permitting conditions (U.S. EPA, 2022a, 2022d), EPA recommended that NPDES stormwater permits contain best management practices (BMPs) to address and minimize AFFF discharges, as included in the Naval Air Station Whidbey Island MS4 NPDES Permit[6]:

- Prohibiting the use of AFFFs other than for actual firefighting.
- Eliminating PFOS- and PFOA-containing AFFFs.
- Requiring immediate clean-up in all situations where AFFFs have been used, including diversions and other measures that prevent discharges via storm sewer systems.

[6]Naval Air Station Whidbey Island MS4 NPDES permit: *https://www.epa.gov/sites/default/files/2020-12/documents/r10-npdes-naval-air-station-whidbey-ms4-was026611-final-permit-2020.pdf*

2.c Soils and biosolids

As required by Clean Water Act Section 405(d), EPA established requirements for the final use or disposal of sewage sludge when it is: (1) applied to land as a fertilizer or soil amendment; (2) placed in a surface disposal site, including sewage sludge-only landfills; or (3) incinerated. The regulation at 40 CFR part 503 defines sewage sludge as "solid, semi-solid, or liquid residue generated during the treatment of domestic sewage in a treatment works." Sewage sludge includes scum or solids removed in primary, secondary, or advanced wastewater treatment processes and any material derived from sewage sludge (e.g., a blended sewage sludge/fertilizer product) but does not include grit and screenings or ash generated by the incineration of sewage. Sewage sludge treated for land application is commonly referred to as "biosolids."

Part 503 considers domestic septage as sewage sludge and sets separate requirements for domestic septage applied to agricultural land, forests, or reclamation sites. Influent containing PFAS that enters wastewater treatment facilities may result in the presence of PFAS in biosolids (Thompson et al., 2023; Helmer et al., 2022; Venkatesan & Halden, 2013). When biosolids are applied to land, there is the potential for leaching or runoff (Schaefer et al., 2022; Navarro et al., 2016; Lindstrom et al., 2011; Sepulvado et al., 2011; Washington et al., 2010). If landfilled instead, there are concerns with PFAS releases via landfill leachate and gaseous emissions, especially for highly organic material (Section 3.b). Alternatively, if incinerated, there is the potential for PFAS to be emitted as PICs (Section 3.a). Research needs and resources on possible emerging PFAS destruction technologies for biosolids (e.g., pyrolysis/gasification coupled with a thermal oxidizer and supercritical water oxidation) are shared in Sections 5 and 6. Across disposal and destruction technologies, there are capacity concerns and more research is needed on potential PICs/products of incomplete destruction (PIDs).

Total nationwide biosolids generation and management statistics in 2021 (reported to EPA's biosolids program as of January 9, 2023) and data considerations are presented in Table 2-3. EPA does not have data on the volume of biosolids that contain PFAS; however, EPA is planning for the next National Sewage Sludge Survey in collaboration with the Effluent Guidelines Program Plan 15's upcoming POTW influent study, both of which will focus on monitoring for PFAS. Additionally, EPA is currently conducting a refined risk assessment for PFOA and PFOS in biosolids (U.S. EPA, 2024), as well as recommending exposure mitigation measures and pretreatment program BMPs to reduce PFAS sources (U.S. EPA, 2022d).

Table 2-3. Biosolids Generation and Management in 2021

NPDES permits issued to POTW[a]	**16,109**
Biosolids NPDES permits[b]	9,834
Biosolids annual reporters (2021)[c]	2,519
Sewage Sludge generated in 2021 (metric tons)[d]	**4,548,462**
Land application of biosolids (metric tons)	1,955,684
Land disposal (also called surface disposal) (metric tons)	94,522
Incineration (metric tons)	633,054
Landfilling (metric tons)	1,808,136
Other management (metric tons)	57,066

[a] An NPDES permit is typically a license for a facility to discharge a specified amount of a pollutant into a receiving water under certain conditions. A POTW is a treatment works treating domestic sewage (TWTDS) that is owned, and usually operated, by a local or regional government agency (U.S. EPA, 2020b).

[b] Biosolids permits apply to any person or treatment works that prepares sewage sludge, applies sewage sludge to the land, or fires sewage sludge in a sewage sludge incinerator and to the owners/operators of surface disposal sites, as well as the exit gas from sewage sludge incinerator stacks (U.S. EPA, 2019).

[c] Annual reports are required for POTW that have a design flow rate equal to or more than one million gallons per day, that can serve populations of 10,000 or more, that are required to have approved pretreatment programs (Class I Sludge Management Facilities), or that are otherwise required to report (U.S. EPA, 2023a).

[d] Total amount of biosolids generated is reported by each TWTDS and may not equal the sum of component amounts reported for each management method. Biosolids totals do not represent PFAS presence in biosolids.

In addition to land application of PFAS-containing materials (e.g., biosolids), there are other scenarios where PFAS release to soils can occur. Direct release of PFAS into soils can occur through use of PFAS-containing products such as AFFF, and the discharge or application of treated industrial or municipal wastewater containing PFAS. PFAS can also be in soils due to unintentional contact, such as spills or leaks during the production, handling, transport, or use of PFAS-containing materials (see Table 2-4 for examples of industrial sources of PFAS in soils). Both direct and indirect soil impacts might also occur via the atmospheric deposition of PFAS released from stack emissions and atmospheric transformation products of volatile precursors, respectively (Davis et al., 2007; Dreyer et al., 2009; Schenker et al., 2008). Remediation wastes such as soils excavated during the cleanup of sites or during decommissioning of facilities where PFAS was manufactured, used, or applied may contain diverse mixtures of PFAS in elevated concentrations.

2.d Textiles, other than consumer goods, treated with PFAS

Because PFAS can repel oil, water, and stains, the textile industry uses these chemicals in a broad range of textile products other than consumer goods (apparel or household textiles). For example:

- PFAS can be used to treat outdoor equipment such as tents and sails (UNEP, 2011).
- Technical or occupational textiles, such as protective clothing for firefighters, can be treated with PFAS or woven from fluoropolymers (OECD, 2013).
- Medical garments can be treated with fluorinated polymers (OECD, 2013).
- Fluoropolymers can be spun into fibers and used to make sailcloth and fabric for fire suppression needs (Tokarsky & Uy, 2003).

- PTFE can be woven to make architectural fabrics, such as roofs, and can also be used to coat fiberglass for tensile structures or long-life structures (Fabric Architect, 2020).
- Textiles made from fiberglass coated with or saturated with PFAS are used for high-temperature or corrosive industrial environments. Kevlar and perfluoroplastic composite textiles are used for similar industrial environments (Robco, 2020).

Examples of typical PFAS-containing waste streams generated from textiles include discarded industrial or commercial textiles such as apparel, carpets, or personal protective equipment; solids coated with PFAS from cuttings and shearings; and fugitive volatiles from spray applications of textile surface treatments. The destruction and disposal technologies used for these waste streams include landfill disposal and thermal treatment. For examples of industrial waste streams from the textiles/apparel manufacturing industry, see Table 2-4.

2.e Spent water treatment materials

EPA published a proposed rule for a PFAS National Primary Drinking Water Regulation (NPDWR) for six PFAS in March 2023 that will require compliance once finalized and promulgated. Additionally, 12 states have established standards limiting the presence of various PFAS in drinking water. EPA has identified "best available technologies" for treating drinking water for PFAS to comply with the NPDWR; these technologies will produce residuals. The 2024 Interim Guidance details options available to manage these residuals while minimizing environmental exposures and releases to the extent feasible. EPA notes that there are multiple approaches for water systems to manage PFAS residuals that are generated when PFAS is removed from drinking water. As of December 2023, there are no specific federal regulatory requirements for PFAS disposal, and the presence of PFAS in treatment residuals does not impose any additional federal requirements, but they may be subject to regulatory attention. EPA's PFAS actions are updated on the webpage *Key EPA Actions to Address PFAS*.

Conventional drinking water treatment methods such as coagulation, flocculation, sedimentation, disinfection, and filtration do not remove PFAS found in source water. Therefore, residues from these processes such as grit, spent membrane, or filter elements are unlikely to contain significant PFAS.

Although novel technologies for removing PFAS from drinking water sources and groundwater are being developed, current processes known to be effective are activated carbon, anion exchange resins, and high-pressure membranes (reverse osmosis [RO] and nanofiltration [NF]) (U.S. EPA, 2022b, 2022c). This section discusses the residual streams of these three processes (see Section 3 for discussions on treatment and disposal considerations, Appendix D for costs and considerations, and Section 5 for discussions of research needs for more novel treatments) and regulatory considerations for drinking water treatment utilities.

Table 2-4 shows the primary residual streams by water treatment process, with options for reactivation, destruction, and disposal of these residuals.

Table 2-4. Destruction and Disposal Options for Drinking Water Treatment Residuals Containing PFAS

Treatment Process	Residuals Generated	Destruction/ Disposal Option(s)	Section Reference
Granular Activated Carbon	Spent GAC	Reactivation	3.a.i.2
		Incineration	3.a
		Landfill	3.b

Treatment Process	Residuals Generated	Destruction/ Disposal Option(s)	Section Reference
Powdered Activated Carbon (PAC)	Spent PAC	Incineration	3.a
		Landfill	3.b
Ion Exchange	Single use and regenerative ion exchange: spent resins	Incineration	3.a
		Landfill	3.b
	Regeneration brine	Interim Storage	1.c
		Underground Injection	3.c.ii.1
RO/NF	Brine	Permitted discharge, direct or indirect	Not described in this guidance. Refer to permit.

The practices discussed in Table 2-4 are not in a hierarchical order and should be chosen based on site-specific conditions. The practices presented in Table 2-4 may help reduce and/or control unintentional PFAS releases to the environment.

2.e.i Activated carbon

Activated carbon (discussed more extensively in Section 3.a.i) is manufactured from carbon-rich sources such as coal or wood and is used to treat water or air by filtering contaminants (U.S. EPA, 2012). Activated carbon can be used in either PAC or GAC form. In PAC treatments, carbon is powdered and added to the water for treatment, and then chemicals in the water stick to the powdered carbon as the water passes through and typically carries the PAC along. In GAC treatments, chemicals in water adsorb to small pieces of carbon in a bed as the water passes through.

PAC application will remove some PFAS from water, though the amount of PFAS removed depends on many factors. For PAC treatment, the residual stream is the sedimentation sludge or the filter backwash solids that contain the PFAS-laden PAC along with the coagulant, coagulant aids (if used), natural particulates, and enmeshed organic carbon (Dudley et al., 2015). Given the many conditions that affect treatment, the weight percent PFAS in a PAC residual stream varies by many orders of magnitude but will be in the same range as GAC treatment, as discussed below.

For GAC, the range of PFAS concentrations on spent media can be estimated in several ways (Crone et al., 2019; U.S. EPA, 2020d). A conservatively high loading of PFAS onto GAC (using a GAC with a high-capacity potential, a strongly adsorbing PFAS, few competitive contaminants, low organic levels, and a high concentration in the feed water treated) is on the order of 2 percent by weight (e.g., 0.02 grams PFOS per gram of GAC). For more common treatment scenarios, or for PFAS that are adsorbed more weakly (i.e., the shorter-chain PFAS), the weight percent of PFAS will be orders of magnitude lower—about $1x10^{-5}$ grams PFAS per gram of GAC.

GAC can be reactivated and reused (see Section 3.a.i.2 for a discussion on GAC reactivation). If the GAC is landfilled, PFAS can desorb off the carbon into the landfill leachate if the GAC comes into contact with a water stream that contains low PFAS, or more strongly adsorbing constituents that can displace the PFAS. Desorption will occur due to the disequilibrium between the liquid and solid phases and will accelerate at higher temperatures where adsorption becomes less favorable for exothermically adsorbed contaminants. Therefore, the resulting PFAS concentrations in the landfill leachate impacted by spent GAC will vary greatly depending on conditions.

Typically, deep bed GAC does not require backwashing because the particulates in the water are removed ahead of the GAC system, but there may be instances where particulates deposit onto the GAC, creating a headloss that will require backwashing. If so, the backwash water may contain a PFAS foam that may need to be managed.

2.e.ii Ion exchange resins

Like GAC, ion exchange resins are utilized in fixed bed adsorbers. Similarly, the residual stream from this treatment is the spent media; however, ion exchange resins cannot be thermally reactivated like GAC. Due to several factors, the market is moving toward single-use media for anion exchange resin treatment with incineration as the final disposal point for the spent resin.

Final PFAS loadings onto resins vary widely. A conservatively high estimate of loading (calculated using a PFAS-selective resin, few competitive constituents, and a PFAS known to adsorb well) is expected to be in the order of 10 percent by weight for the strongly adsorbing PFAS (e.g., 0.1 gram PFOS per gram of resin). The higher percent weight than GAC is due to the high capacities of PFAS-selective resins. Like GAC, more typical scenarios for PFAS that are more weakly adsorbed (i.e., shorter-chain PFAS) will yield much lower average weight percent of PFAS.

Assuming single-use resin is used, landfilling may be less expensive than incineration. If the resin is landfilled, PFAS can desorb off the resin if the resin comes into contact with a water stream whose counter ions can displace the PFAS. The resulting leachate concentrations will vary tremendously depending on conditions. In rare cases, it may be appropriate to regenerate the ion exchange resins with a brine; however, the latest science has shown that an alcohol solvent is needed along with the inorganic exchange ion. This is difficult to do at the full-scale, especially for drinking water facilities. If done in this manner, the resulting regenerate brine will have high concentrations of PFAS and will need to be disposed of appropriately. In rare cases, there may not be a ready disposal option for this regenerate brine and interim storage may be appropriate. Before selecting interim storage, it is important to consider that generating routine volumes of waste can pile up over time, which may become more difficult to store than originally envisioned. Otherwise, regenerate brine may be handled like other high-strength liquid PFAS wastes.

Typically, resin beds do not require backwashing because the particulates in the water are removed ahead of the resin system, but there may be instances where particulates deposit onto the resin, creating a headloss that will require backwashing. If so, the backwash water may contain a PFAS foam that may need to be managed.

2.e.iii High-pressure membranes (reverse osmosis and nanofiltration)

High-pressure membranes are extremely effective for removing many PFAS from water to a high degree (Crone et al., 2019; U.S. EPA, 2020d). As high-pressure membranes are based on a rejection phenomenon, water treatment with them creates a waste stream with potentially high concentrations of PFAS as well as treated water with a lower pH. These waste streams also have high concentrations of salts, other contaminants, and dissolved organic matter.

Treatment of the concentrated residual stream can be challenging, and the cost will likely be high, similar to those for landfill leachates, ion exchange spent regenerates, and waters from highly contaminated sites. Many variables could affect the cost of treating these waste streams. At this time, there is no obvious treatment technology choice, especially given that the concentrated retentate

stream is typically 20 percent of flow (Baruth, 2005). This represents a sizeable flow, especially for large membrane treatment systems, such as those used by large municipalities (e.g., treating 20 million gallons per day [4 million gallons per day] concentrate flow). This large-volume flow would prevent the use of batch treatment processes, which have higher efficiencies because they can process the water multiple times before discharge.

When the membranes lose their integrity or foul to the point of needed replacement, they will need to be disposed of. It is not anticipated that the materials of construction are high in PFAS unless they are specifically made of PFAS materials (e.g., Nafion). Also, the mass of PFAS remaining on the membrane surfaces should be minimal except in extreme cases.

2.e.iv Regulatory considerations for drinking water treatment utilities

Sites with reject water from high pressure membranes typically have a NPDES permit[7]. To meet NPDES statutory requirements to obtain an NPDES permit prior to discharging any pollutant to waters of the United States, drinking water treatment utilities using membranes must follow all NPDES permit or pretreatment program requirements, including those applicable to PFAS, for any permit or control mechanism issued for their facility. For more information, see EPA 2022d.

2.f Landfill leachate containing PFAS

Landfill leachate (discussed in more detail in Section 3.b.iii.5) is the liquid formed by rainwater percolating through waste in landfills. Leachate generation may continue even after a landfill's closure period, as a result of liquids inherent in the waste or if the cap system fails. There are different types of solid waste landfills characterized by the wastes managed, which also dictate the environmental controls employed. Hazardous waste landfills and most MSW landfills are required to collect the liquid leachate captured within the landfill liner and subsequently manage or treat the leachate. While PFAS concentrations in different landfill leachates have been documented (see Table 3-3 in Section 3.b), there are no monitoring or reporting requirements at the federal level for PFAS in landfill waste or leachate. Thus, existing treatment methods are being used to process leachate irrespective of PFAS concentrations.

Landfill leachate can be treated on site or off site. The most prevalent off-site management approach is to export leachate to a TWTDS, where it is mixed with wastewater and treated. However, as noted in Section 2.a.ii, conventional wastewater treatment technologies are generally unable to treat or control PFAS (Schultz et al., 2006). Other off-site treatment methods include incineration and underground injection (see Sections 3.a and 3.c, respectively). The on-site leachate treatment technologies employed at landfills are explored in Table 3-2 in Section 3.b.i. Some management approaches and treatment technologies represent significant pathways for PFAS release. Unlined impoundments, release to constructed wetlands, and land applications can release PFAS and potentially contaminate groundwater. Additional research is needed to determine the efficacy of landfill leachate treatments for PFAS (see Section 5).

[7] The Clean Water Act prohibits anybody from discharging "pollutants from a point source" to a "Water of the United States" unless they have an NPDES permit. See 40 CFR Section 122.1(b)(1). The terms "pollutant," "point source," and "Waters of the United States" are defined at 40 CFR Section 122.2.

2.g Summary

PFAS are synthetic chemicals that are used in a diverse array of industrial and commercial applications. Industrial waste streams containing PFAS stem from two main sources: (1) primary manufacturing facilities of PFAS chemistry and (2) secondary industries that use PFAS-containing products and/or manufacture finished products containing PFAS. Ultimately, PFAS end up in solid, liquid, or gas waste streams from industrial facilities that manufacture or use PFAS and PFAS-containing products. Other common PFAS-containing waste streams include AFFF, biosolids, textiles, spent water treatment materials, and landfill leachate. Any of these waste streams can contribute to PFAS entering environmental media, including soil and groundwater. The potential destruction, disposal, and treatment technologies for processing these streams are discussed in Section 3.

2.h References

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3. Technologies for the Destruction and Disposal of PFAS and PFAS-Containing Materials

EPA has identified three existing and potentially available destruction and disposal technologies that may be effective for managing PFAS and PFAS-containing materials. These technologies are thermal treatment (Section 3.a), landfilling (Section 3.b), and underground injection (Section 3.c). Each subsection describes various considerations for these technological solutions, including types of treatment, potential for releasing PFAS into the environment, and uncertainties. References appear at the end of each subsection. See Section 6 for a discussion of emerging technologies that show potential to destroy PFAS. See Appendix D for a summary of costs and considerations.

3.a Thermal treatment

As the industrial and commercial use of fluorinated organic compounds grew over the past 70 years, their presence, compositions, and concentrations in materials that need to be destroyed or disposed of also grew. Organic fluorine-containing materials including flame suppressants, refrigerants, long- and short-chain PFAS, fluoropolymers, and their precursor chemicals have been commonly treated using a variety of thermal treatment technologies. These range from permitted HWCs and industrial furnaces to MWCs, SSIs, and thermal oxidizers designed to treat material and process gas emissions. Compared to landfills and underground injection, thermal treatment offers the potential advantage of mineralizing PFAS and preventing further release into the environment.

The carbon–fluorine bond is much stronger than the carbon–chlorine bond. Breaking the carbon–fluorine bond requires 1.5 times more energy and therefore higher temperatures and reaction times. Based on calculated bond energies, the most difficult fluorinated organic compound to decompose is carbon tetrafluoride (CF_4), which requires temperatures over 1,400°C (2,550°F) (Tsang et al., 1998). This is due to the compound's four carbon–fluorine bonds and symmetrical structure. The presence of carbon–carbon or carbon–hydrogen bonds (as in hexafluoroethane [C_2F_6] or fluoroform [CHF_3]) provides a weak point in the structure and thus significantly lowers temperatures needed for decomposition. Due to their thermal stabilities, short-chain fluorinated carbons such as CF_4, CHF_3, C_2F_6, and octafluoropropane (C_3F_8) may be good indicators of broader PFAS defluorination. In addition, these compounds may be relatively easy to monitor by Fourier transform infrared spectrometry (FTIR) and are targets measured in whole gas samples by Other Test Method 50 (OTM-50)[8], which makes them potential low-risk candidate surrogates for thermal destructibility trials.

Further, fluorinated organic compounds can be destroyed in flames by free radical initiation, propagation, and branching mechanisms. To increase the efficiency of these processes, it is important to provide high concentrations of hydrogen radicals (as in flames) to promote hydrogen fluoride (HF) formation, reducing the strong flame inhibition effects of fluorine radicals. There is concern that carbon–carbon bonds can be broken at moderate temperatures, leaving carbon–fluorine fragments. The fate of these carbon–fluorine radicals will depend on the local temperatures and concentrations of

[8] EPA recently released the OTM-50 methodology *https://www.epa.gov/emc/emc-other-test-methods*.

other free radicals. If the local energies and free radical concentrations are low, these carbon–fluorine fragments may recombine to form fluorinated PICs. However, if the local temperatures and concentrations of free radicals (particularly hydrogen radicals) are high, as occurs in flames, the carbon–fluorine species is more likely to degrade further to CO_2 and HF.

The stability of perfluorinated radicals and their propensity to recombine present the potential for the creation of PFAS PICs distinctive from the original fluorinated compounds. These reactions are promoted by partial combustion caused by insufficient temperatures, time, and turbulence. Many PFAS are composed of very stable fluorinated carbon chains and relatively weak non-fluorinated functional groups. Often, the functional group is easily removed, allowing the fluorinated chain to react with other radicals and create a variety of compounds, which complicates the determination of destruction and removal efficiencies (DREs) and the identification of PICs (Wang et al., 2015). In addition, the presence of catalytic surfaces, often metals, may promote further reaction and PIC formation in post-combustion regions. PFAS PICs may be smaller in molecular weight than the original species or larger in molecular weight when formed via the recombination of two large radicals.

Thermal treatment units generally use high-temperature combustion and incineration to destroy organic materials and control organic pollutants. Incineration is an effective and permitted method for destroying certain halogenated organic chemicals including chlorinated solvents, polychlorinated biphenyls (PCBs), dioxin-laden wastes, brominated flame retardants, refrigerants, and ozone-depleting substances.

Combustion is described by complex sets of physical processes and elementary reactions involving many intermediate and free radical species. Even combustion of a simple fuel, such as methane, involves several hundred elementary reactions. For more complex fuels, or systems that include additional elements, such as fluorine, elementary reactions can number in the thousands or more. These elementary reactions describe pathways or mechanisms whereby large molecules, such as PFAS, decompose and transform during combustion.

Thermal treatment of halogenated organic compounds generates the corresponding halogen acid when sufficiently high temperatures and long residence times break the carbon–halogen bonds and the resulting halogen atoms react with available hydrogen. Thermodynamically, for a reacting system containing carbon, hydrogen, fluorine, and oxygen, chemical equilibrium favors the formation of carbon dioxide, water, and hydrogen fluoride. Unfortunately, complete decomposition is almost impossible to achieve due to kinetic (temperature) and transport (mixing) limitations. If temperatures are insufficient or mixing is incomplete at any point, complete conversion to hydrogen fluoride and carbon dioxide may not be achieved. This incomplete decomposition is the genesis of many pollutants from hydrocarbon fuels PICs. Certain thermal technologies can potentially achieve the operating conditions necessary to break down fluorinated organic compounds, but not every unit typically operates at these conditions. Data are lacking about the effectiveness of the characteristic operating conditions of the different thermal destruction technologies currently used to treat PFAS materials. Also, more measurement methods are needed to evaluate technology performance for both the destruction of specific chemical species and the formation of byproducts. As a result, EPA does not know if/which thermal treatment technologies are fully able to achieve adequate PFAS destruction and minimization of PFAS PICs.

Hazardous waste combustion technologies (commercial incinerators, cement kilns, and LWAKs) can potentially achieve temperatures and residence times sufficient to break apart the PFAS contained in the waste stream being thermally treated, but not every unit currently operates at those conditions and

those that do may not operate at those conditions at all times. To assess the capability of HWCs to destroy the materials fed into them and ensure that harmful emissions into the environment are minimized, it is standard practice to evaluate the DRE of one or several principal organic hazardous constituents (POHCs) during a trial burn. POHCs are selected to be at least as difficult to destroy as the feed material, based on an incinerability index scale of how difficult a compound is to destroy. The existing index does not include PFAS compounds. Permitted hazardous waste facilities have stringent regulatory controls on temperatures and other important operating parameters and require greater than 99.99 percent DRE for each selected POHC. Data from pilot-scale experiments conducted by EPA researchers indicate that "several operating conditions above 1090 °C resulted in high destruction efficiency (DE)[9] and few detectable fluorinated PIC emissions. However, several conditions below 1,000 °C produced DEs greater than 99.99 percent for the quantifiable PFAS and mg/m^3 emission concentrations of several nonpolar PFAS PICs. These results suggest that DE alone may not be the best indication of total PFAS destruction, and additional PIC characterization may be warranted" (Shields et al., 2023).

For HWCs, DRE is calculated based on the amount of a specific chemical entering an incinerator and the amount of that same chemical exiting the incinerator. While the compound selected for DRE determination in an HWC is chosen based on the difficulty of destroying it, DRE does not account for chemical reactions or transformations into new/different organic compounds (i.e., PICs). Because DRE does not account for chemical transformations, it is not an accurate indicator, by itself, of complete PFAS destruction. Recent research (Shields et al., 2023) has demonstrated that PIC formation can occur even when high DRE is measured at temperatures below 1,000 C. It is not accurate to state that a high DRE by itself indicates complete destruction of PFAS and that no other PFAS are being emitted. Complete PFAS destruction, also referred to as "complete mineralization," results in the production of only HF and CO_2 and does not include the production of other PFAS or other fluorinated organic compounds. The term "adequate destruction" is used in this guidance because it is not feasible to measure complete mineralization due to methodological limitations.

To fully evaluate whether adequate destruction is occurring, EPA and other investigators need to sample and analyze for a range of chemicals exiting a thermal treatment device, including the specific PFAS in the feed material, other PFAS beyond those in the feed (i.e., potential PICs), and HF. EPA currently lacks test methods for measuring most PFAS before and after the incineration process, but new methods are being advanced that will provide more information in the coming years. Currently, standard methods are available for HF (e.g., EPA Method 26A) and a targeted list of semivolatile PFAS (i.e., Other Test Method 45 [OTM-45]), and other test methods are in development for a relatively small percentage of PFAS as surrogates for the class of PFAS compounds (see Section 3.a.iv).

For this second version of the interim guidance, PFAS destruction is defined as the severing of all carbon–fluorine bonds in a PFAS molecule and the mineralization of carbon and fluorine to CO_2, HF, and water. HF and some of the other products of combustion can be effectively removed in pollution control devices designed for acid gas removal. The PFAS Thermal Treatment Database (PFASTT) is a growing database that contains more than 2,000 records of 80 sources documenting the treatability of PFAS in different media via various thermal processes.

[9] Destruction efficiency differs from destruction removal efficiency because samples were collected before any pollution control devices.

EPA currently has limited data characterizing emissions from HWCs that burn PFAS (Focus Environmental, 2021, 2022), along with data from a pilot-scale study at an EPA research facility (Shields et al., 2023). These studies are summarized below and provide some insight on the potential efficacy of thermal treatment in an HWC. To date, the commercial facility studies have focused on the removal of easily transformed PFAS target compounds, which is a significant limitation.

Clean Harbors Environmental Services. Two testing campaigns (2021 and 2022) were conducted at Clean Harbors Environmental Services' hazardous waste incinerator located in Aragonite, Utah (EA Engineering, Science, and Technology, Inc., PBC., 2021, 2022). Each test campaign was divided into three test conditions: Baseline (Condition 1), Augmented PFAS Feed (Condition 2, where known amounts of four or five target PFAS were fed to the incinerator), and Post-spiking Normal Operations (Condition 3, where AFFF was included in the incinerator feed). Clean Harbors used OTM-45 (*Measurement of Selected Per- and Polyfluorinated Alkyl Substances from Stationary Sources*) for stack gas sampling and analysis. The 49 targeted PFAS analytes were measured using liquid chromatography/tandem mass spectrometry (LC/MS/MS) with isotope dilution. Utilizing the available analytical methods at the time, the Clean Harbors study demonstrated a DRE for the legacy PFAS in the 99.9999 percent range at high temperatures. Of the 49 compounds detected by OTM-45, 34 were non-detect in the stack gas and only 15 were detectable at extremely low concentrations. The tests appear to demonstrate that the facility's primary objective was met: "demonstrate the ability of the high-temperature incineration system at Aragonite to effectively destroy PFAS in the waste feed during normal process operating conditions." Although a high DRE was achieved (greater than 99.999 percent) for the five selected PFAS compounds that were subjects of these studies, these tests did not collect data to fully support demonstration of adequate destruction as the production of PICs were not evaluated due to methodology limitations.

EPA PFAS thermal destruction research. Recently, EPA's Office of Research and Development (ORD) completed an initial study examining the relationship between destruction efficiency (DE) and PICs (Shields et al., 2023). This study used a pilot-scale research furnace and atomized a PFOS-dominant AFFF at various furnace locations and combustion environments. These included atomization through the flame with the natural gas auxiliary fuel and at various post-flame locations so that the AFFF would experience decreasing peak temperature exposures ranging from ~1,200°C to ~800°C (2,192°F to 1,472°F). Stack measurements included OTM-45 and a new draft GC/MS-based Other Test Method for non-polar volatile PFAS (OTM-50). Instead of DREs, this study calculated DEs, because samples were collected before any pollution control, i.e., removal equipment, including fabric filter and alkaline wet scrubber. Analysis of the AFFF and OTM-45 samples was used to calculate DEs for 10 PFAS quantified in the AFFF. OTM-50 was used to quantify concentrations of 30 PFAS PICs. Results indicate that several idealized operating conditions with peak temperatures above 1,090°C (1,976°F) resulted in high DEs (greater than 99.99 percent) and low PIC emissions that were near or below the detection limits. However, several conditions below 1,000°C (1,832°F) produced DEs greater than 99.99 percent for the quantifiable PFAS while simultaneously measuring emission concentrations of several volatile PFAS (mg/m^3). The study concluded that DE alone may not be the best indication of PFAS destruction.

Additional studies are necessary to more fully measure and evaluate the ability to approach complete mineralization and eliminate potential formation of PICs. EPA is working to advance new measurement methodologies (see Section 3.a.iv.1) and gather additional information to conclude whether potential PICs are adequately controlled (see Appendix A).

This section focuses on considerations associated with thermal treatment of PFAS and PFAS-containing materials. It discusses:

- The types of thermal treatment units that manage PFAS-containing materials and their ability to effectively destroy PFAS.
- The potential for environmental releases during these thermal treatment operations.
- PFAS monitoring methods relevant to these thermal treatment operations.
- Uncertainties and unknowns associated with thermally treating PFAS-containing materials, including the ability to effectively measure and monitor thermal treatment performance.

3.a.i Types of thermal treatment

The following subsections describe the types of thermal treatment devices that may have directly or indirectly received and treated the PFAS-containing materials identified in Section 2.a. These subsections focus on design and operation parameters that are important for destroying PFAS. Treatment devices described here include HWCs, non-hazardous waste combustors, carbon regeneration and reactivation units, and thermal oxidizers. Waste incinerators are typically classified by the type of waste they combust. Suggested resources for locating such facilities are included in Section 1.c.ii.

3.a.i.1 Hazardous waste combustors

HWCs include hazardous waste incinerators (HWIs), cement kilns, LWAKs, boilers, and hydrochloric acid production furnaces that burn hazardous waste. Two types of HWCs that have treated PFAS waste in the United States are commercial[10] incinerators and LWAKs.

All HWIs, LWAKs, and cement kilns are subject to RCRA and Clean Air Act (CAA) permitting requirements[11,12] that provide additional regulatory oversight and include operating requirements and emission limitations to safely and effectively treat regulated hazardous contaminants that may not be required for non-permitted facilities. These types of HWCs are subject to CAA Title V permitting requirements, and to maximum achievable control technology standards pursuant to Section 112 of the CAA (40 CFR part 63, subpart EEE). These standards include emission limitations for metals, dioxins/furans, particulate matter, hydrogen chloride and chlorine gas, and carbon monoxide or hydrocarbons, as well as limits on minimum organic DRE, but do not include emission limitations for PFAS. Also, under the authority of RCRA's "omnibus" clause (Section 3005(c)(3); see 40 CFR 270.32(b)(2)), RCRA permit writers may impose additional terms and conditions on a site-specific basis as may be necessary to protect human health and the environment. Due to these additional safeguards, permitted HWCs may operate under conditions more conducive to destroying PFAS and controlling related PICs relative to thermal treatment units that do not have both RCRA and CAA permits. Further, incinerators permitted to incinerate liquid PCBs are required to meet even more stringent operating requirements with respect to time and temperature (40 CFR 761.70(a)(1)) when incinerating PCBs, and these operating conditions may be even more conducive to destroying PFAS and controlling PIC

[10] Commercial thermal treatment units primarily treat waste received from other facilities.

[11] Hazardous waste is regulated pursuant to RCRA authority. See 42 U.S.C. 6903. The regulatory definition is found in 40 CFR 261.3. PFAS is currently not a listed or characteristic hazardous waste, but a PFAS-containing waste may meet the regulatory definition of hazardous waste if PFAS is mixed with a listed hazardous waste or if a PFAS-containing mixture exhibits a hazardous characteristic (e.g., corrosivity or another characteristic stemming from the material that is mixed with PFAS) Waste managers must comply with RCRA hazardous waste requirement for the specific regulated hazardous waste, including storage limits, land disposal restrictions, and utilizing disposal and destruction facilities that are permitted to receive hazardous wastes.

[12] Hazardous-waste-burning cement kilns and LWAKs are a small subset of the total cement kiln and LWAK universe—i.e., most kilns do not burn hazardous waste.

formation. The following sections generally describe design and operational parameters of commercial HWIs, LWAKs, and cement kilns, focusing on parameters that are important for destroying PFAS. Design and operation information summarized for HWCs is generally based on background support documents for previous rulemakings (U.S. EPA, 2005b). Information on the numbers of HWC facilities is based on a 2019 national hazardous waste capacity assessment report (U.S. EPA, 2019). The capacity assessment report also provides a list of these facilities.

3.a.i.1.1 Commercial hazardous waste incinerators

Currently, there are 10 commercial HWI facilities operating in the United States. HWIs are designed to optimize temperatures, residence times, turbulence, and other parameters to ensure compliance with organic DRE requirements.[13] Three of these facilities are also permitted for incineration of PCBs. Most commercial HWIs use rotary kilns as primary combustion chambers to facilitate the thermal treatment of containerized wastes (e.g., in drums) and solid wastes such as contaminated soils. Low-heating-value aqueous wastes may also be pumped into the rotary kiln. The kiln maintains a continuous standing flame fueled by high-heating-value wastes and auxiliary fuels that maintain high temperatures. Typically, solids retention time in the kiln is 0.5 to 1.5 hours, while gas residence time through the kiln is usually around two seconds. Kiln flame/solids temperatures range from 650°C to 1,650°C (1,200°F to 3,000°F) (U.S. EPA, 2005b).

After combustion in the rotary kiln, wastes may be moved to an afterburner or secondary chamber. If an afterburner is used, additional high-heating-value gaseous and liquid wastes and auxiliary fuels may be added. The afterburner/secondary chamber is typically operated at about 1,100°C to 1,370°C (2,000°F to 2,500°F) with a gas residence time from one to three seconds to maximize organic destruction and minimize the formation of PICs (U.S. EPA, 2005b). Depending on the fuels used and waste streams introduced, combustion products might include carbon dioxide, water, nitric oxide, a variety of acid gases (sulfur dioxide, hydrochloric acid, HF, etc.), organic PICs, fly ash, and bottom ash constituents. Bottom ash is removed at the end of the kiln and from the bottom of the afterburner/secondary chamber and is typically disposed of in hazardous waste landfills (see Section 3.b). Fly ash entrained in the gas is removed downstream by fabric filters (FFs) or electrostatic precipitators, and acid gases are removed by gas scrubbers. Some HWIs use activated carbon injection systems to control mercury, dioxin/furan, and other emissions, and some also use TMT-15 (1,3,5-triazine-2,4,6(1H,3H,5H)-trithione, trisodium salt) injection for additional mercury and metals emissions control.

Limited studies of PFAS treatment in HWIs have been conducted (EA Engineering, Science, and Technology, Inc., PBC., 2021, 2022). These studies demonstrated treatment of five selected PFAS compounds in the feed material. However, these test data are not able to demonstrate adequate destruction of PFAS (Taylor, 2022) due to the lack of data concerning PIC formation.

3.a.i.1.2 Hazardous-waste-burning cement kilns

Currently, there are 11 hazardous-waste-burning cement kiln facilities in the United States. A cement kiln is a long, cylindrical, slightly inclined rotating furnace designed to calcine a blend of raw materials such as limestone, shale, clay, or sand to produce a key ingredient of Portland cement. These cement kilns burn hazardous-waste-derived fuels to replace some of the fossil fuels. Most burn liquid waste; some may also burn solids and small containers containing viscous or solid hazardous waste fuels.

[13] DRE = $[1 - (W_{out} / W_{in})] \times 100\%$, where: W_{in} = mass feed rate of an organic compound into the combustion device and W_{out} = mass emission rate of the same organic compound in exhaust emissions. HWCs are required to achieve at least 99.99% DRE.

Extreme combustion conditions (e.g., temperatures above 1,650°C [3,000°F]), turbulent mixing, and long gas phase residence time (from four to as high as 16 seconds in long kilns) may effectively treat hazardous waste. Cement kilns use either electrostatic precipitators or baghouses to collect particulate and metal emissions, referred to as cement kiln dust (CKD). Portions of the CKD can be fed back into the kiln as a raw material feed or used in other industries as neutralizers or additives, but usually the excess CKD is land-disposed. Add-on acid gas air pollution control devices, such as wet or dry scrubbers, are typically not used. The high alkaline content of the raw material feeds already prevents or minimizes the formation and release of acid gases by providing for in situ absorption of chlorine and other halogens and sulfur.

Several laboratory studies have examined the potential benefit of treating PFAS wastes in cement kilns where the calcium in the raw limestone can react with fluorine to form calcium fluoride (CaF_2). These studies indicate that calcium species react readily with PFAS at relatively low temperatures (400-800°C) to promote mineralization of PFAS waste (Wang et al., 2011, 2013, 2015; Reidel et al., 2021). However, the currently available lab-scale data are insufficient to determine with confidence the effectiveness of treating PFAS waste in commercial cement kiln operations.

3.a.i.1.3 Hazardous-waste-burning lightweight aggregate kilns

There is one LWAK facility operating in the United States that burns hazardous waste. LWAKs thermally process raw material (clay, shale, and slate) in slightly inclined, rotating furnaces to produce a coarse aggregate used in lightweight concrete products. In hazardous-waste-burning LWAKs, liquid wastes are either blended directly with conventional fuels burned in the hot end of the kiln or pumped separately into the hot end flame. High combustion gas flame temperatures (above 1,650°C [3,000°F]) and high kiln gas residence times (over two seconds) are used to destroy hazardous organics. Kiln exhaust gases leave the cold upper end of the kiln at a temperature from 205°C to 540°C (400°F to 1,000°F). LWAKs use FFs to control dust contained in the exhaust gas. The collected dust can be recycled back into the kiln (at the hot or cold end) or mixed into the lightweight aggregate product. Some LWAKs also use wet or dry scrubbing for acid gas emissions control. For example, the currently permitted hazardous-waste-burning LWAK has dry scrubbing for acid gas control. There are insufficient data to determine the effectiveness of commercial LWAK facilities in treating PFAS waste.

3.a.i.2 Carbon regeneration and reactivation units

Carbon reactivation units or “furnaces” use high temperatures to thermally desorb contaminants from GAC, which allows for the carbon to be used again. Over a dozen large-scale companies and utilities in the United States reactivate sizeable quantities of GAC. In all, these entities operate about 17 commercial furnaces (Roskill Information Services Ltd., 2017). Four of these commercial furnaces operate under RCRA permits and applicable air permits. RCRA permits provide additional regulatory oversight and include operating requirements and emission limitations to promote safe and effective treatment of the hazardous contaminants; non-RCRA-permitted carbon reactivation furnaces may not be subject to such requirements. Due to these additional safeguards, RCRA-permitted furnaces may operate under conditions more conducive to destroying PFAS and controlling related PICs, relative to furnaces without RCRA permits. This discussion focuses on RCRA-permitted furnaces because EPA has more design and operational information on these devices as a result of the RCRA permitting process.

Reactivation[14] of spent carbon is generally carried out in multiple-hearth ("multi-hearth") or rotary kiln furnaces, although fluidized bed and infrared furnaces are also options. While the furnace designs vary, they all use high temperatures and residence times designed to eliminate the adsorbed contaminants and return the carbon to a virgin state for reuse.

During reactivation, spent GAC is typically exposed to drying, desorption, pyrolysis, and oxidation as it moves through the furnace. The drying stage eliminates moisture via evaporation and occurs when hot combustion gases at temperatures ranging from 100°C to 110°C (212°F to 230°F) contact the carbon. During the desorption stage, hydrocarbons, hydrogen, and water vapor escape the pores of the granular carbon at temperatures of 315°C to 400°C (600°F to 750°F). The pyrolysis stage eliminates any volatile compounds adsorbed within the carbon porosity, including residual moisture, and thermally decomposes other less-volatile compounds. Pyrolysis occurs when the carbon is exposed to temperatures up to 800°C (1,472°F) under inert conditions (i.e., low oxygen). A residue of carbonized char is formed from the adsorbed compounds during pyrolysis, which occupies some of the carbon porosity and must be removed in the next stage. The oxidative stage involves the controlled gasification of the pyrolyzed carbon at temperatures of around 800°C (1,472°F) in the presence of a mildly oxidizing atmosphere, usually steam or carbon dioxide or a mixture of both. This results in the elimination of most of the charred residue and exposes the original carbon-pore structure (Miguel et al., 2001).

The residence times required for carbon reactivation vary by unit design and the contaminant loads and concentrations. For example, two facilities using multi-hearth furnaces have residence times of at least 38 minutes at one facility and between 90 and 120 minutes at the second facility (Kentucky Energy and Environment Cabinet, 2019; U.S. EPA, 2018).[15]

To ensure adequate destruction and removal of any remaining contaminants, reactivation furnaces are typically equipped with afterburners/thermal oxidizers. To meet regulatory requirements, afterburners are designed to achieve 99.99 percent DRE via thermal oxidation. DRE for a reactivation/regeneration furnace is evaluated on a "total hydrocarbon" basis rather than for a specific most-difficult-to-destroy component, as is done for HWCs. This adds additional uncertainty to the question of whether a thermal treatment unit can effectively destroy PFAS. The temperature required to achieve 99.99 percent DRE depends on residence time as well as on the concentration of contaminants, but minimum temperatures are around 885°C (1,625°F) and maximum temperatures are as high as 1,316°C (2,400°F), with a minimum residence above one second (U.S. EPA, 2018).

Depending on the process or waste streams treated, a reactivation furnace can be equipped with add-on air pollution control equipment to ensure environmental standards are met. Equipment can include venturi scrubbers for particulate matter control, packed-bed scrubbers for acid gas and particulate matter control, and wet electrostatic precipitators or baghouses for additional particulate matter control. As discussed in Section 3.a.ii, carbon reactivation systems can degrade PFAS even at the lower temperatures (150°C–700°C, or 302°F–1,292°F).

[14] "Reactivation" refers to a regeneration process that requires high temperatures. Regeneration also includes low-temperature processes, such as those using brines, solvents, oxidants, biological treatment, etc. These processes may not be as effective as reactivation for GAC (AWWA, 2018); therefore, they are not considered for this discussion.

[15] These two references are RCRA permits for Evoqua Water Technologies LLC and Calgon Carbon Corporation, two companies that accept spent activated carbon from off-site sources. They each hold RCRA permits, which allow them to treat spent carbon that meets the definition of hazardous waste.

3.a.i.3 Non-hazardous-waste combustion sources

Non-hazardous-waste incineration units in the United States includes SSIs at wastewater treatment facilities and MWCs. Under Section 129 of the CAA, these units are regulated for emissions of particulate matter, carbon monoxide, dioxins/furans, sulfur dioxide, nitrogen oxides, hydrogen chloride, lead, mercury, and cadmium. Because of this, the air pollution control devices employed by these units are intended to control these nine pollutants primarily. The following sections generally describe these incinerator types and their design and operational parameters that may influence the treatment of PFAS-containing waste (U.S. EPA, 1995).

3.a.i.3.1 Sewage sludge incinerators

An SSI unit is a combustion device that is used to burn dewatered sewage sludge. There are currently 170 SSI units operating at 86 facilities in the United States (U.S. EPA, 2016). The main types of SSIs are multi-hearth furnaces and fluidized bed combustors (FBCs).

The basic multi-hearth furnace is a vertical cylinder divided into zones. The sludge is dried at temperatures from 425°C to 760°C (800°F to 1,400°F) (U.S. EPA, 1995). Sludge combustion occurs as the temperature is increased to about 925°C (1,700°F) in successive zones. The gas residence times are typically four or five seconds. Emission controls on multiple hearths can include wet scrubbers, wet electrostatic precipitators, afterburners, and regenerative thermal oxidizers.

An FBC consists of a vertically oriented outer steel shell with nozzles designed to deliver fluidizing air at the base of the furnace within a refractory-lined grid. Air is injected into the furnace to fluidize the sludge and the sand. The combustion of the sludge occurs at temperatures from 750°C to 925°C (1,400°F to 1,700°F). The gas residence times are typically two to five seconds. Emission controls on FBCs can include venturi scrubbers, multicyclones, FFs, activated carbon injection, and carbon bed absorbers. There is substantial variation in the design and operation of SSIs and it has not been shown that standard operating conditions are effective for the treatment of PFAS. The data currently available are insufficient to make a determination about the effectiveness of SSIs in treating PFAS.

3.a.i.3.2 Municipal waste combustors

There are 193 MWC units operating at 75 facilities in the United States (Michaels & Krishnan, 2018). Three main classes of technologies are used to combust MSW: mass burn, refuse-derived fuel (RDF), and modular combustors. Mass burn and RDF combustors are the predominant designs.

With mass burn units, the MSW is combusted without any preprocessing other than removal of items too large to go through the feed system or hazardous materials, such as pressurized containers. In a typical mass burn combustor, refuse is placed on a grate that moves the waste through the combustor. The grates typically have three sections. On the initial grate section, referred to as the drying grate, the moisture content of the waste is reduced before ignition. Evaporated moisture is combined with the moisture from combustion and treated with the resulting combustion gases. The second grate section, referred to as the burning grate, is where most of the active burning takes place. The third grate section, referred to as the burnout or finishing grate, is where remaining combustibles in the waste are burned. Typical combustion temperatures for mass burn units can range from 800°C to 1,100°C (1,500°F to 2,012°F) (Reddy, 2016).

RDF combustors burn waste that has been processed to varying degrees to raise its heating value and provide a more uniform fuel. Most boilers designed to burn RDF use spreader stokers and typically

operate at around 680°C (1,250°F). RDF-fired FBCs typically operate at bed temperatures of around 815°C (1,500°F) (Reddy, 2016).

Residence times of gases within MSW combustors vary from unit to unit, depending on design and operational factors such as furnace volume, excess combustion air percentage, whether flue gas recirculation is employed, and combustor operating load parameters (Scavuzzo, 1990; Themelis & Reshadi, 2009). Overall combustion air residence times have been calculated in the 7- to 10-second range for a small sampling of MWC design loads (Themelis & Reshadi, 2009). At temperatures above 980°C (1,800°F), the approximate residence time is about two seconds for full combustor loads (Scavuzzo, 1990).

Emission controls on MWCs can include spray dryer or dry sorbent injection, electrostatic precipitators, FFs, selective or non-selective catalytic reduction, and activated carbon injection.

There is substantial variation in the design and operation of MSW combustors. The data currently available are insufficient to make a determination about the effectiveness of MSW combustors to treat PFAS.

3.a.i.4 Thermal oxidizers

Thermal oxidizers are used to treat process gases and destroy volatile organic compounds (VOCs) and organic hazardous air pollutants (HAPs) at a manufacturing or production facility. Some types of thermal oxidizers are also able to accept liquid feed streams. Each thermal oxidizer is designed to optimize temperatures, residence times, and turbulence to address the composition of the particular feed stream at that unit and meet the requirements of a regulation or permit. Depending on the type of thermal oxidizer (direct-fired, regenerative, recuperative, or flameless), the operating temperature ranges from 760°C to 1,200°C (1,400°F to 2,190°F). The residence time of thermal oxidizers ranges from 0.5 to two seconds, depending on site-specific criteria. Catalytic thermal oxidizers operate at lower temperatures than other types of thermal oxidizers—typically at about 400°C (750°F) with similar residence times to non-catalytic thermal oxidizers—while achieving the same efficiency in destruction of VOCs.

Emission controls for thermal oxidizers vary widely depending on the facility and the composition of the feed streams. Existing thermal oxidizers may have no additional pollution controls or may use prefiltration (to prevent fouling of the oxidizer) and/or wet or dry scrubbers or FFs. Thermal oxidizers have historically not been designed with destruction of PFAS as the primary focus, so most currently installed thermal and catalytic oxidizers may not be optimized for PFAS destruction. However, some thermal oxidizers are being used for halogenated wastes, including PFAS precursors. The operating conditions for some thermal oxidizers have the potential to effectively treat PFAS, but the data currently available are insufficient to make a determination about effectiveness.

Chemours. Two testing campaigns (2020 and 2022) were conducted on a thermal oxidizer installed to control PFAS emissions at a Chemours chemical and plastics manufacturing plant located in Fayetteville, North Carolina. The objective of the tests was to determine how the thermal oxidizer and 4-stage scrubber system would achieve a 99.99 percent emission reduction, including the use of a surrogate for all PFAS, such as the hexafluoropropylene oxide (HFPO). The test reports demonstrate a PFAS destruction efficiency above the 99.99 percent for the specific compounds that were tested. HFPO was tested as a surrogate for all species of PFAS, but there are no concrete data showing the performance of the thermal oxidation process for PFAS species outside of the compounds tested. There was also no discussion of possible products of incomplete combustion in the reports. The thermal oxidizer at the

Chemours facility appropriately destroys the five PFAS compounds of interest that have been identified at the facility. However, removal processes for products of incomplete combustion or of destruction of potential compounds not studied but potentially found in the facility waste streams are still unclear.

3.a.ii Limitations and considerations in thermal treatments

Incinerator designs vary, resulting in differing operational and waste feed approaches (see Section 3.a.i). HWIs typically operate at very high average temperatures and employ auxiliary, primary, and secondary flames. MWCs typically operate at lower temperatures and often do not employ auxiliary primary or secondary flames. SSIs vary in design, often operating as dryers with very low temperatures. Even within the same incinerator, wastes can be introduced at different locations and can experience different time, temperature, and mixing histories. PFAS introduced into a hazardous waste rotary kiln incinerator's main burner, along with auxiliary fuel, may experience very different conditions than the same waste introduced to the kiln as contained charges with solid wastes. These factors are expected to affect PFAS destruction and PIC formation. Limited studies have investigated the influence of various factors on PFAS destruction and PIC formation (see Section 3.a).

Carbon reactivation systems can degrade PFAS even at the lower temperatures (150°C–700°C, or 302°F–1,292°F) seen in bench-scale research studies (Watanabe et al., 2018). Experimental data suggest that thermal destruction of PFAS occurs in two stages: first during reactivation of the GAC, and then when the off-gas is introduced into a high-temperature zone with temperatures as high as 1,000°C (Forrester, 2018; Watanabe et al., 2016, 2018; Xiao et al., 2020). DiStefano et al., (2022) showed >99.99 percent destruction of PFAS at a full-scale commercial reactivation facility with a large percentage of the PFAS destruction occurring in the furnace. The fluoride mass balance was reported to be 61.4 percent. Although this low percentage could be due to a number of factors, it may be due to the formation of PICs that were not quantitated in the analytical method. While carbon reactivations systems, with the concomitant use of off-gas incineration (i.e., afterburners) and gas scrubbing units, have the potential to remove PFAS from the activated carbon and destroy it, there is insufficient data to make a determination on its effectiveness. However, as discussed elsewhere in this interim guidance, more work is needed for confirmation, particularly with regard to full-scale reactor conditions, differing carbons, and PICs.

Thermal oxidizers have historically not been designed with destruction of PFAS as the primary focus, so most currently installed thermal and catalytic oxidizers may not be optimized for PFAS destruction. However, some thermal oxidizers are being used for halogenated wastes, including PFAS precursors. Thermal oxidizers are being employed to destroy PFAS-containing and gaseous streams, but the data are insufficient to allow conclusions on the overall efficiency of thermal oxidizers in PFAS destruction. For example, the two tests at Chemours Company's Fayetteville Works (Focus Environmental, Inc., 2020, 2022) focused on five specific PFAS of interest found in waste gas streams at the facility. While the tests seem to prove a DRE of 99.999 percent for the five PFAS compounds, they do not present data on overall PFAS destruction or potential formation of PICs. (See Appendices B and C for summaries of the Clean Harbors and Chemours test data.) EPA is currently unaware of any catalytic oxidizers being used specifically for the destruction of PFAS, particularly in light of their site-specific design and optimization. Though the efficacy of thermal and catalytic oxidizers in destroying PFAS is currently unknown, a properly optimized thermal oxidizer can readily achieve a DRE of 99.99 percent for VOCs.

In addition to incinerators and thermal oxidizers, cement kilns are also used for the destruction of hazardous wastes. Cement kilns operate at very high temperatures (exceeding 1,800°C [3,270°F]),

exhibit very large gas and solid residence times, and have the added advantage of providing a caustic environment for halogen reaction and acid neutralization. A cement kiln in Australia has received an operating permit from the government of Queensland to burn PFAS wastes. The permit requires annual monitoring for 21 PFAS (Department of Environment and Heritage Protection, 2018). Test results from 2017 indicate that a DRE in excess of 99.99 percent is possible for specific PFAS compounds at the conditions of the test (Cement Australia, 2017), but the results do not present data on overall PFAS destruction or potential formation of PICs.

Processes involving calcium and alumina may have benefits and may require lower energies to destroy PFAS. Recent research (Wang et al., 2011, 2013, 2015) has investigated PFAS interactions with calcium oxide (CaO) and calcium hydroxide ($Ca(OH)_2$) at moderate temperatures (200°C to 900°C, or 390°F to 1,650°F) and found that these calcium species exhibit a pseudo-catalytic effect promoting PFAS destruction and fluorine capture at relatively low temperatures. Similar studies by the electronics industry indicate that alumina catalysts can effectively convert residual CF_4 and related species (generated by plasma arc techniques for etching purposes) to CO_2 and HF at comparable temperatures.

It is not well understood how effective high-temperature combustion is at completely destroying PFAS or whether the process can form fluorinated or mixed halogenated organic byproducts. Few experiments have been conducted under oxidative and temperature conditions representative of different field-scale incineration devices used for PFAS destruction. Limited studies on the thermal destructibility of fluorotelomer-based polymers found no detectable levels of PFOA after two seconds of residence time at 1,000°C (1,830°F) (Yamada et al., 2005; Taylor et al., 2014). Two recent field studies examining the performance of a specially designed thermal oxidizer in destroying PFAS in process gases at a Chemours production facility near Fayetteville, North Carolina, indicated greater than 99.999 percent destruction of five PFAS (Focus Environmental, Inc., 2020, 2022). Similarly, the recent Clean Harbors studies appear to show that the facility was able to achieve its primary objective: “demonstrate the ability of the high-temperature incineration system at Aragonite to effectively destroy PFAS in the waste feed during normal process operating conditions.” A high DRE was achieved (> 99.999 percent) for the five selected PFAS compounds that were subjects of these studies (EA Engineering, Science, and Technology, Inc., PBC., 2021, 2022). However, these test data did not include characterization of potential PICs due to previous methodology limitations.

The Chemours and Clean Harbor test results indicate the PFAS compounds measured were changed by the treatment, but the tests did not investigate whether the compounds were changed to carbon dioxide and hydrofluoric acid to show adequate destruction of the original PFAS. As discussed previously, emission studies, particularly for potential PICs, are largely incomplete due to lack of validated sampling and measurement methods for the potentially large number of fluorinated and mixed halogenated organic compounds that might be formed. EPA continues to seek information on PFAS that may be present in air pollution control device media (e.g., scrubber water, particulate matter control device media) and the presumed effectiveness of these air emission controls. (See Section 5 for more information about ongoing research and research needs.)

Incineration may be a viable PFAS destruction technology if done under certain conditions. The preliminary research on thermal treatment of PFAS suggests that the minimum conditions for PFAS destruction include well mixed environments with temperatures greater than 1,100°C and optimized combustion stoichiometry (Shields et al., 2023). These conditions may adequately destroy PFAS and minimize PICs. However, evaluations have only been done on liquid fired materials at a pilot scale. No comparative studies that include PIC measurements on solids or containerized wastes have been

performed. Liquids should preferably have direct flame contact or at least well mixed exposure to temperatures greater than 1,100°C.

EPA encourages additional tests be performed to demonstrate the ability of a thermal treatment technologies to mineralize PFAS. EPA encourages testing to demonstrate destruction of parent compounds (OTM-45) and volatile PICs (OTM-50) based on suggested procedures in Appendix A) to show efficient thermal destruction of atmospherically transformative PICs of health and environmental concern.

3.a.ii.1 Other thermal treatment byproducts of concern

Thermal treatment systems including HWIs, MWCs, and SSIs are configured such that gas phase effluent from the combustion chambers passes through a series of pollution control devices to remove acid gases, particulate matter, and sometimes mercury and other specific HAPs. The behavior of PFAS and PFAS-related PICs in these unit operations is largely unknown (see Section 5 for more information about research on this topic and others). Additionally, these control devices produce secondary waste streams in the form of fly ash and scrubber blowdown solutions, and PFAS and PFAS-related PICs may be present in these solid and liquid effluents depending on their vapor pressure and solubility.

Thermal oxidation processes used for treating PFAS-containing waste, such as incineration, generate HF as the most stable product from combustion of fluorocarbon compounds. HF is a CAA-listed HAP. Highly corrosive, HF creates significant operation and maintenance issues by damaging thermal system materials such as furnace refractory and metal ductwork. Both wet scrubbing and semi-dry scrubbing processes have been applied to control HF emissions from thermal treatment sources.

A thermal oxidizer with a potential for HF emissions typically uses a wet scrubber integrated with the oxidizer to control HF emissions. Hot flue gas exiting the oxidizer is cooled rapidly in a quenching unit, and HF (which has high water solubility) is removed by the quenching water. The cooled flue gas then flows up through a multistep wet scrubbing tower for further HF removal by scrubbing water. Flue gas is scrubbed by a sodium hydroxide solution to neutralize the residual HF as the final scrubbing step. After exiting the tower, flue gas is emitted through a stack. All effluents, including those from the quenching unit and scrubber tower, are mixed with a $Ca(OH)_2$ solution in a reactor where calcium is combined with fluorine and precipitation of water-insoluble calcium fluoride (CaF_2) occurs. After dewatering, dry CaF_2 is sent to a landfill for disposal (see Section 3.b) or used to produce fluorine gas for new PFAS production, and wastewater is discharged from the plant after it is treated by activated carbon to remove trace fluorinated contaminants. Rapid cooling of hot flue gas is known to be effective in reducing catalytic reformation of chlorinated PICs such as dioxins during cooling of incineration flue gas.

A thermal oxidizer equipped with a quenching unit to treat PFAS-containing wastes may also limit catalytic reformation of fluorinated PICs if they are actually formed in the oxidizer. Those PICs may be subsequently transferred into the liquid phase in the wet scrubber. Those PICs could then be partitioned between solid CaF_2 and water in the precipitator, and most of the PICs retained in water are then removed by activated carbon adsorption. EPA is not aware of peer-reviewed studies that measure levels of fluorinated contaminants remaining in both the treated scrubber water stream and the dry CaF_2 stream. Such measurements could be useful for evaluating the potential environmental impacts of byproducts and residuals generated from thermal oxidation of PFAS-containing wastes.

Spray dryer absorber (SDA) technology has been applied to control emissions of halogen acids (including HCl and HF) from both MWCs and HWIs. This semi-dry scrubbing process is designed to inject an alkaline

slurry, typically lime, to control acid and fly ash. Water in the fine slurry droplets is vaporized by heat carried by the flue gas, and the lime carried in the droplets neutralizes the halogen acids simultaneously in this two-phase reaction process. The cooled flue gas carries the dried acid neutralization product downstream to a particle collection device, typically an FF. PAC may also be injected into flue gas upstream of the FF to control emissions of mercury and chlorinated dioxins/furans from both MWCs and HWIs. Fly ash, dried acid neutralization product, and PAC are captured by the FF. The SDA/FF with PAC injection flue gas cleaning train produces no scrubber water. The addition of lime (a calcium compound) into the flue gas is known to be effective for forming CaF_2 through hydro-defluorination of PFOS at a moderate temperature of about 350°C (660°F) (Wang et al., 2015); this suggests that the SDA may provide a potential co-benefit of controlling fluorinated PICs. The injection of PAC upstream of the FF may create another potential co-benefit for capturing fluorinated PICs. Studies evaluating PFAS mitigation via SDA/FF with PAC injection (e.g., see research activities in Section 5) will help develop data on this potentially viable technology option.

3.a.iii Potential for releases for thermal treatment technologies

Thermal treatment devices that may be used to treat PFAS-containing waste (see Section 3.a.i for descriptions of these devices) are located in both rural and more densely populated areas throughout the United States. Two possible sources of PFAS emissions from thermal treatment are stack emissions and the management of scrubber water and bottom ash/fly ash. As previously discussed, emissions from thermal treatment activities may contain PFAS if adequate combustion conditions are not achieved or if adequate acid gas scrubbers or other pollution control devices are not used. EPA is aware of a limited number of peer-reviewed studies that have investigated the extent to which emitted PFAS are transported and deposited to surrounding areas (see Section 2.a.iii). EPA plans to conduct research in this area to better characterize the extent to which PFAS deposition may occur near thermal treatment devices (see Section 5 for more information about these types of research activities).

Volatile PFAS releases from thermal treatment equipment (e.g., fugitive emissions) and waste storage activities are another potential source of PFAS releases, if they are not adequately controlled.[16] There is also the potential for releases from management of thermal treatment process residuals, such as air pollution control device media, incinerator bottom ash, and liquids from acid gas scrubbers. For example, incinerator bottom ash disposed of in a landfill would be a possible source of release if the ash contained PFAS and the landfill lacked adequate controls (see Section 3.b for more information on landfill types and controls, and Section 4 for more information about environmental releases and vulnerable populations). HWIs, however, typically dispose of incinerator bottom ash in hazardous waste landfills, minimizing the potential for the release of PFAS to the environment. Again, information on partitioning of PFAS in control technology residuals is lacking at present.

Potential impacts to communities from releases from HWIs should also be considered. Considerations should include which media may be impacted by releases, and the potential exposure pathways resulting from releases. The characteristics of the potential exposed communities (demographics, socioeconomic status, other environmental exposures, health conditions, etc.) provide information regarding the potential for disproportionate and adverse impacts.

[16] For example, CAA and RCRA regulations applicable to HWCs require controls to prevent/minimize combustion system leaks (e.g., due to positive pressure events in the combustion chamber), as well as controls to minimize releases from equipment and tanks that store or manage hazardous waste. See 40 CFR 63.1206(c)(5) and 264.1050–1090.

3.a.iv Testing and monitoring

Validated measurement methods are limited and under development for reliably identifying and quantifying if PFAS are released into the air from stationary sources. The limited scope of standardized methods to measure PFAS emissions and the limited availability of data using these methods to evaluate PFAS destruction at a variety of treatment devices introduce uncertainty in the understanding of the efficacy of thermal treatment approaches for destroying PFAS.

Ongoing method development by EPA has a broad focus that will address PFAS in various waste streams, stack and fugitive emissions, and ambient air (see Section 5 and Appendix A). Method development also includes identification of PFAS that are potential PICs, not targeted in established water methods such as Methods 533 and 537.1. Due to the number and complexity of PFAS that have already been identified, a simpler class-specific measurement is also being investigated to determine if measurement of a single compound or a small group of compounds is adequate to characterize the completeness of thermal treatment for both targeted PFAS and potential PICs.

These factors raise important risk communication challenges, particularly for communicating the potential significance of testing and monitoring results to adjacent communities. But the importance of encouraging appropriate information access for and dialogue with communities—and, in particular, with potentially vulnerable communities —cannot be overemphasized. Suggestions for risk communication are included in Section 4.

3.a.iv.1 Semivolatile PFAS sampling and analysis

Initial development of methods to measure PFAS in air or air sources has focused on compounds whose physical state is liquid or solid at room temperature. Methods for air have included the target compounds commonly found in water methods. The basis for measuring PFAS compounds from air or air sources involves evaluating and then modifying existing sampling and analysis methods for semivolatile organic compounds.

Filtered particulate, solid sorbent, and aqueous impinger media sampling procedures have been used to collect and recover a wider range of PFAS and associated byproducts in ambient air and source emissions (Barber et al., 2007; Martin et al., 2002). However, these sampling methods have some limits, including poor retention or chemical conversion of the PFAS during sampling and poor recovery during sample preparation prior to chemical analysis (Arp & Goss, 2008).

Ambient sampling for semivolatile PFAS roughly follows either the high-volume air sampling protocol described in EPA compendium Method TO-13a (U.S. EPA, 1999) or the National Atmospheric Deposition Program (NADP) approaches for wet and dry deposition sampling (NADP, 2020). High-volume air samples collect both water-soluble PFAS acids and salts and water-insoluble telomer alcohols. NADP sampling has focused on condensable and particulate-bound targeted PFAS captured in polypropylene buckets to evaluate deposition due to rain.

Current sampling of stationary source air emissions for semivolatile PFAS targets is based on EPA SW-846 Method 0010. Analysis of samples for a list of PFAS compounds is based on EPA Method 533—modified to include collection of both targeted and non-targeted PFAS in a single sampling system. Sampling includes heated or stack temperature probe extraction of emission gases followed by collection on filters, XAD sorbent media, and aqueous impingers. EPA released OTM-45, *Measurement of Selected Per- and Polyfluorinated Alkyl Substances from Stationary Sources,* in January 2021 (U.S. EPA,

2021). OTM-45 experience indicates the method works well to measure targeted PFAS. OTM-45 is an effective and sensitive method to measure a list of PFAS included in the method. In coordination with the release of this guidance, EPA recently released OTM-50, which enables analysis of non-polar volatile PFAS.

These field sampling procedures collect samples that are subsequently transported to a laboratory for extraction and analysis. Analysis procedures include established water methods for targeted compounds and/or non-targeted analysis (NTA) for unknown PFAS. NTA is a research tool that can be used on OTM-45 and OTM-50 samples to investigate semivolatile polar and nonpolar PFAS recovered by these methods. High-resolution mass spectrometry can be used for both targeted analysis and NTA. Qualitative identification of PFAS by NTA reveals PICs/degradants formed during the thermal treatment of PFAS-contaminated media (Aleksandrov et al., 2019; McCord & Strynar, 2019; Newton et al., 2020). NTA currently relies on mass spectrometry, which generates qualitative information about the molecular formula of unknown PFAS. NTA is an important component in characterizing thermal treatment emissions because it provides an approach for identifying unknown PFAS or PICs.

3.a.iv.2 Gaseous volatile PFAS sampling and analysis

Volatile PFAS targets and thermal treatment byproducts from ducted emissions or in ambient air have been sampled using a variety of whole gas sample collection approaches, such as Tedlar® bags and passified canisters, as well as sorbent traps and cryogenic solvents. EPA and private sector investigators have sampled with specialized commercial sorbent traps and Tedlar® bags in laboratory-scale thermal destruction and ambient volatile PFAS measurement of targeted and non-targeted PFAS (Wang et al., 2013, 2015; Yamada et al., 2005). Issues such as sample reactivity, breakthrough volumes, and quantitative transfer to the analysis instrument complicate these approaches. Direct instrumental methods for measuring volatile PFAS (such as FTIR) can suffer from lack of sensitivity compared with extractive methods that allow concentration prior to analysis.

To develop more sensitive methods for measuring volatile fluorocarbon compounds, EPA has evaluated the use of steel canisters for targeted volatile PFAS as well as PICs during multiple-source emissions tests, including at a thermal treatment facility for AFFF-contaminated soil (U.S. EPA, 2020b). Canisters have been used to sample source emissions and perform targeted measurements for PFAS, including both industrially produced compounds (such as FC-116, HFC-23, HFC-125, E1, and E2) and potential PIDs (such as CF_4 through octadecafluorooctane). EPA is developing the ability to characterize a more diverse set of possible PFAS PICs and measure volatile PFAS at trace concentrations. The ability to measure trace concentrations will allow investigators to introduce a known concentration of a hard-to-destroy fluorocarbon and evaluate the behavior of this compound when exposed to specific thermal environments. EPA's preferred approach is to introduce a known concentration of a hard-to-destroy fluorocarbon and evaluate the behavior of this compound when exposed to specific thermal environments in order to characterize a more diverse set of possible PFAS PICs. Though insufficient by itself, this approach is consistent with the EPA approach for determining the DRE of fluorinated greenhouse gas abatement equipment in electronics manufacturing (U.S. EPA, 2010). NTA has also been performed on the same samples. Simultaneous with the release of this 2024 interim guidance, EPA will release OTM 50, which provides an approach and procedures for using silanized steel canisters to sample and analyze volatile PFAS compounds from thermal treatment sources. This and OTM-45 are examples of monitoring methods that could be considered for addition to a potential PFAS waste management system or permit (e.g., RCRA).

EPA continues to evaluate FTIR as a suitable real-time measurement technique for CF_4 and C_2F_6 in stationary source emissions. In addition, in ambient air, EPA also investigated chemical ionization mass spectrometry (CI/MS) to monitor individual PFAS in real time (Riedel et al., 2019). EPA found the technique sufficiently sensitive for fugitive emissions measurements or leak detection.

The quantitative measurement of total organic fluorine (TOF) is also being evaluated to represent all, or most, of the PFAS class of compounds. This approach represents a simpler and more comprehensive measurement alternative to target list approaches that focus on a limited number of PFAS. Several potential techniques warrant consideration and additional evaluation, including (but not limited to) combustion–ion chromatography (CIC), particle-induced gamma emission spectrometry (PIGE), and X-ray photo-electron spectroscopy (XPS). For air and thermal treatment emissions, TOF must measure highly volatile as well as semivolatile PFAS. TOF analysis is an ongoing research area: data users must recognize the benefits of receiving general screening data for a wide array of potentially present PFAS, while also recognizing the limitations and uncertainties inherent in not knowing which PFAS or class of PFAS is present in the sample, including uncertainties associated with potential health risk. In addition, to minimize the risk of PFAS false positives, techniques within a validated method or methods must be developed that demonstrate effective separation and removal of inorganic fluorine from organic fluorine (Koch et al., 2020). TOF is not specific to PFAS, and any fluorine-containing compounds (e.g., pesticides, pharmaceuticals) that are retained during extraction would be included in the organic fluorine measurement.

HF measurement is included in stationary source measurements to evaluate control efficiency of HF as a HAP at emission outlets. Multiple studies of PFAS thermal decomposition and HF monitoring have demonstrated that EPA compliance methods for HF measurement are adequate to meet this need. However, HF is difficult to transport through treatment and control equipment and is therefore not a candidate for mass balance to evaluate PFAS destruction efficiency.

3.a.v Uncertainties/unknowns

EPA is planning to collect additional information and conduct additional research to better understand PFAS destruction and evaluate emission control efficiency (see Section 5). EPA is working to develop a standardized validated methodology for measuring PFAS gaseous emissions (see Section 3.a.iv); however, EPA also lacks detailed information on the amounts and concentrations of PFAS-containing materials that are generated and managed in thermal treatment devices.

Development of sampling and analytical methodologies must continue (see Section 5 research activities) so that emissions and other media from thermal treatment devices burning PFAS-containing materials can be adequately characterized.

When evaluating thermal treatment options, decision-makers (e.g., permit writers, waste managers) should consider potential impacts to communities, including vulnerable populations. Uncertainties such as the overestimation of destruction and disposal capabilities, or a failure to account for potential PIC production and subsequent transformation back to PFAS of concern could have consequences for all nearby communities, including for vulnerable communities that may be disproportionately impacted. EPA recommends decision-makers screen communities located in the vicinity of potential releases from the destruction, disposal, and storage options (considering fate and transport) in order to consider the potential for adverse and disproportionate impacts (see Section 4), and to consider potential measures to prevent, reduce, or address such impacts. Depending on site-specific circumstances (e.g., PFAS

concentrations, impacted media, and potential exposure pathways), the size and shape of this area (vicinity) will vary.

In order to provide more detailed recommendations, further data on the ability of thermal treatment units to completely mineralize PFAS-containing materials is necessary (see Appendix A and Section 5.a). Performance testing of all types of combustors is lacking. EPA recommends that full-scale thermal destruction facilities conduct performance testing prior to accepting and processing PFAS materials. Objectives of the testing should include:

- Demonstrate destruction of parent compounds and potential production of semivolatile and volatile PICs (using OTM-45 and OTM-50) to show efficient thermal destruction.
- Characterize PFAS being fed, including the feed rate, whether it is a concentrated or diluted stream, and how the PFAS are fed (e.g., containerized or direct feed).
- Demonstrate utilization of key combustion parameters (temperature, residence time, other conditions that impact mineralization) and include relevant data in the report.
- Characterize release of PFAS and PICs through the system by sampling at multiple points in the process (e.g., before and after a scrubber) and sampling all relevant effluent streams (e.g., scrubber blowdown and fly ash). Document and report operating parameters for all relevant control devices to EPA and relevant state, territorial, or Tribal authorities.

Further details on suggested parameters and procedures are provided in Table 5-1 and Appendix A.

3.a.vi Treatment commercial availability

Section 3.a.i describes the commercial availability of thermal treatment devices. The United States has about 22 commercial hazardous waste combustion facilities[17] in operation; over a dozen large-scale, commercial carbon reactivation companies with about 17 furnaces; 193 MSW incineration units at 75 facilities; and 170 SSIs at 86 facilities.

3.a.vii Summary

PFAS-containing waste can potentially be treated in several types of thermal treatment devices, including HWCs, MWCs, SSIs, and activated carbon reactivation furnaces, but further research is needed to gain a better understanding of what may be possible in practice. These treatment devices operate differently and handle different types of PFAS-containing media with varying concentrations. Even within the same category of thermal treatment device, designs and operating conditions may vary across sources in a way that could affect PFAS treatment efficiency. As a general approach, EPA encourages decision-makers to prioritize the use of destruction and disposal technologies that have a lower potential for PFAS release to the environment, over destruction and disposal technology options with a greater potential for environmental release of PFAS. EPA acknowledges that each destruction and disposal technology has limitations. However, managing PFAS to minimize environmental releases during destruction and disposal activities supports protection of human health and the environment.

There are limited characterizations of potential PFAS emissions from thermal treatment devices that burn PFAS-containing media, and EPA is not aware of any full emission characterizations that have been conducted at HWCs, MWCs, SSIs, or activated carbon reactivation furnaces or any studies to evaluate

[17] This includes commercial incinerators, cement kilns, and LWAKs that are permitted to burn hazardous waste.

the formation of products of incomplete combustion resulting from inefficiency to convert PFAS into carbon dioxide and hydrogen fluoride. EPA is also not aware of any studies that have been conducted on the extent to which PFAS contaminants partition to air pollution control device residuals or bottom ash, which may also be a concern. This is likely (in part) because PFAS emission measurement methods and other analytical methodologies (e.g., for fly ash, other waste streams) are limited and continue to be developed. Data, however, from pilot-scale experiments conducted by EPA researchers suggest that temperatures above approximately 1,100°C / 2,012°F may result in high destruction efficiencies and few detectable fluorinated products of incomplete combustion.

Given the unique characteristics of fluorine combustion chemistry (particularly the strength of the carbon–fluorine bond), adequate thermal destruction of PFAS requires high temperatures and long residence times and likely benefits from direct flame contact. Hazardous waste combustion technologies (commercial incinerators, cement kilns, and LWAKs) can potentially achieve temperatures (~1,100°C / 2012°F) and residence times sufficient to break all carbon-fluorine bonds in the PFAS contained in the waste stream being thermally treated. Permitted hazardous waste facilities have stringent regulatory controls on temperatures and other important operating parameters to achieve a 99.99 percent destruction efficiency for other (non-PFAS) organic chemicals. Such facilities also have air pollution control devices to prevent certain gaseous and particulate pollutants from entering the atmosphere. However, information on the efficacy of PFAS destruction in these facilities is currently limited. EPA has limited data characterizing PIC emissions from these sources when they burn PFAS. EPA is working to develop measurement methodologies as well as to gather information to conclude whether PICs are adequately controlled. EPA recognizes that PICs are inevitable (even for nonfluorinated compounds); however, based on the unique characteristics of fluorine combustion chemistry, EPA believes it is important to determine whether thermal treatment devices and their associated post-combustion control devices are adequately controlling PICs, especially fluorinated PICs. Given all these factors, there is a current need to continue research activities investigating incineration of PFAS. After sufficient research has been completed to address the related knowledge and data gaps, EPA can make a more informed recommendation on disposal of PFAS compounds and PFAS-containing substances using incineration.

Similar conclusions can be drawn for activated carbon reactivation furnaces as for HWCs. Experimental data suggest that thermal destruction of PFAS will occur in two stages: first during reactivation of the GAC, and then when the off-gas is introduced into a high-temperature zone with temperatures as high as 1,000°C/1,832°F. As referenced in Section 3.a.i, an activated carbon reactivation furnace can be equipped with an afterburner to treat off-gases at high temperatures to achieve 99.99 percent DRE of VOC. In addition, scrubbers can be installed to remove acid gases. This is a promising treatment method, but more information is needed, including confirmation that PICs are controlled based on actual operations, establishment of standard operating conditions for activated carbon reactivation furnaces of various designs to ensure optimal destruction of PFAS, and an understanding of how thermal treatment influences the physical and chemical properties of GAC (in ways that can affect GAC's adsorption behavior and sorption capacity for PFAS).

Research and testing of PFAS destruction performance within MWCs is extremely limited, primarily comprising laboratory and pilot-scale studies (Aleksandrov et al., 2019; Taylor et al., 2014). For example, to assess whether PTFE is destroyed or reformed as PFAS, the Aleksandrov et al. study uses a pilot-scale rotary MWC with afterburner chamber combusting PTFE granules added to wood pellets (also firing natural gas). This study looked at a half-load scenario of 870°C (1,600°F) with a four-second residence time and a full-load scenario of 1,020°C (1,870°F) with a 2.7-second residence time. The study analyzed

for 31 PFAS compounds within the flue gas samples collected, assumed to represent a broad range of PFAS. While the laboratory and pilot-scale studies conclude that MSW incineration of PTFE is not a significant source of PFAS, the laboratory thermal reactor and the pilot incinerator used in these studies may not be representative of the design of MWC units currently operating in the United States. For example, the pilot-scale unit in the Aleksandrov et al. study is a rotary combustion chamber followed by an upflow afterburner. No MWC units operating in the United States have a similar configuration. In addition, while several PFAS species were analyzed for in these studies, it is important to note that there are far more PIC species possible, and no studies have thoroughly evaluated the types and quantities of PICs.

As noted earlier in this section, Wang et al. (2013) investigated PFAS interactions with CaO and $Ca(OH)_2$ at moderate temperatures (200°C to 900°C, or 390°F to 1,650°F), both with and without sewage sludge. These experiments were conducted in a laboratory (i.e., combustion in a crucible within a muffle furnace) and found that these calcium species exhibit a pseudo-catalytic effect promoting PFAS destruction and fluorine capture at relatively low temperatures. The study did not investigate the evolution of PICs during the thermal treatment process. While this study shows promising results for the use of catalysts to achieve PFAS destruction and fluorine capture at low temperatures, along with the potential for full-scale application (since lime is occasionally added to sewage sludge to control odor at SSIs), it is important to note that, as with the MWC studies described above, there are caveats for applying these results to the real-world design and operation of SSIs.

More research is needed to address these issues and develop reliable measurement techniques. Section 5 summarizes EPA's continuing PFAS research, as well as a general proposal to collaborate with stakeholders to address these uncertainties promptly. See Appendix D for a summary of costs and considerations.

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3.b Landfills

RCRA regulations define a landfill as “an area of land or an excavation in which wastes are placed for permanent disposal, and that is not a land application unit, surface impoundment, injection well, or waste pile” (40 CFR 257.2). The goal of solid waste landfills is to contain waste, and thereby restrict the release of contaminants within the landfill from entering the environment. Landfills are commonly classified by the types of waste they accept and/or by their ownership status.

Landfills control waste and corresponding pollutants through containment. Because of their many and varied uses, PFAS enter landfills as part of the general MSW stream, in industrial waste, or in other PFAS-containing wastes, with a range of concentrations. Types of landfills include MSW, hazardous waste, industrial, C&D, and ash monofill.

Some landfills are available disposal options for PFAS and PFAS-containing materials. MSW landfills commonly receive large quantities of wastes with significant PFAS concentrations (e.g., biosolids, septage, soils excavated during remediation, and phytoremediation biomass). These concentrations are much lower than some industrial or commercial wastes with high PFAS content (e.g., leather tannery, chrome plating, and textile industry waste). Permitted hazardous waste landfills employ the most extensive set of environmental controls (e.g., double liner systems with leachate collection/treatment and leak detection) and practices (e.g., comprehensive record keeping) that are currently available for the containment of commercial and industrial wastes with high PFAS content (see Table 3-2). Hazardous waste landfills are more effective at minimizing PFAS release into the environment than other landfill types. Note that hazardous wastes that are typically landfilled are not usually biodegradable; thus, most hazardous waste landfills do not have LFG collection and control systems installed and, therefore, may not be appropriate for disposal of biodegradable PFAS-containing wastes (e.g., biosolids) that may produce LFG. It is also noted that leachate from hazardous waste landfills is designated as hazardous waste (F039) and must be managed in accordance with specific requirements for hazardous waste. Nonetheless, some leachate treatment or management options cannot treat or destroy PFAS.

Most modern MSW landfills, when constructed and operated with appropriate controls (e.g., a flexible membrane liner system and leachate and LFG collection and management systems), can also help contain PFAS. However, research indicates that although MSW landfills contain PFAS for the most part, up to 5 percent of the PFAS may be released in the LFG and, 11 percent may be released in the leachate annually (Tolaymat et al., 2023). Also, leachate may be sent to wastewater treatment plants that are not capable of treating or destroying PFAS; thus, the PFAS in landfill leachate may be released to the environment. While some information is available, EPA plans to research to understand the effects of PFAS on liner integrity, gaseous emissions from landfills, the effectiveness of leachate treatment for PFAS removal, and the levels and types of PFAS in landfill leachate (see Section 5). MSW landfills present a good opportunity to dispose of lower-concentration PFAS waste such as biosolids, phytoremediation biomass, and contaminated soils. However, there is a need for additional research regarding the effectiveness of emerging PFAS separation and destruction treatment technologies on a landfill leachate matrix.

While landfills might serve as long-term containment sites, it is unclear how effective they are at containing PFAS. Because landfills are a containment method and do not destroy PFAS, PFAS are anticipated to persist in landfills for the life of the compounds, which could be many centuries.

Section 3.b focuses on the viability of landfilling as a means of containing PFAS and PFAS-containing material, discussing:

- The types of PFAS waste and how they behave within a landfill.
- The types of landfills that receive PFAS-containing waste.
- The types of environmental controls and their ability to effectively contain PFAS.
- The potential for environmental releases of PFAS associated with landfilling.
- Recommendations for deciding what type of landfill is most appropriate for PFAS-containing waste.

- Methods to monitor PFAS at landfills and corresponding waste streams.
- Uncertainties and unknowns associated with landfilling PFAS-containing waste.

3.b.i Types of PFAS and PFAS-Containing Materials

As described in the Executive Summary and Section 2, "PFAS" is a broad term for a wide range of chemicals. When disposed of in a landfill, these different types of PFAS may behave differently from one another and may require different considerations for the types of landfills that are suitable for disposal and the types of emissions controls that are necessary to contain the PFAS over time.

PFAS Polymers. Stable polymeric PFAS (e.g., PTFE or Teflon™) are less likely to migrate within a landfill by partitioning to the gas or liquid (i.e., leachate) phase and should not be confused with side-chain fluorinated polymers; however, physical abrasion of the polymers can lead to the creation of fluorinated microplastics that may be suspended in landfill leachate. Although fluoropolymers are highly resistant to chemical and thermal degradation, they can still break down into microplastics due to weathering and physical strain, increasing their dispersion and bioavailability (Lohmann et al., 2020). Fluorinated microplastics have been measured in landfill leachate (He et al., 2019), but because UV radiation and resulting oxidation in landfills is negligible and the physical processes that lead to abrasion and the development of microplastics are expected to be relatively low in landfills, the bulk of stable fluoropolymers would be expected to be retained for a long duration. Additionally, soluble, and volatile PFAS have been identified in fluoropolymers as synthesis byproducts or incomplete reactants.

Soluble PFAS. Soluble PFAS and precursors that degrade to soluble PFAS, including perfluoroalkyl acids such as PFOA, PFOS, PFHxS, PFBA, and HFPO-DA, can more easily and quickly partition to the liquid phase and become part of the landfill leachate. Landfills without an appropriate liner and leachate collection system (see Section 3.b.iii) will allow this PFAS-containing leachate to enter and contaminate the groundwater. It is therefore not appropriate to dispose of soluble PFAS in landfills without these controls in place.

Volatile PFAS. Volatile PFAS and forms that degrade to volatile PFAS, including alcohols such as FTOHs and FASEs may be released through uncollected or fugitive LFG. These types of PFAS should therefore be disposed of in landfills with LFG collection and control systems in place and operating (see Section 3.b.iii.6). Furthermore, PFAS waste that is comingled or combined with other waste determined to be biodegradable (see EPA Test Methods for biodegradability listed at *https://www.epa.gov/pesticide-labels/criteria-biodegradability-claims-products-registered-under-fifra*) should only be disposed of at landfills with gas collection and control systems in place. The biodegradable fraction of waste will produce LFG as it decomposes and allow volatile PFAS to be emitted from the landfill.

Mechanisms and rates of transformation. The behavior of PFAS in landfills is a complex and multifaceted process that involves various abiotic and biotic transformation and partitioning pathways. While the exact mechanisms and rates of transformation are not fully understood, both aerobic and anaerobic conditions in landfills can facilitate the conversion of PFAS precursors into more persistent terminal PFAS species. It is notable that fluorotelomer carboxylic acids (FTCAs) are a soluble intermediate transformation product which has been well documented to accumulate in biologically active anaerobic landfill conditions and is considered a landfill leachate contamination indicator. FTCAs result from the degradation of volatile PFAS such as FTOHs. For this reason, volatile PFAS-containing wastes should be managed in landfill environments which are also appropriate for soluble PFAS disposal.

As the environmental and health risks of PFAS are the subject of emerging research, it is essential to consider PFAS management strategies that minimize their release and transformation in landfills and other waste management systems. For example, Subtitle D MSW landfills are required to install a low-permeability liner prior to waste disposal, and LFG at MSW landfills is collected and managed based on the landfill's permitted design capacity and emission rate of nonmethane organic compounds (NMOCs) (40 CFR part 60, subparts Cf and XXX). Given these potential pathways for release, Table 3-1 summarizes the recommended engineering controls at landfills accepting PFAS waste based on the type of PFAS and the biodegradability of any comingled waste. It is noted that non-biodegradable stable polymeric PFAS do not require specific controls since they are not anticipated to migrate or biodegrade and generate gas, can be disposed in any type of landfill, and are not included in the table below. Also note that soluble PFAS comingled with biodegradable waste should have both control liquid and gaseous emissions, i.e., leachate collection and gas control.

Table 3-1. Recommendations for Disposal of PFAS-Containing Waste by Type of PFAS, Waste Biodegradability, and Type of Landfill Controls to Reduce Risks of Environmental Release

Type of PFAS	Degradability of comingled waste	Landfills with liner and leachate collection	Landfills with gas collection and control
Stable polymeric PFAS (e.g., PTFE)	Biodegradable		✓
Soluble PFAS (e.g., PFAAs)	Biodegradable	✓	✓
	Non-biodegradable	✓	
Volatile PFAS (e.g., FTOHs)	Biodegradable		✓
	Non-biodegradable		✓

3.b.ii Types of landfills

Landfills are typically classified by ownership status and by the type of solid waste they are permitted to receive, which determines the types of environmental controls they must employ. Depending on the type of waste disposed, a landfill could be subject to regulation and permitting under RCRA, the CAA, and/or TSCA. RCRA regulates two types of landfills: Subtitle C facilities receive hazardous waste, while Subtitle D landfills are primarily intended for the management of non-hazardous waste and can include MSW landfills, industrial non-hazardous waste landfills, C&D waste landfills, and coal combustion residual landfills. The CAA regulates air emissions from MSW landfills and TSCA regulates landfills that contain PCBs. The requirements under RCRA, CAA, and TSCA determine how the landfill must be constructed, operated, controlled, maintained, monitored, and closed when it reaches its final capacity.

Although categories and environmental controls vary from state to state, the following categories of landfills exist in most states. Landfills tend to have similar environmental controls within each category:

- Hazardous waste
- MSW
- Ash monofill
- Industrial
- C&D debris

Table 3-2 identifies the environmental controls required by RCRA and CAA for the landfill types defined in RCRA Subtitles C and D. RCRA regulations focus on the containment of solid waste and leachate, while CAA regulations focus on collection and control (destruction) of components in LFG. The landfill types

differ in how they are constructed, operated, controlled, monitored, and closed, reflecting the different types of waste they are allowed to receive.

- Subtitle C hazardous waste landfills that meet the minimum technology requirements set under RCRA.
- Subtitle C hazardous waste landfills are permitted to receive hazardous wastes that pose potential risk to humans and the environment and therefore have the most stringent environmental controls.
- Subtitle D MSW landfills are allowed to receive MSW and are required to install a low-permeability liner prior to waste disposal. LFG must be collected and managed according to the requirements of the CAA standards of performance (40 CFR part 60 subparts Cc, Cf, WWW, and XXX) at landfills that meet the regulatory requirement thresholds. Subtitle D landfills that receive non-hazardous and non-biodegradable waste tend to have environmental controls commensurate with the waste they receive. These controls can vary from state to state; for example, bottom liner, leachate collection, and final cover requirements for industrial and C&D landfills range from none to rigorous.

Note that certain small MSW landfills in arid or remote locations are exempt from RCRA design and groundwater monitoring requirements (40 CFR 258.1). See Sections 3.b.ii.2 and 3.b.iii.1 for additional exemptions.

Table 3-2. Required Environmental Controls by Landfill Type

Landfill Type	Federally Regulated Under	Bottom Liner and Leachate Collection System	Gas Collection and Control System	Final Cover
Hazardous waste	RCRA Subtitle C	Yes (double liner or better)	No	Flexible membrane liner (FML) cap
MSW	RCRA Subtitle D 40 CFR part 258 CAA 40 CFR parts 60 and 63	Yes (composite liner or better)	Yes (when regulatory size and emission thresholds are triggered)	FML cap
Ash monofills	RCRA Subtitle D 40 CFR part 257	Yes (composite liner or better)	No	Clay cap
Industrial	RCRA Subtitle D 40 CFR part 257	Varies by state, from no liner requirement to composite liner	No	Varies by state, from no requirements to FML cap
C&D debris	RCRA Subtitle D 40 CFR part 257	Varies by state, from no liner requirement to composite liner	No	Varies by state, from no requirements to FML cap

PFAS concentrations have been detected in landfill leachates from various types of landfills, as shown in Table 3-3.

Table 3-3. Range of PFAS Concentrations in Different Types of Landfill Leachate Reported in Published Studies. Adapted from Tolaymat et al., 2023.

Matrix	Number of PFAS Detected (Number of PFAS in Method)	∑PFAS Range (ng L^{-1})	Country	Reference
MSW Leachate	25 (26)	300 – 58,000	USA	Chen et al. (2023)
	10 (11)	15,000 – 18,000	USA	Solo-Gabriele et al. (2020)
	24 (24)	2,700 – 7,400	USA	Huset et al. (2011)
	30 (70)	2,000 – 29,000	USA	Lang et al. (2017)
	2 (2)	330 – 2,600	USA	Clarke et al. (2015)
	28 (28)	230 – 29,000	USA	Helmer et al. (2022)
	2 (2)	47 – 3,400	USA	Michigan Waste & Recycling Association (2019)
	22 (25)	1,400 – 125,000	USA	NWRA (2020)
	31 (40)	BDL – 104,000	USA	California Water Boards (2023)
	14 (14)	33 – 15,000	Australia	Gallen et al. (2016)
	9 (9)	210 – 46,000	Australia	Gallen et al. (2017)
	15 (15)	Not reported	Australia	Simmons (2019)
	25 (43)	31 – 13,000	Germany	Busch et al. (2010)
	24 (24)	2,500 – 36,000	Canada	Benskin et al. (2012)
	16 (18)	700 – 6,400	Canada	Li (2009)
	2 (2)	50 – 2,300	Canada	Gewurtz et al.
	16 (27)	2,200 – 6,100	Norway	Eggen et al. (2010)
	7 (8)	200 – 1,500	Norway	Kallenborn et al. (2004)
	4 (4)	210 – 610	Finland	Perkola & Sainio (2013)
	7 (10)	14 – 17,500	Ireland	Harrad et al. (2019)
	8 (16)	640 – 1,400	Spain	Fuertes et al. (2017)
	28 (30)	320 – 11,000	Norway	Knutsen et al. (2019)
	17 (26)	0.3 – 1,300	Sweden	Gobelius et al. (2018)
	11 (14)	7,300 – 290,000	China	Yan et al. (2015)
	33 (57)	3,040 – 109,000	China	Liu et al. (2022)
	17 (17)	1,800 – 43,300	China	Huang et al. (2022)
	18 (18)	1,270 – 7,660	Singapore	Yin et al.
C&D Leachate	8 (9)	4,200 – 11,000	Australia	Gallen et al. (2017)
	24 (26)	270 – 30,500	USA	Chen et al. (2023)
	11 (11)	14,000 – 16,000	USA	Solo-Gabriele et al. (2020)
MSW Incineration Ash Leachate	9 (11)	2,800 – 3,400	USA	Solo-Gabriele et al. (2020)
	26 (26)	39 – 54,500	USA	Chen et al. (2023)
MSW Gas Condensate	26 (26)	199 – 80,900	USA	Chen et al. (2023)
	53 (92)	3,000 – 50,000	USA	Smallwood et al. (2023)
Hazardous Waste Leachate (Primary)	17 (28)	570 – 377,000	USA	California Water Boards (2023)
Hazardous Waste Leachate (Secondary)	13 (24)	25 – 3,700	USA	California Water Boards (2023)

BDL = below detection limit; ng/L = nanograms per liter

The following subsections describe the types of landfills that are expected to contain PFAS or PFAS-containing materials, with a focus on design and operation aspects that EPA considers important from a PFAS-treatment perspective.

3.b.ii.1 Hazardous waste landfills

Hazardous waste landfills are permitted to receive waste that is defined as "hazardous" under RCRA. This waste either is explicitly listed as hazardous or demonstrates at least one of certain characteristics (i.e., toxicity, corrosivity, reactivity, or ignitability) as defined by RCRA. Hazardous waste landfills (Subtitle C landfills) can be used for non-hazardous wastes like PFAS, when the generator wants a more controlled solution. These landfills are required to have a double liner system and must adhere to waste pretreatment standards prior to disposal (40 CFR part 268). Most hazardous waste landfills are not biologically active because they do not typically accept biodegradable wastes (e.g., biosolids, food scraps), and therefore typically do not need to have gas collection systems. Conditions in these landfills are not expected to cause PFAS emissions via LFG, although gas collection systems could be installed if a problem arises related to gas release or gas emissions. Any hazardous waste landfill must have a final cover consisting of an FML covered by soil.

PFAS-containing waste is and will likely continue to consume a fraction of hazardous waste landfill capacity. Although waste containing PFAS is currently not defined as hazardous waste under RCRA (unless the particular wastes are hazardous for some other reason), some waste generators, perhaps to be cautious, have been sending PFAS-containing wastes to hazardous waste facilities. To ensure hazardous waste landfill capacity is available in the future, EPA reviews and analyzes the Biennial Hazardous Waste Report and other data to develop and then publish an assessment of national capacity for hazardous waste management.[18] The last such capacity assessment indicated that there is adequate capacity nationwide through 2044, and a new assessment is currently underway to incorporate new information and extend the time horizon. EPA will continue to work with states to monitor hazardous waste treatment and disposal capacity and report on the status.

3.b.ii.2 Municipal solid waste landfills

EPA estimates that in 2018, 50 percent of the MSW generated was landfilled (U.S. EPA, 2024). An MSW landfill typically has at least a bottom liner and an extensive gas collection and control system (GCCS) to collect the LFG generated when the biodegradable waste, such as food scraps and office paper, degrades over time. Some MSW landfills have accepted PFAS manufacturing wastes, and as a result, PFAS concentrations in leachate are higher than the typical range expected in MSW landfill leachate (NWRA, 2020). Under RCRA, MSW landfills that received waste after 1993 must install a composite liner that meets design criteria (40 CFR 258.40). However, MSW landfills that receive less than 20 tons of waste per day are exempt from installing a liner and monitoring groundwater under RCRA (40 CFR 258.1(f)(1)). MSW landfills that exceed specified thresholds for size (based on design capacity) and emissions of NMOCs are required to install gas collection systems under current CAA regulations. Therefore, a subset of MSW landfills do not have gas collection and could release fugitive PFAS associated with LFG emissions. Similarly, unlined MSW landfills are ineffective at managing the release of mobile PFAS to groundwater (see Section 4.a). The fate of PFAS in LFG that is managed through on-site GCCS including on-site flares, engines, boilers, and turbines is unknown.

[18] Background information and links to related documents are available at *https://www.epa.gov/hwpermitting/assessment-national-capacity-hazardous-waste-management*.

3.b.ii.3 Ash monofills

Ash monofills are a subtype of MSW landfills that mainly receive ash from MSW incinerators but could also receive smaller volumes of other waste streams such as biosolids from WWTPs. Ash monofills typically have a bottom liner and final cover requirements similar to other MSW landfills. However, they usually do not require GCCSs due to the use of incineration to remove biodegradable waste. Solo-Gabriele et al., (2020) found that ash monofills have lower PFAS concentrations than other landfill types, as shown in Table 3-3. While some landfill wastes, such as MSW ash, may be low in PFAS concentrations, the inclusion of higher-PFAS waste types may increase PFAS release. With limited data on the presence of PFAS in ash monofill leachate, more research is needed to determine the PFAS and precursor content of ash from different incineration technologies and air pollution control systems.

3.b.ii.4 Industrial landfills

Industrial landfills receive solid waste from industrial operations (non-municipal). Industrial landfills are often designed to manage specific waste streams (e.g., furnace slag, fly ash, plastics). The designs of industrial landfills vary widely based on the characteristics of the waste they receive. Requirements for environmental controls at these landfills also vary from state to state. Depending on the waste types and size of the landfill, some states do not require a liner. If a liner is required, a membrane cap is often also required. Due to the variability in control technologies, industrial landfills may not be an effective disposal option for managing uncontrolled releases of PFAS. Landfills that have historically received PFAS manufacturing wastes have been associated with high concentrations in leachate and reported environmental impacts (MPART, 2020). Some waste types received at industrial landfills, including plastics and materials with polishes or coatings, are associated with high concentrations of PFAS (OECD, 2013). Specifically, industrial processes such as the leather tanning, chrome plating, and textile industries contribute to PFAS in the industrial solid waste stream (ITRC, 2022).

3.b.ii.5 Construction and demolition landfills

C&D landfills receive waste from construction, renovation, and demolition projects, as well as other inert materials that are generated in high volumes, comparable to MSW waste (U.S. EPA, 2020b). The requirements for environmental controls at these landfills vary widely from state to state, ranging from no liner to a required composite liner. C&D landfills receive some PFAS-containing wastes (e.g., building materials and carpeting with PFAS treatments) (OECD, 2013; Solo-Gabriele et al., 2020; Bečanová et al., 2016), and soluble PFAS have been noted in the leachate from C&D landfills that are equipped with a leachate collection system (Chen et al., 2023). If a liner is required, a membrane cap could also be required. GCCSs are not required in C&D landfills due to low levels of biodegradable waste received compared to MSW landfills. Therefore, PFAS-containing wastes that also include a biodegradable component should not be disposed of at C&D landfills. A GCCS may sometimes be necessary to remediate a specific issue, such as gases generated from the decay of drywall. C&D landfills that lack composite liners and leachate management systems are unlikely to prevent the migration of soluble or volatile PFAS types; however, longer chain PFAS polymers such as PTFE, which is found in some electrical insulation and membrane material, are less mobile and likely to be contained in the waste mass for a longer period.

3.b.iii Ability of engineered landfill components to contain PFAS

PFAS are emitted from landfills via two possible routes: landfill leachate and LFG. Landfill leachate is liquid that has come into contact with solid waste and is either derived from the waste itself or the result of rainwater intrusion into the waste mass. Leachate contains soluble, suspended, or miscible

materials removed from such waste. LFG is the result of the natural decomposition of organic material in landfills under anaerobic conditions. LFG is composed of roughly 50 percent methane, 50 percent carbon dioxide, and a small amount of NMOCs.

Existing efforts to manage contaminants in landfills focus on controlling leachate and LFG emissions. As shown in Figure 3-1, landfills constructed with environmental controls (e.g., bottom liner, leachate collection system, gas collection system, final cover system) manage the release of contaminants into the environment.

The uses of the engineered landfill controls shown in Figure 3-1 vary by landfill type due to differences in types of waste accepted, operating practices, site conditions, and federal and state regulations.

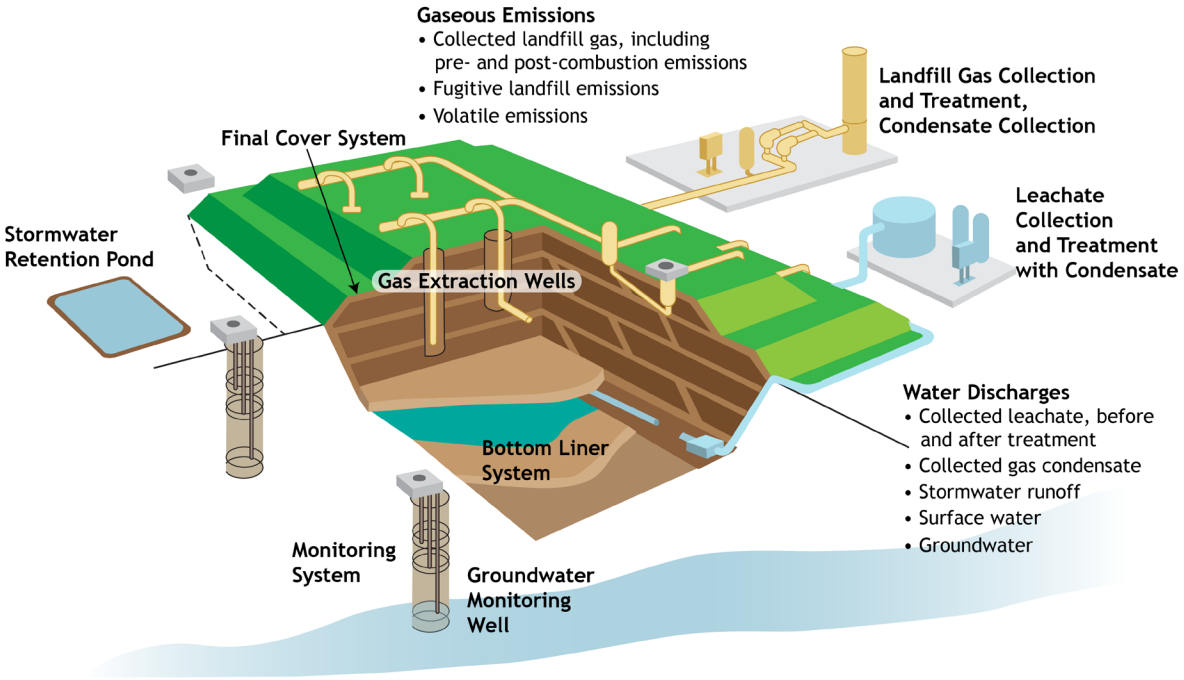


Figure 3-1. Engineered landfill components and potential PFAS release pathways.

3.b.iii.1 Bottom liner and leachate collection system

Under RCRA, any new landfill, replacement of an existing landfill, or lateral expansion of an existing landfill must have a double liner and leachate collection system if it receives hazardous waste, to limit leachate and gas release through the bottom of the landfill. Most landfills that are regulated under RCRA to receive non-hazardous waste are subject to design criteria that require either a composite liner and leachate collections system or a state-approved design that satisfies performance standards to ensure that regulated chemicals do not migrate beyond a specified distance from the landfill.

The variation of landfill requirements among states poses a challenge for the long-term containment of existing PFAS in landfills: state requirements for landfill liners vary, and some landfills are not required to have liners because of waste type or quantities received. Additionally, RCRA-exempt sites do not require liners (40 CFR 258.1). Because PFAS are not a RCRA-regulated hazardous waste, existing unlined

landfills could include PFAS-containing wastes that are easily emitted into the environment. Depending on their mobility, PFAS compounds could impact groundwater if disposed of in an unlined landfill.

A liner is built of layers of clay and/or polymers (i.e., FMLs) designed to withstand the weight of waste and soil. Leachate will collect on top of the liner, so its design must include a leachate collection system contoured to collect leachate through a network of pipes leading to a low point called a sump. The collected leachate is pumped from the landfill and managed as liquid wastes (see Section 2.f and Section 3.b.iii.5). Uncontrolled leachate could result in the release of PFAS into the environment.

PFAS interactions with landfill liner materials have been the subject of limited studies. The most common types of FML are made with polyethylene geomembranes. PFAS diffusion through linear low-density polyethylene is reported below detection diffusion rates (Di Battista et al., 2020), and diffusion through high-density polyethylene may be even slower due to differences in material structure. PFAS likely pass through clay liners, which are required to have low hydraulic conductivity on the order of 10^{-6} centimeters per second, at the same rate as leachate and other constituents such as chloride (Li et al., 2015).

The most significant pathway for leachate (and PFAS) transmission through geomembrane or composite liners is via imperfections (e.g., flaws or holes) (Di Battista et al., 2020). A review of landfill liner performance reported median leakage rates of 44 and 33 liters per hectare per day for geomembrane and composite liners, respectively, and overall liner collection efficiency of 98 percent (Jain et al., 2023). Even if liners successfully prevent leachate from reaching groundwater, very few data exist on whether concentrated PFAS waste interacts with the different types of geotextiles used for landfill liners, thus affecting the performance of the liner. While the performance of clay liners may not be affected drastically, there is currently no research on the long-term stability of FML in the presence of PFAS.

3.b.iii.2 Landfill gas collection system

Landfills use GCCSs to manage gas generated from decomposing organic waste. A GCCS consists of a network of perforated pipes sunken into the waste mass. These “gas wells” are connected to a central blower that pulls gas from the wells. Despite the presence of a GCCS, gas can still migrate both through the surface of the landfill and underground through the bottom of the landfill. The gas produced by MSW landfills contains about 50 percent methane that is usually collected and burned off at the site via flares or for energy recovery; however, those systems for LFG destruction (e.g., flares, engines, boilers) typically operate up to 85°C—below the minimum temperature required to destroy PFAS.

Not all landfills are required to collect LFG (see Table 3-2). As noted in Section 3.b.iii.6.1, research has found that soluble PFAS with relatively high vapor pressures can be emitted into the atmosphere via the gas generated at landfills (Ahrens et al., 2011; Hamid et al., 2018; Wang et al., 2020; Weinberg et al., 2011). Direct sampling methodologies are currently under development, and published concentrations of PFAS measured *in situ* are limited to a single study (Titaley et al., 2023). LFG flares generally operate at approximately 650°C to 850°C, lower than the 1,100°C necessary to achieve the mineralization of PFAS. Nonetheless, LFG collection and management systems (e.g., flares) must be present at MSW landfill sites that accept PFAS waste, especially biodegradable PFAS waste such as biosolids. The use of GAC prior to the flaring of LFG can be effective in removing PFAS from LFG. Data on the fate of PFAS in LFG that is managed through on-site GCCS, including on-site flares, are still limited. See Section 5 for potential research needs.

3.b.iii.3 Final cover system

After a regulated landfill has reached its designed and permitted capacity, it must be capped with a cover system. This system typically consists of some combination of soil and membrane materials and is primarily intended to reduce infiltration of rainwater into the landfill to minimize leachate generation. The cover system also helps increase the efficiency of the GCCS and reduce uncontrolled gas emissions. Synthetic membranes and caps are more effective at controlling release of PFAS than earthen covers. Earthen covers are more subject to wet/dry cycles and cracking and are more likely to result in uncontrolled LFG emissions, which could contain PFAS (Ahrens et al., 2011; Tian et al., 2018; Wang et al., 2020; Weinberg et al., 2011).

3.b.iii.4 Other environmental controls and monitoring systems

In addition to the major infrastructure discussed above, solid waste landfills implement other practices and systems to protect human health and the environment. In active landfill cells, daily application of a material such as soil or inert waste covers exposed solid waste. Daily cover reduces leachate generation, gas emissions, and direct exposure to humans and wildlife. Access control for a landfill site, such as a fence, is typically also required to reduce direct human and ecological exposure to waste. Extensive monitoring networks are generally required to measure the landfill's impact on surface water and groundwater. RCRA Subtitle C requires all hazardous waste landfills to install groundwater monitoring wells. See Section 3.b.iii.5 for leachate discharge controls.

3.b.iii.5 Leachate discharge controls

3.b.iii.5.1 Leachate characteristics

Leachate is the liquid effluent from landfills primarily generated through the percolation or infiltration of rainwater through waste. Leachate often contains high concentrations of biodegradable and non-biodegradable organic matter, dissolved and suspended solids, heavy metals, ammonia, and sulfur compounds (Mukherjee et al., 2015; Renou et al., 2008). The waste type, age, climate at the landfill site, and methods of landfill operation dictate the characteristics of leachate. These factors result in highly variable leachate characteristics across landfill types and on a site-by-site basis. Subtitle C hazardous waste landfills are required to use a leachate collection system during their active and post-closure care periods to mitigate adverse impact to human health and the environment. Since the use of leachate collection systems at Subtitle D landfills varies by landfill type and state requirements, the efficacy of leachate management and PFAS emissions depends on the controls implemented. PFAS containment in landfills is expected to be indefinite, so the generation of PFAS-containing leachates remains a probability during the post-closure care period and beyond. Ideally, the leachate collected at the bottom of the landfill is removed and managed to minimize impacts to human health and the environment. See Section 2 for more a more detailed discussion on the types of leachates and associated PFAS.

3.b.iii.5.2 Off- and on-site management of leachate

The most common method for non-hazardous waste leachate disposal is off-site treatment at municipal WWTPs, where leachate is mixed with wastewater and treated. The dynamic nature of leachate characteristics, the presence of non-biodegradable compounds and ammonia, and the presence of emerging contaminants (such as PFAS) in the leachate may make it difficult for WWTPs to effectively treat the influent water. In some cases, a landfill with elevated PFAS in its leachate may burden a WWTP's ability to treat, remove, or destroy these compounds before discharge to the environment (Masoner et al., 2020). As mentioned in Section 2.a.ii, wastewater treatment technologies used at most municipal WWTPs are generally ineffective at destroying or controlling PFAS (Schultz et al., 2006) and as

a result may also be ineffective at treating PFAS-containing landfill leachate. Furthermore, in WWTPs, PFAS may bind to and accumulate in biosolids, which can lead to PFAS entering the environment if biosolids are applied to land. Some WWTPs have requested that landfill operators pretreat leachate on site, and some WWTPs are implementing surcharges based on the leachate quality. Underground injection is another form of off-site leachate management (see Section 3.c).

Table 3-4 presents leachate management or treatment methods for the on-site management of leachate. Multiple technologies in the table are marked for further research—technologies for which, at the time of publishing, data do not exist to support or reject application for PFAS treatment. "Secondary treatment required" refers to the remaining concentrated or captured PFAS, which must be disposed of after treatment. "Potential secondary release" refers to the potential for PFAS release or breakthrough during the treatment process. Leachate characteristics are site-specific; therefore, the effectiveness of leachate technology should be evaluated on a site-by-site basis. (Table 3-3 lists average PFAS concentrations observed in different types of landfill leachate.)

Landfill operators should identify management or treatment methods that: (1) are suitable for the leachate at their specific sites and (2) meet the leachate discharge standards for chemicals and characteristics of leachate that are regulated. For example, leachate from a hazardous waste landfill is regulated as a listed hazardous waste (F039) and must be managed appropriately but that does not necessarily mean that treatment technologies are adequate to control the release of PFAS into the environment. For example, evaporation is often used to reduce leachate volume, which could lead to uncontrolled PFAS volatilization. The geography of the landfill site and cost-effectiveness of the methods play a crucial role in the identification of viable approaches to leachate management or treatment. For example, evaporation is often used to reduce dependence on treating a specific chemical(s) (e.g., ammonia). The use and effectiveness of leachate management strategies in removing or destroying PFAS during treatment varies (and, as noted in Section 3.b.vi, methods to quantify effectiveness are still under development). Leachate treatment technologies can be largely categorized into physiochemical processes, physical processes, biological processes, natural processes, and other management methods, as grouped in Table 3-4. Considering that leachate contains a variety of chemicals, a combination of physiochemical treatment processes can be used to narrowly target specific parameters for pre-treatment, or as part of a multi-step treatment strategy.

Table 3-4. Existing Landfill Leachate Treatment Technologies for PFAS Removal or Destruction

Treatment Technology	Treatment Mechanism	Pros for PFAS Treatment	Cons for PFAS Treatment	References
Physiochemical Processes				
GAC	Adsorption	• Familiar technology • Effective for long-chain PFAS	• Secondary treatment required • Short-chain PFAS breakthrough • Potential secondary release • Cost	• McCleaf et al. (2017), Pan et al. (2016), Ross et al. (2018)
PAC with coagulation	Adsorption	• Effective for long-chain PFAS	• Secondary treatment required • Costly for high-volume leachate • Potential secondary release	• Bao (2014), Pan et al. (2016)
Polymeric adsorption	Adsorption	• Tailored for specific compounds	• Secondary treatment required • Potential secondary release	• Liu (2017)
Ion exchange resin	Ion exchange adsorption	• Specified for certain compounds • More effective than GAC for long-chain compounds	• Secondary treatment required • Less effective for short-chain PFAS • Potential secondary release	Dickenson & Higgins (2016), McCleaf et al. (2017), Ross et al. (2018)
Zeolite	Ion exchange adsorption	• Inexpensive	• Secondary treatment required • Low surface area compared to GAC • Unknown reaction with short-chain PFAS	Chiang et al. (2017), Ochoa-Herrera & Sierra-Alvarez (2008)
Ozonation/ ozofraction-ation	Oxidation	• Potentially effective multi-contaminant removal	• Potential side-product formation • Secondary treatment required	Franke et al. (2019), Lin et al. (2012), Rahman et al. (2014), Ross et al. (2018)
Fenton oxidation	Oxidation	*Limited data available*	*Limited data available*	*None identified*

Treatment Technology	Treatment Mechanism	Pros for PFAS Treatment	Cons for PFAS Treatment	References
Photocatalytic advance oxidation process	Oxidation	• Permanent degradation	• Potential side-product formation • Cost	Lockwood (2018), Ross et al. (2018)
Coagulation-flocculation	Precipitation	*Limited data available*	*Limited data available*	Bao (2014), Dickenson & Higgins (2016), ITRC (2018), Rahman et al. (2014)
Chemical precipitation	Precipitation	*Limited data available*	*Limited data available*	*None identified*
Air stripping	Volatilization	*More research needed*	• Potential secondary emissions	*None identified*
Physical Processes				
RO	Physical separation	• Commonly used • Effective for short and long-chain PFAS	• Secondary treatment required for high-volume concentrate • Membrane fouling	Dickenson & Higgins (2016), Ross et al. (2018)
NF	Physical separation	• Uses less energy than RO • Effective for short- and long-chain PFAS	• Secondary treatment required	Boo et al. (2018), Dickenson & Higgins (2016)
Ultrafiltration (UF); microfiltration (MF)	Physical separation	*N/A*	• Not effective for PFAS	U.S. EPA (2020a)
Climatic evaporation; thermal evaporation; mist evaporation	Volume reduction	*Limited data available*	• Potential secondary emissions	*None identified*
Other On-Site Management Methods				
Recirculation	Containment	• Co-location with landfill	• Oversaturation • Potential surface water contamination • Dependent on climate	*None identified*
Underground injection	Containment	• Potential solution for PFAS concentrate	• Dependent on site geology • Regulatory approval	ITRC (2018)

Treatment Technology	Treatment Mechanism	Pros for PFAS Treatment	Cons for PFAS Treatment	References
Incineration	Thermal destruction	• PFAS destruction	• Potential secondary emissions (incomplete destruction) • Regulatory approval	ITRC (2017), Yamada et al. (2005)
Solidification	Containment	• Co-location with landfill • Reduces PFAS mobility	• Consumes air space in landfill • Unrealistic for large leachate volume	*None identified*
Biological Processes				
Activated sludge process sequencing; batch reactor; anaerobic; digestor; membrane bioreactor	Biological processes	*Limited data available*	• Limited by high concentrations of non-biodegradable organic matter	Ross et al. (2018), Saez et al. (2008), U.S. EPA (2020a)
Natural Processes				
Constructed wetlands; aerated ponds; phytoremediation; land application	Environmental release	*N/A*	• Direct release of PFAS	U.S. EPA (2020a)

3.b.iii.5.3 Leachate management and treatment technologies

Membrane treatments separate compounds from the leachate using mechanical filtration and pressure. Leachate passes through selective membranes (such as RO, NF, UF, and MF membranes) that divide it into two parts: permeate (which has passed through the membrane) and concentrate (which has not). The permeate and concentrate can then be treated as independent streams. The primary difference between these membranes is the pore size, which in turn affects the operating pressure and removal efficiency for different types of contaminants. RO is the most commonly used type of membrane for leachate treatment, while NF, UF, and MF are generally used in combination with other treatment technologies including RO. RO and NF are known to be effective in concentrating some PFAS, but UF and MF have pores that are too large to limit the release of most water-bound PFAS across the filtration membrane. Membrane fouling and a large amount of concentrate generation are two of the major drawbacks observed in implementing the membrane treatment system for landfill leachate and may be further complicated by high concentrations of PFAS (Dickenson & Higgins, 2016; ITRC, 2018; Ross et al., 2018).

An adsorption process with activated carbon is used for targeted removal of organic matter at some landfill sites. Activated carbon is known to be effective at trapping some PFAS, but it may need to be combined with other treatment methods to manage the range of PFAS found in landfill leachate. Activated carbon is a non-selective treatment method, expected to be less effective in nutrient-laden effluents such as landfill leachate. Once saturated, activated carbon needs to be reactivated through a pyrolysis process (see Section 3.a), collected as solid waste, or otherwise treated, with consideration of

the PFAS concentration (ITRC, 2017). Leachate also may need to be pretreated before activated carbon treatment to avoid rapidly saturating the carbon.

Methods using ozone, Fenton's reagent, or advanced photocatalytic technologies are used to oxidize organic matter in the leachate. Ozone and hydrogen peroxide (H_2O_2) in combination with other oxidizing agents have been observed to remove up to 99 percent of chemical oxygen demand from leachate at different operating conditions (Renou et al., 2008) and may be effective at reducing or modifying certain PFAS in leachate (Ross et al., 2018). Ultraviolet photocatalytic advanced oxidation is known to be capable of destroying PFAS, but additional studies are needed to understand the subsequent products associated with this method (Lockwood, 2018; Ross et al., 2018).

Ion exchange processes using zeolite and magnetic ion exchange resin remove ammonia and organic matter, respectively. Ion exchange can be flexibly designed to address different compounds and may be effective at reducing PFAS in leachate; however, performance data are not currently available. As with activated carbon, the leachate may require pretreatment and the spent media would need to be handled as solid waste or otherwise treated.

Air stripping of landfill leachate is used for ammonia removal. While air stripping could be effective in the treatment pathway of leachate, if it is used before the removal of PFAS, it would likely lead to emissions of more volatile PFAS to air.

Recirculation of leachate within a landfill—a management strategy unique to MSW landfills—keeps the leachate within the landfill. Although recirculation can filter heavy metals and improve leachate quality, it is primarily used as a management option that may also help accelerate biodegradable waste decomposition. The recirculation of leachate in the landfill would return any PFAS to containment within the landfill. However, recirculation tends to increase the PFAS content of the landfill and may result in larger fugitive PFAS emissions since the waste will degrade faster.

Underground injection, specifically Class I injection, has also been used to manage landfill leachate in the United States (see Section 3.c).

Natural processes (such as constructed wetlands and phytoremediation) and biological processes (degradation, nitrification, and denitrification) are expected to be ineffective at treating and preventing release of many PFAS into the environment. Current biological treatment processes such as the activated sludge process and sequencing batch reactor have not been shown to be effective at treating many PFAS, but future research may show biological treatment can play a role in controlling some PFAS or converting them into other types of PFAS. Note that biological treatment does not necessarily result in PFAS releases directly into the environment, because the outputs can be further treated.

Leachate treatment through evaporation reduces the volume of leachate. Open-air evaporation methods may be effective at concentrating leachate but are a pathway for secondary PFAS releases to air. Commercial evaporators operated through the heat generated by the LFG combustion or other fuel sources are sometimes used at landfills. Exhaust gases emitted from the evaporators may be exposed to high temperatures, but those temperatures may not be high enough or last long enough to destroy PFAS (see Section 3.a).

3.b.iii.6 Landfill gas emission controls

3.b.iii.6.1 Landfill gas characteristics

Under the anaerobic conditions that dominate landfill environments, organic waste (e.g., food waste, paper, cardboard) decomposes and generates LFG. LFG in MSW landfills consists mostly of methane and carbon dioxide. In most landfills where gas is collected, it is burned for energy or to destroy the methane and other organic chemicals it contains. Even at sites that actively collect LFG, a fraction (EPA estimates 25% on average; AP-42, Vol.1, Chapter 2.4) of the LFG is emitted directly to the environment through the landfill surface and other routes. These uncontrolled emissions are referred to as fugitive losses.

Research has found that soluble PFAS with relatively high vapor pressures can be emitted into the atmosphere via the gas generated at landfills (Ahrens et al., 2011; Hamid et al., 2018; Wang et al., 2020; Weinberg et al., 2011). EPA is currently researching direct LFG sample evaluation for PFAS concentrations (see Section 5).

Unlike waste in MSW landfills, the C&D landfill waste that contributes most to LFG production is generally dominated by gypsum drywall (Yang et al., 2006). Gypsum drywall results in C&D LFG largely consisting of hydrogen sulfide, a highly pungent gas, with a smaller fraction of methane. Because C&D landfills generate a lower volume of gas than MSW landfills, LFG from C&D landfills is typically not collected and is often emitted to the environment without treatment.

3.b.iii.6.2 On- and off-site management of landfill gas

LFG collection and management are regulated under the CAA through New Source Performance Standards (NSPS) and National Emission Standards for Hazardous Air Pollutants (NESHAP) programs. After collection, LFG can be managed on site and burned using a flare. There are two basic types of flares common at MSW sites: open (candlestick) and enclosed flares. LFG can also be managed off site, where it is usually piped from the landfill to a nearby gas-fired system to generate heat or power.

On-site open flares must operate in accordance with key parameters for exit velocity and flare diameter for non-assisted flares (in 40 CFR 60.18). Additionally, a heat-sensing device must be installed to indicate continuous flame presence (but no specific temperature level). A landfill with an enclosed flare must reduce NMOCs to 20 parts per million by volume (ppmv) outlet or reduce NMOCs by 98 percent via a one-time performance test. Lowered emissions are maintained by following operating parameters set during the test for the requisite flare temperature and flow rate.

Combustion temperatures and duration may prove to be critical factors for destruction of PFAS in LFG. While on-site flare systems average 850°C (1,550°F) (U.S. EPA, 2008), power-generating engines and boiler systems may run even cooler. None of these systems operate at the temperature necessary to mineralize PFAS (1,100°C) as indicated in the EPA boiler database. See Section 3.a for a more complete discussion on conditions required for PFAS destruction.

Under the CAA regulations, if LFG is treated for sale or use in a beneficial energy recovery device, it must be treated according to a site-specific treatment plan before being utilized on site or piped out of the facility. Like liners, active gas collection systems are not required at all landfills, depending on the landfill size and level of NMOCs in the LFG. The presence or absence of an LFG collection and control system directly affects the release of PFAS via fugitive emissions.

LFG condensate is a liquid that has condensed in the LFG collection system piping during the extraction of gas from the landfill. In gases with high concentrations of water vapor, water condenses in pipes and

accumulates in traps staged throughout the collection network. This condensate contains volatile compounds and accounts for a relatively small percentage of flow from a landfill. Gas condensate is commonly collected and managed with the landfill leachate as liquid waste. PFAS has been detected in LFG condensate, with perfluorobutanesulfonic acid (PFBS) being the dominant species at a concentration of 1,000 nanograms per liter (Li, 2011).

3.b.iv Potential for releases during landfilling

Over time, there is increased potential for certain pollutants to move into the environment beyond the footprint of the landfill. As water passes through the landfill, it may leach pollutants from the waste—including PFAS—and move them deeper into the waste mass. Controlled landfill leachate is collected and either reinjected, treated on site, or sent for off-site treatment. In the absence of leachate management systems, uncontrolled leachate releases occur when water travels through the waste, out of the landfill, and into groundwater or surface water (see Section 4).

LFG can also contain heavy metals, organic chemicals, and greenhouse gases and can produce explosive mixtures of gas in the vicinity of the landfill if not properly controlled (ATSDR, 2001). LFG capture technologies are widely used to control gaseous emissions from landfills. However, not all landfills are required to install and operate LFG collection systems. Even when an LFG collection system is installed and properly operated, a fraction of LFG is emitted directly to the environment through the landfill surface and other routes as fugitive losses (see Section 4).

Even years after landfill closure, direct human and ecological exposure to PFAS is possible if PFAS are emitted through the air, groundwater, or surface water, or if remaining waste is disturbed. Because landfills contain PFAS but are not designed to destroy these compounds, they represent a potential source of PFAS release well beyond the period in which landfills receive waste and the post-closure care period. Ideally, landfill areas could be used for other beneficial purposes after closure; this requires that potential risks from landfill contents be adequately managed. Direct exposure to PFAS from landfilled waste is possible for people living or working near landfills due to gaseous or water releases from the site. Additionally, if PFAS-containing wastes are present on trucks and moved to active cells, landfill employees are directly exposed to PFAS on the job. Research has shown elevated concentrations of PFAS in landfill ambient air (Hamid et al., 2018).

Potential impacts to vulnerable communities from landfill releases should also be considered. Considerations should include which media may be affected by releases, and the potential exposure pathways resulting from releases. The characteristics of the potential exposed communities (e.g., demographics, socioeconomic status, ambient exposures, health conditions) provide information regarding the potential for disproportionate and adverse health and environmental effects, including cumulative impacts.

Ensuring that PFAS-containing waste is properly disposed of in lined landfills with active gas collection systems where volatile PFAS or MSW landfills are involved, is instrumental in reducing PFAS emissions into the environment. Care must also be taken to avoid disposal of PFAS wastes in landfills that are known to have compromised liners, as PFAS in the leachate will migrate and contaminate groundwater.

3.b.v Testing and monitoring

Currently, there are no federal requirements for the monitoring of PFAS in landfill waste, leachate, condensate, or LFG. EPA and others are conducting studies to evaluate the effectiveness of landfills in

containing or managing PFAS (see Section 3.b). Landfill operators should include PFAS measurements with the regular leachate monitoring parameters that are regularly evaluated. Analyzing PFAS in LFG may pose a challenge for comparison across destruction/control technologies and types of landfills due to the lack of standardized testing (see Section 5.a for research needs).

The lack of testing and monitoring limit effective risk communication and dialogue with adjacent communities. The importance of dialogue with communities, and in particular, communities that may be more vulnerable to PFAS exposures, cannot be overemphasized. Suggestions for risk communication are included in Section 4.

3.b.vi Uncertainties/unknowns

EPA plans to conduct further research on PFAS within landfills, including the potential for PFAS to migrate to leachate or LFG without adequate controls. As with thermal treatment, EPA lacks detailed information on the amounts and concentrations of PFAS and precursor compounds in wastes that are landfilled. Limited research is providing some information on PFAS fate and portioning in landfill environments. The bulk of PFAS seems to remain with the solid waste mass, with quantifiable percentages in LFG and leachate. However, there is high level of uncertainty in these values and more data are needed. Sampling and analytical methodologies must be developed to quantify potential PFAS flows out of landfills—an effort that may be complicated by the long lifespan of some PFAS. Additionally, the efficacy of treatment options for PFAS captured by leachate and LFG systems is not well understood and is in some cases intrinsically entwined with TWTDS and thermal treatment options. EPA continues to research these complex and important issues. Refer to Section 5 for a summary of EPA and DoD's planned research activities specific to landfill containment, wastewater treatment, and thermal treatment of PFAS.

When evaluating landfill options, decision-makers (e.g., managers of PFAS-containing materials and PFAS-containing waste) should consider potential impacts to communities, including vulnerable populations. Uncertainties regarding LFG or leachate releases could have consequences for communities that are exposed and may be disproportionately affected. EPA recommends that decision-makers screen communities located in the vicinity of potential releases from the destruction, disposal, and storage options (considering fate and transport) in order to consider the potential for adverse or disproportionate impacts (see Section 4) and to consider potential measures to prevent, reduce, or address such impacts. Depending on site-specific circumstances (e.g., PFAS concentrations, impacted media, and potential exposure pathways), the size and shape of this area (vicinity) will vary.

3.b.vii Summary

Due to widespread use and disposal through typical waste management pathways, many PFAS-containing wastes are currently managed through containment in landfills. Though landfills (except C&D landfills) are designed for permanent waste containment and management of liquid and gas production, it is currently unclear if all landfills used to dispose of PFAS and PFAS-containing materials have controls that are effective for managing PFAS discharges and emissions from waste streams. PFAS-containing waste should not be placed in C&D landfills that don't have liner systems to intercept and manage the leachate. Even with appropriate liners, research has shown an overall collection efficiency of 98 percent for geomembrane and composite liners. Given the chemical makeup of PFAS, some compounds are expected to persist in landfills for years.

As leachate passes through landfills, PFAS are released from degrading wastes. PFAS have been detected in the leachate for all types of landfills, and improper management of landfill leachate would result in PFAS releases. To date, research on the efficacy of wastewater treatment technologies in capturing or destroying PFAS in leachate is limited, as most landfills are not currently required to treat leachate for PFAS. The existing data suggest that adsorption and separation treatment mechanisms have been shown to concentrate or capture PFAS from landfill leachate (see Table 3-4). Other leachate management options can control the release of PFAS, including recirculation and solidification, which return PFAS to the landfill. Leachate treatment through natural processes such as constructed wetlands, land application, or ponds is ineffective for preventing the release of PFAS into the environment. More data are needed on the volatilization of PFAS during leachate handling and treatment.

PFAS can be emitted with LFG that is generated as waste decomposes over time. On-site and off-site LFG management commonly uses flares, engines, or boilers to combust LFG. Combustion temperature and duration could be critical factors for the destruction of PFAS in LFG, as discussed in Section 3.a.

To varying degrees, hazardous waste or MSW landfills are feasible and effective disposal options for PFAS and PFAS-containing materials. Permitted hazardous waste landfills employ the most extensive set of environmental controls (e.g., double liner systems with leachate collection and leak detection) and practices (e.g., extensive record keeping) that are currently available for the containment of PFAS waste (see Table 3-2) and as a result would be more effective at minimizing PFAS release into the environment than other landfill types. Hazardous waste landfills do not typically accept biodegradable wastes (e.g., biosolids, food scraps), and are not biologically active, so they do not generally need to be equipped to collect and control LFG. Therefore, hazardous waste landfills are not expected to cause PFAS emissions via LFG and may not be appropriate options for biodegradable PFAS-containing wastes.

Modern MSW landfills, when constructed with appropriate controls (e.g., liner system and leachate and gas collection and management systems), can also control the release of PFAS into the environment. Even with these controls in place, the proper management of landfill gaseous and liquid releases needs to be applied to minimize PFAS release into the environment (see Section 3.b.iii). Care must be taken to apply the leachate control technologies that are effective at containing (e.g., solidification, recirculation) or destroying PFAS (see Table 3-4).

Given the high level of uncertainty associated with PFAS behavior in landfills, the effects of PFAS on liner integrity, gaseous emissions from landfills, the effectiveness of leachate treatment for PFAS removal, and the levels and types of PFAS in landfill leachate—additional research will help to further evaluate this disposal method for PFAS and PFAS-containing wastes. See Figure 3-2 for a summary of recommended landfill disposal options for PFAS-containing wastes and considerations for selecting the best option based on both waste and landfill characteristics.

See Appendix D for a summary of costs and considerations.

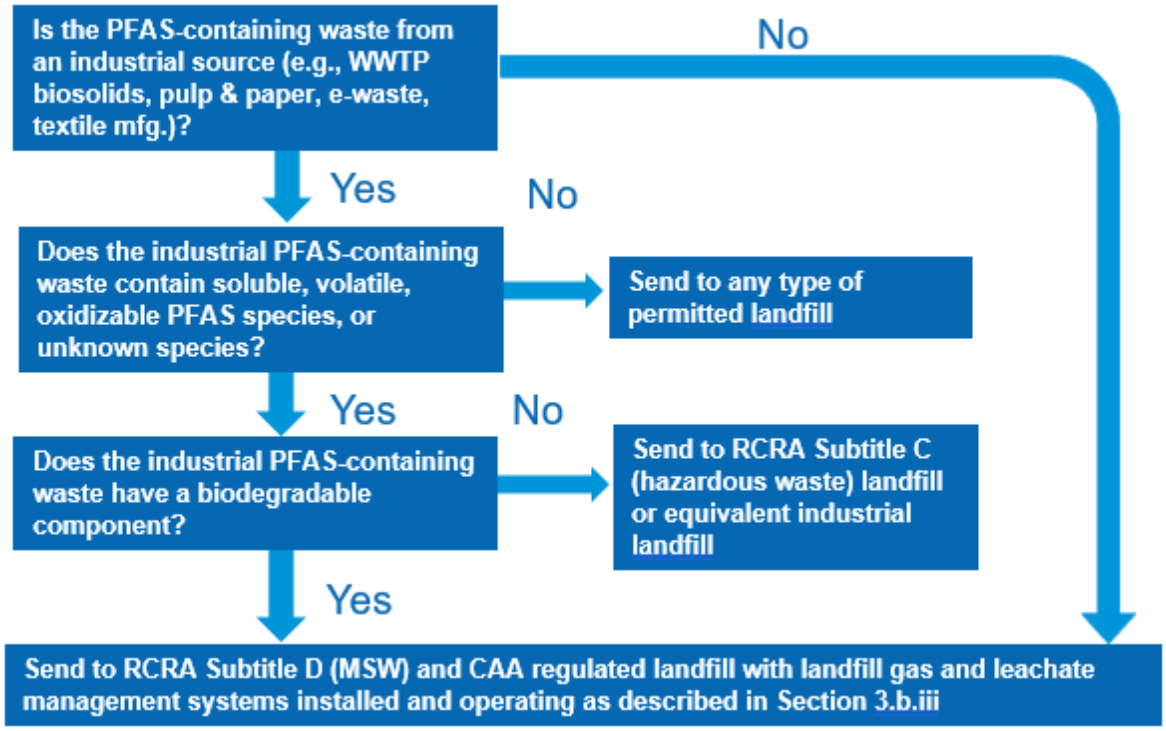


Figure 3-2. Decision framework for selection of landfill disposal for PFAS-containing industrial waste.

3.b.viii References for Section 3.b

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3.c Underground injection

Underground injection wells are a feasible and effective disposal option that normally should minimize release of PFAS into the environment. However, underground injection wells are only suited for the disposal of liquids and are restricted to locations with suitable geology. PFAS-containing fluids are currently handled similarly to other waste streams that are injected deep into geologic formations in Class I non-hazardous industrial and hazardous waste disposal wells, while shallow Class V wells would be inappropriate for the management of PFAS-containing fluids. The limited number of wells currently receiving PFAS and accepting off-site waste, well location, and waste transportation logistics may significantly limit the type and quantity of PFAS-containing fluids appropriate for underground injection.

3.c.i Types of wells

Underground injection is generally defined as the subsurface emplacement of fluids through a well. Under the Safe Drinking Water Act (SDWA), EPA is authorized to regulate the permitting of injection wells—including construction, operation, monitoring, and proper closure—for the purpose of protecting USDWs. Underground injection control (UIC) regulations are found in 40 CFR parts 144 to 148.

EPA's UIC program shares information for owners and operators of injection wells, regulators, and the public about safe injection well operations to prevent the contamination of USDWs. Under the UIC program, EPA regulates the permitting of the following well types:

- **Class I** wells inject into geologic formations below the lowermost USDW and are further subdivided into four categories: municipal wastewater, radioactive waste, hazardous waste, and non-hazardous industrial waste disposal wells (see Figure 3-3).
- **Class II** wells are used for injection activities associated with oil and gas production and hydrocarbon storage.
- **Class III** wells are solution mining wells used to inject fluids for the purposes of dissolving and extracting minerals.
- **Class IV** wells, with limited exceptions, have been banned by EPA since 1984 and were used to inject hazardous or radioactive waste into or above geologic formations containing USDWs.

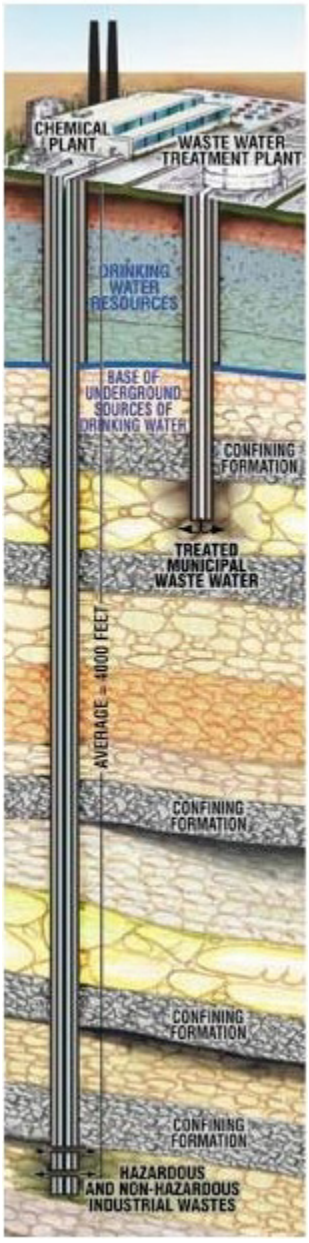


Figure 3-3. Class I wells.

- **Class V** wells include injection wells that are not included in Classes I, II, III, IV, or VI. EPA has identified multiple subtypes, including stormwater drainage wells, septic system leach fields, and agricultural drainage wells.
- **Class VI** wells are used to inject and geologically sequester carbon dioxide.

EPA has determined the use of Class I non-hazardous industrial waste and hazardous waste wells for high concentration liquid PFAS waste has a lower potential for environmental release when compared to other PFAS destruction and disposal options, and there is relatively low uncertainty in this

determination. Class I underground injection wells are designed to dispose of and isolate liquid waste below the land surface and beneath USDWs. The standards associated with the construction, operation, and monitoring of Class I wells are designed to ensure protection of USDWs. These standards include at least one confining layer between the zone in which the fluid will be emplaced and the lowest USDW. While Class I wells are an option for managing PFAS-containing fluids, this technology may not be appropriate everywhere. The suitability of a site for injection is dependent on the geologic formations in the area. Sites need to be evaluated to ensure that there is an appropriate confining zone and that there are geologic formations that can receive fluids.

Class I wells for non-hazardous industrial and hazardous waste are currently being used for disposal of PFAS-containing fluids and are the focus of this section. PFAS-containing fluids that may be disposed of via Class I underground injection wells may originate from industrial activities such as chemical production (e.g., products and byproducts) and waste management operation (e.g., landfill leachate).

The standards associated with the permitting, construction, operation, and monitoring of Class I hazardous waste wells, which are regulated under RCRA and SDWA, are more stringent than for non-hazardous industrial waste disposal wells.

3.c.ii Siting, engineering, and operational controls

3.c.ii.1 Overview of the regulatory framework

Underground injection through Class I non-hazardous industrial and hazardous waste wells is a long-standing, well-regulated disposal technology. Underground injection has been used as a waste disposal practice in the United States since the 1930s, beginning with disposal of brines from oil production activities. Underground injection of wastewater from industrial facilities has been in practice since the 1950s. In response to concerns around underground injection activities and incidents of well failure, in 1974 (the same year the SDWA was enacted), “EPA issued a policy statement in which it opposed underground injection without strict control and clear demonstration that the wastes will not adversely affect groundwater supplies” (U.S. EPA, 2001).

Final UIC regulations were published six years later, and federal and state regulation and oversight has been informed by extensive reviews of injection practices and the associated risks over time (U.S. EPA, 2001). The requirements for Class I wells under 40 CFR part 146 and 40 CFR part 148 (which applies to hazardous waste wells only) are designed to ensure that injected fluids cannot migrate into USDWs through either of two potential pathways: loss of waste confinement or “improperly plugged or completed wells or other pathways near the well” (U.S. EPA, 2001). These requirements include, but are not limited to:

- Proper siting.
- Conducting geologic and hydrogeologic studies that demonstrate that injected fluids will not endanger USDWs.
- Implementing specific design, construction, and operation requirements.
- Implementing continuous monitoring and periodic monitoring and testing requirements.
- Performing appropriate well closure and plugging.

Specific components of these requirements are discussed further below.

3.c.ii.2 Class I non-hazardous industrial and hazardous waste wells

Underground injection to Class I non-hazardous industrial and hazardous waste wells reduces the potential risks of human exposure to injected materials, avoiding discharge to surface and shallow groundwater and generating little or no air emissions. When injected into non-hazardous industrial or hazardous waste Class I wells, fluids are placed below the lowermost USDW. The area into which waste is injected is referred to as the injection zone. Injection zones of Class I wells typically range from 1,700 to more than 10,000 feet in depth (U.S. EPA, 2001). Injection zones are porous and permeable geologic formations. They are separated from USDWs by one or more confining layers of impermeable rock. The confining layers prevent injected fluids from migrating vertically into a USDW.

Class I wells are sited in geological areas that are conducive to injection operations. Siting considerations include ensuring that injected fluids will not migrate through natural fractures and faults from the injection zone into USDWs. Likewise, well operators are required to demonstrate the absence of non-natural pathways (e.g., abandoned wells) or other nearby active wells that could allow for movement of injected fluids into USDWs, within a prescribed area surrounding the well (known as the area of review). In addition to the safeguards offered by siting, engineering, and operating requirements, well design and construction requirements incorporate redundant safety features, and construction materials are "corrosion-resistant and compatible with the wastewater and the formation rocks and fluids into which they come in contact" (U.S. EPA, 2001). Class I wells might also use multiple strings of well casing, inject through tubing set on a packer, and be constructed with adequate cement alongside the entire well string to protect any USDWs.

3.c.ii.3 Potential for Release

Permitted underground injection of fluids through Class I non-hazardous industrial and hazardous waste wells ensures that injected fluids are confined and cannot enter USDWs—the pathway of concern for this waste disposal technology. In its 2001 study of risks associated with Class I wells, EPA stated that the "probability of Class I well failures, both non-hazardous and hazardous, has been demonstrated to be low. In the unlikely event that a well would fail, the geology of the injection and confining zones serves as a final safety net against movement of wastewaters to USDWs" (U.S. EPA, 2001).

Injection well operators invest millions of dollars in the permitting, construction, and operation of wells. Development of Class I non-hazardous industrial and hazardous waste wells is a resource-intensive process, with the geologic limitations noted previously. In addition, siting requirements limit the areas in the country where Class I wells can be located (see Section 3.c.iii). Routine operation and maintenance include addressing requirements for extensive mechanical integrity testing, monitoring, and periodic submission of permit/no-migration petitions.

3.c.ii.4 Additional requirements for Class I hazardous waste wells

Class I hazardous waste wells are highly protective of USDWs and include additional requirements beyond those of Class I non-hazardous industrial waste wells. The 1984 Hazardous and Solid Waste Amendments to RCRA prohibited land disposal of hazardous waste, including via underground injection, with limited exceptions. Hazardous waste disposal via Class I injection wells is permitted if the operator can demonstrate that the waste will remain where it has been injected for as long as it remains hazardous (defined under regulation as a period of up to 10,000 years). To demonstrate this, Class I hazardous waste well operators must receive approvals of "no-migration petitions" from EPA.

No-migration petitions present information and modeling results using data on local and regional geology, waste characteristics, geochemical conditions of the well site, injection history, and many other factors, which EPA reviews to determine whether the petitioner has adequately demonstrated that the waste will not migrate from the disposal site for as long as it remains hazardous. Furthermore, Class I hazardous waste well facilities are subject to inspections and well operators must conduct annual testing and analysis to demonstrate they are meeting the conditions of the permit and that all assumptions, projections, and models are still appropriate and valid. This includes performing mechanical pressure tests and geophysical logging tests to assess well integrity both internally and externally to ensure injected fluids are being emplaced and are remaining within the injection zone.

3.c.iii Availability

The United States currently has 925 Class I wells. Slightly less than half (48 percent) are permitted for non-hazardous industrial waste injection. Approximately 15 percent are permitted for hazardous waste disposal. The remainder are permitted for municipal wastewater disposal. Currently, EPA is aware of both non-hazardous industrial and hazardous Class I wells that manage PFAS-containing fluids. However, there is currently no national information on the number and location of Class I non-hazardous industrial or hazardous waste wells that could accept PFAS-containing waste or are willing to accept waste not generated on site.

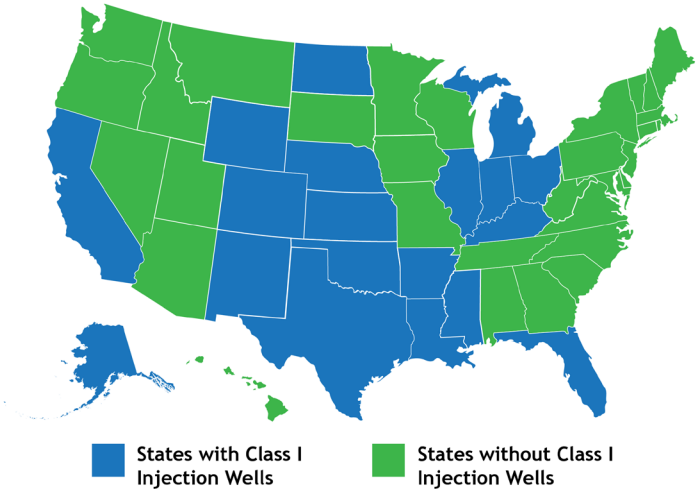


Figure 3-4. States with Class I non-hazardous or hazardous waste injection wells.

Figure 3-4 shows all states that currently have at least one permitted Class I injection well of any type (non-hazardous industrial or hazardous waste). Table 3-5 provides a more detailed breakdown of the number of permitted Class I wells by state.

Table 3-5. Inventory of Permitted Class I Non-Hazardous and Hazardous Waste Wells in the United States (FY 2022; Source: EPA)[19]

Location of Wells		Number of Wells	
EPA Region	**State/Tribe**	**Class I Non-Hazardous**	**Class I Hazardous**
4	Florida	343	-
	Kentucky	2	-
	Seminole Tribe	3	-
	Mississippi	4	4
5	Illinois	5	2

[19] EPA's inventory of Class I non-hazardous waste wells consists of all non-hazardous waste wells, including municipal and industrial waste wells. However, the municipal waste wells are located exclusively in Florida.

Location of Wells		Number of Wells	
EPA Region	**State/Tribe**	**Class I Non-Hazardous**	**Class I Hazardous**
	Indiana	13	4
	Michigan	40	7
	Ohio	7	11
6	Arkansas	8	4
	Louisiana	16	19
	New Mexico	6	-
	Oklahoma	6	-
	Osage Nation	1	-
	Texas	83	77
7	Kansas	58	7
	Nebraska	11	-
8	Colorado	13	-
	North Dakota	8	-
	Wyoming	89	-
9	California	52	-
10	Alaska	22	-
Total		**790**	**135**

3.c.iii.1 Class I non-hazardous industrial waste wells

Non-hazardous industrial waste wells are currently located across 19 states and two Tribal lands, though the majority are in five states—Wyoming, Texas, Kansas, California, and Michigan. There are currently no Class I non-hazardous waste wells in EPA Regions 1, 2, or 3 (see Figure 3-4 and Table 3-5). Disposal to this type of well requires well operators to apply for and receive a permit for specific waste streams. Current Class I injection wells may have limited capacity for PFAS-containing fluids, as many of them are used for specific purposes and disposal of waste generated on site. To begin accepting PFAS-containing fluids, existing well permits would have to be modified to recognize that the facility is accepting waste from other entities and authorize the facility to inject modified waste streams.

Well operators must also consider the well's capacity to accept additional volumes of waste and the compatibility of the PFAS-containing fluids with the well material, the geochemistry of the injection formation and formation fluids, and the properties of other injected wastes.

3.c.iii.2 Class I hazardous waste wells

Hazardous waste wells are currently located in nine states; however, most are in Texas and Louisiana. Most Class I hazardous waste wells are sited at industrial facilities and dispose of waste generated on site (U.S. EPA, 2016). There are currently no Class I hazardous waste wells in EPA Regions 1, 2, 3, 8, 9, or 10 (see Figure 3-4 and Table 3-5). Because of this geographic concentration of Class I hazardous waste wells, waste producers may face transportation and logistical challenges.

EPA anticipates the number of current Class I hazardous waste wells that would begin accepting new sources of PFAS-containing waste to be very limited due to the necessary modifications of Class I

hazardous waste well permits (e.g., increased injection volumes, changes to waste streams, no-migration petitions). These modifications would involve engineering and scientific evaluations, modeling, and public hearings.

3.c.iv Testing and monitoring

Class I non-hazardous industrial waste and hazardous waste disposal wells are subject to extensive testing and monitoring requirements established under federal regulations. Requirements for hazardous waste wells are more stringent than for non-hazardous industrial waste wells. Additionally, by law, states with primary enforcement authority for Class I wells may have more stringent testing and monitoring requirements. Class I monitoring and testing requirements are designed to ensure there are no leaks within or out of the well and that all injected fluid is contained in the injection zone.

Broadly, Class I well operators must:

- Analyze characteristics of injected fluids at a frequency that results in representative data.
- Continuously monitor and record injection pressure, annulus pressure, flow rate, and volume.
- Conduct internal and external mechanical integrity testing.
- Monitor for fluid release into the USDW within the area of review.

Class I hazardous waste wells operators must conduct mechanical integrity testing more often than Class I non-hazardous industrial waste operators. They also must establish and follow additional procedures for reporting and correcting mechanical integrity problems. Class I hazardous waste well operators must also develop and follow a waste analysis plan and conduct annual tests of cement at the base of the well (U.S. EPA, 2015).

Additional information on testing and monitoring requirements, including mechanical integrity testing, is included in EPA's summary document *Requirements for All Class I Wells and Class I Hazardous Waste Wells* and EPA Region 5's guidance on *Determination of the Mechanical Integrity of Injection Wells*.

Communicating testing and monitoring results with adjacent communities is particularly important given the high-profile nature of PFAS. The importance of encouraging dialogue with communities, especially communities that may be more vulnerable to PFAS exposures, cannot be overemphasized. Suggestions for risk communication are included in Section 4.

3.c.v Uncertainties/unknowns

The fate and transport of PFAS in the subsurface depends on the chemical and physical properties of specific PFAS and the geochemical properties of the injection zone. Understanding of the long-term fate and transport properties of PFAS (including precursors) in the injection zone is currently limited. Studies have shown wide ranges in PFAS properties, and these can be altered by mixture effects and interactions with co-contaminants. This creates uncertainty in predictions of PFAS contaminant release and longevity in the injection zone. These uncertainties need to be considered in the development of the required no-migration petition for disposal of PFAS in Class I hazardous waste wells.

When evaluating underground injection options, decision-makers (managers of PFAS-containing materials and PFAS-containing waste) should consider potential impacts to communities, including vulnerable populations. Underestimation of the potential for PFAS release could increase impacts to local communities, including vulnerable communities. EPA recommends that decision-makers screen

communities located in the vicinity of the destruction, disposal, and storage options in order to consider the potential for adverse, including any cumulative and disproportionate impacts (see Section 4), and potential measures to prevent, reduce, or address such impacts. Depending on site-specific circumstances (e.g., PFAS concentrations, impacted media, and potential exposure pathways), the size and shape of this area (vicinity) will vary.

3.c.vi Summary

EPA has determined the use of Class I non-hazardous industrial waste and hazardous waste wells for high concentration liquid PFAS waste has a lower potential for environmental release when compared to other PFAS destruction and disposal options. Permitted underground injection of fluids through Class I non-hazardous industrial and hazardous waste wells ensures that injected fluids are confined and cannot enter USDWs—the pathway of concern for this waste disposal technology. Additional Class I wells may need to be constructed where geologically suitable, and existing well permits may need to be modified to meet the capacity needs for PFAS disposal. Research on the long-term fate and transport of PFAS (including precursors) to predict release potential in the injection zone could support future permits.

See Appendix D for a summary of costs and considerations.

3.c.vii References for Section 3.c

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4. Considerations for Potentially Vulnerable Populations Living Near Likely Destruction or Disposal Sites

The FY 2020 NDAA specifies that the interim guidance consider:

- The potential for releases of PFAS during destruction or disposal, including through volatilization, air dispersion, or leachate.
- Potentially vulnerable populations living near likely destruction or disposal sites.

This section describes potential releases and exposure pathways associated with destruction or disposal sites. It defines potentially vulnerable populations and provides interim guidance on considering vulnerable populations when assessing the potential impacts of releases. This section also suggests approaches to screen communities located in the vicinity of the destruction, disposal, and storage options in order to consider the potential for adverse and disproportionate impacts and potential measures to prevent, reduce, or address such impacts. Depending on site-specific circumstances (e.g., PFAS concentrations, impacted media, and potential exposure pathways), the size and shape of this area (vicinity) will vary. Focusing on impacts and potential ways to protect vulnerable populations, such as children who are more vulnerable than adults to chemicals like PFAS, will ensure the protection of all populations exposed to PFAS.

The primary audience of this interim guidance is managers of PFAS-containing materials and PFAS-containing waste and regulators who need to identify the most effective means for destroying or disposing of these materials, including to protect nearby communities who may include vulnerable populations. Others may find it useful, such as communities themselves, as well as community relations personnel, and the public. This section is not a primer on risk assessment and risk communication; rather, it contains pointers and references to existing information.

4.a Potential releases from destruction and disposal facilities

EPA develops regulations, guidance, and policies that ensure the safe management and cleanup of waste.[20] Nonetheless, it is possible for destruction or disposal activities to release PFAS.

For example, as described in Section 3.a.iii, thermal treatment activities could potentially release PFAS to the environment via stack emissions if adequate combustion conditions are not achieved or adequate pollution control devices are not used. Releases can also occur from the management of thermal treatment process residuals such as liquid discharges from acid gas scrubbers, air pollution control device media, and incinerator bottom ash. In addition, if uncontrolled, leachate can travel out of landfills (see Section 3.b.i) and into groundwater or surface water. Disposal of PFAS could also result in potential

[20] Information on EPA's actions to address PFAS can be found here: *https://www.epa.gov/pfas/key-epa-actions-address-pfas*

releases from increased transport, management, and handling of waste associated with all the available technologies.

Risk assessment and communication are important tools to help protect communities and the environment from potential releases of harmful substances. Tools from the risk assessment process may also be useful when considering whether a facility is an appropriate option for receiving PFAS-containing waste. Risk communication and community engagement are important for building trust and addressing concerns about potential releases. EPA has developed resources for assessing, managing, and communicating environmental risks, including interim guidance and tools available to stakeholders and the public. These resources are summarized in Section 4.c.

4.b Potentially vulnerable populations

To consider potential impacts on vulnerable populations in PFAS destruction and disposal decision contexts, it is helpful to keep in mind that vulnerability refers to characteristics of individuals or populations that place them at increased risk of an adverse health effect. Vulnerability includes the cumulative impacts of economic, demographic, social, cultural, psychological, and physical states of the individual person or population that influence patterns of exposure to environmental contaminants and alter the relationship between the exposure of the environmental agent and adverse health outcomes (U.S. EPA, 2019a).

Described in Guidelines for Exposure Assessment (U.S. EPA, 2019), EPA's Framework for Cumulative Risk Assessment (U.S. EPA 2003) describes four properties of vulnerability:

- Differential susceptibility: An increased likelihood of sustaining an adverse effect from exposure to an agent. For example, an individual, group, or population might be more likely to show a response to an agent at a lower dose than the general population because of a preexisting health condition (e.g., asthma, cardiovascular disease, disability), genetic variation, prior damage from exposure, concurrent exposures to other stressors or developmental or life stage [e.g., children, older adults, pregnant women]).
- Differential exposure: Differences in exposure (e.g., magnitude, duration, frequency, pathway, route) from a variety of factors, including life stage, socioeconomic status, and cultural characteristics. For example:
    - Children might have a higher exposure and proportionally higher body burden of pesticides than adults because of their behavior patterns or food consumption (Moya et al., 2004; NRC, 1993).
    - When neighborhoods are racially or economically segregated, low-income people and people of color might live in neighborhoods or conditions where pollution sources are more likely to be sited and concentrated and thus where they experience higher exposures to air pollution (Lopez, 2003).
    - Studies on fish consumption and subsistence fishing patterns have documented racial/ethnic differences that can increase exposure from persistent, bioaccumulative chemicals in fish or wildlife (Burger, 2000; Burger 2002a; Burger 2002b; Burger et al. 2001; Burger et al., 1999a; Burger et al., 1998; Burger et al., 1993; Burger et al., 1999b; Corburn, 2002).
    - Tribal Nations and Native Americans can be exposed differentially to toxicants when dietary patterns involve consumption of locally caught fish or game for traditional or religious reasons (Fitzgerald et al., 1999; Fitzgerald et al., 1995; Fitzgerald et al., 1998; Fitzgerald et al., 2001; Harper et al., 2002; Schell et al., 2003).

- Differential preparedness: The regulatory frameworks, protections, and resources that an individual, community or population uses or can access to withstand the insult of agents.
- Differential ability to recover: Refers to resources, repair, and resilience systems, such as income level, ability to move from an affected area or access to health care, which can affect recovery from the effects of an agent.

Communities with environmental justice concerns experience disproportionate and adverse human health or environmental burdens. These burdens may arise from a number of causes, including the cumulative impacts of inequitable access to clean water, clean air, natural places, and resources for other basic human health and environmental needs; the concentration of pollution, hazardous waste, and toxic exposures; and underinvestment in affordable housing that is safe and healthy and in basic infrastructure and services to support such housing, including safe drinking water and effective sewage management. The cumulative impacts of exposure to those types of burdens and other stressors, including those related to climate change and the environment, are also important indicators of vulnerable populations (Executive Order 14096, 2023).

The following sections provide examples of tools, methods, and approaches to identifying and considering vulnerable populations.

4.c Considering vulnerability

Executive Order (EO) 14096, "Revitalizing Our Nation's Commitment to Environmental Justice for All"" (issued by President Biden in April 2023), directs federal agencies to advance the goal of environmental justice, which means: "the just treatment and meaningful involvement of all people, regardless of income, race, color, national origin, Tribal affiliation, or disability, in agency decision-making and other federal activities that affect human health and the environment so that people:

(i) are fully protected from disproportionate and adverse human health and environmental effects (including risks) and hazards, including those related to climate change, the cumulative impacts of environmental and other burdens, and the legacy of racism or other structural or systemic barriers; and

(ii) have equitable access to a healthy, sustainable, and resilient environment in which to live, play, work, learn, grow, worship, and engage in cultural and subsistence practices."[21]

To advance this charge and apply the best available science on the vulnerability of exposure to children and other sensitive populations, EPA has developed tools, methods, and approaches to identify and assess the potential for adverse and disproportionate impacts, including risks, to potentially vulnerable populations, including communities with environmental justice concerns.

4.c.i Identifying potentially vulnerable populations

The consideration of potentially vulnerable populations living near likely PFAS destruction or disposal sites starts with identifying and characterizing adjacent and potentially exposed communities and populations. EPA provides the following tools to assist with this task:

[21] See EO 14096, section 2(b), 88 FR 25251 (2023). EO 14096 builds on and complements EO 12898, "Federal Actions to Address Environmental Justice in Minority Populations and Low-Income Populations" (1994).

4.c.i.1 EJScreen

This EJ mapping and screening tool (*EJScreen*) provides EPA with a nationally consistent dataset and approach for combining environmental and demographic indicators. EJScreen users choose a geographic area; the tool then provides demographic and environmental information for that area. This is EPA's principal tool for characterizing and identifying communities with environmental justice concerns next to facilities or sources of pollution. Users can download reports that summarize search findings.

To summarize how environmental indicators and demographics come together in the same location, EJScreen 2.2 uses 13 EJ indexes that reflect the following 13 environmental indicators:

- Particulate Matter 2.5
- Ozone
- Diesel Particulate Matter
- Toxic Releases to Air
- Air Toxics Cancer Risk
- Air Toxics Respiratory Hazard Index
- Traffic Proximity
- Lead Paint
- Risk Management Plan (RMP) Facility Proximity
- Hazardous Waste Proximity
- Superfund Proximity
- Underground Storage Tanks (USTs) and Leaking USTs
- Wastewater Discharge.

The EJ Indexes combine environmental indicators with information about the low-income and racial and ethnic minority population in a Census block group. A Census block group is the smallest level of geography you can get basic demographic data for, such as total population by age, sex, and race (U.S. Census Bureau, 2023). For example, the EJ Index for traffic combines:

- The traffic indicator
- The low-income population
- The racial and ethnic minority population.

EJScreen presents results in terms of percentiles, allowing community comparisons with the rest of the state, an EPA Region, or the nation, and to identify which communities may be potentially overburdened.

Users can also look at the factors independently. In addition, EJScreen offers several more maps based on demographic and socioeconomic data (e.g., U.S. Census data, the American Community Survey) as well as indicators of other stressors, such as certain health indicators and access to medical care.

4.c.i.2 Enforcement Compliance and History Online (ECHO)

EPA's *Enforcement Compliance and History Online* (ECHO) website lets users search for facilities in communities to assess their compliance with environmental regulations that protect human health and the environment. Permit writers may consider susceptibility and vulnerability when establishing permit

conditions and ECHO is a useful tool to evaluate whether a facility is currently violating or has previously violated those conditions. Users access ECHO to:

- Search for facilities
- Investigate pollution sources
- Search for EPA enforcement cases
- Examine and create enforcement-related maps
- Analyze trends in compliance and enforcement data
- Identify facilities on or near Tribal land.

4.c.i.3 PFAS Analytic Tools

The *PFAS Analytic Tools* integrate 11 national datasets on PFAS into an interactive, web-based software. Users can filter data by PFAS or geographic area, explore application charts and maps, or download and open the files to explore the data. Information includes Clean Water Act discharges from permitted sources, a log of spills reported containing PFAS constituents, lists of facilities historically manufacturing and importing PFAS, federally owned locations where PFAS is being investigated, a history of known transfers of PFAS waste and how those wastes were managed, facilities that EPA knows is processing, releasing, and disposing of PFAS, PFAS detections in the environment (e.g., surface water, fish tissue), and drinking water testing results.[22] The data cover a broad list of PFAS and represent EPA's ongoing efforts to consolidate the growing amount of testing information that is available.

4.c.i.4 Private Domestic Well Map

EPA's *Private Drinking Water Wells* collection of online mapping tools provides information on drinking water providers and sources (including domestic [i.e., private] water wells and how many people are using them), as well as potential sources of contamination. Understanding the density and geospatial location of private domestic wells, and housing units relying on them, improves detection and response efforts.

EPA developed this mapping tool based on two methods to estimate private well density. The maps combine reported wells in 20 states with available well log data and the net housing unit method that is available in all 50 states for estimating domestic well use. The maps do not display the location of private wells. The value of this mapping tool is its identification of areas that may be vulnerable to groundwater contamination based on the presence of private wells. The source of contamination widget allows users to identify potential sources of contamination in defined locations.

[22] Information available to EPA is limited. For example, EPA has identified transfers of PFAS waste in EPA's eManifest system that tracks shipments of federally regulated hazardous wastes. Since PFAS are not currently regulated as hazardous waste, though, this information is not required to be reported – resulting in an incomplete picture of PFAS waste transfers in the United States. What EPA is able to identify in eManifest comes from querying free text fields where PFAS information is mentioned or a state has regulated PFAS as a hazardous waste and has established a state hazardous waste code. The tool also shows release and transfer information from the Toxics Release Inventory. However, the number of reporting facilities and reported recipient facilities has been low since the beginning of PFAS TRI Reporting likely associated with the *de minimis* exemption. A final rulemaking removing that exemption may lead to a more complete picture of releases and transfers in future reporting years. The PFAS Analytic Tools does not currently include information on PFAS destruction efficiency or efficacy.

4.c.i.5 Risk-Screening Environmental Indicators (RSEI) model

EPA's Risk-Screening Environmental Indicators (RSEI) model helps policy makers, researchers, and communities explore data on toxic chemicals being managed by industrial and federal facilities. RSEI incorporates information from the TRI on the amount of toxic chemicals released or transferred from facilities, together with factors such as the chemicals' fate and transport through the environment, each chemical's relative toxicity, and potential human exposure. RSEI model results can be used to help establish priorities for further investigation and to look at changes in potential human health impacts over time.

4.c.i.6 EnviroAtlas

EPA's *EnviroAtlas* highlights relationships between communities, land use, and environmental quality. The mapping functions help identify potential uses of land that, when combined with site contaminant data, may indicate possible exposure pathways. Such land use relationships can trigger deeper investigation into land uses to characterize relevant activities to inform site sampling and risk assessment.

The information gathered can be used to modify Conceptual Site Models, which are descriptions of contaminant sources, releases, transport pathways, and potential receptors. Accurate and complete Conceptual Site Models are required to ensure that samples are collected in appropriate media and risks are analyzed for potentially exposed receptors.

4.c.i.7 Climate and Economic Justice Screening Tool (CEJST)

The *Climate and Economic Justice Screening Tool* (CEJST) helps federal agencies identify disadvantaged communities geographically that are marginalized by underinvestment and overburdened by pollution. These communities are in Census tracts that are at or above the thresholds in one or more of eight categories of burden or are located on the lands of Federally Recognized Tribes.[23] The current version of the tool evaluates the likelihood of disadvantage based on climate change, energy, health, housing, legacy pollution, transportation, water and wastewater, and workforce development factors, each in combination with low-income or educational attainment status. The current version of the tool will be updated based on more feedback and research.

4.c.i.8 Centers for Disease Control and Prevention (CDC) EJ Dashboard

The Centers for Disease Control and Prevention (CDC) *EJ Dashboard* provides contextual public health messaging and allows users to evaluate several screening factors, including community characteristics, environmental exposures, health burden, and indexes. Assessments are based on ZIP code. and census tracts. Evaluating communities surrounding a facility may require the use of multiple ZIP codes and census tracts. Community characteristics include demographics and social vulnerability, as well as a description of the built environment (e.g., access to parks). The Social Vulnerability Index (SVI) uses U.S. Census data to determine the social vulnerability of every Census tract. Census tracts are subdivisions of counties for which the U.S. Census collects statistical data. The SVI ranks each tract across 15 social factors, including poverty, lack of vehicle access, and crowded housing, and groups them by theme.

[23] Census tracts that are completely surrounded by disadvantaged communities and are at or above the 50th percentile for low-income are also identified as disadvantaged by the CEJST.

The EJ Dashboard also provides more information on environmental exposures, health, and EJ. The Environmental Exposures tab presents environmental pollutant data, including water quality, age of housing, $PM_{2.5}$, and ozone. It also includes factors related to climate change. Under "Health Burden," users can examine health vulnerability based on access to health insurance and hospitals, disability, infant mortality, and percentage of low birth weights. The *Environmental Justice Index* is a calculation based on the combined rankings of the Environmental Burden Module, the Social Vulnerability Module, and the Health Vulnerability Module. The Environmental Justice Index represents a measure of cumulative impacts on human health and well-being.

4.c.i.9 Tribal Data

Tribal populations and Tribal lands can be identified in EJScreen, ECHO, PFAS Analytic Tools (U.S. EPA, 2023) or with Housing and Urban Development's Tribal Directory Assessment Tool (TDAT): *https://egis.hud.gov/TDAT/*. For example, by clicking on a specific state, the user can identify Bureau of Indian Affairs Tribal boundaries and areas along with environmental media and other mapping layers. In addition, all lands of Federally Recognized Tribes, including Alaska Native Villages, are identified as disadvantaged in the CEJST.

4.c.ii Screening strategies to support waste management decision-making

There is no singularly definitive way to screen for the presence of and potential impacts on vulnerable populations. However, combining information on demographics and environmental and enforcement compliance could provide a useful indication of already overburdened or potentially vulnerable populations to inform decision-making. To avoid adding to disproportionate impacts, decisions regarding the destruction/disposal of PFAS-containing materials should be supported by an evaluation of communities located in the vicinity of the destruction, disposal, and storage. For example, less preferred facilities could be identified as those having a history of significant Clean Air Act violations, in an area with poor air quality and a high percentage of low-income people with significant air-related health disparities (e.g., asthma, lung cancer). This type of screening-level analysis can be used to identify communities with adverse and disproportionate impacts so as not to further exacerbate those impacts. It could also trigger questions for the facility regarding additional strategies to reduce the release of PFAS and other substances into the surrounding environment, including additional health protection, mitigation, and monitoring measures needed.

EPA has created several tools that can be helpful for screening, as have other federal and state agencies. Each tool has strengths and limitations. Options and suggestions for conducting a screening are described below.

EPA's EJScreen (see Section 4.c.i.1) collates social and demographic information that provides insights into non-chemical stressors to communities and subpopulations. EJ screen provides data from the U.S. Census and the American Community Survey[24], as well as information on race, income, unemployment rate, limited English proficiency, educational attainment, and age. Users can drill down to learn about additional demographics, including population, health, housing, and other points of interest. These data can be particularly useful to characterize community resilience and limitations. EJScreen also has

[24] The American Community Survey is an ongoing survey that annually provides vital demographic information about the United States (*https://www.census.gov/programs-surveys/acs*). Data are updated annually as a 5-year average. EJScreen version 2.2 includes 2017-2021 ACS 5-year summary file data.

important information about existing pollution levels. It is most robust for air quality, although it does have information on releases to water via NPDES permits and other sources.

EJScreen also provides indicators on health disparities. Other sources on health include CDC's EJ Dashboard. States may include additional details on health burdens on their websites and tools.

Collectively, this information can provide a snapshot of communities with environmental justice concerns, allowing the user to flag communities that may be stressed due to multiple factors. Decision-makers, for example, could perform additional screening at destruction and disposal facilities with communities exceeding the 80th (or another appropriate) percentile in multiple indicators, and therefore more closely consider vulnerable populations while evaluating destruction, disposal, and storage facilities.

Regarding compliance, EPA's ECHO tool (see Section 4.c.i.1) provides enforcement and compliance data on specific candidate destruction and disposal facilities. A history of Clean Water Act violations, for example, could be an indicator of disproportionately exposed communities, particularly if they rely on public water systems using a surface water source downstream of the facility. In addition, communities using private wells for drinking water may be more vulnerable to releases regulated under RCRA or CERCLA.

4.c.iii Considerations for community engagement

In certain cases, community engagement is required under law. For example, facilities must hold public meetings before submitting part B RCRA permit applications (U.S. EPA, 2013a) and public hearings if they are requested for CAA Title V permits. In some cases, EPA's policy is to consult and coordinate with Tribes (U.S. EPA, 2013b). Community engagement is not merely a matter of meeting requirements, though. Advancing the goal of meaningful involvement is a core principle under EO 14096[25]. Reaching out to the community and providing notification, information, and an opportunity for input before deciding whether to accept PFAS-containing waste for destruction or disposal will help build trust and support for operations and can reduce the likelihood of negative reactions stemming from unresolved concerns.

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Meaningful community engagement typically includes two key elements:

- Public outreach to disseminate relevant and accessible information to the community, including in relevant local languages and in formats accessible to individuals with disabilities.

[25] *https://www.federalregister.gov/documents/2023/04/26/2023-08955/revitalizing-our-nations-commitment-to-environmental-justice-for-all*

- Public participation, which generally entails a dialogue with the community to ascertain information and viewpoints. This dialogue is particularly important because the community can provide local knowledge of health and existing conditions, identify concerns and issues that may not be readily apparent outside the community, and offer contextual/cultural perceptions and experience (U.S. EPA, 2016).

Although presenting highly technical information is always a challenge, involving vulnerable populations in a meaningful way may present different challenges and opportunities from those in a general public involvement effort. To foster meaningful participation of all community members, it may be important to address issues that could hinder a community's participation in the decision-making process. These may include time and resource constraints, language barriers, and lack of trust (U.S. EPA, 2016).

Examples of effective practices to engage diverse and vulnerable populations include the following:

- Early notification so that community input can be offered in a timely way and incorporated into the decision-making process.
- Conveying issues in ways that are tailored (for example, translation, timing, location) to each specific population.
- Bridging cultural and economic differences that affect participation.
- Developing trust between the government and potentially affected populations.
- Working closely with state and local partners, as well as other federal agencies, to present a unified, consistent message to communities.
- Developing stakeholder capacity or providing technical assistance to effectively participate in future decision-making processes (U.S. EPA, 2015b).

EPA has developed tools to assist the federal government, states, and private entities with community engagement and outreach. For example, the *Superfund Community Involvement Toolkit* (CI Toolkit, available at *https://www.epa.gov/superfund/superfund-community-involvement-tools-and-resources*) provides practical information to design and enhance community involvement activities. While the CI Toolkit is designed for users to quickly review and adapt a variety of community involvement tools to engage the community during all stages of the Superfund processes, the same tools can be adapted to engage communities adjacent to destruction and disposal facilities.

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5. Research Needs and Data Gaps for Destruction and Disposal Technologies

EPA has a better understanding of potential PFAS releases from destruction and disposal activities, but more research is needed. This section identifies high-priority data gaps that researchers across government, academia, and industry need to address. This additional research will help EPA provide meaningful updates on effective destruction and disposal practices for PFAS-containing materials. This section outlines research needs to inform future guidance updates and provides an overview of existing research activities to address these gaps.

5.a Research needs to inform future guidance updates

EPA has translated the uncertainties and data gaps identified throughout this interim guidance into research needs, which are presented in Table 5-1. The research needs in Table 5-1 are focused on advancing our ability to measure PFAS in materials and releases from destruction and disposal technologies and improving our understanding of the performance of thermal treatment units, landfills, and underground injection control wells for managing PFAS and PFAS-containing materials. Individually, the research needs identified in Table 5-1 are complex to address because of the variety of substances within the PFAS class, the range of PFAS-containing materials discussed in this interim guidance, and the variability in technology designs and operating conditions. Collectively, some of the research needs identified in Table 5-1 are dependent upon other research needs. For example, fully characterizing PFAS releases from destruction and disposal technologies is dependent upon the availability of methods for measuring PFAS in air, water, and solids. Because of the complexity of the research needs and their inter-dependence, EPA anticipates that it will take years for the research needs in Table 5-1 to be fully addressed.

EPA has also prioritized the research needs in Table 5-1 as either *high*, *medium*, or *low* based on the potential for results to inform future versions of this interim guidance. In general, EPA designated the characterization of potential PFAS releases from thermal treatment units and landfills as high priority because these data are essential for determining whether communities surrounding destruction and disposal sites could be exposed to PFAS at unreasonable levels from these facilities. At the same time, EPA recognizes that current laboratory methods for identifying and quantifying PFAS are limited. As such, the agency also considers the development of sampling and analytical methods to be medium or high priority. The agency does not, however, believe that characterizing PFAS releases from PFAS destruction and disposal activities should wait until additional analytical methods are available. Rather, EPA expects that characterizing PFAS releases from destruction and disposal sites will be iterative and that future characterization efforts will be informed and guided by past efforts and the availability of new methods.

EPA designated research needs associated with the underground injection of PFAS and PFAS-containing materials via Class I wells as a low priority. The agency made this designation because the standards associated with the construction, operation, and monitoring of Class I wells are designed to isolate liquid wastes deep below the land surface and ensure protection of underground sources of drinking water. As

a result, EPA expects underground injection of liquid PFAS waste to have a lower potential for environmental release, compared to managing PFAS wastes through thermal treatment or landfills. While data on the performance of Class I wells for managing liquid PFAS waste would be informative, the highest priority continues to be on characterizing PFAS releases from thermal treatment units and landfills.

Table 5-1. Prioritized Research Needs to Inform Future Guidance Updates

Topic	Research Needs	Priority
Thermal Treatment	Methods for sampling and analyzing PFAS in solid-, liquid-, and gas-phase waste streams from thermal treatment units[a]	
	• Organic fluorine (all phases)	Low
	• Targeted methods for non-polar, semivolatile PFAS (gas-phase)	High
	• Targeted methods for cationic, zwitterionic, and nonionic PFAS (solid- and liquid-phase)	High
	Full-scale performance testing—following the parameters and procedures suggested in Appendix A—at:	
	• Hazardous waste combustors	
	○ Commercial hazardous waste incinerators	High
	○ Hazardous-waste-burning cement kilns	High
	○ Hazardous-waste-burning lightweight aggregate kilns	Medium
	• Carbon regeneration and reactivation units	High
	• Non-hazardous waste combustors	
	○ Sewage sludge incinerators	High
	○ Municipal waste combustors	Medium
	○ Thermal oxidizers	High
	Characterization of PFAS releases from thermal treatment units operating under different conditions/designs	
	• Gas-phase emissions	High
	• Air pollution control device discharges	High
	• Bottom ash	High
	• Biosolids, remediated soil	High
Landfills	Methods for sampling and analyzing PFAS in liquid- and gas-phase waste streams from landfills[a]	
	• Organic fluorine (liquid- and gas-phase)	Medium
	• Targeted methods for non-polar, semivolatile PFAS (gas-phase)	Medium
	• Targeted methods for cationic, zwitterionic, and nonionic PFAS (liquid-phase)	Medium
	Approaches for solidification and stabilization of PFAS-containing materials[a]	
	• Effectiveness of differing solidification and stabilization techniques	High
	• Performance over time	High
	Fate and transport of PFAS—including partitioning between solid-, liquid-, and gas-phases in:[a]	
	• Hazardous waste landfills	High
	• Industrial waste landfills	High
	• Municipal solid waste landfills	Medium
	• Ash monofill landfills	Low
	• Construction and demolition (C&D) landfills	High
	Full-scale performance testing of engineered landfill components	
	• Bottom liner and leachate collection systems	Low
	• On-site leachate treatment systems	High

Topic	Research Needs	Priority
	• Gas collection and emission control systems • Final cover systems	Low Low
	Characterization of PFAS releases from landfills operating in different regions and under different conditions/designs • Gas-phase emissions • Groundwater contamination	 High High
	Efficacy and potential release of PFAS during leachate treatment • Conventional technologies • Emerging technologies	 High Medium
Underground Injection (Class I)	Performance data from wells that are managing PFAS-containing liquids • Surface management practices • Effects on injection performance	 Low Low
	Long-term fate and transport of PFAS in injection zones of Class I wells • Mixture effects • Interactions with co-contaminants	 Low Low
	Data on the presence/absence of PFAS in groundwater near Class I wells receiving PFAS-containing liquids	Low
Emerging Technologies	Lab-, pilot-, and field-scale research to test the effectiveness of emerging technologies for different PFAS-containing materials and to characterize the outputs for PFAS and non-PFAS constituents.[b]	High

[a.] EPA is conducting research on this topic. See Section 5.b.ii for a high-level overview of EPA research and development activities.

[b.] See Section 6 for discussion of emerging technologies. EPA encourages technology developers to generate and publicly release data that can be used to answer the questions presented in the technology evaluation framework in Section 6.b.

5.b Current research efforts to address data gaps

Addressing the research and data gaps described in Section 5.a will require effort across government, academic, and private institutions. This section provides an overview of resources to learn more about prospective research activities.

5.b.i Governmental, academic, and industry research activities

In late 2021, the Office of Science and Technology Policy (OSTP) formed the interagency PFAS Strategy Team as part of the National Science and Technology Council (NSTC) to coordinate federal research on PFAS. In early 2023, the PFAS Strategy Team published the *Per- and Polyfluoroalkyl Substances (PFAS) Report*. The PFAS Report provides a high-level overview of research on PFAS as a chemical class by addressing the following strategic areas: removal and destruction; safer alternatives; sources and pathways of exposure; and toxicity. This document is a state-of-the-science report that includes research activities, gaps, and opportunities for the federal government. Following release of the PFAS Report, the PFAS Strategy Team initiated efforts to develop a federal strategic plan to address data gaps

identified in the PFAS Report. Additional information is available in the *CEQ Report on Biden-Harris Administration Progress on PFAS* (March 2023).[26]

While this interim guidance only outlines major DoD and EPA research programs, a more complete overview of existing federal PFAS research activities is available in Appendix A of the OSTP PFAS Report. DoD is the largest federal funder of PFAS destruction and disposal research (see Appendix B, OSTP 2023). Within DoD, the Strategic Environmental Research and Development Program (SERDP) and the Environmental Security Technology Certification Program (ESTCP) have been funding significant research on PFAS for several years. The goals of this research include:

- Improving PFAS analytical methods and AFFF site characterization
- Understanding PFAS ecotoxicological effects
- Developing tools for assessing the fate of PFAS in the subsurface
- Developing and validating *in situ* and *ex situ* PFAS treatment technologies.

In 2022, SERDP-ESTCP published its *Summary Report: Strategic Workshop on Management of PFAS in the Environment* (Leeson et al., 2022). This report identifies research and demonstration needs for PFAS fate and transport, sampling and analysis, thermal treatment, non-thermal destructive treatments, and concentration technologies. To address these research needs, SERDP and ESTCP fund a robust research program, with annual solicitations for proposals. Descriptions of all PFAS-related statements of need and funded projects are available at *https://www.serdp-estcp.org/Featured-Initiatives/Per-and-Polyfluoroalkyl-Substances-PFASs*. Additionally, the Defense Innovation Unit has begun funding opportunities for innovation in technology for on-site PFAS destruction and remediation.

Colleges and universities, who are the recipients of federal and other research funding, conduct cutting-edge research related to PFAS destruction and disposal. These institutions often bring together expertise from many disciplines. This collaboration enables the rapid development of innovative approaches for managing the constantly changing set of PFAS-containing materials.

Other entities have strong interests in expanding into the area of PFAS material management, disposal, and destruction. These entities include private industry, the operators of waste disposal operations and technologies, municipal water utilities, professional and trade associations, and relevant research foundations. These organizations are actively developing and marketing solutions to address PFAS material management, disposal, and destruction.

5.b.ii EPA research and development activities

As described above, EPA is one of many governmental, academic, and industry organizations engaged in research on PFAS destruction and disposal. EPA's ORD is conducting research to characterize PFAS-contaminated sites and sources, and to understand the fundamental mechanisms of PFAS destruction and disposal technologies. This section provides a high-level overview of EPA research activities to support future interim guidance updates.

[26] See also White House Fact Sheet: Biden-Harris Administration Takes New Action to Protect Communities from PFAS Pollution (March 14, 2023): *https://www.whitehouse.gov/briefing-room/statements-releases/2023/03/14/fact-sheet-biden-harris-administration-takes-new-action-to-protect-communities-from-pfas-pollution/*.

5.b.ii.1 Characterizing PFAS-containing materials

EPA is developing laboratory-based aqueous leaching methods to characterize leaching behavior of organic constituents, including PFAS, from solid materials under a range of environmental conditions. These methods are based on existing SW-846 Leaching Environmental Assessment Framework (LEAF) methods for inorganic constituents of potential concern (U.S. EPA, 2023). The methods produce a source term (i.e., as a concentration or release rate). Screening-level or scenario-based assessments can use the source term to model transport in the subsurface environment (Garrabrants et al., 2021a and 2021b).

EPA is continuing to develop and validate robust analytical methods to detect and measure PFAS in aqueous, solid, and gas-phase samples. The data generated with these methods will improve the understanding of environmental fate and transport and help evaluate the effectiveness of various treatment and destruction technologies. The suite of PFAS analytical methods under development includes targeted methods validated for quantitative analysis of a wider range of target analytes and matrices; class-specific methods, such as adsorbable organic fluorine and the total oxidizable precursors assay, to screen for a range of PFAS precursors; and non-targeted analytical methods to identify novel PFAS.

5.b.ii.2 Thermal treatment

ORD continues to conduct laboratory- and pilot-scale research to describe the behavior and mechanisms of destruction of PFAS-containing materials subject to thermal treatment. ORD facilities include a pilot-scale incinerator (the Rainbow furnace) and rotary kiln. This research examines the operating conditions (time, temperature, and combustion parameters) necessary for adequate PFAS destruction using conventional thermal treatment. The goal is to develop a mechanistic understanding of PFAS behavior and help interpret results from full-scale field studies. This includes measuring the generation of PICs from different PFAS-containing materials.

ORD is also interested in opportunities to partner with thermal treatment facilities to conduct field-scale research. The goals of this research would be to determine if representative field conditions achieve adequate thermal destruction of PFAS as defined by conventional DRE; characterize potential PICs/PIDs; and evaluate alternate indicators of destruction performance. The details of this thermal treatment field testing request are outlined in Appendix A.

5.b.ii.3 Landfills

ORD is developing novel sampling and modeling approaches to identify PFAS and characterize fate and transport at contaminated sites and source areas, including landfills. ORD is also evaluating solidification and stabilization techniques for PFAS applications.

5.b.ii.4 Extramural research

EPA supports PFAS research and development efforts through several programs, including *research grants*, the *Small Business Innovation Research* (SBIR) program, *challenges and prizes*, and the *P3 Student Design Competition*. For example:

- In 2019, *EPA awarded grants to eight universities* to better understand the environmental risks posed by PFAS and identify practical approaches to manage their potential environmental impacts. Several of these projects focused on characterizing PFAS in landfills. Research

completed under these projects contributed to EPA's improved understanding of the potential for PFAS releases from landfills.

- In 2021, EPA collaborated on the *Innovative Ways to Destroy PFAS* challenge. Partners included SERDP-ESTCP, the Environmental Council of States and the Environmental Research Institute of the States, the Michigan Department of Environment, Great Lakes and Energy, and the Colorado Department of Public Health and Environment. The goal of the challenge was to discover new technologies and approaches that have the potential to remove at least 99 percent of PFAS in unused AFFF without creating harmful byproducts.
- EPA's SBIR program has funded many small businesses developing technologies to test and treat PFAS. These companies are developing products to destroy PFAS in a range of media using technologies including plasma reactors, electrochemical processes, sonolysis, hydrothermal alkaline treatment, and mechanochemical destruction. More information on technologies funded by EPA's SBIR program is available at *https://www.epa.gov/sbir/test-and-treat-pfas-epa-sbir-technologies*.

Information on open solicitations can be found at *https://www.epa.gov/chemical-research/pfas-research-extramural-funding-opportunities*.

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6. Emerging Technologies for PFAS Destruction and Disposal

Because of their chemical and physical properties, PFAS can pose unique challenges for end-of-life materials management. As described in Section 3, the effectiveness of commonly used waste management technologies, such as thermal treatment and landfilling, for managing PFAS materials can vary. Additionally, current operating conditions and practices at some thermal treatment facilities and landfills may not be sufficient to limit releases of PFAS to the environment. Concerns about the effectiveness of widely-used technologies for managing PFAS materials call for innovative research and development efforts toward new technologies as well as testing to apply existing destruction technologies to PFAS materials at various scales.

Development of new technologies typically involves the following stages:

- Technology development often starts with exploring and understanding the basic scientific principles and processes that underly the technology. At this early stage, the primary focus is on gaining understanding of how the process works and whether it shows promise for a particular application (e.g., PFAS destruction).
- If the results of early experiments are promising, the next stage of development generally focuses on prototype development and testing under controlled conditions. This stage allows developers to better understand whether the technology is practical and effective at larger scales. It also provides developers with information on costs associated with manufacturing and operating units.
- In the final stage of development, the focus generally shifts to field-scale demonstration projects that allow developers to test the technology under a variety of real-world conditions and to develop better cost estimates for operation and maintenance. During this stage, developers may identify the best and most cost-effective applications for the technology. In the case of PFAS destruction and disposal, this may include identifying the types of PFAS materials best suited for the technology (e.g., high-concentration materials versus low-concentration materials).

At any stage of the technology development process, developers may determine that the technology is not effective or is not cost-effective and development can stop.

In addition to research to identify and develop novel technologies for PFAS destruction, efforts are underway to apply existing destruction technologies—other than thermal treatment—to PFAS materials. In these cases, the scientific principles and processes underlying a technology may already be understood, but there is little information on the performance of the technology or its applicability to PFAS materials. Evaluating the applicability of an existing technology to PFAS materials can occur at various scales:

- Laboratory- and pilot-scale studies can be used to evaluate the effectiveness of a technology under controlled conditions and to determine how operating conditions can affect PFAS destruction.
- Field studies can provide data from full-scale applications that reflect the variability seen in real-world operating conditions.

The purpose of this section is to: (1) provide an overview of EPA efforts to identify and test emerging technologies for PFAS destruction and (2) introduce the PFAS destruction and disposal technology evaluation framework, which provides a transparent, consistent approach for evaluating destruction and disposal technologies for PFAS materials.

6.a EPA's PFAS Innovative Treatment Team

EPA's ORD initiated the PFAS Innovative Treatment Team (PITT) in 2020 as a short-term dedicated, cross-ORD effort to identify, review, and conduct preliminary research on potential treatment technologies. Over six months, the multi-disciplinary PITT conducted state-of-the-science reviews of proposed methods to dispose and/or destroy PFAS in media and wastes. Several criteria were evaluated using available information, including efficacy, feasibility, performance, and costs of each technology, while also considering potentially hazardous byproducts. The PITT partnered with other agencies and states to conduct a competitive public challenge to identify solutions that were potentially overlooked and to encourage further development of potential technologies.

The PITT chose to focus on the effectiveness of four technologies: mechanochemical degradation, electrochemical oxidation, gasification and pyrolysis, and supercritical water oxidation. These four technologies showed theoretical promise of the sought-after criteria: PFAS destruction, production of few to no hazardous residuals or byproducts, commercial availability, and cost effectiveness for various contaminated matrices. References and discussion for each technology can be accessed in the four PITT Research Briefs at *https://www.epa.gov/chemical-research/pfas-innovative-treatment-team-pitt*. The identification of these technologies by the PITT should not be considered an endorsement or recommendation to use these technologies to destroy PFAS. Additional research is needed to determine the effectiveness of these technologies for different PFAS-containing materials and to characterize the outputs for PFAS and non-PFAS constituents. An overview of the results of the PITT's state-of-the-science review is available in *Developing Innovative Treatment Technologies for PFAS-Containing Wastes* (Berg et al., 2022).

After the state-of-the-science reviews, the PITT selectively encouraged development and research of the four chosen emerging technologies to better understand the potential for PFAS destruction, specifically AFFF, while avoiding creation or transfer of environmental hazards. The PITT conducted in-house research and partnered with industry and academia to collect data regarding the technologies and to encourage further development of the methods required to ascertain PFAS destruction efficacy. Although the effort originally was allotted six months in 2020, the work initiated by the PITT continues to produce journal articles, fact sheets, and ORD reports. Some of the results from this continued work are described below. When considering the timeline, including navigating the initial restrictions of the COVID-19 pandemic, note there were several hurdles to obtaining necessary data, such as limited access to industrial field sites, analytical methods development, and availability of standards.

6.a.i PITT-investigated emerging technologies

To further explore the potential for mechanochemical degradation of PFAS, ORD scientists partnered with colleagues in New Zealand to conduct a proof-of-concept case study using a benchtop ball mill, AFFF-spiked sand, and AFFF-contaminated soil (Gobindlal et al., 2023). Mechanochemical degradation is a treatment technology that uses a high-energy ball-milling device, with the option of co-milling reagents, to produce highly reactive conditions to degrade contaminants. Results from the AFFF-spiked sand showed a 99.99% destruction efficiency for the sum of all measured PFAS after 960 min, and results from the AFFF-contaminated soil indicate that measured PFAS were below the limit of detection

after 1440 min. Because this study analyzed PFAS in the solids after different ball milling time intervals, from 15 to 1440 min, the authors were able to identify increases in some of the measured PFAS before concentrations decreased, indicating the transformation of PFAS during ball milling. An extractable organic fluorine method was also used to determine the reduction in extractable organic fluorine before and after ball milling. In the case of AFFF-spiked sand, the authors reported a 97.8% reduction in extractable organic fluorine. A 53.5% reduction in extractable organic fluorine was reported for the AFFF-contaminated soil. Air emissions were not characterized as part of this study, making it difficult to determine whether full mineralization of PFAS occurred, and more work is needed in this regard. As noted in the study, more work is also needed to identify primary operations variables to inform the scalability of mechanochemical destruction.

In 2020, ORD conducted a pilot study at a biosolids treatment facility using pyrolysis (Thoma et al., 2022). Pyrolysis is a process that decomposes materials at moderately elevated temperatures in an oxygen-free environment. During the pilot study, dried biosolids were fed into a pyrolysis reactor operating at approximately 610 °C (1100 °F). The dried biosolids and the resulting biochar were sampled and tested for 41 target PFAS. Twenty-one PFAS were detected in the input biosolids, and none of the target PFAS were detected in the biochar. The pyrolysis system's target PFAS removal efficiencies were estimated to range between >81.3% and >99.9%. The pilot study also included limited analysis of the air emission control system, with results indicating no transmission of the target PFAS to the air. It is important to note, however, that full characterization of PFAS using non-targeted methods was not performed and more work is needed to understand PFAS transformations and the destruction mechanism.

ORD also completed studies to test the effectiveness of supercritical water oxidation for treating AFFF. Supercritical water oxidation is a process that occurs when the temperature and pressure of water is above the critical point. Above the critical point, oxidation processes are accelerated. In 2020, ORD partnered with three companies to perform tests of their supercritical water oxidation systems on dilute, PFOS-based AFFF (Krause et al., 2022). During these demonstration tests, each system was operated under slightly different conditions, and each company analyzed samples of the influent and effluent for slightly different PFAS. The overall destruction efficiency of each system was calculated by summing the concentrations of the measured PFAS. In all cases, the overall destruction efficiency was found to be greater than 99%. It is important to note, however, that the sum of the PFAS measured in the effluent ranged from approximately 10 µg/L to approximately 100 µg/L, suggesting that further treatment of the effluent may be necessary. It is also important to note that non-targeted analysis was not conducted, and air emissions were not monitored, making it difficult to assess the fate, transport, and transformation of PFAS with the supercritical water oxidation systems.

In 2021, ORD also partnered with a company to conduct tests at industrial-scale supercritical water oxidation system (Sahle-Demessie et al., 2022). These tests used a dilute, FTS-based AFFF. The primary objective of the study was to determine the destruction efficacy of the PFAS in the input material. Analysis of the influent and effluent showed greater than 99.99% destruction of the total PFAS measured. The average sum of the targeted PFAS in the effluent was approximately 55 µg/L, but PFAS were also detected in the tap water used as quench water in the system, suggesting that the overall performance of the system could be improved if non-PFAS-contaminated water is used in the system. A secondary objective of the study was to characterize degradation products using non-targeted analysis and stack sampling for nonpolar volatile fluorochemicals. Non-targeted analysis of the effluent tentatively identified the shorter carbon-chain PFAS on the targeted analyte list, and the authors suggested a possible destruction mechanism that would be consistent with this result. Further work is

needed, however, to determine the destruction mechanism. The air emissions sampling did not detect targeted PFAS in air emissions above background levels; further work with finalized air methods (e.g., OTM-50) is needed to confirm this observation.

At this time, EPA is neither recommending nor discouraging the use of any emerging technology for managing PFAS-containing materials, including the technologies studied by the PITT. While the PITT studies generally indicated potential for PFAS destruction, further work using newly available methods is needed to more fully characterize the outputs of these processes and to evaluate their performance for PFAS-containing materials beyond AFFF.

6.b PFAS Destruction and Disposal Technology Evaluation Framework

Researchers have proposed metrics to evaluate PFAS destruction and disposal technologies, including technology readiness levels (TRLs), DREs of target compounds, fluorine mass balances, and others (Berg et al., 2022; Deeb et al., 2021; Krause et al., 2021). When used individually, these metrics fail to accurately capture comprehensive system performance or provide a holistic understanding of the potential for PFAS transformation and/or release to the environment. For example, DREs calculated from targeted analyses using existing methods can result in underreporting of the total PFAS in a matrix. That's because existing detection methods can only measure a fraction (e.g., 50 individual PFAS) of the total number of PFAS that may be present in a sample. Similarly, TRLs can fail to accurately reflect the current development stage of a technology by either reporting the overall maturity of the technology, instead of its specific PFAS application, or by relying on a niche, small-scale application to justify an overall readiness level.

These limitations drive the need for a comprehensive framework for evaluating a technology and its application to PFAS-containing materials. Such a framework should allow the user to prioritize the collection and consideration of different pieces of information. To meet these needs, EPA developed a technology evaluation framework that uses a multiple-lines-of-evidence approach to evaluate a technology/PFAS material combination (Table 6-1). The framework provides a transparent approach for evaluating and selecting a technology to reduce PFAS releases to the environment. EPA recommends that managers of PFAS-containing materials use the framework to evaluate emerging technologies, and to inform decisions about destruction and disposal of PFAS-containing materials.[27] EPA encourages technology developers to generate and publicly release data that can be used to answer the questions presented in the framework.

The framework comprises several topical sections: technology, material, analytical methods, disposal/destruction efficacy, community considerations, and regulatory requirements. Within each section, EPA has identified key pieces of information that, when considered together, provide a more holistic evaluation of a technology and its applicability for PFAS disposal or destruction.

The framework is intended to be an adaptable tool that allows the user to prioritize the collection and consideration of different pieces of information via the "priority" column in the framework. This allows users to address questions most relevant to their priorities and with respect to their decision context

[27] The Interstate Technology and Regulatory Council (ITRC) PFAS Team developed the *PFAS Technical and Regulatory Guidance Document* (available at *https://pfas-1.itrcweb.org/*) to support state and federal environmental staff, as well as others, to gain a working knowledge of the current state of PFAS science and practice. Section 12 of the guidance document provides information on PFAS treatment technologies, including destruction technologies. Managers of PFAS-containing materials may find this resource useful when completing Table 6-1.

and the shared priority of preventing and mitigating potential exposures. Users may also choose to prioritize addressing certain questions before others to streamline their evaluation of a technology/material combination. For example, if a user of the framework is evaluating the performance of a destruction technology, the questions in the disposal efficacy section of the framework are not needed and could be removed from the framework. As another example, a user may prioritize answering questions under the technology section of the framework because those questions may rule out a technology for a particular application (e.g., the technology may not be available at the scale needed to process the PFAS-containing material of interest). Once a technology is ruled out, lower priority questions do not need to be answered. While users may not answer each question in the framework, EPA encourages users of the framework to address as many questions as possible to inform decisions about the destruction and disposal of PFAS materials.

Table 6-1 shows this approach, followed by more detailed considerations for each question.

Table 6-1. Framework for Evaluating Destruction/Disposal Technologies for PFAS-Containing Materials

Category of Information	Key Questions	Priority[a]	Answer and Associated Notes for PFAS Material of Interest
Technology	If available, what is the TRL for the technology/material combination of interest?		
	Is the technology of interest available at the scale needed for the intended application?		
	Is a treatment train needed to meet the desired destruction/disposal outcome?		
Material	What PFAS-containing material has been used to test the performance of the technology of interest?		
	Which PFAS, and at what concentration ranges, have been used to test the performance of the technology of interest?		
	Are there non-PFAS constituents that may affect the performance of the technology of interest?		
Analytical methods	Which targeted analytical methods (e.g., OTM-45, draft EPA Method 1633) have been used to characterize the performance of the technology for the PFAS-containing material of interest?		
	Has non-targeted analysis been performed on waste streams from the technology/material combination of interest?		
	What other analytical approaches have been used to characterize the performance of the technology for the PFAS-containing material of interest?		
	Are there any concerns about the quality of the data generated during testing?		
Disposal efficacy	Have potential transformation and partitioning pathways been identified for the technology/material combination of interest?		
	Are engineering controls in place to prevent or minimize release of PFAS to the environment?		

Category of Information	Key Questions	Priority[a]	Answer and Associated Notes for PFAS Material of Interest
	What is the longevity of the disposal technology?		
	Has a fluorine mass balance been determined?		
	Does the disposal of the PFAS-containing material contribute to the potential release of non-PFAS constituents to the environment?		
Destruction efficacy	Is the mechanism of destruction understood?		
	Have all process inputs and outputs been characterized for PFAS to the extent possible given current analytical methods?		
	What is the reported destruction efficiency?		
	Have any PIDs (or PICs) been identified?		
	Has a fluorine mass balance been determined?		
	Have all process outputs been characterized for non-PFAS constituents to the extent possible given current analytical methods and understanding of the composition of the PFAS-containing material?		
Community Considerations	Has a destruction/disposal site been identified?		
	What are the characteristics of the surrounding community?		
	Are there potentially vulnerable populations and/or communities with environmental justice concerns near the destruction/disposal site?		
	Are PFAS releases anticipated from the technology/material combination of interest?		
	Has the surrounding community been engaged?		
Regulatory requirements	Are there state or federal regulations that control emissions or releases from the technology of interest?		
	Are there state or federal regulations that control the management of the PFAS-containing material of interest?		

[a] Users can prioritize key questions using a variety of approaches. For example, questions could be labeled as *high, medium*, or *low* priority. Questions could also be prioritized using a numerical ranking system with *1* indicating questions to answer first, *2* indicating questions to answer second, and so forth.

6.b.i Key questions for technology evaluation

Technology. The first section of the framework focuses on the destruction or disposal technology. Key questions include:

- **If available, what is the TRL for the technology/material combination of interest?** TRLs are generally assigned using the *definitions developed by NASA* (NASA, 2023), where level 1 corresponds to observation and reporting of basic scientific principles and level 9 corresponds to "flight proven"

technologies. TRLs may be determined through technology readiness assessments[28] or through other methods. If other methods are used, EPA recommends that users consider the source of reported TRLs and prioritize TRLs determined through independent technology readiness assessments over TRLs assigned by the technology developer.

- **Is the technology of interest available at the scale needed for the intended application?** Some technologies may only be available as mobile, containerized units, which may be suitable for high-concentration, low-volume materials. These units may not be appropriate for low-concentration, high-volume materials, where a stationary treatment unit with greater capacity is needed.
- **Is a treatment train needed to meet the desired destruction/disposal outcome?** If so, describe the treatment train needed and whether such technology is available and effective for the PFAS-containing material of interest. Note that treatment trains may produce materials that will also need to be managed.

Material. The second section of the framework focuses on the PFAS-containing material of interest. The six types of PFAS-containing materials discussed in this interim guidance vary in terms of phase, PFAS composition and concentration, physical and chemical properties, and overall composition (e.g., the presence of other contaminants). To understand technology performance across the range of variations within a material type, the following key questions should be addressed:

- **What PFAS-containing material has been used to test the performance of the technology of interest?** The description of the material(s) used to evaluate a technology/material combination of interest should be specific as possible (e.g., 3M Litewater versus AFFF), and links to bulk material characterization should be included, if available. If the material evaluated is artificially contaminated (i.e., spiked with known PFAS species and concentrations), that should be noted because it may not be an accurate reflection of the performance of the technology for the intended application.
- **Which PFAS, and at what concentration ranges, have been used to test the performance of the technology of interest?** To best inform decisions on technology use, the PFAS and concentrations used to test performance should match the PFAS and concentrations anticipated for the intended application.
- **Are there non-PFAS constituents that may affect the performance of the technology of interest?** This could include constituents in the original PFAS-containing material or constituents that may be created or introduced during a treatment train or by the technology of interest. For example, hydrofluoric gas may be produced during PFAS destruction, which may cause corrosion.

Analytical methods. PFAS are a large class of substances, and there are various approaches for identifying and measuring PFAS. EPA recommends using a combination of approaches to characterize the performance of the technology for the PFAS-containing material of interest, including characterizing PFAS in emissions or waste streams from the technology of interest. Key questions to be addressed under this section include:

- **Which targeted analytical methods (e.g., OTM-45, draft EPA Method 1633) have been used to characterize the performance of the technology for the PFAS-containing material of interest?** When possible, EPA recommends the use of standardized and/or validated methods for targeted analysis.

[28] See the *Technology Readiness Assessment Guide* for best practices for evaluating technology maturity. The guide, developed by the U.S. Government Accountability Office and released in January 2020, provides organizations with best practices for conducting high-quality assessments.

- **Has non-targeted analysis been performed on waste streams from the technology/material combination of interest?** Non-targeted analysis is an important tool for characterizing PFAS in waste streams from a technology/material combination of interest because it can tentatively identify PFAS beyond those measured using targeted analytical methods.
- **What other analytical approaches have been used to characterize the performance of the technology for the PFAS-containing material of interest**? Other analytical approaches may include total oxidizable precursor assays and total organic fluorine assays for PFAS and analysis for non-PFAS substances (e.g., HF).
- **Are there any concerns about the quality of the data generated during testing?** For any quality assurance/quality control concerns identified, the user should determine whether it is appropriate to use the data to inform decisions about destruction and disposal of the PFAS-containing material using the technology of interest.

Disposal efficacy. Effective disposal of PFAS-containing material should prevent or minimize re-release of PFAS to the environment. Key questions for evaluating the efficacy of a disposal technology for a particular PFAS-containing material are identified below. (Questions included in this section should be skipped when evaluating a destruction technology.)

- **Have potential transformation and partitioning pathways been identified for the technology/material combination of interest?** The fate and transport of PFAS within a disposal technology can help inform whether PFAS may be present in emissions or releases from the technology. Note that the characteristics of the receiving matrix and environmental conditions can affect the fate and transport of PFAS within the disposal technology.
- **Are engineering controls in place to prevent or minimize release of PFAS to the environment?** If so, is there a monitoring system in place to evaluate the performance of the engineering controls?
- **What is the longevity of the disposal technology?** Because of the strength of the carbon–fluorine bond, PFAS are persistent. It is important to consider the lifespan of the disposal technology and whether the technology can contain PFAS for very long timeframes.
- **Has a fluorine mass balance been determined?** If so, is there fluorine that is unaccounted for? In some cases, a fluorine mass balance may be impractical.
- **Does the disposal of the PFAS-containing material contribute to the potential release of non-PFAS constituents to the environment?** PFAS-containing materials may contain other, non-PFAS constituents. Depending on the identity of those constituents and their potential to impact human health and the environment, it may be important to consider whether appropriate engineering controls are in place to prevent or minimize releases of non-PFAS constituents.

Destruction efficacy. Destruction of PFAS should result in breaking carbon–fluorine bonds without reforming PFAS and releasing PFAS to the environment. Key questions for evaluating the efficacy of a destruction technology for a particular PFAS-containing material are identified below. (Questions included in this section should be skipped when evaluating a disposal technology.)

- **Is the mechanism of destruction understood?** If so, has the destruction mechanism been experimentally confirmed, and have the reaction kinetics been determined? A thorough understanding of the destruction mechanism improves confidence in the performance of the technology of interest for PFAS.
- **Have all process inputs and outputs been characterized for PFAS to the extent possible given current analytical methods?** If not, what information may be missing?

- **What is the reported destruction efficiency?** Destruction efficiencies (or DREs) are generally reported for specific PFAS (e.g., PFOA or PFOS). As noted earlier, the destruction efficiency (or DRE) alone may not be sufficient to determine whether other PFAS may be released to the environment during the destruction process.
- **Have any PIDs (or PICs) been identified?** If so, which PFAS have been detected and at what levels? Are those PFAS regularly detected for this technology/material combination?
- **Has a fluorine mass balance been determined?** If so, is there fluorine that is unaccounted for? In some cases, a fluorine mass balance may be impractical.
- **Have all process outputs been characterized for non-PFAS constituents to the extent possible given current analytical methods and understanding of the composition of the PFAS-containing material?** The destruction of PFAS-containing materials is expected to result in non-PFAS products. Depending on the identify of those products and their potential to impact human health and the environment, it may be important to consider whether appropriate engineering controls are in place to prevent or minimize releases of non-PFAS constituents. It may also be important to consider the effect of the destruction process on non-PFAS constituents in the PFAS-containing material of interest. Thus, the characterization of process outputs beyond PFAS may be warranted.

Community considerations. When considering destruction and disposal technologies for PFAS-containing materials, it is important to consider the community that may be exposed to potential PFAS releases from intended destruction or disposal. Key questions to consider include:

- **Has a destruction/disposal site been identified?** Some destruction technologies may be mobile, allowing for treatment and destruction to occur where PFAS-containing materials are generated or stored. Other technologies may be stationary, requiring PFAS-containing materials to be brought on site for destruction or disposal. The use of mobile technologies may allow flexibility in siting to avoid potential impacts on overburdened and vulnerable communities.
- **What are the characteristics of the surrounding community?** See Section 4 for tools and approaches that can be used to characterize communities near destruction/disposal sites.
- **Are there potentially vulnerable populations and/or communities with environmental justice concerns near the destruction/disposal site?** Section 4 provides tools and strategies that can be used to screen nearby communities for potentially vulnerable populations and/or communities with environmental justice concerns.
- **Are PFAS releases anticipated from the technology/material combination of interest?** If so, describe which PFAS may be released, at what levels, and over what duration. Also note whether there are controls in place to mitigate releases to avoid or minimize potential impacts to surrounding communities.
- **Has the surrounding community been engaged**? Sharing information about planned destruction or disposal activities for PFAS-containing materials allows community members to take actions to address concerns they may have. There may also be opportunities to collaborate with government officials and community leaders on engagement activities.

Regulatory requirements. Regulations regarding the destruction or disposal of PFAS-containing materials can vary from state to state and may change over time. Key questions to consider include:

- **Are there state or federal regulations that control emissions or releases from the technology of interest?** Such regulations may limit the amount of PFAS or other pollutants present in emissions or other releases from the technology of interest.

- **Are there state or federal regulations that control the management of the PFAS-containing material of interest?** Such regulations may specify or restrict destruction or disposal options for the PFAS-containing material of interest.

6.c References

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Appendix A: EPA Guidance to Conduct PFAS Emissions Field Testing at Commercial Thermal Destruction Sources

The U.S. Environmental Protection Agency (EPA) Office of Research and Development (ORD) is conducting per- and polyfluoroalkyl substances (PFAS) thermal destruction emissions characterization research to improve our understanding of the behavior of PFAS with respect to waste incineration and thermal treatment (e.g., hazardous waste, municipal waste, biosolids, contaminated soils, spent media), as well as thermal destruction emissions control technologies (e.g., afterburners, thermal and catalytic oxidizers). The following could be used by any entity evaluating thermal treatment of PFAS-containing materials either in collaboration with EPA or independently.

Overall goals of the field tests are as follows:

- Confirm/determine if thermal treatment under representative conditions can result in the thermal destruction of PFAS as defined by conventional destruction and removal efficiency (DRE) testing.
- Characterize potential products of incomplete combustion/destruction (PICs/PIDs) and evaluate the use of alternative, or surrogate, indicators of destruction performance.
- Confirm that any treated material is appropriate for any defined beneficial reuse (e.g., treated soils, reactivated granular activated carbon [GAC]).

To achieve these goals, it is necessary to determine the specific conditions (i.e., temperature, residence time, and turbulence) required to maximize DREs, minimize PICs/PIDs, and produce products appropriate for beneficial reuse. Because bench- and pilot-scale testing have limitations, actual test data under full-scale thermal treatment conditions are necessary to determine if representative thermal processes can achieve these goals.

Sampling Details:

- EPA is available to collaborate with the host site on the technical approach for source characterization. Testing would require multiple stack sampling methodologies performed by commercial stack testing companies, including the host's regular tester, if desired.
- All gas, liquid, and solid inlet, intermediate, and outlet streams will be collected and analyzed. This includes:
    - At the stack and including all air pollution control devices
    - All inlet streams (e.g., materials to be thermally treated, air or gas inlets, water), including samples taken prior to testing
    - All residual streams (e.g., bottom ash, baghouse and/or electrostatic precipitator [ESP] solids, scrubber effluent, spent carbon)
    - All final products (e.g., reactivated carbon, soils), if applicable
- A comprehensive air emission source characterization would include:
    - Samples would be collected for semivolatile PFAS, using method OTM-45 targeted (known) PFAS, or method established endpoints. Samples would be collected for volatile PFAS, using

method OTM-50 and analyzed for targeted (known) PFAS in samples and the identification of unknown detected volatile fluorocarbons.

- Samples would be collected for other semivolatile target compounds using methods SW-846 Methods 0010/3542/8270 with the inclusion of the Method 8270 procedure. Carbon hexafluoride (C_2F_6) and carbon tetrafluoride (CF_4) could be injected during one day of testing, ideally at multiple injection locations, as a surrogate measure of destruction efficiency if appropriate and permitted.
- Tests would be run under a single, representative process condition including the waste that would be co-fired with PFAS-containing material.
- EPA would prioritize testing several PFAS-containing materials, including but not limited to:
  - Incineration of aqueous film forming foam (AFFF)
  - Incineration of wastewater sludges
  - Reactivation of spent GAC
  - Incineration of municipal solid waste
  - Thermal treatment of contaminated soils
  - Incineration of spent ion exchange resins and their concentrated regeneration liquids
- All sampling would be conducted under a well-documented sampling plan and quality assurance project plan.
- Final testing results would be shared with EPA, the state, local authorities.

Additional details on emission testing approaches can be provided upon request.

Supplemental Information

Emissions sampling and analytical approaches

Emissions samples would be collected for polar, nonpolar, volatile, and semivolatile PFAS compounds and analyzed for both targeted PFAS compounds, or method established endpoints.

EPA's Other Test Method (OTM) 45 would be used to collect polar semivolatile PFAS compounds for targeted analyses. The method's 50+ target analytes include many PFAS commonly found in AFFF. This would enable DE, DRE, and emission rate determination as appropriate for a known list of PFAS compounds.

The OTM-50 canister sampling method would be used to collect nonpolar volatile PFAS compounds for targeted compound analyses. The current target list includes CF_4 and C_2F_6 as well as a procedure to identify unknown volatile fluorocarbons.

Non-targeted analyses (NTA) are an optional analytical tool for characterizing potential PICs/PIDs. High resolution mass spectrometry, combined with chromatographic separation and multiple ionization techniques, is used to identify compounds present in collected samples. NTA could be performed on the OTM-45 samples. Fourier transform infrared (FTIR) spectroscopy would be used to measure a variety of compounds in real-time, including hydrogen fluoride (HF), hydrogen chloride (HCl), carbon dioxide (CO_2), and water (H_2O); multiple PFAS compounds including CF_4 and C_2F_6; and multiple known PICs/PIDs. FTIR is particularly critical during surrogate compound injection.

ORD research questions

The following section describes detailed elements of the research questions ORD will ultimately work to address, as well as the input information that is needed. ORD requests an opportunity to engage with the host facility prior to developing a full sampling plan to better understand site-specific conditions and requirements.

Process characterization

- **Question:** How does the process operation affect the destruction and removal of PFAS compounds present in the feed?
- Requires understanding of waste feed (e.g., description, concentration, other wastes), feed approach (e.g., liquid feed, through flame/post flame, co-fired, drums/barrels), and feed rate
- Requires data on combustor operations and conditions (e.g., temperature, residence time)
- Requires data on air pollution control devices (APCD) and their operation: configuration, operating parameters (e.g., temperature, pressure drop, pH of scrubbing liquid, gas velocity)

Determine destruction removal efficiency (DRE)

- **Question:** What is the DRE and emission rate of the specific PFAS compounds present in the feed
- material?
- Requires known input rate and identity of PFAS compounds in waste
- Requires evaluation of the extent of PFAS mineralization
- Requires replicate sampling of polar semivolatile organics using OTM-45 for quantitative measurement of feed PFAS compounds in emissions
- Requires sampling across multiple APCDs

Products of incomplete combustion/destruction (PICs/PIDs)

- **Question:** Are PICs/PIDs formed during thermal treatment and measured by OTM-50 present and, if so, what are they and under what conditions?
- Requires replicate sampling of polar, nonpolar, semivolatile, and volatile organics for non-targeted compound analysis using OTM-45, OTM-50, and Method 0010/3542/8270.
- Requires sampling across multiple APCDs.

Alternative indicators of destruction performance

Question: Can easily measured/monitored, hard-to-destroy compounds serve as alternative (surrogate) indicators for PFAS adequate destruction or PID formation conditions?

- Requires injection of known concentrations/masses of CF_4 and C_2F_6 (approximately 10 parts per million by volume stack equivalent) at representative waste injection locations
- Requires on-line FTIR monitoring of the surrogate compounds, PICs, HF, HCl and other compounds of interest
- Requires initial background signal baseline
- Requires concurrent OTM-50 sampling for analysis of surrogate compounds and known PICs

Analysis of fluorinated compounds in waste/effluent streams

Question: What is the fate of PFAS compounds across the entire thermal system?

- Requires sampling of all discharges
    - Gas sampling prior to and after APCDs (e.g., spray dryer, wet scrubber, baghouse and/or ESP, activated carbon injection)
    - Sampling of solid and liquid discharges (e.g., bottom ash, baghouse and/or ESP solids, scrubber effluent analyses, spent carbon)

Analysis of fluorinated compounds in final product materials, where applicable

Question: What PFAS remain in the material treated, such as reactivated GAC or treated soils?

- Requires sampling of influent and effluent materials
    - Sampling of solid materials (e.g., spent carbon and reactivated carbon)

Appendix B: Summary of the Clean Harbors Test Data

Summary

Two testing campaigns were conducted at Clean Harbors Environmental Services' (CHES) hazardous waste combustor (HWC) located in Aragonite, Utah. Each test campaign was divided into three test conditions: Baseline (Condition 1), Augmented Per- and Polyfluorinated Alkyl Substances (PFAS) Feed (Condition 2, where known amounts of four or five target PFAS were fed to the incinerator), and Post-spiking Normal Operations (Condition 3, where aqueous film-forming foam [AFFF] was included in the incinerator feed). Clean Harbors used the *Other Test Method 45 (OTM-45) Measurement of Selected Per- and Polyfluorinated Alkyl Substances from Stationary Sources* (OTM-45) from the U.S. Environmental Protection Agency (EPA) for stack gas samples. The 49 targeted PFAS analytes were measured using liquid chromatography/tandem mass spectrometry (LC/MS/MS) with isotope dilution. The tests appear to demonstrate that the primary objective, "demonstrate the ability of the high-temperature incineration system at Aragonite to effectively destroy PFAS in the waste feed during normal process operating conditions," was achieved for the five selected PFAS compounds that were subjects of these studies.

Introduction

Two testing campaigns were conducted at CHES HWC located in Aragonite, Utah. The first campaign was conducted from June 17 to 19, 2021 and the second from February 17 to 19, 2022. The stated principal objective of the test program was to "demonstrate the ability of the high-temperature incineration system at Aragonite to effectively destroy PFAS in the waste feed during normal process operating conditions," with secondary objectives of conducting a mass balance of PFAS in process streams and demonstrating high hydrogen fluoride (HF) removal that would be associated with a high destruction of PFAS. This summary presents a high-level overview of both tests' final reports as well as a third-party review of the 2021 test performed by Dr. Philip H. Taylor under contract to CHES.

General Process Description

The HWC tested at the CHES Aragonite facility consists of a slagging rotary kiln incinerator followed by an afterburner. Solid waste materials and containerized wastes are fed into the rotary kiln only, while liquid wastes may be injected directly into rotary kiln or the afterburner. The air pollution control device system for the HWC as tested[29] consists of a spray dryer/gas quench followed by activated carbon injection upstream of a fabric filter (particulates, dioxin/furan, and mercury control). Following the fabric filter, the gases enter a sodium carbonate saturator/wet scrubber (sulfur dioxide, hydrogen chloride, hydrogen fluoride control) with TMT-15 (1,3,5-triazine2,4,6(1H,3H, 5H)-trithione, trisodium salt, used for additional mercury and metals control) injection.

[29] Stack testing was performed in the exit duct of the wet scrubber instead of at the stack for safety reasons (lack of scaffolding). The HWC is equipped with a wet electrostatic precipitator (WESP) following the wet scrubber and prior to the stack, but the WESP was decommissioned in 2004 and is no longer in service. These tests were conducted prior to the WESP although the stack location is the test location for compliance performance tests for the facility.

Operational Description

Each test campaign was divided into three test conditions: Baseline (Condition 1), Augmented PFAS Feed (Condition 2), and Post-Spiking Normal Operations (Condition 3), with the waste feed matrices as follows:

1. **Condition 1.** Normal matrix of liquid materials (waste fuel, clean fuels, sludge, and aqueous wastes) and solid waste materials (containerized, shredded, and bulk solid wastes). Following the 2021 testing, CHES determined that the sludge stream fed to the kiln during all three conditions included some AFFF. During the first run of the 2022 testing, CHES determined that AFFF was inadvertently being fed into the afterburner's corrosive waste port. The testing team decided to be consistent with runs 2 and 3 and continued the AFFF feed, with additional amounts of AFFF fed during Condition 3.
2. **Condition 2.** Normal matrix of liquid materials (waste fuel, clean fuels, sludge, and aqueous wastes) and solid waste materials (containerized, shredded, and bulk solid wastes), with the addition of augmented feed rates of four (2021) or five (2022) PFAS (or their equivalent potassium salt). The four PFAS are perfluorooctanioic acid (PFOA), perfluorooctanesulfonic acid (PFOS), perfluorohexane sulfonic acid (PFHxS), hexafluoropropylene oxide-dimer acid (HFPO-DA), and perfluorobutanesulfonate (PFBS, 2022 only). The target feed rate of the PFAS was estimated to be .0816 pounds per hour or around 0.62 grams per minute in 2021 for all PFAS, but the HFPO-DA was increased to 2.2 grams per minute in the 2022 testing to reflect the method detection limit (MDL) for this compound. Similarly, the PFBS feed rate was 0.33 grams per minute on average to reflect the MDL. The PFAS were fed in boxed containers to the rotary kiln via the containerized solid conveyor in approximately 10-gram portions of all PFAS compounds about every 15 minutes for the duration of these test runs.
3. **Condition 3.** Normal matrix of liquid materials (waste fuel, clean fuels, sludge, and aqueous wastes) and solid waste materials (containerized, shredded, and bulk solid wastes). For this test condition, AFFF concentrate was also fed (neat) to the incinerator. In 2021, the AFFF was fed via the containerized solids in 5-gallon buckets at a rate of 176 pounds per hour. During the 2022 test, the AFFF was labeled as Ansulite and was fed through the educt station to the incinerator directly from a tote bin. The AFFF feed rate ranged from 244 pounds per hour to 316 pounds per hour during the 2022 test.

The rotary kiln was operated in a temperature range between 1,893°F and 2,008°F for the 2021 test and between 1,949°F and 2,219°F for the 2022 test. The afterburner was operated at 2,052°F to 2,110°F during the 2021 test and 2,048°F to 2,075°F for the 2022 test. Oxygen content (free) at the exit of the rotary kiln was maintained between 8 and 9 percent and ranged from 6 to 7 percent for the afterburner for both test campaigns. Clean Harbors estimates that non-combustible material is retained within the combustor for approximately 60 minutes, while combustion gas residence time of the afterburner is two to three seconds.

For the 2021 test, the temperature of the flue gas was reported as 369°F following the spray dryer; between 341°F and 343°F following the fabric filter; between 166°F and 168°F at the saturator outlet; and 148°F to 153°F at the stack. For the 2022 test, the temperature of the flue gas was reported as

363°F following the spray dryer; between 168°F and 170°F at the saturator outlet; and 149°F to 151°F at the stack. The following table lists the air pollution control device operational parameters during the testing campaigns.

Test Campaign	Stack gas flow (acfm)	Soda Ash Flow (lb/hr)	Carbon Injection (lb/hr)	TMT-15 flow (lb/hr)
2021	66,793–68,761	452–789	30.7–31.5	3.95–4.48
2022	51,053–53,640	411–1594	32.0–33.1	3.64–4.00

Test Methods and Scope

During both testing campaigns, samples of stack gases and process streams were taken and analyzed for 49 PFAS target compounds, with the stated analytical objective of characterizing as many streams entering or exiting the incinerator as possible. Clean Harbors used EPA's *Other Test Method 45 (OTM-45) Measurement of Selected Per- and Polyfluorinated Alkyl Substances from Stationary Sources* (OTM-45) for stack gas samples (as noted earlier, "stack" meaning the exit duct from the wet scrubber as it was deemed a safer sampling location than the actual stack). The 49 targeted PFAS analytes were measured using LC/MS/MS with isotope dilution.

Process streams sampled included waste fuels, clean fuels, waste feeds, process water, and residuals (liquids and solids), as well as some pre-test sampling of chemical inputs (soda ash, activated carbon, and TMT-15). As noted in the reports, however, due to the waste profile matrix complexity and the heterogeneity of all three solid waste streams, they were not sampled or analyzed for PFAS. Absent any final EPA-approved methods for media other than drinking water, LC/MS/MS analysis was used to measure the targeted PFAS compounds in the process stream samples.

In addition to the 49 PFAS analytes, the HF concentration of the stack gas was measured using EPA Method 26A. The AFFF-containing material in the 2021 test was analyzed for the 49 target PFAS both before and after chemical oxidation as a total oxidizable precursor assay (TOPA). During the 2022 test, a TOPA and total organic fluorine assay (TOFA) were performed on the AFFF-containing materials. The objective of performing TOPA is to "reveal perfluoroalkyl acid (PFAA), fluorotelomer sulfonate (FTS), and possibly other precursors that are not identifiable with targeted PFAS analysis." TOFA, as described in the 2022 report, identifies the mass of compounds containing carbon-fluorine bonds, and was performed using combustion ion chromatography following a laboratory in-house method for adsorbable organo-fluorine.

Results

According to the tests and third-party review, the primary objective of determining the destruction and removal efficiency (DRE) of each of the spiked (augmented feed) PFAS was achieved. The calculated DREs for each test (triplicate test run average) and PFAS compound are as follows:

PFAS Analyte	2021 Test DRE (%)	2022 Test DRE (%)
PFOA (perfluorooctanioic acid)	99.999943	99.99990
PFOS (perfluorooctanesulfonic acid)	99.999955	99.99977
PFHxS (perfluorohexane sulfonic acid)	99.999977	99.99996

PFAS Analyte	2021 Test DRE (%)	2022 Test DRE (%)
HFPO-DA (hexafluoropropylene oxide – dimer acid)	99.999979	99.99984
PFBS (perfluorobutanesulfonate)	n/a	99.99997

Both test reports note that none of the other 45 (or 44, in 2022) target PFAS analytes were present in the waste feed at levels sufficient to demonstrate 99.9999 percent DRE without spiking, even though stack gas concentrations were non-detect in many cases. According to the 2021 test report, there were three other PFAS present in the waste feeds at sufficient mass feed rate without spiking to enable demonstration of DRE's exceeding 99.999 percent. These included PFOS, 6:2 fluorotelomer sulfonate (FTS), and 8:2 FTS. The report stated that the FTS compounds are common constituents of AFFF.

As for the secondary objectives, the results are, as stated within Dr. Taylor's review, "most difficult to achieve as they involve more complex sampling issues." With respect to the secondary PFAS mass balance objective, he notes that "some of the PFAS that was not sampled in the infeed may have been more difficult to gasify and burn, contrary to the assertions in the report." However, he concurs with the report statements that the PFAS DRE is conservative.

The objective of demonstrating high HF removal that would be associated with a high destruction of PFAS is seemingly not achieved, primarily due to analytical issues. Dr. Taylor notes that "the sinks for the HF were not accurately quantitated leading to some questions about the validity of the reported HF removal efficiency." He further notes that the assumption that all fluorine in the waste if converted to HF is challenged, as there would be a required excess of hydrogen to fluorine to be demonstrated, which are not provided for in the data. Further, he stated that there is a "demonstrated lack of accuracy of the analytical methods for measuring inorganic fluorine in the residual streams," which, in his opinion, leads to a very low fluorine recovery mass balance. He concluded that, "development of better analysis methods for complex matrices for inorganic fluorine are needed before high HF removal efficiencies can be reported at full scale."[30]

Conclusions

The tests appear to demonstrate that the primary objective, "demonstrate the ability of the high-temperature incineration system at Aragonite to effectively destroy PFAS in the waste feed during normal process operating conditions," was achieved for the five selected PFAS compounds that were subjects of these studies. However, this study did not evaluate the formation of PICs due to previous methodology limitations. Dr. Taylor, in his review, noted this as well: "The larger question from an environmental viewpoint is the complete mineralization of these substances and prevention of emission of highly stable C_1-C_2 PFCs (perfluorocarbons)." Dr. Taylor concludes that CHES' results, although promising and demonstrating reduction of a limited selection of specific PFAS compounds, did not support complete mineralization.

[30] Although not stated by Dr. Taylor in his assessment, another possible reason for lack of finding inorganic fluorine (HF) could be the result of poor conversion (mineralization) of PFAS to HF and carbon dioxide during incineration. This may indicate formation of non-target PFAS or fluorocarbons during incineration that were not measured by these tests.

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Appendix C: Summary of the Chemours Thermal Oxidizer Test Data

Introduction

Chemours Company FC, LLC, located in Fayetteville, North Carolina, manufactures chemicals and plastics and was required to install a thermal oxidizer to control per- and polyfluoroalkyl substances (PFAS) emissions. After installation, tests were conducted in March 2020 and March 2022. The objective of the tests was to determine how "the Thermal Oxidizer and 4-stage Scrubber system will achieve the emission reduction [of 99.99 percent], including the use of a surrogate for all PFAS, such as the hexafluoropropylene oxide (HFPO)." This summary presents a high-level review of the two tests' reports.

General Process Description

The thermal oxidizer at the Chemours facility operates at 10 million BTU per hour and is powered by natural gas. Waste gases from both polymer and monomer production are fed into the thermal oxidizer. The gaseous emissions from the oxidizer are fed into the four-stage packed bed column scrubber system while solid calcium fluoride (CaF_2) is removed, collected, and disposed of off site. The scrubber has three water scrubbing stages and one caustic scrubbing stage and is stated to remove hydrogen fluoride (HF) from the final emissions.

Operational Description

Test Methods and Scope

The tests done on the thermal oxidizer focused on five PFAS compounds: HFPO, hexafluoropropylene oxide-dimer acid (HFPO-DA), hexafluoropropylene oxide-dimer acid fluoride (HFPO-DAF), carbonyl fluoride (COF_2), and Flouroether E-1. These compounds were selected because they are handled and used at the Fayetteville facility. The compounds have varying chemical structures and properties and cannot be sampled and tested in the same manner.

Modified Method 18 sampling was used to capture the PFAS compounds. This method uses six fluoropolymer (PFA) impingers charged with methanol arranged in a series and captures the compounds using chemical reactions and condensation.

To capture HFPO-A, the facility needed to use Modified Method 0010 sampling with a heated probe and filter, condenser coil, XAD-2 resin cartridge, deionized water impingers, and a silica gel impinger. In addition to the normal Modified Method 0010 sampling process, this test added a second XAD-2 resin cartridge to monitor possible target PFAS breakthrough.

Three compounds, HFPO, HFPO-DAF, and COF_2, were sampled by chemical reaction. The sample gas was passed through the series of methanol impingers. The compounds then react with the methanol, and each produced unique ether compounds that were monitored and quantified using SW-846 Method 8260.

The strategy used to sample Flouroether E-1 and HFPO-DA was to capture the compounds through condensation and dissolution in the same methanol impingers. The volatile organic compound, Flouroether E-1, was then quantified using SW-846 Method 8260b. The semivolatile organic compound HFPO-DA was quantified using EPA Method 537.

Samples were taken from the polymer production and monomer production waste gas feed lines to capture the initial amount of PFAS compounds they produced as waste and give them a baseline concentration of PFAS prior to treatment. The other sampling location was in the stack after the thermal oxidizer and scrubber. This location would show how effective the process was at destroying the targeted PFAS compounds.

The sampling and testing methods only captured the amount of target PFAS compounds and did not measure any products of incomplete combustion. Although the four-stage scrubber was implemented to reduce the possible HF emissions, there was no sampling of HF.

Results

The results of the tests showed that the mandated 99.99 percent destruction of targeted PFAS compounds had been achieved. In both testing years the average PFAS destruction and removal efficiency (DRE) was above 99.999 percent with no result being lower than 99.999 percent. Results were presented as a total FPAS destruction efficiency with the amount of each target PFAS compound summed to provide an aggregate destruction efficiency. The total PFAS destruction efficiencies for each run and each year are as follows:

Year	Run 1	Run 2	Run 3	Average
2020	99.99982%	99.99974%	99.99986%	99.99981%
2022	99.99951%	99.99966%	99.99967%	99.99961%

Conclusions

The test reports demonstrate a PFAS destruction efficiency above the 99.99 percent that was mandated for the Chemours facility for the specific compounds that were tested. HFPO was tested as a surrogate for all species of PFAS, but there are no concrete data showing the performance of the thermal oxidation process for PFAS species outside of the compounds tested. There was also no discussion of possible products of incomplete combustion in the reports. The thermal oxidizer at the Chemours facility appropriately destroys the five PFAS compounds of interest that have been identified at the facility. However, removal processes for products of incomplete combustion or of destruction of potential compounds not studied but potentially found in the facility waste streams are still unclear.

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Appendix D: Summary of Costs and Considerations

Introduction

This appendix provides some estimates of costs for end-of-life management of PFAS materials by thermal treatment, landfilling, and underground injection. The cost information presented is relatively uncertain and is likely most suitable to make comparisons between techniques.

The cost to destroy or dispose of PFAS and PFAS-containing materials can vary widely depending on a number of factors. Waste management infrastructure's design and operation are heavily influenced by regional needs, local regulations, and technological advancements. As a result, waste management costs vary by the type of waste and by location due to factors like availability and transportation costs. In addition, technological advances specifically related to the management of PFAS-containing materials can potentially reshape the cost and operational landscape of PFAS waste management. Multiple factors and limitations contribute to cost variability and should be considered when using the information in this appendix.

- Market conditions such as supply and demand change frequently, resulting in fluctuating costs.
- Location of destruction and disposal options and transportation affect case-by-case costs.
- Transportation costs or any special handling costs for accepting and treating additional waste are not included in most of these estimates, thus actual costs would vary.
- Costs may be affected by permitting and state/local requirements for PFAS-containing material, which vary depending on state/local regulations, public input, and possible environmental justice concerns.

Thermal Treatment

Hazardous Waste

Table D-1. Estimated Costs to Incinerate Different Types of Hazardous Waste (U.S. EPA, 2005a)

Hazardous Waste Type	Estimated Thermal Treatment Cost per Ton[a]
Liquids, sludges, solids (halogenated)	$1,300-1,889
Liquids, sludges, solids (non-halogenated)	$381-1,040
Lab packs	$6,448
Containerized gases	$3,120

[a] Costs were developed for hazardous waste combustors (HWC) that were affected by the 2005 National Emission Standards for Hazardous Waste Pollutants (NESHAP), including commercial incinerators, cement kilns, lightweight aggregate kilns, and commercial HWC boilers. Cost per ton is assumed to be in 2002 dollars, the same year basis of the regulation from which these costs were obtained. Costs were normalized to 2022 dollars using the Bureau of Economic Analysis (BEA) gross domestic product (GDP) deflator (U.S. BEA, 2023) for waste management and remediation services using a base year of 2002. Note that applying the BEA GDP deflator for this industry sector is a top-down approach. For regulatory purposes, a bottom-up approach is typically used, accounting for capital costs based on the Chemical Engineering Plant Cost Index (CEPCI), energy prices typically based on Energy Information Administration (EIA) data, and labor costs based on industry segment indices using Bureau of Labor Statistics (BLS) data.

Limitations:

- Costs have likely changed since 2002 and scaling costs to 2022 contributes to uncertainty.

- Does not include transportation costs or any special handling costs for accepting and treating additional waste materials.

Table D-2. Example Disposal/Reactivation Costs for Drinking Water Treatment Residuals (Derived from U.S. EPA, 2020a)

Method	Cost of Disposal ($/Pound of Media)	Estimated Cost of Disposal Plus Replacement Media ($/Pound of Media)[a]
Reactivated GAC—off-site	$0	$1.74
Disposal via landfill	$0.06	$2.35
Disposal via incineration	$1.28	$3.57

[a] Cost per pound is in 2022 dollars. For GAC, on-site reactivation is possible. However, the utility or site would have to have ample workforce, managerial, and financial (both capital and operating) resources to justify this choice. It is likely to be cost-effective only for very large facilities and would require consideration of other factors including availability of land and public opinion. Due to the complex analysis needed, a full comparison of off-site versus on-site is beyond the scope of this document.

Limitations:

- These estimates are presented as examples only to illustrate tradeoffs. Actual site-specific costs are likely to vary based on factors including the quantity of media reactivated/replaced and are used as an example of trends. In this example, reactivated GAC and virgin GAC are estimated to be $1.51 per pound and $2.29 per pound, respectively.
- All unit costs assume 21,000 lbs of GAC reactivated/replaced.
- For reactivated GAC, it is assumed that the "replacement" media is the returned reactivated media plus the replacement of the GAC lost during reactivation with virgin media (assumed to be 30 percent).
- Disposal costs assume 10 miles of transportation to the disposal site.
- Incineration costs are based on incineration of ion exchange resin media. Incineration of GAC could be somewhat more expensive, depending on the relative fuel value of the spent media.
- The analysis is complex and a number of issues need to be considered at the site level, such as those that affect costs (cost of energy, shipping, labor, construction, operation, sampling, etc.) and those that affect other matters (practicality, public versus private ownership, contract availability, regional reactivation availability, off-gas permitting, public opinion, etc.).

Landfills

Hazardous Waste

Table D-3. Estimated Costs to Landfill A Representative Type of PFAS-Containing Material (Defense Logistics Agency (DLA), 2023)

Hazardous Waste Type	Estimated Cost per Ton of Waste
Aqueous film-forming foam (AFFF) and other PFAS-related Solids/Debris, Non-RCRA, >100 parts per million (ppm) PFAS for direct disposal at RCRA Subtitle C Landfill (e.g., contract line item number (CLIN) PF06B1)	$1,300-3,660 ($0.65-1.83/lb)

Limitations:

- The DLA pricing web application lists current government contracts for destroying or disposing of PFAS and PFAS-containing materials and may be useful for government contract cost estimates. The estimated cost per ton (lb) of waste appears to include removal and disposal costs for the specific material, although a portion of transportation costs may be attributed to other cost elements of each respective contract so transportation costs may not be fully reflected in these costs.
    - **Per the DLA pricing web application:** The prices contained in the database were awarded based on contract terms and conditions, procurement requirements, and price reasonableness determinations. The prices and contract information in the database are for reference only.
- Users of the DLA pricing web application are cautioned that each contract was developed to meet specific requirements for specific geographic regions or military installations. There are multiple variables that result in the pricing information displayed, including contract award date, proximity to DLA-approved disposal facilities, quantities of PFAS material estimated for disposal in proportion to other hazardous waste on the contract, reoccurring need for services, and whether PFAS material is disposed of in bulk or whether there are small, limited quantities.
- Pricing is provided here for bulk waste types for disposal at a Subtitle C hazardous waste landfill. Pricing for multiple waste types with varying units of measure for disposal, including containerized waste, are provided in the DLA pricing web application.
- The estimated cost in the table above only applies to a limited number of military installations and only considered bulk pricing from 600 to 10,000 pounds. Pricing is expected to differ for containerized waste and should be considered when relying on this cost information.

Non-Hazardous Waste (Municipal solid waste)

Table D-4. Average MSW Tipping Fees per Ton by U.S. Region (EREF, 2022)

Region	Average Tipping Fee ($/ton)	Region	Average Tipping Fee ($/ton)
Pacific	$69.02	Northeast	$75.92
Mountains/Plains	$50.84	Southeast	$48.70
Midwest	$62.02	South Central	$50.84

Limitations:

- MSW landfills emit more PFAS than originally thought in 2020. Research estimates that although MSW landfills contain PFAS for the most part, up to 5 percent of the PFAS may be released in the landfill gas and, 11 percent may be released in the leachate annually (Tolaymat et al., 2023). Thus, MSW tipping fees may not be a relevant data point for cost information.

Non-Hazardous Waste (Liquid waste)

Table D-6. Average Industrial Wastewater Rates by U.S. Region (Unger et al, 2023)

Region	$/1,000 gal	Region	$/1,000 gal
Pacific	$11.90	Northeast	$4.82
Mountains/Plains	$3.12	Southeast	$7.52
Midwest	$4.77	South Central	$5.20

[a] Data are consolidated by region and the number of data points varies by region. American Water Works Association (AWWA) water and wastewater rate surveys were used to gather historical rate data for water and wastewater utilities in the United States. These datasets were compiled and assessed to produce a single dataset of time series rate data for 112 water utilities and 76 wastewater utilities located throughout the United States.

Limitations:

- These data do not include specific information on extra treatment considerations that may be required by a national pollutant discharge elimination system (NPDES) permit or by an industrial user permit for a discharge into a POTW to control the release of PFAS. Thus, similar to the cost of landfilling PFAS waste, the associated treatment of PFAS-laden leachate is difficult to assess because the available data are associated with typical industrial wastewater generators or typical landfill leachate.

One report examined the cost to manage landfill leachate on site (U.S. EPA, 2021). Based on review of the treatment processes and technologies that are currently used, the cost to manage leachate on site is highly variable but generally ranges between $10-150/1,000 gallons ($0.01-0.15/gallon). Capital costs can range from hundreds of thousands to several million dollars. The report lists multiple limitations and does not specify treatment for PFAS.

Underground Injection

Class I Wells (hazardous and non-hazardous)

Table D-6. Estimated Costs for Class I Wells

Underground Injection Cost	$/1,000 gal[a]
Deep well injection	$190-270 (deSilva, 2019)
Trucking costs	$10-200 (McCurdy, 2011)

[a] Costs were normalized to 2022 dollars using the Bureau of Economic Analysis (BEA) gross domestic product (GDP) deflator (U.S. BEA, 2023) for waste management and remediation services using a base year of 2019.

Limitations:

- Class I well capacity is limited, which may affect the costs associated with deep well injection.
- Because of the geographic concentration of Class I wells, waste producers may face transportation and logistical challenges.
- To begin accepting PFAS-containing fluids, owners/operators of existing Class I wells may incur costs to modify their permits to accept waste from other entities or new waste streams.

Another source includes costs for disposing of sewage sludge and indicates that among fees and transportation costs is a $37.50 per ton fee for deep well injection of leachate (CDM Smith, 2020).

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> **Per the report:** *This study develops alternatives to remove and destroy per- and polyfluoroalkyl substances (PFAS) from water resource recovery facility (WRRF) effluent, biosolids, mixed municipal solid waste (MSW) landfill leachate, and compost contact water (waste streams) using currently feasible technologies (i.e., could be built today). The report summarizes estimated capital and operations and maintenance (O&M) cost ranges for the two highest-ranking PFAS management alternatives for four waste streams (WRRF effluent, WRFF biosolids, landfill leachate, and compost contact water).*

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> The pricing web application lists Department of Defense contracts for destroying or disposing of PFAS and PFAS-containing materials. The website is organized by multiple contract line item numbers (CLIN), each of which describes a material or waste stream and a unit of measure for disposal. The site lists contracts and pricing for available disposal technologies, including solidification and disposal in a hazardous waste landfill. The application may be useful as a reference for historical pricing for Department of Defense contracts.
>
> **Per the DLA pricing web application:** *The prices contained in the database were awarded based on contract terms and conditions, procurement requirements, and price reasonableness determinations. The prices and contract information in the database apply to Department of Defense requirements and are for reference only.*

Interim Guidance on the Destruction and Disposal of Perfluoroalkyl and Polyfluoroalkyl Substances and Materials Containing Perfluoroalkyl and Polyfluoroalkyl Substances—2026 Version

INTERIM GUIDANCE FOR PUBLIC COMMENT
APRIL 20, 2026

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Abbreviations

acfm	actual cubic feet per minute
AFFF	aqueous film-forming foam
AOF	absorbable organofluorine
APCD	air pollution control device
ARFF	aircraft rescue firefighting
BDL	below detection limit
BMP	best management practice
BRAC	base realignment and closure
BTU	British thermal unit
C	Celsius
CAA	Clean Air Act
CaF_2	calcium fluoride
CaO	calcium oxide
$Ca(OH)_2$	calcium hydroxide
C&D	construction and demolition
CDC	Centers for Disease Control and Prevention
CDR	Chemical Data Reporting
CERCLA	Comprehensive Environmental Response, Compensation, and Liability Act
CF_4	carbon tetrafluoride
C_2F_6	hexafluoroethane
C_3F_8	octafluoropropane
CFR	Code of Federal Regulations
CHES	Clean Harbors Environmental Services
CHF_3	fluoroform
CIC	combustion–ion chromatography
CIMS	chemical ionization mass spectrometry
CKD	cement kiln dust
CLIN	contract line item number
COD	chemical oxygen demand
CO_2	carbon dioxide
CV	coefficient of variation
CWA	Clean Water Act
cm	centimeter
DE	destruction efficiency
DoD	Department of Defense
DOE	Department of Energy
DoW	Department of War
DRE	destruction and removal efficiency
EOF	extractable organofluorine
EPA	United States Environmental Protection Agency
ESP	electrostatic precipitator
ESTCP	Environmental Security Technology Certification Program
F	Fahrenheit
FAA	Federal Aviation Administration
FBC	fluidized bed combustor

FF	fabric filter
FML	flexible membrane liner
FTCA	fluorotelomer carboxylic acids
FTIR	Fourier transform infrared spectrometry
FTOH	fluorotelomer alcohol
FTS	fluorotelomer sulfonate
FUDS	formerly used defense sites
FY 2020 NDAA	National Defense Authorization Act for Fiscal Year 2020
GAC	granular activated carbon
GCCS	gas collection and control system
HALT	hydrothermal alkaline treatment
HAP	hazardous air pollutant
HCl	hydrogen chloride
HDPE	high-density polyethylene
HF	hydrogen fluoride
HFPO-DA	hexafluoropropylene oxide-dimer acid, 2,3,3,3-Tetrafluoro-2-(1,1,2,2,3,3,3-heptafluoropropoxy) propanoic acid, ($C_6F_{11}O_3H_2$)
hr	hour
HRMS	high-resolution mass spectrometry
HWC	hazardous waste combustor
HWI	hazardous waste incinerator
H_2O_2	hydrogen peroxide
ITRC	Interstate Technology and Regulatory Council
kg	kilogram
L	liter
lb	pound
LC/MS/MS	liquid chromatography/tandem mass spectrometry
LFG	landfill gas
LWAK	lightweight aggregate kiln
MDL	method detection limit
MF	microfiltration
mg	milligram
MnO_2	manganese(IV) oxide
MSW	municipal solid waste
MWC	municipal waste combustor
m^3	cubic meter
NADP	National Atmospheric Deposition Program
NASA	National Aeronautics and Space Administration
NDAA	National Defense Authorization Act
NESHAP	National Emission Standards for Hazardous Air Pollutants
NF	nanofiltration
ng	nanogram
NIOSH	National Institute for Occupational Safety and Health
NMOC	nonmethane organic compound
NPDES	National Pollutant Discharge Elimination System
NPDWR	National Primary Drinking Water Regulation
NSPS	New Source Performance Standards

NTA	non-targeted analysis
OECD	Organization for Economic Cooperation and Development
OSTP	Office of Science and Technology Policy
OTM	Other Test Method
PAC	powdered activated carbon
PA/SI	preliminary assessments/site inspections
PBPK	physiologically based pharmacokinetic
PCB	polychlorinated biphenyl
PFAA	perfluoroalkyl acid
PFAS	perfluoroalkyl and polyfluoroalkyl substances
PFASTT	PFAS Thermal Treatment Database
PFBS	perfluorobutanesulfonic acid ($C_4F_9SO_3H$)
PFC	perfluorocarbon
PFHxS	perfluorohexanesulfonic acid ($C_6F_{13}SO_3H$)
PFOA	perfluorooctanoic acid ($C_8F_{15}O_2H$)
PFOS	perfluorooctanesulfonic acid ($C_8F_{17}SO_3H$)
PIC	product of incomplete combustion
PID	product of incomplete destruction
PIGE	particle-induced gamma emission spectrometry
PITT	PFAS Innovative Treatment Team
POHC	principal organic hazardous constituent
POTW	publicly owned treatment works
ppb	parts per billion
ppmv	parts per million by volume
PTFE	polytetrafluoroethylene
PVDF	polyvinylidene fluoride or polyvinylidene difluoride
RCRA	Resource Conservation and Recovery Act
RDF	refuse-derived fuel
RO	reverse osmosis
SBIR	Small Business Innovation Research
SDA	spray dryer absorber
SDWA	Safe Drinking Water Act
SERDP	Strategic Environmental Research and Development Program
SFP	side-chain fluorinated polymers
SSI	sewage sludge incinerator
S/S	solidification/stabilization
TD-GC/MS	thermal desorption-gas chromatography/mass spectrometry
TMC	thermomechanochemical
TOC	total organic carbon
TOF	total organic fluorine
TOP	total oxidizable precursor
TRI	Toxics Release Inventory
TRL	technology readiness level
TSCA	Toxic Substances Control Act
TWTDS	treatment works treating domestic sewage
UF	ultrafiltration
UIC	underground injection control

U.S.C.	United States Code
USDW	underground source of drinking water
UST	underground storage tank
VFC	volatile fluorinated compounds
VOC	volatile organic compound
WESP	wet electrostatic precipitator
WWTP	wastewater treatment plant
XPS	X-ray photo-electron spectroscopy
µg	microgram
$	United States dollar

Executive Summary

This 2026 update of the U.S. Environmental Protection Agency (EPA) interim guidance presents currently available information on the destruction and disposal of perfluoroalkyl and polyfluoroalkyl substances (PFAS) and PFAS-containing materials. This guidance focuses on updates to the current state of science and associated uncertainties for three large-scale capacity technologies that can destroy PFAS or control PFAS release into the environment: thermal treatment, landfills, and underground injection. This guidance document also discusses emerging technologies and provides a framework for evaluating these technologies for PFAS destruction or disposal. This guidance is non-binding and does not have the force and effect of law.

EPA published the first edition of this guidance on December 18, 2020, as required by the National Defense Authorization Act (NDAA) of 2020, and the second edition of this guidance on April 16, 2024. The NDAA directs EPA to address the destruction and disposal of PFAS and specific PFAS-containing materials, including aqueous film-forming foam (AFFF), contaminated media, textiles (other than consumer goods), and various wastes from water treatment. The NDAA also requires EPA to review and update the guidance as appropriate, but no less frequently than every three years. On April 28, 2025, the EPA Administrator committed to providing more frequent updates to the interim guidance—changing from every three years to annually—as EPA continues to assess the effectiveness of available treatment technologies.

The primary audience of this guidance are decision-makers (e.g., managers of PFAS and PFAS-containing materials making decisions about destruction and disposal) who need to identify effective means for destroying or disposing of PFAS-containing materials and wastes. The audience may also include regulators, waste managers, and the public, including affected communities. While this document highlights a few emerging approaches for PFAS destruction and disposal, EPA acknowledges that alternatives not discussed in this document may also be appropriate. EPA is recommending that managers of PFAS wastes consider the nature of the waste, location, potential for environmental release, and other factors to determine the most appropriate destruction, disposal, or storage method. As a general approach, EPA recommends that decision-makers prioritize the use of destruction and disposal technologies that have a lower potential for PFAS release to the environment over destruction and disposal technology options with a greater potential for environmental release of PFAS given similar costs or other considerations. EPA acknowledges that each destruction and disposal technology has limitations. This guidance is intended to assist with managing PFAS to minimize environmental releases during destruction and disposal activities supports protection of human health and the environment.

This guidance highlights effective PFAS destruction and disposal technologies to prevent or minimize environmental releases, while acknowledging the important distinction between a potential environmental release and human exposure and risk.

EPA has developed a fact sheet for the public, which provides a more concise and less technical presentation of key materials (see Appendix E).

Key Updates

EPA evaluated recent information pertaining to destruction and disposal options based on each technology's potential to destroy PFAS or control PFAS release into the environment. This approach is

consistent with EPA's mission to protect human health and the environment. This guidance describes several different types of technologies that are widely used and commercially available for PFAS treatment and disposal, and their potential to destroy PFAS or control PFAS releases is described herein (see Sections 1 and 3). The following technologies (listed in no relative order) generally have a lower potential for environmental release of PFAS compared to other technologies:

- **Underground Injection - Permitted Class I non-hazardous industrial or hazardous waste injection wells** are one of the regulatory waste management approaches that can provide reasonable assurance that injected fluids will remain isolated and not migrate into underground sources of drinking water (USDWs). However, over long timescales, uncertainties remain, including the long-term fate and transport properties of PFAS in the injection zone. These wells may help ensure that injected fluids are confined and cannot enter underground sources of drinking water. However, the limited number of wells currently receiving off-site PFAS and waste transportation logistics may significantly limit the type and quantity of PFAS-containing fluids appropriate for underground injection.
- **Landfills - Permitted RCRA Subtitle C hazardous waste landfills** incorporate the most stringent engineering controls and practices for containment and are expected to be more effective at minimizing PFAS release into the environment than other landfill types. EPA recommends landfills subject to Subtitle C of the Resource Conservation and Recovery Act (RCRA) when PFAS levels of the waste are relatively high, and landfill disposal is the selected option. However, for all landfill types, new information demonstrates that landfilling could result in higher PFAS releases to the environment than previously thought in 2024 (see Section 3.b).
- **Thermal Treatment - Permitted hazardous waste combustors** (HWCs) such as commercial incinerators, cement kilns, and lightweight aggregate kilns (LWAKs) and granular activated carbon (GAC) reactivation units with thermal oxidizers may operate under conditions more conducive to destroying PFAS and controlling related products of incomplete combustion (PICs). Research available since release of the 2024 Interim Guidance suggests that the use of higher temperatures, well mixed combustion environments, and longer residence times may be more conducive to destroying PFAS and controlling related PICs. Although newer, limited data have been obtained since the 2024 version of the interim guidance, uncertainties remain about the effectiveness of thermal treatment to adequately destroy PFAS or control release to the environment. EPA encourages additional testing with EPA-approved or EPA-evaluated methods by waste managers of thermal treatment operations (including testing for PICs present at the treatment device outlet and the presence of PFAS in all associated process and waste streams) to evaluate whether thermal treatment technologies are minimizing potential environmental releases. EPA's new analytical methods for a targeted list of polar semivolatile PFAS (Other Test Method 45 [OTM-45]) and volatile fluorinated compounds (OTM-50), as well as additional EPA methods under development for evaluating treatment of hard-to-destroy PFAS will allow better characterization of PICs which will help to address some of these uncertainties when additional data are collected as recommended in this guidance (Appendix A).

This list includes the three technology categories described in Section 3 and does not preclude the use of other new or emerging technologies. When considering the bullets above, it is important to note that real-world performance and testing data are generally limited. Additional performance and testing data—including data on destruction and removal of PFAS in thermal treatment devices, ongoing research activities, and long-term performance data for landfills and underground injection—may

change EPA's understanding of the ability of each technology to control PFAS. Section 4 of this updated guidance summarizes the data needs and priorities for research to inform future updates.

EPA continues to seek collaboration with thermal treatment facilities to conduct air emission testing during thermal treatment of PFAS and PFAS-containing materials (see Appendix A). In addition to performance and testing data, the 2026 guidance continues to identify other, high-priority data gaps that need to be addressed by researchers across government, academia, and industry for EPA to provide meaningful updates on effective destruction and disposal practices.

EPA partnered with industry and academia to collect data on emerging technologies for PFAS destruction, such as mechanochemical degradation, gasification and pyrolysis, and supercritical water oxidation. While the results from these studies show promise for PFAS destruction, further work using newly available methods is needed to more fully characterize the outputs of these processes and to evaluate their performance for a wider range of PFAS-containing materials. Managers of PFAS and PFAS-containing materials may consider these or other emerging technologies for PFAS disposal or destruction. To assist PFAS material managers with evaluating whether an emerging destruction (or disposal) technology is suitable for a particular PFAS-containing material, EPA developed a technology evaluation framework. The framework provides a transparent, consistent approach for evaluating destruction and disposal technologies for PFAS and PFAS-containing material and includes considerations for communities. EPA recommends that managers of PFAS-containing materials use the technology evaluation framework to evaluate emerging destruction and disposal technologies and to inform their decisions about managing PFAS-containing materials. EPA also encourages technology developers to generate and publicly release data that can be used to complete the framework (see Section 5).

Public Feedback and Next Steps

EPA and other government, academic, and private institutions will continue to conduct research to better understand PFAS destruction and disposal (see Section 4). EPA will consider public comments it receives on this version of the guidance, as well as additional advancements in PFAS research and science to publish annual updates.[1] This guidance will be published with a 60-day comment period. EPA will publish a Federal Register Notice with Instructions on how to submit comments. During the public comment period, EPA encourages the public to submit studies and data related to management of the PFAS-containing waste described in Section 2. Additionally, continued collaboration with industry researchers is encouraged to conduct emissions testing of PFAS treatment devices, including evaluation of PICs and products of incomplete destruction (PIDs).

[1] On April 28, 2025, the EPA Administrator committed to providing more frequent updates to the interim guidance—changing from at least every three years as required by the NDAA to annually.

1. Introduction

This 2026 version of the interim guidance provides information on technologies that may be feasible and appropriate for the destruction or disposal of perfluoroalkyl and polyfluoroalkyl substances (PFAS) and PFAS-containing materials. This guidance is non-binding and represents the current state of science and the associated uncertainties for large-capacity technologies that can destroy PFAS or control PFAS release into the environment. This is an update to the interim guidance the EPA issued in 2024 and utilizes information and data reviewed up until September 2025.

EPA published the second version of this interim guidance and made it available for public comment.[2] This updated version incorporates the feedback EPA received from the 37 unique comments on the interim guidance submitted by states, Tribes, industries, trade organizations, environmental organizations, and members of the public.

The 2026 interim guidance contains the following new information:

Section 1: Introduction

- Updated review of PFAS destruction and disposal technologies.

Section 2: Description of PFAS-Containing Materials Identified in the FY 2020 NDAA

- Provided information regarding updates and planned revisions to the PFAS 8(a)(7) reporting rule under the Toxic Substances Control Act (TSCA [88 FR 70516, October 11, 2023]).
- Updated biosolids generation and management information.
- Updated information on the PFAS National Primary Drinking Water Regulations.

Section 3: Technologies for the Destruction and Disposal of PFAS and PFAS-Containing Materials

- Incorporated information from 2024 tests evaluating the PFAS destruction at hazardous waste incinerators in Aragonite, Utah and Port Arthur, Texas in Section 3.a.
- Added information from recent studies on thermal destruction of HFPO-DA into Section 3.a.i.1, laboratory- and facility-scale investigations of PFAS thermal treatment in sewage sludge incinerators (SSIs) into Section 3.a.i.3.1, and the efficacy of thermal oxidizers and carbon adsorption beds as PFAS control technologies at a fluoropolymer manufacturing facility into Sections 3.a.i.4 and 3.a.ii.
- Included published data evaluating landfill liner permeability and implications for PFAS transport and transformation in Section 3.b.ii.1, and added newly available published data on gas collection systems and implications for PFAS transport and transformation to Section 3.b.ii.2.
- Incorporated published ranges of PFAS concentrations measured in leachate samples from different types of landfills and municipal solid waste (MSW) landfill gas (LFG) condensate to Table 3-3 and data and information regarding PFAS concentrations in LFG samples from Subtitle D landfills to Section 3.b.ii.3.
- Updated Section 3.b.iii.6.2 regarding on- and off-site treatment technologies for landfill leachate that remove PFAS, and added new information on non-targeted analysis, uncertainties, and unknowns in Section 3.b.vi.
- Updated the information showing the locations of Class I wells in the United States using 2023 data in Section 3.c.iii.

[2] Interim PFAS Destruction and Disposal Guidance; Notice of Availability for Public Comment. U.S. EPA. 89 Federal Register 26879. April 16, 2024.

Section 4: Research Needs and Data Gaps for Destruction and Disposal Technologies

- Revised the table in Section 4.a based on new information presented in this document and public comments received on the 2024 interim guidance.
- Replaced discussion of current research efforts with links to ongoing, relevant federal research activities.

Section 5: Emerging Technologies for PFAS Destruction and Disposal

- Added information on the remaining PFAS Innovative Treatment Team (PITT) outputs in Section 5.a.
- Revised information on the technology evaluation framework in Section 5.b to incorporate suggestions from public comments received on the 2024 interim guidance.

Appendix A: EPA Guidance to Conduct PFAS Emissions Field Testing at Commercial Thermal Destruction Sources

- Expanded the list of inlet, intermediate, and outlet streams for sample collection and analysis to include process materials.
- Clarified the target analytes to include in an air emission source characterization.

Appendix B: Summary of the 2021 and 2022 Clean Harbors Test Data

- Added the test years (2021 and 2022) for the two source test campaigns conducted at an HWC to the appendix title.

Appendix C: Summary of the Chemours Thermal Oxidizer Test Data

- Expanded the table of destruction and removal efficiency (DRE) values to include the results for 2021, 2023, and 2024 from the test campaign conducted at a chemical manufacturer.

Appendix D: Summary of Costs and Considerations

- Updated the cost estimates for end-of-life management of PFAS materials by thermal treatment, landfilling, and underground injection from 2022 to 2024 dollars.

Appendix E: Fact Sheet for the 2026 Interim Guidance on the Destruction and Disposal of PFAS

- Added the fact sheet for the 2026 interim guidance document.

1.a Purpose and audience of this interim guidance

The purpose of this interim guidance is to describe technologies for managing the destruction and disposal of PFAS-containing materials and to recommend practices associated with these technologies that minimize PFAS releases to the environment. This purpose is consistent with EPA's mission to protect human health and the environment and the NDAA for Fiscal Year 2020, Public Law No: 116-92 (hereafter, "FY 2020 NDAA"), signed into law on December 19, 2019.

Section 7361 of the FY 2020 NDAA (see Figure 1-1) directs EPA to publish interim guidance on the destruction and disposal of PFAS and PFAS-containing materials no later than one year from the date of enactment of the FY 2020 NDAA. EPA published the initial interim guidance on December 18, 2020. The FY 2020 NDAA also requires EPA to review and revise the interim guidance at least every three years, if appropriate. The previous update was published in April 2024. On April 28, 2025, the EPA Administrator committed to changing the frequency of the interim guidance updates from every three years to annually as EPA continues to assess the effectiveness of available treatment technologies. This 2026 update fulfills that commitment.

The primary audience of this guidance are decision-makers who need to identify effective means for destroying or disposing of PFAS-containing materials and wastes based on their site-specific conditions, costs and other considerations. The audience may also include regulators, waste managers, and

technology developers who can use the technology evaluation framework in Section 5 to evaluate the applicability of emerging technologies to PFAS destruction or disposal. This guidance may also interest the general public, particularly communities near PFAS destruction and disposal sites. EPA has developed a fact sheet for the public that provides a more concise and less technical presentation of this guidance (see Appendix E).

SEC. 7361. PFAS DESTRUCTION AND DISPOSAL GUIDANCE.

(a) IN GENERAL.—Not later than 1 year after the date of enactment of this Act, the Administrator shall publish interim guidance on the destruction and disposal of perfluoroalkyl and polyfluoroalkyl substances and materials containing perfluoroalkyl and polyfluoroalkyl substances, including—

(1) aqueous film-forming foam;
(2) soil and biosolids;
(3) textiles, other than consumer goods, treated with perfluoroalkyl and polyfluoroalkyl substances;
(4) spent filters, membranes, resins, granular carbon, and other waste from water treatment;
(5) landfill leachate containing perfluoroalkyl and polyfluoroalkyl substances; and
(6) solid, liquid, or gas waste streams containing perfluoroalkyl and polyfluoroalkyl substances from facilities manufacturing or using perfluoroalkyl and polyfluoroalkyl substances.

(b) CONSIDERATIONS; INCLUSIONS.—The interim guidance under subsection (a) shall—

(1) take into consideration—
(A) the potential for releases of perfluoroalkyl and polyfluoroalkyl substances during destruction or disposal, including through volatilization, air dispersion, or leachate; and
(B) potentially vulnerable populations living near likely destruction or disposal sites; and
(2) provide guidance on testing and monitoring air, effluent, and soil near potential destruction or disposal sites for releases described in paragraph (1)(A).

(c) REVISIONS.—The Administrator shall publish revisions to the interim guidance under subsection (a) as the Administrator determines to be appropriate, but not less frequently than once every 3 years.

Figure 1-1. FY 2020 NDAA Section 7361.

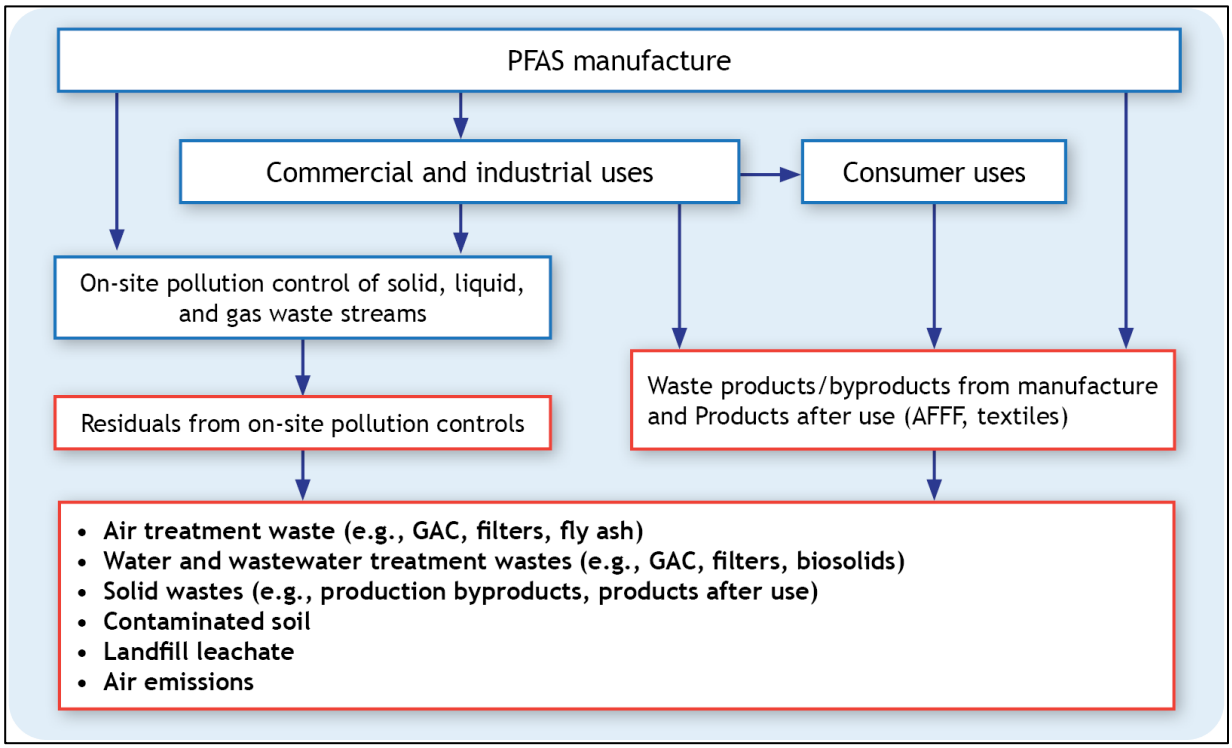
1.b Scope and use of interim guidance

1.b.i PFAS and PFAS-containing materials in this interim guidance

Section 7361 of the FY 2020 NDAA (see Figure 1-1) lists six types of PFAS-containing materials and wastes[3]. Although the information included in this interim guidance is likely to be suitable for other types of PFAS and PFAS-containing materials, this interim guidance addresses destruction and disposal centering on these six material types, which are described in more detail in Section 2. In this document, the PFAS-containing materials covered by the FY 2020 NDAA that will be destroyed or disposed of are referred to as "waste."

Figure 1-2 shows conceptually how manufacturing activities could result in material streams that are within the intended scope of this interim guidance, including GAC and AFFF.

[3] While there is no current EPA consensus definition of PFAS, perfluoroalkyl and polyfluoroalkyl substances can be generally characterized as a very large group of synthetic chemicals that due to their strong carbon-fluorine bonds, can be very persistent in the environment. EPA's ***CompTox Chemicals Dashboard*** maintains a master list of more than 12,000 chemicals that are potential PFAS, including 10,200 chemicals containing a specific defined structure and an additional 1,300 without explicit structures (polymers and other chemical substances of unknown or variable composition).



Note: The red-outlined portions show where the FY 2020 NDAA material types occur during manufacture, use, and disposal of PFAS and PFAS-containing materials that are within the scope of this interim guidance.

Figure 1-2. Generation of PFAS materials identified in the FY 2020 NDAA.

1.b.ii Destruction and disposal technologies addressed in this interim guidance

This interim guidance presents three widely-used, commercially available destruction and disposal technologies that may be effective for managing PFAS and PFAS-containing materials. Advances in scientific understanding allow EPA to make some recommendations; however, filling information gaps and addressing uncertainties will improve the scientific understanding and could lead to additional guidance in the future.

This interim guidance describes technologies with the potential to destroy PFAS or control releases of PFAS into the environment. Other waste management options are not discussed, because they are not within the intended scope of this interim guidance. For example, land application of biosolids containing PFAS does not control releases of PFAS into the environment.

Current technologies. In developing this third version of the interim guidance, EPA focused on the three widely-used, commercially available destruction and disposal technologies that may be effective for managing PFAS wastes: thermal treatment, landfills, and underground injection. EPA also focused on reviewing recent data and information about the ability of each of these technologies to manage PFAS-containing materials through containment or destruction (i.e., breaking carbon–fluorine bonds and mineralization) while limiting releases of PFAS to the environment. In doing so, EPA prioritized addressing the scientific issues and uncertainties highlighted in the 2024 interim guidance and, when possible, relied on publicly available, peer-reviewed data and information (see Section 3).

Emerging technologies. Concerns about the effectiveness, availability, capacity, or cost of current, widely-used technologies for managing PFAS and PFAS-containing materials have motivated efforts to develop new technologies and apply existing technologies to PFAS materials. EPA is aware of and, in some cases, has participated in efforts to identify, develop, and test non-thermal destruction technologies for PFAS-containing materials (see Section 5).

Note that EPA is aware of additional information and technologies that were not considered for evaluation and are not mentioned in this guidance. EPA encourages continued collaboration with other government, academic, and private institutions to continue conducting research to better understand destruction and disposal of waste containing PFAS, and to be potentially included in future iterations of this guidance. EPA encourages technology developers to generate and publicly release data that can be reviewed, and to ensure the data are consistent with the tenets of gold standard science established in Executive Order 14303 (*Restoring Gold Standard Science*[4]).

In some cases, managers of PFAS and PFAS-containing materials may consider emerging technologies for PFAS destruction. When considering the use of emerging technologies, managers of PFAS-containing materials should work with state, territorial, or Tribal government agencies. To assist PFAS material managers with evaluating whether an emerging destruction (or disposal) technology is suitable for a particular PFAS-containing material, EPA developed a technology evaluation framework (see Section 5). The framework provides a transparent, consistent approach for evaluating destruction and disposal technologies for PFAS materials.

In 2026, EPA is neither recommending nor discouraging the use of any emerging technology for managing the PFAS-containing materials specified in the FY 2020 NDAA. Instead, the Agency recommends that managers of PFAS-containing materials use the technology evaluation framework to evaluate emerging destruction and disposal technologies and to inform their decisions about managing PFAS-containing materials. EPA also encourages technology developers to generate and publicly release data that can be used to complete the framework (see Section 5).

Generally, destruction and disposal activities for PFAS-containing materials are currently not federally regulated. PFAS-containing materials may be managed in non-hazardous and hazardous waste treatment and disposal systems. Some exceptions exist, such as risk management requirements in cases where permits for production of new chemicals specify destruction or disposal methods following the review of a new chemicals notice under TSCA. Unless governing regulations require specific actions to the contrary, this guidance recommends selecting an approach that minimizes the potential for releases of PFAS into the environment.

Table 1-1 presents examples of the PFAS destruction and disposal technologies discussed in this guidance document for materials in solid, liquid, and gas phases.

4 Available at: https://www.govinfo.gov/content/pkg/FR-2025-05-29/pdf/2025-09802.pdf.

Table 1-1. Destruction and Disposal Technologies Discussed in This Interim Guidance, with Examples of PFAS-Containing Materials

Destruction and Disposal Technology, by Physical Phase of Materials	Examples of PFAS-Containing Materials (within the Scope of the FY 2020 NDAA) That Could Be Managed Using These Technologies

Solid phase:
Landfill disposal.
Thermal treatment.
	• Drinking water, groundwater, and wastewater treatment residuals:
	  ○ Biosolids/sewage sludge.
	  ○ Spent GAC.
	  ○ Ion exchange resins.
	• Air waste stream treatment residuals:
	  ○ Spent GAC.
	  ○ Fly ash.
	• Contaminated soil.
	• End-of-life products (e.g., textiles).
	• Solidified liquid wastes.

Liquid phase: Underground injection. Thermal treatment.	• Sewage sludge (liquid). • Landfill leachate. • AFFF (spent or concentrate). • End-of-life products (e.g., spent cleaning solvents). • Pollution control residuals (e.g., concentrates) from PFAS production and use.
Gas phase: Thermal treatment.	• LFG. • Emissions from manufacture, use, or destruction of PFAS.

1.b.iii Use of this interim guidance

This interim guidance provides information and suggested considerations for evaluating destruction and disposal options. This third version of the interim guidance is based on recent research and information available in 2025 and is responsive to the scope of the FY 2020 NDAA. This interim guidance presents background information on the manufacture and uses of PFAS, as well as solid, liquid, and gas waste streams containing PFAS, including those materials identified in the FY 2020 NDAA: AFFF, soils and biosolids, textiles, spent water treatment materials, landfill leachate, and PFAS waste streams from facilities manufacturing or using PFAS (see Section 2). Most significantly, it provides up-to-date information on potential releases during the destruction and disposal of PFAS and PFAS-containing materials (see Section 3) and identifies data gaps to be filled that can inform future EPA guidance (see Section 4). This version also incorporates information received during the public comment period for the 2024 interim guidance document.

This version of the interim guidance:

- Is based on currently available information on technology performance and capabilities for destruction and disposal of the PFAS and PFAS-containing materials specified in the FY 2020 NDAA (see Section 1.b.i).

- Focuses on three widely-used, commercially available destruction and disposal technologies that may be effective for managing PFAS wastes (i.e., thermal treatment, landfills, underground injection) (see Section 3).
- Identifies research needs to inform future updates to this interim guidance (see Section 4).
- Discusses the development of emerging technologies and includes a framework for evaluating technology/PFAS material combinations (see Section 5).
- Does *not* establish what concentrations of PFAS in wastes, spent products, or other materials or media would necessitate destruction or disposal. Regulatory mechanisms are more appropriate for establishing such concentrations.
- Provides references to destruction and disposal facility locators that decision-makers can use to request cost estimates. EPA recommends obtaining cost estimates from applicable destruction and disposal facilities as described in Section 1.c.ii. In addition, Appendix D provides some cost information that PFAS material managers may want to consider when evaluating different techniques to destroy or dispose of PFAS and PFAS-containing materials. Note that cost information presented in this appendix is associated with a relatively high level of uncertainty. Moreover, the cost of PFAS destruction and disposal may change based on demand and evolution of available technologies. Additionally, overall costs may vary widely based on the type of materials or the distance to a facility.

This interim guidance is not a regulation, is non-binding, and does not pose any specific requirements on users. Discussion in this guidance of EPA's regulatory authorities is for the purpose of describing controls relevant to the destruction or disposal of PFAS and should not be considered a description of the applicability of those authorities or affect EPA's or any other agency's ability to enforce under these authorities in the future.

1.c Considerations for managing PFAS and PFAS-containing materials

The information presented in this interim guidance can help managers of PFAS or PFAS-containing materials make informed decisions regarding destruction and disposal. To assist decision-makers, EPA reviews destruction and disposal options for thermal treatment, landfills, and underground injection based on the relative potential for releasing PFAS to the environment. This approach highlights destruction and disposal options with lower potential for PFAS releases to the environment compared to other options. Also, this approach is consistent with EPA's mission to protect human health and the environment and is designed to facilitate decisions that protect human health and the environment as much as possible.

EPA recognizes that the potential to control PFAS releases to the environment is one of several factors to consider when selecting among options for destroying or disposing of PFAS and PFAS-containing materials in a way that is consistent with maximizing protection to public health and the environment. Other factors include whether it is imperative to destroy or dispose of the waste immediately, the availability of interim storage or destruction and disposal options, the type and volume of waste materials, the concentrations of PFAS in the waste, and the presence of and potential impacts to nearby communities. In the following sections, EPA provides guidance for these decisions in terms of technology considerations and infrastructure considerations.

1.c.i Technology considerations - Relative potential for PFAS releases to the environment

Using the information presented in Section 3, and considering the significant uncertainties that remain, EPA listed destruction and disposal options in terms of relative potential for releasing PFAS to the environment. When considering the data and information presented in Section 3, EPA considered the type of data available (e.g., laboratory-, pilot-, or field-scale studies), the amount of data available (e.g., data from different operating conditions), and the quality of the data.

When considering the order, it is important to note that available performance and testing data are still generally limited. Additional performance and testing data—including data on destruction and removal of PFAS in thermal treatment devices, and long-term performance data for landfills and underground injection—are needed to support more specific guidance. When these data and information become available, EPA may reconsider and revise the order presented below.

EPA is not endorsing any technology, but it is presenting the technologies' relative potential to minimize PFAS release; EPA encourages those that minimize PFAS release; managers should choose based on site specific conditions. As a general approach, EPA encourages managers of PFAS and PFAS-containing materials to prioritize destruction and disposal options with a lower potential for releasing PFAS to the environment over the use of destruction and disposal technology options with a higher potential for environmental release of PFAS given similar costs or other considerations. EPA also encourages testing with EPA-approved methods of thermal treatment operations, including PICs and the presence of PFAS in all associated waste streams (see Appendix A), to evaluate whether site-specific conditions are minimizing potential environmental releases.

While the purpose of an effective destruction and disposal technology is to prevent or minimize environmental releases, it is also important to distinguish between a potential environmental release and direct human exposure and risk.

EPA encourages managers of PFAS and PFAS-containing materials to consider the factors discussed in this guidance and select current or future disposal or destruction technologies that protect human health and the environment as much as possible. Based on currently available information cited in this guidance, EPA has listed the following disposal and destruction options from lowest to highest based on their relative potential to release PFAS to the environment:

- **Interim storage with controls.** While not a destruction or disposal method, interim storage may be a short-term option, if the destruction or disposal of PFAS and PFAS-containing materials is not imperative, on-site storage capacity is readily available, and interim storage has proper controls in place to reduce releases into the environment. Storage may be more appropriate for some PFAS materials than others. For example, EPA recommends interim storage of low volumes of containerized or high PFAS-concentration materials. In contrast, some materials may be less appropriate for storage because they are continuously generated or are typically high volume. With proper engineering controls in place, interim storage can control PFAS releases in the short term.

- **Underground injection (Class I non-hazardous industrial and hazardous waste wells).** EPA has determined the use of Class I non-hazardous industrial waste and hazardous waste wells for high-concentration liquid PFAS waste has a lower potential for environmental release when

compared to other PFAS destruction and disposal options, and there is relatively low uncertainty in this determination. The standards associated with the construction, operation, and monitoring of these Class I wells are designed to isolate liquid wastes deep below the land surface and ensure protection of USDWs. Operators of Class I hazardous waste wells must conduct mechanical integrity testing more often than Class I non-hazardous industrial waste operators. They also must establish and follow additional procedures for reporting and correcting mechanical integrity problems. Class I hazardous waste well operators must also develop and follow a waste analysis plan and conduct annual tests of the cement in place at the base of the well. While the standards associated with Class I hazardous wells are more stringent than for non-hazardous industrial wells, EPA has determined that the probability of failures for both has been demonstrated to be low. While Class I wells are an option for managing PFAS-containing fluids, this technology may not be appropriate everywhere. The limited number of wells currently receiving PFAS, well location, and waste transportation logistics may significantly limit the type and quantity of PFAS-containing fluids appropriate for underground injection. Additional Class I wells may need to be constructed where geologically suitable, and existing well permits may need to be modified to meet the capacity needs for PFAS disposal (see Section 3.c.v).

- GAC reactivation units with thermal oxidizers operating under certain conditions (applies only to GAC used for PFAS removal from drinking water treatment systems). GAC reactivation systems, with the associated use of off-gas thermal oxidizers (i.e., afterburners operating at temperatures greater than 1,100°C[5]) and gas scrubbing units, can potentially destroy PFAS without significant environmental releases, or without significant PFAS remaining on the reactivated carbon. However, as discussed in Section 3.a.i, more data are needed for confirmation, particularly regarding reactor operating conditions, differing GACs, evaluating GAC used for industrial processes and site remediation, and further characterization of PICs and PIDs. There is uncertainty associated with this option because it is based on limited research. While recent studies have improved understanding, additional research is needed to resolve uncertainties on PFAS emissions from full-scale reactivation facilities (see Section 3.a.v).
- **Thermal treatment units operating under certain conditions.** HWCs, including commercial incinerators, cement kilns, LWAKs, and thermal oxidizers, that operate under certain conditions (i.e., feeding liquid PFAS-containing materials, higher temperatures greater than 1,100°C[4], well mixed, and adequate residence time) may be more effective at adequately destroying (mineralizing) PFAS and minimizing PICs. There is uncertainty associated with this option because it is based on limited research and observations, there are limited data for the treatment of solid materials or containerized wastes, and there are limited emissions data (e.g., few test reports containing data for PIC formation and the presence of PFAS in air pollution control device [APCD] residuals) at full-scale facilities (see Section 3.a.v) due to previous methodology limitations. Site-specific considerations impact the ability of any thermal treatment unit to destroy PFAS; therefore, EPA encourages site-specific testing (see Appendix A) to determine if a particulate thermal treatment unit is suitable.
- **Hazardous waste landfills (RCRA Subtitle C).** This class of landfills has the most stringent environmental controls in place for minimizing environmental releases and migration of some

[5] Maximum PIC destruction was observed at and above 1,090°C as described by Shields et al., 2023.

PFAS from disposed waste. EPA recommends Subtitle C landfills when PFAS levels of the waste are relatively high and landfill disposal is the selected option. Permitted hazardous waste landfills have relatively low quantities of landfill leachate[6], which lessens the probability of mobilizing PFAS that is reversibly bound to the waste. These landfills are required to handle and treat leachate as listed hazardous waste (hazardous waste number F039 per 40 Code of Federal Regulations [CFR] 261.31), lessening the likelihood of environmental release. Because hazardous waste landfills generally do not receive organic wastes that decompose, PFAS migration via LFG is anticipated to be relatively low but LFG collection and destruction equipment could be installed if anaerobic decomposition is demonstrated (see Section 3.b). While recent studies have improved understanding of PFAS migration, additional research is needed to resolve uncertainties (see Section 3.b.vi).

- **Landfill disposal of stable polymeric PFAS (all landfill types)**. Stable polymeric PFAS, such as polytetrafluoroethylene (Teflon™) and fluorinated ethylene propylene (FEP), are large molecules that are not susceptible to hydrolysis or oxidation. They are neither volatile nor water soluble and, as a result, they are expected to remain within the waste mass for long periods of time. This PFAS category tends not to migrate with the leachate and is unlikely to volatilize with the LFG; therefore, permitted hazardous and MSW landfills may provide a good disposal option. Some data are available to support this assessment, but more data are needed to resolve uncertainties (see Sections 3.b.i and 3.b.vi).

- Disposal of volatile or water soluble PFAS in MSW landfills that have composite liners and leachate and gas collection and treatment systems. MSW landfills receive non-hazardous waste and most have composite liners and gas collection systems. Soluble PFAS in these landfills are likely to be released over time in landfill leachate, if they are not adequately bound or sequestered in immobile solids. Further research is needed on the ability of various solidification techniques to retain soluble PFAS for extended periods under the environmental conditions found in MSW landfills. Although MSW landfills generally have leachate collection and management processes in place, the typical practices employed are not expected to prevent further PFAS migration. In addition, MSW landfills generally receive significant quantities of organic wastes (e.g., food scraps) that decompose and thereby can cause PFAS migration via LFG. Multiple studies have reported elevated levels of PFAS in leachate and LFG. Studies estimate that up to 15 percent of mobile PFAS disposed in MSW landfills is emitted to the environment over time. EPA does not recommend MSW landfill disposal of media containing relatively high concentrations of soluble or volatile PFAS. While recent studies have improved understanding of PFAS migration in MSW landfills, additional research is needed to resolve uncertainties (see Section 3.b).

- **Thermal treatment at lower temperatures** including municipal waste combustors (MWCs), SSIs, or HWCs operating at lower temperatures or feeding solid or containerized PFAS materials. Some HWCs operate at temperatures near or below 1,100°C, while MWCs typically operate between 850 and 1,000°C, and SSIs typically operate at even lower temperatures. There is uncertainty associated with this option because it is based on very limited research. Limited data indicate the possibility of air emissions of some PFAS and PICs above the detection limits at

[6] EPA defines landfill leachate as the liquid formed when rainwater filters through wastes placed in a landfill. When this liquid contacts buried wastes, it leaches, or draws out, chemicals or constituents from those wastes (*https://www.epa.gov/landfills/municipal-solid-waste-landfills*).

lower temperatures (Shields et al., 2023). No data are available regarding the treatment of PFAS-containing solid materials or containerized wastes at lower temperatures. There are currently insufficient data and information to conclude anything about PIC formation or PFAS air emissions from full-scale combustion units operating at these lower temperatures or when feeding solid materials or containerized wastes. Because insufficient data are available, there is low confidence in the reliability of this technology to control PFAS releases (see Section 3.a).

- **Disposal of volatile, water soluble, or oxidizable PFAS in construction and demolition (C&D) landfills.** This class of landfills may not have a composite liner or leachate collection system. Because of the potential for environmental releases, EPA does not recommend that PFAS waste, other than wastes containing stable polymeric PFAS, be disposed of in this type of landfill. While few studies have documented emissions of PFAS from C&D landfills, the known mobility of PFAS in landfills and lack of engineered controls at C&D landfills make them unsuitable for PFAS disposal. For these reasons, EPA does not expect that C&D landfills will minimize PFAS releases to the environment (see Section 3.b).

1.c.ii Infrastructure considerations – Communities, costs, and capacity

Waste management infrastructure in the United States is a complex and varied system, crucial for managing the vast amounts of waste generated, including PFAS-containing waste. Therefore, in addition to considering potential for environmental release, decision-makers should consider site- or project-specific factors that may influence decisions about managing PFAS-contaminated wastes. Specific factors include, but are not limited to, the composition of the waste, the types and concentrations of PFAS, transportation costs, destruction and disposal costs, and potential impacts to communities.

EPA emphasizes the importance for decision-makers (managers of PFAS-containing materials making decisions about destruction and disposal) to consider potential impacts to nearby communities when evaluating destruction, disposal, and storage options. In deciding how to address PFAS waste, site-specific considerations and waste characteristics should factor into the decision.

The waste management infrastructure's design and operation are heavily influenced by regional needs, local regulations, and technological advancements. As a result, waste management options vary by the type of waste and by location due to factors like availability, feasibility, and transportation requirements. For instance, urban areas may incur higher disposal costs due to limited space while rural areas may face higher transportation costs due to distant disposal facilities. Furthermore, technological advances specifically related to the management of PFAS-containing materials can potentially reshape the cost and operational landscape of PFAS waste management. EPA recommends that decision-makers request cost estimates directly from potentially applicable destruction and disposal facilities. In addition, decision-makers can refer to Appendix D, which summarizes the estimated costs and factors to consider when evaluating technologies for destroying or disposing of PFAS and PFAS-containing materials.

Decision-makers seeking lists of waste management sites in the United States can access various resources depending on the type of waste and location. It is important to note that often the most direct source is local government websites, such as county or city environmental services or public works departments. These websites typically provide comprehensive lists and maps of local facilities, along with their types, hours of operation, and accepted waste materials. For specialized waste like hazardous or medical waste, state environmental protection agencies or EPA are valuable resources.

EPA websites, in particular, offer databases and tools such as *RCRAInfo*[7], which provides detailed information about facilities managing hazardous waste.

A general yet incomplete list of potential treatment and disposal facilities may also be available through the EPA Incident Waste Decision Support Tool (*I-WASTE DST*[8]). This list includes landfills (hazardous waste, MSW, industrial, and C&D), HWCs, and nonhazardous combustors (sewage sludge and municipal waste), but does not include all technologies available for PFAS destruction and disposal (e.g., cement kilns, carbon reactivation facilities, and underground injection facilities). *I-WASTE DST* is updated periodically and is limited to information available online.

Similar to cost estimation, developing management capacity estimates can also be challenging. Facilities that can potentially manage PFAS-containing waste are not evenly distributed and their number and capacity vary by region. Some areas across the United States face challenges due to limited available landfill space or capacity limits. Incineration and underground injection capacities can also be greatly influenced by technological capabilities and local regulations.

[7] Available at: *https://enviro.epa.gov/envirofacts/rcrainfo/search*.

[8] Available at: *https://iwaste.epa.gov/*.

2. Description of PFAS-Containing Materials Identified in the FY 2020 NDAA

The FY 2020 NDAA identifies six types of materials that commonly contain PFAS (see Figure 1-1 and the Executive Summary). This section discusses each FY 2020 NDAA material type, its origin, potential sources of PFAS, current disposal and treatment methods, and potential releases to the environment.

What's New in Section 2 of the 2026 Guidance Document?

- Information on updates and planned revisions to the PFAS 8(a)(7) reporting rule under TSCA (88 FR 70516, October 11, 2023).
- Updated biosolids generation and management information.
- Updated information on the PFAS National Primary Drinking Water Regulations.

Data on FY 2020 NDAA-relevant material types come from a variety of sources, and more PFAS data will be available from EPA-managed datasets in the near future. For example, EPA's Chemical Data Reporting (CDR) dataset includes production volumes for manufactured and imported amounts and conveys certain industrial processing and use activities. One CDR data element distinguishes the amounts that are recycled instead of discharged or released to a waste stream. In the 2020 CDR reporting cycle, submitters began using Organization for Economic Cooperation and Development (OECD)-based industrial processing and use codes to better harmonize those data (U.S. EPA, 2020a).

Additionally, EPA's *Toxics Release Inventory (TRI) program*[9] collects data related to industrial releases and waste management of certain chemicals. The TRI program covers many, but not all, industry sectors and chemicals. Additionally, some facilities with a covered sector may not meet TRI reporting criteria. The TRI dataset includes, among other information, quantities of environmental releases to all media above certain thresholds (including on-site disposals and land application), as well as quantities transferred to off-site waste management facilities, including POTW. Under Section 7321 of the FY 2020 NDAA, certain PFAS were added to the TRI list beginning with Reporting Year 2020 (U.S. EPA, 2020b). As of Reporting Year 2023 (reporting forms due by July 1, 2024), a total of 189 PFAS are on the TRI list. TRI reporting data can be accessed at the *TRI Toolbox*[10].

In October 2023, EPA finalized the PFAS 8(a)(7) reporting rule under TSCA (88 FR 70516, October 11, 2023). In accordance with obligations under TSCA, as amended by the FY 2020 NDAA, the PFAS 8(a)(7) reporting rule requires any person that manufactures (including imports) or has manufactured (including imported) PFAS or PFAS-containing articles in any year since January 1, 2011, to electronically report information regarding PFAS uses, production volumes, disposal, exposures, and hazards. The PFAS 8(a)(7) rule will help EPA better understand and develop the most comprehensive dataset of PFAS sources and quantities manufactured in the United States. In May 2025, EPA announced an interim final rule which extended the dates of the reporting period for data submissions to April 13, 2026,

[9] Available at: *https://www.epa.gov/toxics-release-inventory-tri-program/what-toxics-release-inventory*.

[10] Available at: *https://www.epa.gov/toxics-release-inventory-tri-program/tri-data-and-tools*.

through October 13, 2026. On November 13, 2025, EPA proposed revisions and exemptions to the PFAS 8(a)(7) reporting rule and also proposed to amend the start date of the submission period to 60 days after the effective date of a future final rule. On April 13, 2026, EPA published a final rule that amended the submission period for the PFAS 8(a)(7) reporting rule to begin on January 31, 2027, or 60 days following the effective date of a forthcoming final action, whichever date is earlier. This final rule solely amends the start date of the data submission period; the proposed revisions in the November 2025 proposed rule will be addressed in a future final rule.

2.a Solid, liquid, or gas waste streams containing PFAS from facilities manufacturing or using PFAS

PFAS do not occur naturally. They are synthesized for use in a diverse array of industrial and commercial applications and may be released into the environment at every step in the production process, including during synthesis, polymerization, application, transport, usage, waste stream management, and disposal (3M Company, 2000b). Industrial waste streams containing PFAS stem from two main sources: (1) primary manufacturing facilities of PFAS, and (2) secondary industries that use PFAS or manufacture finished products that contain PFAS. A 2009 survey by OECD identified 27 primary manufacturers and processors of PFAS globally (OECD, 2011). At the time of that survey, more than 90 percent of the global annual production of PFAS was generated by eight manufacturers (Posner et al., 2009), all of which participated in EPA's 2010/2015 Perfluorooctanoic Acid (PFOA) Stewardship Program (U.S. EPA, 2006).

The goal of the PFOA Stewardship Program was to reduce PFOA facility emissions and their use in products by 95 percent by 2010, compared with 2006 baseline levels, and to eliminate PFOA from all facility emissions and products by 2015 (U.S. EPA, 2006). Companies that did not participate in the PFOA Stewardship Program likely continue to use these chemical substances. Long-chain PFAS and their precursors may still be produced as unintentional byproducts and may persist in facility emissions and as product impurities in small quantities (3M Company, 1999; Boucher et al., 2019; Lehmler, 2009; Kissa, 2001). Perfluoroalkyl sulfonic acids containing six or more carbons and perfluoroalkyl carboxylic acids with seven or more carbons are generally considered to be long-chain PFAS. PFOA and PFOS are examples of long-chain PFAS, and perfluorobutanesulfonic acid (PFBS) and PFHxA are examples of short-chain PFAS. Furthermore, information on the toxicity and environmental fate and transport of alternative PFAS chemistries is limited (Sun et al., 2016; Wang et al., 2014).

For the 2020 CDR reporting cycle, 52 sites reported a total domestic manufacture and import production volume of 339,000 tons for PFAS in 2019. This represents the aggregate production volume for PFAS produced and imported into the United States across all industries. Although the aggregate production volume might not include all PFAS sources (for instance, a specific chemical or site may not meet reporting obligations), it provides a proxy quantity of all PFAS domestically produced and imported.

Table 2-1 lists examples of PFAS uses and the resulting solid, liquid, and gas waste streams for primary and secondary manufacturers of PFAS-containing materials and certain service sectors as indicated by industry, national and global inventories, and research. However, this list is not exhaustive or representative of all current uses, applications, recovery and recycling practices, or treatment technologies that could affect the volume and characteristics of the resulting waste streams. EPA recognizes the need for continued research to better characterize the multi-media PFAS-containing materials targeted for destruction or disposal, as discussed in Section 4.a.

2.a.i Solid-phase wastes

Primary manufacturing and secondary industrial use of PFAS can generate solid waste streams with PFAS-containing materials (OECD, 2011, 2015). For example, some PFAS synthesis processes can produce tars and polymers consisting of high-molecular weight byproducts that are either fully or partially fluorinated. These byproducts can be recycled back into the process or treated as waste (3M Company, 2000a, 2000b). Solid wastes such as fly ash (e.g., some thermal treatment) or spent GAC (e.g., drinking water treatment) can also be produced from PFAS incineration and other treatment processes.

Other important solid-phase materials include sludges and biosolids (see Section 2.c) that result from stabilizing or treating process waters and wastewaters, either on site or at a municipal wastewater treatment plant (WWTP)[11] that receives influent from industrial sources (Venkatesan and Halden, 2013). In addition to solids produced via treatment, spent water treatment media (such as activated carbon and ion exchange resins) are part of this waste stream (see Section 2.e). Other direct industrial sources of solid wastes containing PFAS include intentional residuals, such as cuttings and fibers from textile manufacturing (see Section 2.d), and materials unintentionally produced outside of product specification. The concentrations and composition of PFAS in solid wastes generated from primary and secondary industrial sources vary by facility and depend on factors such as facility- or industry-specific production processes and the types and quantities of PFAS produced or used (ITRC, 2020).

The characteristics of these solid-phase, PFAS-containing wastes vary significantly, depending on several factors. As discussed in Section 3.b, the type of PFAS (e.g., polymeric, soluble, or volatile PFAS) as well as the type of waste with which the PFAS is comingled (e.g., biodegradable or non-biodegradable) dictates the potential and pathways for environmental release. The type of environmental release will, in turn, inform the most appropriate disposal option(s) for each type of PFAS-containing solid waste.

2.a.ii Liquid-phase wastes

Primary manufacturers and secondary industrial users of PFAS can generate liquid-phase wastes in the form of: (1) liquid byproducts of PFAS synthesis to be recycled or disposed of (e.g., to a landfill) following stabilization, solidification, or another management method; (2) wastewater resulting from activities using PFAS as a processing aid (e.g., surfactant, emulsifier, mist suppressant, sizing agent) and production of finished products containing PFAS; (3) spills or unintentional releases of liquid wastes and products containing PFAS; and (4) AFFF/water/foam mixtures from the use of fire-extinguishing agents (see Section 2.b) for emergency response activities and emergency response training activities at industrial facilities. Table 2-1 provides examples of liquid wastes containing PFAS generated by industrial sources and their uses. This is not an all-inclusive list of industries nor waste streams.

Another liquid-phase waste stream is wastewater effluent discharged directly from a primary manufacturer or secondary industrial PFAS user. Effluent from wastewater treatment facilities that receive wastewater from industrial PFAS sources may also contain PFAS. According to several studies, conventional wastewater treatment technologies are generally ineffective at destroying or controlling PFAS (Schultz et al., 2006) and may result in higher measurable PFAAs (e.g., PFOA, perfluorooctane sulfonate [PFOS], and their homologues) when precursor compounds (e.g., fluorotelomers) are degraded during the treatment process (Buck et al., 2011; Dauchy et al., 2017a; Schultz et al., 2006;

[11] Regulations at 40 CFR part 122.2 and part 403.3 identify these “municipal wastewater treatment plants" as treatment works treating domestic sewage (TWTDS) or publicly owned treatment works (POTW), dependent on their ownership.

Sinclair and Kannan, 2006). Less often, primary industrial PFAS manufacturers have opted to transport liquid wastes off site for incineration (North Carolina Department of Environmental Quality, 2017).

2.a.iii Gas-phase wastes

Studies suggest that PFAS in air emissions from manufacturing facilities are a source of both localized (i.e., within a short radius of the facility) and long-distance (i.e., global) transport of PFAS within the environment (Davis et al., 2007; Dreyer et al., 2009). Non-volatile forms of PFAS, such as the anionic PFAAs (e.g., PFOA, PFOS, their homologues), are associated with airborne particulates when emitted as aerosols from stack emissions at primary manufacturing facilities (Barton et al., 2006; Dreyer et al., 2015). Gas phase emissions of volatile and semivolatile PFAS, and the subsequent transformation of precursor compounds into persistent PFAAs, are a potential mechanism for the atmospheric transport of PFAS. For example, volatile fluorotelomer alcohols (FTOHs) and perfluoroalkyl sulfonamides can transform into perfluoroalkyl carboxylic acids (e.g., PFOA and homologues) and perfluoroalkyl sulfonates (e.g., PFOS and homologues) which can be deposited at significant distances from their origin, and may result in soil and groundwater contamination (Dreyer et al., 2009; Ellis et al., 2004; Martin et al., 2006; Schenker et al., 2008).

Table 2-1. Examples of PFAS Waste Streams by Industry Type.
This is not an all-inclusive list of industries or waste streams

Industry Type	Uses	Examples of Waste Streams[a]			Source
		Solid	Liquid	Gas	
Primary chemical manufacturing	PFAS synthesis, feedstocks for primary products, feedstocks for secondary users, processing aids (fluoropolymers).	Process byproducts (tars), sludges/residuals, off-spec materials[b], treatment residuals (GAC/anion exchange resins), spill residues (replacement and legacy), *particulate emissions.*	Degraded/stabilized process wastes, wastewater effluent, *stack emissions condensate.*	Stack emissions, fugitive volatiles.	3M Company (1999, 2000b)
Secondary Manufacturing (Industry Users of PFAS-Containing Materials)[c]					
Adhesives manufacturing	Component of solvent- and water-based adhesives, rubber to allow bonding to steel, and urea-formaldehyde adhesive resins for wood particleboard bonding.	*Used filter media and filter residues, residues of cured adhesives, empty containers, used shop rags (from cleaning), contaminated soil (from spill cleanup residues).*	*Residues of liquid adhesives, off-spec products[b], contaminated wastewater (from spill cleanup residues).* *For cleaning: equipment startup, cleaning, and flushing wastes; spent cleaning solvents; and contaminated wastewater.*	Stack emissions, fugitive volatiles.	ASC (n.d.), RadTech International North America (2010)

Industry Type	Uses	Examples of Waste Streams[a]			Source
		Solid	Liquid	Gas	
Cleaning product manufacturing	Component of household cleaners; car wash products; automobile waxes; wiper fluid; strongly acidic or basic cleaners for concrete, masonry, airplanes. Used for removal of adhesives, dry cleaning of textiles or metal surfaces, machine parts cleaning after nickel plating, and removal of calcium sulfate from reverse osmosis membranes.		*Off-spec products[b], liquid residues from empty containers, and spills.*	Stack emissions, fugitive volatiles.	3M Company (1999)
Computers/ electronics manufacturing	Component of sealant for electric circuits, zinc battery electrolyte, wetting agents in solders, polar solvents used before welding. Used for removal of cured epoxy resins from integrated circuit modules, treatment of insulated wire, alkaline manganese battery MnO_2 cathode treatment, production of polymer electrolyte membrane for fuel cells, cleaning of electronic components, and coating of the surface of magnetic recording devices.	*Collected airborne particulates for cleaning/surface preparation.*	*Spent acid solution for cleaning/surface preparation, liquid residues from empty containers, and spills.*	Stack emissions, fugitive volatiles.	U.S. EPA (1990)
Film/ lithography manufacturing	Used in coatings for surface tension, static discharge, and adhesion control for films, papers, and printing plates, and as a surfactant in mixtures used to process imaging films.	Rags and wipes discarded by applicator; solids coated with PFAS from processing, sampling, quality assurance; off-spec products.[b]	Solvent waste, liquid residues from empty containers, spills, and unused application mix.	Coating application exhaust	3M Company (1999), Bowden et al. (2002)

Industry Type	Uses	Examples of Waste Streams[a]			Source
		Solid	Liquid	Gas	
Metal plating/ fabrication	Used as a surfactant, wetting agent, and mist suppressing agent; as a wetting agent fume suppressant for chromium plating and chromium anodizing; as a dispersion product used to coat metals; as a blocking agent for aluminum foil; in plating baths; and to treat metal surfaces.	*Off-spec products.*[b]	Spent plating or etching baths, rinse water effluent, liquid residues from empty containers, and spills.	Stack emissions, fugitive volatiles.	3M Company (1999), U.S. EPA (2009b)
Oil and gas drilling/ extraction/ refinery/ support	Component of chemical barrier used for containing oil spills. Used as a surfactant for recovery in oil/gas recovery wells, a jet fuel/hydrocarbon solvent, and in hydraulic oils. Used as a gasoline/petroleum product evaporation inhibitor in storage tanks in the following forms: a floating layer of cereal grains treated with PFAS, an aqueous layer containing PFAS.		Applied product[d] (oil spills, oil and gas recovery wells), *liquid residues from empty containers, and spills.*		UNEP (2011), Kissa (2001)
Paint/coating manufacturing	Component of coatings, paints, varnishes, dyes, ink jet printer inks, and ski waxes.	*Pigment dust*	*Unused paint products, off-spec products*[b]*, liquid residues from empty containers, and spills.*	*Fugitive volatiles, atomized paint.*	Waste Management and Research Center (1992)

Industry Type	Uses	Examples of Waste Streams[a]			Source
		Solid	Liquid	Gas	
Paper products/ packaging manufacturing	Waterproofing and grease proofing for products including food contact paper (e.g., plates, popcorn bags, pizza box liners, food containers, wraps), non-food contact applications (e.g., folding cartons, carbonless forms, masking papers).	Dusts; *solids coated with PFAS from processing, sampling, quality assurance; off-spec products.*[b]	Spillage, cleanup, and releases during opening, rinsing, and cleaning of PFAS totes.	Fugitive volatiles	U.S. EPA (2009a)
Pesticide/ fertilizer/ other agriculture chemical manufacturing	Pesticide and herbicide ingredients and packaging.	*Particulate emissions*	*Liquid residues from empty containers, spills, off-spec products*[b]*, cleaning of equipment, and process wastewaters.*	*Fugitive volatiles*	U.S. EPA (2023)
Plastic materials/ resins/ rubber product manufacturing	Used to make membranes used in fuel cells; chlor-alkali cells; water, caustic soda, and caustic potash electrolyzers; silicone rubber sealants; composite resins; polytetrafluoroethylene (PTFE, or Teflon); and polyvinylidene fluoride or polyvinylidene difluoride (PVDF). Processing aid for PVDF manufacture, mold-release agent in foam molding, and antiblocking agent for rubbers.	Dusts, spillage (micropowders and resins), cuttings, scrap, debris, and off-spec products[b]; particulate emissions.	*Liquid residues from empty containers, spills, and unused application mix.*	Fumes from PTFE heating (volatile)	Ebnesajjad (2015)
Textiles/ apparel/ leather/ carpets/ fiber manufacturing	Dispersion products that coat fabrics: jackets, shoes, umbrellas, carpets, upholstery, leather, tents, sails.	Solids coated with PFAS from cutting, shearing, packaging, laboratory and color sampling, quality assurance; flakes or dust containing PFAS; *off-spec products.*[b]	Spills, wastewater effluent from product adhering to inside of drum, and unused application mix.	Releases of vapors and aerosols during application of surface treatment and mechanical finishing.	U.S. EPA (2009a)

Industry Type	Uses	Examples of Waste Streams[a]			Source
		Solid	Liquid	Gas	
Aerospace component manufacturing	Mechanical components such as tubing, hoses, and seals; brake and hydraulic fluid additive; wire and cable insulation; used in coating and paint.		*Wastewater effluent*		FluoroCouncil (2019)
Automotive component manufacturing	Mechanical components such as tubing, hoses, and seals; brake and hydraulic fluid additive; anti-mist film on windshields; used in coating/paint; used in coatings or surface treatments of textiles and upholstery.		*Wastewater effluent*		FluoroCouncil (2019), ITRC (2020)
Semiconductor manufacturing	Etching solutions for photolithography, glass etching, plastics etching, fused silica, aluminum; liquid etchant in photo mask rendering.		Spent plating or etching baths, PFOA residues from photoresist developers associated with semiconductor liquid waste streams, liquid residues from empty containers, and spills. Photoresists and antireflective coatings stripped off from semiconductor devices before shipment are present in waste solvent streams.	Photoresists and antireflective coatings stripped off from semiconductor devices before shipment are present in waste gas streams.	Bowden et al. (2002), Tremblay (2015)
Building and construction materials manufacturing	Component of cement and primers used to coat cement mortar; used in wire and cable insulation and coatings for wood particleboards.	*Cuttings and debris, off-spec materials.*[b]	*Wastewater effluent*		Buck et al. (2012), FluoroCouncil (2019), U.S. EPA (2009a)

Industry Type	Uses	Examples of Waste Streams[a]			Source
		Solid	Liquid	Gas	
Mining industry	Surfactant for recovery of metals from ores; used in ore flotation to separate metal salts from soil, electrowinning of metals, and nitrogen flotation to recover uranium.	Contaminated rock from applied product.[d]	Applied product[d]		ITRC (2020)
Medical uses	Video endoscopes; catheters; saline solutions for in vitro diagnostics; treatment/coatings for textiles such as hospital gowns, curtains, drapes; dialysis machines.	*Laboratory/medical solid wastes (e.g., tubing, filters, films).*			FluoroCouncil (2019), Posner (2012)
Cosmetics and personal care product manufacturing	Used in cosmetics, hair conditioning formulations, hair creams, and toothpaste.	*Off-spec materials*[b]	*Wastewater effluent*		Danish EPA (2018), FluoroCouncil (2019), Schultes et al. (2018)
Fire suppression systems[e]	AFFF and certain dry fire-extinguishing agents.	Contaminated soil and debris from applied product.[d]	Applied product[d]		See Section 2.b for more information. Gaines (2023)

[a] The italicized waste streams may contain PFAS, given the wastes the relevant industry sectors are known to generate and given applications of PFAS. The presence and concentration of PFAS have not been quantified.

[b] "Off-spec materials" or "off-spec products" are materials or products that do not meet specified standards or requirements and are discarded rather than sold or used.

[c] Some industries listed under "Secondary Manufacturing" may also include primary manufacturing of PFAS. The waste streams resulting from manufacture of PFAS in these industry sectors are addressed in the first row, "Primary chemical manufacturing."

[d] "Applied product" refers to the intentional application of a PFAS-containing product to the environment.

[e] Fire suppression systems, which include AFFF, are commonly found in manufacturing, storage, extraction and refining, and national defense facilities, as well as airports, fire departments, and other federal facilities (e.g., facilities operated by the National Aeronautics and Space Administration [NASA] and the U.S. Department of Energy [DOE]). Although this is an industrial use, not an industrial sector, the prevalence of AFFF in the above primary and secondary PFAS manufacturing industries warrants highlighting these waste streams.

2.b Aqueous film-forming foam

AFFFs are a group of PFAS-containing fire extinguishing agents for low-flashpoint hydrocarbon fuel fires (Tuve et al., 1964) and are intended for use where a significant flammable liquid fire hazard exists (FFFC, 2016).

AFFFs are based on synthetic fluorosurfactants that provide unique low surface tension and positive spreading coefficient characteristics. When mixed with water and applied, AFFFs form an aqueous film and a foam solution to coat the liquid fuel, seal fuel vapor, and reduce oxygen availability which extinguishes the fire and prevents burnback (FFFC, 2016; SERDP, 2020; Sheinson et al., 2002).

Until application, AFFF is managed as a concentrated product containing less than 2 percent PFAS fluorosurfactants, by weight, for a typical 3 percent AFFF concentrate (ITRC, 2020). AFFF is stored in either fixed, structural dispensing systems, such as those in hangars and aboard vessels, or in mobile, vehicle-based systems (i.e., aircraft rescue firefighting [ARFF] vehicles) (Field et al., 2017). Reserve AFFF concentrate inventory may be stored in hangars or warehouses. The amount of AFFF concentrate in the finished foam varies by manufacturer and application circumstances, but is usually between 1 and 6 percent, meaning the fluorosurfactants are diluted to less than a fraction of a percent (FFFC, 2016; ITRC, 2020).

A 2004 inventory estimated that there were 4.6 million gallons of legacy PFOS-containing AFFF in the United States (Darwin, 2011). AFFF inventory depletion is determined by frequency of use for firefighting, training, or testing; transfers between locations; and other factors. However, AFFF's characteristically long shelf life means there should be little disposal due to expiration (FFFC, 2016), increasing the possibility that legacy PFOS-containing AFFF concentrate remains in service or reserve inventories. Fluorotelomer-based AFFF surfactant products became available in the 1970s (Prevedouros, 2006). In 2017, the U.S. Department of War (DoW) edited its military specification for AFFF to include no more than 800 parts per billion (ppb) of PFOA and PFOS in the concentrate, the limit set by the DoW *Quality Systems Manual 5.1*[12]. However, this specification does not mention any other PFAS standards.

In the United States, AFFF and associated systems are or have been in service at federal facilities, civil airports, and oil refineries. Civilian fire departments also use or have used AFFF. The federal government is working to identify areas of current and former federal properties where PFOS- or PFOA-containing AFFFs have been used (Darwin, 2011; DoD, 2020). Through March 31, 2025, DoW has determined that 723 active military installations, base realignment and closure (BRAC) locations, National Guard facilities, and formerly used defense site (FUDS) properties require an assessment of PFAS use or potential release. DoW is performing preliminary assessments/site inspections (PA/SIs) at these installations, which is the first phase of the cleanup process and may take two to three years to complete. As of March 31, 2025, DoW has completed the PA/SI phase at 703 installations. The Department has determined that no further action is required at 117 of these installations, while 586 installations are proceeding to the next step in the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) process (DoW, 2025).

The FY 2020 NDAA prohibits AFFF use at any military installation, effective October 1, 2024, or up to two years later if the Secretary of War deems a waiver necessary. DoW issued a policy in January 2016 to discontinue land-based AFFF training and testing activities, unless the AFFF could be captured and properly disposed. Since then, DoW has managed any mission-critical AFFF use in response to an emergency event as a spill response to mitigate impacts to the environment (DoD, 2020; DoW, 2019). DoW, among other entities, is also investing in research and development for fluorine-free AFFF alternatives (SERDP, 2020). In 2023, DoW issued a new military specification for fluorine-free foam (MIL-

[12] Available at: *https://www.denix.osd.mil/edqw/denix-files/sites/43/2017/04/FINAL-QSM-5.1-SIGNED-010517-1.pdf*.

PRF-32725) (DoD, 2023). On *August 11, 2025*[13], DoW extended its deadline to end the use of AFFF until October 1, 2026, to provide more time to successfully transition to PFAS-free alternatives.

Examples of AFFF users and locations in the United States are listed in Table 2-2. Note that the list of sources in the table is non-exhaustive.

Table 2-2. Examples of AFFF Users and Locations in the United States

AFFF User	Locations	Comments
DoW	• Hundreds of military installations (active and former) with AFFF use. • In-service systems installed at an estimated 1,350 locations in aircraft hangars and on an estimated 3,000 ARFF vehicles.	• In 2016, DoW discontinued AFFF use for military installation training and testing unless the AFFF could be captured and properly disposed and is investing in fluorine-free alternatives. • The FY 2020 NDAA, asa modified by the FY 2026 NDAA, requires DoW to end use of AFFF at military installations by October 1, 2026 (with extensions possible until 2028). • Sources: DoD (2017, 2020); DoW (2019); CBO (2019); SERDP (2020)
NASA	• Multiple properties with AFFF apparatus. • Five properties with fixed systems.	• In late 2018, NASA issued order to cease training with AFFF. • Source: SERDP (2020)
DOE	• Lawrence Berkeley National Laboratory. • Brookhaven National Laboratory. • Los Alamos National Laboratory. • Strategic Petroleum Reserve facilities (Gulf of America).	• Sources: DOE (2021 *DOE Initial Assessment of PFAS at DOE Sites*[14]); Darwin (2011)
Airports and supporting facilities	• 523 Federal Aviation Administration (FAA)-certified civilian airports in 2018. • Since 2006, an annual average of nearly 600 certified airports. • Supporting facilities include firefighting training sites, such as the FAA Technical Center's Fire Training Area.	• Starting in 1972, FAA regulations allowed for the use of AFFF among a number of different extinguishing agents at civilian airports; since 2006, FAA guidance (FAA, 2006) provides that AFFF must conform to the applicable military specification (AC 150/5210-6 and MIL-F-23485F) at most civilian airports.[a] • 80 percent of respondents to a 2017 survey of U.S. and Canadian airports reported training-related discharge directly to the ground; two-thirds reported testing-related discharge directly to the ground. • FAA has taken steps to reduce AFFF discharges during testing since 2019. • Sources: DOT (2019); FAA (2006, 2019); Thalheimer et al. (2017); U.S. EPA (2020c)

[13] Available at: *https://texaschemistry.org/aws/TCC/pt/sd/news_article/599913/_PARENT/TCC_layout_details/true*.

[14] Available at: *https://www.energy.gov/sites/default/files/2022-11/DOE%20Initial%20PFAS%20Assessment%20-508.pdf*.

AFFF User	Locations	Comments
Fire departments	• At civilian fire departments throughout the United States.	• AFFF may be present at fire departments. • Some states have begun inventorying and reporting having AFFF (e.g., Michigan, New Hampshire). • Some states (e.g., Vermont) have take-back programs to help local fire departments identify AFFF in inventory and assist with removal and disposal. • Sources: Michigan PFAS Action Response Team (n.d.); New Hampshire Department of Environmental Services (2020); Vermont Agency of Natural Resources (n.d.)
Ships and other vessels	• Ships and other marine vessels, including the United States Coast Guard.	• Little information is available about AFFF quantities on ships. • There has been a shift toward non-fluorinated AFFF for some uses including testing and training, though the Coast Guard has indicated that certain uses (e.g., required inspections) must continue to use fluorinated AFFF. • Source: U.S. EPA (2020e)

[a] An exception exists for airports with low departure traffic and serving aircraft less than 90 feet in length. See 14 CFR 139.317 for more information (FAA, 2006).

Fate and transport of PFAS in AFFF after use depends on the release circumstances and chemical-specific properties. Though sometimes classified as incidental releases (Thalheimer et al., 2017), equipment failure, accidental releases, or operator error can result in substantial leaks (Anderson et al., 2016; Resolution Consultants, 2016; Leidos, 2016).

Engineering controls (such as dikes, barriers, or basins) may be installed at facilities with significant flammable liquid hazards to contain foam solution and runoff for later disposal (FFFC, 2016). Where such hazards do not significantly exist, or installed engineering controls are otherwise not practicable, firefighting personnel may block sewer drains or deploy portable dikes as containment measures (FFFC, 2016). Runoff can then be pumped out, and the affected environmental media can be removed for disposal (ITRC, 2020). C&D debris originating from facilities where AFFF was historically released may also be a source of PFAS in landfills and groundwater (Solo-Gabriele et al., 2020).

Though subject to site-specific characteristics and conditions, studies demonstrate AFFF use at airports is a source of PFAS in soil and groundwater (Ahrens et al., 2015; Dauchy et al., 2017b; Høisaeter et al., 2019). Further, PFAA precursors from original AFFF concentrate products may transform in the environment to more mobile products over time (Houtz et al., 2013), expanding plumes long after AFFF use is discontinued.

2.c Soils and biosolids

As required by Clean Water Act (CWA) Section 405(d), EPA established requirements for the final use or disposal of sewage sludge when it is: (1) applied to land as a fertilizer or soil amendment, (2) placed in a surface disposal site (e.g., sewage sludge-only landfill), or (3) incinerated. The regulation at 40 CFR part 503 defines sewage sludge as "solid, semi-solid, or liquid residue generated during the treatment of domestic sewage in a treatment works." Sewage sludge includes scum or solids removed in primary, secondary, or advanced wastewater treatment processes and any material derived from sewage sludge (e.g., a blended sewage sludge/fertilizer product) but does not include grit and screenings or ash generated by the incineration of sewage. Sewage sludge treated for land application is commonly referred to as "biosolids." Part 503 considers domestic septage as sewage sludge and sets separate requirements for domestic septage applied to agricultural land, forests, or reclamation sites.

Influent that contains PFAS entering wastewater treatment facilities may result in the presence of certain PFAS in biosolids (Thompson et al., 2023; Helmer et al., 2022; Venkatesan and Halden, 2013). When biosolids are applied to land, there is the potential for leaching or runoff (Schaefer et al., 2022; Navarro et al., 2016; Lindstrom et al., 2011; Sepulvado et al., 2011; Washington et al., 2010). If landfilled instead, there are potential concerns with PFAS releases via landfill leachate and gaseous emissions, especially for highly organic material (Section 3.b). Alternatively, if incinerated, there is the potential for PFAS to be emitted as PICs (Section 3.a). Research needs and resources on possible emerging PFAS destruction technologies for biosolids (e.g., pyrolysis/gasification coupled with a thermal oxidizer and supercritical water oxidation) are discussed in Sections 4 and 5. Across disposal and destruction technologies, there are capacity concerns, and more research is needed on potential PICs/PIDs.

Total nationwide biosolids generation and management statistics in 2024 (reported to the EPA as of September 25, 2025) and data considerations are presented in Table 2-3. EPA does not have data on the volume or percentage of biosolids that contain PFAS. Additionally, EPA released the Draft Sewage Sludge Risk Assessment for PFOA and PFOS in January 2025 for public comment (U.S. EPA, 2025a).

Table 2-3. Biosolids Generation and Management in 2024

NPDES permits issued to POTW[a]	**16,109**
Biosolids NPDES permits[b]	9,834
Biosolids annual reporters (2024)[c]	2,350
Sewage Sludge generated in 2024 (dry metric tons)[d]	**4,008,886**
Land application of biosolids (dry metric tons)	2,386,861
Land disposal (also called surface disposal) (dry metric tons)	102,456
Incineration (dry metric tons)	558,360
Landfilling (dry metric tons)	879,721
Other management (dry metric tons)	81,488

[a] An NPDES permit is typically a license for a facility to discharge a specified amount of a pollutant into a receiving water under certain conditions. A POTW is a treatment works treating domestic sewage (TWTDS) that is owned, and usually operated, by a local or regional government agency (U.S. EPA, 2020b).

[b] Biosolids permits apply to any person or treatment works that prepares sewage sludge, applies sewage sludge to the land, or fires sewage sludge in a sewage sludge incinerator and to the owners/operators of surface disposal sites, as well as the exit gas from sewage sludge incinerator stacks (U.S. EPA, 2019).

[c] Annual reports are required for POTWs that have a design flow rate equal to or more than one million gallons per day, that can serve populations of 10,000 or more, that are Class I Sludge Management Facilities (POTWs required to have approved pretreatment programs or facilities classified as such by the EPA or state), or that are otherwise required to report (U.S. EPA, 2025b).

[d] The total amount of biosolids generated is reported by each treatment works treating domestic sewage (TWTDS) and may not equal the sum of component amounts reported for each management method. Biosolids totals do not represent PFAS presence in biosolids.

Release of PFAS into soils can occur through use of PFAS-containing products such as AFFF, and the discharge or application of treated industrial or municipal wastewater containing PFAS. Unintentional contact, such as spills or leaks during the production, handling, transport, or use of PFAS-containing materials can also be the source of PFAS in soils (see Table 2-4 for examples of industrial sources of PFAS in soils). Remediation wastes such as soils excavated during the cleanup of sites or during decommissioning of facilities where PFAS was manufactured, used, or applied may contain diverse mixtures of PFAS in elevated concentrations.

2.d Textiles, other than consumer goods, treated with PFAS

Because PFAS can repel oil, water, and stains, the textile industry uses these chemicals in a broad range of textile products other than consumer goods (apparel or household textiles). For example:

- PFAS can be used to treat outdoor equipment such as tents and sails (UNEP, 2011).
- Technical or occupational textiles, such as protective clothing for firefighters, can be treated with PFAS or woven from fluoropolymers (OECD, 2013).
- Medical garments can be treated with fluorinated polymers (OECD, 2013).
- Fluoropolymers can be spun into fibers and used to make sailcloth and fabric for fire suppression needs (Tokarsky and Uy, 2003).
- Textiles made from fiberglass coated or saturated with PFAS are used for high-temperature or corrosive industrial environments. Kevlar and perfluoroplastic composite textiles are used for similar industrial environments (Robco, 2020).

Examples of typical PFAS-containing waste streams generated from textiles include discarded industrial or commercial textiles such as apparel, carpets, or personal protective equipment; solids coated with PFAS from cuttings and shearing; and fugitive volatiles from spray applications of textile surface treatments. The destruction and disposal technologies used for these waste streams include landfill disposal and thermal treatment. For examples of industrial waste streams from the textiles/apparel manufacturing industry, see Table 2-4.

2.e Spent water treatment materials

On April 10, 2024, EPA announced the final National Primary Drinking Water Regulations (NPDWR) for six PFAS. On May 14, 2025, EPA announced that the Agency will keep the NPDWR for PFOA and PFOS. EPA also announced its intent to extend the PFOA and PFOS Maximum Contaminant Level compliance deadlines. Additionally, EPA announced its intent to rescind the regulations and reconsider the regulatory determinations for PFHxS, PFNA, HFPO-DA (commonly known as GenX), and the Hazard Index mixture of these three PFAS plus PFBS to ensure the determinations and any resulting drinking water

regulation follow the Safe Drinking Water Act process. Additionally, as of March 2023, 9 states have established separate state level MCLs limiting PFAS in drinking water

EPA has identified "best available technologies" for treating drinking water for PFAS to comply with the NPDWR, and these technologies will produce residuals. The 2026 Interim Guidance details options available to manage these residuals while minimizing environmental exposures and releases to the extent feasible. EPA notes that there are multiple approaches for water systems to manage PFAS residuals that are generated when PFAS is removed from drinking water. As of January 2026, there are no specific federal regulatory requirements for PFAS disposal, and the presence of PFAS in treatment residuals does not impose any additional federal requirements.

Conventional drinking water treatment methods such as coagulation, flocculation, sedimentation, disinfection, and filtration do not remove PFAS found in source water. Therefore, residues from these processes such as grit, spent membranes, or filter elements are unlikely to contain significant PFAS.

Although novel technologies for removing PFAS from drinking water sources and groundwater are being developed, current processes known to be effective are activated carbon, anion exchange resins, and high-pressure membranes (reverse osmosis [RO] and nanofiltration [NF]) (U.S. EPA, 2022a, 2022b). This section discusses the residual streams of these three processes (see Section 3 for discussions on treatment and disposal considerations, Appendix D for costs and considerations, and Section 4 for discussions of research needs for more novel treatments) and regulatory considerations for drinking water treatment utilities.

Table 2-4 shows the primary residual streams by water treatment process, with options for reactivation, destruction, and disposal of these residuals.

Table 2-4. Destruction and Disposal Options for Drinking Water Treatment Residuals Containing PFAS

Treatment Process	Residuals Generated	Destruction/ Disposal Option(s)	Section Reference
Granular Activated Carbon (GAC)	Spent GAC	Reactivation	3.a.i.2
		Incineration	3.a
		Landfill	3.b
Powdered Activated Carbon (PAC)	Spent PAC	Incineration	3.a
		Landfill	3.b
Ion Exchange	Single use and regenerative ion exchange: spent resins	Incineration	3.a
		Landfill	3.b
	Regeneration brine	Interim Storage	1.c.
		Underground Injection	3.c.ii.1
RO/NF	Brine	Permitted discharge, direct or indirect	Not described in this guidance. Refer to permit.

The practices discussed in Table 2-4, which may help reduce and/or control unintentional PFAS releases to the environment, are not presented in hierarchical order and should be chosen based on site-specific conditions.

2.e.i Activated carbon

Activated carbon (discussed more extensively in Section 3.a.i) is manufactured from carbon-rich sources such as coal or wood and is used to treat water or air by filtering contaminants (U.S. EPA, 2012). Activated carbon can be used in either PAC or GAC form. In PAC treatments, carbon is powdered and added to the water to be treated, and chemicals in the water stick to the powdered carbon as the water passes through and typically carries the PAC along. In GAC treatments, chemicals in water adsorb to small pieces of carbon in a bed as the water passes through.

PAC application will remove some PFAS from water, though the amount of PFAS removed depends on many factors. For PAC treatment, the residual stream is the sedimentation sludge or the filter backwash solids that contain the PFAS-laden PAC along with the coagulant, coagulant aids (if used), natural particulates, and enmeshed organic carbon (Dudley et al., 2015). Given the many conditions that affect treatment, the weight percent of PFAS in a PAC residual stream varies by many orders of magnitude but will be in the same range as GAC treatment, as discussed below.

For GAC, the range of PFAS concentrations on spent media can be estimated in several ways (Crone et al., 2019; U.S. EPA, 2020d). A conservatively high loading of PFAS onto GAC (using a GAC with a high-capacity potential, a strongly adsorbing PFAS, few competitive contaminants, low organic levels, and a high concentration in the feed water treated) is on the order of 2 percent by weight (e.g., 0.02 grams PFOS per gram of GAC). For more common treatment scenarios, or for PFAS that are adsorbed more weakly (i.e., the shorter-chain PFAS), the weight percent of PFAS will be orders of magnitude lower—about $1x10^{-5}$ grams PFAS per gram of GAC.

GAC can be reactivated and reused (see Section 3.a.i.2 for a discussion on GAC reactivation). If the GAC is landfilled, PFAS can desorb off the carbon into the landfill leachate if the GAC contacts a water stream that contains low PFAS, or more strongly adsorbing constituents that can displace the PFAS. Desorption will occur due to the disequilibrium between the liquid and solid phases and will accelerate at higher temperatures where adsorption becomes less favorable for exothermically-adsorbed contaminants. Therefore, the resulting PFAS concentrations in the landfill leachate impacted by spent GAC will vary greatly depending on conditions.

Typically, deep-bed GAC does not require backwashing because the particulates in the water are removed ahead of the GAC system, but there may be instances where particulates deposit onto the GAC, creating a headloss that will require backwashing. If so, the backwash water may contain a PFAS foam that may need to be managed.

2.e.ii Ion exchange resins

Like GAC, ion exchange resins are utilized in fixed-bed adsorbers. Similarly, the residual stream from this treatment is the spent media; however, ion exchange resins cannot be thermally reactivated like GAC. Due to several factors, the market is moving toward single-use media for anion exchange resin treatment with incineration as the final disposal point for the spent resin.

Final PFAS loadings onto resins vary widely. A conservatively high estimate of loading (calculated using a PFAS-selective resin, few competitive constituents, and a PFAS known to adsorb well) is expected to be in the order of 10 percent by weight for the strongly adsorbing PFAS (e.g., 0.1 grams of PFOS per gram of resin). The higher percent weight than GAC is due to the high capacities of PFAS-selective resins. Like

GAC, more typical scenarios for PFAS that are adsorbed more weakly (i.e., shorter-chain PFAS) will yield much lower average weight percent of PFAS.

Assuming single-use resin is employed, landfilling may be less expensive than incineration. If the resin is landfilled, PFAS can desorb off the resin if the resin contacts a water stream whose counter ions can displace the PFAS. The resulting leachate concentrations will vary tremendously depending on conditions. In rare cases, it may be appropriate to regenerate the ion exchange resins with a brine; however, the latest science has shown that an alcohol solvent is needed along with the inorganic exchange ion. This is difficult to do at full scale, especially for drinking water facilities. If done in this manner, the resulting regenerate brine will have high concentrations of PFAS and will need to be disposed of appropriately. In rare cases, there may not be a ready disposal option for this regenerate brine and interim storage may be appropriate. Before selecting interim storage, it is important to consider that the volume of waste which is generated routinely can accumulate up over time, and the additional waste may become more difficult to store than originally envisioned. Otherwise, regenerate brine may be handled like other high-strength liquid PFAS wastes.

Typically, resin beds do not require backwashing because the particulates in the water are removed ahead of the resin system, but there may be instances where particulates deposit onto the resin, creating a headloss that will require backwashing. If so, the backwash water may contain a PFAS foam that may need to be managed.

2.e.iii High-pressure membranes (reverse osmosis and nanofiltration)

High-pressure membranes are extremely effective for removing many PFAS from water to a high degree (Crone et al., 2019; U.S. EPA, 2020d). As high-pressure membranes are based on a rejection phenomenon, water treatment using these systems creates a waste stream with potentially high concentrations of PFAS as well as treated water with a lower pH. These waste streams also have high concentrations of salts, other contaminants, and dissolved organic matter.

Treatment of the concentrated residual stream can be challenging, and the cost will likely be high, similar to those for landfill leachates, ion exchange spent regenerates, and waters from highly contaminated sites. Many variables could affect the cost of treating these waste streams. At this time, there is no obvious treatment technology choice, especially given that the concentrated retentate stream is typically 20 percent of flow (Baruth, 2005). This represents a sizeable amount, especially for large membrane treatment systems, such as those used by large municipalities (e.g., treating 20 million gallons per day [4 million gallons per day concentrate flow]). This large-volume flow would prevent the use of batch treatment processes, which have higher efficiencies because they can process the water multiple times before discharge.

When the membranes lose their integrity or foul to the point of needed replacement, they must be disposed. It is not anticipated that the materials of construction are high in PFAS unless they are specifically made of PFAS materials (e.g., Nafion™). Also, the mass of PFAS remaining on the membrane surfaces should be minimal except in extreme cases.

2.f Landfill leachate containing PFAS

Landfill leachate (discussed in more detail in Section 3.b.iii.5) is the liquid formed by rainwater percolating through waste in landfills. Leachate generation may continue even after a landfill's closure

period, due to liquids inherent in the waste or if the cap system fails. There are different types of solid waste landfills characterized by the types of wastes managed, which also dictate the environmental controls employed. Hazardous waste landfills and most MSW landfills are required to collect the liquid leachate captured within the landfill liner and subsequently manage or treat the leachate. While PFAS concentrations in different landfill leachates have been documented (see Table 3-3 in Section 3), there are no monitoring or reporting requirements at the federal level for PFAS in landfill waste or leachate. Thus, existing treatment methods are being used to process leachate irrespective of PFAS concentrations.

Landfill leachate can be treated on site or off site. The most prevalent off-site management approach is to export leachate to a POTW or TWTDS, where it is mixed with wastewater and treated. However, as noted in Section 2.a.ii, conventional wastewater treatment technologies are generally unable to treat or control PFAS (see Table 3-4). Other off-site treatment methods include incineration and underground injection (see Sections 3.a and 3.c, respectively). The on-site leachate treatment technologies employed at landfills are explored in Table 3-2 in Section 3.b.i. Additional research is needed to determine the efficacy of landfill leachate treatments for PFAS (see Section 4).

2.g Summary

PFAS are synthetic chemicals used in a diverse array of industrial and commercial applications. Industrial waste streams containing PFAS stem from two main sources: (1) primary manufacturing facilities of PFAS chemistry, and (2) secondary industries that use PFAS-containing products and/or manufacture finished products containing PFAS. Ultimately, PFAS can end up in solid, liquid, or gas waste streams from industrial facilities that manufacture or use PFAS and PFAS-containing products. Other common PFAS-containing waste streams include AFFF, biosolids, textiles, spent water treatment materials, and landfill leachate. Any of these waste streams can contribute to PFAS entering environmental media, including soil and groundwater. The potential destruction, disposal, and treatment technologies for processing these streams are discussed in Section 3.

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3. Technologies for the Destruction and Disposal of PFAS and PFAS-Containing Materials

EPA has identified three existing and potentially available destruction and disposal technologies that may be effective for managing PFAS and PFAS-containing materials. These technologies are thermal treatment (Section 3.a), landfilling (Section 3.b), and underground injection (Section 3.c). Each subsection describes various considerations for these technological solutions, including types of treatment, potential for releasing PFAS into the environment, and uncertainties. References appear at the end of each subsection. See Section 5 for a discussion of emerging technologies that show potential to destroy PFAS. See Appendix A for EPA guidance on conducting PFAS emissions field testing at commercial thermal destruction sources. See Appendix D for a summary of costs and considerations.

What's New in Section 3 of the 2026 Guidance Document?

- Section 3.a:
  - Added 2024 test reports on PFAS destruction at two incinerators (Clean Harbors and Veolia) and a recent study on treatment of HFPO-DA.
  - Discussed recent studies using sewage sludge incinerators (SSIs) and thermal oxidizers with carbon adsorbers for PFAS treatment.
- Section 3.b:
  - Discussed recent studies on using sewage sludge incinerators for PFAS treatment.
  - Added PFAS concentration data for MSW and ash landfill leachate, MSW LFG condensate, and LFG from RCRA Subtitle D landfills.
  - Added data on landfill liner permeability and gas collection systems and the implications for PFAS transport and transformation.
  - Updated the section regarding on- and off-site PFAS treatment technologies for landfill leachate.
  - Added new information on non-targeted analysis, uncertainties, and unknowns.
- Section 3.c:
  - Updated Class I well inventory using 2023 data.

3.a Thermal treatment

As the industrial and commercial use of fluorinated organic compounds has grown over the past 70 years, so has their presence and concentration in materials that need to be destroyed or disposed. Organic fluorine-containing materials including flame suppressants, refrigerants, long- and short-chain PFAS, fluoropolymers, and their precursor chemicals have been treated using a variety of thermal treatment technologies. These range from permitted HWCs and industrial furnaces to MWCs, SSIs, and thermal oxidizers designed to treat materials and process gas emissions. Compared to landfills and underground injection, thermal treatment offers the potential advantage of mineralizing PFAS and preventing further release into the environment.

Thermal treatment units generally use high-temperature combustion and incineration to destroy organic materials and control organic pollutants. Incineration is an effective and permitted method for destroying certain halogenated organic chemicals including chlorinated solvents, polychlorinated

biphenyls (PCBs), dioxin-laden wastes, brominated flame retardants, refrigerants, and ozone-depleting substances.

Combustion is described by complex sets of physical processes and elementary reactions involving many intermediate and free radical species. Even combustion of a simple fuel, such as methane, involves several hundred elementary reactions. For more complex fuels or systems that include additional elements, such as fluorine, elementary reactions can number in the thousands or more. These elementary reactions describe pathways or mechanisms whereby large molecules, such as PFAS, decompose and transform during combustion. Generally, breaking carbon-fluorine (C-F) bonds require more energy and higher temperatures than needed to break C-C, C-Cl, and C-H in analogous molecules. Due to their thermal stabilities, short-chain perfluoroalkanes such as hexafluoroethane (C_2F_6) may be good indicators of broader PFAS decomposition (Guzman et al., 2025; Shields et al., 2025a; Troxler et al., 2025; Ram et al., 2025). In addition, these short-chain perfluoroalkanes may be relatively easy to monitor by FTIR (Weber et al., 2025) and are targets measured in whole gas samples by OTM-50 making them candidates for broad PFAS thermal decomposition. The exceptional stability of CF_4 requires much higher temperatures (above 1,400°C) to destroy compared to other fluorinated organics. Thus, while unrepresentative of broader PFAS destruction, CF_4 may be a useful species during PFAS thermal destructibility trials to confirm recovery of gases by FTIR or OTM-50.

Thermal treatment of halogenated organic compounds yields the corresponding halogen acid when utilizing sufficiently high temperatures, residence times, and mixing to promote mineralization. In the case of fluorinated organics, mineralization yields hydrogen fluoride (HF), carbon dioxide (CO_2), and water. If any of these parameters are insufficient, this treatment may lead to the formation of fluorinated PICs. Some of the PICs produced in the decomposition process may have higher thermal and chemical stabilities than the parent PFAS.

Introducing PFAS directly into a hydrocarbon-based flame can assist in their adequate destruction and mineralization. The flame may provide reactive free radicals which facilitate the initial decomposition of PFAS. Hydrogen containing radicals (e.g., H and OH) and water vapor facilitate the breakdown of remaining species resulting in HF formation (Weber et al., 2025). Fluorocarbon radicals generated from incomplete thermal treatment of PFAS contribute to fluorinated PICs due to their thermal stability and propensity to undergo radical-radical recombination (Krug et al., 2022; Xu et al., 2025; Guzman et al., 2025). Thus, it is important for hydrogen to be in stoichiometric excess relative to fluorine to minimize the formation of fluorinated PICs.

Many PFAS are composed of highly stable fluorinated carbon chains and relatively weakly-bonded, non-fluorinated functional groups. Often, the functional group is easily removed, allowing the fluorinated chain to decompose or react with other radicals and create a variety of intermediates and products. For example, Shields et al. (2023) observed temperature-dependent production of 1H-perfluoroalkanes and other volatile fluorinated PICs during pilot-scale incineration of an AFFF containing PFOS and PFHxS. Additionally, Rocchio et al. (2025) and Weber et al. (2025) recently demonstrated that inadequate thermal treatment of PFOA or HFPO-DA, respectively, may lead to regeneration of shorter perfluorocarboxylic acids as PICs.

Certain thermal technologies can potentially achieve the operating conditions (e.g., temperature, residence time, turbulence) needed to adequately destroy and mineralize fluorinated organic compounds, but not every unit typically operates at these conditions. Recent data shows promise for two facilities, but data are lacking about the effectiveness of the operating conditions in different

thermal destruction technologies and facilities. Further methods development to evaluate more classes of potential PICs and testing at individual facilities are needed to help determine the overall effectiveness of thermal treatment. As a result, EPA does not have specified conditions for the adequate destruction of PFAS, but promising results inform the recommendation for comprehensive testing following procedures in Appendix A at any thermal treatment facility planning to treat PFAS-containing waste.

Hazardous waste combustion technologies (e.g., commercial incinerators, cement kilns, and LWAKs) can potentially achieve temperatures and residence times sufficient to break apart the PFAS contained in the waste stream being thermally treated, but not every unit currently operates at those conditions and those that do may not operate at those conditions at all times. To assess the capability of HWCs to destroy the materials fed into them and ensure that harmful emissions into the environment are minimized, it is standard practice to evaluate the DRE of one or several principal organic hazardous constituents (POHCs) during a trial burn. POHCs are selected to be at least as difficult to destroy as the feed material, based on an incinerability index scale (i.e., the relative level of difficulty for destroying a particular compound); however, the existing index does not include PFAS compounds. Permitted hazardous waste facilities have stringent regulatory controls on temperatures and other important operating parameters and require greater than 99.99-percent DRE for each selected POHC. Some HWC are also permitted under TSCA to incinerate PCBs and have a further requirement to demonstrate a DRE of 99.9999 percent for PCBs as well as even more stringent operating requirements with respect to residence time and temperature (40 CFR 761.70(a)(1)) when incinerating PCBs.

For HWCs, DRE is calculated based on the amount of a specific chemical (e.g., POHC) entering a thermal treatment device compared to the amount of that chemical present at the exit. While the compound selected for DRE determination in an HWC is chosen based on the difficulty of destroying it, DRE does not account for chemical transformations into different organic compounds (i.e., PICs). Thus, DRE is not an accurate indicator, by itself, of complete PFAS mineralization. Shields et al. (2023) demonstrated that PIC formation can occur even when high DRE is measured at temperatures below 1,000°C. Therefore, it is not accurate to state that a high DRE of the parent molecule by itself indicates complete destruction of all PFAS. It is difficult to ascertain the complete destruction of the added PFAS, and subsequent PICs produced due to measurement limitations. Instead, the term “adequate destruction” is used in this guidance to represent appropriate PFAS and PIC attenuation, given the gaps in accuracy of current measurement methodologies. To fully evaluate whether adequate destruction is occurring, EPA and other investigators need to sample and analyze a range of chemicals present at the outlet of a thermal treatment device, including the specific PFAS in the feed material, other PFAS beyond those in the feed (i.e., potential PICs), and other fluorinated compounds. EPA is developing test methods for measuring most PFAS before and after the incineration process, and new methods are being advanced that will provide more information in the coming years. Currently, OTM-45 is available for a targeted list of polar semivolatile PFAS and OTM-50 for volatile fluorinated compounds (VFCs). Test methods are in development for semivolatile nonpolar PFAS as surrogates for the class of PFAS compounds and fluorinated compounds (see Section 3.a.iv).

For this version of the interim guidance, PFAS destruction is defined as the severing of all carbon–fluorine bonds in a PFAS molecule and the mineralization of carbon and fluorine to HF, CO_2, and water. HF and some of the other products of combustion can be effectively removed in pollution control devices designed for acid gas removal, although more research regarding the efficacy of these technologies for controlling PFAS PICs is warranted (see Section 4).

EPA currently has limited data characterizing emissions from HWCs that burn PFAS (Focus Environmental, 2021, 2022) (see Appendix B), a comprehensive test including an evaluation of PICs at Clean Harbors in Aragonite, Utah (Troxler, et al. 2025), a comprehensive test including an evaluation of PICs at Veolia in Port Arthur, Texas (Veolia North America, 2025), and data from a pilot-scale study at an EPA research facility (Shields et al., 2023, 2025a; Weber et al., 2025). These recent tests at Clean Harbors in Aragonite, Utah (Troxler et al., 2025) and Veolia in Port Arthur, Texas (Veolia North America, 2025), which include determination of PICs, are promising and indicate that some HWCs can operate under conditions where high mineralization of PFAs and minimal formation of PIC are possible. As each HWC is optimized for the type of waste it treats, additional testing is necessary to determine if and what site-specific conditions are necessary to achieve high DRE with a corresponding minimization of PIC formation. Additional studies are necessary to more fully measure and evaluate the ability to approach complete mineralization and eliminate potential formation of PICs. EPA is working to advance new measurement methodologies (see Section 3.a.iv.1) and gather additional information to conclude whether potential PICs are adequately controlled (see 6.cAppendix A).

This section focuses on considerations associated with thermal treatment of PFAS and PFAS-containing materials and discusses:

- The types of thermal treatment units that manage PFAS-containing materials and their ability to effectively destroy PFAS.
- The potential for environmental releases during these thermal treatment operations.
- PFAS monitoring methods relevant to these thermal treatment operations.
- Uncertainties and unknowns associated with thermally treating PFAS-containing materials, including the ability to effectively measure and monitor thermal treatment performance.

3.a.i Types of thermal treatment

The following subsections describe the types of thermal treatment devices that may have directly or indirectly received and treated the PFAS-containing materials identified in Section 2.a. These subsections focus on design and operation parameters that are important for destroying PFAS. Treatment devices described here include HWCs, non-hazardous waste combustors, carbon regeneration and reactivation units, and thermal oxidizers. Waste incinerators are typically classified by the type of waste they combust. Suggested resources for locating such facilities are included in Section 1.c.ii.

3.a.i.1 Hazardous waste combustors

HWCs include hazardous waste incinerators (HWIs), cement kilns, LWAKs, boilers, and hydrochloric acid production furnaces that burn hazardous waste. Two types of HWCs that have treated PFAS waste in the United States are commercial[15] incinerators and LWAKs. Currently, no LWAKs fire hazardous waste.

[15] Commercial thermal treatment units primarily treat waste received from other facilities.

All HWCs are subject to RCRA and Clean Air Act (CAA) permitting requirements[16,17] that provide additional regulatory oversight and include operating requirements and emission limitations to safely and effectively treat regulated hazardous contaminants which may not be required for non-permitted facilities. These types of HWCs are subject to CAA Title V permitting requirements, and to maximum achievable control technology standards pursuant to Section 112 of the CAA (40 CFR part 63, subpart EEE). These standards include emission limitations for metals, dioxins/furans, particulate matter, hydrogen chloride (HCl) and chlorine gas, and carbon monoxide or hydrocarbons, as well as limits on minimum organic DRE, but do not include emission limitations for PFAS. Also, under the authority of RCRA's "omnibus" clause (Section 3005(c)(3); see 40 CFR 270.32(b)(2)), RCRA permit writers may impose additional terms and conditions on a site-specific basis as may be necessary to protect human health and the environment. Due to these additional safeguards, permitted HWCs may operate under conditions more conducive to destroying PFAS and controlling related PICs relative to thermal treatment units that do not have both RCRA and CAA permits. Further, incinerators permitted to fire liquid PCBs are required to meet even more stringent operating requirements with respect to time and temperature (40 CFR 761.70(a)(1)) when incinerating PCBs, and these operating conditions may be even more conducive to destroying PFAS and controlling PIC formation. The following sections generally describe the design and operational parameters of commercial HWIs, LWAKs, and cement kilns, focusing on parameters that are important for destroying PFAS. Design and operation information summarized for HWCs is generally based on background support documents for previous rulemakings (U.S. EPA, 2005b). Information on the numbers of HWC facilities is based on a 2019 national hazardous waste capacity assessment report (U.S. EPA, 2019) which also provides a list of these facilities. In addition to the commercial HWCs, many chemical facilities operate captive hazardous waste boilers and incinerators that only burn waste generated by that particular site or other sites owned by that parent company. These captive units may operate at temperatures lower than that of typical commercial units because of their narrow waste profile. Data gathered from commercial incinerators may not be indicative of potential control of a particular captive unit due to site-specific design and operating conditions.

3.a.i.1.1 Commercial hazardous waste incinerators

Currently, there are 10 commercial HWI facilities operating in the United States. HWIs are designed to optimize temperatures, residence times, turbulence, and other parameters to ensure compliance with organic DRE requirements.[18] Three of these facilities are also permitted for incineration of PCBs. Most commercial HWIs use rotary kilns as primary combustion chambers to facilitate the thermal treatment of containerized wastes (e.g., in drums) and solid wastes such as contaminated soils. Aqueous wastes that have low heating values may also be pumped into the rotary kiln.

The kiln maintains a continuous standing flame fueled by wastes with high heating values and auxiliary fuels that maintain high temperatures. Typically, solids retention time in the kiln is 0.5 to 1.5 hours,

[16] Hazardous waste is regulated pursuant to RCRA authority. See 42 U.S.C. 6903. The regulatory definition is found in 40 CFR 261.3. PFAS is currently not a listed or characteristic hazardous waste, but a PFAS-containing waste may meet the regulatory definition of hazardous waste if PFAS is mixed with a listed hazardous waste or if a PFAS-containing mixture exhibits a hazardous characteristic (e.g., corrosivity or another characteristic stemming from the material that is mixed with PFAS). Waste managers must comply with RCRA hazardous waste requirement for the specific regulated hazardous waste, including storage limits, land disposal restrictions, and utilizing disposal and destruction facilities that are permitted to receive hazardous wastes.

[17] Hazardous-waste-burning cement kilns and LWAKs are a small subset of the total cement kiln and LWAK universe—i.e., most kilns do not burn hazardous waste.

[18] DRE = $[1 - (W_{out} / W_{in})] \times 100\%$, where: W_{in} = mass feed rate of an organic compound into the combustion device and W_{out} = mass emission rate of the same organic compound in exhaust emissions. HWCs are required to achieve at least 99.99% DRE.

while gas residence time through the kiln is usually around two seconds. Kiln flame/solids temperatures range from 650°C to 1,650°C (1,200°F to 3,000°F) (U.S. EPA, 2005b).

After combustion in the rotary kiln, wastes may be moved to an afterburner or secondary chamber. If an afterburner is used, additional gaseous and liquid wastes with high heating values and auxiliary fuels may be added. The afterburner/secondary chamber is typically operated at about 1,100°C to 1,370°C (2,000°F to 2,500°F) with a gas residence time from one to three seconds to maximize organic destruction and minimize the formation of PICs (U.S. EPA, 2005b). Depending on the fuels used and waste streams introduced, combustion products might include CO_2, water, nitric oxide, a variety of acid gases (sulfur dioxide, hydrochloric acid, HF, etc.), organic PICs, fly ash, and bottom ash constituents. Bottom ash is removed at the end of the kiln and from the bottom of the afterburner/secondary chamber and is typically disposed of in hazardous waste landfills (see Section 3.b). Fly ash entrained in the gas is removed downstream by fabric filters (FFs) or electrostatic precipitators (ESPs), and acid gases are removed by gas scrubbers. Some HWIs use activated carbon injection systems to control mercury, dioxin/furan, and other emissions, and some also use TMT-15 (1,3,5-triazine-2,4,6(1H,3H,5H)-trithione, trisodium salt) injection for additional control of mercury and metal emissions.

EPA currently has limited data characterizing emissions from HWI that burn PFAS:

- Multi-year tests of a specially designed thermal oxidizer at a Chemours production facility (Focus Environmental, 2021, 2022, 2023, 2024), see Appendix C.
- A comprehensive test including an evaluation of PICs at Clean Harbors in Aragonite, Utah (Troxler et al., 2025).
- A pilot-scale study at an EPA research facility (Shields et al., 2023, 2025a; Weber et al., 2025).
- A comprehensive test including an evaluation of PICs at Veolia in Port Arthur, Texas (Veolia North America, 2025).

The Clean Harbors, EPA pilot-scale, and Veolia studies summarized below, and the Chemours production facility data summarized in Appendix C, provide some insight on the potential efficacy of thermal treatment in an HWI. To date, the commercial facility studies have focused on the removal of easily transformed PFAS target compounds, which is a significant limitation.

Clean Harbors Environmental Services. Two testing campaigns (2021 and 2022) were conducted at Clean Harbors Environmental Services' hazardous waste incinerator located in Aragonite, Utah (EA Engineering, Science, and Technology, Inc., PBC., 2021, 2022). These tests are summarized in detail in Appendix B. The incinerator is permitted under both the RCRA and TSCA programs. Each test campaign was divided into three test conditions: Baseline (Condition 1), Augmented PFAS Feed (Condition 2, where known amounts of four or five target PFAS were fed to the incinerator), and Post-spiking Normal Operations (Condition 3, where AFFF was included in the incinerator feed). OTM-45 (Measurement of Selected Per- and Polyfluorinated Alkyl Substances from Stationary Sources) was used for stack gas sampling and analysis. The 49 targeted PFAS analytes were measured using liquid chromatography/tandem mass spectrometry (LC/MS/MS) with isotope dilution. Utilizing the available analytical methods at the time, the Clean Harbors study demonstrated a DRE for the legacy PFAS in the 99.9999-percent range at temperatures around 1,100°C. Of the 49 compounds detected by OTM-45, 34 were non-detectable in the stack gas and only 15 were detectable at extremely low concentrations. The tests appear to demonstrate that the facility's primary objective was met: "demonstrate the ability of the high-temperature incineration system at Aragonite to effectively destroy PFAS in the waste feed

during normal process operating conditions." Although a high DRE was achieved (greater than 99.999 percent) for the five selected PFAS compounds that were subjects of these studies, these tests did not collect data to fully support demonstration of adequate destruction as the production of PICs were not evaluated due to methodology limitations.

A third test campaign (conducted by EPA, Clean Harbors, and SERDP in 2024 at Clean Harbors in Aragonite, Utah) performed the comprehensive testing outlined in Appendix A (Troxler et al., 2025). During the test, PFAS-containing AFFF and nine additional PFAS were added to the incinerator along with the typical hazardous waste feeds. The gaseous, liquid, and solid end products were sampled and analyzed for the presence of PFAS and other fluorinated compounds. The kiln exit temperature during testing was greater than 1,097°C (2,006°F) and the secondary combustion chamber was operated at greater than 1,120°C (2,048°F). Results showed that the measures of the incinerator's DRE ranged from 99.95 to 99.9999 percent for the added PFAS tested under these conditions. Additionally, the analyses of the gas phase emissions showed low to non-detectable levels of VFCs that could be considered destruction of PFAS PICs using OTM-50 and SW-846 Method 0010/8270. Finally, test runs were performed with the quantitative addition of C_2F_6 with the waste streams to investigate its potential use as a surrogate compound. The resulting DRE for C_2F_6 ranged from 99.999 to 99.9999 percent and were similar to those of the other spiked compounds. These results suggest correlation between high C_2F_6 DRE and the absence of PICs. While this result is promising, more field data is required to establish C_2F_6 as an appropriate surrogate compound. There were uncertainties and limitations to the data that should be considered when evaluating the overall effectiveness of thermal treatment for PFAS.

EPA PFAS thermal destruction research. EPA completed an initial study examining the relationship between destruction efficiency (DE) and PICs (Shields et al., 2023). This study used a pilot-scale research furnace and atomized a PFOS-dominant AFFF at various furnace locations and combustion environments. These included atomization through the flame with the natural gas auxiliary fuel and at various post-flame locations so that the AFFF would experience decreasing peak temperature exposures ranging from approximately 1,200°C to 800°C (2,192°F to 1,472°F). Stack measurements included OTM-45 and OTM-50. Instead of DREs, this study calculated DEs because samples were collected before any pollution control (i.e., removal equipment), including fabric filter and alkaline wet scrubber. Analysis of the AFFF and OTM-45 samples was used to calculate DEs for 10 PFAS quantified in the AFFF. OTM-50 was used to quantify concentrations of 30 PFAS PICs. Results indicate that several idealized operating conditions with peak temperatures above 1,090°C (1,976°F) resulted in high DEs (greater than 99.99 percent) and low PIC emissions that were near or below the detection limits. However, several conditions below 1,000°C (1,832°F) yielded substantial concentrations (milligrams per cubic meter [mg/m^3]) of volatile fluorinated PICs, despite achieving over 99.99-percent DE for PFAS quantified in the AFFF. The study concluded that DE alone may not be the best indication of PFAS destruction. Additionally, EPA recently assessed thermal treatment of atomized aqueous solutions of HFPO-DA (Weber et al., 2025). Here, HFPO-DA was destroyed efficiently (DE above 99.99 percent) at peak furnace temperatures at or above 750°C, but yielded fluorinated PICs whose concentrations decreased with increasing peak temperatures.

Veolia Environment SA. Veolia conducted two test campaigns at their HWI located in Deer Park, Texas in Summer and Fall 2024 (Veolia North America, 2025). The two campaigns consisted of multiple test conditions, firing of AFFF, AFFF-contaminated soil, AFFF with lime injection (1 test run), spent activated carbon, and an assessment of CF_4 as a potential POHC (1 run). Prior to each test campaign feed variation, a baseline test run was conducted while no PFAS were fed to the combustor. In addition to

stack samples analyzed by OTM-45 and OTM-50, liquid and solid residues (scrubber blowdown, ash, slag, and filter cake) were sampled and analyzed. The kiln exit temperature during testing was greater than 816°C (1,500°F) and the secondary combustion chamber was operated at temperatures greater than 1,093°C (2,000°F). During the baseline tests, even without detectable PFAS in the waste feeds, small amounts of some PFAS were detected at the stack. Some of these were detected in the process water used in the quench, the scrubbers, and the wet ESP (WESP) indicating the possibility of the process water being a contributing source to emissions. DRE was determined for all potential PFAS, but with decreasing input, DRE determinations become less reliable indicators, as the potential noise and detection limits become dominating factors in the calculations. DRE was assessed for every target PFAS according to OTM-45. In many cases, these evaluations were not indicative of performance due to the low feed rate and detection limits at the stack. The DRE results of 20 PFAS with the highest feed rates ranged from 99.807 to 99.9999 percent. For all PFAS introduced at a feed rate greater than 0.02 lb/hr, the DRE exceeded 99.99 percent. Results of the OTM-50 sampling for volatile, nonpolar PFAs showed detectable emissions of CF_4 in only 3 of 15 sample runs (including the DRE test run where CF_4 was spiked) and a single test run where heptafluoropropyl 1,2,2,2-tetrafluoroethyl ether was detected near the method detection limit (MDL). The single spiked run of CF_4 seems to indicate that it is not appropriate as a POHC with a DRE of 43 percent for CF_4 under identical conditions to those tests showing high DRE of select PFAS and minimal PIC formation (as demonstrated by the results of the OTM-50 analysis).

These recent tests at Clean Harbors in Aragonite, Utah (Troxler et al., 2025) and Veolia in Port Arthur, Texas (Veolia Environment SA, 2025) including determination of PICs are promising, indicating that some HWCs are capable of operating under conditions where high mineralization of PFAS and minimal formation of PIC are possible. As each HWI is optimized for the type of waste it treats, additional site-specific testing is necessary to determine if, and what site-specific conditions are necessary to achieve high DRE with a corresponding minimization of PIC formation. Additional studies are necessary to more fully measure and evaluate the ability to approach complete mineralization and eliminate potential formation of PICs. EPA is working to advance new measurement methodologies (see Section 3.a.iv.1) and gather additional information to conclude whether potential PICs are adequately controlled (see Appendix A).

3.a.i.1.2 Hazardous-waste-burning cement kilns

Currently, there are 11 hazardous-waste-burning cement kiln facilities in the United States. A cement kiln is a long, cylindrical, slightly inclined rotating furnace designed to calcine a blend of raw materials such as limestone, shale, clay, or sand to produce a key ingredient of Portland cement. These cement kilns burn hazardous-waste-derived fuels to replace some of the fossil fuels needed for combustion. Most kilns burn liquid waste, although some may also burn solids and small containers containing viscous or solid hazardous waste fuels.

Extreme combustion conditions such as temperatures above 1,650°C (3,000°F), turbulent mixing, and extended gas-phase residence time (e.g., from four to as high as 16 seconds in long kilns) may effectively treat hazardous waste. Cement kilns use either ESPs or baghouses to collect particulate and metal emissions, referred to as cement kiln dust (CKD). Portions of the CKD can be fed back into the kiln as a raw material feed or used in other industries as neutralizers or additives, but usually the excess CKD is land-disposed. Add-on acid gas APCDs, such as wet or dry scrubbers, are typically not used. The high alkaline content of the raw material feeds already prevents or minimizes the formation and release of acid gases by providing for in situ absorption of chlorine and other halogens and sulfur.

Several laboratory studies have examined the potential benefit of treating PFAS wastes in cement kilns where the calcium in the raw limestone can react with fluorine to form calcium fluoride (CaF_2). These studies indicate that calcium species react readily with PFAS at relatively low temperatures (400 to 800°C) to promote mineralization of PFAS waste (Wang et al., 2011, 2013, 2015; Riedel et al., 2021). However, the currently available laboratory-scale data are insufficient to determine with confidence the effectiveness of treating PFAS waste in commercial cement kiln operations.

A cement kiln in Australia has received an operating permit from the government of Queensland to burn PFAS wastes. The permit requires annual monitoring for 21 PFAS (Department of Environment and Heritage Protection, 2018). Test results from 2017 indicate that a DRE greater than 99.99 percent is possible for specific PFAS compounds at the conditions of the test (Cement Australia, 2017), but the results do not present data on overall PFAS destruction or potential formation of PICs.

3.a.i.1.3 Hazardous-waste-burning lightweight aggregate kilns

The one LWAK facility in the United States that burned hazardous waste ceased operation in early 2024. LWAKs thermally process raw material (clay, shale, and slate) in slightly inclined, rotating furnaces to produce a coarse aggregate used in lightweight concrete products. In hazardous-waste-burning LWAKs, liquid wastes are either blended directly with conventional fuels burned in the hot end of the kiln or pumped separately into the hot end flame. High combustion gas flame temperatures (above 1,650°C [3,000°F]) and high kiln gas residence times (over two seconds) are used to destroy hazardous organics. Kiln exhaust gases leave the cold upper end of the kiln at a temperature from 205°C to 540°C (400°F to 1,000°F). LWAKs use FFs to control dust contained in the exhaust gas. The collected dust can be recycled back into the kiln (at the hot or cold end) or mixed into the lightweight aggregate product. Some LWAKs also use wet or dry scrubbing for acid gas emissions control. For example, the currently permitted hazardous-waste-burning LWAK has dry scrubbing for acid gas control. There are insufficient data to determine the effectiveness of commercial LWAK facilities in treating PFAS waste.

3.a.i.2 Carbon regeneration and reactivation units

Carbon reactivation units or "furnaces" use high temperatures to thermally desorb contaminants from GAC, which allows for the carbon to be used again. Over a dozen large-scale companies and utilities in the United States reactivate sizeable quantities of GAC. In all, these entities operate about 17 commercial furnaces (Roskill Information Services Ltd., 2017). Four of these commercial furnaces operate under RCRA permits and applicable air permits. RCRA permits provide additional regulatory oversight and include operating requirements and emission limitations to promote safe and effective treatment of the hazardous contaminants; non-RCRA-permitted carbon reactivation furnaces may not be subject to such requirements. Due to these additional safeguards, RCRA-permitted furnaces may operate under conditions more conducive to destroying PFAS and controlling related PICs, relative to furnaces without RCRA permits. This discussion focuses on RCRA-permitted furnaces because EPA has more design and operational information on these devices because of the RCRA permitting process. Reactivation[19] of spent carbon is generally carried out in multiple-hearth ("multi-hearth") or rotary kiln furnaces, although fluidized bed and infrared furnaces are also options. While the furnace designs vary,

[19] "Reactivation" refers to a regeneration process that requires high temperatures. Regeneration also includes low-temperature processes, such as those using brines, solvents, oxidants, biological treatment, etc. These processes may not be as effective as reactivation for GAC (AWWA, 2018); therefore, they are not considered for this discussion.

they all use high temperatures and residence times designed to eliminate the adsorbed contaminants and return the carbon to its original state for reuse.

During reactivation, spent GAC is typically exposed to drying, desorption, pyrolysis, and oxidation as it moves through the furnace. The drying stage eliminates moisture via evaporation and occurs when hot combustion gases at temperatures ranging from 100°C to 110°C (212°F to 230°F) contact the carbon. During the desorption stage, hydrocarbons, hydrogen, and water vapor escape the pores of the granular carbon at temperatures of 315°C to 400°C (600°F to 750°F). The pyrolysis stage eliminates any volatile compounds adsorbed within the carbon porosity, including residual moisture, and thermally decomposes other less-volatile compounds. Pyrolysis occurs when the carbon is exposed to temperatures up to 800°C (1,472°F) under inert conditions (i.e., low oxygen). A residue of carbonized char is formed from the adsorbed compounds during pyrolysis, which occupies some of the carbon porosity and must be removed in the next stage. The oxidative stage involves the controlled gasification of the pyrolyzed carbon at temperatures of around 800°C (1,472°F) in the presence of a mildly oxidizing atmosphere, usually steam or CO_2 or a mixture of both. This results in the elimination of most of the charred residue and exposes the original carbon-pore structure (Miguel et al., 2001).

The residence times required for carbon reactivation vary by unit design and the contaminant loads and concentrations. For example, two facilities using multi-hearth furnaces have residence times of at least 38 minutes at one facility and between 90 and 120 minutes at the second facility (Kentucky Energy and Environment Cabinet, 2019; U.S. EPA, 2018).[20]

To ensure adequate destruction and removal of any remaining contaminants, reactivation furnaces are typically equipped with afterburners/thermal oxidizers. To meet regulatory requirements, afterburners are designed to achieve 99.99-percent DRE via thermal oxidation. DRE for a reactivation/regeneration furnace is evaluated on a "total hydrocarbon" basis rather than for a specific most-difficult-to-destroy component, as is done for HWCs. This adds additional uncertainty to the question of whether a thermal treatment unit can effectively destroy PFAS. The temperature required to achieve 99.99-percent DRE depends on residence time as well as on the concentration of contaminants, but minimum temperatures are around 885°C (1,625°F) and maximum temperatures are as high as 1,316°C (2,400°F), with a minimum residence above one second (U.S. EPA, 2018).

Depending on the process or waste streams treated, a reactivation furnace can be equipped with add-on air pollution control equipment to ensure environmental standards are met. Equipment can include venturi scrubbers for particulate matter control, packed-bed scrubbers for acid gas and particulate matter control, and WESPs or baghouses for additional particulate matter control. As discussed in Section 3.a.ii, carbon reactivation systems can degrade PFAS even at the lower temperatures (150°C to 700°C, or 302°F to 1,292°F)

A study on a full-scale carbon reactivation furnace (DiStefano, 2022) consisting of OTM-45 and EPA Method 26A measurements of HF conducted before and after the thermal oxidizer showed DRE greater than 99.99 percent for select PFAS, with most of the destruction occurring prior to the thermal oxidizer. However, these test data do not demonstrate adequate destruction of PFAS due to the lack of data concerning PIC formation. Another recent study on a full-scale carbon reactivation furnace

[20] These two references are RCRA permits for Evoqua Water Technologies LLC and Calgon Carbon Corporation (two companies that accept spent activated carbon from off-site sources). They each hold RCRA permits, which allow them to treat spent carbon that meets the definition of hazardous waste.

(Mayerberger et al., 2025) utilized OTM-45 to calculate DREs of GAC-bound PFAS. Temperatures and operating conditions tested at this study yielded DREs greater than 99.99 percent for most detected PFAS (excluding GenX), with the majority of PFAS destruction occurring within the reactivation furnace, (i.e., prior to thermal oxidizer and other pollution control devices). The authors reported high levels of GenX in the OTM-45 test blanks, possibly due to contamination or method interference, complicating a reliable GenX DRE calculation. Additionally, OTM-50 and EPA Method 0010/8720 were employed to characterize fluorinated PICs/PIDs, resulting in no detectable PICs/PIDs at the kiln outlet and stack (e.g., pre- and post-pollution control device) locations. These results serve as a successful (albeit limited) example of adequate PFAS destruction within a GAC reactivation furnace, though subsequent demonstrations of destruction and removal of GAC-bound PFAS and secondary PICs/PIDs at similar facilities are warranted.

3.a.i.3 Non-hazardous-waste combustion sources

Non-hazardous-waste incineration units in the United States includes SSIs at wastewater treatment facilities and MWCs. Under Section 129 of the CAA, these units are regulated for emissions of particulate matter, carbon monoxide, dioxins/furans, sulfur dioxide, nitrogen oxides, HCl, lead, mercury, and cadmium. Because of these requirements, the APCDs employed by these units are intended to control these nine pollutants primarily. The following sections generally describe these incinerator types and their design and operational parameters that may influence the treatment of PFAS-containing waste (U.S. EPA, 1995).

3.a.i.3.1 Sewage sludge incinerators

An SSI is a combustion device used to combust dewatered sewage sludge. As of a 2016 EPA inventory, there are 170 SSI units operating at 86 facilities in the United States (U.S. EPA, 2016). The main types of SSIs are multi-hearth furnaces (MHFs) and fluidized bed combustors (FBCs).

The basic MHF is a vertical cylinder divided into zones. The sludge is dried at temperatures from 425°C to 760°C (800°F to 1,400°F) (U.S. EPA, 1995). Sludge combustion occurs as the temperature is increased to about 925°C (1,700°F) in successive zones. The gas residence times are typically four or five seconds. Emission controls on multiple hearths can include wet scrubbers, WESPs, afterburners, and regenerative thermal oxidizers.

An FBC consists of a vertically oriented outer steel shell with nozzles designed to deliver fluidizing air at the base of the furnace within a refractory-lined grid. Air is injected into the furnace to fluidize the sludge and the sand. The combustion of the sludge occurs at temperatures from 750°C to 925°C (1,400°F to 1,700°F). The gas residence times are typically two to five seconds. Emission controls on FBCs can include venturi scrubbers, multicyclones, FFs, activated carbon injection, and carbon bed absorbers.

There is substantial variation in the design and operation of SSIs, and it has not been shown that standard operating conditions are effective for the treatment of PFAS. A study of a laboratory-scale continuous bubbling FBC operating at 1,562°F (850°C) examined DRE for 5 PFAS. While the DRE of individual PFAS was not assessed, the total PFAS DRE (with respect to the 5 compounds) was greater than 99.9 percent. However, these test data do not demonstrate adequate destruction of PFAS due to the lack of data concerning PIC formation (Urciuolo et al., 2025). A second study of PFAS emissions from two full-scale SSIs yielded unusable OTM-45 results due to issues with the analysis of samples, and the DRE estimates provided (95 percent) are overstated due to the analytical issues. This study also showed measurable PFAS in the process water supplied to the wet scrubber, and those same PFAS in the stack

samples, but could not determine if the emissions were a result of stripping in the scrubber. Emissions of short-chain volatile PFAS (CF_4, C_2F_6, and C_3F_8) were detected using EPA Method 320, suggesting incomplete mineralization of PFAS was occurring (Winchell et al., 2024). The data currently available are insufficient to determine the effectiveness of SSIs in treating PFAS.

3.a.i.3.2 Municipal waste combustors

There are 193 MWC units operating at 75 facilities in the United States (Michaels and Krishnan, 2018). Three main classes of technologies are used to combust MSW: mass burn, refuse-derived fuel (RDF), and modular combustors. Mass burn and RDF combustors are the predominant designs. Emission controls on MWCs can include spray dryer or dry sorbent injection, ESPs, FFs, selective or non-selective catalytic reduction, and activated carbon injection.

With mass burn units, the MSW is combusted without any preprocessing other than removal of items too large to go through the feed system or hazardous materials, such as pressurized containers. In a typical mass burn combustor, refuse is placed on a grate that moves the waste through the combustor. The grate typically has three sections. On the initial grate section, referred to as the drying grate, the moisture content of the waste is reduced before ignition. Evaporated moisture is combined with the moisture from combustion and treated with the resulting combustion gases. The second grate section, referred to as the burning grate, is where most of the active burning takes place. The third grate section, referred to as the burnout or finishing grate, is where remaining combustibles in the waste are burned. Typical combustion temperatures for mass burn units can range from 800°C to 1,100°C (1,500°F to 2,012°F) (Reddy, 2016).

RDF combustors burn waste that has been processed to varying degrees to raise its heating value and provide a more uniform fuel. Most boilers designed to burn RDF use spreader stokers and typically operate at around 680°C (1,250°F). RDF-fired FBCs typically operate at bed temperatures of around 815°C (1,500°F) (Reddy, 2016).

Residence times of gases within MSW combustors vary from unit to unit, depending on design and operational factors such as furnace volume, excess combustion air percentage, use of flue gas recirculation, and combustor operating load parameters (Scavuzzo, 1990; Themelis and Reshadi, 2009). Overall combustion air residence times have been calculated in the 7- to 10-second range for a small sampling of MWC design loads (Themelis and Reshadi, 2009). At temperatures above 980°C (1,800°F), the approximate residence time is about two seconds for full combustor loads (Scavuzzo, 1990).

Research and testing of PFAS destruction performance within MWCs is extremely limited, primarily comprising laboratory- and pilot-scale studies (Aleksandrov et al., 2019; Taylor et al., 2014). For example, to assess whether PTFE is destroyed or reformed as PFAS, the Aleksandrov et al. (2019) study uses a pilot-scale rotary MWC with afterburner chamber combusting PTFE granules added to wood pellets (also firing natural gas). This study looked at a half-load scenario of 870°C (1,600°F) with a four-second residence time and a full-load scenario of 1,020°C (1,870°F) with a 2.7-second residence time. The study analyzed the flue gas samples collected for 31 PFAS compounds, which were assumed to represent a broad range of PFAS. While the laboratory- and pilot-scale studies conclude that MSW incineration of PTFE is not a significant source of PFAS, the laboratory thermal reactor and the pilot incinerator used in these studies may not be representative of the design of MWC units currently operating in the United States. These studies determined emissions, but did not assess the destruction efficiency of the laboratory thermal reactor and the pilot incinerator. For example, the pilot-scale unit in the Aleksandrov et al. (2019) study is a rotary combustion chamber followed by an upflow afterburner.

No MWC units operating in the United States have a similar configuration. In addition, while these studies conducted analysis for several PFAS species, it is important to note that there are far more PIC species possible, and no studies have thoroughly evaluated the types and quantities of PICs.

There is substantial variation in the design and operation of MSW combustors. The data currently available are insufficient to determine the effectiveness of MSW combustors to treat PFAS.

3.a.i.4 Thermal oxidizers

Thermal oxidizers are used to treat process gases and destroy volatile organic compounds (VOCs) and organic hazardous air pollutants (HAPs) at a manufacturing or production facility. Some types of thermal oxidizers are also able to accept liquid feed streams. Each thermal oxidizer is designed to optimize temperatures, residence times, and turbulence to address the composition of the particular feed stream at that unit and to meet the requirements of a regulation or permit. Depending on the type of thermal oxidizer (direct-fired, regenerative, recuperative, or flameless), the operating temperature ranges from 760°C to 1,200°C (1,400°F to 2,190°F). The residence time of thermal oxidizers ranges from 0.5 to two seconds, depending on site-specific criteria. Catalytic thermal oxidizers operate at lower temperatures than other types of thermal oxidizers—typically at about 400°C (750°F) with similar residence times to non-catalytic thermal oxidizers—while achieving the same efficiency in destruction of VOCs.

Emission controls for thermal oxidizers vary widely depending on the facility and the composition of the feed streams. Existing thermal oxidizers may have no additional pollution controls or may use prefiltration (to prevent fouling of the oxidizer) and/or wet or dry scrubbers or FFs. Thermal oxidizers have historically not been designed with destruction of PFAS as the primary focus, so most currently installed thermal and catalytic oxidizers may not be optimized for PFAS destruction. However, some thermal oxidizers are being used for halogenated wastes, including PFAS precursors. The operating conditions for some thermal oxidizers have the potential to effectively treat PFAS, but the data currently available are insufficient to determine effectiveness.

Annual testing campaigns (2020 and through 2025 tests reports are available and summarized in Appendix C) were conducted on a thermal oxidizer installed to control PFAS emissions at a Chemours chemical and plastics manufacturing plant located in Fayetteville, North Carolina. The objective of the tests was to ensure the thermal oxidizer and 4-stage scrubber system achieve a 99.99-percent emission reduction for hexafluoropropylene oxide dimer acid (HFPO-DA) and other PFAS. The test reports demonstrate a PFAS destruction efficiency above 99.99 percent for the specific compounds that were tested, although there was no discussion of possible PICs in the reports. The thermal oxidizer at the Chemours facility appropriately destroys the five PFAS compounds of interest that have been identified at the facility.

The thermal oxidizer at Chemours' Fayetteville works was sampled using OTM-50 for nonpolar VFCs that may be potential PICs. The published results and compliance data show that tetrafluoromethane was the only volatile fluorinated compound measured in the thermal oxidizer emissions (Shields et al., 2025b). The high concentrations of fluorinated compounds in the influent create high concentrations of fluorine radicals that can bond to trifluoromethyl radicals to form tetrafluoromethane that is too stable to be destroyed. From the percent-level PFAS and fluorochemical fed into the thermal oxidizer, only 2 to 4 parts per million by volume (ppmv) of tetrafluoromethane were measured. However, removal processes for more polar PICs or destruction of potential compounds not studied but potentially found in the facility waste streams are still unclear.

A test was performed in November 2021 on the regenerative thermal oxidizer at the Saint-Gobain Performance Plastics Merrimack, New Hampshire facility (Barr Engineering, 2022). This test included evaluations of OTM-45 at the inlet and the outlet of the thermal oxidizer and showed a DRE of 92 percent for PFOA and 75 percent for PFOS. However, these test data do not demonstrate adequate destruction of PFAS due to the lack of data concerning PIC formation. Further data are needed to assess the variety of configurations and operating conditions associated with thermal oxidizers, as exemplified by the broad range of DRE determinations and the lack of data on PIC formation.

3.a.ii Limitations and considerations in thermal treatments

Incinerator designs vary, resulting in differing operational and waste feed approaches (see Section 3.a.i). HWIs typically operate at very high average temperatures and employ auxiliary, primary, and secondary flames. The primary focus of hazardous-waste-burning cement kilns and LWAK is the production of cement or lightweight aggregate and not the destruction of waste, so product considerations may limit the usage of these types of units for destruction of PFAS-containing materials. MWCs typically operate at lower temperatures and often do not employ auxiliary primary or secondary flames. SSIs vary in design, often operating as dryers with very low temperatures. Even within the same incinerator, wastes can be introduced at different locations and can experience different residence time, temperature, and mixing histories. PFAS introduced into the main burner of a hazardous waste rotary kiln incinerator, along with auxiliary fuel, may experience very different conditions than the same waste introduced to the kiln as contained charges with solid wastes. These factors are expected to affect PFAS destruction and PIC formation. Limited studies have investigated the influence of various factors on PFAS destruction and PIC formation (see Section 3.a).

Carbon reactivation systems can degrade PFAS even at the lower temperatures (150°C to 700°C, or 302°F to 1,292°F) seen in bench-scale research studies (Watanabe et al., 2018). Experimental data suggest that thermal destruction of PFAS occurs in two stages: first during reactivation of the GAC, and then when the off-gas is introduced into a high-temperature zone with temperatures reaching 1,000°C (Forrester, 2018; Watanabe et al., 2016, 2018; Xiao et al., 2020). DiStefano et al., (2022) showed greater than 99.99 percent destruction of PFAS at a full-scale commercial reactivation facility with a large percentage of the PFAS destruction occurring in the furnace. The fluoride mass balance was reported to be 61.4 percent. Although this low percentage could be due to a number of factors, it may be due to the formation of PICs that were not quantitated in the analytical method. While carbon reactivation systems, with the concomitant use of off-gas incineration (i.e., afterburners) and gas scrubbing units, have the potential to remove and destroy PFAS from the activated carbon, there is insufficient data to make a determination on its effectiveness. However, as discussed elsewhere in this interim guidance, more work is needed for confirmation, particularly with regard to full-scale reactor conditions, differing carbons, and PICs.

Thermal oxidizers have historically not been designed with destruction of PFAS as the primary focus, so most currently installed thermal and catalytic oxidizers may not be optimized for PFAS destruction. However, some thermal oxidizers are being used for halogenated wastes, including PFAS precursors. Thermal oxidizers are being employed to destroy PFAS-containing and gaseous streams, but the data are insufficient to allow conclusions on the overall efficiency of thermal oxidizers, in general, for PFAS destruction. For example, annual testing at Chemours Company's Fayetteville Works (Focus Environmental, Inc., 2020, 2021, 2022, 2023, and 2024) focused on five specific PFAS of interest found in waste gas streams at the facility. While the tests seem to prove a DRE of 99.999 percent for the five PFAS compounds, they do not present data on overall PFAS destruction and the potential formation of

PICs was investigated with OTM-50 only one time (Shields et al., 2025b). The results are promising but more studies would be beneficial (see Appendix B and Appendix C for summaries of the Clean Harbors and Chemours test data, respectively). EPA is currently unaware of any catalytic oxidizers being used specifically for the destruction of PFAS, particularly considering their site-specific design and optimization. Though data on the efficacy of thermal and catalytic oxidizers in destroying PFAS is currently limited, a properly optimized thermal oxidizer can readily achieve a DRE of 99.99 percent for VOCs.

In addition to incinerators and thermal oxidizers, cement kilns are also used for the destruction of hazardous wastes. Cement kilns operate at very high temperatures (exceeding 1,800°C [3,270°F]), exhibit very large gas and solid residence times, and have the added advantage of providing a caustic environment for halogen reaction and acid neutralization. A cement kiln in Australia has received an operating permit from the government of Queensland to burn PFAS wastes. The permit requires annual monitoring for 21 PFAS (Department of Environment and Heritage Protection, 2018). Test results from 2017 indicate that a DRE greater than 99.99 percent is possible for specific PFAS compounds at the conditions of the test (Cement Australia, 2017), but the results do not present data on overall PFAS destruction or potential formation of PICs.

Processes involving calcium and alumina may have benefits and may require lower energies to destroy PFAS. Recent research (Wang et al., 2011, 2013, 2015) has investigated PFAS interactions with calcium oxide (CaO) and calcium hydroxide ($Ca(OH)_2$) at moderate temperatures (200°C to 900°C, or 390°F to 1,650°F) and found that these calcium species exhibit a pseudo-catalytic effect promoting PFAS destruction and fluorine capture at relatively low temperatures. Similar studies by the electronics industry indicate that alumina catalysts can effectively convert residual CF_4 and related species (generated by plasma arc techniques for etching purposes) to CO_2 and HF at comparable temperatures.

It is not well understood how effective high-temperature combustion is at completely destroying PFAS or whether the process can form fluorinated or mixed halogenated organic byproducts. Few experiments have been conducted under oxidative and temperature conditions representative of different field-scale incineration devices used for PFAS destruction. Limited studies on the thermal destructibility of fluorotelomer-based polymers found no detectable levels of PFOA after two seconds of residence time at 1,000°C (1,830°F) (Yamada et al., 2005; Taylor et al., 2014). Recent field studies examining the performance of a specially designed thermal oxidizer in destroying PFAS in process gases at a Chemours production facility near Fayetteville, North Carolina, indicated greater than 99.999 percent destruction of five PFAS (Focus Environmental, Inc., 2020, 2021, 2022, 2023, and 2024). Similarly, the 2021 and 2022 Clean Harbors studies appear to show that the facility was able to achieve its primary objective: “demonstrate the ability of the high-temperature incineration system at Aragonite to effectively destroy PFAS in the waste feed during normal process operating conditions.” A high DRE was achieved (greater than 99.999 percent) for the five selected PFAS compounds that were subjects of these studies (EA Engineering, Science, and Technology, Inc., PBC., 2021, 2022). However, these test data did not include characterization of potential PICs due to previous methodology limitations. More recent testing at Veolia Port Arthur (Veolia North America, 2025) and Clean Harbors (Troxler et al., 2025) included an examination of volatile PIC formation using OTM-50 and have promising results, indicating both high DRE of specific PFAS as well as minimal volatile PIC formation.

The Chemours annual tests and Clean Harbors 2021 and 2022 test results indicate the PFAS compounds measured were changed by the treatment, but the tests did not investigate PIC formation to show

adequate destruction of the original PFAS. As discussed previously, emission studies, particularly for potential PICs, are largely incomplete due to lack of validated sampling and measurement methods for the potentially large number of fluorinated and mixed halogenated organic compounds that might be formed. EPA continues to seek information on PFAS that may be present in APCD media (e.g., scrubber water, particulate matter control device media) and the presumed effectiveness of these air emission controls. The Veolia Port Arthur (Veolia North America, 2025), and 2025 Clean Harbors test (Troxler et al., 2025) both examined residuals from the control devices as well as the process water. These tests suggest that contaminated process water is a potential source of remission of PFAS into the environment. See Section 4 for more information about ongoing research and research needs.

Incineration may be a viable PFAS destruction technology if done under certain conditions. The preliminary research on thermal treatment of PFAS suggests that the minimum conditions for PFAS destruction include well mixed environments with temperatures greater than 1,100°C and optimized combustion stoichiometry (Shields et al., 2023). Full sale testing at Veolia, with the kiln operating at greater than 816°C and the secondary combustion chamber operating at greater than 1,093°C, provide results with high DRE and minimal PIC formation (Veolia North America, 2025). The Clean Harbors 2025 testing at Aragonite, Utah (Troxler et al., 2025), with the kiln operating at greater than 1,097°C and the secondary combustion chamber operating at greater than 1,120°C, also showed high DRE with minimal PIC formation. These conditions may adequately destroy PFAS and minimize PICs. However, evaluations have only been done on liquid-fired materials. No comparative studies that include PIC measurements on solids or containerized wastes have been performed. Liquids should preferably have direct flame contact or at least well mixed exposure to temperatures sufficient for adequate destruction of PFAS.

EPA encourages additional tests be performed to demonstrate the ability of thermal treatment technologies to mineralize PFAS. EPA encourages testing to demonstrate destruction of parent compounds (OTM-45) and volatile PICs (OTM-50) based on suggested procedures in Appendix A) to show efficient thermal destruction of atmospherically transformative PICs of health and environmental concern.

3.a.ii.1 Other thermal treatment byproducts of concern

Thermal treatment systems including HWIs, MWCs, and SSIs are configured such that gas-phase effluent from the combustion chambers passes through a series of pollution control devices to remove acid gases, particulate matter, and sometimes mercury and other specific HAPs. The behavior of PFAS and PFAS-related PICs in these unit operations is largely unknown (see Section 4 for more information about research on this topic and others). Additionally, these control devices produce secondary waste streams in the form of fly ash and scrubber blowdown solutions, and PFAS and PFAS-related PICs may be present in these solid and liquid effluents depending on their vapor pressure and solubility.

Thermal oxidation processes used for treating PFAS-containing waste, such as incineration, generate HF as the most stable product from combustion of fluorocarbon compounds. HF is a CAA-listed HAP and is highly corrosive, which creates significant operation and maintenance issues by damaging thermal system materials such as furnace refractory and metal ductwork. Both wet scrubbing and semi-dry scrubbing processes have been applied to control HF emissions from thermal treatment sources.

A thermal treatment system with a potential for HF emissions typically uses a wet scrubber integrated with the oxidizer to control HF emissions. In a typical system, hot flue gas exiting the thermal treatment is cooled rapidly in a quenching unit, and HF (which has high water solubility) is removed by the

quenching water. The cooled flue gas then flows up through a multistep wet scrubbing tower for further HF removal. Flue gas is scrubbed by a sodium hydroxide solution to neutralize the residual HF as the final scrubbing step. After exiting the tower, flue gas is emitted through a stack. All effluents, including those from the quenching unit and scrubber tower, are mixed with a $Ca(OH)_2$ solution in a reactor where calcium is combined with fluorine and precipitation of water-insoluble CaF_2 occurs. After dewatering, dry CaF_2 is sent to a landfill for disposal (see Section 3.b) or used to produce fluorine gas for new PFAS production, and wastewater is discharged from the plant after it is treated by activated carbon to remove trace fluorinated contaminants. Rapid cooling of hot flue gas is known to be effective in reducing catalytic reformation of chlorinated PICs such as dioxins during cooling of incineration flue gas.

A thermal treatment system equipped with a quenching unit to treat PFAS-containing wastes may also limit catalytic reformation of fluorinated PICs, if they are formed in the oxidation/combustion chamber. Those PICs may be subsequently transferred into the liquid phase in the wet scrubber and then be partitioned between solid CaF_2 and water in the precipitator (most of the PICs retained in water are then removed by activated carbon adsorption). EPA is not aware of peer-reviewed studies that measure levels of fluorinated contaminants remaining in both the treated scrubber water stream and the dry CaF_2 stream. Such measurements could be useful for evaluating the potential environmental impacts of byproducts and residuals generated from thermal oxidation of PFAS-containing wastes.

Spray dryer absorber (SDA) technology has been applied to control emissions of halogen acids (including HCl and HF) from both MWCs and HWIs. This semi-dry scrubbing process is designed to inject an alkaline slurry, typically lime, to control acid and fly ash. Water in the fine slurry droplets is vaporized by heat carried by the flue gas, and the lime carried in the droplets neutralizes the halogen acids simultaneously in this two-phase reaction process. The cooled flue gas carries the dried acid neutralization product downstream to a particle collection device, typically an FF. PAC may also be injected into flue gas upstream of the FF to control emissions of mercury and chlorinated dioxins/furans from both MWCs and HWIs. Fly ash, dried acid neutralization product, and PAC are captured by the FF. The SDA/FF with PAC injection flue gas cleaning train produces no scrubber water. The addition of lime (a calcium compound) into the flue gas is known to be effective for forming CaF_2 through hydro-defluorination of PFOS at a moderate temperature of about 350°C (660°F) (Wang et al., 2015), which suggests that the SDA may provide a potential co-benefit of controlling fluorinated PICs. The injection of PAC upstream of the FF may create another potential co-benefit for capturing fluorinated PICs. Studies evaluating PFAS mitigation via SDA/FF with PAC injection (e.g., see research activities in Section 4) will help develop data on this potentially viable technology option.

3.a.iii Potential for releases for thermal treatment technologies

Thermal treatment devices that may be used to treat PFAS-containing waste (see Section 3.a.i for descriptions of these devices) are located in both rural and more densely populated areas throughout the United States. Two possible sources of PFAS emissions from thermal treatment are stack emissions and the management of scrubber water and bottom ash/fly ash. As previously discussed, emissions from thermal treatment activities may contain PFAS if adequate combustion conditions are not achieved or if adequate acid gas scrubbers or other pollution control devices are not used. EPA is aware of a limited number of peer-reviewed studies that have investigated the extent to which emitted PFAS are transported and deposited to surrounding areas (see Section 2.a.iii). EPA plans to conduct research in this area to better characterize the extent to which PFAS deposition may occur near thermal treatment devices (see Section 4 for more information about these types of research activities).

Volatile PFAS releases from thermal treatment equipment (e.g., fugitive emissions) and waste storage activities are another potential source of PFAS releases, if they are not adequately controlled.[21] There is also the potential for releases from management of thermal treatment process residuals, such as APCD media, incinerator bottom ash, and liquids from acid gas scrubbers. For example, incinerator bottom ash disposed of in a landfill would be a possible source of release if the ash contained PFAS and the landfill lacked adequate controls (see Section 3.b for more information on landfill types and controls). HWIs, however, typically dispose of incinerator bottom ash in hazardous waste landfills, minimizing the potential for the release of PFAS to the environment.

3.a.iv Testing and monitoring

Validated measurement methods are limited and under development for reliably identifying and quantifying whether PFAS are released into the air from stationary sources. The limited scope of standardized methods to measure PFAS emissions and the limited availability of data using these methods to evaluate PFAS destruction at a variety of treatment devices introduce uncertainty in the understanding of the efficacy of thermal treatment approaches for destroying PFAS.

Ongoing method development by EPA has a broad focus that will address PFAS in various waste streams, stack and fugitive emissions, and ambient air (see Section 4 and Appendix A). Method development also includes identification of PFAS that are potential PICs, not targeted in established water methods such as Methods 533 and 537.1. Due to the number and complexity of PFAS that have already been identified, surrogates are also being investigated to determine if measurement of a single compound or a small group of compounds is adequate to characterize the completeness of thermal treatment for both targeted PFAS and potential PICs.

3.a.iv.1 Semivolatile PFAS sampling and analysis

Initial development of methods to measure PFAS in ambient air or emissions sources has focused on compounds whose physical state is liquid or solid at room temperature. Methods for measuring gas concentrations have included the target compounds commonly found in water methods. The basis for measuring PFAS compounds in ambient air or from emissions sources involves evaluating and then modifying existing sampling and analysis methods for semivolatile organic compounds.

Filtered particulate, solid sorbent, and aqueous impinger media sampling procedures have been used to collect and recover a wider range of PFAS and associated byproducts in ambient air and source emissions (Barber et al., 2007; Martin et al., 2002). However, these sampling methods have some limits, including poor retention or chemical conversion of the PFAS during sampling and poor recovery during sample preparation prior to chemical analysis (Arp and Goss, 2008).

Ambient sampling for semivolatile PFAS roughly follows either the high-volume air sampling protocol described in EPA compendium Method TO-13a (U.S. EPA, 1999) or the National Atmospheric Deposition Program (NADP) approaches for wet and dry deposition sampling (NADP, 2020). High-volume air samples collect both water-soluble PFAS acids and salts and water-insoluble telomer alcohols. NADP

[21] For example, CAA and RCRA regulations applicable to HWCs require controls to prevent/minimize combustion system leaks (e.g., due to positive pressure events in the combustion chamber), as well as controls to minimize releases from equipment and tanks that store or manage hazardous waste. See 40 CFR 63.1206(c)(5) and 264.1050–1090.

sampling has focused on condensable and particulate-bound PFAS captured in polypropylene buckets to evaluate deposition due to rain.

Current sampling of stationary source air emissions for semivolatile PFAS targets is based on EPA SW-846 Method 0010. Analysis of samples for a list of PFAS compounds is based on EPA Method 533, modified to include collection of both targeted and non-targeted PFAS in a single sampling system. Sampling includes heated or stack temperature probe extraction of emission gases followed by collection on filters, XAD sorbent media, and aqueous impingers. EPA released OTM-45 (Measurement of Selected Per- and Polyfluorinated Alkyl Substances from Stationary Sources) in January 2021 and revised the method in January 2025 (U.S. EPA, 2025a). Experience indicates that OTM-45 is an effective and sensitive approach to measure a list of PFAS included in the method.

These field sampling procedures collect samples that are subsequently transported to a laboratory for extraction and analysis. Analysis procedures include those based on established water methods for targeted compounds and/or non-targeted analysis (NTA) for unknown PFAS. NTA is a research tool that can be used on OTM-45 and OTM-50 samples to investigate semivolatile polar and volatile nonpolar PFAS recovered by these methods. High-resolution mass spectrometry can be used for both targeted analysis and NTA. Qualitative identification of PFAS by NTA reveals PICs/degradants formed during the thermal treatment of PFAS-contaminated media (Aleksandrov et al., 2019; McCord and Strynar, 2019; Newton et al., 2020). NTA currently relies on mass spectrometry, which generates qualitative information about the molecular formula of unknown PFAS, is an important component in characterizing thermal treatment emissions because it provides an approach for identifying unknown PFAS or PICs.

3.a.iv.2 Gaseous volatile PFAS sampling and analysis

Volatile PFAS targets and thermal treatment byproducts from ducted emissions or in ambient air have been sampled using a variety of whole gas sample collection approaches, such as Tedlar® bags and passivated canisters, as well as sorbent traps and cryogenic solvents. EPA and private sector investigators have sampled with specialized commercial sorbent traps and Tedlar® bags in laboratory-scale thermal destruction and ambient volatile PFAS measurement of targeted and non-targeted PFAS (Wang et al., 2013, 2015; Yamada et al., 2005). Issues such as sample reactivity, breakthrough volumes, and quantitative transfer to the analysis instrument complicate these approaches. Direct instrumental methods for measuring volatile PFAS (such as FTIR) can suffer from lack of sensitivity compared with extractive methods that allow concentration prior to analysis.

EPA released OTM-50 (Sampling and Analysis of Volatile Fluorinated Compounds from Stationary Sources Using Passivated Stainless-Steel Canisters) in January 2025 (U.S. EPA, 2025b; Wallace et al., 2025) which enables analysis of non-polar VFCs and PFAS. OTM-50 utilizes silanized steel canisters to measure targeted VFCs and PICs emitted from thermal treatment facilities, as well as industrially-produced compounds (such as FC-116, HFC-23, HFC-125, E1, and E2) and potential PIDs (such as CF_4 through octadecafluorooctane). EPA is also developing the ability to characterize a more diverse set of possible PFAS PICs and measure VFCs at trace concentrations. The ability to measure trace concentrations will allow investigators to introduce a known concentration of a hard-to-destroy fluorocarbon and evaluate the behavior of this compound when exposed to specific thermal environments which is EPA's preferred approach to characterize a more diverse set of possible PFAS PICs. Though insufficient by itself, this approach is consistent with the EPA approach for determining the DRE of fluorinated greenhouse gas abatement equipment in electronics manufacturing (U.S. EPA, 2010).

NTA has also been performed on the same samples. These tools and OTM-45 are examples of monitoring methods that could be considered as additions to a potential PFAS waste management system or permit (e.g., RCRA).

EPA continues to evaluate FTIR as a suitable real-time measurement technique for volatile, non-polar fluorinated compounds (e.g., CHF_3, C_2HF_5, C_3F_8). Measurement of CF_4 and C_2F_6 by FTIR in stationary source emissions has been successfully demonstrated at pilot- (Krug et al., 2022) and commercial-scale (Troxler et al., 2025) operations. Additionally, the aluminum industry has used FTIR for several decades to monitor CF_4 and C_2F_6 formation (World Aluminium 2020). Favorable results have been noted between non-polar fluorinated PICs where both FTIR and OTM-50 were used (Weber et al., 2025; Troxler et al., 2025).

EPA has also recently investigated online chemical ionization mass spectrometry (CIMS) for measuring gas-phase PFAS compounds (Riedel et al., 2019). This technique was applied toward real-time monitoring of short-chain perfluorocarboxylic acids and other fluorinated PICs produced during pilot-scale experiments incinerating AFFFs (Mattila et al., 2024), and single-component aqueous solutions of PFOA (Rocchio et al., 2025) and HFPO-DA (Weber et al., 2025). A recent EPA-led study also used online CIMS to investigate CaO assisted thermal treatment of fluorotelomer alcohols (FTOHs) (Riedel et al., 2021). This technique performed comparably to thermal desorption-gas chromatography/mass spectrometry (TD-GC/MS) for monitoring temperature-dependent FTOH removal efficiencies. Additionally, this technique may be sufficiently sensitive for PFAS fugitive emissions and fenceline monitoring, though further research is needed to determine its efficacy for these applications.

The quantitative measurement of total organic fluorine (TOF) is also being evaluated to represent all, or most, of the PFAS class of compounds. This approach represents a simpler and more comprehensive measurement alternative to target list approaches that focus on a limited number of PFAS (Forster et al., 2023; Idjaton et al., 2024). Several potential techniques warrant consideration and additional evaluation, including (but not limited to) combustion–ion chromatography (CIC), particle-induced gamma emission spectrometry (PIGE) (Smith, et al., 2024), and X-ray photo-electron spectroscopy (XPS). For air and thermal treatment emissions, TOF must measure highly volatile as well as semivolatile PFAS. TOF analysis is an ongoing research area and data users must recognize the benefits of receiving general screening data for a wide array of potentially present PFAS, while also recognizing the limitations and uncertainties inherent in not knowing which PFAS or class of PFAS is present in the sample, including uncertainties associated with potential health risk. Non-targeted methods like extractable organofluorine/absorbable organofluorine (EOF/AOF) have higher quantitation limits (e.g., in the ppb range), which may be insufficient for capturing low PFAS concentrations in the environment. High-resolution mass spectrometry (HRMS) is crucial for identifying PFAS byproducts and degradation pathways, offering structural insight at low concentrations that EOF/AOF assays lack (Smith, et al., 2024). In addition, to minimize the risk of PFAS false positives, techniques within a validated method or methods must be developed that demonstrate effective separation and removal of inorganic fluorine from organic fluorine (Koch et al., 2020). TOF is not specific to PFAS, and any fluorine-containing compounds (e.g., pesticides, pharmaceuticals) that are retained during extraction would be included in the organic fluorine measurement.

HF measurement is included in stationary source measurements to evaluate emissions and control efficiency of HF as a HAP at emission outlets. Multiple studies of PFAS thermal decomposition and HF monitoring have demonstrated that EPA compliance methods for HF measurement are adequate to

meet this need. However, HF is difficult to transport through treatment and control equipment and is therefore not a candidate for using a mass balance approach to evaluate PFAS destruction efficiency. GC-HRMS is essential for analyzing volatile organofluorine compounds in gaseous emissions due to the difficulty of direct quantification without reference standards. Consideration should be given to closing the fluorine mass balance by combining non-targeted methods like EOF and total oxidizable precursor (TOP) analyses with targeted studies (Smith et al., 2024).

3.a.v Uncertainties/unknowns

EPA is planning to collect additional information and conduct additional research to better understand PFAS destruction and evaluate emission control efficiency (see Section 4). EPA is also working to develop a standardized validated methodology for measuring PFAS gaseous emissions (see Section 3.a.iv); however, EPA lacks detailed information on the amounts and concentrations of PFAS-containing materials that are generated and managed in thermal treatment devices.

Development of sampling and analytical methodologies must continue (see Section 4 research activities) so that emissions and other media from thermal treatment devices burning PFAS-containing materials can be adequately characterized.

Uncertainties such as the overestimation of destruction and disposal capabilities, or a failure to account for potential PIC production and subsequent transformation back to PFAS of concern, could have consequences for all nearby communities.

To reduce uncertainties and provide more detailed recommendations, further data on the ability of thermal treatment units to mineralize PFAS-containing materials is necessary (see Appendix A and Section 4.a). Performance testing of all types of combustors is lacking. EPA recommends that full-scale thermal destruction facilities conduct performance testing prior to accepting and processing PFAS materials. Objectives of the testing should include:

- Demonstrating destruction of parent compounds and potential production of semivolatile and volatile PICs (using OTM-45 and OTM-50) to show efficient thermal destruction.
- Characterizing PFAS being fed, including the feed rate, whether it is a concentrated or diluted stream, and how the PFAS are fed (e.g., containerized or direct feed).
- Demonstrating utilization of key combustion parameters (temperature, residence time, other conditions that impact mineralization) and including relevant data in the report.
- Characterizing release of PFAS and PICs through the system by sampling at multiple points in the process (e.g., before and after a scrubber) and sampling all relevant effluent streams (e.g., scrubber blowdown and fly ash). Documenting and reporting operating parameters for all relevant control devices to EPA and relevant state, territorial, or Tribal authorities.

Further details on suggested parameters and procedures are provided in Table 4-1 and Appendix A.

3.a.vi Treatment commercial availability

Section 3.a.i describes the commercial availability of thermal treatment devices. The United States has about 22 commercial HWC facilities[22] in operation, including over a dozen large-scale, commercial

[22] This includes commercial incinerators, cement kilns, and LWAKs that are permitted to burn hazardous waste.

carbon reactivation companies with approximately 17 furnaces, 193 MSW incineration units at 75 facilities (as of 2018), and 170 SSIs at 86 facilities (as of 2016).

3.a.vii Summary

PFAS-containing waste can potentially be treated in several types of thermal treatment devices, including HWCs, MWCs, SSIs, and activated carbon reactivation furnaces, but further research is needed to gain a better understanding of what may be possible in practice. These treatment devices operate differently and handle different types of PFAS-containing media with varying concentrations. Even within the same category of thermal treatment device, designs and operating conditions may vary across sources ways that could affect PFAS treatment efficiency. As a general approach, EPA encourages decision-makers to prioritize the use of destruction and disposal technologies that have a lower potential for PFAS release to the environment, over destruction and disposal technology options with a greater potential for environmental release of PFAS given similar costs or other considerations. EPA acknowledges that each destruction and disposal technology has limitations. However, managing PFAS to minimize environmental releases during destruction and disposal activities supports protection of human health and the environment.

There are limited characterizations of potential PFAS emissions from thermal treatment devices that burn PFAS-containing media, with a few tests at HWIs and a single test at a thermal oxidizer with a more complete emissions characterization, but EPA is not aware of any full emission characterizations that have been conducted at HWCs, MWCs, SSIs, or activated carbon reactivation furnaces or any studies to evaluate the formation of PICs resulting from inefficiency to convert PFAS into CO_2 and HF. EPA is also not aware of any studies that have been conducted on the extent to which PFAS contaminants partition to APCD residuals or bottom ash, which may also be a concern. This is likely (in part) because PFAS emission measurement methods and other analytical methodologies (e.g., for fly ash, other waste streams) are limited and continue to be developed. Data, however, from pilot-scale experiments conducted by EPA researchers suggest that temperatures above approximately 1,100°C (2,012°F) may result in high destruction efficiencies and few detectable fluorinated PICs.

Given the unique characteristics of fluorine combustion chemistry (particularly the strength of the carbon–fluorine bond), adequate thermal destruction of PFAS requires high temperatures and long residence times and likely benefits from direct flame contact. Hazardous waste combustion technologies (commercial incinerators, cement kilns, and LWAKs) can potentially achieve temperatures (~1,100°C [2,012°F]) and residence times sufficient to break all C-F bonds in the PFAS contained in the waste stream being thermally treated. Permitted hazardous waste facilities have stringent regulatory controls on temperatures and other important operating parameters to achieve a 99.99-percent and greater destruction efficiency for other (non-PFAS) organic chemicals. These facilities also have APCDs to prevent certain gaseous and particulate pollutants from entering the atmosphere. However, information on the efficacy of PFAS destruction in these facilities is currently limited and EPA has limited data characterizing PIC emissions from these sources when they burn PFAS. EPA is working to develop measurement methodologies as well as to gather information to conclude whether PICs are adequately controlled. EPA recognizes that PICs are inevitable (even for nonfluorinated compounds); however, based on the unique characteristics of fluorine combustion chemistry, EPA believes it is important to determine whether thermal treatment devices and their associated post-combustion control devices are adequately controlling PICs, especially fluorinated PICs. Given all these factors, there is a current need to continue research activities investigating incineration of PFAS. After sufficient research has been

completed to address the related knowledge and data gaps, EPA can make a more informed recommendation on disposal of PFAS compounds and PFAS-containing substances using incineration.

Similar conclusions can be drawn for activated carbon reactivation furnaces as for HWCs. Experimental data suggest that thermal destruction of PFAS will occur in two stages: first during reactivation of the GAC, and then when the off-gas is introduced into a zone with temperatures as high as 1,000°C (1,832°F). As referenced in Section 3.a.i, an activated carbon reactivation furnace can be equipped with an afterburner to treat off-gases at high temperatures to achieve 99.99 percent DRE of VOC. In addition, scrubbers can be installed to remove acid gases. This is a promising treatment method, but more information is needed, including confirmation that PICs are controlled based on actual operations, establishment of standard operating conditions for activated carbon reactivation furnaces of various designs to ensure optimal destruction of PFAS, and an understanding of how thermal treatment influences the physical and chemical properties of GAC (in ways that can affect GAC's adsorption behavior and sorption capacity for PFAS).

Research and testing of PFAS destruction performance within MWCs is extremely limited, primarily comprising laboratory- and pilot-scale studies (Aleksandrov et al., 2019; Taylor et al., 2014). While the laboratory- and pilot-scale studies conclude that MSW incineration of PTFE is not a significant source of PFAS, the laboratory thermal reactor and the pilot incinerator used in these studies may not be representative of the design of MWC units currently operating in the United States. In addition, while these studies analyzed several PFAS species, it is important to note that there are far more PIC species possible, and no studies have thoroughly evaluated the types and quantities of PICs.

As noted earlier in this section, Wang et al. (2013) investigated PFAS interactions with CaO and $Ca(OH)_2$ at moderate temperatures (200°C to 900°C, or 390°F to 1,650°F), both with and without sewage sludge. These experiments were conducted in a laboratory (i.e., combustion in a crucible within a muffle furnace) and found that these calcium species exhibit a pseudo-catalytic effect promoting PFAS destruction and fluorine capture at relatively low temperatures. The study did not investigate the evolution of PICs during the thermal treatment process. While this study shows promising results for the use of catalysts to achieve PFAS destruction and fluorine capture at low temperatures, along with the potential for full-scale application (since lime is occasionally added to sewage sludge to control odor at SSIs), it is important to note that, as with the MWC studies described above, there are caveats for applying these results to the real-world design and operation of SSIs.

More research is needed to address these issues and develop reliable measurement techniques. Section 4 summarizes EPA's continuing PFAS research, as well as a general proposal to collaborate with stakeholders to address these uncertainties promptly. See Appendix D for a summary of costs and considerations.

3.a.viii References for Section 3.a

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3.b Landfills

RCRA regulations define a landfill as "an area of land or an excavation in which wastes are placed for permanent disposal, and that is not a land application unit, surface impoundment, injection well, or waste pile" (40 CFR 257.2). The goal of solid waste landfills is to contain waste and thereby restrict the release of pathogens and contaminants within the landfill from entering the environment. Landfills are commonly classified by the types of waste they accept and/or by their ownership status.

Landfills control waste and corresponding pollutants through containment. Because of their many and varied uses, PFAS enter landfills as part of the general MSW stream, in industrial waste, or in other PFAS-containing wastes, with a range of concentrations. The two major categories of landfills under RCRA are Subtitle C hazardous waste landfills and Subtitle D non-hazardous waste landfills. Subtitle D landfills include MSW landfills, ash monofils, C&D debris landfills, and industrial landfills. These landfills are required to have different types of control technologies depending on the types of wastes they are designed to contain (see Table 3-2) and, therefore, they can vary in their effectiveness to control releases of PFAS. In addition, there is a subclass of small landfills in the United States that are exempt from RCRA requirements 40 CFR 258.1(f).

The PFAS loading into different types of landfills will also vary markedly depending on the sources and types of wastes they receive. MSW landfills, for example, can include a diverse array of wastes with highly variable PFAS concentrations (e.g., household waste, biosolids, remediation wastes). In contrast, concentrations in wastes from industries that use PFAS directly in their operations (e.g., leather tannery, chrome plating, textile industry waste) can be considerably and consistently higher (USCPSC and RTI International, 2023; Gilchrist, 2022).

The behavior of PFAS in landfills is a complex and multifaceted process that involves various abiotic and biotic transformation and partitioning pathways. While the exact mechanisms and rates of transformation are not fully understood, both aerobic and anaerobic conditions in landfills have been demonstrated to facilitate conversion of PFAS precursors into more persistent, terminal PFAS species. The behavior of these terminal PFAS can differ markedly from precursors. While some information is available, EPA continues to collaborate with industry and the academic community to conduct research to address information and data gaps (see Section 4), including efforts to better understand the effects of PFAS on liner integrity, gaseous emissions from landfills, the effectiveness of leachate treatment for PFAS removal, and the levels and types of PFAS in landfill leachate.

As described in more detail in Section 3.b.i, while generalizations can be made for the properties of classes of PFAS (e.g., perfluoroalkyl acids, fluorotelomer carboxylic acids [FTCAs]), each class encompasses a wide range of physical-chemical properties based on the molecular structure of the individual PFAS, such as chain length. For example, while many perfluoroalkyl acids have high aqueous solubility, the degree of solubility decreases markedly at higher chain lengths. FTCAs are likewise regarded as soluble, but have also been measured in LFG and LFG condensate. Work is still ongoing to better understand the constraints of and conditions that promote transformation and partitioning.

While landfills are designed to serve as long-term containment sites, it is unclear how effective they are at containment of water-soluble or volatile PFAS for an extended duration. PFAS will not break down under the environmental conditions found inside a landfill; consequently, PFAS will persist in landfills for the life of the compounds. Therefore, since landfills are a containment rather than destruction method, landfills will emit volatile and soluble PFAS as long as LFG and leachate generation continue.

Section 3.b focuses on the viability of landfilling as a means of controlling releases of PFAS from contaminated wastes, including consideration of:

- The types of PFAS in wastes and how they behave within a landfill.
- The types of landfills that receive PFAS-containing waste.
- The types of environmental control technologies used for landfills and their ability to effectively contain or remove PFAS.
- The potential for environmental releases of PFAS associated with landfilling.
- Recommendations for deciding what type of landfill is most appropriate for PFAS-containing waste.
- Methods to monitor PFAS at landfills and corresponding waste streams.
- Uncertainties and unknowns associated with landfilling PFAS-containing waste.

3.b.i Types of PFAS and Potential for Release from Landfills

As described in Section 1 ,the term PFAS refers to very large group of synthetic chemicals that due to their strong carbon-fluorine bonds, can be very persistent in the environment. EPA's ***CompTox Chemicals Dashboard*** [23] maintains a master list of more than 12,000 chemicals that are potential PFAS, including 10,200 chemicals containing a specific defined structure and an additional 1,300 without explicit structures (polymers and other chemical substances of unknown or variable composition). It is not known how many of these compounds are in production or have already come to be disposed of in landfills. NTA techniques have identified as many as 651 different PFAS compounds in landfill leachate from China (Liu et al., 2022a). This number may increase as the ability to detect and measure PFAS further evolves. When disposed of in a landfill, different types of PFAS compounds may behave differently from one another and may require different considerations for the types of landfills that are suitable for disposal and the types of emissions controls that are necessary to contain the PFAS over time.

Descriptions of the different types of PFAS provided below are specific to the landfills section and are intended to provide context regarding PFAS release from landfills (e.g., in landfill leachate or in LFG).

PFAS Polymers. Stable PFAS polymers (e.g., PTFE or Teflon™) are generally inert, insoluble, and non-volatile and are unlikely to be released via landfill leachate or LFG. Stable polymers are less likely to migrate within a landfill by partitioning to the gas or liquid (i.e., leachate) phases. However, soluble and volatile PFAS have been identified in fluoropolymers as synthesis byproducts or incomplete reactants. Although stable polymers are highly resistant to chemical and thermal degradation, they can still be broken down by weathering and physical strain, increasing their dispersion and bioavailability (Lohmann et al., 2020). For example, studies have shown that physical abrasion of the polymers can create fluorinated microplastics that are then suspended in landfill leachate (He et al., 2019). However, because exposure to ultraviolet radiation and physical disturbance of the waste are expected to be relatively low after final placement in landfills, the bulk of stable polymers would be expected to be retained for a long duration. Side-chain fluorinated polymers (SFPs), which can be fluorotelomer- or perfluoroalkyl sulfonyl fluoride-based, are distinct from stable PFAS polymers in that they include fluorocarbon groups attached to a non-fluorinated backbone. The fluorinated side chains on SFPs can

[23] Available at: *https://comptox.epa.gov/dashboard/*.

cleave to form volatile or soluble PFAS. As such, EPA recommends using similar controls as are recommended for soluble PFAS. Information about the fate and transport of SFPs is limited.

Soluble PFAS. For the purposes of this section, soluble PFAS are those that would be found in landfill leachate. PFAS monomers, including perfluoroalkyl acids (e.g., PFAAs, PFOA, PFOS, PFHxS, PFBA, HFPO-DA) and precursors that can ultimately degrade to these acids, more readily and rapidly partition into landfill leachate than other types of PFAS due in part to ready dissociation of these compounds into anions over the range of pH values typically encountered within landfills. Landfills without an appropriate liner and leachate collection system (see Section 3.b.iii) may allow leachate containing these soluble PFAS to flow into the subsurface and enter groundwater. Note that longer-chain PFAS are less soluble (Lyu et al., 2022) and have higher affinity for solids. Therefore, C_9 (perfluorononanoic acid) and greater chain length perfluoroalkyl acids are less likely to stay in the aqueous phase (Kurwadkar et al., 2022).

Volatile PFAS. For the purposes of this section, volatile PFAS are those that would be found in LFG. Alcohols (e.g., FTOHs and FASEs) and precursors that degrade to volatile PFAS can partition into the gas phase and be emitted from landfills in LFG due to the shorter chain length and non-ionic structure associated with higher vapor pressures. The rate of volatilization may be influenced by site conditions, such as landfill temperature, and co-occurrence of other LFG. Landfills without appropriate LFG collection and control systems in place and operating (see Section 3.b.iii.6) may result in fugitive emission to surrounding ambient air.

For example, FTCAs are soluble intermediate transformation products of volatile precursors such as FTOHs (Wang et al., 2025). Accumulation of FTCAs has been documented under biologically active, anaerobic landfill conditions, and their presence in groundwater is considered an indicator of landfill leachate contamination. For this reason, volatile PFAS-containing wastes should be managed in landfill environments which are also appropriate for soluble PFAS disposal.

The environmental and health risks associated with different methods for PFAS destruction remain the subject of emerging research. In the interim, while alternate management methods are developed, EPA recommends that the focus of land disposal strategies be on minimizing releases from landfills. This includes low-permeability liner and gas collection systems installed prior to disposal of biodegradable PFAS-containing waste. Given these different potential pathways for release, Table 3-1 summarizes the recommended engineering controls at landfills accepting PFAS waste based on both the type of PFAS and the biodegradability of any comingled waste. Also note that soluble PFAS that are comingled with biodegradable waste should have both liquid and gaseous emissions controls (i.e., leachate collection and gas control).

Table 3-1. Recommendations for Disposal of PFAS-Containing Waste by Type of PFAS, Waste Biodegradability, and Type of Landfill Controls to Reduce Risks of Environmental Release

Type of PFAS	Degradability of Comingled Waste	Landfills with Liner and Leachate Collection	Landfills with Gas Collection and Control
PFAS Polymers (e.g., PTFE)	Biodegradable		✓
Soluble PFAS (e.g., PFAAs)	Biodegradable	✓	✓
	Non-biodegradable	✓	
Volatile PFAS (e.g., FTOHs)	Biodegradable	✓	✓

Type of PFAS	Degradability of Comingled Waste	Landfills with Liner and Leachate Collection	Landfills with Gas Collection and Control
	Non-biodegradable	✓	✓

3.b.ii Types of landfills

Landfills are typically classified by ownership status and by the type of solid waste they are permitted to receive, which determines the types of environmental controls they must employ. Depending on the type of waste disposed, a landfill could be subject to regulation and permitting under RCRA, the CAA, and/or TSCA. RCRA regulates two types of landfills: Subtitle C facilities receive hazardous waste, while Subtitle D landfills are primarily intended for the management of non-hazardous waste and can include MSW landfills, industrial waste landfills, C&D waste landfills, and ash landfills. The CAA regulates air emissions from MSW landfills and TSCA regulates landfills that contain PCBs. The CWA (40 CFR Part 503) regulates management practices for the disposal of treated sewage sludge (i.e., biosolids) in monofills. The requirements under RCRA, CAA, TSCA, and CWA determine how the landfill must be constructed, operated, controlled, maintained, monitored, and closed when it reaches its final capacity.

Table 3-2 identifies the environmental controls required by RCRA and CAA for the landfill types defined in RCRA Subtitles C and D. RCRA regulations focus on the containment of solid waste and leachate, while CAA regulations focus on collection of LFG and control (i.e., destruction) of targeted pollutants. The landfill types differ in how they are constructed, operated, controlled, monitored, and closed. Subtitle C landfills and Subtitle D MSW landfills are subject to specific federal design standards, resulting in greater consistency in construction across the country. Controls at other Subtitle D landfills can vary more widely from state to state, and even from unit to unit. Requirements for bottom liners, leachate collection, and final cover requirements at industrial and C&D landfills may range from unspecified to comparable to federal standards.

Table 3-2. Required Environmental Controls by Landfill Type

Landfill Type	Federally Regulated Under	Bottom Liner and Leachate Collection System	Gas Collection and Control System	Final Cover
Hazardous waste	RCRA Subtitle C	Yes (double liner or better)	No	Flexible membrane liner (FML) cap
MSW	RCRA Subtitle D 40 CFR part 258 CAA 40 CFR parts 60 and 63	Yes (composite liner or better)	Yes (when regulatory size and emission thresholds are triggered)	FML cap
Ash monofills	RCRA Subtitle D 40 CFR part 258	Yes (composite liner or better)	No	FML cap
Industrial	RCRA Subtitle D 40 CFR part 257	Varies by state, from no liner requirement to composite liner	No	Varies by state, from no requirements to FML cap
C&D debris	RCRA Subtitle D 40 CFR part 257	Varies by state, from no liner requirement to composite liner	No	Varies by state, from no requirements to FML cap

PFAS concentrations have been detected in landfill leachates from various types of landfills, as shown in Table 3-3. Variation between studies for similar landfill types can be partly attributed to differences in the number and types of PFAS measured. Further, PFAS concentration data reported for landfills outside of the United States may be grossly different as the amounts and compositions of PFAS-containing materials disposed of, as well as weather, and landfill operations and controls may not be comparable and thus are provided for outside reference.

Table 3-3. Range of PFAS Concentrations in Different Types of Landfill Leachate Reported in Published Studies. Adapted from Tolaymat et al., 2023.

Matrix	Number of PFAS Detected (Number of PFAS in Method)	∑PFAS Range (ng/L)	Country	Reference
MSW Leachate	26 (26)	~2,000 – ~20,000	USA	Chen et al. (2024)
	44 (92)	36,000 – 74,000	USA	Smallwood et al. (2023)
	36 (91)	8,000 – 39,900	USA	Cerlanek et al. (2024)
	26 (26)	8,650 – 12,922	USA	Zhang et al. (2024)
	25 (26)	300 – 58,000	USA	Chen et al. (2023)
	10 (11)	15,000 – 18,000	USA	Solo-Gabriele et al. (2020)
	24 (24)	2,700 – 7,400	USA	Huset et al. (2011)
	30 (70)	2,000 – 29,000	USA	Lang et al. (2017)
	2 (2)	330 – 2,600	USA	Clarke et al. (2015)
	28 (28)	230 – 29,000	USA	Helmer et al. (2022)
	2 (2)	47 – 3,400	USA	Michigan Waste & Recycling Association (2019)
	22 (25)	1,400 – 125,000	USA	NWRA (2020)
	31 (40)	BDL – 104,000	USA	California Water Boards (2023)
	48 (92)	15,000 – 70,300	Puerto Rico	Robey et al. (2024)
	14 (14)	33 – 15,000	Australia	Gallen et al. (2016)
	9 (9)	210 – 46,000	Australia	Gallen et al. (2017)
	15 (15)	Not reported	Australia	Simmons (2019)
	25 (43)	31 – 13,000	Germany	Busch et al. (2010)
	24 (24)	2,500 – 36,000	Canada	Benskin et al. (2012)
	16 (18)	700 – 6,400	Canada	Li (2009)
	2 (2)	50 – 2,300	Canada	Gewurtz et al.
	16 (27)	2,200 – 6,100	Norway	Eggen et al. (2010)
	7 (8)	200 – 1,500	Norway	Kallenborn et al. (2004)
	4 (4)	210 – 610	Finland	Perkola & Sainio (2013)
	7 (10)	14 – 17,500	Ireland	Harrad et al. (2019)
	8 (16)	640 – 1,400	Spain	Fuertes et al. (2017)
	28 (30)	320 – 11,000	Norway	Knutsen et al. (2019)
	17 (26)	0.3 – 1,300	Sweden	Gobelius et al. (2018)
	11 (14)	7,300 – 290,000	China	Yan et al. (2015)
	33 (57)	3,040 – 109,000	China	Liu et al. (2022b)
	17 (17)	1,800 – 43,300	China	Huang et al. (2022)
	18 (18)	1,270 – 7,660	Singapore	Yin et al.

Matrix	Number of PFAS Detected (Number of PFAS in Method)	∑PFAS Range (ng/L)	Country	Reference
C&D Leachate	8 (9)	4,200 – 11,000	Australia	Gallen et al. (2017)
	24 (26)	270 – 30,500	USA	Chen et al. (2023)
	11 (11)	14,000 – 16,000	USA	Solo-Gabriele et al. (2020)
MSW Incineration Ash Leachate	9 (11)	2,800 – 3,400	USA	Solo-Gabriele et al. (2020)
	26 (26)	1,906 – 3,483	USA	Zhang et al. (2024)
	26 (26)	39 – 54,500	USA	Chen et al. (2023)
MSW Gas Condensate	26 (26)	199 – 80,900	USA	Chen et al. (2023)
	26 (26)	9,300 – 45,000	USA	Chen et al. (2024)
	26 (26)	14,000 – 18,000	USA	Zhang et al. (2024)
	53 (92)	3,000 – 50,000	USA	Smallwood et al. (2023)
Hazardous Waste Leachate (Primary)	17 (28)	570 – 377,000	USA	California Water Boards (2023)
Hazardous Waste Leachate (Secondary)	13 (24)	25 – 3,700	USA	California Water Boards (2023)

BDL = below detection limit; ng/L = nanograms per liter.

The following subsections describe the types of landfills that are expected to contain PFAS or PFAS-containing materials, with a focus on design and operation aspects that EPA considers important from a PFAS-treatment perspective.

3.b.ii.1 Hazardous waste landfills

Subtitle C hazardous waste landfills are permitted to receive waste that is defined as "hazardous" under RCRA. Hazardous wastes include certain listed manufacturing or industrial process wastes or discarded commercial chemical products and wastes that exhibit one or more hazardous characteristics (i.e., toxicity, corrosivity, reactivity, or ignitability) as defined by RCRA (40 CFR Part 261 Subparts C and D). Hazardous wastes are generally required to be treated with specific technologies to reduce or eliminate the hazards their disposal would otherwise present, and many types of treated hazardous wastes are also required to meet numeric standards for underlying hazardous constituents prior to land disposal (40 CFR part 268). Federal RCRA regulations do not identify any PFAS-containing wastes as hazardous, but most states are authorized to implement RCRA Subtitle C regulations, and their regulations may be more stringent.

Subtitle C hazardous waste landfills are required to have control technologies (e.g., a double liner with a leachate collection/treatment system and leak detection) and practices (e.g., comprehensive record keeping of waste inputs) to minimize the risk of groundwater contamination. Additionally, when closed, these types of landfills must have a final cover consisting of an FML covered by soil. Hazardous waste landfills are generally not required to have gas collection and control systems because they are not intended to accept biodegradable wastes (e.g., biosolids, food scraps). As such, they are generally not biologically active and do not evolve large amounts of gases that could entrain volatile PFAS and lead to high levels of air emissions. However, LFG collection systems could be installed in a Subtitle C landfill to minimize risks related to gas release or gas emissions.

Although few states have proposed regulating PFAS-containing wastes as hazardous wastes, some states have recommended the use of specific treatment technologies for certain PFAS-containing wastes. For instance, some states such as Washington, encourage solidification/stabilization (S/S) of waste AFFF in a neutral material such as concrete prior to landfilling (Washington State Department of Ecology, 2024). A study by the United States Department of Transportation found that the addition of powered activated carbon to S/S pozzalonic binders significantly reduced PFAS leachate concentrations from PFAS-contaminated soil from a historic aviation fire training facility (Mishewitz and Ruehl, 2022).

Hazardous and non-hazardous solid wastes can contain PFAS, and hazardous waste landfills have been used for disposal of non-hazardous wastes containing PFAS. Therefore, it is likely that PFAS-containing wastes will continue to consume a fraction of hazardous waste landfill capacity. Although waste containing PFAS is currently not defined as hazardous waste under RCRA (unless the particular wastes are hazardous for some other reason), some waste generators, perhaps to be cautious, have been sending PFAS-containing wastes to hazardous waste facilities. To ensure hazardous waste landfill capacity is available in the future, EPA reviews and analyzes the *Biennial Hazardous Waste Report*[24] and other data to develop and then publish an assessment of national capacity for hazardous waste management.[25] The last such capacity assessment indicated that existing nationwide capacity remains adequate through 2044, and a new assessment is currently underway to incorporate new information and extend the time horizon. EPA will continue to work with states to monitor hazardous waste treatment and disposal capacity and report on the status.

3.b.ii.2 Municipal solid waste landfills

EPA estimates that in 2018, 50 percent of the MSW generated was landfilled (U.S. EPA, 2024). Wang et al. (2025) identified PFAS in common consumer products that ultimately are disposed in MSW landfills, and this type of landfill may routinely receive lower-concentration PFAS waste such as biosolids, phytoremediation biomass, and contaminated soils.

An MSW landfill typically has at least a bottom liner and an extensive gas collection and control system (GCCS) to collect the LFG generated when the biodegradable waste, such as food scraps and office paper, degrades over time. Also, under RCRA, MSW landfills that received waste after 1993 must install a composite liner that meets design criteria (40 CFR 258.40). However, MSW landfills that receive less than 20 tons of waste per day are exempt from installing a liner and monitoring groundwater under RCRA (40 CFR 258.1(f)(1)). MSW landfills that exceed specified thresholds for size (based on design capacity) and emissions of non-methane organic compounds (NMOCs) are required to install gas collection systems under current CAA regulations. LFG must be collected and managed according to the requirements of the CAA standards of performance (40 CFR part 60 subparts Cc, Cf, WWW, and XXX). Therefore, a subset of MSW landfills do not have gas collection and could release fugitive PFAS associated with LFG emissions. Similarly, unlined MSW landfills are ineffective at managing the release of mobile PFAS to groundwater (see Section 3.b.iii.1). The fate of PFAS in LFG managed through on-site GCCS including on-site flares, engines, boilers, and turbines is the topic of ongoing investigation.

Most modern Subtitle D MSW landfills, when constructed and operated with appropriate environmental controls (e.g., a flexible membrane liner system and leachate and LFG collection and management

[24] Available at: *https://www.epa.gov/hwpermitting/assessment-national-capacity-hazardous-waste-management*.

[25] Background information and links to related documents are available at *https://www.epa.gov/hwpermitting/assessment-national-capacity-hazardous-waste-management*.

systems), can help to contain PFAS. However, research indicates that up to 5 percent of the PFAS may be released in the LFG and 11 percent may be released in the leachate annually (Tolaymat et al., 2023). Some MSW landfills have accepted PFAS manufacturing wastes, and as a result, PFAS concentrations in leachate are higher than the typical range expected in MSW landfill leachate (NWRA, 2020). Landfill leachate may be sent to wastewater treatment plants (WWTPs) that are incapable of treating or destroying PFAS. Thus, the PFAS in landfill leachate, unless pretreated before entering the WWTP, may be discharged in WWTP effluent or may end up in biosolids that are subsequently land-applied, incinerated, or returned to a landfill for disposal. There is a need for additional research regarding the effectiveness of emerging PFAS separation and destruction treatment technologies on a landfill leachate matrix. There also is a need for additional research on the physical and chemical properties, fate and transport, and transformation processes for PFAS precursors and intermediate and terminal degradation products.

3.b.ii.3 Ash monofills

Ash monofills are a subtype of Subtitle D landfills that mainly receive ash from MSW combustors, but could also receive smaller volumes of ash from other combusted waste streams, such as biosolids from POTWs. Ash monofills typically have a bottom liner and final cover requirements similar to other MSW landfills. However, they usually do not require LFG collection and control systems due to the use of combustion to remove biodegradable waste. Solo-Gabriele et al. (2020) found that ash monofills have lower PFAS concentrations than other landfill types, as shown in Table 3-3. While some landfilled wastes, such as MSW combustion ash, may have low PFAS concentrations, the inclusion of higher-concentration PFAS waste types in MSW combustion feed streams may increase MSW combustion ash PFAS content and potential for PFAS release. The PFAS in MSW combustion ash are remnants of incomplete combustion, since these facilities are generally designed to recover heat and generate electricity and not (currently) PFAS contaminant destruction.

New comparative studies show that the total detected PFAS concentration in MSW leachate samples ($\sum_{26}$PFAS mean: 9,641 ng/L) was higher than in MSW incinerator ash leachate samples ($\sum_{26}$PFAS mean: 2,621 ng/L) (Zhang et al., 2024). The variability of total detected PFAS concentrations in leachate was also higher for the MSW landfill (coefficient of variation, CV = 43 percent) compared to the MSW incinerator ash landfill (CV = 16 percent) (Zhang et al., 2024).

Studies have also examined PFAS concentrations in leachate at landfills where ash was co-disposed with other types of waste. A comprehensive study of co-disposal of MSW combustion ash (30 percent) with MSW (70 percent) and at a separate landfill with sewage sludge (4 percent) and ash (96 percent) found that PFAS concentrations were closer to those found in MSW landfill leachate or sludge leachate regardless of the amount of co-disposed ash ($\sum_{26}$PFAS). This phenomenon was hypothesized to occur due to preferential flow of leachate through the wastes instead of the ash which had lower hydraulic conductivity (Liu et al., 2022b).

With limited data on the breadth and concentrations of PFAS in ash monofill leachate, more research is needed to determine the PFAS and precursor content of ash from different combustion technologies and air pollution control systems.

3.b.ii.4 Industrial landfills

Industrial landfills receive non-hazardous solid waste from industrial operations (i.e., non-municipal) and are often designed to manage specific waste streams (e.g., furnace slag, pulp and paper mill sludges,

plastics) and the designs of industrial landfills vary widely based on the characteristics of the waste they receive. Requirements for environmental controls at these landfills also vary from state to state. Depending on the waste types and size of the landfill, some states do not require a liner; however, if a liner is required, a membrane cap can also be required.

Landfills that have historically received PFAS manufacturing wastes have been associated with high concentrations of PFAS in the leachate and environmental impacts (MPART, 2020). Some waste types received at industrial landfills, including plastics and materials with polishes or coatings, contain PFAS (OECD, 2013). Specifically, industrial processes such as leather tanning, chrome plating, and textile manufacture contribute to PFAS in the industrial solid waste stream (ITRC, 2022). Due to the variability in control technologies, industrial landfills may not be effective at containing PFAS and so are generally not believed to be an appropriate disposal option for PFAS-containing wastes.

3.b.ii.5 Construction and demolition landfills

C&D landfills receive waste from construction, renovation, and demolition projects, as well as other inert materials that are generated in high volumes, comparable to MSW waste generation (U.S. EPA, 2020b). GCCSs are not federally required in C&D landfills, due to the low levels of biodegradable waste received compared to MSW landfills, and there are also no federal regulations for C&D landfills to be equipped with a liner. States create their own requirements for environmental controls at these landfills, so requirements vary widely from state to state, ranging from no liner to a required composite liner. However, if a liner is required, a membrane cap may also be required.

C&D landfills are known to receive PFAS-containing wastes (e.g., building materials and carpeting with PFAS treatments) (OECD, 2013; Solo-Gabriele et al., 2020; Bečanová et al., 2016; Liu et al., 2024), and PFAS have been measured in the leachate from C&D landfills equipped with a leachate collection system (Chen et al., 2023). Thelusmond et al. (2025) estimated that C&D landfills in North Carolina generated more than double the PFAS mass flow (5.1 kg/year) from leachate production than MSW landfills (2.3 kg/year).

Due to the variability in state requirements for GCCSs, PFAS-containing wastes that also include a biodegradable component should not be disposed of at C&D landfills and C&D landfills that lack composite liners, leachate management systems, and GCCSs are unlikely to prevent release of soluble or volatile PFAS. Because stable PFAS polymers (e.g., PTFE found in some electrical insulation and membrane materials) are not expected to undergo chemical transformation in a C&D landfill, these types of PFAS compounds are likely to be contained in the C&D waste mass for a long period. However, C&D landfills may not be suitable for more mobile PFAS, such as non-polymer PFAS and SFPs, because these types of PFAS compounds are expected to release soluble PFAS and are a concern for C&D landfills.

3.b.iii Ability of engineered landfill components to contain PFAS

PFAS are emitted from landfills via LFG and landfill leachate, which is any liquid that has contacted solid waste and is typically derived from the breakdown of waste itself or infiltration of rainwater into the waste. As such, controls to manage contaminants in landfills focus primarily on these two releases.

LFG results from the natural decomposition of organic material in landfill wastes under anaerobic conditions. Traditionally, LFG is composed of roughly 50 percent methane, 50 percent CO_2, and a small amount of NMOCs. Leachate can contain a combination of soluble, suspended, and miscible materials

extracted from the waste. As shown in Figure 3-1, landfills constructed with environmental controls (e.g., bottom liner, leachate collection system, gas collection system, final cover system) manage the release of contaminants into the environment. This figure is designed to be inclusive of all controls commonly encountered at landfills. However, it should be noted that the actual configuration of a particular landfill can vary widely by site due to differences in types of waste accepted, operating practices, site conditions, and state regulations.

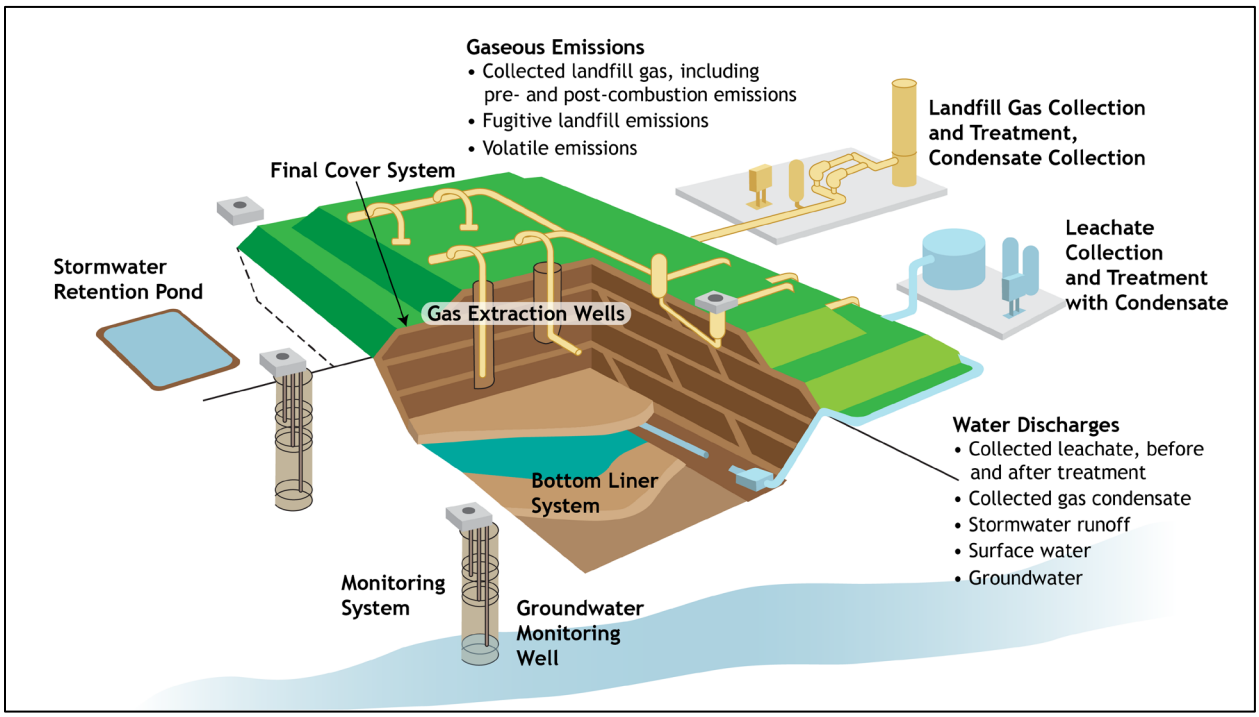


Figure 3-1. Engineered landfill components and potential PFAS release pathways.

3.b.iii.1 Bottom liner and leachate collection system

Under RCRA (Subtitle C), any new landfill, replacement of an existing landfill, or lateral expansion of an existing landfill that received hazardous waste must have a double liner and leachate collection system to limit leachate through the bottom of the landfill. A liner is built of layers of clay and/or polymers (i.e., FMLs) designed to withstand the weight of waste and soil. Leachate generated inside the landfill will collect on top of the liner, so its design includes a leachate collection system contoured to collect leachate through a network of pipes leading to a low point called a sump. The collected leachate is pumped from the landfill and managed as liquid waste (see Section 2.f and Section 3.b.iii.5).

Most landfills regulated under RCRA (Subtitle D) to receive non-hazardous waste are subject to design criteria that require either a composite liner and leachate collection system or a state-approved design that satisfies performance standards to ensure that regulated chemicals do not migrate beyond a specified distance from the landfill.

Uncontrolled leachate could result in the release of PFAS into the environment. Because state requirements for landfill liners vary and some landfills are not required to have liners because of waste type or quantities received, the variation of landfill requirements among states poses a challenge for the

long-term containment of existing PFAS in landfills. Additionally, RCRA-exempt sites may not require any liner system (40 CFR 258.1). Because PFAS are not a RCRA-regulated hazardous waste, existing unlined landfills could receive PFAS-containing wastes that are readily released into the underlying groundwater.

PFAS interactions with landfill liner materials have been the subject of limited studies. The most common types of FML are made with polyethylene geomembranes. PFAS diffusion through linear low-density polyethylene is reported below detection diffusion rates (Di Battista et al., 2020), and diffusion through high-density polyethylene (HDPE) may be even slower due to differences in material structure. PFAS likely pass through clay liners, which are required to have low hydraulic conductivity on the order of 10^{-6} centimeters per second, at the same rate as leachate and other constituents such as chloride (Li et al., 2015).

The most significant pathway for leachate (and PFAS) transmission through geomembrane or composite liners is via imperfections (e.g., flaws or tears) (Di Battista et al., 2020). A review of landfill liner performance reported median leakage rates of 44 and 33 liters per hectare per day for geomembrane and composite liners, respectively, and overall liner collection efficiency of 98 percent (Jain et al., 2023). Even if liners successfully prevent leachate from reaching groundwater, very few data exist on whether concentrated PFAS waste interacts with the different types of geotextiles used for landfill liners, thus affecting the performance of the liner. While the performance of clay liners may not be affected drastically, there is currently no research on the long-term stability of FML in the presence of PFAS.

New research on double HDPE geomembrane liner systems indicates that PFAS were detected in all primary and secondary leachate samples, at concentrations not significantly different from one another, suggesting their transport to secondary leachate collection systems (Robey et al., 2025). HDPE liner permeation experiments with PFAS (specifically PFAAs) indicate migration via permeation an unlikely possibility, given the quickest breakthrough times for 60-millimeter HDPE liners were estimated at approximately 1,500 years (Ahmad et al., 2024). Based on comparing chloride concentrations, PFAS concentrations in secondary leachate were often higher than expected if having been leaked from the primary liner, with PFAAs more likely to exceed expected levels (Robey et al., 2025). These disproportionately higher concentrations suggest that conditions within the secondary leachate collection system itself may be contributing to increased PFAS transformation, and/or that PFAS may be derived from another source, such as LFG (Robey et al., 2025). Wang et al. (2025) and Zhang et al. (2013) showed that FTOHs, the predominant PFAS found in LFG (Titaley et al., 2023), can biodegrade to FTCAs and eventually to PFCAs, both of which were found to be in higher proportions within the secondary leachate (Robey et al., 2025). Further, groundwater PFAS concentrations were found to represent a very small fraction (on average, 0.3 percent) of the total PFAS in secondary leachate, suggesting that the PFAS in the secondary leachate are mainly derived from within the landfill itself (Robey et al., 2025). Thus, PFAS migration through landfill liners and potential groundwater impacts remains a concern, highlighting the need for diligent leachate management and landfill liner quality control (Robey et al., 2025). The study underscores the importance of continued research into PFAS migration mechanisms and the potential environmental impacts of unidentified precursor PFAS in landfills (Robey et al., 2025).

3.b.iii.2 Landfill gas collection system

Landfills use GCCSs to manage gas generated from decomposing organic waste; however, not all landfills are required to collect LFG (see Table 3-2). A GCCS consists of a network of perforated pipes sunken into the waste mass. These “gas wells” are connected to a central blower that pulls gas from the wells. Despite the presence of a GCCS, gas can still migrate both through the surface of the landfill and

underground through the bottom of the landfill. The gas produced by MSW landfills contains about 50 percent methane that is usually collected and burned at the site via flares or for energy recovery.

As noted in Section 3.b.iii.6.1, research has found that soluble PFAS with relatively high vapor pressures can be emitted into the atmosphere via the gas generated at landfills (Ahrens et al., 2011; Hamid et al., 2018; Wang et al., 2020; Weinberg et al., 2011). Direct sampling methodologies are currently under development, and published concentrations of PFAS measured in situ are limited (Titaley et al., 2023; Lin et al., 2024). Many LFG destruction systems (e.g., flares, engines, boilers) generally operate lower than the 1,100°C necessary to achieve the mineralization of PFAS. Nonetheless, LFG collection and management systems (e.g., flares) must be present at MSW landfill sites that accept PFAS waste, especially biodegradable PFAS waste such as biosolids (Tansel et al., 2024) and some paper-based food packaging (Ye et al., 2024). The use of GAC prior to the flaring of LFG can be effective in removing contaminants of concern from LFG (Smallwood et al., 2024). Data on the fate of PFAS in LFG that is managed through on-site GCCS, including flares, are still limited. See Section 4 for potential research needs.

LFG is identified as a significant pathway for the release of volatile PFAS, as noted in studies by Lin et al. (2024) and Titaley et al. (2023). Research has detected various PFAS in landfill leachate, including perfluoroalkyl acids (PFAA), FTCAs, perfluoroalkyl acid precursors, and fluorotelomer alcohols (FTOHs), according to Lang et al. (2017), Robey et al. 2024 and 2025, Zhang et al. (2024), and Smallwood et al. (2023). Among these, volatile FTOHs are predominant in gas-phase PFAS within LFG, with concentrations ranging from 0.46 micrograms per cubic meter (µg/m³) to 14.0 µg/m³, as reported by Titaley et al. (2023) and Lin et al. (2024). These concentration levels are approximately two orders of magnitude greater than FTOH levels reported in ambient air collected near landfills. FTOHs are the most common neutral PFAS, constituting 87 to 97 percent of the total neutral PFAS concentration, as noted by Lin et al. (2024). Specifically, the 8:2 FTOH accounts for 50 to 79 percent of the total neutral PFAS in LFG (Lin et al., 2024).

The observed concentrations of neutral PFAS in LFG range from 22,000 to 33,000 ng/m³, with extrapolated total concentrations (i.e., beyond their calibration range) reaching between 210,000 and 940,000 ng/m³, which is significantly higher than previously reported levels, as highlighted by Lin et al. (2024). Even with conservative estimates, the mass of fluorine released in LFG (32 to 76 percent) is comparable to or exceeds the mass released in landfill leachate (24 to 68 percent), as stated by Lin et al. (2024).

PFAS are typically released early in the decomposition cycle of packaging materials, within about 10 days for high-fluorine materials, indicating that some PFAS may be released before LFG collection systems are installed, as Ye et al. (2024) suggest.

3.b.iii.3 Final cover system

After a regulated landfill has reached its designed and permitted capacity, it must be capped with a cover system. This system typically consists of some combination of soil and membrane materials and is primarily intended to reduce infiltration of rainwater into the landfill to minimize further leachate generation. The cover system also helps increase the efficiency of the GCCS and reduce uncontrolled gas emissions by acting as a barrier. Synthetic membranes and caps are more effective at controlling release of PFAS than earthen covers because of the lower overall hydraulic conductivity. Earthen covers are also more subject to wet/dry and freeze/thaw cycles and cracking that disrupt the compaction of the soil and

can result in greater LFG emissions (Ahrens et al., 2011; Tian et al., 2018; Wang et al., 2020; Weinberg et al., 2011).

3.b.iii.4 Other environmental controls and monitoring systems

In addition to the major design infrastructure discussed above, solid waste landfills implement other practices and systems to protect human health and the environment. In active landfill cells, soil or other inert material covers are applied daily to exposed solid waste. Daily cover reduces leachate generation, gas emissions, and direct exposure to humans and wildlife. Access control for a landfill site, such as a fence, is typically also required to reduce direct human and ecological exposure to waste. Extensive monitoring networks are generally required by states to measure the landfill's impact on groundwater. RCRA Subtitle C requires all hazardous waste landfills to install groundwater monitoring wells. See Section 3.b.iii.5 for leachate discharge controls.

3.b.iii.5 Leachate discharge controls

3.b.iii.5.1 Leachate characteristics

Leachate is the liquid effluent from landfills primarily generated through the percolation or infiltration of rainwater through waste. Leachate often contains high concentrations of biodegradable and non-biodegradable organic matter, dissolved and suspended solids, heavy metals, ammonia, and sulfur compounds (Mukherjee et al., 2015; Renou et al., 2008). PFAS concentrations in leachate are correlated with several physicochemical parameters, including leachate-specific conductivity, alkalinity, total organic carbon (TOC), ammonia, and chemical oxygen demand (COD) (Zhang et al., 2023). However, correlation between leachate PFAS and these parameters does not necessarily indicate causation (Zhang et al., 2023).

The waste type, age, climate at the landfill site, and methods of landfill operation all influence the characteristics of leachate. Some studies found that fresh leachate (from raw waste in transportation vehicles without any processing) initially had no measurable PFAS, but aged leachate showed higher PFAS concentrations, implying that non-target PFAS precursors may transform to measurable intermediate and terminal PFAS degradation products during the aging process (Busch et al., 2010; Eggen et al., 2010; Zhang et al., 2023). Liu et al. (2021) found significantly greater PFAS concentrations in landfill leachate than in leachate from waste transportation vehicles which also could have resulted from transformation processes (Liu et al., 2021). Moreover, landfills in wet, warm climates are most likely to have higher PFAS concentrations in the leachate, as heavy rainfall facilitates the leaching process (Lang et al., 2017; Tolaymat et al., 2024; Wei et al., 2019). Higher PFAS concentrations have also been measured in leachate from an elevated temperature MSW landfill (up to or exceeding 100°C) as compared to concentration in another cell from the same landfill at a lower temperature (less than 60°C), implying temperature-dependent increase in PFAS release and/or precursor transformation rates (Collins et al., 2024).

Leachate from older accumulated wastes (more than 10 years old) contained lower levels of PFAA precursors due to progressive biotransformation over time (Lang et al., 2017; El-Barkaoui et al., 2025). Similarly, waste subjected to prolonged anaerobic degradation exhibited higher PFAS concentrations and higher concentrations of terminal PFAAs relative to measurable PFAS precursors (Liu et al., 2021b; Tolaymat et al., 2023; El-Barkaoui et al., 2025). During the methanogenic phase, organic matter degradation can elevate pH, consequently deprotonating waste surfaces (e.g., organic matter), thereby

reducing their sorption capacity for PFAS and eventually increasing PFAS concentrations in the leachate (Allred et al., 2014; El-Barkaoui et al., 2025).

These factors result in highly variable leachate characteristics across landfill types and on a site-by-site basis. Subtitle C hazardous waste landfills are required to use a leachate collection system during their active and post-closure care periods to mitigate adverse impact to human health and the environment. Since the use of leachate collection systems at Subtitle D landfills varies by landfill type and state requirements, the efficacy of leachate management and PFAS emissions depends on the controls implemented. Because of the persistence of PFAS, the potential remains for leaching of PFAS during the post-closure care period and far beyond. Ideally, leachate collected at the bottom of the landfill is removed and managed to minimize impacts to human health and the environment. See Section 2 for a more detailed discussion on the types of leachates and associated PFAS.

3.b.iii.5.2 Off- and on-site management of leachate

The most common method for non-hazardous waste leachate disposal is off-site treatment at municipal WWTPs, where leachate is mixed with wastewater and treated. The dynamic nature of leachate characteristics, the presence of non-biodegradable compounds and ammonia, and the presence of emerging contaminants (such as PFAS) in the leachate may make it difficult for WWTPs to effectively treat the influent water. In some cases, a landfill with elevated PFAS in its leachate may burden the ability of a WWTP to treat, or remove, these compounds before discharge to the environment (Masoner et al., 2020). Thelusmond et al. (2025) calculated that, generally, leachate is a small contributor to PFAS mass loading of WWTPs; however, for small WWTPs (approximately 3,800 m^3/day), a large landfill receiving more than 51 centimeters (cm) of annual rainfall may contribute comparable PFAS mass loadings as those received from the domestic wastewater.

As mentioned in Section 2.a.ii, wastewater treatment technologies used at most municipal WWTPs are generally ineffective at destroying or controlling PFAS (Schultz et al., 2006). As a result, PFAS is more likely to accumulate in biosolids or be discharged to surface water in plant effluent, which can lead to uncontrolled release of PFAS into the environment. To date, no federal requirements exist for landfill leachate to be monitored or treated for PFAS prior to WWTP consideration for acceptance. However, WWTPs may need to establish additional monitoring and controls to ensure compliance with state-issued permits. For example, local limits developed at the municipal level in accordance with 40 CFR 403.5(c) to prevent the pass through of PFAS through the WWTP or prevent the inhibition of WWTP processes may require contributing sources (e.g., landfills) to install treatment, meet numeric discharge quality levels, or follow best management practices to reduce PFAS prior to introducing the leachate to the WWTP. The selected controls may be tailored to site specific end uses of the WWTP biosolids or effluent. For these reasons, some WWTPs have requested that landfill operators pretreat leachate on site, while others are implementing surcharges based on the leachate quality. Underground injection is another form of off-site leachate management in the United States (see Section 3.c).

Table 3-4 presents leachate management or treatment methods for the on-site management of leachate. Multiple technologies in the table are marked for further research—technologies for which, at the time of publishing, are under development (Sabba et al., 2025). “Secondary treatment required” refers to the remaining concentrated or captured PFAS, which must be disposed of after treatment. “Potential secondary release” refers to the potential for PFAS release or breakthrough during the treatment process. Leachate characteristics are site-specific; therefore, the effectiveness of leachate

technology should be evaluated on a site-by-site basis. (Table 3-3 lists average PFAS concentrations observed in different types of landfill leachate.)

Landfill operators should identify management or treatment methods that: (1) are suitable for the leachate at their specific sites, and (2) meet the leachate discharge standards for chemicals and characteristics of leachate that are regulated. For example, leachate from a hazardous waste landfill is regulated as a listed hazardous waste (F039) and must be managed appropriately, but that does not necessarily mean that treatment technologies are adequate to control the release of PFAS into the environment. The geography of the landfill site and cost-effectiveness of the methods play a crucial role in the identification of viable approaches to leachate management or treatment. The use and effectiveness of leachate management strategies in removing or destroying PFAS during treatment varies (and, as noted in Section 3.b.vi, methods to quantify effectiveness are still under development). Leachate treatment technologies can be largely categorized into physiochemical processes, physical processes, biological processes, natural processes, and other management methods, as grouped in Table 3-4. Considering that leachate contains a variety of chemicals, a combination of physiochemical treatment processes can be used to narrowly target specific parameters for pre-treatment, or as part of a multi-step treatment strategy.

Recirculation of leachate within a landfill—a management strategy unique to MSW landfills—keeps the leachate within the landfill. Although recirculation can filter heavy metals and improve leachate quality, it is primarily used as a management option that may also help accelerate biodegradable waste decomposition. The recirculation of leachate in the landfill would return any PFAS to containment within the landfill. Although recirculation tends to increase PFAS content and may result in larger fugitive emissions due to faster waste degradation, it can also decrease long-chain PFAS concentrations. This process potentially transforms landfills into 'PFAS sinks' rather than 'PFAS sources,' thereby minimizing the environmental footprint of these substances (Saha et al., 2024). Strategic placement of PFAS-containing waste in the upper landfill layers, where degradation can be controlled once the final cover is in place, can further reduce PFAS leaching (Saha et al., 2024). This method limits the likelihood of precipitation reaching the waste, thereby minimizing PFAS migration into the leachate (Saha et al.,2024).

Table 3-4. Existing Landfill Leachate Treatment Technologies for PFAS Removal or Destruction

Treatment Technology	Treatment Mechanism	Pros for PFAS Treatment	Cons for PFAS Treatment	References
Physiochemical Processes				
GAC	Adsorption	• Familiar technology • Effective for long-chain PFAS	• Secondary treatment required • Short-chain PFAS breakthrough • Potential secondary release • Cost	• McCleaf et al. (2017), Pan et al. (2016), Ross et al. (2018)
PAC with coagulation	Adsorption	• Effective for long-chain PFAS	• Secondary treatment required • Costly for high-volume leachate • Potential secondary release	• Bao (2014), Pan et al. (2016)
Polymeric adsorption	Adsorption	• Tailored for specific compounds	• Secondary treatment required • Potential secondary release	• Liu (2017)
Ion exchange resin	Ion exchange adsorption	• Specified for certain compounds • More effective than GAC for long-chain compounds	• Secondary treatment required • Less effective for short-chain PFAS • Potential secondary release	Dickenson and Higgins (2016), McCleaf et al. (2017), Ross et al. (2018)
Zeolite	Ion exchange adsorption	• Inexpensive	• Secondary treatment required • Low surface area compared to GAC • Unknown reaction with short-chain PFAS	Chiang et al. (2017), Ochoa-Herrera and Sierra-Alvarez (2008)
Ozonation/ ozofractionation	Oxidation	• Potentially effective multi-contaminant removal	• Potential side-product formation • Secondary treatment required	Franke et al. (2019), Lin et al. (2012), Rahman et al. (2014), Ross et al. (2018)
Fenton oxidation	Oxidation	Limited data available	Limited data available	None identified

Treatment Technology	Treatment Mechanism	Pros for PFAS Treatment	Cons for PFAS Treatment	References
Photocatalytic advance oxidation process	Oxidation	• Permanent degradation	• Potential side-product formation • Cost	Lockwood (2018), Ross et al. (2018)
Coagulation-flocculation	Precipitation	Limited data available	Limited data available	Bao (2014), Dickenson and Higgins (2016), ITRC (2018), Rahman et al. (2014)
Chemical precipitation	Precipitation	Limited data available	Limited data available	None identified
Air stripping	Volatilization	More research needed	• Potential secondary emissions	None identified
Foam fractionation	Separation	More research needed	• High PFAS emissions to the air	Smith et al. (2022 and 2023)
Physical Processes				
Reverse osmosis (RO)	Physical separation	• Commonly used • Effective for short and long-chain PFAS	• Secondary treatment required for high-volume concentrate • Membrane fouling	Dickenson and Higgins (2016), Ross et al. (2018)
Nanofiltration (NF)	Physical separation	• Uses less energy than RO • Effective for short- and long-chain PFAS	• Secondary treatment required	Boo et al. (2018), Dickenson and Higgins (2016)
Ultrafiltration (UF); microfiltration (MF)	Physical separation	Not available	• Not effective for PFAS	U.S. EPA (2020a)
Climatic evaporation; thermal evaporation; mist evaporation	Volume reduction	Limited data available	• Potential secondary emissions	Cerlanek et al. (2024), Goukeh et al. (2025)
Other On-Site Management Methods				
Recirculation	Containment	• Co-location with landfill	• Oversaturation • Potential surface water contamination • Dependent on climate	None identified

Treatment Technology	Treatment Mechanism	Pros for PFAS Treatment	Cons for PFAS Treatment	References
Underground injection	Containment	• Potential solution for PFAS concentrate	• Dependent on site geology • Regulatory approval	ITRC (2018)
Incineration	Thermal destruction	• PFAS destruction	• Potential secondary emissions (incomplete destruction) • Regulatory approval	ITRC (2017), Yamada et al. (2005)
Solidification	Containment	• Co-location with landfill • Reduces PFAS mobility • Could be used in combination with other treatment technologies	• Consumes air space in landfill • Unrealistic for large leachate volume	None identified
Biological Processes				
Activated sludge process sequencing; batch reactor; anaerobic; digestor; membrane bioreactor	Biological processes	Limited data available	• Limited by high concentrations of non-biodegradable organic matter	Ross et al. (2018), Saez et al. (2008), U.S. EPA (2020a)
Natural Processes				
Constructed wetlands; aerated ponds; phytoremediation; land application	Environmental release	Not available	• Direct release of PFAS	U.S. EPA (2020a)

3.b.iii.5.3 Leachate management and treatment technologies

Membrane treatments separate compounds from the leachate using mechanical filtration and pressure. Leachate passes through selective membranes (such as RO, NF, UF, and MF membranes) that divide it into two parts: permeate (which has passed through the membrane) and concentrate (which has not). The permeate and concentrate can then be treated as independent streams. The primary difference between these membranes is the pore size, which in turn affects the operating pressure and removal efficiency for different types of contaminants. RO is the most commonly used type of membrane for leachate treatment, while NF, UF, and MF are generally used in combination with other treatment technologies including RO. Although RO and NF are known to be effective in concentrating some PFAS, UF and MF have pores that are too large to limit the release of most water-bound PFAS across the filtration membrane. Membrane fouling and a large amount of concentrate generation are two of the major drawbacks observed in implementing the membrane treatment system for landfill leachate and

may be further complicated by high concentrations of PFAS (Dickenson and Higgins, 2016; ITRC, 2018; Ross et al., 2018).

An adsorption process with activated carbon is used for targeted removal of organic matter at some landfill sites. Activated carbon is known to be effective at trapping some PFAS, but it may need to be combined with other treatment methods to manage the full range of PFAS found in landfill leachate. Activated carbon is a non-selective treatment method, expected to be less effective in higher strength effluents, such as landfill leachate, that contain nutrients and other compounds that compete for adsorption sites within the carbon. Once saturated, activated carbon needs to be reactivated through a pyrolysis process (see Section 3.a), collected as solid waste, or otherwise treated, with consideration of the PFAS concentration (ITRC, 2017). Leachate also may need to be pretreated before activated carbon treatment to avoid rapidly saturating the carbon.

Methods using ozone, Fenton's reagent, or advanced photocatalytic technologies are used to oxidize organic matter in the leachate. Ozone and hydrogen peroxide (H_2O_2) in combination with other oxidizing agents have been observed to remove up to 99 percent of COD from leachate at different operating conditions (Renou et al., 2008) and may be effective at reducing or modifying certain PFAS in leachate (Ross et al., 2018). Ultraviolet photocatalytic advanced oxidation is known to be capable of destroying PFAS, but additional studies are needed to understand the subsequent products associated with this method (Lockwood, 2018; Ross et al., 2018).

Ion exchange processes using zeolite and magnetic ion exchange resin remove ammonia and organic matter, respectively. Ion exchange can be flexibly designed to address different compounds and may be effective at reducing PFAS in leachate; however, performance data are not currently available. As with activated carbon, the leachate may require pretreatment and the spent media would need to be handled as solid waste or otherwise treated.

Air stripping of landfill leachate is used for ammonia removal. While air stripping could be effective in the treatment pathway of leachate, if it is used before the removal of PFAS, it would likely lead to emissions of more volatile PFAS to air.

Foam fractionation is a water treatment method that takes advantage of the surfactant-like behavior of many PFAS compounds. As air bubbles rise through contaminated water, PFAS molecules attach to their surfaces. These bubbles accumulate and create a surface foam that becomes concentrated with PFAS. By collecting and collapsing this foam, the process separates PFAS into a smaller, more concentrated volume (called foamate), leaving behind treated water with significantly reduced PFAS levels. Foam fractionation is emerging method for removing PFAS from contaminated waters, particularly from sources like AFFF and leachate. Smith et al. (2022) performed a pilot-scale study that evaluated continuous foam fractionation as a treatment for PFAS-contaminated landfill leachate. The process achieved around 60 percent total PFAS removal, with long-chain compounds removed at rates exceeding 90 percent, while short-chain PFAS showed much lower removal efficiencies (less than 30 percent). Operational factors like airflow rate, foam volume, and contact time influenced performance, but differences between batch and continuous modes were minimal. However, further research is needed to test for PFAS aerosolization and volatilization to better understand safety and prevention of PFAS emissions to the atmosphere (Smith et al., 2023).

Natural processes (such as constructed wetlands and phytoremediation) and biological processes (degradation, nitrification, and denitrification) are expected to be ineffective at treating and preventing

release of many PFAS into the environment. Current biological treatment processes such as the activated sludge process and sequencing batch reactor have not been shown to be effective at treating many PFAS, but future research may show biological treatment can play a role in controlling some PFAS or converting them into other types of PFAS. Note that biological treatment does not necessarily result in PFAS releases directly into the environment, because the outputs can be further treated.

Leachate treatment through evaporation reduces the volume of leachate. Open-air evaporation methods may be effective at concentrating leachate but are a pathway for secondary PFAS releases to air. Commercial evaporators operated through the heat generated by the LFG combustion or other fuel sources are sometimes used at landfills. Exhaust gases emitted from the evaporators may be exposed to high temperatures, but those temperatures may not be high enough or last long enough to destroy PFAS (see Section 3.a). Two full-scale thermal landfill leachate evaporation systems were evaluated by Cerlanek et al. (2024). Samples analyzed included the feed leachate, residual evaporated leachate, sludge, and condensed vapor. PFAS concentrations were measured in both the evaporation vapors and residuals. It was estimated that the vapor pathway can emit 9 to 24 percent of feed leachate PFAS mass. Similarly, Goukeh et al. (2025) found that approximately 99.9 percent of FTOHs and between 0.14 to 6.8 percent of PFCAs were lost in a bench-scale rotary evaporator treating three landfill leachates and two synthetic leachates.

3.b.iii.6 Landfill gas emission controls

3.b.iii.6.1 Landfill gas characteristics

Under the anaerobic conditions that dominate landfill environments, organic waste (e.g., food waste, paper, cardboard) decomposes and generates LFG. In MSW landfills, LFG consists mostly of methane and CO_2. In most landfills where gas is collected, it is burned for energy or to destroy the methane and other organic chemicals it contains. Even at sites that actively collect LFG, a fraction (EPA estimates 25 percent, on average; U.S. EPA, 2025) of the LFG is emitted directly to the environment through the landfill surface and other routes. These uncontrolled emissions are referred to as fugitive losses.

Research has found that soluble PFAS with relatively high vapor pressures can be emitted into the atmosphere via the gas generated at landfills (Ahrens et al., 2011; Hamid et al., 2018; Wang et al., 2020; Weinberg et al., 2011). EPA is currently conducting research on direct LFG sample evaluation for PFAS concentrations (see Section 4).

Simulations show that methane, a major component of LFGs, can significantly affect PFAS behavior in landfill settings by competitively adsorbing with vapor-phase PFAS, particularly FTOHs, in montmorillonite-rich clay barriers, potentially leading to their accumulation within the barrier materials (Xu et al., 2025). As moisture content increases, methane's competitive advantage diminishes, allowing PFAS to maintain distinct adsorption regions within unsaturated montmorillonite pores, slowing its mobility, according to Xu et al. (2025). These findings may provide insights to improve landfill barrier soil designs to reduce PFAS fugitive emissions. However, the challenge of effectively destroying PFAS within collected LFG remains unresolved, as discussed in Section 3.b.iii.2.

Unlike waste in MSW landfills, the C&D landfill waste that contributes most to LFG production is generally dominated by gypsum drywall (Yang et al., 2006). Gypsum drywall results in C&D LFG largely consisting of hydrogen sulfide, a highly pungent gas, with a smaller fraction of methane. Because C&D landfills generate a lower volume of gas than MSW landfills, LFG from C&D landfills is typically not collected and is often emitted to the environment without treatment.

3.b.iii.6.2 On- and off-site management of landfill gas

LFG collection and management are regulated under the CAA through New Source Performance Standards (NSPS) and National Emission Standards for Hazardous Air Pollutants (NESHAP) programs. After collection, LFG can be managed on site and burned using a flare. There are two basic types of flares common at MSW sites: open (candlestick) and enclosed flares. LFG can also be managed off site, where it is usually piped from the landfill to a nearby gas-fired system to generate heat or power.

On-site open flares must operate in accordance with key parameters for exit velocity and flare diameter for non-assisted flares (in 40 CFR 60.18). Additionally, a heat-sensing device must be installed to indicate continuous flame presence (but no specific temperature level). A landfill with an enclosed flare must reduce NMOCs to 20 ppmv at the outlet, or reduce NMOCs by 98 percent via a one-time performance test. Lowered emissions are maintained by following operating parameters set during the test for the requisite flare temperature and flow rate.

Combustion temperatures and duration may prove to be critical factors for destruction of PFAS in LFG. While the operating temperature of on-site flare systems averages around 850°C (1,550°F) (U.S. EPA, 2008), power-generating engines and boiler systems may run even cooler. None of these systems operate at the temperature necessary to mineralize PFAS (1,100°C). The use of GAC prior to the combustion of LFG can be effective in removing contaminants of concern from LFG (Smallwood et al., 2024) and could be used to reduce PFAS flows to on-site LFG thermal destruction devices, reducing the potential for PFAS and PICs emissions. See Section 3.a for a more complete discussion on conditions required for PFAS destruction.

Under the CAA regulations, if LFG is treated for sale or use in a beneficial energy recovery device, it must be treated according to a site-specific treatment plan before being utilized on site or piped out of the facility. Like liners, active gas collection systems are not required at all landfills, depending on the landfill size and level of NMOCs in the LFG. The presence or absence of an LFG collection and control system directly affects the release of PFAS via fugitive emissions.

LFG condensate is a liquid that has condensed in the LFG collection system piping during the extraction of gas from the landfill. In gases with high concentrations of water vapor, water condenses in pipes and accumulates in traps staged throughout the collection network. This condensate contains volatile compounds and accounts for a relatively small percentage of flow from a landfill. Gas condensate is commonly collected and managed with the landfill leachate as liquid waste. PFAS has been detected in LFG condensate, with PFBS being the dominant species at a concentration of 1,000 ng/L (Li, 2011) and Chen et al. (2023) finding a maximum total PFAS concentration of 80,900 ng/L ($\sum_{26}$PFAS).

3.b.iv Potential for releases during landfilling

Over time, certain pollutants have increased potential to move into the environment beyond the footprint of the landfill. As water passes through the landfill, it may leach pollutants from the waste—including PFAS—and move them deeper into the waste mass. Controlled landfill leachate is collected and either reinjected, treated on site, or sent for off-site treatment. In the absence of leachate management systems, uncontrolled leachate releases occur when water travels through the waste, out of the landfill, and into groundwater or surface water (see Section 3.b.iii.1).

LFG can also contain heavy metals, organic chemicals, and methane gas and can produce explosive mixtures of gas in the vicinity of the landfill if not properly controlled. LFG capture technologies are

widely used to control gaseous emissions from landfills. However, not all landfills are required to install and operate LFG collection systems. Even when an LFG collection system is installed and properly operated, a fraction of LFG is emitted directly to the environment through the landfill surface and other routes as fugitive losses (see Section 3.b.iii.2).

Even years after landfill closure, direct human and ecological exposure to PFAS is possible if PFAS are emitted through the air or discharged to groundwater or surface water, or if remaining waste is disturbed. Because landfills contain PFAS but are not designed to destroy these compounds, they represent a potential source of PFAS release well beyond the period during which landfills receive waste and the post-closure care period. Ideally, landfill areas could be used for other beneficial purposes after closure; however, this requires that potential risks from landfill contents be adequately managed. Direct exposure to PFAS from landfilled waste is possible for people living or working near landfills due to gaseous or water releases from the site. Additionally, if PFAS-containing wastes are present on trucks and moved to active cells, landfill employees are directly exposed to PFAS on the job. Research has shown elevated concentrations of PFAS in landfill ambient air (Hamid et al., 2018).

Ensuring that PFAS-containing waste is properly disposed of in lined landfills with active gas collection systems where volatile PFAS or MSW landfills are involved is instrumental in reducing PFAS emissions into the environment. Care must also be taken to avoid disposal of PFAS wastes in landfills that are known to have compromised liners, as PFAS in the leachate will migrate and contaminate groundwater.

3.b.v Testing and monitoring

Currently, there are no federal requirements for the monitoring of PFAS in landfill waste, leachate, condensate, or LFG and analyzing for PFAS in LFG may pose a challenge for comparison across destruction/control technologies and types of landfills due to the lack of standardized testing (see Section 4.a for research needs). However, landfill operators should consider including PFAS measurements with the regular leachate monitoring parameters that are regularly evaluated.

EPA and others are conducting studies to evaluate the effectiveness of landfills in containing or managing PFAS (see Section 3.b) and the Agency and collaborators have developed and updated tools for monitoring and testing of landfill PFAS emissions that will provide a better understanding of environmental fate, transport and transformation of PFAs in landfills. EPA published an updated version of Method 1633 (i.e., 1633A) for quantitative analysis of a range of soluble and neutral PFAS in aqueous and solid matrices by liquid chromatography-tandem mass spectrometry (LC/MS/MS), and this method was validated in a variety of matrices including landfill leachate, groundwater, and biosolids. EPA worked with ASTM International to publish ASTM-D8421-11, an additional LC/MS/MS-based analytical method for aqueous matrices that was validated in leachate and groundwater matrices. In collaboration with researchers, EPA also published an integrated, standardized workflow for NTA of PFAS that can be used for identification of non-target PFAS in landfill emissions or in nearby air or groundwater, potentially including novel PFAS precursors for which no certified reference materials are available (Whitehead et al., 2025).

Waste and leachate matrices can present challenges for identification of unknowns due to high levels of background and interferences the limit reliable qualitative identification. This collection of best practices for NTA data acquisition and interpretation will help promote consistency and standardize laboratories' communication of confidence in identification of unknowns.

3.b.vi Uncertainties/unknowns

Additional sampling and analytical methodologies are needed to better characterize the fate, transport, and transformation of PFAS within landfills to provide accurate estimates of PFAS concentrations in waste inputs and landfill emissions and the potential for PFAS releases from leachate or LFG without adequate controls. Standardized analytical methods are needed for volatile PFAS and ultrashort chain PFAS in wastes, leachate and LFG, and aggregate analytical methods such as total organic fluorine and the total oxidizable precursors assay are also needed to simplify testing for a wide range of PFAS precursors and PFAAs.

As with thermal treatment, EPA lacks detailed information on the amounts and concentrations of PFAS and precursor compounds in wastes that are landfilled; however, limited research is providing some information on PFAS fate and partitioning in landfill environments. The bulk of PFAS seems to remain bound with the waste solids, with quantifiable percentages in LFG and leachate. However, there is a high level of uncertainty in these measurements and more data are needed.

A recent study of three MSW landfills in the United States estimated that LFG accounts for approximately 32 to 76 percent of the mass of fluorine/PFAS released from the landfills and 24 to 68 percent via landfill leachate (Lin et al., 2024). Based on data from two MSW landfills, 95 to 97 percent of PFAS leached from MSW was captured by the leachate collection system with 3 to 5 percent found within stormwater and less than 1 percent found in gas condensate and groundwater. These latter data were used in a mass balance to estimate an average mass flux of PFAS leached of 1.67 to 4.21 kg/year, which along with an estimate of the total mass of PFAS within the landfilled waste approximated a 40-year time span for PFAS to be released, assuming constant mass flux (Chen et al., 2024).

EPA has developed peer-reviewed and reliable toxicity data for only a handful of PFAS. Many PFAS precursors and intermediate and terminal degradation products do not have reliable data regarding physicochemical properties that could be used to improve modeling of their fate, transport, and transformation within landfills, or after release in landfill emissions. SFPs can be considered precursors due to their potential to release perfluoroalkyl acid side chains. Much is still unknown about SFP degradation rates in landfills.

Additionally, the efficacy of treatment options for PFAS captured by leachate and LFG collection systems is not well understood and is in some cases intrinsically entwined with TWTDS and thermal treatment options. EPA continues to research these complex and important issues. Refer to Section 4 for a summary of EPA and DoW's planned research activities specific to landfill containment, wastewater treatment, and thermal treatment of PFAS.

Further, current on-site LFG combustion systems—including flares, engines, and boilers—typically operate below the temperatures known to mineralize PFAS compounds (1,100°C and greater). This raises concerns about incomplete destruction and the potential formation of PICs. While GAC pre-treatment may help reduce PFAS concentrations before thermal treatment, the effectiveness of this approach in minimizing PIC formation remains unclear. Further research is needed to evaluate how combustion temperature, residence time, and pre-treatment strategies influence PFAS breakdown and PIC generation in LFG systems, especially under real-world operating conditions.

3.b.vii Summary

Due to widespread use and disposal through typical waste management pathways, numerous PFAS-containing wastes are contained in landfills and some compounds are expected to persist in landfills for many years, given the chemical makeup of PFAS. Though many landfills (except C&D landfills) are designed for permanent waste containment and management of liquid and gas production, it is currently unclear if all landfills used to dispose of PFAS and PFAS-containing materials have controls that are effective for managing PFAS discharges and emissions from waste streams. PFAS-containing waste should not be placed in C&D landfills that do not have liner systems to intercept and manage the leachate. Even with appropriate liners, research has shown an overall collection efficiency of 98 percent for geomembrane and composite liners.

As leachate passes through a landfill, PFAS are released from degrading wastes. PFAS have been detected in the leachate for all types of landfills, and improper management of landfill leachate would result in PFAS releases. To date, research on the efficacy of wastewater treatment technologies in capturing or destroying PFAS in leachate is limited, as most landfills are not currently required to treat leachate for PFAS. The existing data suggest that adsorption and separation treatment mechanisms have been shown to concentrate or capture PFAS from landfill leachate (see Table 3-4). Other leachate management options can control the release of PFAS, including recirculation and solidification, which return PFAS to the landfill. Leachate treatment through natural processes such as constructed wetlands, land application, or ponds is ineffective for preventing the release of PFAS into the environment. More data are needed on the volatilization of PFAS during leachate handling and treatment.

PFAS can be emitted with LFG that is generated as waste decomposes over time. On-site and off-site LFG management commonly uses flares, engines, or boilers to combust LFG. Combustion temperature and duration could be critical factors for the destruction of PFAS in LFG, as discussed in Section 3.a. The use of GAC for treatment of LFG prior to combustion should be explored further for removal of PFAS (Lin et al., 2024).

To varying degrees, hazardous waste or MSW landfills are feasible and effective disposal options for PFAS and PFAS-containing materials. Permitted hazardous waste landfills employ the most extensive set of environmental controls (e.g., double liner systems with leachate collection and leak detection) and practices (e.g., extensive record keeping) that are currently available for the containment of PFAS waste (see Table 3-2) and as a result would be more effective at minimizing PFAS release into the environment than other landfill types. Hazardous waste landfills do not typically accept biodegradable wastes (e.g., biosolids, food scraps), and are not biologically active, so they do not generally need to be equipped to collect and control LFG. Therefore, hazardous waste landfills are not expected to cause PFAS emissions via LFG and may not be appropriate options for biodegradable PFAS-containing wastes.

Modern MSW landfills, when constructed with appropriate controls (e.g., liner system and leachate and gas collection and management systems), can also control the release of PFAS into the environment. Even with these controls in place, the proper management of landfill gaseous and liquid releases needs to be applied to minimize PFAS release into the environment (see Section 3.b.iii). Care must be taken to apply the leachate control technologies that are effective at containing (e.g., solidification, recirculation) or destroying PFAS (see Table 3-4).

Given the high level of uncertainty associated with PFAS behavior in landfills, the effects of PFAS on liner integrity, gaseous emissions from landfills, the effectiveness of leachate treatment for PFAS removal,

and the levels and types of PFAS in landfill leachate, additional research and test data are needed to further evaluate this disposal method for PFAS and PFAS-containing wastes. See Figure 3-2 for a summary of recommended landfill disposal options for PFAS-containing wastes and considerations for selecting the best option based on waste and landfill characteristics.

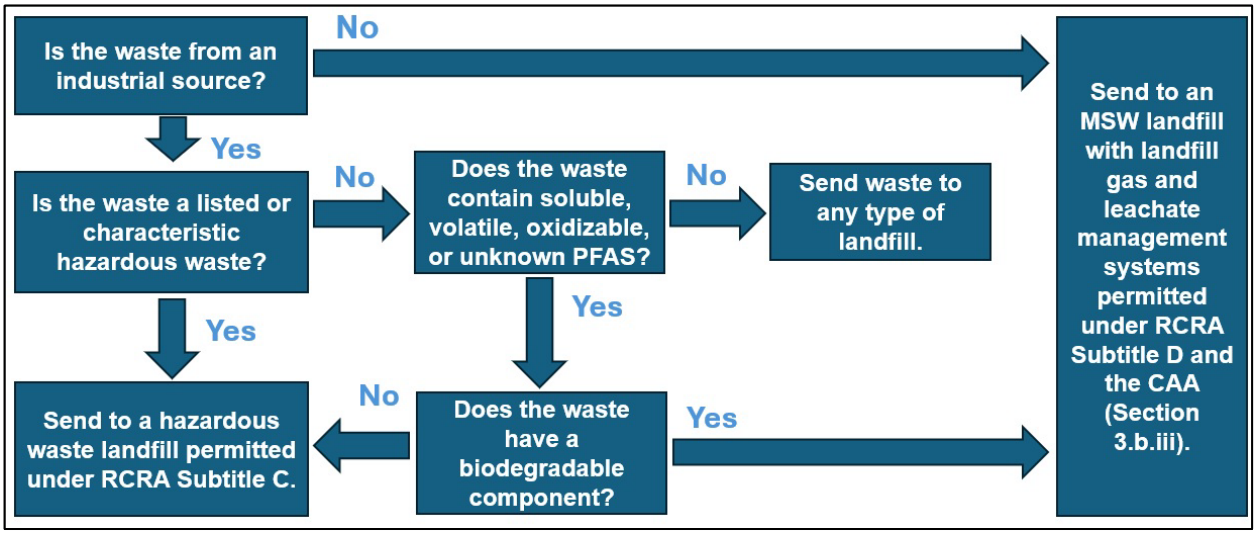


Figure 3-2. Decision framework for selection of landfill disposal for PFAS-containing industrial waste.

3.b.viii References for Section 0

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3.c Underground injection

Underground injection wells are a feasible and effective disposal option that normally should minimize release of PFAS into the environment. However, underground injection wells are only suited for the disposal of liquids and are restricted to locations with suitable geology. PFAS-containing fluids are currently handled similarly to other waste streams that are injected deep into geologic formations in Class I non-hazardous industrial and hazardous waste disposal wells, while shallow Class V wells would be inappropriate for the management of PFAS-containing fluids. The limited number of wells currently receiving PFAS and accepting off-site waste, well location, and waste transportation logistics may significantly limit the type and quantity of PFAS-containing fluids appropriate for underground injection.

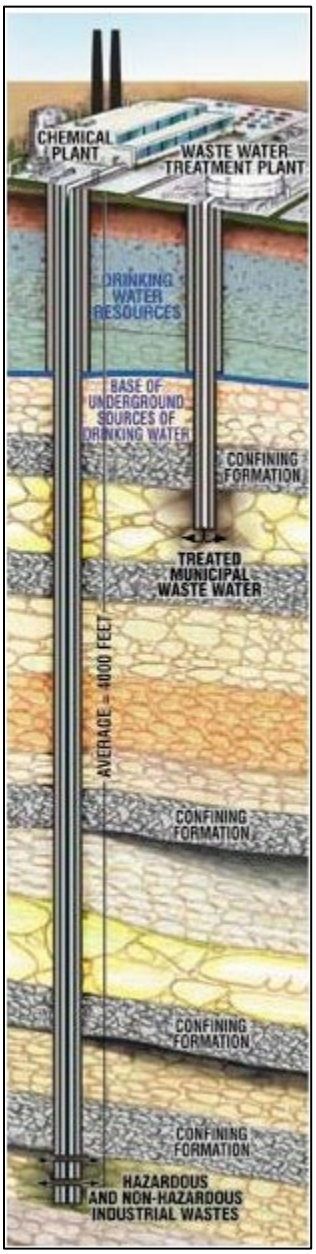


Figure 3-3. Class I wells.

3.c.i Types of wells

Underground injection is generally defined as the subsurface emplacement of fluids through a well. Under the Safe Drinking Water Act (SDWA), EPA is authorized to regulate the permitting of injection wells—including construction, operation, monitoring, and proper closure—for the purpose of protecting USDWs. Underground injection control (UIC) regulations are found in 40 CFR parts 144 to 148.

EPA's UIC program shares information for owners and operators of injection wells, regulators, and the public about safe injection well operations to prevent the contamination of USDWs. Under the UIC program, EPA regulates the permitting of the following well types:

Class I wells inject into geologic formations below the lowermost USDW and are further subdivided into four categories: municipal wastewater, radioactive waste, hazardous waste, and non-hazardous industrial waste disposal wells (see Figure 3-3).

Class II wells are used for injection activities associated with oil and gas production and hydrocarbon storage.

Class III wells are solution mining wells used to inject fluids for the purposes of dissolving and extracting minerals.

Class IV wells, with limited exceptions, have been banned by EPA since 1984 and were used to inject hazardous or radioactive waste into or above geologic formations containing USDWs.

Class V wells include injection wells that are not included in Classes I, II, III, IV, or VI. EPA has identified multiple subtypes, including stormwater drainage wells, septic system leach fields, and agricultural drainage wells.

Class VI wells are used to inject and geologically sequester CO_2.

EPA has determined the use of Class I non-hazardous industrial waste and hazardous waste wells for high-concentration liquid PFAS waste has a lower potential for environmental release when compared to other PFAS destruction and disposal options, and there is relatively low uncertainty in this determination. Class I underground injection wells are designed to dispose of and isolate liquid waste below the land surface and beneath USDWs. The standards associated with the construction, operation, and monitoring of Class I wells are designed to ensure protection of USDWs. These standards include at least one confining layer between the zone in which the fluid will be emplaced and the lowest USDW. While Class I wells are an option for managing PFAS-containing fluids, this technology may not be appropriate everywhere. The suitability of a site for injection is dependent on the geologic formations in the area. Sites need to be evaluated to ensure that there is an appropriate confining zone and that there are geologic formations that can receive fluids.

Class I wells for non-hazardous industrial and hazardous waste are currently being used for disposal of PFAS-containing fluids and are the focus of this section. PFAS-containing fluids that may be disposed of via Class I underground injection wells may originate from industrial activities such as chemical production (e.g., products and byproducts) and waste management operation (e.g., landfill leachate).

The standards associated with the permitting, construction, operation, and monitoring of Class I hazardous waste wells, which are regulated under RCRA and SDWA, are more stringent than for non-hazardous industrial waste disposal wells.

3.c.ii Siting, engineering, and operational controls

3.c.ii.1 Overview of the regulatory framework

Underground injection through Class I non-hazardous industrial and hazardous waste wells is a long-standing, well-regulated disposal technology. Underground injection has been used as a waste disposal practice in the United States since the 1930s, beginning with disposal of brines from oil production activities. Underground injection of wastewater from industrial facilities has been in practice since the 1950s. In response to concerns around underground injection activities and incidents of well failure, in 1974 (the same year the SDWA was enacted), "EPA issued a policy statement in which it opposed underground injection without strict control and clear demonstration that the wastes will not adversely affect groundwater supplies" (U.S. EPA, 2001).

Final UIC regulations were published six years later, and federal and state regulation and oversight has been informed by extensive reviews of injection practices and the associated risks over time (U.S. EPA, 2001). The requirements for Class I wells under 40 CFR part 146 and 40 CFR part 148 (which applies to hazardous waste wells only) are designed to ensure that injected fluids cannot migrate into USDWs through either of two potential pathways: (1) loss of waste confinement, or (2) "improperly plugged or completed wells or other pathways near the well" (U.S. EPA, 2001). These requirements include, but are not limited to:

- Proper siting.
- Conducting geologic and hydrogeologic studies that demonstrate that injected fluids will not endanger USDWs.
- Implementing specific design, construction, and operation requirements.
- Implementing continuous monitoring and periodic monitoring and testing requirements.
- Performing appropriate well closure and plugging activities.

Specific components of these requirements are discussed further below.

3.c.ii.2 Class I non-hazardous industrial and hazardous waste wells

Underground injection to Class I non-hazardous industrial and hazardous waste wells reduces the potential risks of human exposure to injected materials, avoiding discharge to surface and shallow groundwater and generating little or no air emissions. When injected into non-hazardous industrial or hazardous waste Class I wells, fluids are placed below the lowermost USDW. The area into which waste is injected is referred to as the injection zone, which for Class I wells, typically range from 1,700 to more than 10,000 feet in depth (U.S. EPA, 2001). Injection zones are porous and permeable geologic formations and they are separated from USDWs by one or more confining layers of impermeable rock. The confining layers prevent injected fluids from migrating vertically into a USDW.

Class I wells are sited in geological areas that are conducive to injection operations. Siting considerations include ensuring that injected fluids will not migrate through natural fractures and faults from the injection zone into USDWs. Likewise, well operators are required to demonstrate the absence of non-natural pathways (e.g., abandoned wells) or other nearby active wells that could allow for movement of injected fluids into USDWs within a prescribed area surrounding the well (known as the area of review). In addition to the safeguards offered by siting, engineering, and operating requirements, well design and construction requirements incorporate redundant safety features, and construction materials are "corrosion-resistant and compatible with the wastewater and the formation rocks and fluids into which they come in contact" (U.S. EPA, 2001). Class I wells might also use multiple strings of well casing, inject through tubing set on a packer, and be constructed with adequate cement alongside the entire well string to protect any USDWs.

3.c.ii.3 Potential for Release

Permitted underground injection of fluids through Class I non-hazardous industrial and hazardous waste wells ensures that injected fluids are confined and cannot enter USDWs—the pathway of concern for this waste disposal technology. In its 2001 study of risks associated with Class I wells, EPA stated that the "probability of Class I well failures, both non-hazardous and hazardous, has been demonstrated to be low. In the unlikely event that a well would fail, the geology of the injection and confining zones serves as a final safety net against movement of wastewaters to USDWs" (U.S. EPA, 2001).

Injection well operators invest millions of dollars in the permitting, construction, and operation of wells. Development of Class I non-hazardous industrial and hazardous waste wells is a resource-intensive process, with the geologic limitations noted previously. In addition, siting requirements limit the areas in the country where Class I wells can be located (see Section 3.c.iii). Routine operation and maintenance include addressing requirements for extensive mechanical integrity testing, monitoring, and periodic submission of permit/no-migration petitions.

3.c.ii.4 Additional requirements for Class I hazardous waste wells

Class I hazardous waste wells are highly protective of USDWs and include additional requirements beyond those of Class I non-hazardous industrial waste wells. The 1984 Hazardous and Solid Waste Amendments to RCRA prohibited land disposal of hazardous waste, including via underground injection, with limited exceptions. Hazardous waste disposal via Class I injection wells is permitted if the operator can demonstrate that the waste will remain where it has been injected for as long as it remains

hazardous (defined under regulation as a period of up to 10,000 years). To demonstrate this, Class I hazardous waste well operators must receive approvals of "no-migration petitions" from EPA.

No-migration petitions present information and modeling results using data on local and regional geology, waste characteristics, geochemical conditions of the well site, injection history, and many other factors, which EPA reviews to determine whether the petitioner has adequately demonstrated that the waste will not migrate from the disposal site for as long as it remains hazardous. Furthermore, Class I hazardous waste well facilities are subject to inspections and well operators must conduct annual testing and analysis to demonstrate they are meeting the conditions of the permit and that all assumptions, projections, and models are still appropriate and valid. This includes performing mechanical pressure tests and geophysical logging tests to assess well integrity, both internally and externally, to ensure injected fluids are being emplaced and are remaining within the injection zone.

3.c.iii Availability

Currently, the United States has 886 Class I wells. Approximately 50 percent of Class I wells are permitted for non-hazardous industrial waste injection and approximately 16 percent are permitted for hazardous waste disposal. The remainder are permitted for municipal wastewater disposal. EPA is aware of both non-hazardous industrial and hazardous Class I wells that manage PFAS-containing fluids. However, there is currently no national information on the number and location of Class I non-hazardous industrial or hazardous waste wells that could accept PFAS-containing waste or are willing to accept waste not generated on site.

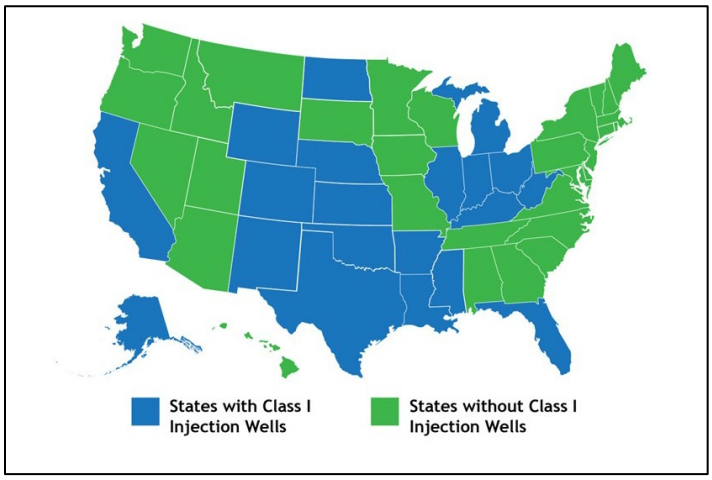


Figure 3-4. States with Class I non-hazardous or hazardous waste injection wells.

Figure 3-4 shows all states that currently have at least one permitted Class I injection well of any type (non-hazardous industrial or hazardous waste). Table 3-5 provides a more detailed breakdown of the number of permitted Class I wells by state.

Table 3-5. Inventory of Permitted Class I Non-Hazardous and Hazardous Waste Wells in the United States (FY 2023; Source: EPA)[26]

Location of Wells		Number of Wells	
EPA Region	State/Tribe	Class I Non-Hazardous	Class I Hazardous
3	West Virginia	1	-
4	Florida	302	-
	Kentucky	2	-

[26] EPA's inventory of Class I non-hazardous waste wells consists of all non-hazardous waste wells, including municipal and industrial waste wells. However, the municipal waste wells are located exclusively in Florida.

Location of Wells		Number of Wells	
EPA Region	State/Tribe	Class I Non-Hazardous	Class I Hazardous
	Seminole Tribe	3	-
	Mississippi	4	4
5	Illinois	5	2
	Indiana	12	4
	Michigan	46	7
	Ohio	9	10
6	Arkansas	8	4
	Louisiana	17	19
	New Mexico	-	6
	Oklahoma	6	-
	Osage Nation	1	-
	Texas	82	78
7	Kansas	58	7
	Nebraska	11	-
8	Colorado	13	-
	North Dakota	8	-
	Wyoming	85	-
9	California	47	-
10	Alaska	25	-
Total		**745**	**141**

3.c.iii.1 Class I non-hazardous industrial waste wells

Non-hazardous industrial waste wells are currently located across 19 states and two Tribal lands, though the majority are in five states—Wyoming, Texas, Kansas, California, and Michigan. There are currently no Class I non-hazardous waste wells in EPA Regions 1 or 2 (see Figure 3-4 and Table 3-5). Disposal to this type of well requires well operators to apply for and receive a permit for specific waste streams. Current Class I injection wells may have limited capacity for PFAS-containing fluids, as many of them are used for specific purposes and disposal of waste generated on site. To begin accepting PFAS-containing fluids, existing well permits would have to be modified to recognize that the facility is accepting waste from other entities and to authorize the facility to inject modified waste streams.

Well operators must also consider the well's capacity to accept additional volumes of waste and the compatibility of the PFAS-containing fluids with the well material, the geochemistry of the injection formation and formation fluids, and the properties of other injected wastes.

3.c.iii.2 Class I hazardous waste wells

Hazardous waste wells are currently located in 10 states; however, most are in Texas and Louisiana. Most Class I hazardous waste wells are sited at industrial facilities and dispose of waste generated on site (U.S. EPA, 2016). There are currently no Class I hazardous waste wells in EPA Regions 1, 2, 3, 8, 9, or

10 (see Figure 3-4 and Table 3-5). Because of this geographic concentration of Class I hazardous waste wells, waste producers may face transportation and logistical challenges.

EPA anticipates the number of current Class I hazardous waste wells that would begin accepting new sources of PFAS-containing waste to be very limited due to the necessary modifications of Class I hazardous waste well permits (e.g., increased injection volumes, changes to waste streams, no-migration petitions). These modifications would involve engineering and scientific evaluations, modeling, and public hearings.

3.c.iv Testing and monitoring

Class I non-hazardous industrial waste and hazardous waste disposal wells are subject to extensive testing and monitoring requirements established under federal regulations. Requirements for hazardous waste wells are more stringent than for non-hazardous industrial waste wells. Additionally, by law, states with primary enforcement authority for Class I wells may have more stringent testing and monitoring requirements. Class I monitoring and testing requirements are designed to ensure there are no leaks within or out of the well and that all injected fluid is contained in the injection zone.

Broadly, Class I well operators must:

- Analyze characteristics of injected fluids at a frequency that results in representative data.
- Continuously monitor and record injection pressure, annulus pressure, flow rate, and volume.
- Conduct internal and external mechanical integrity testing.
- Monitor for fluid release into the USDW within the area of review.

Class I hazardous waste wells operators must conduct mechanical integrity testing more often than Class I non-hazardous industrial waste operators. They also must establish and follow additional procedures for reporting and correcting mechanical integrity problems. Operators of Class I hazardous waste wells must also develop and follow a waste analysis plan and conduct annual tests of cement at the base of the well (U.S. EPA, 2015).

Additional information on testing and monitoring requirements, including mechanical integrity testing, is included in EPA's summary document *Requirements for All Class I Wells and Class I Hazardous Waste Wells*[27] and EPA Region 5's guidance on *Determination of the Mechanical Integrity of Injection Wells*[28].

3.c.v Uncertainties/unknowns

The fate and transport of PFAS in the subsurface depends on the chemical and physical properties of specific PFAS and the geochemical properties of the injection zone. Understanding of the long-term fate and transport properties of PFAS (including precursors) in the injection zone is currently limited. Studies have shown wide ranges in PFAS properties, and these can be altered by mixture effects and interactions with co-contaminants. This creates uncertainty in predictions of PFAS contaminant release

[27] Available at: *https://www.epa.gov/sites/production/files/2015-10/documents/page_uic-class1_summary_class1_reqs_508c.pdf*.

[28] Available at: *https://www.epa.gov/sites/production/files/2015-09/documents/r5-deepwell-guidance5-determation-mechanical-integrity-200802.pdf*.

and longevity in the injection zone. These uncertainties need to be considered in the development of the required no-migration petition for disposal of PFAS in Class I hazardous waste wells.

3.c.vi Summary

EPA has determined the use of Class I non-hazardous industrial waste and hazardous waste wells for high-concentration liquid PFAS waste has a lower potential for environmental release when compared to other PFAS destruction and disposal options. Permitted underground injection of fluids through Class I non-hazardous industrial and hazardous waste wells ensures that injected fluids are confined and cannot enter USDWs—the pathway of concern for this waste disposal technology. Additional Class I wells may need to be constructed where geologically suitable, and existing well permits may require modification to meet the capacity needs for PFAS disposal. Research on the long-term fate and transport of PFAS (including precursors) to predict release potential in the injection zone could support future permits.

See Appendix D for a summary of costs and considerations.

3.c.vii References for Section 3.c

U.S. EPA (Environmental Protection Agency). (2001). *Class I Underground Injection Control Program: Study of risks associated with Class I underground injection wells* (EPA 816-R-01-007). *https://www.epa.gov/sites/production/files/2015-07/documents/study_uic-class1_study_risks_class1.pdf*

U.S. EPA (Environmental Protection Agency). (2015). *Requirements for all Class I wells and Class I hazardous waste wells. https://www.epa.gov/sites/production/files/2015-10/documents/page_uic-class1_summary_class1_reqs_508c.pdf*

U.S. EPA (Environmental Protection Agency). (2016). *Class I industrial and municipal waste disposal wells*. *https://www.epa.gov/uic/class-i-industrial-and-municipal-waste-disposal-wells*

U.S. EPA (Environmental Protection Agency). (2023). *UIC Injection Well Inventory*. *https://www*.epa.gov/uic/uic-injection-well-inventory

4. Research Needs and Data Gaps for Destruction and Disposal Technologies

EPA's understanding of potential PFAS releases from destruction and disposal activities continues to evolve as the results of testing and ongoing research activities become publicly available, but more research is needed to refine EPA's non-binding, interim guidance on the destruction and disposal of PFAS and PFAS-containing materials. This section identifies and prioritizes research needs that, if addressed, are expected to help EPA provide meaningful updates on effective destruction and disposal practices for PFAS-containing materials in future iterations.

What's New in Section 4 of the 2026 Guidance Document?

- Revised Table 4-1 based on new information presented in this document and public comments received on the 2024 interim guidance.
- Replaced discussion of current research efforts with links to ongoing, relevant federal research activities.

Addressing the research needs described in Section 4.a will require sustained effort across government, academic, and private institutions. Within the federal government, EPA, and DoW have been leaders in supporting the research, development, and demonstration of PFAS destruction and disposal technologies. Information on these activities can be found at:

- EPA's PFAS Innovative Treatment Team – Section 5.a
- EPA's Research on PFAS[29]
- DoW's Strategic Environmental Research and Development Program (SERDP) and the Environmental Security Technology Certification Program (ESTCP) PFAS Projects[30]
- DoW's Defense Innovation Unit Prototype Projects[31]

Colleges and universities, who are the recipients of federal and other research funding, conduct cutting-edge research related to PFAS destruction and disposal. These institutions often bring together expertise from many disciplines which enables the rapid development of innovative approaches for managing the constantly changing set of PFAS-containing materials.

Other entities have strong interests in expanding into the area of PFAS material management, disposal, and destruction. These entities include private industry, the operators of waste disposal technologies, municipal water utilities, professional and trade associations, and relevant research foundations. These organizations are actively developing and marketing solutions to address PFAS material management, disposal, and destruction.

[29] Available at: *https://www.epa.gov/chemical-research/research-and-polyfluoroalkyl-substances-pfas*.

[30] Available at: *https://www.serdp-estcp.org/Featured-Initiatives/Per-and-Polyfluoroalkyl-Substances-PFASs*.

[31] Available at: *https://www.diu.mil/pfas*.

EPA encourages all entities conducting PFAS destruction and disposal research to do so in a manner that is consistent with the tenets of gold standard science established in Executive Order 14303 (*Restoring Gold Standard Science*[32]) and further defined in a memorandum from the *Office of Science and Technology Policy dated June 23, 2025*[33].

4.a Research needs to inform future guidance updates

EPA has translated the uncertainties and data gaps identified throughout this interim guidance into research needs, which are presented in Table 4-1. The research needs in Table 4-1 are focused on advancing our ability to measure PFAS in materials and releases from destruction and disposal technologies and improving our understanding of the performance of thermal treatment units, landfills, and underground injection control wells for managing PFAS and PFAS-containing materials. Individually, the research needs identified in Table 4-1 are complex to address because of the variety of substances within the PFAS class, the range of PFAS-containing materials discussed in this interim guidance, and the variability in technology designs and operating conditions. Collectively, some of the research needs identified in Table 4-1 are dependent upon other research needs. For example, fully characterizing PFAS releases from destruction and disposal technologies is dependent upon the availability of methods for measuring PFAS in air, water, and solids. Because of the complexity of the research needs and their inter-dependence, EPA anticipates that it will take years for the research needs in Table 4-1 to be fully addressed.

EPA has also prioritized the research needs in Table 4-1 as either high, medium, or low based on the potential for results to inform future versions of this interim guidance. In general, EPA designated the characterization of potential PFAS releases from thermal treatment units and landfills as high priority because these data are essential for determining whether nearby populations living near destruction and disposal sites could be exposed to PFAS at unreasonable levels from these facilities. At the same time, EPA recognizes that current laboratory methods for identifying and quantifying PFAS are limited. As such, the Agency also considers the development of sampling and analytical methods to be medium or high priority. EPA does not, however, believe that characterizing PFAS releases from PFAS destruction and disposal activities should wait until additional analytical methods are available. Rather, EPA expects that characterizing PFAS releases from destruction and disposal sites will be iterative and that future characterization efforts will be informed and guided by past efforts and the availability of new methods. EPA also expects that data on PFAS releases from PFAS destruction and disposal technologies will be used to inform and prioritize future research efforts.

EPA designated research needs associated with the underground injection of PFAS and PFAS-containing materials via Class I wells as a low priority. The Agency made this designation because the standards associated with the construction, operation, and monitoring of Class I wells are designed to isolate liquid wastes deep below the land surface and ensure protection of USDWs. While data on the performance of Class I wells for managing liquid PFAS waste would be informative, the highest priority continues to be on characterizing PFAS releases from thermal treatment units and landfills.

[32] Available at: *https://www.govinfo.gov/content/pkg/FR-2025-05-29/pdf/2025-09802.pdf*.

[33] Available at: *https://www.whitehouse.gov/wp-content/uploads/2025/03/OSTP-Guidance-for-GSS-June-2025.pdf*.

Table 4-1. Prioritized Research Needs to Inform Future Guidance Updates

Topic	Research Needs	Priority
Thermal Treatment	Methods for sampling and analyzing PFAS in solid-, liquid-, and gas-phase waste streams from thermal treatment units:	
	• Organic fluorine (all phases).	Low
	• Targeted methods for non-polar, semivolatile PFAS (gas-phase).	High
	• Targeted methods for polar, volatile PFAS (gas-phase).	High
	• Targeted methods for cationic, zwitterionic, and nonionic PFAS (solid- and liquid-phase).	High
	Full-scale performance testing—following the parameters and procedures suggested in Appendix A—at:	
	• Hazardous waste combustors:	
	○ Commercial hazardous waste incinerators (HWI).	High
	○ Hazardous-waste-burning cement kilns.	High
	○ Hazardous-waste-burning lightweight aggregate kilns (LWAK).	Medium
	• Carbon regeneration and reactivation units.	High
	• Non-hazardous waste combustors:	
	○ Sewage sludge incinerators.	High
	○ Municipal waste combustors.	Medium
	○ Thermal oxidizers.	High
	Characterization of PFAS releases from thermal treatment units operating under different conditions/designs:	
	• Gas-phase emissions.	High
	• Air pollution control device (APCD) discharges.	High
	• Bottom ash.	High
	• Biosolids, remediated soil.	High
Landfills	Methods for sampling and analyzing PFAS to better characterize PFAS loading to and fate in landfills and releases from landfills to air, municipal WWTPs, and groundwater:	
	• "Aggregate" measurements that capture target and non-target PFAS (e.g., total organic fluorine, total oxidizable precursors).	Medium
	• Non-targeted analysis to better characterize occurrence and prevalence of novel PFAS.	High
	• Quantitative analytical methods for a wider array of precursors and intermediate and terminal transformation products:	Medium
	○ Volatile PFAS (e.g., FTOHs, FTACs).	
	○ PFAA precursors, including cationic, zwitterionic and non-ionic species.	
	○ Ultrashort chain PFAS (e.g., PFPrA).	
	Approaches for solidification and stabilization of PFAS-containing materials:	
	• Effectiveness of differing solidification and stabilization techniques.	High
	• Performance over time.	High
	Fate and transport of PFAS—including modeling transformation processes and partitioning between solid-, liquid-, and gas-phases in:	
	• Hazardous waste landfills.	High
	• Industrial waste landfills.	High
	• Municipal solid waste landfills.	Medium
	• Ash monofill landfills.	Low
	• Construction and demolition (C&D) landfills.	High
	Full-scale performance testing of engineered landfill components:	

Topic	Research Needs	Priority
	• Bottom liner and leachate collection systems. • On-site leachate treatment systems. • Gas collection and emission control systems. • Final cover systems.	Low High Low Low
	Characterization of PFAS releases from landfills operating in different regions and under different conditions/designs: • Gas-phase emissions, including PICs from flaring or incineration of MSW LFG. • Emissions in leachate collected in lined landfills. • Data on groundwater contamination from infiltration at unlined landfills or from permeation through liners at lined landfills.	 High High High
	Efficacy of PFAS removal during LFG and leachate treatment: • Conventional technologies. • Emerging technologies.	 High Medium
Underground Injection (Class I)	Performance data from wells that are managing PFAS-containing liquids: • Surface management practices. • Effects on injection performance.	 Low Low
	Long-term fate and transport of PFAS in injection zones of Class I wells: • Mixture effects. • Interactions with co-contaminants.	 Low Low
	Data on the presence/absence of PFAS in groundwater near Class I wells receiving PFAS-containing liquids and data on known failures or leaks from Class I wells receiving PFAS-containing liquids.	Low
Emerging Technologies	Lab-, pilot-, and field-scale research to test the effectiveness of emerging technologies for different PFAS-containing materials and to characterize the outputs for PFAS and non-PFAS constituents.[a]	High

[a] See Section 5 for discussion of emerging technologies. EPA encourages technology developers to generate and publicly release data that can be used to answer the questions presented in the technology evaluation framework in Section 5.b.

4.b References

Department of War (DoW). *DoD's Environmental Security Technology Certification Program, DIU Partner to Advance Remediation of PFAS as U.S. Defense Installations*. *https://www.diu.mil/pfas*

Department of War (DoW). *Strategic Environmental Research and Development Program (SERDP) and the Environmental Security Technology Certification Program (ESTCP) PFAS Projects*. *https://www.serdp-estcp.org/Featured-Initiatives/Per-and-Polyfluoroalkyl-Substances-PFASs*

Executive Office of the President. *Restoring Gold Standard Science*. Executive Order 14303. 90 Federal Register 102. May 29, 2025. *https://www.govinfo.gov/content/pkg/FR-2025-05-29/pdf/2025-09802.pdf*

U.S. EPA (Environmental Protection Agency). (2025). *Research of Per- and Polyfluoroalkyl Substances (PFAS)*. *https://www.epa.gov/chemical-research/research-and-polyfluoroalkyl-substances-pfas*

5. Emerging Technologies for PFAS Destruction and Disposal

What's New in Section 5 of the 2026 Guidance Document?

- Added information on remaining PITT outputs in Section 5.a.
- Revised information on the technology evaluation framework in Section 5.b to incorporate suggestions from public comments received on the 2024 interim guidance.

Because of their chemical and physical properties, PFAS can pose unique challenges for end-of-life materials management. As described in Section 3, the effectiveness of commonly used waste management technologies, such as thermal treatment and landfilling, for managing PFAS materials can vary. Additionally, current operating conditions and practices at some thermal treatment facilities and landfills may not be sufficient to limit releases of PFAS to the environment. Concerns about the effectiveness of widely-used technologies for managing PFAS materials call for innovative research and development efforts toward new technologies, as well as testing to apply existing destruction technologies to PFAS materials at various scales.

Development of new technologies typically involves the following stages:

- Technology development often starts with exploring and understanding the basic scientific principles and processes that underly the technology. At this early stage, the primary focus is on gaining understanding of how the process works and whether it shows promise for a particular application (e.g., PFAS destruction).
- If the results of early experiments are promising, the next stage of development generally focuses on prototype development and testing under controlled conditions. This stage allows developers to better understand whether the technology is practical and effective at larger scales. It also provides developers with information on costs associated with manufacturing and operating units.
- In the final stage of development, the focus generally shifts to field-scale demonstration projects that allow developers to test the technology under a variety of real-world conditions and to develop better cost estimates for operation and maintenance. During this stage, developers may identify the best and most cost-effective applications for the technology. In the case of PFAS destruction and disposal, this may include identifying the types of PFAS materials best suited for the technology (e.g., high-concentration materials versus low-concentration materials).

At any stage of the technology development process, developers may determine that the technology is not applicable or is not cost-effective and development can stop.

In addition to research to identify and develop novel technologies for PFAS destruction, efforts are underway to apply existing destruction technologies—other than thermal treatment—to PFAS materials. In these cases, the scientific principles and processes underlying a technology may already be understood, but there is little information on the performance of the technology or its applicability to

PFAS materials. Evaluating the applicability of an existing technology to PFAS materials can occur at various scales:

- Laboratory- and pilot-scale studies can be used to evaluate the effectiveness of a technology under controlled conditions and to determine how operating conditions can affect PFAS destruction.
- Field studies can provide data from full-scale applications that reflect the variability seen in real-world operating conditions.

The purpose of this section is to: (1) provide an overview of EPA efforts to identify and test emerging technologies for PFAS destruction, and (2) outline the PFAS destruction and disposal technology evaluation framework, which provides a transparent, consistent approach for evaluating destruction and disposal technologies for PFAS materials. In addition to the EPA-investigated emerging technologies discussed in this section, the Agency is aware of other emerging technologies that are at various stages of development and implementation, such as smoldering combustion, hydrothermal alkaline treatment (HALT), advanced reducing processes (ARP), and non-thermal plasma technologies. For an additional, separate discussion of current PFAS treatment technologies, including emerging technologies, managers of PFAS-containing materials can refer to Section 12 of the *ITRC PFAS Technical and Regulatory Guidance Document*[34].

EPA encourages continued collaboration with other government, academic, and private institutions to continue conducting research to better understand destruction and disposal of waste containing PFAS, and to be potentially included in future iterations of this guidance. EPA encourages that technology developers generate and publicly release data that can be reviewed, and that data are consistent with the tenets of gold standard science established in Executive Order 14303 (*Restoring Gold Standard Science*[35]).

5.a EPA's PFAS Innovative Treatment Team

EPA initiated the PITT in 2020 as a short-term, dedicated, cross-office effort to identify, review, and conduct preliminary research on potential treatment technologies. Over six months, the multi-disciplinary PITT conducted state-of-the-science reviews of proposed methods to dispose and/or destroy PFAS in media and wastes. Several criteria were evaluated using available information, including efficacy, feasibility, performance, and costs of each technology, while also considering potentially hazardous byproducts. The PITT partnered with other agencies and states to conduct a competitive public challenge to identify solutions that were potentially overlooked and to encourage further development of potential technologies.

The PITT chose to focus on the effectiveness of a few technologies, such as mechanochemical degradation, gasification and pyrolysis, and supercritical water oxidation. These technologies showed theoretical promise of the sought-after criteria: PFAS destruction, production of few to no hazardous residuals or byproducts, commercial availability, and cost effectiveness for various contaminated matrices. References and discussion for each technology can be accessed in the PITT Research Briefs at

[34] Available at: *https://pfas-1.itrcweb.org/*.

[35] Available at: *https://www.govinfo.gov/content/pkg/FR-2025-05-29/pdf/2025-09802.pdf*.

PITT Research Briefs[36]. The identification of these technologies by the PITT should not be considered an endorsement or recommendation to use these technologies to destroy PFAS. Additional research is needed to determine the effectiveness of these technologies for different PFAS-containing materials and to characterize the outputs for PFAS and non-PFAS constituents. An overview of the results of the PITT's state-of-the-science review is available in *Developing Innovative Treatment Technologies for PFAS-Containing Wastes* (Berg et al., 2022).

After the state-of-the-science reviews, the PITT selectively encouraged development and research of the four chosen emerging technologies to better understand the potential for PFAS destruction, specifically AFFF, while avoiding creation or transfer of environmental hazards. The PITT conducted in-house research and partnered with industry and academia to collect data regarding the technologies and to encourage further development of the methods required to ascertain PFAS destruction efficacy. Additionally, during the PITT effort, a subsection of the team developed an innovative technology for a thermomechanochemical (TMC) waste treatment processing device. The TMC methodology and apparatus were patented by the U.S. Patent and Trademark Office in 2024 and 2025 (Whitehill et al., 2024; Whitehill et al., 2025).

Although the effort originally was allotted six months in 2020, the work initiated by the PITT results beyond the initial six-month timeframe. The sections below describe some of the results from this work.

5.a.i PITT-investigated emerging technologies

To further explore the potential for mechanochemical degradation of PFAS, EPA scientists partnered with colleagues in New Zealand to conduct a proof-of-concept case study using a benchtop ball mill, AFFF-spiked sand, and AFFF-contaminated soil (Gobindlal et al., 2023). Mechanochemical degradation is a treatment technology that uses a high-energy, ball-milling device (with the option of co-milling reagents) to produce highly reactive conditions to degrade contaminants. Results from the AFFF-spiked sand showed a 99.99-percent destruction efficiency for the sum of all measured PFAS after 960 minutes, and results from the AFFF-contaminated soil indicate that measured PFAS were below the limit of detection after 1,440 minutes. Because this study analyzed PFAS in the solids after different ball-milling time intervals, from 15 to 1,440 minutes, the authors were able to identify increases in some of the measured PFAS before concentrations decreased, indicating the transformation of PFAS during ball milling. An extractable organic fluorine method was also used to determine the reduction in extractable organic fluorine before and after ball milling. In the case of AFFF-spiked sand, the authors reported a 97.8-percent reduction in extractable organic fluorine. A 53.5-percent reduction in extractable organic fluorine was reported for the AFFF-contaminated soil. Air emissions were not characterized as part of this study, making it difficult to determine whether full mineralization of PFAS occurred, and more work is needed in this regard. As noted in the study, more work is also needed to identify primary operations variables to inform the scalability of mechanochemical destruction.

In 2020, EPA conducted a pilot study at a biosolids treatment facility using pyrolysis (Thoma et al., 2022). Pyrolysis is a process that decomposes materials at moderately elevated temperatures in an oxygen-free environment. During the pilot study, dried biosolids were fed into a pyrolysis reactor operating at approximately 610 °C (1,100 °F). The dried biosolids and the resulting biochar were sampled and tested for 41 target PFAS. Twenty-one PFAS were detected in the input biosolids, and none of the target PFAS were detected in the biochar. The pyrolysis system's target PFAS removal efficiencies were estimated to

[36] Available at: *https://www.epa.gov/chemical-research/pfas-innovative-treatment-team-pitt*.

range between greater than 81.3 percent and greater than 99.9 percent. The pilot study also included limited analysis of the air emission control system, with results indicating no transmission of the target PFAS to the air. It is important to note, however, that full characterization of PFAS using non-targeted methods was not performed and more work is needed to understand PFAS transformations and the destruction mechanism.

EPA also completed studies to test the effectiveness of supercritical water oxidation for treating AFFF. Supercritical water oxidation is a process that occurs when the temperature and pressure of water is above the critical point. Above the critical point, oxidation processes are accelerated. In 2020, EPA partnered with three companies to perform tests of their supercritical water oxidation systems on dilute, PFOS-based AFFF (Krause et al., 2022). During these demonstration tests, each system was operated under slightly different conditions, and each company analyzed samples of the influent and effluent for slightly different PFAS. The overall destruction efficiency of each system was calculated by summing the concentrations of the measured PFAS. In all cases, the overall destruction efficiency was found to be greater than 99 percent. It is important to note, however, that the sum of the PFAS measured in the effluent ranged from approximately 10 µg/L to approximately 100 µg/L, suggesting that further treatment of the effluent may be necessary. It is also important to note that non-targeted analysis was not conducted and air emissions were not monitored, making it difficult to assess the fate, transport, and transformation of PFAS with the supercritical water oxidation systems.

In 2021, EPA also partnered with a company to conduct tests at industrial-scale supercritical water oxidation system (Sahle-Demessie et al., 2022). These tests used a dilute, FTS-based AFFF. The primary objective of the study was to determine the destruction efficacy of the PFAS in the input material. Analysis of the influent and effluent showed greater than 99.99-percent destruction of the total PFAS measured. The average sum of the targeted PFAS in the effluent was approximately 55 µg/L, but PFAS were also detected in the tap water used as quench water in the system, suggesting that the overall performance of the system could be improved if non-PFAS-contaminated water is used in the system. A secondary objective of the study was to characterize degradation products using non-targeted analysis and stack sampling for nonpolar volatile fluorochemicals. Non-targeted analysis of the effluent tentatively identified the shorter carbon-chain PFAS on the targeted analyte list, and the authors suggested a possible destruction mechanism that would be consistent with this result. Further work is needed, however, to determine the destruction mechanism. The air emissions sampling did not detect targeted PFAS in air emissions above background levels; further work with finalized air methods (e.g., OTM-50) is needed to confirm this observation.

At this time, EPA is neither recommending nor discouraging the use of any emerging technology for managing PFAS-containing materials, including the technologies studied by the PITT. While the PITT studies generally indicated potential for PFAS destruction, further work using newly available methods is needed to more fully characterize the outputs of these processes and to evaluate their performance for PFAS-containing materials beyond AFFF.

5.b PFAS Destruction and Disposal Technology Evaluation Framework

Researchers have proposed metrics to evaluate PFAS destruction and disposal technologies, including technology readiness levels (TRLs), DREs of target compounds, fluorine mass balances, and others (Berg et al., 2022; Deeb et al., 2021; Krause et al., 2021). When used individually, these metrics fail to accurately capture comprehensive system performance or provide a holistic understanding of the potential for PFAS transformation and/or release to the environment. For example, DREs calculated

from targeted analyses using existing methods can result in underreporting of the total PFAS in a matrix because existing detection methods can only measure a fraction (e.g., 50 individual PFAS) of the total number of PFAS that may be present in a sample. Similarly, TRLs can fail to accurately reflect the current development stage of a technology by either reporting the overall maturity of the technology, instead of its specific PFAS application, or by relying on a niche, small-scale application to justify an overall readiness level.

These limitations drive the need for a comprehensive framework for evaluating a technology and its application to PFAS-containing materials. Such a framework should allow the user to prioritize the collection and consideration of different pieces of information. To meet these needs, EPA developed a technology evaluation framework that uses a multiple-lines-of-evidence approach to evaluate a technology/PFAS material combination (Table 5-1). The framework provides a transparent approach for evaluating and selecting a technology to reduce PFAS releases to the environment. EPA recommends that managers of PFAS-containing materials use the framework to evaluate emerging technologies, and to inform decisions about destruction and disposal of PFAS-containing materials.[37] EPA encourages technology developers to generate and publicly release data that can be used to answer the questions presented in the framework.

The framework comprises several topical sections: technology, material, analytical methods, disposal/destruction efficacy, community considerations, and regulatory requirements. Within each section, EPA has identified key pieces of information that, when considered together, provide a more holistic evaluation of a technology and its applicability for PFAS disposal or destruction.

The framework is intended to be an adaptable tool that allows the user to prioritize the collection and consideration of different pieces of information via the "priority" column in the framework. This allows users to address questions most relevant to their priorities and with respect to their decision context and the shared priority of preventing and mitigating potential exposures. Users may also choose to prioritize addressing certain questions before others to streamline their evaluation of a technology/material combination. For example, if a user of the framework is evaluating the performance of a destruction technology, the questions in the disposal efficacy section of the framework are not needed and could be removed from the framework. As another example, a user may prioritize answering questions under the technology section of the framework because those questions may rule out a technology for a particular application (e.g., the technology may not be available at the scale needed to process the PFAS-containing material of interest). Once a technology is ruled out, lower priority questions do not need to be answered. For further adaptability, if a user has particular needs or concerns that go beyond those currently identified in Table 5-1, the user may decide to add other categories and questions to the framework. For example, a user may want to add a category for pollution prevention and waste minimization considerations, if that is a relevant priority (e.g., Does the technology use toxic chemicals? Is the technology energy or resource intensive; or Does the technology produce large amounts of waste?). While users may not answer each question in the framework or may choose to add other questions according to their needs, EPA encourages users of the framework to

[37] The Interstate Technology and Regulatory Council (ITRC) PFAS Team developed the *PFAS Technical and Regulatory Guidance Document* (available at *https://pfas-1.itrcweb.org/*) to support state and federal environmental staff, as well as others, to gain a working knowledge of the current state of PFAS science and practice. Section 12 of the ITRC guidance document provides information on PFAS treatment technologies, including destruction technologies. Managers of PFAS-containing materials may find this resource useful when completing Table 5-1.

address as many questions as possible to inform decisions about the destruction and disposal of PFAS materials.

Table 5-1 shows this approach, followed by more detailed considerations for each question.

Table 5-1. Framework for Evaluating Destruction/Disposal Technologies for PFAS-Containing Materials

Category of Information	Key Questions	Priority[a]	Answer and Associated Notes for PFAS Material of Interest
Technology	If available, what is the TRL for the technology/material combination of interest?		
	Is the technology of interest available at the scale needed for the intended application?		
	Is a treatment train needed to meet the desired destruction/disposal outcome?		
Material	What PFAS-containing material has been used to test the performance of the technology of interest?		
	Which PFAS, and at what concentration ranges, have been used to test the performance of the technology of interest?		
	Are there non-PFAS constituents that may affect the performance of the technology of interest?		
Analytical methods	Which standardized or validated targeted analytical methods (e.g., OTM-45, EPA Method 1633A) have been used to characterize the performance of the technology for the PFAS-containing material of interest?		
	Has non-targeted analysis been performed on waste streams from the technology/material combination of interest? If so, are there other PFAS detected that should be considered when assessing this technology?		
	What other analytical approaches have been used to characterize the performance of the technology for the PFAS-containing material of interest?		
	Was the study design and the quality of the data generated during testing appropriate to evaluate the technology?		
Disposal efficacy	Have potential transformation and partitioning pathways been identified for the technology/material combination of interest?		
	Are engineering controls in place to prevent or minimize release of PFAS to the environment?		
	What is the longevity of the disposal technology?		

Category of Information	Key Questions	Priority[a]	Answer and Associated Notes for PFAS Material of Interest
	Has a fluorine mass balance been determined?		
	Does the disposal of the PFAS-containing material contribute to the potential release of non-PFAS constituents to the environment?		
Destruction efficacy	Is the mechanism of destruction understood?		
	Have all process inputs and outputs been characterized for PFAS to the extent possible given current analytical methods?		
	What is the reported destruction efficiency?		
	Have any PIDs (or PICs) been identified?		
	Has a fluorine mass balance been determined?		
	Have all process outputs been characterized for non-PFAS constituents to the extent possible given current analytical methods and understanding of the composition of the PFAS-containing material?		
Community Considerations	Has a destruction/disposal site been identified?		
	What are the characteristics of the surrounding community?		
	Are PFAS releases anticipated from the technology/material combination of interest?		
	Has the surrounding community been engaged?		
Regulatory requirements	Are there state or federal regulations that control emissions or releases from the technology of interest?		
	Are there state or federal regulations that control the management of the PFAS-containing material of interest?		

[a] Users can prioritize key questions using a variety of approaches. For example, questions could be labeled as *high, medium*, or *low* priority. Questions could also be prioritized using a numerical ranking system with *1* indicating questions to answer first, *2* indicating questions to answer second, and so forth.

5.b.i Key questions for technology evaluation

Technology. The first section of the framework focuses on the destruction or disposal technology. Key questions include:

If available, what is the TRL for the technology/material combination of interest? TRLs are generally assigned using the *definitions developed by NASA*[38] (NASA, 2023), where level 1 corresponds to observation and reporting of basic scientific principles and level 9 corresponds to "flight proven"

[38] Available at: *https://www.nasa.gov/directorates/heo/scan/engineering/technology/technology_readiness_level*.

technologies. TRLs may be determined through technology readiness assessments[39] or through other methods. If other methods are used, EPA recommends that users consider the source of reported TRLs and prioritize TRLs determined through independent technology readiness assessments over TRLs assigned by the technology developer.

- **Is the technology of interest available at the scale needed for the intended application?** Some technologies may only be available as mobile, containerized units, which may be suitable for high-concentration, low-volume materials. These units may not be appropriate for low-concentration, high-volume materials where a stationary treatment unit with greater capacity is needed.
- **Is a treatment train needed to meet the desired destruction/disposal outcome?** If so, describe the treatment train needed and whether such technology is available and effective for the PFAS-containing material of interest. Note that treatment trains may produce materials that will also need to be managed.

Material. The second section of the framework focuses on the PFAS-containing material of interest. The six types of PFAS-containing materials discussed in this interim guidance vary in terms of phase, PFAS composition and concentration, physical and chemical properties, and overall composition (e.g., the presence of other contaminants). To understand the performance of a technology across the range of variations within a material type, the following key questions should be addressed:

- **What PFAS-containing material has been used to test the performance of the technology of interest?** The description of the material(s) used to evaluate a technology/material combination of interest should be specific as possible (e.g., 3M Light Water™ versus AFFF), and links to bulk material characterization should be included, if available. If the material evaluated is artificially contaminated (i.e., spiked with known PFAS species and concentrations), that should be noted because it may not be an accurate reflection of the performance of the technology for the intended application.
- **Which PFAS, and at what concentration ranges, have been used to test the performance of the technology of interest?** To best inform decisions on technology use, the PFAS and concentrations used to test performance should match the PFAS and concentrations anticipated for the intended application.
- **Are there non-PFAS constituents that may affect the performance of the technology of interest?** This could include constituents in the original PFAS-containing material or constituents that may be created or introduced during a treatment train or by the technology of interest. For example, hydrofluoric gas may be produced during PFAS destruction, which may cause corrosion.

Analytical methods. PFAS are a large class of substances, and there are various approaches for identifying and measuring PFAS. EPA recommends using a combination of approaches to characterize the performance of the technology for the PFAS-containing material of interest, including characterizing PFAS in emissions or waste streams from the technology of interest. Key questions to be addressed under this section include:

[39] See the *Technology Readiness Assessment Guide* for best practices for evaluating technology maturity. The guide, developed by the U.S. Government Accountability Office and released in January 2020, provides organizations with best practices for conducting high-quality assessments.

- Which standardized or validated targeted analytical methods (e.g., OTM-45, EPA Method 1633A) have been used to characterize the performance of the technology for the PFAS-containing material of interest? When possible, EPA recommends the use of standardized and/or validated methods for targeted analysis.
- Has non-targeted analysis been performed on waste streams from the technology/material combination of interest? If so, are there other PFAS detected that should be considered when assessing this technology? Non-targeted analysis is an important tool for characterizing PFAS in waste streams from a technology/material combination of interest because it can tentatively identify PFAS beyond those measured using targeted analytical methods.
- What other analytical approaches have been used to characterize the performance of the technology for the PFAS-containing material of interest? Other analytical approaches may include total oxidizable precursor assays and total organic fluorine assays for PFAS and analysis for non-PFAS substances (e.g., HF).
- **Was the study design and the quality of the data generated during testing appropriate to evaluate the technology?** For any quality assurance/quality control concerns identified, the user should determine whether it is appropriate to use the data to inform decisions about destruction and disposal of the PFAS-containing material using the technology of interest.

Disposal efficacy. Effective disposal of PFAS-containing material should prevent or minimize re-release of PFAS to the environment. Key questions for evaluating the efficacy of a disposal technology for a particular PFAS-containing material are identified below. (Questions included in this section should be skipped when evaluating a destruction technology.)

- **Have potential transformation and partitioning pathways been identified for the technology/material combination of interest?** The fate and transport of PFAS within a disposal technology can help inform whether PFAS may be present in emissions or releases from the technology. Note that the characteristics of the receiving matrix and environmental conditions can affect the fate and transport of PFAS within the disposal technology.
- **Are engineering controls in place to prevent or minimize release of PFAS to the environment?** If so, is there a monitoring system in place to evaluate the performance of the engineering controls?
- **What is the longevity of the disposal technology?** Because of the strength of the C-F bond, PFAS are persistent. It is important to consider the lifespan of the disposal technology and whether the technology can contain PFAS for very long timeframes.
- **Has a fluorine mass balance been determined?** If so, is there fluorine that is unaccounted for? In some cases, a fluorine mass balance may be impractical.
- Does the disposal of the PFAS-containing material contribute to the potential release of non-PFAS constituents to the environment? PFAS-containing materials may contain other, non-PFAS constituents. Depending on the identity of those constituents and their potential to impact human health and the environment, it may be important to consider whether appropriate engineering controls are in place to prevent or minimize releases of non-PFAS constituents.

Destruction efficacy. Destruction of PFAS should result in breaking C-F bonds without reforming PFAS and releasing PFAS to the environment. Key questions for evaluating the efficacy of a destruction technology for a particular PFAS-containing material are identified below. (Questions included in this section should be skipped when evaluating a disposal technology.)

- **Is the mechanism of destruction understood?** If so, has the destruction mechanism been experimentally confirmed, and have the reaction kinetics been determined? A thorough understanding of the destruction mechanism improves confidence in the performance of the technology of interest for PFAS.
- Have all process inputs and outputs been characterized for PFAS to the extent possible given current analytical methods? If not, what information may be missing?
- **What is the reported destruction efficiency?** Destruction efficiencies (or DREs) are generally reported for specific PFAS (e.g., PFOA or PFOS). As noted earlier, the destruction efficiency (or DRE) alone may not be sufficient to determine whether other PFAS may be released to the environment during the destruction process.
- **Have any PIDs (or PICs) been identified?** If so, which PFAS have been detected and at what levels? Are those PFAS regularly detected for this technology/material combination?
- **Has a fluorine mass balance been determined?** If so, is there fluorine that is unaccounted for? In some cases, a fluorine mass balance may be impractical.
- Have all process outputs been characterized for non-PFAS constituents to the extent possible, given current analytical methods and understanding of the composition of the PFAS-containing material? The destruction of PFAS-containing materials is expected to result in non-PFAS products. Depending on the identity of those products and their potential to impact human health and the environment, it may be important to consider whether appropriate engineering controls are in place to prevent or minimize releases of non-PFAS constituents. It may also be important to consider the effect of the destruction process on non-PFAS constituents in the PFAS-containing material of interest. Thus, the characterization of process outputs beyond PFAS may be warranted.

Community considerations. When considering destruction and disposal technologies for PFAS-containing materials, it is important to consider the community that may be exposed to potential PFAS releases from intended destruction or disposal. Key questions to consider include:

- **Has a destruction/disposal site been identified?** Some destruction technologies may be mobile, allowing for treatment and destruction to occur where PFAS-containing materials are generated or stored. Other technologies may be stationary, requiring PFAS-containing materials to be brought on site for destruction or disposal.
- **What are the characteristics of the surrounding community?** Different factors can influence the potential magnitude of community exposures, such as the size and distribution of the community near the destruction/disposal site, the proximity of the community to the destruction/disposal site, geographical characteristics where the community is located, and other considerations.
- **Are PFAS releases anticipated from the technology/material combination of interest?** If so, describe which PFAS may be released, at what levels, and over what duration. Also note whether there are controls in place to mitigate releases to avoid or minimize potential impacts to surrounding communities.
- **Has the surrounding community been engaged?** Sharing information about planned destruction or disposal activities for PFAS-containing materials allows community members to take actions to address concerns they may have. There may also be opportunities to collaborate with government officials and community leaders on engagement activities.

Regulatory requirements. Regulations regarding the destruction or disposal of PFAS-containing materials can vary from state to state and may change over time. Key questions to consider include:

- **Are there state or federal regulations that control emissions or releases from the technology of interest?** Such regulations may limit the amount of PFAS or other pollutants present in emissions or other releases from the technology of interest.
- **Are there state or federal regulations that control the management of the PFAS-containing material of interest?** Such regulations may specify or restrict destruction or disposal options for the PFAS-containing material of interest.

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Appendix A: EPA Guidance to Conduct PFAS Emissions Field Testing at Commercial Thermal Destruction Sources

The U.S. Environmental Protection Agency (EPA) is conducting per- and polyfluoroalkyl substances (PFAS) thermal destruction emissions characterization research to improve our understanding of the behavior of PFAS with respect to waste incineration and thermal treatment (e.g., hazardous waste, municipal waste, biosolids, contaminated soils, spent media), as well as thermal destruction emissions control technologies (e.g., afterburners, thermal and catalytic oxidizers). The following could be used by any entity evaluating thermal treatment of PFAS-containing materials either in collaboration with EPA or independently.

Overall goals of the field tests are as follows:

- Confirm/determine if thermal treatment under representative conditions can result in the thermal destruction of PFAS as defined by conventional destruction and removal efficiency (DRE) testing.
- Characterize potential products of incomplete combustion/destruction (PICs/PIDs) and evaluate the use of alternative, or surrogate, indicators of destruction performance.
- Confirm that any treated material is appropriate for any defined beneficial reuse (e.g., treated soils, reactivated granular activated carbon [GAC]).

To achieve these goals, it is necessary to determine the specific conditions (i.e., temperature, residence time, and turbulence) required to maximize DRE, minimize PICs/PIDs, and produce products appropriate for beneficial reuse. Because bench- and pilot-scale testing have limitations, actual test data under full-scale thermal treatment conditions are necessary to determine if representative thermal processes can achieve these goals.

Sampling Details:

- EPA is available to collaborate with the host site on the technical approach for source characterization. Testing would require multiple stack sampling methodologies performed by commercial stack testing companies, including the host's regular tester, if desired.
- All gas, liquid, and solid inlet, intermediate, and outlet streams would be collected and analyzed. This includes:
    - At the stack and including all air pollution control devices (APCDs).
    - All inlet streams (e.g., materials to be thermally treated, air or gas inlets, water), including samples taken prior to testing.
    - All process materials (e.g., water used for spray dryers and/or scrubbers, carbon, high pH solution for scrubbers) as they may contain PFAS and affect the results, if unknown.
    - All residual streams (e.g., bottom ash, baghouse and/or electrostatic precipitator [ESP] solids, scrubber effluent, spent carbon).
    - All final products (e.g., reactivated carbon, soils), if applicable.
- A comprehensive air emission source characterization would include:

- Samples would be collected for semivolatile polar PFAS using method OTM-45 and analyzed for targeted (known) PFAS. Samples would be collected for nonpolar volatile fluorinated compounds (VFCs) and PFAS using method OTM-50 and analyzed for targeted (known) fluorinated compounds and the identification of unknown detected volatile fluorinated compounds.
- Samples would be collected for other semivolatile nonpolar target compounds using methods SW-846 Methods 0010/3542/8270 with the inclusion of the Method 8270 procedure.
- Carbon hexafluoride (C_2F_6) and carbon tetrafluoride (CF_4) could be injected during one day of testing, ideally at multiple injection locations, as a surrogate measure of destruction efficiency if appropriate and permitted.
- Samples would be collected for other semivolatile target compounds using methods SW-846 Methods 0010/3542/8270 with the inclusion of the Method 8270 procedure.
- Hexafluoroethane (C_2F_6) could be injected during testing, ideally at the location most PFAS would be injected, or multiple injection locations if possible, as a surrogate measure of destruction efficiency if appropriate and permitted. Tetrafluoromethane (CF_4) can also be injected separately, if FTIR is being used, to ensure the injection system is working and to establish the gas residence time of the system to aid in the sampling of C_2F_6.

- Tests would be run under a single, representative process condition including the waste that would be co-fired with PFAS-containing material.
- EPA would prioritize testing several PFAS-containing materials, including but not limited to:
  - Incineration of aqueous film forming foam (AFFF).
  - Incineration of wastewater sludges.
  - Reactivation of spent GAC.
  - Incineration of municipal solid waste.
  - Thermal treatment of contaminated soils.
  - Incineration of spent ion exchange resins and their concentrated regeneration liquids.
- All sampling would be conducted under a well-documented sampling plan and quality assurance project plan.
- Final testing results would be shared with EPA, the state, local authorities.

Additional details on emission testing approaches can be provided upon request.

Supplemental Information

Emissions sampling and analytical approaches

Emissions samples would be collected for nonpolar, volatile, and polar and non-polar semivolatile PFAS compounds and analyzed for both targeted PFAS compounds, or method established endpoints.

EPA's Other Test Method 45 (OTM-45) would be used to collect polar semivolatile PFAS compounds for targeted analyses. The method's 50+ target analytes include many PFAS commonly found in AFFF. This would enable DE, DRE, and emission rate determination as appropriate for a known list of PFAS compounds along with identifying potential PFAS PICs/PIDs.

The OTM-50 canister sampling method would be used to collect nonpolar volatile PFAS compounds for targeted compound analyses. The current target list includes CF_4 and C_2F_6 as well as a procedure to identify unknown volatile fluorocarbons.

Non-targeted analyses (NTA) are an optional analytical tool for characterizing potential PICs/PIDs. HRMS, combined with chromatographic separation and multiple ionization techniques, is used to identify compounds present in collected samples. NTA could be performed on the OTM-45 samples. FTIR spectroscopy would be used to measure a variety of compounds in real-time, including HF, HCl, CO_2, and water; multiple PFAS compounds including CF_4 and C_2F_6; and multiple known PICs/PIDs. FTIR is particularly useful during injection of a surrogate compound.

EPA research questions

The following section describes detailed elements of the research questions EPA will ultimately work to address, as well as the input information that is needed. EPA requests an opportunity to engage with the host facility prior to developing a full sampling plan to better understand site-specific conditions and requirements.

Process characterization

Question: How does the process operation affect the destruction and removal of PFAS compounds present in the feed?

- Requires understanding of waste feed (e.g., description, concentration, other wastes), feed approach (e.g., liquid feed, through flame/post flame, co-fired, drums/barrels), and feed rate.
- Requires data on combustor operations and conditions (e.g., temperature, residence time).
- Requires data on air pollution control devices (APCDs) and their operation: configuration, operating parameters (e.g., temperature, pressure drop, pH of scrubbing liquid, gas velocity).

Determine destruction removal efficiency (DRE)

Question: What is the DRE and emission rate of the specific PFAS compounds present in the feed material?

- Requires known input rate and identity of PFAS compounds in waste.
- Requires evaluation of the extent of PFAS mineralization.
- Requires replicate sampling of polar semivolatile organics using OTM-45 for quantitative measurement of feed PFAS compounds in emissions.
- Requires sampling across multiple APCDs.

Products of incomplete combustion/destruction (PICs/PIDs)

Question: Are PICs/PIDs formed during thermal treatment and measured by OTM-50 present and, if so, what are they and under what conditions?

- Requires replicate sampling of polar, nonpolar, semivolatile, and volatile organics for non-targeted compound analysis using OTM-45, OTM-50, and Method 0010/3542/8270.
- Requires sampling across multiple APCDs.

Alternative indicators of destruction performance

Question: Can easily measured/monitored, hard-to-destroy compounds serve as alternative (surrogate) indicators for PFAS adequate destruction or PID formation conditions?

- Requires injection of known concentrations/masses of C_2F_6 (approximately 10 to 30 parts per million by volume (ppmv) stack equivalent) at representative waste injection locations (CF_4 can be used as a tracer gas to assist in the process).
- The evaluation is greatly benefitted by on-line FTIR monitoring of the surrogate compounds, PICs, HF, HCl, and other compounds of interest.
- Requires initial background signal baseline, normal operation without the addition of the perfluorocarbons.
- Requires concurrent OTM-50 sampling for analysis of surrogate compounds and known PICs.

Analysis of fluorinated compounds in waste/effluent streams

Question: What is the fate of PFAS compounds across the entire thermal system?

- Requires sampling of all discharges:
  - Gas sampling prior to and after APCDs (e.g., spray dryer, wet scrubber, baghouse and/or ESP, activated carbon injection).
  - Sampling of solid and liquid discharges (e.g., bottom ash, baghouse and/or ESP solids, scrubber effluent analyses, spent carbon).

Analysis of fluorinated compounds in final product materials, where applicable

Question: What PFAS remain in the material treated, such as reactivated GAC or treated soils?

- Requires sampling of influent and effluent materials:
  - Sampling of solid materials (e.g., spent carbon and reactivated carbon).

Appendix B: Summary of the 2021 and 2022 Clean Harbors Test Data

Summary

Two testing campaigns were conducted at the Clean Harbors Environmental Services (CHES) hazardous waste combustor (HWC) located in Aragonite, Utah. Each test campaign was divided into three test conditions: Baseline (Condition 1), Augmented Per- and Polyfluorinated Alkyl Substances (PFAS) Feed (Condition 2, where known amounts of four or five target PFAS were fed to the incinerator), and Post-spiking Normal Operations (Condition 3, where aqueous film-forming foam [AFFF] was included in the incinerator feed). Clean Harbors used the Other Test Method 45 (OTM-45) Measurement of Selected Per- and Polyfluorinated Alkyl Substances from Stationary Sources (OTM-45) from the U.S. Environmental Protection Agency (EPA) for stack gas samples. The 49 targeted PFAS analytes were measured using liquid chromatography/tandem mass spectrometry (LC/MS/MS) with isotope dilution. The tests appear to demonstrate that the primary objective, "demonstrate the ability of the high-temperature incineration system at Aragonite to effectively destroy PFAS in the waste feed during normal process operating conditions," was achieved for the five selected PFAS compounds that were subjects of these studies.

Introduction

Two testing campaigns were conducted at CHES HWC located in Aragonite, Utah. The first campaign was conducted from June 17 to 19, 2021, and the second from February 17 to 19, 2022. The stated principal objective of the test program was to "demonstrate the ability of the high-temperature incineration system at Aragonite to effectively destroy PFAS in the waste feed during normal process operating conditions," with secondary objectives of conducting a mass balance of PFAS in process streams and demonstrating high hydrogen fluoride (HF) removal that would be associated with a high destruction of PFAS. This summary presents a high-level overview of both tests' final reports as well as a third-party review of the 2021 test performed by Dr. Philip H. Taylor under contract to CHES.

General Process Description

The HWC tested at the CHES Aragonite facility consists of a slagging rotary kiln incinerator followed by an afterburner. Solid waste materials and containerized wastes are fed into the rotary kiln only, while liquid wastes may be injected directly into rotary kiln or the afterburner. The air pollution control device (APCD) system for the HWC as tested[40] consists of a spray dryer/gas quench followed by activated carbon injection upstream of a fabric filter (particulates, dioxin/furan, and mercury control). Following the fabric filter, the gases enter a sodium carbonate saturator/wet scrubber (sulfur dioxide, hydrogen chloride, hydrogen fluoride control) with TMT-15 (1,3,5-triazine2,4,6(1H,3H, 5H)-trithione, trisodium salt, used for additional mercury and metals control) injection.

[40] Stack testing was performed in the exit duct of the wet scrubber instead of at the stack for safety reasons (lack of scaffolding). The HWC is equipped with a wet electrostatic precipitator (WESP) following the wet scrubber and prior to the stack, but the WESP was decommissioned in 2004 and is no longer in service. These tests were conducted prior to the WESP although the stack location is the test location for compliance performance tests for the facility.

Operational Description

Each test campaign was divided into three test conditions: Baseline (Condition 1), Augmented PFAS Feed (Condition 2), and Post-Spiking Normal Operations (Condition 3), with the waste feed matrices as follows:

1. **Condition 1.** Normal matrix of liquid materials (waste fuel, clean fuels, sludge, and aqueous wastes) and solid waste materials (containerized, shredded, and bulk solid wastes). Following the 2021 testing, CHES determined that the sludge stream fed to the kiln during all three conditions included some AFFF. During the first run of the 2022 testing, CHES determined that AFFF was inadvertently being fed into the afterburner's corrosive waste port. The testing team decided to be consistent with runs 2 and 3 and continued the AFFF feed, with additional amounts of AFFF fed during Condition 3.
2. **Condition 2.** Normal matrix of liquid materials (waste fuel, clean fuels, sludge, and aqueous wastes) and solid waste materials (containerized, shredded, and bulk solid wastes), with the addition of augmented feed rates of four (2021) or five (2022) PFAS (or their equivalent potassium salt). The four PFAS are perfluorooctanioic acid (PFOA), perfluorooctanesulfonic acid (PFOS), perfluorohexane sulfonic acid (PFHxS), hexafluoropropylene oxide-dimer acid (HFPO-DA), and perfluorobutanesulfonate (PFBS, 2022 only). The target feed rate of the PFAS was estimated to be 0.0816 pounds per hour or around 0.62 grams per minute in 2021 for all PFAS, but the HFPO-DA was increased to 2.2 grams per minute in the 2022 testing to reflect the method detection limit (MDL) for this compound. Similarly, the PFBS feed rate was 0.33 grams per minute on average to reflect the MDL. The PFAS were fed in boxed containers to the rotary kiln via the containerized solid conveyor in approximately 10-gram portions of all PFAS compounds about every 15 minutes for the duration of these test runs.
3. **Condition 3.** Normal matrix of liquid materials (waste fuel, clean fuels, sludge, and aqueous wastes) and solid waste materials (containerized, shredded, and bulk solid wastes). For this test condition, AFFF concentrate was also fed (neat) to the incinerator. In 2021, the AFFF was fed via the containerized solids in 5-gallon buckets at a rate of 176 pounds per hour. During the 2022 test, the AFFF was labeled as Ansulite and was fed through the educt station to the incinerator directly from a tote bin. The AFFF feed rate ranged from 244 pounds per hour to 316 pounds per hour during the 2022 test.

The rotary kiln was operated in a temperature range between 1,893°F and 2,008°F for the 2021 test and between 1,949°F and 2,219°F for the 2022 test. The afterburner was operated at 2,052°F to 2,110°F during the 2021 test and 2,048°F to 2,075°F for the 2022 test. Oxygen content (free) at the exit of the rotary kiln was maintained between 8 and 9 percent and ranged from 6 to 7 percent for the afterburner for both test campaigns. Clean Harbors estimates that non-combustible material is retained within the combustor for approximately 60 minutes, while combustion gas residence time of the afterburner is two to three seconds.

For the 2021 test, the temperature of the flue gas was reported as 369°F following the spray dryer; between 341°F and 343°F following the fabric filter; between 166°F and 168°F at the saturator outlet; and 148°F to 153°F at the stack. For the 2022 test, the temperature of the flue gas was reported as 363°F following the spray dryer; between 168°F and 170°F at the saturator outlet; and 149°F to 151°F at the stack. The following table lists the APCD operational parameters during the testing campaigns.

Test Campaign	Stack Gas Flow (actual cubic feet per minute [acfm])	Soda Ash Flow (lb/hr)	Carbon Injection (lb/hr)	TMT-15 Flow (lb/hr)
2021	66,793–68,761	452–789	30.7–31.5	3.95–4.48
2022	51,053–53,640	411–1594	32.0–33.1	3.64–4.00

Test Methods and Scope

During both testing campaigns, samples of stack gases and process streams were taken and analyzed for 49 PFAS target compounds, with the stated analytical objective of characterizing as many streams entering or exiting the incinerator as possible. Clean Harbors used EPA's Other Test Method 45 (OTM-45), *Measurement of Selected Per- and Polyfluorinated Alkyl Substances from Stationary Sources*, for stack gas samples (as noted earlier, "stack" meaning the exit duct from the wet scrubber as it was deemed a safer sampling location than the actual stack). The 49 targeted PFAS analytes were measured using LC/MS/MS with isotope dilution.

Process streams sampled included waste fuels, clean fuels, waste feeds, process water, and residuals (liquids and solids), as well as some pre-test sampling of chemical inputs (soda ash, activated carbon, and TMT-15). As noted in the reports, however, due to the waste profile matrix complexity and the heterogeneity of all three solid waste streams, they were not sampled or analyzed for PFAS. Absent any final EPA-approved methods for media other than drinking water, LC/MS/MS analysis was used to measure the targeted PFAS compounds in the process stream samples.

In addition to the 49 PFAS analytes, the HF concentration of the stack gas was measured using EPA Method 26A. The AFFF-containing material in the 2021 test was analyzed for the 49 target PFAS both before and after chemical oxidation as a total oxidizable precursor assay (TOPA). During the 2022 test, a TOPA and total organic fluorine assay (TOFA) were performed on the AFFF-containing materials. The objective of performing TOPA is to "reveal perfluoroalkyl acid (PFAA), fluorotelomer sulfonate (FTS), and possibly other precursors that are not identifiable with targeted PFAS analysis." TOFA, as described in the 2022 report, identifies the mass of compounds containing carbon-fluorine bonds, and was performed using combustion ion chromatography following a laboratory in-house method for adsorbable organo-fluorine.

Results

According to the tests and third-party review, the primary objective of determining the destruction and removal efficiency (DRE) of each of the spiked (augmented feed) PFAS was achieved. The calculated DREs for each test (triplicate test run average) and PFAS compound are as follows:

PFAS Analyte	2021 Test DRE (%)	2022 Test DRE (%)
PFOA (perfluorooctanioic acid)	99.999943	99.99990
PFOS (perfluorooctanesulfonic acid)	99.999955	99.99977
PFHxS (perfluorohexane sulfonic acid)	99.999977	99.99996
HFPO-DA (hexafluoropropylene oxide – dimer acid)	99.999979	99.99984
PFBS (perfluorobutanesulfonate)	Not available	99.99997

Both test reports note that none of the other 45 (or 44, in 2022) target PFAS analytes were present in the waste feed at levels sufficient to demonstrate 99.9999-percent DRE without spiking, even though stack gas concentrations were non-detect in many cases. According to the 2021 test report, there were three other PFAS present in the waste feeds at sufficient mass feed rate without spiking to enable demonstration of DRE's exceeding 99.999 percent. These included PFOS, 6:2 fluorotelomer sulfonate (FTS), and 8:2 FTS. The report stated that the FTS compounds are common constituents of AFFF.

As for the secondary objectives, the results are, as stated within Dr. Taylor's review, "most difficult to achieve as they involve more complex sampling issues." With respect to the secondary PFAS mass balance objective, he notes that "some of the PFAS that was not sampled in the infeed may have been more difficult to gasify and burn, contrary to the assertions in the report." However, he concurs with the report statements that the PFAS DRE is conservative.

The objective of demonstrating high HF removal that would be associated with a high destruction of PFAS is seemingly not achieved, primarily due to analytical issues. Dr. Taylor notes that "the sinks for the HF were not accurately quantitated leading to some questions about the validity of the reported HF removal efficiency." He further notes that the assumption that all fluorine in the waste if converted to HF is challenged, as there would be a required excess of hydrogen to fluorine to be demonstrated, which are not provided for in the data. Further, he stated that there is a "demonstrated lack of accuracy of the analytical methods for measuring inorganic fluorine in the residual streams," which, in his opinion, leads to a very low fluorine recovery mass balance. He concluded that, "development of better analysis methods for complex matrices for inorganic fluorine are needed before high HF removal efficiencies can be reported at full scale.[41]

Conclusions

The tests appear to demonstrate that the primary objective, "demonstrate the ability of the high-temperature incineration system at Aragonite to effectively destroy PFAS in the waste feed during normal process operating conditions," was achieved for the five selected PFAS compounds that were subjects of these studies. However, this study did not evaluate the formation of PICs due to previous methodology limitations. Dr. Taylor, in his review, noted this as well: "The larger question from an environmental viewpoint is the complete mineralization of these substances and prevention of emission of highly stable C_1-C_2 PFCs (perfluorocarbons)." Dr. Taylor concludes that CHES' results, although promising and demonstrating reduction of a limited selection of specific PFAS compounds, did not support complete mineralization.

References

EA Engineering, Science, and Technology, Inc., PBC. (2021). Report on PFAS destruction testing results at Clean Harbors' Aragonite, Utah hazardous waste incinerator (Final).

EA Engineering, Science, and Technology, Inc., PBC. (2022). Report on PFAS destruction testing results at Clean Harbors' Aragonite, Utah hazardous waste incinerator (Final).

[41] Although not stated by Dr. Taylor in his assessment, another possible reason for lack of finding inorganic fluorine (HF) could be the result of poor conversion (mineralization) of PFAS to HF and carbon dioxide during incineration. This may indicate formation of non-target PFAS or fluorocarbons during incineration that were not measured by these tests.

Taylor, P. (2022). Final report: Assessment of a report on PFAS destruction testing results at Clean Harbors' Aragonite, Utah hazardous waste incinerator. Prepared for Clean Harbors Environmental Services, Inc.

Appendix C: Summary of the Chemours Thermal Oxidizer Test Data

Introduction

Chemours Company FC, LLC, located in Fayetteville, North Carolina, manufactures chemicals and plastics and was required to install a thermal oxidizer to control per- and polyfluoroalkyl substances (PFAS) emissions. Tests have been conducted annually since installation. The objective of the tests was to determine how "the Thermal Oxidizer and 4-stage Scrubber system will achieve the emission reduction [of 99.99 percent], including the use of a surrogate for all PFAS, such as the hexafluoropropylene oxide (HFPO)." This summary presents a high-level review of the two tests' reports.

General Process Description

The thermal oxidizer at the Chemours facility operates at 10 million British thermal units (BTUs) per hour and is powered by natural gas. Waste gases from both polymer and monomer production are fed into the thermal oxidizer. The gaseous emissions from the oxidizer are fed into the four-stage packed bed column scrubber system while solid calcium fluoride (CaF_2) is removed, collected, and disposed of at an off-site location. The scrubber has three water scrubbing stages and one caustic scrubbing stage and is stated to remove hydrogen fluoride (HF) from the final emissions.

Operational Description

Test Methods and Scope

The tests done on the thermal oxidizer focused on five PFAS compounds: HFPO, hexafluoropropylene oxide-dimer acid (HFPO-DA), hexafluoropropylene oxide-dimer acid fluoride (HFPO-DAF), carbonyl fluoride (COF_2), and Fluoroether E-1. These compounds were selected because they are handled and used at the Fayetteville facility. The compounds have varying chemical structures and properties and cannot be sampled and tested in the same manner.

Modified Method 18 sampling was used to capture the PFAS compounds. This method uses six fluoropolymer (PFA) impingers charged with methanol arranged in a series and captures the compounds using chemical reactions and condensation.

To capture HFPO-A, the facility needed to use Modified Method 0010 sampling with a heated probe and filter, condenser coil, XAD-2 resin cartridge, deionized water impingers, and a silica gel impinger. In addition to the normal Modified Method 0010 sampling process, this test added a second XAD-2 resin cartridge to monitor possible target PFAS breakthrough.

Three compounds (HFPO, HFPO-DAF, and COF_2) were sampled by chemical reaction. The sample gas was passed through the series of methanol impingers. The compounds then react with methanol, and each produced unique ether compounds that were monitored and quantified using SW-846 Method 8260.

The strategy used to sample Fluoroether E-1 and HFPO-DA was to capture the compounds through condensation and dissolution in the same methanol impingers. The volatile organic compound, Fluoroether E-1, was then quantified using SW-846 Method 8260b. The semivolatile organic compound HFPO-DA was quantified using EPA Method 537.

Samples were taken from the polymer production and monomer production waste gas feed lines to capture the initial amount of PFAS compounds they produced as waste and to provide a baseline concentration of PFAS prior to treatment. The other sampling location was in the stack after the thermal oxidizer and scrubber. This location would show how effective the process was at destroying the targeted PFAS compounds.

The sampling and testing methods only captured the amount of target PFAS compounds and did not measure any products of incomplete combustion (PICs). Although the four-stage scrubber was implemented to reduce the possible HF emissions, there was no sampling of HF.

Results

The results of the tests showed that the mandated 99.99 percent destruction of targeted PFAS compounds had been achieved. In both testing years the average PFAS destruction and removal efficiency (DRE) was above 99.999 percent with no result being lower than 99.999 percent. Results were presented as a total FPAS destruction efficiency with the amount of each target PFAS compound summed to provide an aggregate destruction efficiency. The total PFAS destruction efficiencies for each run and each year are as follows:

Year	Run 1	Run 2	Run 3	Average
2020	99.99982%	99.99974%	99.99986%	99.99981%
2021	99.99920%	99.99968%	99.99963%	99.99951%
2022	99.99951%	99.99966%	99.99967%	99.99961%
2023	99.99951%	99.99964%	99.9972%	99.9988%
2024	99.99934%	99.99964%	99.99934%	99.99944%

Conclusions

The test reports demonstrate a PFAS destruction efficiency above the 99.99-percent level that was mandated for the Chemours facility for the specific compounds that were tested. HFPO was tested as a surrogate for all species of PFAS, but there are no definitive data showing the performance of the thermal oxidation process for PFAS species outside of the compounds tested. There was also no discussion of possible PICs in the reports. The thermal oxidizer at the Chemours facility appropriately destroys the five PFAS compounds of interest that have been identified at the facility. However, removal processes for PICs or products of incomplete destruction of potential compounds not studied but potentially found in the facility waste streams are still unclear.

References

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Focus Environmental, Inc. (2021). Thermal oxidizer performance test report Chemours Company Fayetteville Works. Prepared for the Chemours Company Fayetteville Works Plant. *https://deq.nc.gov/coastal-management/gis/data/air-sampling/week-1-26-2021-chemours-de-test-report-30-mar-21/download*

Focus Environmental, Inc. (2022). Thermal oxidizer performance test report Chemours Company Fayetteville Works. Prepared for the Chemours Company Fayetteville Works Plant. *https://www.deq.nc.gov/coastal-management/gis/data/air-sampling/chemours-feb-2022-de-test-report-final/download?attachment*

Focus Environmental, Inc. (2023). Thermal oxidizer performance test report Chemours Company Fayetteville Works. Prepared for the Chemours Company Fayetteville Works Plant. *https://edocs.deq.nc.gov/AirQuality/DocView.aspx?id=412229&dbid=0&repo=AirQuality*

Focus Environmental, Inc. (2024). Thermal oxidizer performance test report Chemours Company Fayetteville Works. Prepared for the Chemours Company Fayetteville Works Plant. *https://edocs.deq.nc.gov/AirQuality/DocView.aspx?id=487475&dbid=0&repo=AirQuality*

Appendix D: Summary of Costs and Considerations

Introduction

This appendix provides some estimates of costs for end-of-life management of PFAS materials by thermal treatment, landfilling, and underground injection. The cost information presented has limitations and is highly variable and is likely most suitable to make comparisons between techniques.

The cost to destroy or dispose of PFAS and PFAS-containing materials can vary widely depending on a number of factors. Waste management infrastructure's design and operation are heavily influenced by regional needs, local regulations, and technological advancements. As a result, waste management costs vary by the type of waste and by location due to factors like availability and transportation costs. In addition, technological advances specifically related to the management of PFAS-containing materials can potentially reshape the cost and operational landscape of PFAS waste management. Multiple factors and limitations contribute to cost variability and should be considered when using the information in this appendix.

- Market conditions such as supply and demand and macroeconomics change frequently, resulting in fluctuating costs.
- Location of destruction and disposal options and transportation affect case-by-case costs.
- Transportation costs or any special handling costs for accepting and treating additional waste are not included in most of these estimates, thus actual costs would vary.
- Costs may be affected by permitting and state/local requirements for PFAS-containing material, which vary depending on state/local regulations, public input, and geographic location.

Thermal Treatment

Hazardous Waste

Table D-1. Estimated Costs to Incinerate Different Types of Hazardous Waste (U.S. EPA, 2005a)

Hazardous Waste Type	Estimated Thermal Treatment Cost per Ton[a]
Liquids, sludges, solids (halogenated)	$1,221-1,774
Liquids, sludges, solids (non-halogenated)	$358-976
Laboratory packs	$6,054
Containerized gases	$2,929

[a] Costs were developed for hazardous waste combustors (HWC) that were affected by the 2005 National Emission Standards for Hazardous Air Pollutants (NESHAP), including commercial incinerators, cement kilns, lightweight aggregate kilns, and commercial HWC boilers. Cost per ton is assumed to be in 2002 dollars, the same year basis of the regulation from which these costs were obtained. Costs were normalized to 2024 dollars using the Bureau of Economic Analysis (BEA) gross domestic product (GDP) deflator (U.S. BEA, 2025) for waste management and remediation services using a base year of 2002. Note that applying the BEA GDP deflator for this industry sector is a top-down approach. For regulatory purposes, a bottom-up approach is typically used, accounting for capital costs based on the Chemical Engineering Plant Cost Index (CEPCI), energy prices typically based on Energy Information Administration (EIA) data, and labor costs based on industry segment indices using Bureau of Labor Statistics (BLS) data.

Limitations:

- Costs have likely changed since 2002 and scaling costs to 2024 contributes to uncertainty.
- Does not include transportation costs or any special handling costs for accepting and treating additional waste materials.

Table D-2. Example Disposal/Reactivation Costs for Drinking Water Treatment Residuals (Derived from U.S. EPA, 2020a)

Method	Cost of Disposal ($/lb of Media)	Estimated Cost of Disposal Plus Replacement Media ($/lb of Media)[a]
Reactivated GAC—off-site	$0	$1.63
Disposal via landfill	$0.06	$2.21
Disposal via incineration	$1.20	$3.35

[a] Cost per pound is in 2024 dollars. For GAC, on-site reactivation is possible. However, the utility or site would have to have ample workforce, managerial, and financial (both capital and operating) resources to justify this choice. It is likely to be cost-effective only for very large facilities and would require consideration of other factors including availability of land and public opinion. Due to the complex analysis needed, a full comparison of off-site versus on-site is beyond the scope of this document.

Limitations:

- These estimates are presented as examples only to illustrate tradeoffs. Actual site-specific costs are likely to vary based on factors including the quantity of media reactivated/replaced and are used as an example of trends. In this example, reactivated GAC and virgin GAC are estimated to be $1.42 per pound and $2.15 per pound, respectively.
- All unit costs assume 21,000 lbs of GAC reactivated/replaced.
- For reactivated GAC, it is assumed that the "replacement" media is the returned reactivated media plus the replacement of the GAC lost during reactivation with virgin media (assumed to be 30 percent).
- Disposal costs assume 10 miles of transportation to the disposal site.
- Incineration costs are based on incineration of ion exchange resin media. Incineration of GAC could be somewhat more expensive, depending on the relative fuel value of the spent media.
- The analysis is complex and a number of issues need to be considered at the site level, such as those that affect costs (cost of energy, shipping, labor, construction, operation, sampling, etc.) and those that affect other matters (practicality, public versus private ownership, contract availability, regional reactivation availability, off-gas permitting, public opinion, etc.).

Landfills

Hazardous Waste

Table D-3. Estimated Costs to Landfill a Representative Type of PFAS-Containing Material (Defense Logistics Agency (DLA), 2023)

Hazardous Waste Type	Estimated Cost per Ton of Waste (2024$)
Aqueous film-forming foam (AFFF) and other PFAS-related Solids/Debris, Non-RCRA, >100 parts per million (ppm) PFAS for direct disposal at RCRA Subtitle C Landfill (e.g., contract line item number (CLIN) PF06B1)	$1,301-3,664 ($0.65-1.83/lb)

Limitations:

- The DLA pricing web application lists current government contracts for destroying or disposing of PFAS and PFAS-containing materials and may be useful for government contract cost estimates. The estimated cost per ton of waste appears to include removal and disposal costs for the specific material, although a portion of transportation costs may be attributed to other cost elements of each respective contract so transportation costs may not be fully reflected in these costs.
    - **Per the DLA pricing web application:** The prices contained in the database were awarded based on contract terms and conditions, procurement requirements, and price reasonableness determinations. The prices and contract information in the database are for reference only.
- Users of the DLA pricing web application are cautioned that each contract was developed to meet specific requirements for specific geographic regions or military installations. There are multiple variables that result in the pricing information displayed, including contract award date, proximity to DLA-approved disposal facilities, quantities of PFAS material estimated for disposal in proportion to other hazardous waste on the contract, reoccurring need for services, and whether PFAS material is disposed of in bulk or whether there are small, limited quantities.
- Pricing is provided here for bulk waste types for disposal at a Subtitle C hazardous waste landfill. Pricing for multiple waste types with varying units of measure for disposal, including containerized waste, are provided in the DLA pricing web application.
- The estimated cost in the table above only applies to a limited number of military installations and only considered bulk pricing from 600 to 10,000 pounds. Pricing is expected to differ for containerized waste and should be considered when relying on this cost information.

Non-Hazardous Waste (Municipal solid waste)

Table D-4. Average MSW Tipping Fees per Ton by U.S. Region (EREF, 2022)

Region	Average Tipping Fee (2024$/ton)	Region	Average Tipping Fee (2024$/ton)
Pacific	$64.81	Northeast	$71.28
Mountains/Plains	$47.74	Southeast	$45.73
Midwest	$58.23	South Central	$47.74

Limitations:

- MSW landfills emit more PFAS than originally thought in 2020. Research estimates that although MSW landfills contain PFAS for the most part, up to 5 percent of the PFAS may be released in the landfill gas (LFG) and, 11 percent may be released in the leachate annually (Tolaymat et al., 2023). Thus, MSW tipping fees may not be a relevant data point for cost information.

Non-Hazardous Waste (Liquid waste)

Table D-5. Average Industrial Wastewater Rates by U.S. Region (Unger et al., 2023)

Region	$/1,000 gallon	Region	$/1,000 gallon
Pacific	$11.64	Northeast	$4.71
Mountains/Plains	$3.05	Southeast	$7.35
Midwest	$4.66	South Central	$5.08

[a] Data are consolidated by region and the number of data points varies by region. Cost per gallon is expressed in 2024 dollars. American Water Works Association (AWWA) water and wastewater rate surveys were used to gather historical rate data for water and wastewater utilities in the United States. These datasets were compiled and assessed to produce a single dataset of time series rate data for 112 water utilities and 76 wastewater utilities located throughout the United States.

Limitations:

- These data do not include specific information on extra treatment considerations that may be required by a national pollutant discharge elimination system (NPDES) permit or by an industrial user permit for a discharge into a POTW to control the release of PFAS. Thus, similar to the cost of landfilling PFAS waste, the associated treatment of PFAS-laden leachate is difficult to assess because the available data are associated with typical industrial wastewater generators or typical landfill leachate.

One report examined the cost to manage landfill leachate on site (U.S. EPA, 2021). Based on review of the treatment processes and technologies that are currently used, the cost to manage leachate on site is highly variable but generally ranges between $10.78-161.77/1,000 gallons ($0.01-0.16/gallon). Capital costs can range from hundreds of thousands to several million dollars. The report lists multiple limitations and does not specify treatment for PFAS.

Underground Injection

Class I Wells (hazardous and non-hazardous)

Table D-6. Estimated Costs for Class I Wells

Description	Underground Injection Cost ($/1,000 gallon)[a]
Deep well injection	$178-254 (deSilva, 2019)
Trucking costs	$9-188 (McCurdy, 2011)

[a] Costs were normalized to 2024 dollars using the Bureau of Economic Analysis (BEA) gross domestic product (GDP) deflator (U.S. BEA, 2025) for waste management and remediation services using a base year of 2019.

Limitations:

- Class I well capacity is limited, which may affect the costs associated with deep well injection.
- Because of the geographic concentration of Class I wells, waste producers may face transportation and logistical challenges.
- To begin accepting PFAS-containing fluids, owners/operators of existing Class I wells may incur costs to modify their permits to accept waste from other entities or new waste streams.

Another source includes costs for disposing of sewage sludge and indicates that among fees and transportation costs is a fee ($40.44 per ton) for deep well injection of leachate (CDM Smith, 2020).

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Additional Resources

Barr Engineering Co. and Hazen and Sawyer. (2023). Evaluation of Current Alternatives and Estimated Cost Curves for PFAS Removal and Destruction from Municipal Wastewater, Biosolids, Landfill Leachate, and Compost Contact Water - Prepared for Minnesota Air Pollution Control Agency. *https://www.pca.state.mn.us/sites/default/files/c-pfc1-26.pdf*

> **Per the report:** *This study develops alternatives to remove and destroy per- and polyfluoroalkyl substances (PFAS) from water resource recovery facility (WRRF) effluent, biosolids, mixed municipal solid waste (MSW) landfill leachate, and compost contact water (waste streams) using currently feasible technologies (i.e., could be built today). The report summarizes estimated capital and operations and maintenance (O&M) cost ranges for the two highest-ranking PFAS management alternatives for four waste streams (WRRF effluent, WRFF biosolids, landfill leachate, and compost contact water).*

Defense Logistics Agency (DLA) (2023). DLA Disposition Services hazardous waste disposal pricing web application. *https://public.logisticsinformationservice.dla.mil/hazmat/SearchParameters.aspx* (accessed March 2024).

> The pricing web application lists Department of War (DoW) contracts for destroying or disposing of PFAS and PFAS-containing materials. The website is organized by multiple CLINs, each of which describes a material or waste stream and a unit of measure for disposal. The site lists contracts and pricing for available disposal technologies, including solidification and disposal in a hazardous waste landfill. The application may be useful as a reference for historical pricing for DoW contracts.
>
> **Per the DLA pricing web application:** *The prices contained in the database were awarded based on contract terms and conditions, procurement requirements, and price reasonableness determinations. The prices and contract information in the database apply to DoW requirements and are for reference only.*

Appendix E: Fact Sheet for the 2026 Interim Guidance on the Destruction and Disposal of PFAS

U.S. Environmental Protection Agency has issued an update to the *Interim Guidance on the Destruction and Disposal of Perfluoroalkyl and Polyfluoroalkyl Substances and Materials Containing Perfluoroalkyl and Polyfluoroalkyl Substances (PFAS)*[42].

The non-binding, interim guidance provides recommendations for managers of PFAS and PFAS-containing materials to protect human health and the environment. It also contains a new technology evaluation framework to help analyze the safety and effectiveness of new destruction and disposal (D&D) technologies. The interim guidance does not establish requirements for destruction or disposal of PFAS materials. For more information about PFAS and what EPA is doing to address PFAS, visit EPA's *PFAS website*[43].

The interim guidance summarizes scientific information on current understanding of PFAS and focuses on three widely used and commercially available D&D technologies[44]:

1) Underground injection.
2) Landfills.
3) Thermal treatment under certain conditions, which includes incineration.

The interim guidance summarizes research needs and data gaps and calls for increased collaboration with EPA to collect data and enhance decision-making, as well as describes new EPA test methods.

Key Updates in 2026:

Updated information on destruction and disposal technologies

EPA is not endorsing any technology, but is presenting the technologies' relative potential to minimize PFAS release. As a general approach, EPA encourages managers of PFAS and PFAS-containing materials to consider site-specific conditions and to use D&D options that have a lower potential for releasing PFAS to the environment as described in Section 1 of the interim guidance. In general, the following technologies (in no relative order) have a lower potential for environmental release of PFAS compared to other technologies or operating conditions within the categories of storage, underground injection, landfilling, and thermal treatment:

- **Underground injection–Permitted Class I non-hazardous industrial or hazardous waste injection wells:** The standards associated with the construction, operation, and monitoring of these Class I wells are designed to isolate liquid wastes deep below the land surface and ensure protection of underground sources of drinking water. While Class I wells are an option for managing PFAS-containing fluids, this technology may not be appropriate or available everywhere (Section 3).

[42] Available at: *https://www.epa.gov/pfas/interim-guidance-destroying-and-disposing-certain-pfas-and-pfas-containing-materials-are-not*.

[43] Available at: *http://www.epa.gov/pfas*.

[44] For general information on these technologies, see EPA websites for *incineration*, *landfills*, and *underground injection*. Please note that these resources are not specific to PFAS and not all information in them may be relevant to PFAS D&D.

- **Landfills–Permitted RCRA Subtitle C hazardous waste landfills:** When landfill disposal is selected and PFAS concentration of the waste is relatively high, EPA recommends using a hazardous waste landfill. These types of landfills have the most stringent engineering controls and practices for minimizing environmental release of PFAS from leachate and landfill gas. These controls are especially important for certain types of PFAS-containing materials that break down more easily in landfill conditions. However, for all landfill types, new information demonstrates landfilling could result in higher PFAS releases to the environment than previously thought in 2024 (Section 3).
- **Thermal treatment–Permitted hazardous waste combustors that operate under certain conditions:** New research since 2024 indicates that thermal treatment units operating under certain conditions are more effective at destroying PFAS and minimizing environmental releases or human exposures. Certain hazardous waste combustors and certain granular activated carbon (GAC) reactivation units may operate under these conditions, but uncertainties remain. For example, more information is needed to determine whether harmful products of incomplete combustion (PICs) or PFAS air emissions are formed by units operating at lower temperatures (e.g., municipal waste combustors). EPA's new analytical methods for a targeted list of polar semivolatile PFAS (Other Test Method 45 [*OTM-45*[45]]) and volatile fluorinated compounds (*OTM-50*[46]) that, as well as additional EPA methods under development for evaluating treatment of hard-to-destroy PFAS will allow better characterization of PICs which will help to address some of these uncertainties. The updated interim guidance encourages testing with a range of methods at thermal treatment facilities before accepting large quantities of PFAS-containing materials (Section 3 and Appendix A).
- The interim guidance discusses these and other PFAS D&D technologies, plus testing and research needs to improve technology performance, improve understanding of PFAS behavior, and reduce uncertainties (Section 4).

Emerging Destruction and Disposal Technologies

Federal entities and many companies and researchers are developing and testing new PFAS D&D technologies (e.g., the *PFAS Technical and Regulatory Guidance Document*[47] developed by the Interstate Technology and Regulatory Council, and the Department of War's *Strategic Environmental Research and Development Program (SERDP) and the Environmental Security Technology Certification Program (ESTCP) PFAS Projects*[48] website). EPA encourages technology developers to generate and publicly release data that can be reviewed, and to ensure the data are consistent with the tenets of gold standard science established in Executive Order 14303 (*Restoring Gold Standard Science*[49]). The guidance provides a technology evaluation framework to help analyze the safety and effectiveness of new D&D technologies, and notes the need for innovation, research, and validation (Section 5).

[45] Available at: *https://www.epa.gov/emc/emc-other-test-methods#Other%20Test%20Methods*.

[46] Available at: *https://www.epa.gov/emc/emc-other-test-methods#Other%20Test%20Methods*.

[47] Available at: *https://pfas-1.itrcweb.org/*.

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[49] Available at: *https://www.govinfo.gov/content/pkg/FR-2025-05-29/pdf/2025-09802.pdf*.

Public Input and Participation

EPA has opened a *docket*[50] to take input on the interim guidance. We welcome public input on how to improve the interim guidance and this fact sheet.

Public Feedback and Next Steps

EPA and other government, academic, and private institutions will continue to conduct research to better understand PFAS destruction and disposal (see Section 4). EPA will consider public comments it receives on this version of the guidance, as well as additional advancements in PFAS research and science to publish annual updates[51]. This guidance will be published with a 60-day comment period. EPA will publish a Federal Register Notice with instructions on how to submit comments. During the public comment period, EPA encourages the public to submit studies and data related to management of the PFAS-containing waste described in Section 2. Additionally, continued collaboration with industry and researchers is encouraged to conduct emissions testing of PFAS treatment devices, including evaluation of PICs and products of incomplete destruction (PIDs).

[50] Available at: *https://www.regulations.gov/document/EPA-HQ-OLEM-2020-0527-0003*.

[51] On April 28, 2025, the EPA Administrator committed to providing more frequent updates to the interim guidance—changing from every three years to annually.

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Global Distribution of Perfluorooctane Sulfonate in Wildlife

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Here we report, for the first time, on the global distribution of perfluorooctanesulfonate (PFOS), a fluorinated organic contaminant. PFOS was measured in the tissues of wildlife, including, fish, birds, and marine mammals. Some of the species studied include bald eagles, polar bears, albatrosses, and various species of seals. Samples were collected from urbanized areas in North America, especially the Great Lakes region and coastal marine areas and rivers, and Europe. Samples were also collected from a number of more remote, less urbanized locations such as the Arctic and the North Pacific Oceans. The results demonstrated that PFOS is widespread in the environment. Concentrations of PFOS in animals from relatively more populated and industrialized regions, such as the North American Great Lakes, Baltic Sea, and Mediterranean Sea, were greater than those in animals from remote marine locations. Fish-eating, predatory animals such as mink and bald eagles contained concentrations of PFOS that were greater than the concentrations in their diets. This suggests that PFOS can bioaccumulate to higher trophic levels of the food chain. Currently available data indicate that the concentrations of PFOS in wildlife are less than those required to cause adverse effects in laboratory animals.

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Introduction

Research investigating the environmental fate of halogenated compounds has focused largely on chlorinated and brominated compounds. Fluorinated organic compounds (FOCs) have received less attention because their measurement is more difficult. Because they have surface-active properties, FOCs are more difficult to separate from tissues than the more classical neutral residues that have been studied. Also, because they are nonvolatile and have relatively high molecular weights, they require specialized liquid chromatography/mass spectrometry methods for analysis. Because much of the FOCs were incorporated into polymers, they are perceived as more biologically inert and therefore, less likely to have an impact on human health or the environment.

FOCs have been manufactured for over 50 years. FOCs are used as refrigerants, surfactants, and polymers, and as components of pharmaceuticals, fire retardants, lubricants, adhesives, cosmetics, paper coatings, and insecticides (*1*). Because of their many useful properties, since the 1970s there has been a steady increase in the use of FOCs for a variety of industrial applications.

Although partially fluorinated hydrocarbons may undergo chemical breakdown at functional group bonds (*2*), given the high energy of the carbon−fluorine bond (known to be the strongest of covalent bonds), some perfluorinated compounds are very stable in the environment (*3*). Even though FOCs could be persistent, the magnitude and extent of distribution of FOCs in the environment was unknown. However, the occurrence of FOCs in human blood sera was reported as early as 1968 (*4*). Further reports of FOCs in human sera from various locations in the U.S., South America, and Japan appeared over the next several decades (*5*−*8*). These reports would indicate a relatively widespread distribution of these materials in human sera and would suggest the potential for distribution in the environment. However, no compound-specific information on the extent of the distribution of FOCs in the environment or in wildlife was available. This was due, in part, to the lack of a practical compound-specific method of analysis that could be applied to a wide range of sample types with sufficient sensitivity to measure ecologically relevant concentrations of specific compounds. Recent development of a compound-specific method for the analysis of some FOCs using high-performance liquid chromatography−negative ion electrospray tandem mass spectrometry (*9*) permitted the survey of four FOCs in liver and blood plasma of wildlife on a global scale.

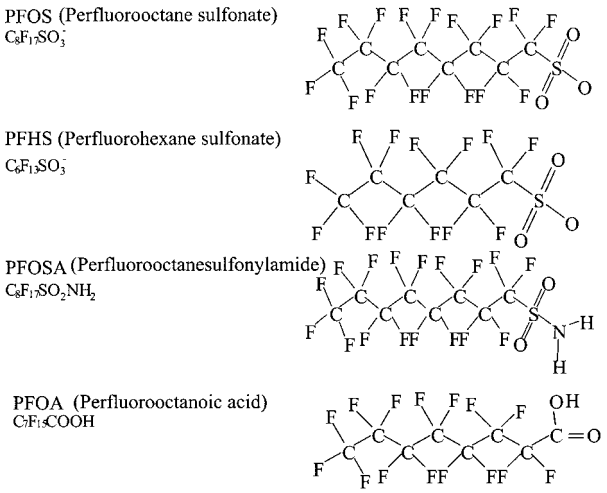


FIGURE 1. Structures of target fluorinated organic compounds.

The number of synthetic FOCs has increased over the past few years. Among them, sulfonyl-based fluorochemicals have been produced and used for over 40 years. Perfluorooctanesulfonyl fluoride (POSF) is the basic building block of a series of perfluorinated alkyl sulfonates that are used as surface protectors in carpets, leather, paper and packaging, fabric, and upholstery. It has been hypothesized that, during their life cycles, POSF and some POSF-based products could ultimately degrade to other fluorochemicals including perfluorooctane sulfonate (*10*) (PFOS; Figure 1).

The studies on which we report here are part of a larger, ongoing, comprehensive program of investigation of global distribution and fate of PFOS in the environment. The objectives of this study were to elucidate the current environmental distribution of PFOS by use of biomonitoring and to evaluate bioaccumulation and biomagnification potential in aquatic and terrestrial food webs. Tissues of a variety of wildlife species, living near urban areas and in more remote areas, including the Arctic and the Antarctic, were studied to monitor for the presence of PFOS. In addition, species from different trophic levels with different feeding

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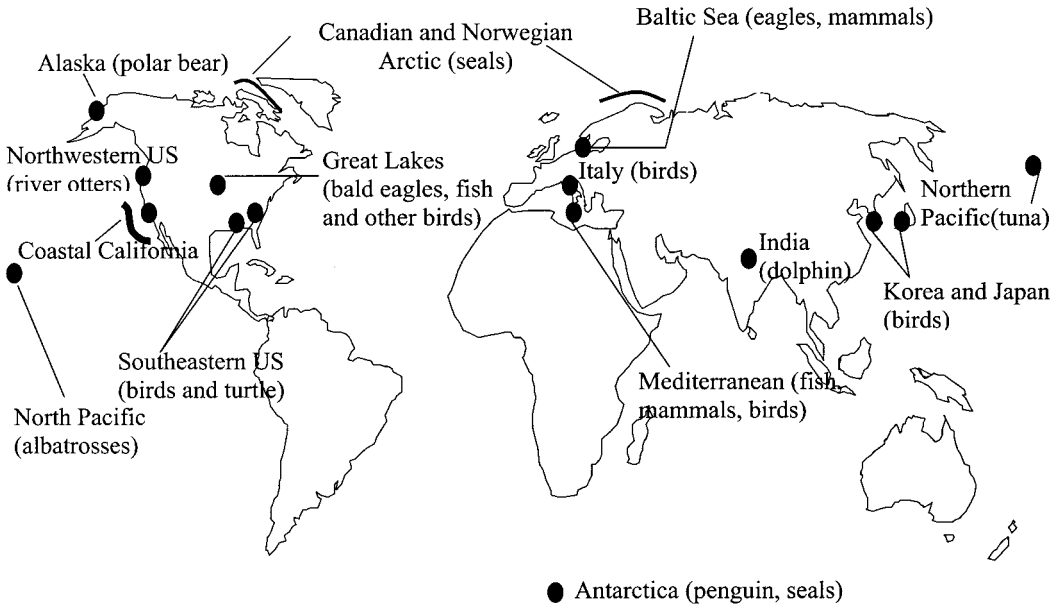


FIGURE 2. Sampling locations of wildlife to monitor fluorinated organic compounds.

strategies were selected to provide some insight into potential environmental pathways.

Materials and Methods

Tissues of various species of aquatic mammals, birds, fish, and amphibians were collected as part of earlier monitoring studies conducted by Michigan State University, East Lansing, Michigan. Samples were collected under permits from state and federal agencies. Tissues of most individuals of aquatic mammals were obtained from stranded dead animals. Samples were also obtained from archived tissues from federal and state agencies. Samples collected in the 1990s were used. Samples were obtained to represent various geographical locations including the Arctic and the Antarctic Oceans (Figure 2).

Concentrations of PFOS and other FOCs in liver, egg yolk, muscle, or blood plasma were determined by use of high-performance liquid chromatography (HPLC) with electrospray tandem mass spectrometry (*9*). The PFOS used as standards and for matrix spikes was purchased as the potassium salt (Fluka, Milwaukee, WI). The purity of the PFOS standard was 86%. The reported concentrations were not corrected for the purity. The internal standard, 1*H*,1*H*,2*H*,2*H*-perfluorooctane sulfonate (THPFOS) was purchased from ICN (Costa Mesa, CA). One mL of sera, 5 μL of internal standard (250 ng), 1 mL of 0.5 M tetrabutylammonium hydrogen sulfate (TBA) solution, and 2 mL of 0.25 M sodium carbonate buffer (adjusted to pH 10) were added to a 15-mL polypropylene tube for extraction. After the solution was thoroughly mixed, 5 mL of methyl-*tert*-butyl ether (MTBE) was added, and the mixture was shaken for 20 min. The organic and aqueous layers were separated by centrifugation, and an exact volume of MTBE (4 mL) was removed from the solution. The aqueous mixture was rinsed with MTBE and separated twice; all rinses were combined in a second polypropylene tube. The solvent was allowed to evaporate under nitrogen before being reconstituted in 1 mL of methanol. The extract was vortex mixed for 30 s and passed through a 0.2-μm nylon mesh filter into an autosampler vial. For the extraction of liver samples, a liver homogenate of 1 g of liver in 5 mL of purified (milli-Q) water was prepared. A 1-mL aliquot of the homogenate was added to a polypropylene tube, and the sample was extracted according to the procedure described above.

Analyte separation was performed using a Hewlett-Packard HP1100 liquid chromatograph modified with low dead-volume internal tubing. Extract (10 μL) was injected onto a 50 × 2 mm (5 μm) Keystone Betasil C_{18} column with a 2 mM ammonium acetate/methanol mobile phase starting at 45% methanol. At a flow rate of 300 μL/min, the gradient was increased to 90% methanol before reverting to the original conditions after 9 min. Column temperature was maintained at 25 °C. The HPLC system was interfaced to a Micromass (Beverly, MA) Ultima or Quattro II atmospheric pressure ionization tandem mass spectrometer operated in the electrospray negative ion mode. In all cases, the capillary was held between 1.6 and 3.2 kV. Cone voltage was 20−60 v and collision energy was 30−45 v. The source temperature was maintained at 250 °C. Instrumental parameters were optimized to transmit the [M-H], [M-K], or [M-NH_4] ion for analytes. When possible, multiple daughter ions were monitored, but quantitation was based on a single product ion. In the electrospray tandem mass spectrometry (ESMSMS) system, the 499 Da → 80 Da transition can be optimized to provide a stronger signal than the 499 Da → 99 Da transition of PFOS. However, in the analysis of tissue samples collected from some species of animals, an unidentified interferent was present in the 499 Da → 80 Da transition. Although this interferent was rarely observed, to ensure complete selectivity, quantitation was based on the 499 Da → 99 Da transition. To further ensure that no interferent compromised the quantitation of PFOS, the transitions 499 Da → 80 and 499 → 99 were compared. If a quantitative deviation of >20% was observed, a third transition, 499 Da → 130 was also monitored. In all cases, the validity of the 499 Da → 99 transition was confirmed by one of the other transitions. Samples were evaluated versus an averaged $^1/_x$ weighted, unextracted external calibration curve. Because of the variety of matrices analyzed, with respect to both species and tissues, and because of evolving analytical methods, the limit of quantitation (LOQ) was variable. Data quality assurance and quality control protocols included matrix spike, surrogate spike, laboratory blank, surrogate matrix blanks, and continuing calibration verification. Recoveries of target analytes spiked to rabbit sera or liver and passed through the analytical procedure ranged from 85 to 101% (*9*). Concentrations of target analytes were not corrected for recoveries of the surrogate standard or matrix spike recoveries.

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TABLE 1. Ranges of Concentrations of PFOS (ng/g, wet wt, for Liver and Muscle Tissues and ng/mL for Blood Plasma) in Wildlife; Mean Concentrations Are Given in Parentheses

species	location	tissue	n	PFOS
	Aquatic Mammals			
ringed seal	Canadian Arctic	plasma	24	<3−12
ringed seal	Baltic Sea	plasma	18	16−230 (110)
ringed seal	Norwegian Arctic	plasma	18	5−14 (9)
gray seal	Baltic Sea	plasma	26	14−76 (37)
gray seal	Canadian Arctic	plasma	12	11−49 (28)
California sea lion	Coastal California	liver	6	<35−49
elephant seal	Coastal California	liver	5	<35
harbor seal	Coastal California	liver	3	<35−57
river otter	Northwestern USA	liver	5	34−990 (330)
sea otter	Coastal California	liver	8	<35
northern furseal	Coastal waters of Alaska	liver	14	<35−120
polar bear	Alaska, USA	liver	17	180−680 (350)
mink	Midwestern USA	liver	18	970−3680 (2630)
bottlenose dolphin	Mediterranean Sea	liver	5	170−430 (270)
striped dolphin	Mediterranean Sea	liver	4	65−160 (100)
weddel Seal	Terra Nova Bay, Antarctica	liver	1	<35
Ganges river dolphin	Ganges River, India	liver	2	<35−81
	Birds			
double-crested cormorant	Lake Huron, Great Lakes	plasma	6	1−270 (170)
herring gull	Lake Huron, Great Lakes	plasma	2	66−79 (73)
polar skua	Terra Nova Bay, Antarctica	plasma	2	<1−1.4
black-tailed gull	Hokkaido, Japan	plasma	24	2−12 (6)
double-crested cormorant	Lake Winnepeg, Canada	egg yolk	4	130−320 (210)
ring-billed gull	Lake Huron, Great Lakes	egg yolk	3	<35−150
Laysan and black-footed albatrosses	Midway Atoll, North Pacific	liver	9	<35
common loon	North Carolina, USA	liver	8	35−690 (290)
brown pelican	Mississippi, USA	liver	2	290−620 (460)
common cormorant	Italy	liver	12	33−470 (96)
black-tailed gull	Korea	liver	15	70−500 (170)
Laysan and black-footed albatrosses	Midway Atoll, North Pacific	plasma	3	9−26 (18)
double-crested cormorant	Lake Huron, Great Lakes	plasma	3	110−430 (260)
herring gull	Lake Huron, Great Lakes	plasma	2	280−450 (370)
bald eagle	Midwestern USA	plasma	26	1−2570 (360)
Laysan and black-footed albatrosses	Midway Atoll, North Pacific	plasma	10	3−39 (9)
	Fish			
lake whitefish	Michigan waters, USA	eggs	2	150−380 (260)
brown trout	Michigan waters, USA	eggs	3	49−75 (64)
Chinook salmon	Michigan waters, USA	liver	6	33−170 (110)
lake whitefish	Michigan waters, USA	liver	5	33−81 (67)
brown trout	Michigan waters, USA	liver	10	<17−26
yellow-fin tuna	Northern North Pacific Ocean	liver	12	<7
blue-fin tuna	Mediterranean Sea	liver	8	21−87 (48)
carp	Saginaw Bay, Michigan, USA	muscle	10	60−300 (120)
Chinook salmon	Michigan waters, USA	muscle	6	7−190 (110)
lake whitefish	Michigan waters, USA	muscle	5	97−170 (130)
brown trout	Michigan waters, USA	muscle	10	<6−46
	Turtles and Frogs			
yellow-blotched map turtle	Mississippi, USA	liver	6	39−700 (190)
green frogs	Southwest Michigan, USA	liver	4	<35−290
snapping turtle	Lake St. Clair, Michigan, USA	plasma	5	1−170 (72)

Results and Discussion

Samples were scanned for four FOCs: PFOS, perfluorooctane sulfonylamide (PFOSA), perfluorohexane sulfonate (PFHS), and perfluorooctanoate (PFOA). However, all concentrations of PFHS were less than the LOQ (LOQ range = 1 to 86 ng/g, wet wt) and only a few samples contained PFOA or PFOSA at levels greater than the LOQ of 2.5 to 180 ng/g, wet wt, and 1 to 38 ng/g, wet wt, respectively. Therefore, only the results for PFOS are presented (Table 1).

When a method detection limit of 1 ng/g, wet wt, was attained, PFOS was detectable in most samples including those from remote marine regions. Concentrations of PFOS in the blood of ringed and gray seals from the Canadian and Norwegian Arctic were in the range of 3 to 50 ng/mL. PFOS concentrations were 2−10 times greater (14−230 ng/mL) in seals from relatively more urbanized locations,such as the Baltic Sea, than those from the Arctic Ocean. Blood plasma of Laysan and black-footed albatrosses collected from remote oceanic locations, such as Midway Atoll in the North Pacific Ocean, contained concentrations of PFOS ranging from 3 to 26 ng/mL. PFOS concentrations in the blood of cormorants and herring gulls from the Great Lakes were approximately 10-fold greater than those in albatrosses from Midway Atoll. Although the liver of yellow-fin tuna from the northern North Pacific did not contain quantifiable concentrations of PFOS (<7 ng/g, wet wt), blue-fin tuna from the Mediterranean Sea contained up to 87 ng/g, wet wt, PFOS in the liver. Livers of polar bears from Alaska contained concentrations of PFOS between 180 and 680 ng/g, wet wt. Although PFOS is distributed in remote marine environments including polar regions, concentrations of PFOS in these regions were severalfold less than those from relatively more industrialized

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locations such as the Baltic Sea and the Great Lakes.

Among various bird species analyzed, blood plasma of bald eagles from the midwestern U.S. contained PFOS concentrations of up to 2570 ng/mL (mean: 360). All plasma samples were obtained from bald eagle chicks less than 200 days old. Other fish-eating water birds such as common loons and brown pelicans contained quantifiable concentrations of PFOS in the liver. All the bird tissues from Canada, Italy, Japan, and Korea contained quantifiable concentrations of PFOS. PFOS concentrations in the blood of black-tailed gulls (2−12 ng/mL) from Hokkaido in Japan were less than those found in albatrosses from Midway Atoll. Occurrence of PFOS in these birds suggests that their potential source of exposure is the fish diet. Tissues of fish from the Great Lakes and the Mediterranean Sea contained measurable concentrations of PFOS. The highest PFOS concentrations observed in fish (300 ng/g, wet wt) were in the muscle of carp from Saginaw Bay, Michigan.

Mink from the midwestern U.S. contained significant concentrations of PFOS in their livers (970−3680 ng/g, wet wt). Mink are opportunistic predators but do eat fish as part of their diet. When mink were fed carp from Saginaw Bay, Michigan, containing an average concentration of 120 ng PFOS/g (wet wt), under laboratory conditions, the estimated biomagnification factor based on the concentrations of PFOS in livers of mink was approximately 22.

Although the results of this study have demonstrated for the first time that PFOS is widely distributed on a global scale and can be persistent and bioaccumulative in various food chains, little information is available on possible toxic effects of PFOS to wildlife. PFOS and related perfluorinated compounds have been shown to affect cell−cell communication, membrane transport and process of energy generation, and proxisome proliferation (*11*, *12*). Currently available data (unpublished reports to U.S. EPA; Docket No. FYI-0500-01378) indicate that concentrations of PFOS in wildlife are less than those required to cause significant adverse effects in laboratory animals. However, as more species-specific information becomes available, a more refined assessment of the risks to wildlife will be possible.

Acknowledgments

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GREENHOUSE GAS PROTOCOL

Global Warming Potential Values

The following table includes the 100-year time horizon global warming potentials (GWP) relative to CO_2. This table is adapted from the IPCC Fifth Assessment Report, 2014 (AR5)[i]. The AR5 values are the most recent, but the second assessment report (1995) and fourth assessment report (2007) values are also listed because they are sometimes used for inventory and reporting purposes. For more information, please see the IPCC website (www.ipcc.ch). The use of the latest (AR5) values is recommended. Please note that the GWP values provided here from the AR5 for non-CO_2 gases do not include climate-carbon feedbacks.

Global warming potential (GWP) values relative to CO_2

Industrial designation or common name	Chemical formula	GWP values for 100-year time horizon		
		Second Assessment Report (SAR)	Fourth Assessment Report (AR4)	Fifth Assessment Report (AR5)
Carbon dioxide	CO_2	1	1	1
Methane	CH_4	21	25	28
Nitrous oxide	N_2O	310	298	265
Substances controlled by the Montreal Protocol				
CFC-11	CCl_3F	3,800	4,750	4,660
CFC-12	CCl_2F_2	8,100	10,900	10,200
CFC-13	$CClF_3$		14,400	13,900
CFC-113	CCl_2FCClF_2	4,800	6,130	5,820
CFC-114	$CClF_2CClF_2$		10,000	8,590
CFC-115	$CClF_2CF_3$		7,370	7,670
Halon-1301	$CBrF_3$	5,400	7,140	6,290
Halon-1211	$CBrClF_2$		1,890	1,750
Halon-2402	$CBrF_2CBrF_2$		1,640	1,470
Carbon tetrachloride	CCl_4	1,400	1,400	1,730
Methyl bromide	CH_3Br		5	2
Methyl chloroform	CH_3CCl_3	100	146	160

GREENHOUSE GAS PROTOCOL

Industrial designation or common name	Chemical formula	GWP values for 100-year time horizon		
		Second assessment report (SAR)	Fourth Assessment Report (AR4)	Fifth Assessment Report (AR5)
HCFC-21	$CHCl_2F$			148
HCFC-22	$CHCLF_2$	1,500	1,810	1,760
HCFC-123	$CHCl_2CF_3$	90	77	79
HCFC-124	$CHClFCF_3$	470	609	527
HCFC-141b	CH_3CCl_2F	600	725	782
HCFC-142b	CH_3CClF_2	1,800	2,310	1,980
HCFC-225ca	$CHCl_2CF_2CF_3$		122	127
HCFC-225cb	$CHClFCF_2CClF_2$		595	525
Hydrofluorocarbons (HFCs)				
HFC-23	CHF_3	11,700	14,800	12,400
HFC-32	CH_2F_2	650	675	677
HFC-41	CH_3F_2	150		116
HFC-125	CHF_2CF_3	2,800	3,500	3,170
HFC-134	CHF_2CHF_2	1000		1,120
HFC-134a	CH_2FCF_3	1,300	1,430	1,300
HFC-143	CH_2FCHF_2	300		328
HFC-143a	CH_3CF_3	3,800	4,470	4,800
HFC-152	CH_2FCH_2F			16
HFC-152a	CH_3CHF_2	140	124	138
HFC-161	CH_3CH_2F			4
HFC-227ea	CF_3CHFCF_3	2,900	3,220	3,350
HFC-236cb	$CH_2FCF_2CF_3$			1,210
HFC-236ea	CHF_2CHFCF_3			1,330
HFC-236fa	$CF_3CH_2CF_3$	6,300	9,810	8,060
HFC-245ca	$CH_2FCF_2CHF_2$	560		716
HFC-245fa	CHF2CH2CF3		1,030	858
HFC-365mfc	CH3CF2CH2CF3		794	804
HFC-43-10mee	CF3CHFCHFCF2CF3	1,300	1,640	1,650

GREENHOUSE GAS PROTOCOL

Industrial designation or common name	Chemical formula	GWP values for 100-year time horizon		
		Second assessment report (SAR)	Fourth Assessment Report (AR4)	Fifth Assessment Report (AR5)
Perfluorinated compounds				
Sulfur hexafluoride	SF_6	23,900	22,800	23,500
Nitrogen trifluoride	NF_3		17,200	16,100
PFC-14	CF_4	6,500	7,390	6,630
PFC-116	C_2F_6	9,200	12,200	11,100
PFC-218	C_3F_8	7,000	8,830	8,900
PFC-318	c-C_4F_8	8,700	10,300	9,540
PFC-31-10	C_4F_{10}	7,000	8,860	9,200
PFC-41-12	C_5F_{12}	7,500	9,160	8,550
PFC-51-14	C_6F_{14}	7,400	9,300	7,910
PCF-91-18	$C_{10}F_{18}$		>7,500	7,190
Trifluoromethyl sulfur pentafluoride	SF_5CF_3		17,700	17,400
Perfluorocyclopropane	c-C_3F_6			9,200
Fluorinated ethers				
HFE-125	CHF_2OCF_3		14,900	12,400
HFE-134	CHF_2OCHF_2		6,320	5,560
HFE-143a	CH_3OCF_3		756	523
HCFE-235da2	$CHF_2OCHClCF_3$		350	491
HFE-245cb2	$CH_3OCF_2CF_3$		708	654
HFE-245fa2	$CHF_2OCH_2CF_3$		659	812
HFE-347mcc3	CH3OCF2CF2CF3		575	530
HFE-347pcf2	CHF2CF2OCH2CF3		580	889
HFE-356pcc3	CH3OCF2CF2CHF2		110	413
HFE-449sl (HFE-7100)	C4F9OCH3		297	421
HFE-569sf2 (HFE-7200)	C4F9OC2H5		59	57
HFE-43-10pccc124 (H-Galden 1040x)	$CHF_2OCF_2OC_2F_4OCHF_2$		1,870	2,820
HFE-236ca12 (HG-10)	CHF2OCF2OCHF2		2,800	5,350

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Industrial designation or common name	Chemical formula	GWP values for 100-year time horizon		
		Second assessment report (SAR)	Fourth Assessment Report (AR4)	Fifth Assessment Report (AR5)
HFE-338pcc13 (HG-01)	$CHF_2OCF_2CF_2OCHF_2$		1,500	2,910
HFE-227ea	$CF_3CHFOCF_3$			6,450
HFE-236ea2	$CHF_2OCHFCF_3$			1,790
HFE-236fa	$CF_3CH_2OCF_3$			979
HFE-245fa1	$CHF_2CH_2OCF_3$			828
HFE 263fb2	$CF_3CH_2OCH_3$			1
HFE-329mcc2	$CHF_2CF_2OCF_2CF_3$			3,070
HFE-338mcf2	$CF_3CH_2OCF_2CF_3$			929
HFE-347mcf2	$CHF_2CH_2OCF_2CF_3$			854
HFE-356mec3	$CH_3OCF_2CHFCF_3$			387
HFE-356pcf2	$CHF_2CH_2OCF_2CHF_2$			719
HFE-356pcf3	$CHF_2OCH_2CF_2CHF_2$			446
HFE 365mcf3	$CF_3CF_2CH_2OCH_3$			<1
HFE-374pc2	$CHF_2CF_2OCH_2CH_3$			627
Perfluoropolyethers				
PFPMIE	$CF_3OCF(CF_3)CF_2OCF_2OCF_3$		10,300	9,710
Hydrocarbons and other compounds - direct effects				
Chloroform	$CHCl_3$	4		16
Methylene chloride	CH_2Cl_2	9	8.7	9
Methyl chloride	CH_3Cl		13	12
Halon-1201	$CHBrF_2$			376

IPCC data sources for more information:

- AR4 values: https://www.ipcc.ch/publications_and_data/ar4/wg1/en/ch2s2-10-2.html
- AR5 values: https://www.ipcc.ch/pdf/assessment-report/ar5/wg1/WG1AR5_Chapter08_FINAL.pdf (p. 73-79)

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Review

Selecting reliable physicochemical properties of perfluoroalkyl and polyfluoroalkyl substances (PFASs) based on molecular descriptors

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ABSTRACT

Perfluoroalkyl and polyfluoroalkyl substances (PFASs) are a class of global environmental pollutants whose environmental fate and adverse effects are of concern. However, data on the basic physicochemical properties of PFASs are scarce. To fill part of the data gaps, improved quantitative structure–property relationship (QSPR) models for prediction of PFAS properties are developed based on the correlation between reported experimental data and molecular descriptors (fluorine number, molar volume and total surface area). Properties include vapor pressure, aqueous solubility, octanol/water partition coefficient, air/water partition coefficient and octanol/air partition coefficient. The fluorine number-descriptor model is based on good statistical results. However, this model cannot distinguish among PFASs with the same number of attached fluorines. Setting aside the fluorine number-descriptor models, models based on molar volume are statistically better than those based on total surface area. Therefore, The PFAS data obtained from the molar volume descriptor model are more reliable than from fluorine number and total surface area descriptor models. These results are intended to improve the understanding of the behavior and fate of PFASs in the environment, at contaminated sites and during remediation.

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1. Introduction

Perfluoroalkyl and polyfluoroalkyl substances (PFASs) are aliphatic materials containing one or more carbon atoms, on which all the hydrogen substituents present in the nonfluorinated analogs from which they are notionally derived have been replaced by fluorine atoms, in such a manner that PFASs contain the perfluoroalkyl moiety C_nF_{2n+1} (Buck et al., 2011). They have been manufactured since the 1950s for use as refrigerants, surfactants, fire retardants, and stain-resistant coatings for fabrics, carpets and paper (Kissa, 2001). Given their high electronegativity and the small size of the fluorine atom, the carbon–fluorine bond possesses strong polarity and large bond energy. The strength of the carbon–fluorine bond, together with the presence of three pairs of non-bonding electrons around each fluorine atom, as well as effective shielding of the carbon atom by fluorine atoms, render PFASs relatively stable. Consequently, PFASs are non-flammable and resist degradation by acids, bases, oxidants, photolytic processes, microbes and metabolic processes (Giesy and Kannan, 2002; Kissa, 2001).

Because of their persistence, PFASs can be detected in all environmental media and in biological samples. Environmental monitoring studies have reported the presence of PFASs in lakes (De Silva et al., 2009), rivers (Eschauzier et al., 2012), precipitation (Kwok et al., 2010), sea sediment (Gomez et al., 2011) and even the Arctic atmosphere (Shoeib et al., 2006). In addition, it has been reported that PFASs are highly accumulated in fish in different countries such as Germany (Schuetze et al., 2010) and Spain (Llorca et al., 2009), as well as different regions of North America (De Silva et al., 2009). In view of their persistence and widespread occurrence in the environment, perfluorooctane sulfonate (PFOS), its salts and perfluorooctane sulfonyl fluoride were added to Annex B of the International Stockholm Convention treaty on Persistent Organic Pollutants (POPs) in May 2009, resulting in global restrictions on production and use of these compounds.

To understand and model how PFASs migrate through the global environment and their potential for multi-media fate and transport, it is necessary to know their fundamental physicochemical properties. Important properties in this regard include: vapor

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pressure and aqueous solubility, as well as their air/water, octanol/water and octanol/air partition coefficients (Wang et al., 2011). Long-range transport of PFASs is strongly affected by vapor pressure and partitioning between air and water (Schenker et al., 2008). The solubility and octanol/air partition coefficient is also useful for predicting the potential for bioaccumulation and toxicity of chemicals in the environment and biota, such as soil, vegetation and humans (Boethling et al., 2004).

Despite their importance, few experimental measurements of physicochemical properties have been reported, with the result that some property values of PFASs are missing (Dreyer et al., 2009; Liu and Lee, 2007; Shoeib et al., 2004; Steele et al., 2002ab). Also, fundamental thermodynamic relationships between the reported properties are commonly not satisfied because of errors and inconsistencies in experimental measurements and predictions. For example, fluorotelomer alcohol (FTOH) and sulfonamide congeners have shown substantial differences (up to 10 orders of magnitude) between experimental and predicted partitioning data (Arp et al., 2006). The calculated concentrations of perfluorooctylsulfonyl fluoride (POSF-based precursors) in air deviate from levels measured in the environment (Schenker et al., 2008). Henry's law constant is often estimated as the ratio of saturation vapor pressure and aqueous solubility, but measured or estimated data are not fully consistent (Beyer et al., 2002).

To fill the gaps, QSPR models have been utilized as powerful tools to predict properties of other compounds (Li et al., 2008; Mansouri et al., 2012). QSPR studies are based on the correlation between the experimental values and different molecular descriptors such as the number of specific atoms, molecular volume, molecular surface area, molecular connectivity indexes and relative retention time. Among the most commonly used molecular descriptors are the number of specific atoms, molar volume and total surface area (Yue and Li, 2013) which are related to the chemical's hydrophobicity, aqueous solubility and octanol/water partitioning (Amidon and Anik, 1981; Gobas, 1991; Mailhot and Peters, 1988).

This paper aims to: (i) review available experimental and estimated data for key basic physicochemical properties of PFASs (vapor pressure (P_L), aqueous solubility (S_L), air/water partition coefficient (K_{AW}), octanol/water partition coefficient (K_{OW}) and octanol/air partition coefficient (K_{OA})). (ii) Evaluate QSPR models based on fluorine number (F_N), molar volume (V_M) and total surface area (TSA) and select the best ones to estimate complete sets of data for PFASs. (iii) Check for internal consistency based on fundamental thermodynamic relationships. With the aid of these steps, more reliable data can be utilized for environmental modeling, and for assessment and remediation of PFAS-contaminated sites.

2. Methodology

2.1. Collection of experimental and estimated data

Experimental and estimated property values of PFASs which include perfluorinated carboxylic acids (PFCAs), perfluorinated sulfonic acids (PFSAs), perfluoroalkane sulfonamide substances (FASAs) and fluorotelomer alcohols (FTOHs) were selected from the literature. These four classes of PFASs have elicited considerable attention because of toxic effects of their degradation products or manufacturing residuals of fluorinated polymers and PFAS precursors (Giesy and Kannan, 2002; Halldorsson et al., 2012). For this reason, a total of 23 PFASs encompassing 10 PFCAs, 3 PFSAs, 6 FASAs and 4 FTOHs congeners were selected for our study (Table 1). Molecular structures of these 23 PFASs are represented in Supplementary information (Table S1). For the selection of reliable data, Yue and Li (2013) critically review properties data and assign values of PBDEs based on several criteria. In this study, the same criteria were applied to filter available data for PFASs physicochemical properties: (i) The available property values were obtained from measured and estimated data at the same conditions (298 K and 760 mm Hg). (ii) Where multiple data were available from the same research group, the most recent values were

Table 1
Molecular descriptors and physical property of PFASs.

Group	CAS[a]	Acronym	Molecular formula	MM (g mol^{-1})[b]	F_N[c]	V_M (cm^3 mol^{-1})[d]	TSA (Å^2)[e]	T_m (K)[f]
PFSAs	375-73-5	PFBS	$C_4HF_9O_3S$	300.1	9	162.3	282.1	310
	355-46-4	PFHxS	$C_6HF_{13}O_3S$	400.1	13	217.2	367.2	314
	1763-23-1	PFOS	$C_8HF_{17}O_3S$	500.1	17	272.1	454.0	363
PFCAs	375-22-4	PFBA	$C_4HF_7O_2$	214.0	7	127.5	201.9	256
	2706-90-3	PFPA	$C_5HF_9O_2$	264.1	9	154.9	244.9	413
	307-24-4	PFHxA	$C_6HF_{11}O_2$	314.1	11	182.4	287.5	287
	375-85-9	PFHpA	$C_7HF_{13}O_2$	364.1	13	209.8	331.9	303
	335-67-1	PFOA	$C_8HF_{15}O_2$	414.1	15	237.3	372.8	329
	375-95-1	PFNA	$C_9HF_{17}O_2$	464.1	17	264.7	416.6	334
	335-76-2	PFDA	$C_{10}HF_{19}O_2$	514.1	19	292.2	459.2	352
	2058-94-8	PFUnA	$C_{11}HF_{21}O_2$	564.1	21	315.1	501.6	372
	307-55-1	PFDoA	$C_{12}HF_{23}O_2$	614.1	23	347.1	544.7	380
	376-06-7	PFTA	$C_{14}HF_{27}O_2$	714.1	27	396.6	630.6	406
FASAs	754-91-6	FOSA	$C_8H_2F_{17}NO_2S$	499.1	17	278.3	461.4	428
	31506-32-8	MeFOSA	$C_9H_4F_{17}NO_2S$	513.2	17	299.9	492.6	
	4151-50-2	EtFOSA	$C_{10}H_6F_{17}NO_2S$	527.2	17	316.4	526.3	353
	1691-99-2	EtFOSE	$C_{12}H_{10}F_{17}NO_3S$	571.3	17	345.4	461.4	333
	24448-09-7	MeFOSE	$C_{11}H_8F_{17}NO_3S$	557.3	17	328.9	567.5	
	25268-77-3	MeFOSEA	$C_{14}H_{10}F_{17}NO_4S$	611.3	17	379.5	632.5	
FTOHs	2043-47-2	4:2 FTOH	$C_6H_5F_9O$	264.1	9	174.3	281.0	230
	647-42-7	6:2 FTOH	$C_8H_5F_{13}O$	364.1	13	229.2	366.8	240
	678-39-7	8:2 FTOH	$C_{10}H_5F_{17}O$	464.1	17	284.1	453.1	323
	865-86-1	10:2 FTOH	$C_{12}H_5F_{21}O$	564.1	21	339.0	538.3	368

[a] Chemical Abstract Service.
[b] Molar mass (g mol^{-1}).
[c] Fluorine number by ChemAxon
[d] Molar volume (cm^3 mol^{-1}) by ACD/Labs' ACD/PhysChem Suite.
[e] Total surface area (Å^2) by ChemAxon
[f] Experimental melting point (K) data obtained from ChemSpider (www.chemspider.com).

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assigned. (iii) Where data were available from different research groups and it was unclear which are more reliable, those based on what appeared to be the more acceptable method were chosen. (iv) Obviously dubious values were excluded. Data were converted to a log scale for modeling.

2.1.1. Vapor pressure

Selected experimental log P_L values (hereafter labeled Ext-Sel. log P_L, with pressure units in Pa) were collected from measured data based on indirect and direct measurement methods. Steele et al. (2002a, b) determined the vapor pressures of perfluoroheptanoic acid (PFHpA) and perfluorobutanoic acid (PFBA) using a twin ebulliometric apparatus. Kaiser et al. (2005) obtained the vapor pressures of perfluorooctanoic (PFOA), -nonanoic (PFNA), -decanoic (PFDA), -undecanoic (PFUnA) and -dodecanoic (PFDoA) acids using a dynamic method developed by Scott (1986). Lei et al. (2004) estimated the liquid-phase vapor pressures of FTOHs and ethyl perfluorooctane sulfonamidoethanol (EtFOSE) as a function of temperature, based on a gas chromatographic retention time (GC-RT) technique which utilizes the consistent retention time of a compound in a GC column at a given temperature. The above methods were successfully employed for reference compounds. The resulting data sets are used for QSPR analysis of PFAS properties.

2.1.2. Aqueous solubility

Experimental data from U.S. EPA (2003), Kaiser et al. (2006) and Liu and Lee (2007) were selected to predict the solubility of all PFASs in this study (Ext-Sel. log S_L, with S_L units in mol L^{-1}). The solubility data reported in the references were determined by the method incorporated as a standard by the Organization for Economic Co-operation and Development. The solubility is affected by the intermolecular forces between solute and solvent, as well as the melting point and enthalpy of the solute. Subcooled liquid solubility is a function of both molecular size and melting point, while solid solubility is a function of only molecular size (Yue and Li, 2013). Therefore, to overcome the difference between congeners due to the melting-point effect, aqueous solubility (S_S, in mol L^{-1}) for solid substances have to be converted into the subcooled liquid using the fugacity ratio (F).

$$1/F = \exp\left[\Delta S_{\mathrm{fus}}/R \times (T_{\mathrm{m}}/T - 1)\right] \quad (1)$$

$$S_{\mathrm{L}}/S_{\mathrm{S}} = P_{\mathrm{L}}/P_{\mathrm{S}} = 1/F \quad (2)$$

where ΔS_{fus} is the entropy of fusion (J K^{-1} mol^{-1}), R the universal gas constant (8.314 J K^{-1} mol^{-1}), T_m the normal melting point (K), and T is the absolute temperature of interest (here 298.15 K). Based on Hinckley et al. (1990), Bidleman and Harner (2000) and Shoeib et al. (2004), ΔS_{fus} = 56.45 J K^{-1} mol^{-1} was adopted in this study. T_m for PFASs was obtained from U.S.EPA's EPI (Estimations Programs Interface) Suite.

2.1.3. Octanol/water partition coefficient

PFASs tend to form three immiscible layers when they are added to an octanol/water mixture because PFASs are simultaneously hydrophobic and lipophobic. Therefore, it is impossible to determine directly their K_{OW} values using 'regular' methods that are common for organic chemicals. Jing et al. (2009) determined K_{OW} of a homologous series of perfluoroalkyl and alkyl carboxylates using ion-transfer cyclic voltammetry. In this study, the selected experimental octanol/water partition coefficient data (Ext-Sel. log K_{OW}) were obtained from Jing et al. (2009).

2.1.4. Air/water partition coefficient

The Air/Water partition coefficient, K_{AW}, which is the concentration ratio of a substance in equilibrium between air and water phases, can be expressed either in dimensionless form or with the same units as the Henry's law constant (H) (Ding and Peijnenburg, 2013; Shiu and Ma, 2000). Because the information on K_{AW} for PFASs was extremely limited, values of K_{AW} (Ext-Sel. log K_{AW}) are estimated from the dimensionless Henry's law constant (H′) according to the equation

$$K_{\mathrm{AW}} = H/RT = H' \quad (3)$$

$$H = P_L/S_L \quad (4)$$

where, P_L and S_L represent Ext-Sel. log P_L and Ext-Sel. log S_L respectively.

2.1.5. Octanol/air partition coefficient

Experimental log K_{OA} (Ext-Sel. log K_{OA}) data for PFASs were selected from Shoeib et al. (2004), Goss et al. (2006) and Dreyer et al. (2009). Shoeib et al. (2004) and Dreyer et al. (2009) measured K_{OA} for perfluoroalkyl sulfonamides (FASAs) and perfluoroalkyl sulfonamidoethanols (FASEs) using a modified generator column. Goss et al. (2006) determined K_{OA} of FTOHs based on the method described by Shoeib et al. (2004). These results were supported by data reported by Thuens et al. (2008).

2.2. Correlations with molecular descriptors

Selected PFAS property data were correlated to F_N, V_M and TSA. F_N is a constitutional descriptor derived from atomic composition, whereas V_M and TSA are geometric descriptors derived from 3-D structures. These molecular descriptors were gathered from the ACD/Labs' ACD/PhysChem Suite and are listed in Table 1.

The single descriptor models correlated with selected molecular descriptors were evaluated from the statistical values of significance test (F), significance level (P), squared correlation coefficient (r^2), and standard error (S.E.) of the estimate and cross-validated coefficient (Q^2 or Rcv^2) (Li et al., 2008; Sabljic et al., 1993; Yue and Li, 2013). Cross-validated coefficients using the leave-one-out technique (LOO-Rcv^2) were calculated from the equation: LOO-Rcv^2 = 1 − PRESS/TSS, where PRESS is the prediction error sum of squares and TSS is the total sum of squares (Gramatica and Pilutti, 2004; Li et al., 2011). Statistical significance was set at $P < 0.05$, and linear regression was performed using Sigmaplot 10 (Systat Software Inc, Point Richmond, CA).

2.3. Incorporating reliability

Basic constraining equations exist for the air, water and octanol system, as described by Cole and Mackay (2000). For internal consistency, all property data from available single-descriptor models were checked against the following basic equations (Beyer et al., 2002; Yue and Li, 2013):

$$\log K_{\mathrm{AW}} = \log S_A - \log S_W \quad (5)$$

$$\log K_{\mathrm{OA}} = \log S_O - \log S_A \quad (6)$$

$$\log K_{\mathrm{OW}} = \log S_{\mathrm{OW}} - \log S_{\mathrm{WO}} \quad (7)$$

$$\log S_O/S_W = \log S_O - \log S_W \quad (8)$$

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where the subscripts A, W and O designate air, water and octanol, respectively. OW and WO denote octanol saturated with water and water saturated with octanol, respectively. S_O/S_W is regarded as the partition coefficient between pure octanol and pure water. However, S_O/S_W is not necessarily equal to K_{OW} because S_O is not equal to S_{OW}, nor is S_W equal to S_{WO} (Harner and Mackay, 1995; Kömp and McLachlan, 1997; Pinsuwan et al., 1995). In addition, Eqs. (5), (6) and (8) can be combined to give;

$$\log S_O/S_W - \log K_{OA} = \log K_{AW} \quad (9)$$

Eq. (9) can be used to determine the deviation (ε) from the ideal relationship when only K_{OW}, K_{AW} and K_{OA} are available, where ε from the ideal relationship ($\log K_{AW} - \log S_A + \log S_W = 0$) is calculated by

$$\log K_{AW} - \log S_A + \log S_W = \varepsilon \quad (10)$$

In the procedure for internal consistency, an uncertainty factor is assigned to each property ranging from 0 (highest accuracy) to 5 (lowest accuracy). This assignment is subjective, based on judgment through proposed model statistical results. This methodology was described in greater detail by Beyer et al. (2002).

In addition, to evaluate the consistency of QSPR models related to molecular descriptor and faulty adjusted property values, estimated data (Est'd-Sel. property) from QSPR models were compared with those from Ext- Cal data. Est'd-Sel. properties of PFASs were

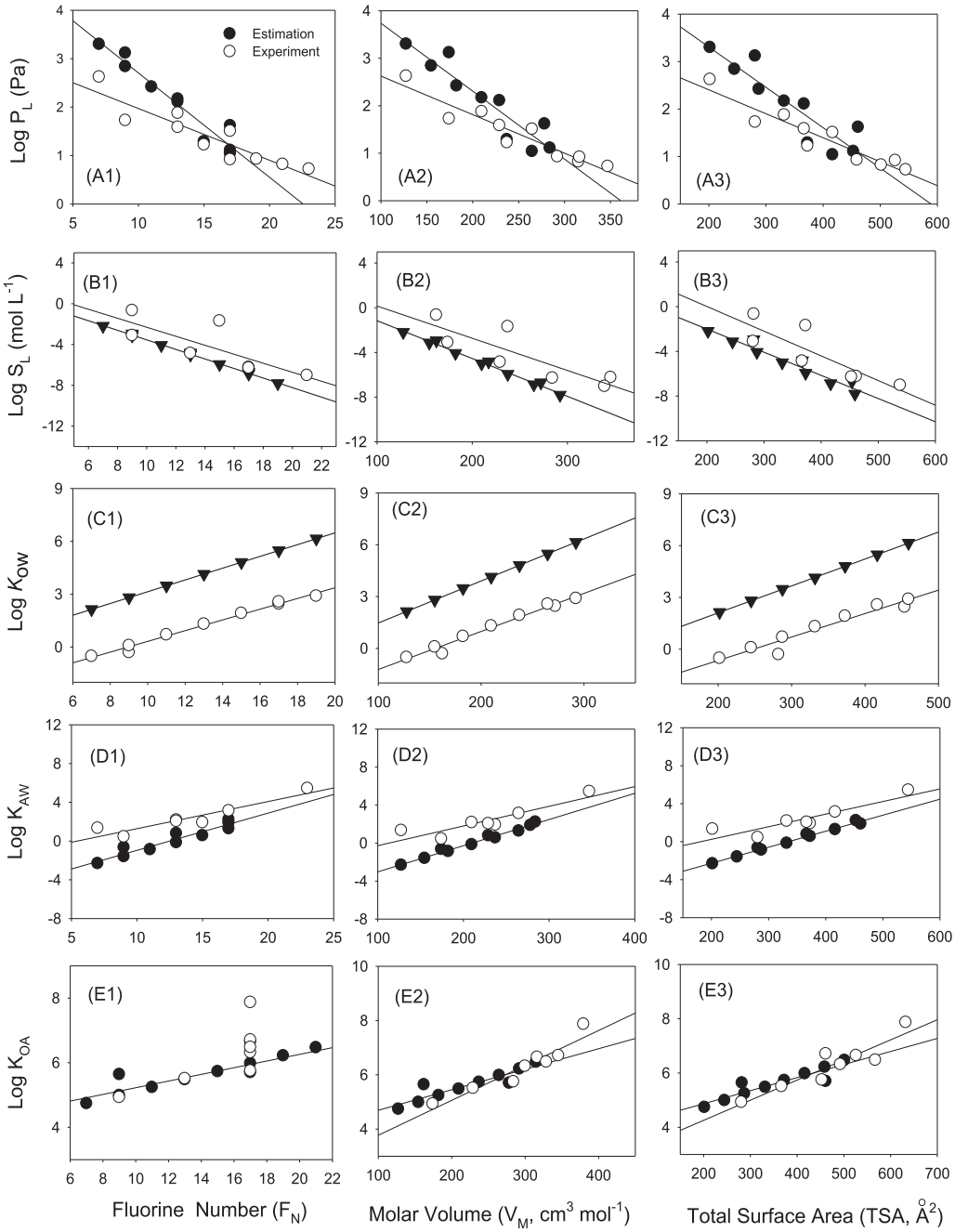


Fig. 1. Correlations between PFASs properties ((A) vapor pressure, (B) aqueous solubility, (C) octanol/water partition coefficient (D) air/water partition coefficient and (E) octanol/air partition coefficient) and molecular descriptors ((1) Fluorine number, (2) molar volume and (3) total surface area).

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obtained from U.S. EPA's EPI. EPI Suite, a set of computer programs for physical property estimations developed by the US EPA's Office of Pollution Prevention Toxics and Syracuse Research Corporation. These programs are useful for QSPRs and prediction of partition parameters (K_{OW}, K_{AW} and K_{OA}), with values available (Dearden and Schuurmann, 2003; Meylan and Howard, 1995).

3. Results and discussion

3.1. Vapor pressure

Vapor pressure determines the exchange rate of compounds across an air/water interface, volatilization from water, soil or plants, and transport of trace organics throughout the global environment. The volatility parameter, log P_L, is plotted against F_N, V_M and TSA of PFASs in Fig. 1. The Ext-Sel. log P_L, correlation equations and statistical results appear in Table S2 of the Supplementary information, with P_L in units of Pa. In the statistical results, the P values of all single descriptor models based on F_N, V_M and TSA were all significant at the <0.05 level of significance for their respective regression models. In the single descriptor models obtained from experimental data, the model based on V_M was statistically better than those based on F_N and TSA because it has the highest Q^2 (0.8597), largest r^2 (0.8981) and smallest S.E. (0.2006). The single descriptor model with V_M is then

$$\log P_L = -0.0081 \times V_M + 3.4361 \text{ with } n = 10,\ r^2 = 0.8981,\ F = 70.48,\ \text{S.E.} = 0.2006,\ Q^2 \text{ or LOO} - \text{Rcv}^2 = 0.8597 \quad (11)$$

Bhhatarai and Gramatica (2011) also provided an equation to estimate the vapor pressure for 20 PFASs and obtained a number of statistical parameters.

$$\log P_L = 7.97(\pm 1.26) - 0.16(\pm 0.02)F03[C-F] - 3.16(\pm 0.92)\text{AAC} - 0.64(\pm 0.40)n\text{DB} \quad (12)$$

with $n = 35$, $r^2 = 0.9093$, $F = 103.6$, S.E. = 0.883, $Q^2 = 0.8821$.

The model consisted of 2D frequency fingerprint descriptor, F03 [C–F], 2D information indices, AAC, and 0D constitutional descriptor, nDB, which represent the frequency of C–F bonds at three distant linkages, the average atomic composition index and the number of double bonds, respectively. This correlation is based on experimental data of Arp et al. (2006). It can be seen that the model based on Eq. (12) has higher $r^2$and more experimental data (35) than the one based on Eq. (11). However, Arp et al. (2006) only compared the estimated P_L (e.g., COSMOtherm., Sparc. and EPI Suite) with experimental data obtained by Krusic et al. (2005), Kaiser et al. (2005) and Shoeib et al. (2004). These gathered experimental data were determined using different methods and were not tested for internal consistency. In addition, the vapor pressures of FTOHs determined by Krusic et al. (2005) were significantly lower than those reported by Lei et al. (2004) and Stock et al. (2004). For these reasons, the descriptor model (Eq. (12)) of Bhhatarai and Gramatica (2011) cannot be used directly to estimate P_L for PFASs. Based on the descriptor model based on V_M (Eq. (11)), the calculated log P_L values (Ext-Cal. log P_L) of all PFASs are compiled in Table S2 of the Supplementary information, and are subjected below to an internal consistency check.

3.2. Aqueous solubility

Aqueous solubility plays an important role when water ecosystems are involved in chemical transport. Aqueous solubility of PFASs is a useful property on its own, but it can also be used to calculate other physicochemical properties such as K_{AW} and Henry's law constant, which can be estimated together with vapor pressure (Staudinger and Roberts, 1996). In our study, Ext-Sel. log S_L values of PFASs were found from U.S. EPA (2003), Kaiser et al. (2006) and Liu and Lee (2005), as recorded in the Supplementary information, Table S3. Single descriptor models were obtained based on F_N, V_M and TSA (Fig. 1). Although all single descriptor models have similar statistics, the model based on TSA,

$$\log S_L = -0.0221 \times \text{TSA} + 4.4391 \text{ with } n = 7,\ r^2 = 0.7313,\ F = 13.6071,\ \text{S.E.} = 1.4095,\ Q^2 \text{ or LOO} - \text{Rcv}^2 = 0.9305 \quad (13)$$

has higher r^2 and lower S.E than those based on F_N and V_M, as shown in Table S3 of the Supplementary information. Bhhatarai and Gramatica (2011) developed QSPR for the aqueous solubility of PFASs based on experimental data for 20 PFASs. The aqueous solubility of PFASs using both molecular descriptors which are bi-dimensional was predicted by

$$\log S_L = -0.418\left(\pm 1.940\right) - 0.003\left(\pm 0.001\right)T\left(\text{F..F}\right) + 5.185\left(\pm 3.849\right)\text{SIC1 with } n = 20, r^2 = 0.7631,\ F = 27.38,\ \text{S.E.} = 0.91,\ Q^2 = 0.6913 \quad (14)$$

where T(F..F), a topological descriptor, represents the sum of distances between pairs of fluorine atoms. SIC1 is a descriptor based on neighbor degrees and edge multiplicity, mainly related to the structural symmetry of the molecule. It can be seen that Eq. (14) is statistically better than Eq. (13), except for Q^2, with higher r^2 and F, lower S.E. and more experimental data. However, the data from Bhhatarai and Gramatica (2011) were not obtained from similar experimental measurements and were not tested for internal consistency. For these reasons, Eq. (13) is used to estimate the solubilities of 25 PFASs. The calculated log S_L values (Ext-Cal. log S_L) are compiled in Table S3 of the Supplementary information.

3.3. Octanol/water partition coefficient

The octanol/water partition coefficient is the ratio of the concentrations of organic compounds in n-octanol and water phases at equilibrium. The octanol/water partition coefficient is the best-known environmental parameter used to characterize the lipophilicity of compounds to evaluate the tendency of compounds to partition between an organic phase (e.g., fish tissue or soil organic matter) and an aqueous phase (Ding and Peijnenburg, 2013). Arp et al. (2006) calculated K_{OW} values from experimental K_{AW} and K_{OA} data of FTOHs. However, Schwarzenbach et al. (2002), and Carmosini and Lee (2008) suggested that the K_{OW} values calculated by Arp et al. (2006) were not obtained with water molecules in the octanol phase and octanol molecules in the water phase at phase equilibrium. Therefore, K_{OW} values from Arp et al. (2006) were excluded in our study. Jing et al. (2009) reported the first experimental K_{OW} for several representative straight-chain PFCA and PFSA congeners. Their data provided the opportunity to assess the predictive abilities of various software programs (e.g., SPARC and ALOGPS 2.1) using the SMILES molecular formula language to predict K_{OW} of PFASs. Based on the experimental data collected by Jing et al. (2009) (Table S4 of the Supplementary information), correlation equations were obtained for three molecular descriptors (Fig. 1):

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$$\log K_{OW} = 0.3047 \times F_N - 2.7258 \quad (15)$$

with $n = 9$, $r^2 = 0.9876$, $F = 557.76$, S.E. $= 0.1548$, LOO-Rcv$^2 = 0.9800$

$$\log K_{OW} = 0.0220 \times V_M - 3.4264 \quad (16)$$

with $n = 9$, $r^2 = 0.9768$, $F = 294.63$, S.E. $= 0.2119$, LOO-Rcv$^2 = 0.9635$

$$\log K_{OW} = 0.0136 \times TSA - 3.3683 \quad (17)$$

with $n = 9$, $r^2 = 0.9322$, $F = 96.17$, S.E. $= 0.3623$, LOO-Rcv$^2 = 0.9031$

Of these, the F_N descriptor model (Eq. (15)) is chosen as the best model because it has the highest r^2 (0.9876), highest LOO-Rcv^2 (0.9800) and smallest S.E. (0.1548). Recently, de Voogt et al. (2012) discussed the relationship between hydrophobicity and number of fluorine-substituted carbon atoms in perfluoros. The contribution of each CF_2 group to the increase in hydrophobicity is equal to 0.66, a value that is close to the values of 0.50 and 0.80 estimated by earlier model calculations using COSMOtherm and SPARC models (Arp et al., 2006) and again close to the value of 0.62 calculated with COSMOtherm (Wang et al., 2011). However, the value in Eq. (15) (F_N correlation) is 0.3047, which is close to half values from previous reports (de Voogt et al., 2012; Jing et al., 2009) because the number of fluorines is equal to nearly twice the number of CF_2 groups. Therefore, values of K_{OW} calculated (Ext-Cal. log K_{OW}) from the F_N descriptor model (Eq. (15)) are provided in Table S4 of the Supplementary information.

In addition, Endo and Goss (2014) predicted log K_{OW} of PFASs using poly-parameter linear free energy relationships (PP-LFER). These equations are

$$\log K_{OW} = c + sS + aA + bB + vV \quad (18)$$

$$\log K_{OW} = c + eE + sS + aA + bB + lL \quad (19)$$

$$\log K_{OW} = c + sS + aA + bB + vV + lL \quad (20)$$

based on three major forms, where, the descriptors are E, excess molar refraction; S, dipolarity/polarizability parameter; A, solute H-bond acidity; B, solute H-bond basicity; V, McGowan's molar volume with units of (cm^3 mol^{-1})/100 and L, the logarithmic hexadecane-air partition coefficient. However, the PP-LFER model cannot respect the K_{OW} for all PFASs because K_{OW} values were only predicted based on experimental FTOH data (4:2 FTOH, 6:2 FTOH and 8:2 FTOH). Therefore, these models are not suitable when selecting the best model in this study.

3.4. Air/water partition coefficient

The air/water partition coefficient is useful for predicting vapor exchange rates between air and water interfaces (Burkhard et al., 1985). Lei et al. (2004) and Goss et al. (2006) measured K_{AW} for the FTOHs (4:2 FTOH, 6:2 FTOH and 8:2 FTOH) using static headspace. However, Lei et al. (2004) were unable to account fully for the intermolecular interaction between target molecules and the water phase because of the limited number of experimental temperatures. Goss et al. (2006) found that the experimental values from Lei et al. (2004) contradicted thermodynamic theory, and the 8:2 FTOH result raised some questions. Therefore, because there is little information on different K_{AW} values for FTOHs and none for PFCAs, PFSAs and FASAs, Ext-Sel. log K_{AW} values were calculated from Ext-Sel. log P_L and Ext-Sel. log S_L in our study.

To select a QSPR model with respect to the log K_{AW} of PFASs, Ext-Sel. log K_{AW} was correlated with single molecular descriptors (Fig. 1) leading to:

$$\log K_{AW} = 0.2804 \times F_N - 1.5251 \quad (21)$$

with $n = 7$, $r^2 = 0.8692$, $F = 33.23$, S.E. $= 0.6284$, LOO-Rcv$^2 = 0.8295$

$$\log K_{AW} = 0.0207 \times V_M - 2.3439 \quad (22)$$

with $n = 7$, $r^2 = 0.8223$, $F = 23.13$, S.E. $= 0.7325$, LOO-Rcv$^2 = 0.7583$

$$\log K_{AW} = 0.0133 \times TSA - 2.4051 \quad (23)$$

with $n = 7$, $r^2 = 0.8103$, $F = 21.35$, S.E. $= 0.7568$, LOO-Rcv$^2 = 0.7493$

Eq. (21) is chosen as the best of these models because it has the highest r^2 (0.8692) and LOO-Rcv^2 (0.8295), as well as the smallest S.E. (0.6284). Calculated K_{AW} values (Ext-Cal. log K_{AW}) are given in Table S5 of the Supplementary information.

3.5. Octanol/air partition coefficient

The octanol/air partition coefficient is a key property when assessing partitioning between the atmosphere and organic phases, such as for organic films on aerosols, organic carbon in soil, the waxy cuticle and lipid portions of vegetation (Ding and Peijnenburg, 2013). However, application of K_{OA} is based on the assumption that interactions between organic chemicals and environmental organic phases closely resemble the interaction between the chemical and octanol (Wania et al., 2002). Shoeib et al. (2004) measured K_{OA} for methyl perfluorooctane sulfonamidoethanol (MeFOSE), EtFOSE and methyl perfluorooctane sulfonamidethylacrylate (MeFOSEA) using a modified generator column method at different temperatures. Goss et al. (2006) estimated K_{OA} of FTOHs as a function of temperature using the identical method. Since then, Thuens et al. (2008) agreed with K_{OA} values for FTOHs published by Goss et al. (2006). Dreyer et al. (2009) determined K_{OA} of methyl perfluorooctane sulfonamide (MeFOSA), ethylperfluorooctane sulfonamide (EtFOSA), MeFOSE and EtFOSE. In our study, K_{OA} data for FASAs and FTOHs are obtained from Shoeib et al. (2004), Goss et al. (2006) and Dreyer et al. (2009). However, log K_{OA} values for PFCA and PFSA are limited, and the QSPR model has never been applied to estimate K_{OA} of all PFASs. Based on Ext-Sel. log K_{OA}, correlation equations with F_N, V_M and TSA were obtained (Fig. 1, Supplementary information Table S6);

$$\log K_{OA} = 0.2234 \times F_N + 2.8150 \quad (24)$$

with $n = 8$, $r^2 = 0.5537$, $F = 7.44$, S.E. $= 0.6447$, LOO-Rcv$^2 = 0.5114$

$$\log K_{OA} = 0.0129 \times V_M + 2.4847 \quad (25)$$

with $n = 8$, $r^2 = 0.8989$, $F = 53.36$, S.E. $= 0.3068$, LOO-Rcv$^2 = 0.8716$

$$\log K_{OA} = 0.0074 \times TSA + 2.7736 \quad (26)$$

with $n = 8$, $r^2 = 0.8485$, $F = 33.60$, S.E. $= 0.3756$, LOO-Rcv$^2 = 0.8133$

Of these correlations, Eq. (25) has the largest r^2 (0.8989) and LOO-Rcv^2 (0.8716), as well as the smallest S.E. (0.3068). Calculated K_{OA} (Ext-Cal log K_{OA}) values from this model are provided in Table S6 of the Supplementary information.

3.6. Reliability based on fundamental thermodynamic relationships

Internal consistency of all Ext-Cal. data was evaluated through fundamental thermodynamic relationships. For reliability or

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uncertainty of all Ext-Cal. values, uncertainty factors of 1 and 4 were assigned to the K_{OW} model and S_w model respectively. The K_{AW} model was assigned an uncertainty factor of 3. Other properties were given an uncertainty factor of 2. The adjusted experimental property data (Ext-Adj. data) based on these uncertainty factors are listed in Supplementary information, Table S7. The Ext-Adj. property data are compared in Fig. 2 with Ext-Cal. property data to evaluate the accuracy of these proposed models for PFAS properties (Table S9 and S10 of the Supplementary information).

Of the PFASs, three classes, PFCAs, PFSAs and FTOHs, showed close linearity between Ext-Adj. data and Ext-Cal. data for all properties of interest (i.e., P_L, S_L, K_{OW}, K_{AW} and K_{OA}). In the case of

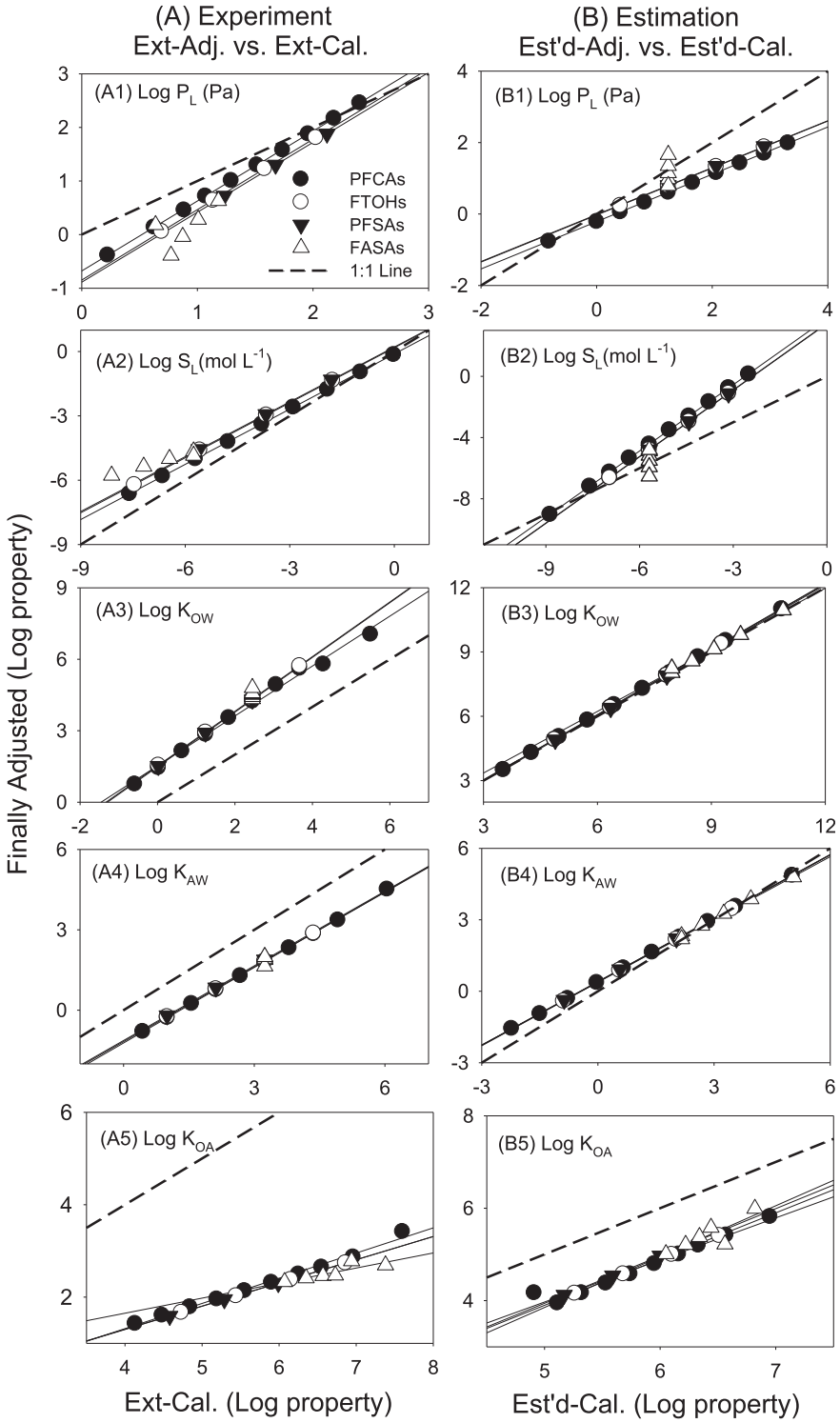


Fig. 2. Relationship between finally adjusted properties and calculated properties of PFASs (PFSA, PFCA, FASA and FTOH) from experimental and estimated data. Calculated properties were obtained by single descriptor model, selected based on statistical results. Adjusted properties were obtained based on fundamental thermodynamic relationships (Beyer et al., 2002).

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FASAs, except for log P_L, log S_L and log K_{OA}, the Ext-Adj. data and the Ext-Cal. data for FASAs (MeFOSA, EtFOSA, EtFOSE, MeFOSE and MeFOSEA) are not described well by linear fits (Fig. 2). The different trend of the dependence of FASAs on K_{OW} and K_{AW} could arise from either: (i) The proposed model related to the molecular descriptor or (ii) Faulty adjusted property values.

In order to determine the reason for this FASA lack of agreement between Ext-Adj. data and Ext-Cal. data, the Est'd-Cal. data from available property models obtained from estimated property data

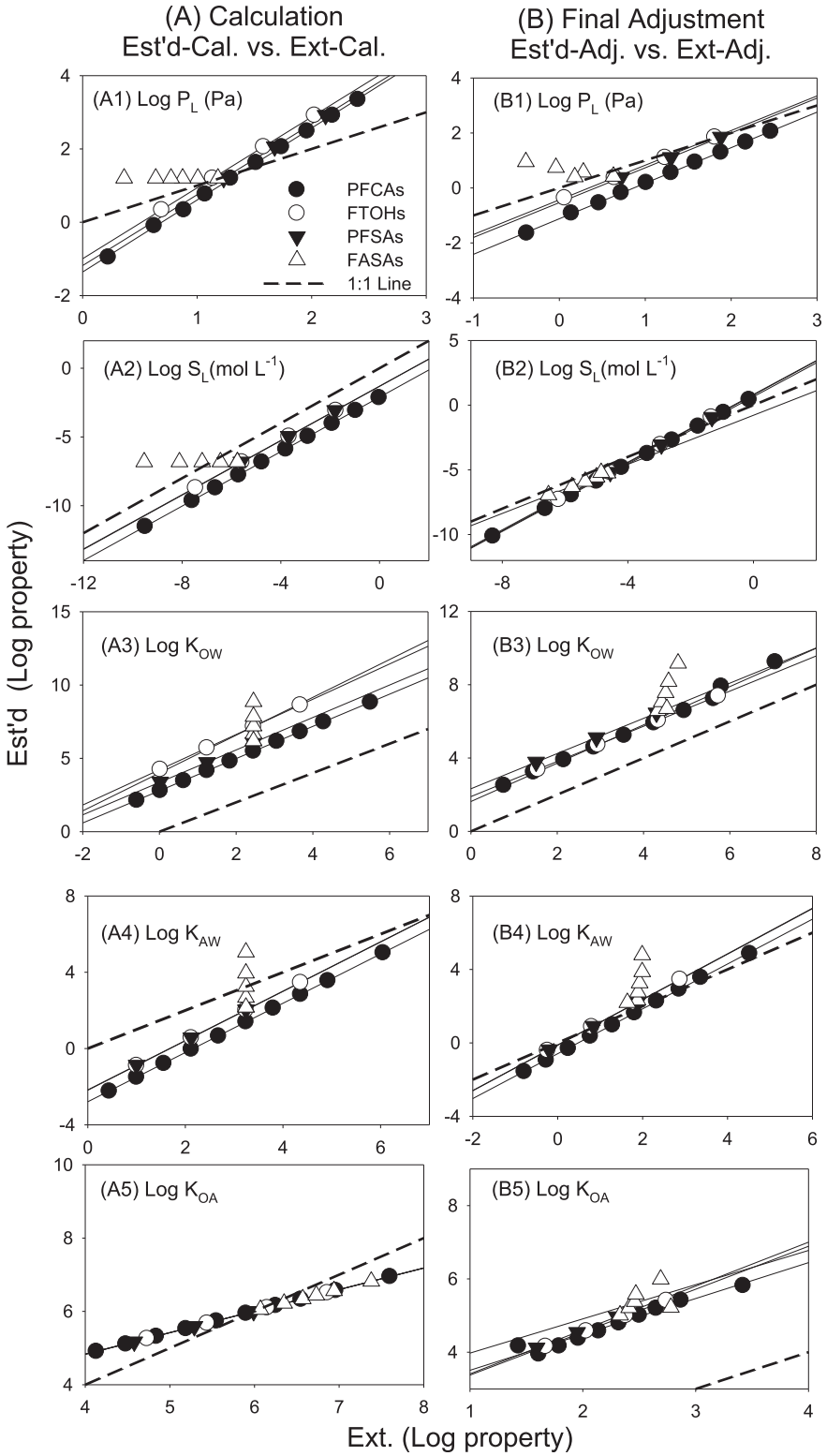


Fig. 3. Comparison of relationship between experimental calculated data and estimated calculated data ((A) Ext-Cal. vs. Est'd-Cal.) and that between finally adjusted experimental data and finally adjusted estimated data ((B) Ext-Adj. vs. Est'd-Adj.).

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Table 2
Quantitative structure property relationships between logarithm of properties (298 K and 760 mmHg) and molar volume (V_M, $cm^3\ mol^{-1}$) for PFASs.

Property	Single molecular model	r^2
Vapor pressure (P_L)	$\log (P_L/Pa) = -0.0081 \times V_M + 3.4361$	0.8981
Water solubility (S_L)	$\log (S_L/mol\ L^{-1}) = -0.0288 \times V_M + 3.0364$	0.7229
Octanol/water partition coefficient (K_{OW})	$\log K_{OW} = 0.0220 \times V_M - 3.4264$	0.9768
Air/water partition coefficient (K_{AW})	$\log K_{AW} = 0.0207 \times V_M - 2.3439$	0.8223
Octanol/air partition coefficient (K_{OA})	$\log K_{OA} = 0.0129 \times V_M + 2.4847$	0.8989

(Est'd-Sel. data) are compared with adjusted estimation data (Est'd-Adj. data) based on the identical procedure. The Est'd.-Cal data and Est'd-Adj. data sets are compared in Fig. 2. The degree of linearity between the Est'd-Adj. log data and Est'd-Cal. log data for FASAs differs significantly from that of the Ext-Adj. data and Ext-Cal. data. log P_L and log S_L do not show a linear relationship. The results suggest that non-linear relationships of the properties are driven by the proposed model with F_N because the FASA family consists of an eight-carbon backbone, fully surrounded by ten fluorines and a terminal sulfonamide functional group. Therefore, although the F_N descriptor model displayed good statistical results, this descriptor model cannot distinguish among PFASs with the same number of fluorines (i.e., FASA).

The relationship between Ext-Cal. data and Est'd-Cal. data is compared with that of Ext-Adj. data and Est'd-Adj. data in Fig. 3. The correlation equations and statistical results for each relationship appear in Tables S11 and S12 of the Supplementary information. Except for FASAs data obtained from the F_N molecular descriptor models, the correlation coefficient of the log–log linear relationship between two sets (i.e., Ext-Cal. data and Est'd-Cal) of data was >0.83. However, linear relationships differ from the ideal relationship (1:1 Line). This could be due to different methods of estimation and unmatching experimental conditions.

The relationship between Ext-Adj. data and Est'd-Adj. data (Ext-Cal. data and Est'd-Cal. data) for FASA tends to be non-linear when the Ext-Cal. data or Est'd-Cal. FASA data are obtained from the F_N molecular descriptor models, indicating that the non-linearity between Ext-Adj. data and Est'd-Adj. data for FASA is driven by faulty Ext-Cal. data and Est'd-Cal. data from the F_N descriptor model. Thus, it is clear that this F_N model, which is constitutional descriptor model derived from fluorine atom composition, is not very accurate for prediction. Meanwhile, V_M and TSA, which are geometric descriptors derived from 3-D structures, are highly correlated with the properties of PFASs (PFCA, PFSA, FASA and FTOH). V_M and TSA have been used mostly as bulk/cavity terms. There is no unique way to define V_M or TSA, but most approaches try to define a surface contour similar to the van der Waals volume. Therefore, V_M and TSA have often been used in QSPR studies to model molecular properties (e.g., Shiu et al., 1988; Valvani et al., 1976). In order to obtain reasonable estimates of the experimental properties for PFASs, simple QSPRs are suggested on the basis of V_M for the PFASs, including the same number of fluorine atoms because, setting aside the F_N descriptor models, models based on V_M are statistically better than those based on TSA. Hence V_M is the most important descriptor for QSPR of PFAS properties (Table 2). The calculated property values from the descriptor model based on V_M are given in Table 3.

Table 3
Physical–chemical properties of selected PFASs calculated from equations in Table 2.

Group	CAS	Acronym	log (P_L/Pa)	log (S_L/mol L^{-1})	log K_{OW}	log K_{AW}	log K_{OA}
PFSAs	375-73-5	PFBS	2.12	−1.64	0.14	1.02	4.58
	355-46-4	PFHxS	1.68	−3.23	1.35	2.15	5.29
	1763-23-1	PFOS	1.23	−4.81	2.56	3.29	5.99
PFCAs	375-22-4	PFBA	2.40	−0.64	−0.62	0.30	4.13
	2706-90-3	PFPA	2.18	−1.43	−0.02	0.86	4.48
	307-24-4	PFHxA	1.96	−2.22	0.59	1.43	4.84
	375-85-9	PFHpA	1.74	−3.01	1.19	2.00	5.19
	335-67-1	PFOA	1.51	−3.81	1.79	2.57	5.55
	375-95-1	PFNA	1.29	−4.60	2.40	3.14	5.90
	335-76-2	PFDA	1.07	−5.39	3.00	3.70	6.25
	2058-94-8	PFUnA	0.88	−6.05	3.51	4.18	6.55
	307-55-1	PFDoA	0.62	−6.97	4.21	4.84	6.96
	376-06-7	PFTA	0.22	−8.40	5.30	5.87	7.60
	754-91-6	FOSA	1.18	−4.99	2.70	3.42	6.07
FASAs	31506-32-8	MeFOSA	1.01	−5.61	3.17	3.86	6.35
	4151-50-2	EtFOSA	0.87	−6.09	3.53	4.21	6.57
	1691-99-2	EtFOSE	0.64	−6.92	4.17	4.81	6.94
	24448-09-7	MeFOSE	0.77	−6.45	3.81	4.46	6.73
	25268-77-3	MeFOSEA	0.36	−7.91	4.92	5.51	7.38
FTOHs	2043-47-2	4:2 FTOH	2.02	−1.99	0.41	1.26	4.73
	647-42-7	6:2 FTOH	1.58	−3.57	1.62	2.40	5.44
	678-39-7	8:2 FTOH	1.13	−5.16	2.82	3.54	6.15
	865-86-1	10:2 FTOH	0.69	−6.74	4.03	4.67	6.86

4. Conclusion

To narrow physicochemical property data gaps, predictive models for P_L, S_L, K_{OW}, K_{AW} and K_{OA} of PFASs are developed based on molecular descriptors (F_N, V_M and TSA). Properties were calculated from single descriptor models selected by statistical results and checked for internal consistency. Each of these properties for PFCAs, PFSAs and FTOHs showed good linearity between finally adjusted properties and calculated properties.

On the other hand, log K_{OW} and log K_{AW} for FASAs do not show a linear relationship. The results were driven by the proposed model with F_N because the FASA families all consisted of an eight-carbon backbone fully surrounded by ten fluorines. It is indicated that although the F_N descriptor model provides good statistical results, this model cannot distinguish among PFASs with the same number of attached fluorines.

Setting aside the F_N descriptor models, models based on V_M were statistically better than those based on TSA. Therefore, in order to obtain reasonable estimates of experimental PFASs properties, simple QSPR models are suggested based on the V_M of the PFASs. As a result, more reliable properties and predictive models are provided to improve the ability to understand and predict the behavior and fate of PFASs in the environment.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.envpol.2014.11.008.

Nomenclature and abbreviations

CAS Chemical Abstracts Service
Est'd-Adj. finally adjusted estimated

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Est'd-Cal.	calculated estimated
Est'd-Sel.	selected estimated
EtFOSA	ethylperfluorooctane sulfonamide
EtFOSE	ethyl perfluorooctane sulfonamido ethanol
Ext-Adj.	finally adjusted experimental
Ext-Cal.	calculated experimental
Ext-Sel.	selected experimental
F	fugacity ratio, dimensionless
F	overall statistical significance
F_N	fluorine number
FASAs	perfluorinated sulfonamides
FASEs	perfluorinated sulfonamido ethanols
FOSA	perfluorooctanesulfonamide
FTOHs	fluorotelomer alcohols
GC-RT	gas chromatographic retention time
K_{AW}	air/water partition coefficient, dimensionless
K_{OA}	octanol/air partitioning coefficient, dimensionless
K_{OW}	octanol/water partition coefficient, dimensionless
LOO	leave-one-out
MeFOSA	methyl perfluorooctane sulfonamide
MeFOSE	methyl perfluorooctane sulfonamido ethanol
MM	molar mass, g mol^{-1}
MP	melting point, K
n	sample number
P	significance level
Pa	pascal
PFBA	perfluorobutanoic acid
PFBS	perfluorobutane sulfonate
PFASs	Perfluoroalkyl and polyfluoroalkyl substances
PFCAs	perfluorinated carboxylic acids
PFDA	perfluorodecanoic acid
PFDoA	perfluorododecanoic acid
PFHpA	perfluoroheptanoic acid
PFHxA	perfluorohexanoic acid
PFHxS	perfluorohexane sulfonate
PFNA	perfluorononanoic acid
FASAs	perfluoroalkane sulfonamide substances
PFOA	perfluorooctanoic acid
PFOS	perfluorooctane sulfonate
PFPA	perfluoropentanoic acid
PFSAs	perfluorinated sulfonic acids
PFUnA	perfluoroundecanoic acid
P_L	subcooled liquid vapor pressure, Pa
PRESS	predictive sum of squares error
P_S	solid vapor pressure, Pa
Q^2 (or Rcv^2)	cross-validated coefficient, dimensionless
QSPR	quantitative structure–property relationships
R	gas constant, 8.314 J mol^{-1} K^{-1}
r^2	squared correlation coefficient, dimensionless
S.E.	standard error
S_L	subcooled liquid solubility in water, mol L^{-1}
T_m	normal melting point, K
TSA	total surface area, $Å^2$
TSS	total sum of squares
V_M	molar volume, cm^3 mol^{-1}
ΔS_{fus}	entropy of fusion, J mol^{-1} K^{-1}

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Global Perspective

Scientific Basis for Managing PFAS as a Chemical Class

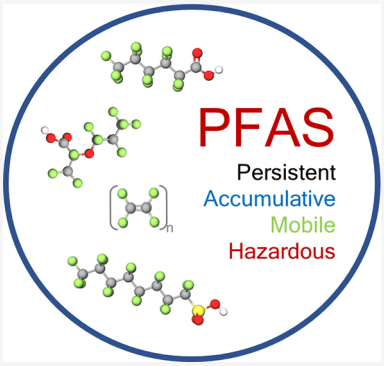
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ABSTRACT: This commentary presents a scientific basis for managing as one chemical class the thousands of chemicals known as PFAS (per- and polyfluoroalkyl substances). The class includes perfluoroalkyl acids, perfluoroalkylether acids, and their precursors; fluoropolymers and perfluoropolyethers; and other PFAS. The basis for the class approach is presented in relation to their physicochemical, environmental, and toxicological properties. Specifically, the high persistence, accumulation potential, and/or hazards (known and potential) of PFAS studied to date warrant treating all PFAS as a single class. Examples are provided of how some PFAS are being regulated and how some businesses are avoiding all PFAS in their products and purchasing decisions. We conclude with options for how governments and industry can apply the class-based approach, emphasizing the importance of eliminating non-essential uses of PFAS, and further developing safer alternatives and methods to remove existing PFAS from the environment.



INTRODUCTION

When chemicals have similar molecular structures, environmental properties, and/or biological hazards, managing them as a class can be an effective means of reducing adverse effects on human and ecological health.[1−4] While a class-based approach to chemical management can pose challenges to the traditional paradigm of individual chemical risk assessment, the extreme persistence and potential for harm from thousands of PFAS (per- and polyfluoroalkyl substances)[5,6] demand a more efficient and effective approach. Examples of cases in which substances with common chemical characteristics are currently managed as a class include organophosphate pesticides, organochlorine pesticides, and organohalogen flame retardants.[1,7] Thus, a class-based approach not only is feasible but also has already been implemented by regulatory agencies globally.

Here we provide scientific justification for why a class-based approach is appropriate and necessary for all PFAS, defined as chemicals with at least one aliphatic perfluorocarbon moiety (e.g., -C_nF_{2n}-).[5,6] We discuss the following major subclasses of PFAS in detail: perfluoroalkyl acids and perfluoroalkylether acids (together termed PFAA) and their precursors, fluoropolymers and perfluoropolyethers, and other (primarily less reactive) PFAS (see Figure 1 for examples). PFAA are nonpolymer PFAS with at least one perfluorocarbon moiety (e.g., -CF_2-, >CF-) directly linked to an acid functional group. The most well-known are perfluorooctanesulfonic acid (PFOS) and perfluorooctanoic acid (PFOA). Many other PFAS may transform and yield PFAA in the environment and biota and are thus regarded as precursors to PFAA. Examples are PFAS derived from fluorotelomers and perfluoroalkanesulfonyl fluorides, including so-called side-chain-fluorinated polymers (i.e., polymers with nonfluorinated backbones and fluorinated side chains). Fluoropolymers and perfluoropolyethers include polymers with backbones being per- or polyfluorinated. Other PFAS in the class include primarily nonpolymeric PFAS with limited chemical reactivity, such as linear and cyclic perfluoroalkanes, perfluoroalkylethers, and perfluoroalkyl amines.

PFAS function in many capacities, including as surfactants, friction reducers, and repellents of water, dirt, and oil. As such, they are used in a wide variety of consumer products to confer nonstick (waterproof, greaseproof, and stainproof) and low-friction properties. Examples of products that contain or are coated with PFAS include carpets, glass, paper, clothing and other textiles, plastic articles, cookware, food packaging, electronics, and personal care products. PFAS are also used directly or as technical aids (dispersants and emulsifiers) in many industrial applications, such as in metal coatings, lubricants for machinery, membranes, and firefighting foams. PFAS are used in the synthesis of or as adjuvants in pesticides,

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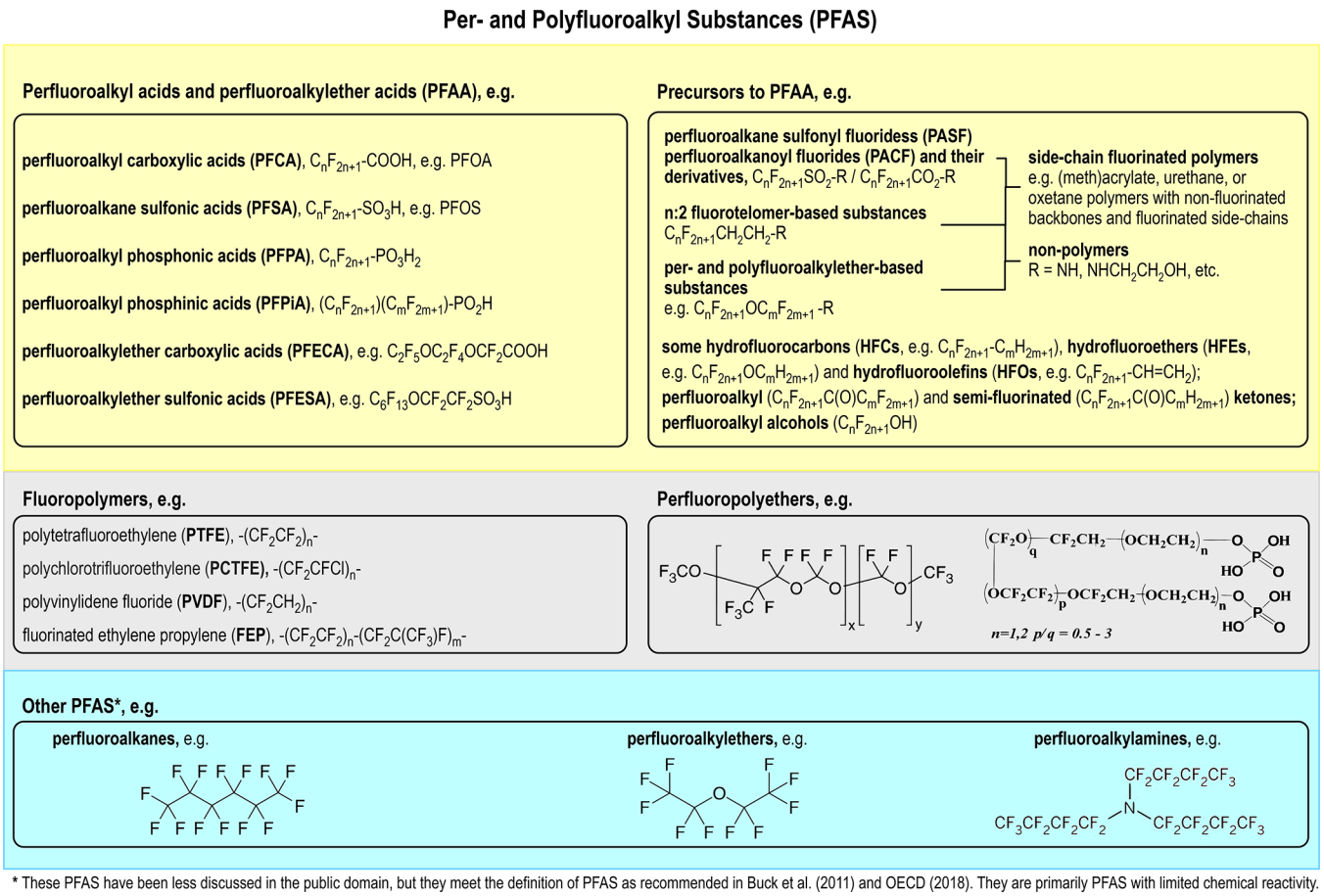


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* These PFAS have been less discussed in the public domain, but they meet the definition of PFAS as recommended in Buck et al. (2011) and OECD (2018). They are primarily PFAS with limited chemical reactivity.

Figure 1. Examples of PFAS chemistries. *These PFAS have been less discussed in the public domain, but they meet the definition of PFAS as recommended in refs 58 and 5. They are primarily PFAS with limited chemical reactivity.

in medical procedures and products, and in many other applications.[8]

The most consistent feature within the class of PFAS is that their perfluorocarbon moieties do not break down, or do so very slowly under natural conditions, which is why PFAS are often termed "forever chemicals".[9] Because PFAS are persistent, they accumulate or concentrate in the environment, including water, air, sediment, soil, and plants.[10] Elevated levels of PFAS and their widespread presence in environmental media and drinking water stem from industrial sites that produce or use PFAS (or have done so in the past), airports, military bases (fire-training and response areas), landfills, wastewater treatment plants, and the spreading of PFAS-contaminated biosolids.[11−13] Some PFAS are highly mobile in either air or water, allowing them to travel long distances from their source.

Environmental and human exposure to PFAS can occur throughout the life cycles of these chemicals and products containing them, including during chemical production, product manufacturing, distribution, use, disposal, and recycling. Many PFAS, particularly PFAA, have been detected globally and are in the bodies of nearly all people living in the United States (US), Europe, and other countries worldwide.[14,15] Major sources of human exposure to PFAS are from contaminated food, water, air, and other media such as consumer products and house dust.[16,17] Limited testing of primarily large public water sources in the US found PFAS in the water supplies serving an estimated 16.5 million people, including 6 million with a combined PFOS and PFOA concentration over the US EPA's lifetime health advisory of 70 ng/L.[11] More recent testing of 25 public water systems in the US identified PFAS in every one, with an average of nearly 10 different PFAS at a combined concentration near 20 ng/L.[18] Such testing is lacking in many other parts of the world, including many European countries. PFAS are also found in a variety of foods.[19,20] The highest levels are found in fish and shellfish, but meat, eggs, and milk may also contain PFAS if animals have consumed contaminated feed or water. Fruits and vegetables have been shown to contain PFAS taken up from the soil and water used to grow them.[21] Food contact materials are another source of exposure,[22,23] as are consumer products and house dust.[24−28]

Exposure to PFAS occurs in complex mixtures of multiple PFAS, yet at present, fewer than 50 individual PFAS (often fewer than 10) are commonly measured in environmental media.[29,30] New analytical methods allow for more comprehensive screening such as measuring total fluorine or extractable/adsorbable organofluorine in the environment,[31−33] products,[22,23,34,35] dust,[27] biota,[36] and humans.[37] These methods reveal evidence that humans and wildlife are exposed to more PFAS than previously estimated. For example, in one study of tap water in five US cities, less than half the total organic fluorine measured in treated drinking water was accounted for by the sum of individually identified PFAS, indicating far more PFAS and other organofluorine com-

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pounds were present in the water than were identified with targeted analysis.[38]

The most well-studied of these substances, PFOA and PFOS, have been linked to a variety of health problems. They are termed "long-chain" PFAS, a designation that includes perfluoroalkylcarboxylic acids (PFCA) with seven or more fluorinated carbons, perfluoroalkanesulfonic acids (PFSA) with six or more fluorinated carbons, and their precursors. When some major manufacturers phased out the production of long-chain PFAS, most industries turned to structurally similar replacements, including homologues with fewer fluorinated carbons (short-chain PFAS) or other less well known PFAS (e.g., per- and polyfluoroalkylether-based substances).[39,40] These replacement PFAS were marketed by producers as safer alternatives because of their presumed lower toxicity and lower level of bioaccumulation in human blood.[41] However, several lines of evidence suggest that short-chain PFAS are not safer alternatives. Research has demonstrated that short-chain PFAS can be equally environmentally persistent and are even more mobile in the environment and more difficult to remove from drinking water than long-chain PFAS.[33,42,43] Bioaccumulation of some short-chain PFAS occurs in humans and animals,[44−46] and research in fish suggests they can do so in excess of the long-chain compounds they aimed to replace.[47,48] Short-chain PFAS also can be more effectively taken up by plants.[49−51] Because short-chain PFAS have, to a large extent, replaced the long-chain PFAS in commerce, the levels of short-chain PFAS, such as perfluorobutanoic acid (PFBA), perfluorobutanesulfonic acid (PFBS), and perfluorohexanoic acid (PFHxA), have increased in environmental media.[37,43,52,53] To date, relatively little is known about possible health effects of long-term exposure to short-chain PFAS. However, a growing body of evidence suggests they are associated with similar adverse toxicological effects as long-chain PFAS.[54−57] The ongoing accumulation of persistent chemicals that are known or potentially hazardous increases risks to human and environmental health over an indefinite period of time.

Fluoropolymers consist of molecular segments (monomers) that are linked together, with up to hundreds of thousands of linked monomers in high-molecular weight polymers. While they are commonly regarded as PFAS,[58] fluorochemical producers now argue that fluoropolymers should be separated from other PFAS for hazard assessment or regulatory purposes.[59] However, the production of fluoropolymers and perfluoropolyethers is responsible for extensive environmental PFAS contamination, including releases of both intentionally added PFAA processing aids and unintentional PFAS by-products.[13,43,60−65] It is estimated that the vast majority (~80%) of PFCA in the environment is from fluoropolymer manufacture and use.[60] Below, we discuss reasons why fluoropolymers and perfluoropolyethers should be included in the class approach to managing PFAS.

To date, managing the risk of PFAS has focused primarily on one chemical at a time, or a small group of PFAS. This approach has not been effective at controlling widespread exposure to this large group of chemicals with known and potential hazards. Below, we present scientific justification for managing PFAS as a single chemical class, and we suggest ways in which government and industry can reduce PFAS-related risks. For example, a class-based approach can be implemented to more effectively eliminate non-essential uses of PFAS, develop safer alternatives, and clean up highly contaminated areas. Ultimately, this will reduce and prevent further accumulation of these hazardous chemicals in people and the environment and avoid replacing them with other related and harmful substances.

HEALTH AND ENVIRONMENTAL HAZARDS

With regard to biological activity and the potential for human health impacts, PFAA, particularly PFOA and PFOS, are the most well studied PFAS. Data from toxicokinetic studies of PFAA indicate that they are generally well-absorbed after ingestion.[66] After absorption, they distribute from blood to organs and tissues that receive high blood flow, such as the liver, kidney, lung, heart, skin, testis, brain, bone, and spleen.[46,66−70] Because PFAA can occupy sites on multiple receptors, proteins, and cell interfaces in the body, they can produce physiological effects across a range of tissues.[66] Toxicological (*in vitro* and *in vivo*) and epidemiological (in occupational, highly exposed, and general populations) studies have identified a broad range of adverse health outcomes associated with exposure to PFAA in people and animals. In studies of exposed humans, elevated blood levels of PFAA have been associated with kidney and testicular cancer, elevated cholesterol, liver disease, decreased fertility, thyroid problems, changes in hormone functioning, changes in the immune system, and adverse developmental effects.[54,71,72] Studies of experimental animals provide biological support for associations seen in human epidemiological studies, and mechanistic studies increase confidence in a causal relationship between PFAA and health effects in humans.[72,73] To understand the potential of every PFAS to adversely affect health would require testing across the entire range of different biological end points.

Effects on the immune system are some of the most well studied health effects of PFAA. Multiple lines of evidence support PFAA as immunotoxicants and, more specifically, immunosuppressants at small administered doses in rodents, and measured serum concentrations in humans. Findings of suppressed vaccine response in humans and T cell-dependent antibody response in experimental animals led the US National Toxicology Program (NTP) to classify PFOA and PFOS as presumed immune hazards to humans.[72] In a recent draft toxicological profile, the US Agency for Toxic Substances and Disease Registry (ATSDR) extended this finding to PFHxS and perfluorodecanoic acid (PFDeA), identifying all four compounds as suppressants of antibody response in humans.[54] These reviews provide strong evidence for immunotoxicity, especially when seen across multiple compounds, species, and studies. Notably, suppressed vaccine response in children indicates the period of early life as an exposure window of specific concern.[74,75] As such, developmental toxicity has been used as the basis for managing PFAS in drinking water and food contact materials.[76−80] Although the immune system, particularly during development, appears to be sensitive to these chemicals, few PFAS have been studied for such effects.

To date, a majority of human epidemiological studies have focused on long-chain PFAA. In experimental animal models, however, short-chain PFAA have shown effects similar to those of long-chain PFAA. For example, exposure to GenX has been associated with hepatic and renal effects[81,82] and suppressed immune function in mice.[83] A study of PFBA in rats indicated changes to liver weight, serum cholesterol, and thyroid hormones,[84] and a two-generation study of PFBS in rats demonstrated increased liver weight and pathological changes

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in kidneys.[85] Recent reports by NTP found that both PFBS and PFHxA had numerous adverse effects, including decreased thyroid hormones in male and female rats.[55,56] Effects on kidneys[86] and on reproduction and development[87] also have been reported for PFHxA. Notably, effects observed with other PFAA may occur at larger administered doses compared to the long-chain PFAA. However, humans are exposed to multiple PFAS at once, and there is little research to date on the effects of combined exposures. To account for such effects, an additive model for PFAS toxicity is used by the US EPA for two PFAS and in several US states for five to six PFAS. In Europe, an additive model is used by the European Food Safety Authority (EFSA) for four PFAS,[88] by the EU drinking water directive listing 20 PFAS, and by individual European countries, including Sweden and Denmark.

Some manufacturers have proposed that fluoropolymers should not be grouped with other PFAS for regulatory purposes, arguing that they are biologically inert because of their high molecular weight.[59] However, these chemicals can release low-molecular weight PFAS and other hazardous substances to the environment throughout their life cycle. Thus, we argue for the inclusion of fluoropolymers and perfluoropolyethers in the overall class approach for PFAS, specifically for the following reasons.

(1) During production of fluoropolymers and perfluoropolyethers, low-molecular weight PFAS used as raw materials, processing agents, or additives, or generated as intermediates, can be released into different waste streams (air and water) and current emission filters do not completely capture them, nor is there an effective means of disposing of captured PFAS.[13,89−91] For example, it was the production of fluoropolymers and the associated use and release of PFOA that led to the widespread contamination of the US mid-Ohio river valley and its residents.[92] In addition, potent greenhouse gases such as HFC-23 [trifluoromethane (CHF_3)] can be formed during fluoropolymer production, and emissions to the atmosphere have been reported.[93,94]

(2) During use, low-molecular weight PFAS may be released, for example, PFCA in personal care products that contain PTFE.[95]

(3) During disposal, PFAA and other hazardous byproducts may be generated and released, such as when they are incinerated at an insufficiently high temperature for insufficient time.[96−100] For example, when PTFE is heated above 350−400 °C, it decomposes and releases various gases that cause the so-called "Teflon fever" in workers.[101]

Other important considerations are that (1) some perfluoropolyethers (e.g., Krytox 157FS[102]) are mixtures of PFAA with molecular weights of only several thousand grams per mole and thus potentially biologically active; (2) in the EU and many other countries, substances registered as polymers can consist of fewer than 10 monomers, which are likely to be small and bioavailable molecules;[103] (3) fluorine is 19 times heavier than hydrogen, and therefore high-molecular weight PFAS can be relatively small molecules, compared to hydrocarbon molecules of the same weight; and (4) fluoropolymer microplastics contribute to global plastic and microplastics debris,[104,105] thus adding to ongoing environmental plastic and PFAS pollution.

Similar to fluoropolymers, other PFAS such as perfluoroalkanes and perfluoroalkylamines are generally inert,[106] but they can be very potent greenhouse gases, up to 3 orders of magnitude more potent than CO_2.[107−113]

In sum, scientific evidence supports the possibility that adverse effects of PFAS can occur in several bodily systems, with the developing immune system being particularly sensitive. Health effects have been demonstrated for several PFAS, including long- and short-chain PFAA, and chemicals associated with polymers. However, <1% of all PFAS have been tested for their hazardous effects. Proceeding with the approach of testing one chemical at a time will cause substantial delays in the effort to protect health and the environment from this large class of potentially hazardous chemicals.

■ ENVIRONMENTAL EXPOSURE: PERSISTENCE, ACCUMULATION, AND MOBILITY

An overarching property of all PFAS is that they have highly stable perfluorocarbon moieties in their molecular structure. Thus, all PFAS either are extremely persistent in the environment and biota or partially transform into extremely persistent PFAS.[114−117] Studies have estimated that PFAS such as perfluoroalkanes have lifetimes in the thousands of years.[113,118] Thus, PFAS will be present in the environment for centuries or longer, even if environmental releases cease immediately.

The high persistence of PFAS results in long-term accumulation in the environment and living organisms, which increases the risk of harm. A key concern in recent years is that some replacement PFAS, such as PFBA, PFBS, and GenX, have been widely detected in surface water and groundwater.[43,65,119] PFAS can concentrate in plants, including food crops, when grown in contaminated soil or irrigated with contaminated water.[20,120−122] Bioaccumulation occurs through the food chain, with top predators (e.g., whales, bald eagles, and humans) having the highest levels.[123−127] Most concerning is that when PFAS accumulate, they can reach concentrations where hazardous effects are observed in humans and ecosystems, particularly when the effects of combined exposure to multiple PFAS are considered.[128,129]

The high mobility of many PFAS further exacerbates the concern.[130] Many PFAS can travel long distances from their sources. PFAA, particularly short-chain types, are very water-soluble, being distributed readily in groundwater, surface waters, and the oceans.[53,131,132] They can be difficult, costly, and sometimes even impossible to remove from water with conventional and even advanced treatment processes.[13,43,133,134] Many other PFAS may be highly mobile in air, including the volatile perfluoroalkanes, fluorotelomer alcohols (FTOH), and many other (semi)volatile PFAA precursors.[116]

The extreme persistence of the fluorocarbon chain, combined with the propensity for accumulation and mobility of many PFAS, has resulted in PFAS being ubiquitous globally, even in remote regions like the Arctic.[36,53,135,136] The continued use of PFAS will result in increasing concentrations of PFAS, increasing numbers of exposed organisms,[137] and increasing probabilities of harm. Once adverse effects are identified, it will take decades, centuries, or even longer to reverse contamination and reduce the harm to our health and the environment.

■ MANAGING RISK

Risk management consists of various actions to minimize the chance of harm. Chemical risk management can be carried out

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by governments and businesses and includes the cessation or restriction of production and use of the chemicals, and efforts to clean up contamination.

Regulatory Approaches. Many different regulatory frameworks are used for managing the risk of exposure to hazardous chemicals. While traditionally PFAS have been regulated one chemical at a time, subgroups of PFAS have also been regulated, with a focus on PFAA and their precursors.[79,138−142] An advantage of targeting chemical subgroups is that the toxicological end points are often assumed to be similar, which allows for extrapolation from well-studied chemicals to those less studied. However, assessing only small subgroups systematically ignores the majority of PFAS and underestimates the overall risk, particularly when many of the chemicals are unknown. For example, the EU drinking water directive, which addresses a relatively large subgroup, covers only 20 PFAS.[143]

Governments are increasingly using broader management approaches to control PFAS exposure, such as targeting all PFAS within certain use categories. For example, the US states of Maine and Washington banned all PFAS in food contact materials[144,145] and Denmark banned PFAS from paper and paperboard food packaging.[146,147] South Australia and Washington state (and other US states) enacted bans on PFAS in firefighting foam.[148,149] California has proposed to regulate any PFAS used in carpets and rugs.[150] In the case of drinking water, a "PFAS - Total" limit was recently adopted by the European Commission.[143] Regulatory agencies in Europe and the US are working to advance, validate, and standardize currently available methods to measure total PFAS in certain media.

A more comprehensive risk management approach that has been gaining traction is to limit the uses of hazardous chemicals to only those considered "essential", while fostering development of safer alternatives. In 1987, the Montreal Protocol defined essentiality (in the case of ozone-depleting chlorofluorocarbons) as being necessary for health or safety, or critical for the functioning of society, and without technically and economically feasible alternatives or substitutes that are acceptable from the standpoint of environment and health.[151] In the 2015 "Madrid Statement", more than 200 scientists advocated using a similar approach for PFAS, i.e., limiting the production and use of the entire class of PFAS, including polymers, to essential uses.[152] A more recent publication applied the essentiality concept to specific PFAS use categories and described examples of current PFAS-free alternatives, as well as uses where alternatives still need to be developed.[153] In 2019, several European countries committed to phasing out all non-essential uses of PFAS by 2030.[154] Limiting PFAS to essential uses would incentivize further development of alternatives that do not require fluorinated chemicals. Focusing on pollution prevention is critical because remediation of PFAS-impacted media, such as polluted groundwater aquifers, is costly, is energy-intensive, and cannot fully reverse the damage.

Managing PFAS as a class has additional benefits. It reduces the likelihood of replacing well-studied hazardous chemicals with poorly studied but structurally similar PFAS that have the potential to be similarly hazardous (i.e., "regrettable substitution"). It can be simpler and less expensive to implement: for example, for premarket regulation of uses for the entire class, for setting procurement standards, for testing for compliance and communicating test results through the supply chain, and for authorities monitoring the extent of PFAS contamination of humans, products, food, water, and the environment. Simpler, cheaper, class-based methods also typically result in more frequent testing, which improves compliance and detection of emerging risks. Methods to screen for fluorine already exist, for example, extractable organic fluorine methods coupled to combustion ion chromatography (EOF-CIC) and particle-induced γ-ray emission (PIGE).[23,155,156] Hence, focusing on risk management tools that address PFAS as a class has the potential not only to prevent pollution by known PFAS but also to prevent regrettable substitution, to improve the efficiency and effectiveness of chemical management, and to encourage the selection of treatment approaches that effectively reduce total PFAS exposure when remediating PFAS-contaminated sites.

Marketplace Approaches. Compared to governments, retailers and manufacturers can make more rapid changes to reduce their use of chemical classes of concern. For instance, home retailer IKEA committed to a complete phase-out of all PFAS in its textile products and reported achieving this goal as of 2016.[157] Recently, H&M, Danish COOP, and ChemSec's corporate initiative called to end the use of PFAS in products and the supply chain.[158] Numerous factors are encouraging companies to stop using the entire class of PFAS. Increasing demand for products containing fewer harmful chemicals is one driver. For example, demand from retailers for food contact materials, from textile brands for sportswear, or from large purchasers and green builders for carpets has resulted in safer PFAS-free products on the market. Pressure from environmental groups is another driver. One prominent campaign contributed to the decision of many apparel companies to eliminate PFAS in their textile treatments.[159] Companies' values can also play a role, such as when member-owned retailer COOP Denmark announced a phase-out of all PFAS-containing cosmetics "on the basis of a precautionary principle".[160] Similarly, Kaiser Permanente, Levi Strauss & Co., and Crate and Barrel are phasing out all PFAS based on the companies' environmental and health values.[3]

New international, national, state, and local regulations focusing on PFAS in certain products are additional influences,[150] as are threats of future litigation and liability.[161] While some companies may find it challenging to eliminate all PFAS from their products, others view it as important for mitigating business risks, or as a business opportunity. For example, treating PFAS as a class can help companies avoid multiple cycles of reformulations due to regrettable substitutions. Regulatory action addressing the class of PFAS will encourage further preventive actions from companies and help "level the playing field" by reducing the financial disadvantage of industry front-runners developing safer alternatives while incentivizing even further innovation toward safer alternatives.

■ OPTIONS MOVING FORWARD

Thousands of PFAS have already been documented across multiple industries and business sectors, and the list is growing.[5,6] Managing PFAS one by one is neither feasible nor cost-efficient. More comprehensive solutions are needed, given that traditional approaches have failed to control widespread exposures to PFAS and resulted in inadequate public health protection. For Europe alone, the annual health costs linked to exposure to just a few PFAS are estimated at 52−84 billion Euros, and environmental remediation costs at roughly 17 billion Euros.[162] Here we suggest class-based

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options to more comprehensively and efficiently reduce PFAS exposure.

Government policy makers have already begun limiting PFAS through bans in certain product categories. However, to more effectively manage PFAS, governments can apply the essential uses framework. Examples of essential and non-essential uses of PFAS have already begun to be described.[153] To make the criteria fully operational for inclusion in legislation, a more precise set of decision criteria is needed to guide the categorization. Such decisions involve both scientific and ethical considerations and thus require input from a broad set of scientists, civil society, industry, and policymakers.

Limiting the entire class of PFAS, including fluorinated polymers, to essential uses is critical, given that currently, remediating PFAS, once released to the environment, is at best extremely costly and, in some cases, impossible.[114,163] Governments can take a class-based approach to cleanup efforts, for example, by prioritizing research and development funding for treatment and disposal/destruction methods that are effective for the entire class of PFAS. Such an approach would ensure that treatment strategies remove all PFAS from all impacted environmental media (water, air, and soil) and that treatment residuals (for example, spent activated carbon and reverse osmosis concentrate) are managed such that the entire PFAS class is destroyed and its degradation products (or minerals) captured, so that unknown fluorinated reaction intermediates and harmful levels of organofluorines and hydrogen fluoride are not reintroduced into the environment. A class approach can also be used in developing cleanup standards, so that responsible industries are held accountable for remediation of all PFAS, not just a few. Additionally, governments can hold responsible parties accountable for exposure and health monitoring in heavily exposed populations, in order to promote effective and lasting solutions.

Regulatory agencies can also adopt class-based strategies to reduce exposure and minimize health risk. For example, they may extrapolate risk from well-understood PFAS when limiting uses of PFAS in commerce or setting protective cleanup levels. They can also assess combined exposures to PFAS (e.g., in drinking water, food, air, consumer products, and waste) as a basis to set regulatory limits and treatment standards. Establishing limits to the class rather than doing so on a chemical-by-chemical basis would result in lower exposure values that better protect vulnerable populations such as pregnant women, children, and workers. In addition, systems that can track historic, current, and future uses of all PFAS, and releases to the environment, could help to guide and prioritize monitoring, for instance, for emerging risk detection and compliance/enforcement testing. The further development, use, and interlaboratory standardization of analytical methods to measure total PFAS would complement this effort, improving the accuracy, speed, and cost of screening for PFAS in the environment, consumer products, and people. Collaboration within and across national and international policy and regulatory bodies to foster class-based strategies would be beneficial. Such concerted efforts could help prevent shifting burdens from one geographical location to another and may evolve into "de facto" industry standards as international actors attempt to minimize costs of complying with multiple different regulations.

Solutions are also available in the marketplace. Chemical manufacturers can move quickly to develop safer non-fluorinated alternatives for PFAS with current essential uses. They can also work with product manufacturers and businesses to rapidly replace all PFAS uses that have technically and economically feasible alternatives that are acceptable from the standpoint of environment and health. Chemical and product manufacturers can be transparent about the use of any PFAS chemistries in the supply chain and monitor and strictly control releases of all PFAS into the environment until their use can be phased out. In addition, PFAS manufacturers can assist in developing better methods to detect, remove, and destroy PFAS, although regulatory incentives or pressures may be needed.

The more we study PFAS, the more we learn about the harm they can do to our health and the environment. However, it is not possible to thoroughly assess every individual PFAS, or combination of PFAS, for their full range of effects in a reasonable time frame. Without effective risk management action around the entire class of PFAS, these chemicals will continue to accumulate and cause harm to human health and ecosystems for generations to come. As demonstrated above, managing PFAS as a class is scientifically sound, will provide business innovation opportunities, and will help protect our health and environment now and in the future.

Box Key Messages

1. Per- and polyfluoroalkyl substances (PFAS) make up a class of extremely persistent chemicals, numbering in the thousands, that accumulate in the environment and living organisms and can be highly mobile, leading to global contamination.
2. The use of PFAS in numerous consumer and industrial applications has led to widespread human and environmental exposure from, for example, drinking water, food, and consumer products.
3. Toxicological and epidemiological studies have identified a broad range of adverse health outcomes associated with exposure to PFAS in people and animals.
4. We suggest a class-based approach to managing the human and environmental risks associated with all PFAS, including polymers.
5. We provide options for how governments and industry can apply the class-based approach, emphasizing the importance of eliminating non-essential uses of PFAS, and further developing safer alternatives and methods to remove all existing PFAS from the environment.

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Notes

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Letter

Landfill Gas: A Major Pathway for Neutral Per- and Polyfluoroalkyl Substance (PFAS) Release

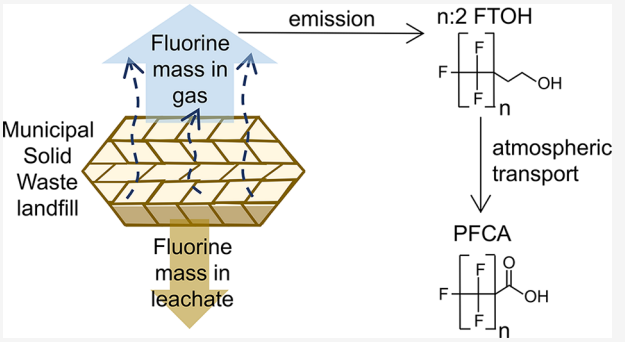
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ABSTRACT: The undisclosed and ubiquitous use of perfluoroalkyl and polyfluoroalkyl substances (PFAS) in consumer products has led to a growing issue of environmental pollution, particularly within the solid waste community, where the fate of volatile (neutral) PFAS in landfilled refuse is not well understood. Here, three municipal solid waste landfills in Florida were assessed for neutral PFAS in landfill gas and ionic PFAS in landfill leachate to compare the relative mobility between the two pathways. Landfill gas was directly sampled using a high volume, XAD-2 resin based sampling approach developed for adsorption and analysis of 27 neutral PFAS. Across sites, 13 neutral PFAS were identified from fluorotelomer alcohol (FTOH), fluorotelomer olefin (FTO), secondary FTOH, fluorotelomer acetate (FTOAc), and fluorotelomer methyl acrylate (FTMAc) classes; however, FTOHs dominated concentrations (87−97% total neutral PFAS), with most detections surpassing utilized calibration levels. Even under conservative assumptions, the mass of fluorine leaving in landfill gas (32−76%) was comparable to or greater than the mass leaving in landfill leachate (24−68%). These findings suggest that landfill gas, a less scrutinized byproduct, serves as a major pathway for the mobility of PFAS from landfills.



KEYWORDS: *volatile, emissions, GC, fluorotelomer alcohol*

1. INTRODUCTION

Widespread per- and polyfluoroalkyl substance (PFAS) contamination has been a mounting environmental concern due to their chemical persistence and toxicity to human and biotic health.[1−4] While numerous industries are being confronted with PFAS-related management challenges, the burden of remediation and PFAS removal has often fallen on downstream industries—namely, the solid waste sector.[5−9] Discarded, PFAS-laden consumer products including textiles, wood products, and packaging and commonly landfilled industrial byproducts like MSW incineration ash and wastewater biosolids are known contributors to PFAS loading in landfills.[10−16] Existing research suggests most discarded PFAS mass is retained within landfills[9,17] with liquid-phase byproducts of waste decomposition, leachate and gas condensate, currently considered prevalent pathways for PFAS mobilization.[2,7,9] However, the extent of PFAS release to another major byproduct, landfill gas (LFG), has remained largely unscrutinized.

The bulk of PFAS characterization studies focus on nonvolatile/semivolatile (ionic) perfluoroalkyl acids (PFAAs) measured in liquid and solid matrices, in part because of a high presence and awareness of these species within the PFAS community but largely because analytical capabilities for ionic PFAS measurement are better established.[18−21] Volatile (neutral) PFAS are also utilized in consumer products[13,22−27,27] and have been determined in a few studies on ambient air surrounding landfills and near wastewater treatment plants,[28−32] but a lack of volatile analytical standards and latency in methodological development has hindered the progression of gas phase research in environmental matrices. Whereas PFAS characterization in leachate is established, concentrations ranging from thousands to tens of thousands of nanograms per liter are commonly encountered;[33−38] only two studies characterize volatile PFAS directly in LFG.[39,40] Titaley et al. identified fluorotelomer alcohol (FTOH), fluorotelomer acrylate (FTAc) and fluorotelomer olefin (FTO) homologues in LFG with combined concentrations ranging from 4,600 to 14,000 ng m^{-3} across three landfills. Goukeh et al., only assessing FTOHs, identified higher combined concentrations than Titaley et al., finding ∼18,000 ng m^{-3} (sum of 6:2 and 8:2

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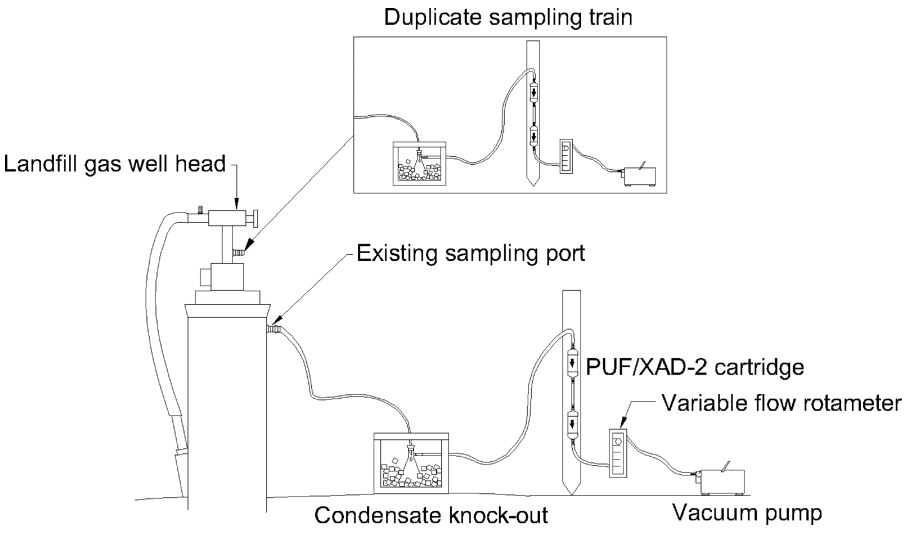


Figure 1. Developed system for sampling neutral PFAS directly from landfill gas well heads.

FTOH) in the one LFG sample examined. These studies suggest PFAS variability in LFG, which motivates further investigation, deploying higher sampling volumes[39] and larger analyte lists[40] to understand the potential presence of other neutral PFAS and distribution among landfills of different regions, compositions, and sizes.

With the ongoing development of PFAS regulation,[19] understanding the partitioning behavior of PFAS in major repositories like MSW landfills grows increasingly critical to minimize environmental and human risk. Unlike leachate, LFG is not always captured by collection systems, and management varies broadly across landfills, ranging from no treatment (i.e., passive venting) to some treatment (i.e., flaring, LFG to energy projects), but current treatment, if any, is not intended for PFAS.[41,42] Emerging research suggests the toxicity of volatile species (specifically 6:2 FTOH) to be significantly higher than their ionic counterparts via the inhalation pathway (a main route of exposure for volatile compounds).[43−47] Further, degradation of neutral species to ionic PFAAs once emitted to the atmosphere is well established.[48−58] The potential for long-range atmospheric transport of PFAS from landfills underscores the importance of considering neutral species and their fate during management to prevent further environmental contamination of highly scrutinized PFAAs such as perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS). As the only studies on LFG primarily identified FTOHs in LFG, the magnitude and significance of other neutral species remains unclear.

Here, LFG was sampled directly from gas well heads at three MSW landfill locations in Florida using a higher volume sampling protocol. XAD-2 resin sandwiched between polyurethane foam (PUF) was utilized for PFAS capture, then samples were analyzed for 27 volatile/semivolatile (neutral) PFAS via targeted gas chromatography high resolution mass spectrometry (GC-HRMS). To contextualize release in the gas phase, leachate was also collected at each landfill and analyzed for ionic PFAS (n = 93) using ultrahigh pressure liquid chromatography tandem mass spectrometry (UHPLC-MS/MS). The observed LFG and leachate concentrations were normalized on a mass of fluorine basis to compare the potential mobility in gas versus leachate matrices. This study provides foundational data critical for understanding the role of landfills in anthropogenic PFAS release and for informing LFG management.

2. METHODS AND MATERIALS

For brevity, materials and methods associated with ionic PFAS analysis in landfill leachate are provided in section 1 of the Supporting Information (Tables S-1 through S-4).

2.1. Standards and Reagents. Targeted neutral PFAS (≥97% purity, n = 27) were purchased from Wellington Laboratories Inc. (Guelph, ON, Canada), SynQuest Laboratories (Alachua, FL), and Chiron (Stiklestadveien, Trondheim, Norway). Nine classes of neutral PFAS (perfluoroalkane sulfonamides (FASAs), perfluoroalkane sulfonamidoethanols (FASEs), fluorotelomer acetates (FTOAcs), fluorotelomer methyl acrylates (FTMAcs), fluorotelomer iodides (FTIs), fluorotelomer secondary alcohols (sFTOHs), FTOHs, FTAcs, and FTOs) were measured using eight isotopically labeled internal standards (IS) from FASA, FASE, FTOH, and FTMAc classes for quantitation (Table S-5).

2.2. Sample Preparation and Collection. Polyvinyl chloride (PVC) cartridges filled with 4−5 g of Amberlite XAD-2 resin retained between two polyurethane foam (PUF) discs were utilized for PFAS capture.[29,59,60] Before use, XAD-2 sorbent was made PFAS-free through sequential Soxhlet extractions.[61] All cartridge components, sampling vessels, and tubing were sonicated in a mixture of Liquinox and PFAS-free water, rinsed, and then sonicated in methanol and methanol rinsed before use. Once dried and assembled, cartridges were stored in individually sealed polyethylene bags at 4 °C until sampling.

As neutral compounds were the focus of this investigation, aerosolized/particulate-bound PFAS were not specifically targeted for capture; however, a condensate collection system was included to prevent moisture interference. The developed sampling system (Figure 1) consisted of a condensate knockout (borosilicate, barbed Erlenmeyer flask contained in a cold box), two PUF/XAD-2 cartridges (installed in-series), a rotameter for flow control, a portable vacuum pump, and PFAS-free Tygon tubing. Before each sampling event, gas well head connection to the larger landfill gas collection system was disabled to create a neutral to positive pressure, workable for flow through the sampling system, then gas composition/

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Table 1. Average Concentrations (n = 2) of 13 Neutral PFAS (ng m^{-3}) from Three Municipal Solid Waste Landfills in Florida (Site Characteristics Are Provided in Table S-9)[a]

	concentration (ng m^{-3})					
analyte	landfill 1	E	landfill 2	E	landfill 3	E
4:2 FTOH	220		ND		57	
6:2 FTOH	*>9,900*	**170,000**	*>6,000*	**22,000**	*>6,500*	**62,000**
8:2 FTOH	*>6,800*	**200,000**	*>6,000*	**140,000**	*>6,500*	**740,000**
10:2 FTOH	*>5,100*	**14,000**	*>3,000*	**23,000**	*>5,000*	**120,000**
12:2 FTOH	860		1,400		5,000	
5:2 sFTOH	*>2,900*	**8,800**	*>1,700*	**9,000**	*>1,900*	**5,900**
7:2 sFTOH	320		*>1,300*	**13,000**	*>1,400*	**11,000**
8:2 FTO	2,500		1,300		550	
10:2 FTO	650		840		540	
12:2 FTO	97		580		160	
8:2 FTOAc	610		90		490	
10:2 FTOAc	99		19		140	
6:2 FTMAc	3,800		56		150	

[a]Concentrations of 6:2, 8:2, and 12:2 FTOH and 5:2 and 7:2 sFTOH consistently exceeded the upper limit of developed calibration ranges; therefore, both a minimum concentration (assuming the highest calibration concentration) and a maximum extrapolated concentration are provided. Italicized values denote a minimum concentration. Column "E" presents average maximum concentrations. "ND" denotes non-detect measurements. FTAcs, FASAs, FASEs, FTIs, and 8:2 FTMAc were not detected in any samples. Analyte acronyms and details are provided in Table S-5.

temperature was recorded using an Optimax Biogas analyzer (MRU Instruments, Humble, TX). Duplicate sampling trains were connected to existing gas well sampling ports. Approximately 1,200 L was sampled through each train at a flow rate of 5 L min^{-1}. After sampling, PUF/XAD-2 cartridges were sealed and individually stored at ≤4 °C for transport/storage. Quality control (QC) procedures are provided in the SI, section 2.

2.3. Extraction and Analysis. Spent XAD-2 from each cartridge was weighed and transferred to a 50 mL polypropylene centrifuge tube and vortexed, and approximately 2 g aliquoted for extraction. Samples were spiked with a mixture of mass labeled IS (Table S-5), rotated end-over-end for 18 h in 4 mL of 75/25% (v/v) ethyl acetate and methanol, and centrifuged for 10 min at 4,000 rpm. Supernatants were transferred to 15 mL centrifuge tubes, and the extraction process was repeated, combining supernatants from the two-fold extraction. Extracts were concentrated to 3 mL via gentle nitrogen evaporation, aliquoted, and stored no more than 30 days at −20 °C until analysis. QC details are provided in the SI, section 2 (Table S-6 and Figure S-1).

Targeted analysis of 27 neutral PFAS by positive chemical ionization (PCI) with selected ion monitoring (SIM) was conducted using a Thermo Scientific TRACE 1310 gas chromatograph coupled to a Thermo Scientific Orbitrap Exploris GC 240 mass spectrometer (GC-HRMS; see SI, section 2 for details regarding GC separations and instrumentation). A 12-point external calibration curve (from 1 to 2,000 pg μL^{-1}) was developed for quantitation, prepared through serial gravimetrically derived dilutions of primary stock solutions. A mixture of mass labeled IS at concentrations of 150 pg μL^{-1} was added to each calibration level. When a labeled standard was not available for a compound, a labeled standard with a similar retention time or structure was utilized for quantitation (Table S-5).

3. RESULTS AND DISCUSSION

Unexpectedly, several neutral PFAS concentrations in LFG exceeded the implemented calibration levels. Because of considerable exceedance for some compounds, dilution would reduce IS below instrument detection; therefore, in instances where sample concentrations exceeded calibration limits, two concentrations are presented (Equation S-1): a minimum value which assumes the highest calibration concentration and a maximum extrapolated concentration. Fluorine mass release calculations utilize minimum values, preventing overextrapolation while providing a conservative estimate for leachate comparison. Even under these assumptions, substantial concentrations of neutral PFAS, higher than those previously observed, were identified. Future assessments should deploy shorter sampling durations to refine findings.

3.1. Neutral PFAS in Landfill Gas. Except for 4:2 FTOH in one landfill, 13 PFAS were detected in duplicate samples across the three sites (site characteristics are provided in Table S-9). Observed concentrations are displayed in Table 1. At minimum, combined concentrations of neutral PFAS in LFG ranged from 22,000 to 33,000 ng m^{-3}. Considering extrapolated values, total concentrations ranged from 210,000 to 940,000 ng m^{-3}, an order of magnitude higher than those previously reported in LFG.[39]

3.1.1. FTOHs and sFTOHs in Landfill Gas. Like previous studies on LFG and air surrounding landfills, FTOHs dominated neutral PFAS concentrations;[28,31,32,39,40] however, extrapolated concentrations in this study surpassed previous reports in LFG, in some cases by 2 orders of magnitude, and were more comparable (although much lower) to concentrations recently identified in soil vapor near a PFAS manufacturing facility.[55] While there are uncertainties given the degree of extrapolation, the magnitude of FTOHs found in this study compared to existing research suggests fundamental differences potentially related to sampling methodology (e.g., much larger sampling volumes) and/or sampled landfill characteristics (e.g., waste type, age, air intrusion), although these data were not available for comparison. Across the three sites, 6:2, 8:2, and 10:2 FTOH, combined, made up 87 to 97% of total concentrations, but 8:2 FTOH alone constituted 50 to 79%. The shortest and longest analyzed homologues, 4:2 and

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12:2 FTOH, were significantly lower in concentration (Table S-10). This is supported by previous FTOH distributions determined from source fluoro-telomer polymers[62] and observations in LFG, urban air, and air surrounding wastewater treatment/landfill sites.[28−31,39,40,63] Concentrations of 12:2 FTOH were of similar magnitude to those in Titaley et al., but 4:2 FTOH has not been detected in LFG, suggesting MSW landfills to be a previously unidentified potential source of atmospheric 4:2 FTOH.[39]

Secondary FTOHs have not been targeted in gas-phase landfill research but have been identified in condensate associated with LFG collection systems.[37] As intermediary byproducts of 6:2 and 8:2 FTOH biodegradation to PFAAs, 5:2 and 7:2 sFTOH, were unsurprisingly elevated, they were at least an order of magnitude lower than respective parent FTOH homologues.[64−66] All detections of 5:2 sFTOH and two out of three detections of 7:2 sFTOH were above calibration, combined sums attributed to 2 to 10% of total concentrations.

3.1.2. Other Neutral PFAS in Landfill Gas. Other neutral PFAS fell within acceptable calibration ranges and together accounted for 0.22 to 1.9% of total concentrations. FTO homologues have been encountered in other LFG and ambient air studies, but in past assessments 8:2 and 10:2 FTO were below limits of quantitation and 12:2 FTO concentrations were consistently an order of magnitude higher than those reported here.[39,59] To the authors' knowledge, 8:2 and 10:2 FTOAc and 6:2 FTMAc have not been determined in LFG. FTOAcs are not commonly assessed analytes but are associated with fluoropolymer textile treatments and have been identified in one indoor air study from Japan.[67,68] Similarly, 6:2 FTMAc has only been analyzed in a few studies on cosmetics and wastewaters but at lower concentrations.[69−71]

3.2. Comparative Fluorine Mass Release between Landfill Byproducts. Normalizing PFAS concentrations on a fluorine basis allows comparisons to be drawn between different matrices and PFAS types (e.g., gas−liquid, neutral−ionic, precursor−terminal). This methodology is widely used to assess the "mass balance" of PFAS within systems, given that the long-term environmental fate of measurable PFAS is transient, whereas the mass of fluorine is conserved.[17,72,73]

Here, the same approach is utilized to compare the PFAS mobility in leachate versus LFG pathways. Neutral (Table 1, minimum values) and ionic (Table S-4) PFAS concentrations in LFG and leachate from this study were individually normalized to a mass of fluorine (Equation S-2) using compound specific fluorine mass fractions (Table S-8). Summed fluorine masses in leachate and LFG were then scaled according to site-specific annual generation volumes reported for each landfill (Table S-9).[41] A caveat of this comparison is the absence of measurements for neutral species in leachate and ionic species in LFG; however, the literature suggests FTOHs (the dominant neutral class identified) predominantly exist in the gaseous phase, while PFAAs exist in liquid or particulate phases.[28,74] Subsequent research should assess neutral and ionic compounds in both matrices to validate findings and further elucidate the PFAS behavior in landfills.

Even utilizing minimum concentrations observed in LFG, equal magnitudes of fluorine release are observed between LFG and leachate at each site (Figure 2)—contrasting from existing estimates of PFAS mass flow from landfills.[9] Existing

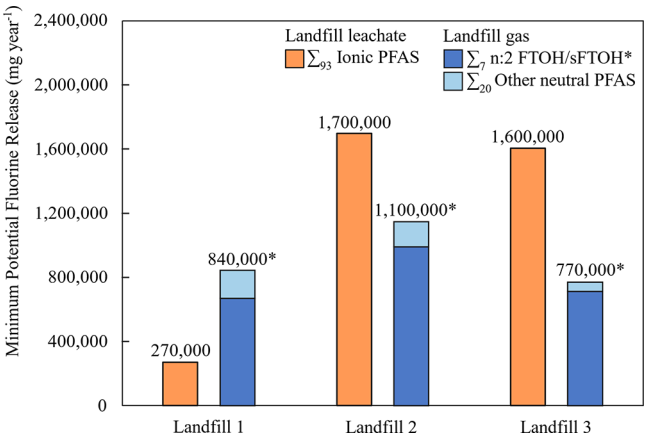


Figure 2. Annual fluorine mass release in landfill leachate versus landfill gas (LFG) from three municipal solid waste landfills in Florida. Fluorine masses in leachate are derived from ionic PFAS ($\sum_{93}$ PFAS) concentrations measured in leachate from each site (Table S-4) multiplied by the annual leachate generation volume and scaled using each detected compound's fluorine mass fraction (Tables S-8, S-9). The same methodology was applied for neutral PFAS ($\sum_{27}$ PFAS) in LFG by using the average of minimum concentrations (Table 1). Asterisked (*) values denote input FTOH/sFTOH concentrations which were above calibration levels developed for this study and therefore assumed to be at the highest calibration concentration. Consequently, these findings should be viewed as minimum values which conservatively estimate the magnitude of PFAS mobility in leachate versus LFG.

estimates, based on limited data, suggest that most PFAS mass mobilized from landfills releases through leachate (~62%).[9] However, our data from Landfill 1, showing over 76% fluorine release in LFG, along with substantial masses released by LFG in Landfills 2 and 3 (at minimum 40% and 32%, respectively), indicate that LFG may serve as an equal, likely greater, conduit of PFAS mobility from landfills than leachate, concurring with previous reactor studies on FTOH volatilization and neutral/ionic PFAS assessments of select waste materials.[17,75,76]

At least 79 to 92% of the fluorine mass in LFGs were derived from FTOH/sFTOH classes, with minimal contribution from FTOs, FTOAcs, and FTMAcs. In this conservative assessment, fluorine from LFG surpassed leachate in only Landfill 1. Although actual fluorine emission from LFG is higher than reported here, the elevated ratio of gas-to-leachate generation at Landfill 1 likely caused this difference (Table S-9). Landfill 2, the largest site, demonstrated the highest combined fluorine release from leachate and LFG, followed by Landfill 3, and then Landfill 1, corresponding to descending waste mass in place at each location.

4. IMPLICATIONS

This study provides fundamental data about neutral PFAS in LFG from MSW landfills. Unexpectedly, FTOH/sFTOH detections in LFG from this study exceeded implemented calibration levels; subsequent research should deploy shorter sampling durations. Regardless, even under more conservative assumptions these findings suggest that LFG, largely unscrutinized for PFAS, contains similar or greater magnitudes of PFAS compared to leachate, mostly attributed to midlength FTOH homologues. As landfills can be viewed as unabating PFAS repositories, the significance of LFG management in mitigating the long-term, long-range atmospheric transport of

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neutral PFAS, and subsequently derived PFAAs, cannot be understated. Unlike landfill leachate, LFG collection systems (when in place) are not fully efficient, collecting an estimated ~50−70% of generated biogases.[77] Though this is a considerable collection efficiency of biogas and presumably neutral PFAS, management of captured LFG fractions varies globally, from no treatment to degrees of carbon filtration and thermal treatment (i.e., flaring, advanced renewable natural gas technologies). Because the feasibility of PFAS destruction through thermal treatment remains unclear, research is needed to determine the treatment/removal efficiency of existing LFG management technologies. Considering the range of LFG capture efficiency, the retention and emission of neutral PFAS via fugitive emissions (i.e., migration through the waste layer) should also be examined, along with the role of landfill waste type, age, and temperature in neutral PFAS variability.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.estlett.4c00364.

Acronyms, structures, and instrumental parameters for neutral PFAS via GC-HRMS and ionic PFAS via LC-MS/MS; detailed QC information and results; and fluorine mass balance/extrapolation details and assumptions (PDF)

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Notes

The authors declare no competing financial interest.

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KD2190

LOGAN CITY COUNCIL

Technical Report

Loganholme Wastewater Treatment Plant:

Biosolids Gasification Demonstration Plant

(PBE-075)

March 2021

KD2191

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APPENDICES

Appendix A: Char Characteristics, Potential Value and Reuse Options 68

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The views expressed herein are not necessarily the views of the Australian Government, and the Australian Government does not accept responsibility for any information or advice contained herein.

Acronyms and Abbreviations

ARENA	Australia Renewable Energy Agency
BFP	Belt Filter Press
CEMS	Continuous Emission Monitoring Systems
CO	Carbon Monoxide
DM	Document management system
EA	Environmental Authority
EoW	End of waste Code
ESP	Electrostatic Precipitator
FTIR	Fourier Transform Infrared
HBCD	Hexabromocyclododecane
ID	Identification
LCC	Logan City Council
MJ	Megajoules
Mg(OH)2	Magnesium Hydroxide
NATA	National Association of Testing Authorities
NEMP	National Environmental Management Plan
NPV	Net Present Value
NOx	Nitrogen Oxide
O_2	Oxygen
OWT	Online waste tracking
PBDE	Polybrominated diphenyl esters
PFAS	Per- and poly-fluoroalkyl substances
PFFA	Total perfluoro alkyl acids
PFHxS	Perfluorohexane sulfonate
PFOA	Perfluorooctanoic acid
PFOS	Perfluorooctanesulfonic acid
POEO	Protection of the environment policies
POPs	Persistent organic pollutants
SOx	Oxidised sulfurs
STP	Sewage Treatment Plant
TOPA	Total oxidisable precursor analysis
VS	Volatile Solids
WAS	Waste Activated Sludge
WESP	Wet Electrostatic Precipitator
w/w	Weight per weight

Executive Summary

Logan City Council, via the Logan Water Partnership, conducted the *Loganholme Wastewater Treatment Plant (WWTP) Gasification Demonstration Plant* that illustrates how to treat biosolids with gasification to produce a more environmentally friendly product, called biochar, and renewable energy while reducing the operation and maintenance costs of treating biosolids.

Gasification or carbonisation of biosolids produces biochar that is classified as a Grade A product. Gasification of biosolids destroys persistent organic pollutants (POPs), micro-plastics, and nano-plastics, while retaining phosphorus in a plant-available form. The final biochar product is sterile, does not generate odours, is not subject to a restricted storage time before application, and is easier to handle than the current biosolids form. Biochar also reduces the volume of biosolids (dewatered sludge cake) by more than 90%, requiring fewer truck movements and significantly reducing disposal volumes and costs.

Logan Water developed an industry-first demonstration facility at Loganholme Wastewater Treatment Plant (WWTP) to confirm that the production of biochar from biosolids is viable technology and that associated waste streams can meet the environmental license requirements. The overall project is split into two stages:

1. Stage 1: Demonstration plant designed to showcase both the technology process of gasification on dewatered sludge and its reliability for incorporation into a WWTP to assist obtaining all relevant approvals for the full-scale gasification facility.
2. Stage 2: Full-scale gasification facility will process sludge dewatering, drying and gasification at the Loganholme WWTP, including centrifuges, paddle dryers and gasifiers.

To approve the construction of Stage 2, the demonstration plant had five key objectives to validate or measure:

1. Reliability: to demonstrate reliable operation of the facility (see Section 3: Reliability)
2. Air emissions: to measure the air emissions from the facility and confirm compliance with Queensland regulations (see Section 4: Air emissions)
3. Persistent organic pollutants (POPs): to measure the quality of the biochar product and confirm the destruction of POPs (see Section 5: Biosolids and biochar characterisation and Section 6: Emerging contaminates of concern)
4. Heat balance: to confirm the heat balance for an integrated drying facility (see Section 7: Heat balance)
5. Operating costs: to assess operating costs of the facility (see Section 8: Operating costs).

Biosolids were sourced from two WWTP in Victoria (Source 1 and Source 2) for the demonstration plant as the gasification system requires a biomass that is 90% dry. In the full-scale facility, biosolids will be dewatered through centrifuges and then dried through belt dryers before being treated in the gasifier. In the demonstration plant, the sourced biosolids were from WWTPs that have waste activated sludge (WAS) that has been dried to 90% weight per weight (w/w) and then transported to the Loganholme WWTP.

In total, 12 runs of the demonstration plant were completed between 22 January and 11 August 2020. This consisted of small 4–6 hour runs ramping up to runs that were over 100 hours. The gasification plant had over 450 hours of run time on two sources of biosolids, demonstrating its reliability.

Reliability

Reliability is a major requirement for incorporating a permanent biosolids gasification facility at Loganholme WWTP. Loganholme WWTP is Logan City Council's (LCC) largest WWTP. In the future, Beenleigh WWTP could be diverted to Loganholme WWTP, which would mean that the WWTP will treat over 97% of LCC's wastewater network. The reliability of the demonstration plant was tested over two 100-hour trial runs, successfully processing biochar, and producing heat energy throughout these periods. The trial runs complied with the air emission limits set by the Logan City Council Environmental Authority (EA) (EPPR00867913). However, the trial runs were not completed at full operational threshold, as detailed in Section 3; throughput was reduced to ensure compliance with the requirements of the EA.

Air emissions

The requirements of air emission regulations in relation to the demonstration plant relate to emissions from the 7.1m stack. The stack allowed the discharge air/gas to mix and disperse quickly into the atmosphere, as well as allowing sampling and measuring of the quality. Most of the footprint of the demonstration plant is used to purify the off gas/biogas produced and for heat recovery. The demonstration plant was required to adhere to strict conditions as part of the EA Condition LGN-A2.

Two types of air emissions were monitored:

1. Basic air emissions: monitored on a continuous basis by the continuous emission monitoring systems (CEMS) unit for every run, including parameters for oxygen (O_2), nitrous oxide (NOx), carbon monoxide (CO), and oxidised Sulphur's (SOx).
2. Full air emissions: monitored by specialist samplers and equipment at times to measure all parameters required by the EA, including particulates, heavy metals and emerging contaminates of concern, such as per- and poly-fluoroalkyl substances (PFAS).

In summary, the air emissions passed most of the limits when an external NATA-accredited laboratory analysed the results. The only exceedance of the limits was the particulates for the second 100-hour test when the throughput was increased.

The CEMS unit concluded that the demonstration plant was unreliable, with condensate causing blockages; therefore, an additional monitoring unit was used throughout the trial period. The data from this second CEMS unit shows that the air emissions can be controlled when dosing magnesium hydroxide (MgOH2) to control SOx in the air stream and urea to control NOx. The levels of CO and particulates that occurred with increased throughput required modifications to the throughput; however, these modifications introduced complications with particulates. Solutions have been identified and outlined in Section 6 to ensure this issue will be engineered out in the full-scale facility design.

Persistent organic pollutants

Persistent organic pollutants (POPs) were a major driver for this demonstration plant, with the inclusion of PFAS into the Queensland Department of Environment and Science's (DES) *End of Waste Code for Biosolids* (DES, 2020). In the *End of Waste Code for Biosolids*, target limits have been set for soil application. The measurement of other POPs, such as microplastics, are under consideration and it is anticipated they will also be incorporated in some form into the *End of Waste Code for Biosolids* (DES, 2020).

Both sources of dried biosolids came from Victoria and contained PFAS limits that would have triggered the limits set for soil application. On average, Source 1 dried biosolids had a concentration of 0.173 mg/kg of PFOS and PFHxS. Source 2 dried biosolids had a concentration of 0.367mg/kg of PFOS and PFHxS, on average. The biochar produced from the demonstration plant had a concentration of <0.005 mg/kg of PFOS and PFHxS on average or were non-detectable. This means that the dried biosolids had PFAS that would not meet soil trigger values and, once processed through the gasifier, the biochar did not contain any PFAS. This shows that through the gasification process, the PFAS compounds are made volatile and are then treated through the oxidation process.

Microplastics are an emerging contaminant of concern and were analysed as part of the demonstration plant. Source 1 dried biosolids contained 8.8 particles/gram, on average. Once processed through the gasifier, the

biochar contained 3.3 particles/gram, or a 63% reduction of this contaminant. Source 2 dried biosolids contains 8.8 particle/gram on average. Once processed through the gasifier, the biochar contained 5.06 particles/gram, or a 43% reduction of this contaminant. This source material was small and dense because it was pelletised; however, in the full-scale facility design, the belt dryers will produce a more uniform crumb rather than a pellet. Therefore, it is expected that the destruction of microplastics will be greater when processed through the belt dryer than what was achieved with the Source 1 and Source 2 dried biosolids.

Heat balance

One of the main requirements of the demonstration plant trials was to confirm that sufficient heat could be produced for the permanent plant belt dryers. The heat recovery achieved was approximately 700kW at 85% of design rate, on the Source 2 dried biosolids. Scaling to 100% of the design rate (565 kg/hr) and 80% efficiency, the heat recovery is forecast to be greater than 1.1 MW, measured against a design requirement of 1 MW.

Operating costs

The main operational consumables and costs of the gasification facility include power, potable water, and chemical dosing. During the demonstration plant trials, the following consumables were monitored and optimised, where possible:

1. Chemical dosing for SOx control was initially via hydrated lime. Hydrated lime did not perform well and was replaced with Mg(OH)2 The dosing locations and rates were optimised throughout the trials, with the optimal dosing rate was determined to be 0.4% of the weight of dried biosolids processed, or about 2.6 litres per tonne.
2. Chemical dosing for NOx control was via urea. The dosing locations and rates were optimised throughout the trials and the optimal dosing rate was determined to be around 20 litres per tonne.
3. Potable water and power usage were both as expected.

The demonstration plant confirmed that gasification of biosolids is a reliable process that can be incorporated into a WWTP. Each of the objectives set for the demonstration plant in terms of reliability, air emissions, persistent organic pollutants, heat balance and operating costs were achieved.

Therefore, it is recommended to proceed with the full-scale facility at Loganholme WWTP.

1. Introduction

Biosolids treatment and disposal is a significant component of wastewater treatment plant operation and operating costs. In Australia, most biosolids are mechanically dewatered and disposed to land (land application) under programs aimed at 'beneficial re-use'. Biosolids are a source of organic material, nitrogen, and phosphorus; however, land application often requires dewatered biosolids to be transported long distances from metropolitan areas to farming areas. Transport is costly and impacts on communities via truck movements, fuel usage, fumes and odours, noise, and high greenhouse gas emissions.

In Queensland, land application of biosolids is regulated by the Department of Environment and Science (DES). The *End of Waste Code for Biosolids* (DES, 2020) require operators to meet stabilisation quality criteria and limits on land application uses and rates.

An emerging threat to existing practices of land application of biosolids is organic micro-pollutants and persistent organic pollutants (POPs). POPs are toxic chemicals that are not biodegradable and can accumulate up the food chain and within soils and within sludge produced by WWTP processes. Examples of POPs include polybrominated diphenyl esters (PBDEs), hexabromocyclododecane (HBCD), and perfluorooctanesulfonic acid (PFOS).

Another emerging issue for the land application of biosolids is microplastic and nanoplastic pollutants. These pollutants are shed during machine washing of certain fabrics such as those used to produce active wear. Plastic microfibres from the fabric enter the wastewater network. Where sewage is treated by activated sludge processes, the microfibres become enmeshed in the biosolids. These microfibres are not destroyed by anaerobic or aerobic digestion biosolid treatment processes. Consequently, there are concerns about the potential for these pollutants to enter the terrestrial food chain when biosolids are applied to land used for agricultural production.

While POPs and microplastic and nanoplastic pollutants are present in Australian biosolids, they are not listed as contaminants in Australian biosolids guidelines. In Europe, 50% of biosolids do not meet POPs limits set for land application. Switzerland and Germany have banned land application of biosolids, with incineration a more common practice of disposal (European Commission, 2008) (German Federal Environment Ministry, 2016). Many Australian biosolids would not meet the limits established in Europe. The Australian water industry is also not prepared for this threat to the land application of biosolids.

Logan City Council, via the Logan Water Partnership, has been investigating how to better treat biosolids to produce biochar a more environmentally friendly product, while reducing the operation and maintenance costs of treating biosolids. This work recommended treatment via gasification, with other upstream processes to obtain a biochar product and produce a renewable energy.

Thermally treating biosolids produces biochar, a Grade A pathogen product that meets the criteria set in the *End of Waste Code for Biosolids* (DES, 2020). Thermal treatment of biosolids destroys POPs, micro-plastics, and nano-plastics, while retaining phosphorus in a plant-available form. The final biochar product is sterile, does not generate odours, is not subject to a restricted storage time before application, and is easier to handle than the current biosolids form. Biochar also reduces the volume of biosolids (dewatered sludge cake) by more than 90%, requiring fewer truck movements and significantly reducing disposal volumes and costs.

Logan Water's industry-first demonstration plant confirmed that the production of biochar from biosolids is a viable technology and associated waste streams can meet the environmental regulations. Funding was made available for the Loganholme Wastewater Treatment Plant: Demonstration Biosolids Gasification Facility (demonstration plant) and the Loganholme WWTP Gasification Facility (full-scale).

1.1 Objectives

Five key objectives were set for the demonstration plant:

1. Reliability: to demonstrate reliable equipment operation
2. Air emissions: to measure the air emissions from the facility and confirm compliance with Queensland regulations
3. Persistent organic pollutants (POP): to measure the quality of the biochar product and confirm the destruction of POP
4. Heat balance: to confirm the heat balance for an integrated drying facility
5. Operating costs: to assess operating costs of the facility.

1.2 Components of the demonstration plant

Because the demonstration plant is a first of its kind in Australia, it was critical to demonstrate proof of concept using a sole supply of biosolids not a blend and therefore be able to move forward with the full-scale Loganholme WWTP Gasification facility.

The demonstration plant consists of eight key components that were part of Pyrocal's scope: dried biosolids feed system, hearth, biochar system, thermal oxidisers, heat recovery system, scrubber, air steam release and control room. Figure 1-1 illustrates these key components of the demonstration plant.

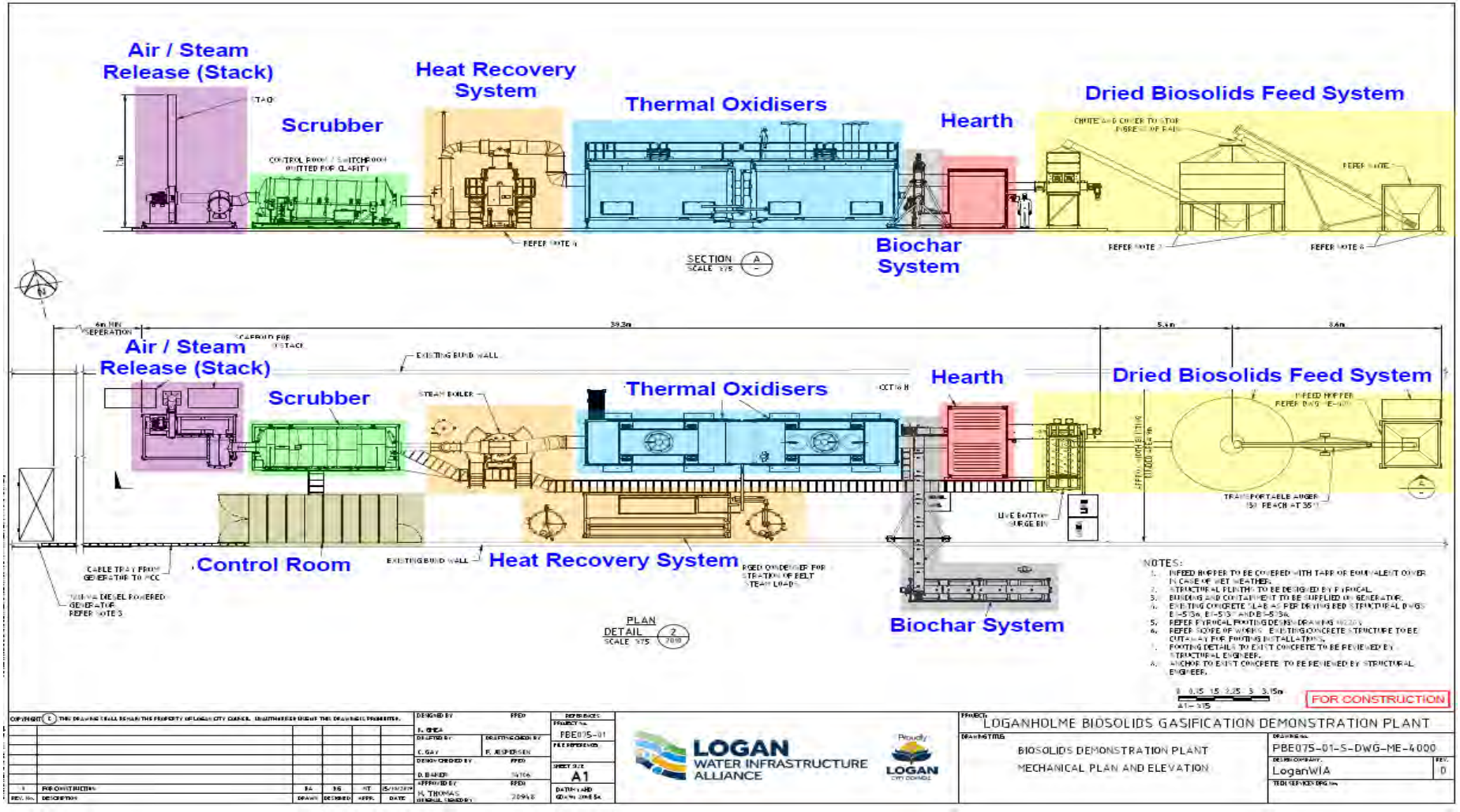


Figure 1-1: Key components of the Loganholme Wastewater Treatment Plant: Biosolids Gasification Demonstration Plant

2. Source of Biosolids

Biosolids were sourced from Victoria for the demonstration plant as the gasification hearth requires a biomass that is 90% dry. In the full-scale facility, biosolids will be dewatered through centrifuges and then dried through the belt dryers before being treated in the gasifier. In the demonstration plant, the sourced biosolids were from WWTPs that have waste-activated sludge (WAS) that has been dried to 90% w/w and then were transported to the Loganholme WWTP.

Two types of biosolids were processed in the demonstration plant. To de-identify the sources of these biosolids, throughout this chapter they are referred to as Source 1 and Source 2. This chapter details the biosolids from each source and outlines the approval process to move biosolids from Victoria to Queensland. The biochar produced that is available for beneficial reuse is also quantified.

Certain parameters of biosolids were required for the demonstration plant, as outlined in the Dryer Technology Review (LoganWIA, 2020; DT0007). These parameters include particle size, dust ratio, moisture content and volatile solids:

- The ideal particle size range for the carbonisation process gasifier unit is 3–10 mm
- Both regular and irregular particle shapes are acceptable
- Larger particles are also acceptable
- Concentration of fines/dust must comply with the specification for the carbonisation equipment that less than 2.5%, by mass, of dried particles must have an aerodynamic equivalent diameter below 3 mm.

2.1 Source 1 biosolids

The Source 1 facility uses a thermal sludge dryer to process waste. The sludge goes through a process of centrifuge, like the planned Loganholme WWTP full-scale design, through to a feed screw directly into the thermal dryer, which has a gas fired burner (up to 600 °C) that tumbles the product. The end-product is 90% dry biosolids. The biosolid are cooled in a water-cooled auger to 40 °C.

However, the biosolids from Source 1 are processed through a third-party and the batch of ~60T received was several months old and had degraded with a lower calorific value of volatile solid (VS) around 73% and moisture content of 85% dry. The particle size was less than 5mm, and this meant that the hearth had to be retrofitted to receive smaller particles, which in turn affected the airflow. Figure 2-1 shows the Source 1 biosolids.



Figure 2-1: Source 1 biosolids

Although the batch that was received did not fit the specific design parameters for particle size set for the gasification unit, these biosolids were still able to be processed and provide assurance that the hearth can be adapted to fit the performance of the dryer units. This created confidence that although technical specifications for the dryer have been set, slight variations to particle size and distribution can be adapted so that the gasification unit can process slightly irregular material.

2.2 Source 2 biosolids

Source 2 biosolids uses a drum dryer to produce a biosolid pellet that is >88% dry. The sludge is dewatered through belt presses and then is brushed on a series of heated revolving turntables. The heat treatment kills all micro-organisms, resulting in a small, pelletised product that is safe to handle. As shown in Figure 2-2, the biosolids were pellet sizes of around 8–10 mm, with a VS 81% and greater than 90% dry.



Figure 2-2: Source 2 biosolids—pelletised

The Source 2 biosolids granules were approximately ten times the size of the Source 1 biosolids. The size of the dry crumb produced by the belt dryers in the full-scale facility is expected to be between the size of the biosolids from Source 1 and Source 2, and similar to the Source 2 biosolids in terms of volatile solids and approximately 10% moisture. Table 2-1 shows the average calorific value of the biosolids from Source 2.

Table 2-1: Average calorific value of biosolids from Source 2

Calorific value	Unit	Source 2
Net	MJ/kg dry matte (DM)	19.26
Gross	MJ/kg (calc)	2.23

2.3 Loganholme biosolids

In the full-scale facility design, strict criteria have been set for the dryers, outlined in the Dryer Technology Review (LoganWIA, 2020; DT0007) report. However, the Eliquo Stultz low-temperature dryer is the preferred supplier because it can produce the biosolid to 88% w/w solids at a particle size between 3–10mm. Also, a low- temperature dryer allows hot water to be used rather than thermal oil, providing improved efficiency in the heat–energy balance and reduced long-term operational costs. Because the process in the Loganholme gasification facility will be continuous (8000 hours per year), there will not be issues with the source material degrading during storage as it will be immediately transferred between the belt dryers and gasification unit.

As noted, the gasifier works optimally with biosolids ranging from 3–10mm due to the design of the rotating plates within the hearth and the ability to gain as much energy as possible from the sludge (greater than 70% volatile solids). The design has been optimised from paddle dryers to belt dryers due to the high dust fraction produced by paddle dryers.

The calorific value or net energy value of biosolids from Loganholme and Beenleigh WWTPs are shown in Table 2-2. This compares favourably with the calorific value of the biosolids from Source 2.

Table 2-2: Average calorific value of biosolids from Loganholme and Beenleigh WWTPs

Calorific value	Unit	Loganholme WWTP	Beenleigh WWTP
Net	MJ/kg dry matter (DM)	18.80	18.4
Gross	MJ/kg (calc)	2.25	2.20

2.4 Approvals process for movement of biosolids

Transporting biosolids from Victoria has a lengthy approvals process, which was heightened with the COVID-19 pandemic. Figure 2-3 provides an overview of the process.

Loganholme WWTP

Environmental approvals issued by Department of Environment and Science (DES) (Queensland Environmental Protection Agency).

Loganholme WWTP has an Environmental Approval (EA EPP00867913).

The environmental authority includes ERA633 to conduct wastewater activity.

The license includes the prescribed ERA61-Waste incineration and thermal treatment.

Under ERA61, Loganholme WWTP can accept regulated waste, such as biosolids.

Consignment Authority

Department of Science—Waste Track team issue consignment authorities for prescribed waste such as biosolids.

Movement of biosolids interstate requires a consignment authority.

The Waste Track team check that:

- the waste transporter holds the appropriate license to transport regulated waste
- the waste generator material is not going to cause environmental harm—for example they will check the biosolids' characteristics are within the PFAS NEMP 2.0
- the waste receiver (in this case Loganholme WWTP) is allowed to receive the waste.

Waste track then approves the consignment authority and LCC is issued with a consignment authorisation number.

Statutory Approval

Statutory Approval Document for the transport of waste from Victoria is issued by the Victorian EPA

LCC applies to the Victorian EPA for approval for the movement of prescribed industrial waste from Victoria (F1007), providing the Queensland DES Consignment Authority number

Victoria EPA provide an R26 Assessment Report—the assessment is based on the authority being satisfied that the proposed transport of the prescribed waste is for the purposes of reuse or recycling in accordance with the principal waste hierarchy

Victoria EPA then provide an approval for the movement of non-liquid prescribed industrial waste, issued to LCC.

Figure 2-3: Approvals process for transporting biosolids interstate

Until both approvals from Queensland DES and Victorian EPA were granted, the biosolids could not be moved between states. Because the biosolids moved through NSW, the NSW EPA was informed.

> *As NSW is only a transit state, NSW should be advised of the movement of trackable waste by the receiving jurisdiction when the consignment authorisation is generated. The NSW EPA waste tracking system (online waste tracking (OWT) system) is not used to track the waste. A waste transport certificate is used to track each load and would capture that the waste has passed through NSW as a transit state.*

2.5 COVID-19

During the Phase 2 of the demonstration plant, when biosolids from Source 2 were tested, COVID-19 impacted the project, especially the freight component. Transporting biosolids from Victoria to Queensland became increasingly difficult from July, with several restrictions put in place. To transport freight from Victoria, the following documentation had to be obtained:

1. **NSW Border Pass:** NSW temporarily imposed border restrictions with Victoria. Only persons authorised under the Public Health Order could enter NSW if they had been in Victoria in the previous 14 days
2. **Queensland Border Pass:** Entry to Queensland was temporarily restricted under the Border Restrictions Direction. Personnel were only permitted to enter Queensland in accordance with the Border Restrictions Direction (No. 8), which required additional documentation and a completed Queensland Border Declaration Pass
3. **Letter of Exemption:** Letters of exemption were sent to the company transporting the biosolids stating that the transport of the material was an ‘essential activity’ as per the Queensland Border Directive No.8.

A journey plan was developed for each run of biosolids from Victoria and protocols were put in place at Loganholme WWTP to ensure minimum contact with drivers and written records of close contacts were maintained.

During this time, four runs of biosolids (160 t) were received at the Loganholme WWTP without issues or delays.

3. Reliability

The demonstration plant ran continuously for a series of shorter tests, including 6-hour, 24-hour and 48-hour tests, as well as two 100-hour tests. Stable reliability of the demonstration plant and the associated equipment was expected; however, some reliability issues were encountered.

During commissioning, a number of issues were experienced, and some modifications were required to optimise the operation to match the dried biosolids that were being used for testing, including modifications to cater for the different properties of the biosolids received from the two sources.

Table 3-1 outlines the reliability lessons learned, the implications for the permanent biosolids gasification facility and the modifications undertaken in the preparation phase and during the demonstration plant trials.

Table 3-1: Plant performance and operations and lessons learnt

Component/performance parameter	Findings	Implications for the permanent biosolids gasification facility
Bulk feed handling	Bulk handling via a modified grain silo was abandoned early in the trials due to blockages. A steeper-angled transfer hopper was found to be more suitable. Dust release during the transfer of biosolids to the feed metering system was a concern to consider for the permanent plant.	Control of fugitive dust release in the bulk feed handling systems will need to be minimised and managed. Minimising product handling and storage is a constraint of the design, to reduce the generation of fines in the feed. Handling and road transport of biosolids over 3000 km for the demonstration plant trial increased the dust fraction in the already partly degraded material.
Particle size	Source 1: particle size <5 mm Source 2: particle size 8–10 mm	The gasification unit can process particle sizes that vary against the optimum range of 3–7 mm. It does require a change to the hearth and can affect the heat exchange, but the process is robust enough to accommodate changes in particle size and, therefore, reduces the risk for the belt dryers to meet this requirement 100% of the time.
Oxidisers	The demonstration plant has two oxidisers to increase the retention time as part of destruction of POPs such as PFAS. To determine if the two oxidisers were necessary, air emissions were measured between the oxidisers, which proved that the second oxidiser was not necessary.	The second oxidiser was contributing more to heat loss than heat gain. As such, it provided additional residence time for the flue gases, but otherwise was not obviously performing a useful function.
Feed metering	The drop-out hatch burn-back prevention system proved to be unreliable (false trips) and was bypassed before the trials commenced. The feed metering system required minor modification to deal with the fine free-flowing biosolids that were initially used for the trials. Some logic changes were also required to alter the way the live bottom screws fed the metering screw, to avoid	Pyrocal has developed a new live bottom feed bin that uses a slow-moving agitator and metering flap, rather than multiple floor-mounted screws. This system also has a burn-back prevention arrangement that does not use a drop-out hatch. The system also includes online mass flow measurement.

	over filling. Once these changes were put in place, the feed system achieved a typical reliability of 98%. On rare occasions, the live bottom screws experienced over-torque protection events, causing temporary disruption to the feed and the need for operator intervention.	The revised system is being evaluated by Pyrocal, so that an optimised system is available for the permanent plant that incorporates all the lessons learned from this demonstration plant.
Hearth	Slagging, which is the partial or complete melting of ash species in contact with equipment surfaces, is of concern in the gasification, combustion, or incineration of fossil fuels, biomass, and wastes. Slags can cause fuel agglomeration, damaging ash deposits (e.g. clinker) or corrosion of equipment, which shortens the achievable run time between maintenance shutdowns. Pyrocal's rotary hearth and oxidiser are operated at temperatures well below the ash softening point for most feedstocks, typically 500–800 °C for the hearth and 750–900 °C for the oxidiser, relative to typical ash softening points of 1000–1400 °C. Where a feedstock has an exceptionally low ash softening point, the positive rotary action of the rake system in the hearth tends limit any the build-up. Flux agents, such as lime, are also used by Pyrocal to raise the ash softening point of problematic feeds, such as cotton gin trash. In the case of biosolids, lime dosing into the feed is used to control hot corrosion caused by the presence of Sulphur in the feed. Lime dosing also assists to raise the ash softening point, although the softening point is reported to be 980–1100 °C for sewage-derived biomass.	Fouling of duct work is a common concern for updraft gasifiers, as they are known to generate high concentrations of soot and tars (condensates with a condensation point as high as 400 °C). Pyrocal's systems use a close coupling between the hearth and the oxidiser, which ensures that the off-gas released from the hearth is maintained at temperatures exceeding 600 °C until they enter the oxidiser, where the tars are mixed with air and burned off at temperatures in the range of 750–900 °C and 2–4 seconds residence time. This leaves a hot flue gas that contains a moderate concentration of non-sticky carbon/ash dust to pass through to the downstream heat recovery system. This dust is periodically blown off heat exchange surfaces in a soot- blowing process. Soot and tar build-up in the hearth became apparent during the two longest test runs. This restricted the primary air manifolds, which significantly reduced the capacity of the equipment. The soot and tar build-up readily burned off during a pause in feed or shutdown. Inspections revealed that the hearth and associated equipment remained in good order throughout the tests. The dry crumb from the permanent plant is intended to have a median particle size greater than 6 mm, so that the feed is compatible with the standard hearth decks. For soot and tar build-up, the primary control measure will be to ensure that a hearth-operating temperature exceeding 600 °C is maintained. The permanent system will also include logic and a hearth primary air pressure sensor to detect fouling of the primary air manifolds, to enable a scheduled burn-off sequence to be triggered. The net result is expected to be 30 minutes of lost production every 50–80 hours.
Char Thermal combustion of char (4-day rule)	The char handling and quench system performed reliably for the duration of the tests, except for two incidents where the base of the char transfer conveyor flooded with quench fluid, causing char to float and back up. No quench failures or spontaneous combustion events were experienced during the trials.	Pyrocal's transfer conveyor design will be amended to ensure quench water flooding cannot occur in the permanent plant.
Radiant heat exchanger	The radiant heat exchanger in the shell of the thermal oxidiser removed excessive heat when the Source 1 biosolids were trialed. This exchanger performed according to design on the Source 2 biosolids feed and recovered approximately 360kW of heat at 85% of design rate. The design intent for this first	No changes are required for the permanent plant, as the desired 400 kW of recovery at the nominal design rate has been demonstrated.

	stage heat recovery is 400 kW at a nominal design rate (565 kg/hr).	
Heat recovery steam boiler	The boiler performance was hindered by a relatively low inlet temperature (i.e. ~600 °C rather than the intended 800 °C and well below typical values of 1200 °C during conventional operation with a fossil fuel burner). In addition, fouling of the heat transfer surfaces was evident by the progressive increase in exhaust temperature from 205–300 °C during extended operation. This performance was typically restored via soot blowing operations, which could only be performed on shutdown or start-up. Hence, the typical heat recovery efficiency of this unit was 51–66% compared to an intended value of greater than 80%. Despite this, the heat recovery achieved by the boiler was approximately 700kW at 85% of design rate, on the Source 2 biosolids. Scaling to 100% of the design rate (565 kg/hr) and at 80% efficiency, the heat recovery is forecast to be greater than 1.1 MW, compared to a design requirement of 1 MW. Attempts to operate the system above 85% of the design feed rate led to the heat rejection system starting to reach capacity, which created condensate flow surging, resulting in signal noise in the heat recovery measurement.	The heat recovery steam generator will be replaced with a heat recovery water heating unit to better suit the requirements of the permanent plant. The proposed unit has in-built soot blowing arrangements (online compressed air type). The proposed heat exchanger is also designed to achieve a heat recovery exceeding 80% at the expected flue gas inlet temperatures (600–800 °C). Pyrocal recommends the addition of a downstream condensing heat exchanger (200 kW rating) to ensure that the overall heat recovery is achieved with a suitable design margin.
Scrubber	The absorption scrubber was upgraded with a venturi stage after the initial emissions tests, when it became apparent that the dust loads could not be catered for. This improved the emissions performance, at the expense of limiting the ID fan flow capacity by 15%, due to increased pressure differential. This directly impacted on the achievable feed rate of biosolids. As a result of very high dust loads, it was necessary to drain liquid from the scrubber, back into the treatment plant, on a continuous basis.	Pyrocal estimates that the PM10 and PM2.5 dust loading from the biosolids were four to ten-fold greater than that typical of plant derived biomass. Therefore, the permanent plant design has been amended to allow for a high pressure drop venturi and a downstream PM2.5 capture device (refer to the filter section in this table) and upgraded capacity ID fan.
Filter	The barrier type filter installed on the demonstration plant proved to be unsuitable as it fouled too rapidly in service.	Pyrocal recommends implementing a wet electrostatic precipitator (WESP) on the permanent unit, to cater for the high PM2.5 loads that the biosolids release into the flue gas. The benefit of this approach is improved reliability, lower operating costs and complexity compared to a barrier filter, as well as reduced ID fan pressure differential relative to barrier filters.

Continuous Emissions Monitoring Systems (CEMS)	The online CEMS was installed as part of Pyrocal's scope. The unit that was installed by Aquagas was a MAMOS CEMS designed for online measurement of O, CO_2, SO_2, NO, NO_2. NOx and O_2 as well as stack gas temperature and velocity. However, this unit had several issues including blockages due to the high moisture content. The internal fix was to install a gravity head drain instead of the peristatic drain pump. This allowed a continuous monitoring, although this solution would not be suitable for the full-scale facility.	The appropriate CEMS monitoring system for this type of analysis would be an extractive system or Fourier Transform Infrared (FTIR). FTIRs can operate in a high moisture environment because every component of the system is heated above dew point, including the analyser itself; therefore, a condenser is not required. This recommendation is included in the scope of the full-scale facility.
Processing rate	The maximum processing rate achieved during the trials was 600 kg/hr. The absorption scrubber was upgraded with a venturi stage when it became apparent that the dust loads could not be catered for. This improved the emissions performance at the expense of increased pressure differential and reduced fan flow capacity. This addition of the venturi directly impacted on the achievable feed rate of biosolids.	Modifications to the thermal oxidiser and upgrading of the ID fan pressure and low capacity are planned to ensure that a capacity of 650 kg/hr is achievable.
Particulates	Venturi was added after running 2 of the full air emissions to mitigate particulates. When the threshold was increased, the venturi and fans could not cope and increased the particulates in the air stream. Another run was planned for September with the addition of a cyclone and modified oxidiser. This is so that the threshold can be optimised at the same time to obtain reliable results on dosing and chemical rates while still being within the limits set for air emissions and particulates.	In the full-scale facility, the issues surrounding the particulates has been engineered out with the inclusion of an ESP into Pyrocal's scope. An ESP is a filtration device that removes fine particles, like dust and smoke, from a flowing gas using the force of an induced electrostatic charge, minimally impeding the flow of gases through the unit.
Carbon monoxide (CO) Carbon monoxide exceedances related to particulates	The performance with respect to carbon monoxide slip vs excess O_2 and visual inspection of the swirl pattern suggested that turbulent mixing was less than desired. Therefore, a swirl vane was installed at the oxidiser inlet and a directional nozzle implemented for the secondary air inlet. This reduced the carbon monoxide slip; however, it was evident that further reduction in short circuiting was necessary as higher feed rates were required.	It is proposed to include a deflector baffle before the oxidiser outlet to reduce the ability for flue gas to short circuit from the inlet to outlet. Improved turbulence in the oxidiser is expected to reduce the residual oxygen concentration required to achieve the desired destruction in the thermal oxidiser. This will, in turn, reduce the necessary ID fan flow demand. Both solutions worked in the October trial and will be replicated on the full-scale facility.

4. Air Emissions

4.1 Background

The air emissions from the gasification plant will be substantially less than a typical incineration plant because the carbonisation process is not destroying 100% of the mass, but rather removing the volatile fraction and treating that fraction, while carbonising the non-volatile fraction. The volatile fraction represents 40–60% of the total dry mass. Consequently, imposing best practice emission limits for incinerators on the plant for the demonstration plant was sufficient to protect environmental values while actual release data is gathered.

The gas residence time and temperature in the thermal oxidiser is 2 seconds at 800–850 °C. This residence time and temperature has been demonstrated in previous tests on a range of biomasses, sufficient to exceed 99% destruction of the organic matter presented to the oxidiser. The percentage destruction or alteration of halogenated species is not known and measuring this percentage was an objective of the demonstration plant.

After the thermal oxidiser, the next air emission control unit is a venturi through to a wet scrubber. The scrubber is a spray absorber with extended residence time for particulate-seeded condensation capture of the PM10 fraction. This allows rapid quenching of the gas to avoid the dioxin reformation temperature range. The gas/liquid contact allows acid gases and other species to be physically or chemically captured (Bouzaza, Laplanche, & Marsteau, 2003). Fine mist that carries over in the exit gas is captured in a wire mesh mist eliminator and recirculated to the scrubber sump.

The accumulated solids from the scrubber are discharged to the biochar from the carbonisation stage, to perform the quench duty. This results in no liquid emissions from the system.

In the first phases of the trial, lime was added to the biosolids feed and to the scrubber as many of the potential air pollutants can be are sequestered as calcium salts. This includes acid gas species such as HCl, HF, H2SO4, metals such as Hg, As, Cd, Pb, Zn, Cu and Cr and many of the halogenated species (Busca & Pistarino, 2003) (Dias, Alvim-Ferraz, Almeida , Rivera-Utrilla, & Sanchez-Polo, 2007) (Kailiva & Smith, 1983).

A change to Mg (OH)2 was made (5/05/2020) for the SOx control because although the lime dosing can hold the SOx concentration at around 30–150 mg/Nm^3 (not oxygen corrected). The lime contributes to fouling in the wet scrubber, so it is prudent not to dose any more than necessary to protect the steel in the hot zones. Therefore, it was determined to use Mg (OH)2 dosing at the wet scrubber for mitigating SOx in the air emissions. The results are shown in Section 4.6.

In the original design, a moisture-tolerant, 5-micron-pleated filter element (barrier filter) followed the scrubber and captured the residual PM5 matter before the flue gases were drawn into an induced draft fan and discharged to the atmosphere. Experience proves that the action of the scrubber to remove the bulk of the particulate matter allows the barrier filter to be used without incurring impractical service intervals.

However, the Source 2 biosolids were extremely dusty materials and demonstrated that although the filters worked, they would have to be cleaned more often than expected compared to other types of materials processed through the gasification facility. Therefore, the filter element was removed, and a venturi device added at the entrance of the scrubber (12/05/2020). This configuration was used for all tests after this date, including the 100-hour reliability runs.

4.2 Regulation requirements

The regulation requirements mostly relate to the environmental harm perceived from the air emissions via the stack. Therefore, when designing the full-scale facility, 75% of the facility will be focused on purifying the biogas produced, with the dual purpose of measuring the heat recovery. The demonstration plant was run under strict conditions imposed as part of the Environmental Authority (EA) (EPPR00867913) Condition LGN-A2: Contaminants must only be released from the Biosolids Gasification Demonstration Plant to air via point release sources in accordance with Table 4—Point Source Air Release Limits and associated monitoring requirements.

Table 4-1 is the point source air release limits set for the demonstration plant.

Table 4-1: Point source air release limits for the demonstration plant

Contaminant	NSW EPA Protection of the environment policies (POEO) Regulation (see Note 2)	European Commission (2000/76/EC) (see Note 3)	Target limits for the demonstration plant
Total solid particulate	50 mg/Nm^3 (dry)	30 mg/Nm^3 (dry)	30 mg/Nm^3 (dry) at 11% O_2
Carbon monoxide (CO)	125 mg/Nm^3 (dry)	-	125 mg/Nm^3 (dry) 11% O_2
Oxides of nitrogen as NO_2	350 mg/Nm^3 (dry)	400 mg/Nm^3 (dry)	400 mg/Nm^3 (dry) at 11% O_2
Sulphur dioxide (SO_2)	-	200 mg/Nm^3 (dry)	200 mg/Nm^3 (dry) at 11% O_2
Hydrogen chloride (HCl)	-	60 mg/Nm^3 (dry)	60 mg/Nm^3 (dry) at 11 % O_2
Total fluoride (as HF)	50 mg/Nm^3 (dry)	4 mg/Nm^3 (dry)	4 mg/Nm^3 (dry) at 11 % O_2
Total volatile organic compounds	20 mg/Nm^3 (dry)	20 mg/Nm^3 (dry)	20 mg/Nm^3 (dry) at 11% O_2
Cadmium and its compounds	0.2 mg/Nm^3 (dry)	0.05 mg/Nm^3 (dry)	0.05 mg/Nm^3 (dry) at 11% O_2
Mercury and its compounds	0.2 mg/Nm^3 (dry)	0.05 mg/Nm^3 (dry)	0.05 mg/Nm^3 (dry) at 11% O_2
Total heavy metals (see Note 1)	1 mg/Nm^3 (dry)	0.5 mg/Nm^3 (dry)	0.5 mg/Nm^3 (dry) at 11% O_2
Dioxins and furans (I-TEQ for PCDDs and PCDFs, including half LOD)	0.1 ng/Sm^3 (dry) at 11% O_2	0.1 ng/Sm^3 (dry)	0.1 ng I-TEQ/Nm3 (dry) at 11 % O_2
Polycyclic aromatic hydrocarbons (PAH)	-	-	-
PFAS extended suite containing 28 compounds (see Note 4)	-	-	-

Table notes:

Note 1: Total heavy metals limit is for the total of antimony, arsenic, cadmium, lead, mercury, beryllium, chromium, cobalt, manganese, nickel, selenium, and vanadium and their compounds.

Note 2: The concentrations limits for solid waste incineration refer to 7% O_2, except dioxins that refers to 11% O_2.

Note 3: All concentrations limits (100% compliance) refer to 11% O_2 reference level and average over the sample period of 30 minutes.

Note 4: The samples should be analysed to quantify the PFAS, by applying the total oxidisable precursor analysis (TOPA), to analyse the PFAS extended suite of 28 compounds. To determine PFAS concentrations, at least the PFAS extended suite containing 28 compounds should be analysed from the following sources:

- sewage sludge used in the demonstration plant
- stack emissions during the selected demonstration plant where emissions are expected to be maximum.

4.3 Status of air pollution control equipment used for the demonstration plant

The online CEMS was installed as part of Pyrocal's scope. The unit installed by Aquagas was a MAMOS CEMS designed for online measurement of O, CO2, SO2, NO, NO2. NOx and O2 as well as stack gas temperature and velocity. However, this unit had several issues including blockages due to the high moisture content and a gravity-head drain in place of the peristatic drain pump. This allowed continuous monitoring, although labour intensive and would not be suitable for the full-scale facility.

The appropriate CEMS monitoring system for this type of analysis would be an extractive system or FTIR as both systems can withstand a high-moisture environment because every component of the system is heated above dew point, including the analyser; therefore, a condenser is not required. This approach is included in the scope of the full-scale facility.

4.4 Stack emission results

Two types of air emissions analysis were undertaken for the demonstration plant:

1. Basic air emissions: This analysis is performed by the CEMS unit for every run and including parameters: O2, NOx, CO, and SOx. All data is stored, but a summary is in Section 4.6.
2. Full air emissions: This analysis includes all parameters including particulates, heavy metals and emerging contaminates of concern, such as PFAS. Three runs were performed, and a summary is in Section 4.6 and full reports supplied by Assured Environmental are included in Appendix D.

Stack monitoring was conducted by Assured Environmental, an external third party. This approach ensured that all air emission stack monitoring was conducted by an experienced provider holding current National Association of Testing Authorities (NATA) accreditation. Due to the CEMS unit having issues, for example blockages, some of the results for the continuous monitoring were also undertaken by Assured Environmental. This approach ensured that the demonstration plant was meeting all required parameters, not causing environmental harm, and meeting all conditions stipulated in the license to operate issued by DES.

4.5 Timeline

Table 4-2 lists of all the runs that were performed, and the analysis undertaken.

Table 4-2: Timeline of runs of the demonstration plant

		Date	Comment
4–6-hour run			
Run 1	SOx, NOx, and heat balance	22/01/2020	CEMS unit
Run 2	SOx, NOx, and heat balance	04/03/2020	CEMS unit
Run 3	SOx, NOx, and heat balance	17/03/2020	CEMS unit
6-hour run			
Run 4	SOx, NOx, and heat balance	23/03/2020	CEMS unit
Run 5	SOx, NOx, and heat balance	02/04/2020	CEMS unit
Run 6	SOx, NOx, and heat balance	11/06/2020	CEMS unit
Run 7	SOx, NOx, and heat balance	11/08/2020	CEMS unit
24-hour run			
Run 8	SOx, NOx, and heat balance	10/08/2020	Assured Environmental
Run 9	Full air emissions, SOx, and NOx	02/04/2020	Assured Environmental—full air emissions data
48-hour run			
Run 10	SOx, NOx, and heat balance	21/07/2020	CEMS unit
100-hour run			
Run 11	Full air emissions, SOx, NOx, and heat balance	22/7/2020	Assured Environmental—full air emissions data
Run 12	SOx, NOx, and heat balance	27/07/2020	CEMS Unit supplied by Assured Environmental

4.6 Summary of continuous stack monitoring results.

Figure 4-1 to Figure 4-13 provide an overview of each of the runs performed. The data shown is the chemical parameters for the basic air emissions: NOx, SOx, and CO. Each run highlights the set limit in red.

This section presents the data from the online CEMS unit. As discussed, this unit had issues and was found not to be fit-for-purpose and therefore will be replaced for the full-scale facility; however, it does give an overall perspective of the range of each analyte: NOx, Sox and CO.

Figure 4-1 was one of the first runs completed using the Source 1 biosolids after the hearth had been fitted for the difference in the material size. However, this run was also completed when the throughput was not at full capacity; rather, it was gradually increased starting at 320kg/hour. This run illustrates that the gasification unit can meet the criteria set in the license limits.

With regards to start-up and shutdowns, there will be a spike in air emissions until the system is stable (~1 hour) and this should be factored for license limits. However, the full-scale facility is being designed to run with fewer shutdowns limiting the start-up and shutdowns and to integrate with the heat energy balance, keeping the continuous loop. Therefore, this spike would not be a daily occurrence, but rather once every few couple of weeks.

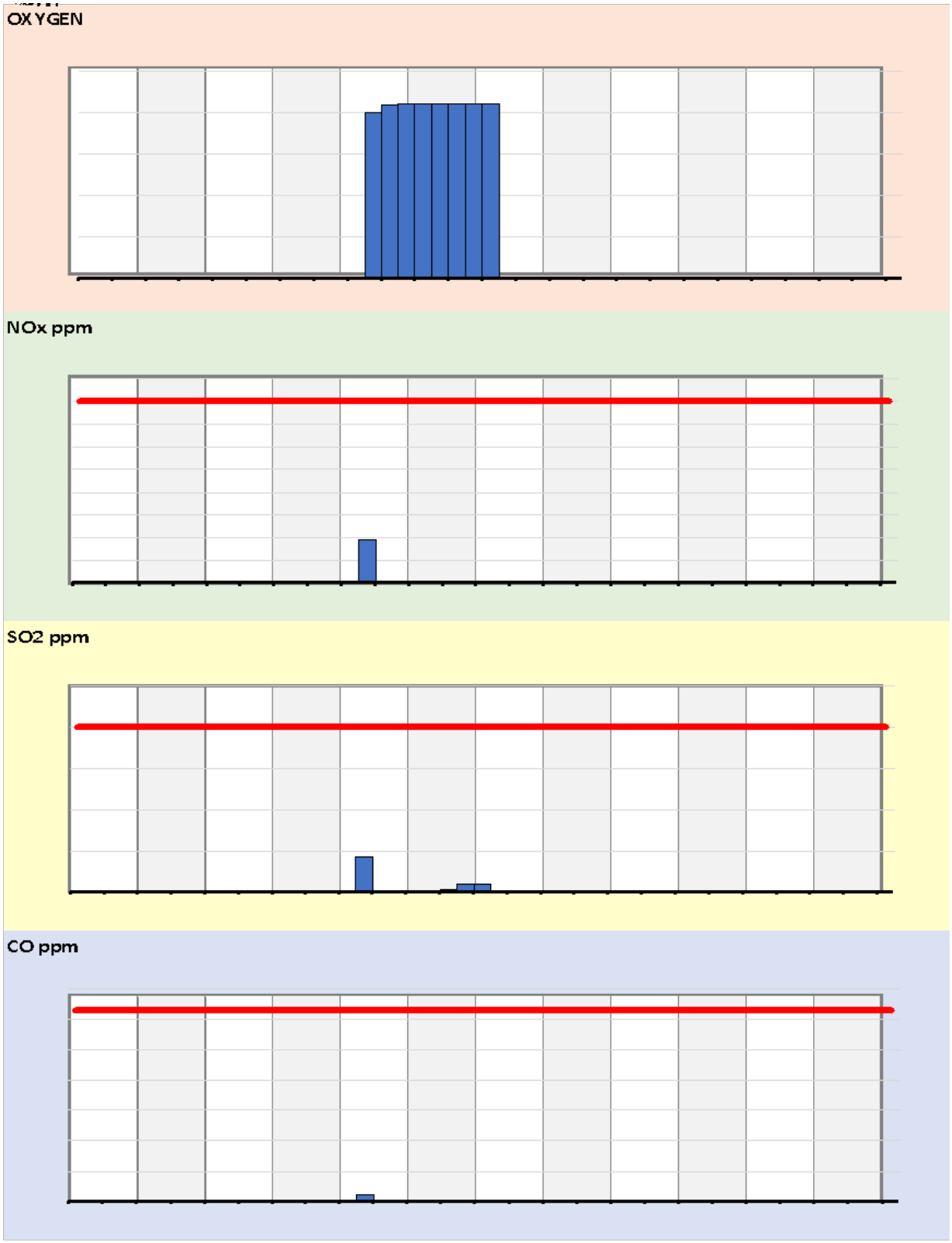


Figure 4-1: Run 1: 4–6 hour run (22/01/2020)

Run 2 (Figure 4-2) was completed using Source 1 biosolids and again illustrates that the license limits can be achieved on biosolids through a gasification system. There was an apparent spike in SOx concentration levels for this run; however, this was the result of a blockage in the CEMS unit.

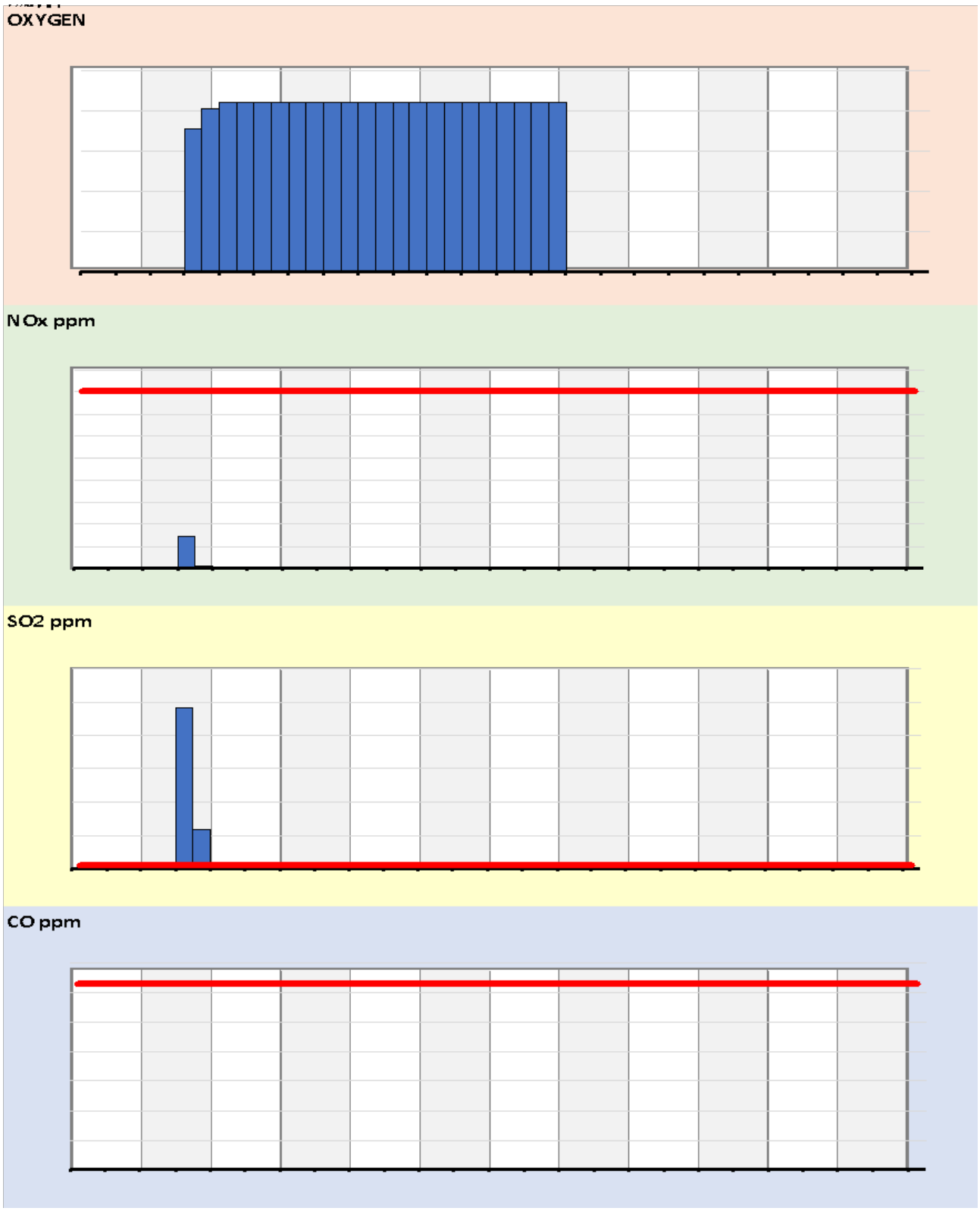


Figure 4-2: Run 2: 4–6 hour run (04/03/2020)

Run 3 (Figure 4-3) was completed using Source 1 biosolids and illustrates that the license limits can be achieved on biosolids through a gasification system. There was a spike in SOx, firstly on start-up, which is to be expected, and then an hour later, which was brought under control. This result is discussed in the lessons learnt in Table 3-1 and is one of the reasons for the change from lime dosing to MgOH because the lime dosing system consistently had blockages and was not the best agent for mitigating SOx.

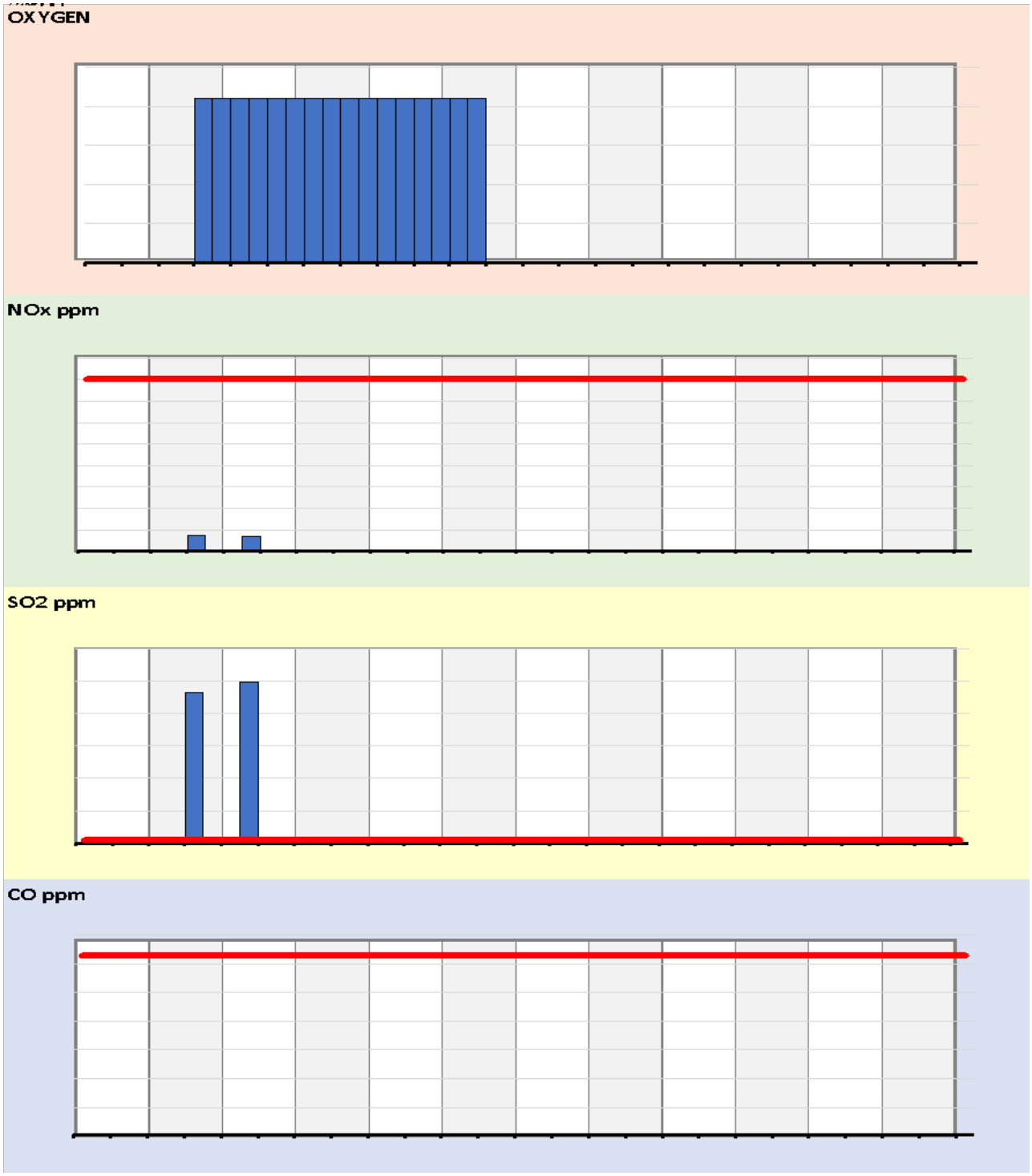


Figure 4-3: Run 3: 4–6 hour run (17/03/2020)

The following data is from the four 6-hour runs and provides an overview of the early lessons learnt on the reliability of the demonstration plant.

Figure 4-4 demonstrates a typical run that occurred on the Source 1 biosolids with spikes in SOx but relatively low NOx and CO. The NOx levels were mostly due to the nature of the biosolids and because they had been stored for such a long period. Ammonia (NH_3) is oxidised, which promotes the simultaneous oxidation of NO to NO_2 (Leppaelahti, Simell, & Kurkela, 1991). If not controlled, this would result in an undesirable visible brownish colour of the plume from the stack. Another nitrogen species that can be present is di-nitrous oxide (N2O), which is a greenhouse gas. This run gave the indication that, for SOx mitigation, the chemical agent did need to be changed to MgOH.

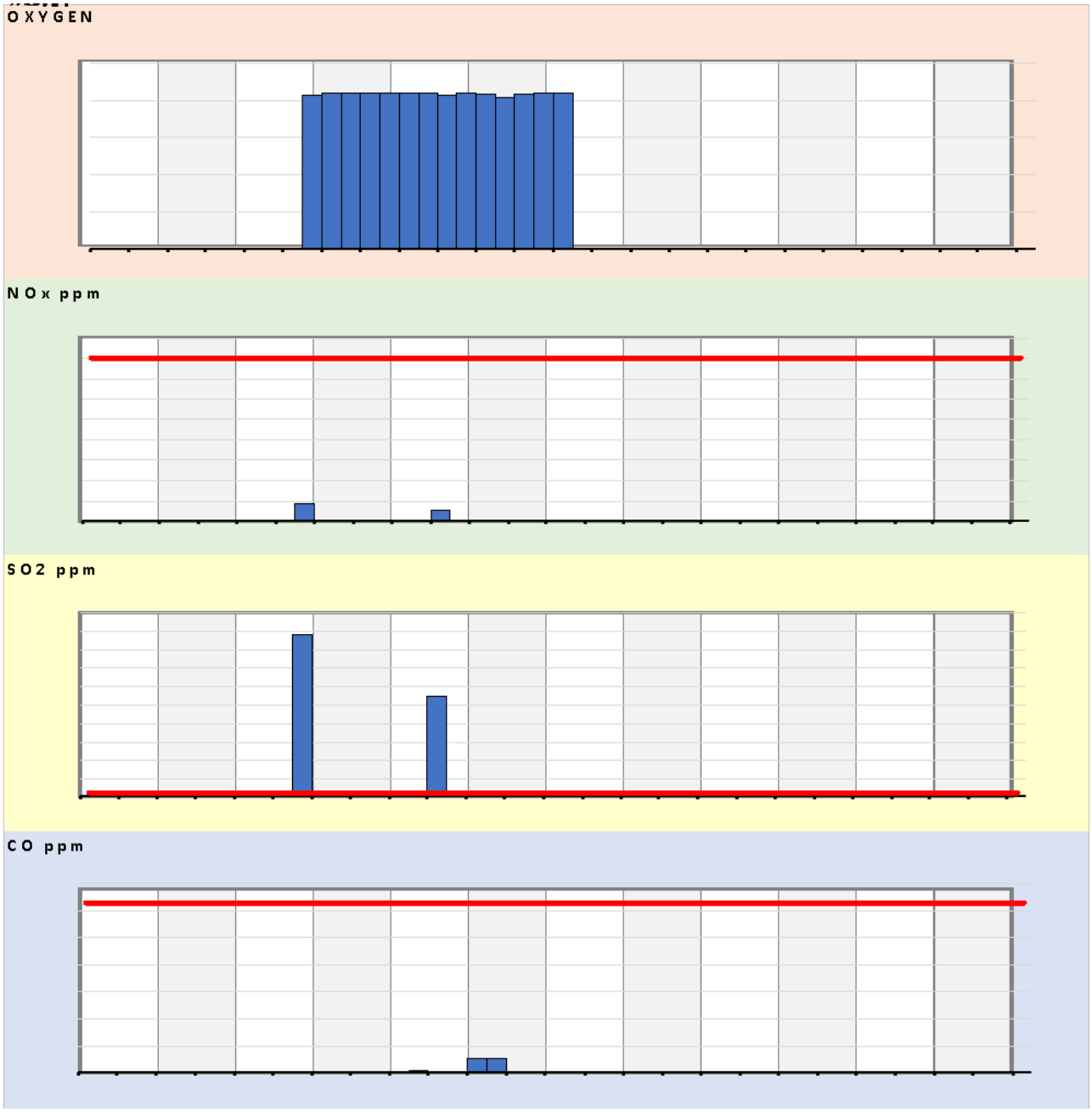


Figure 4-4: Run 4: 6-hour run (23/03/2020)

Run 5 (Figure 4-5) was completed using the Source 1 biosolids and the data reinforces the findings found in the previous runs.

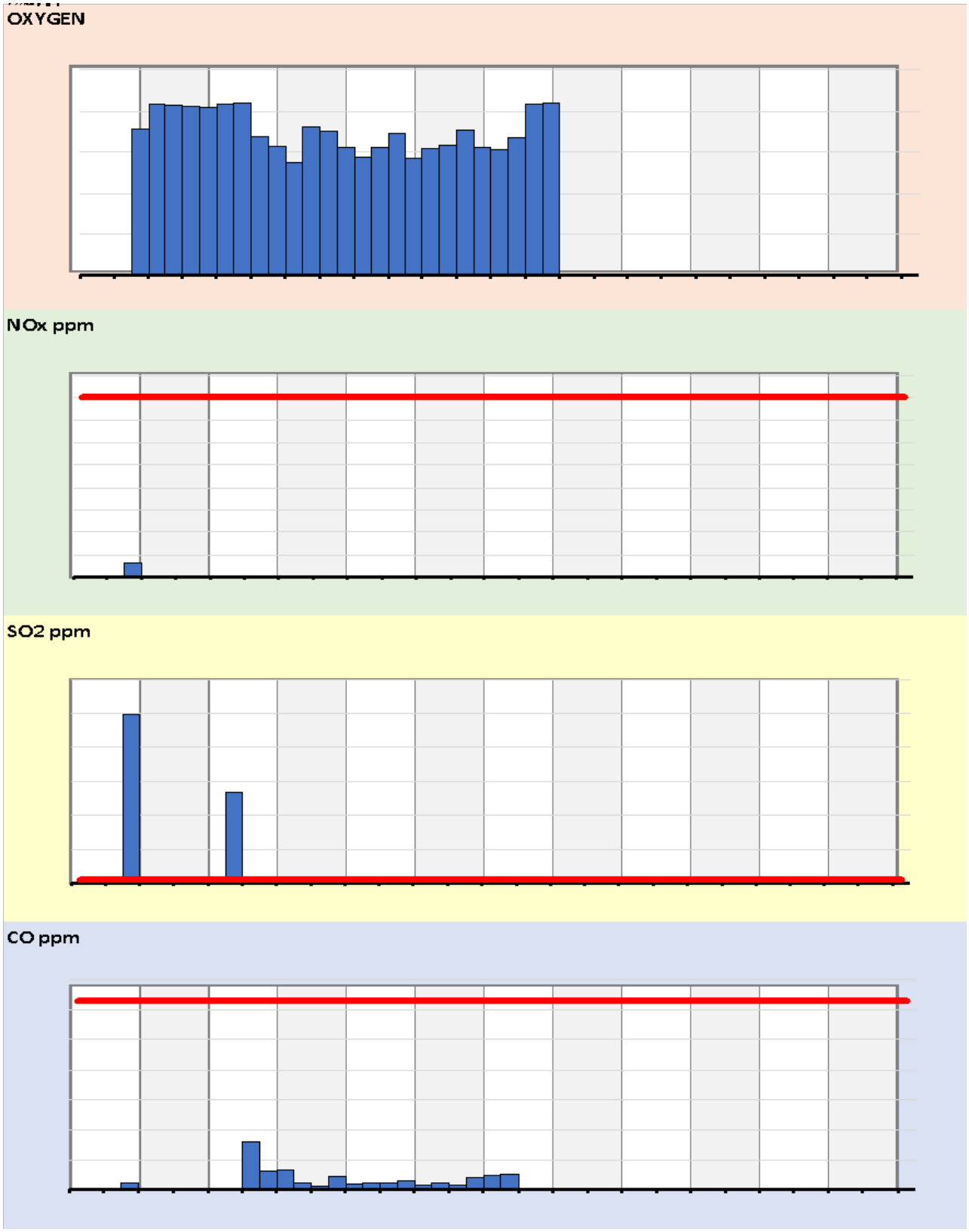


Figure 4-5: Run 5: 6-hour run (02/04/2020)

Run 6 (Figure 4-6) was a longer run on Source 2 biosolids, but demonstrates that on start-up there could be exceedances in the air emissions until the unit has stabilised, which will need to be considered in the scope of the full-scale facility. It also illustrates that the facility can meet the license requirements over a longer period. However, this run was still undertaken at a lower throughput due to the particle sizing of the biosolids.

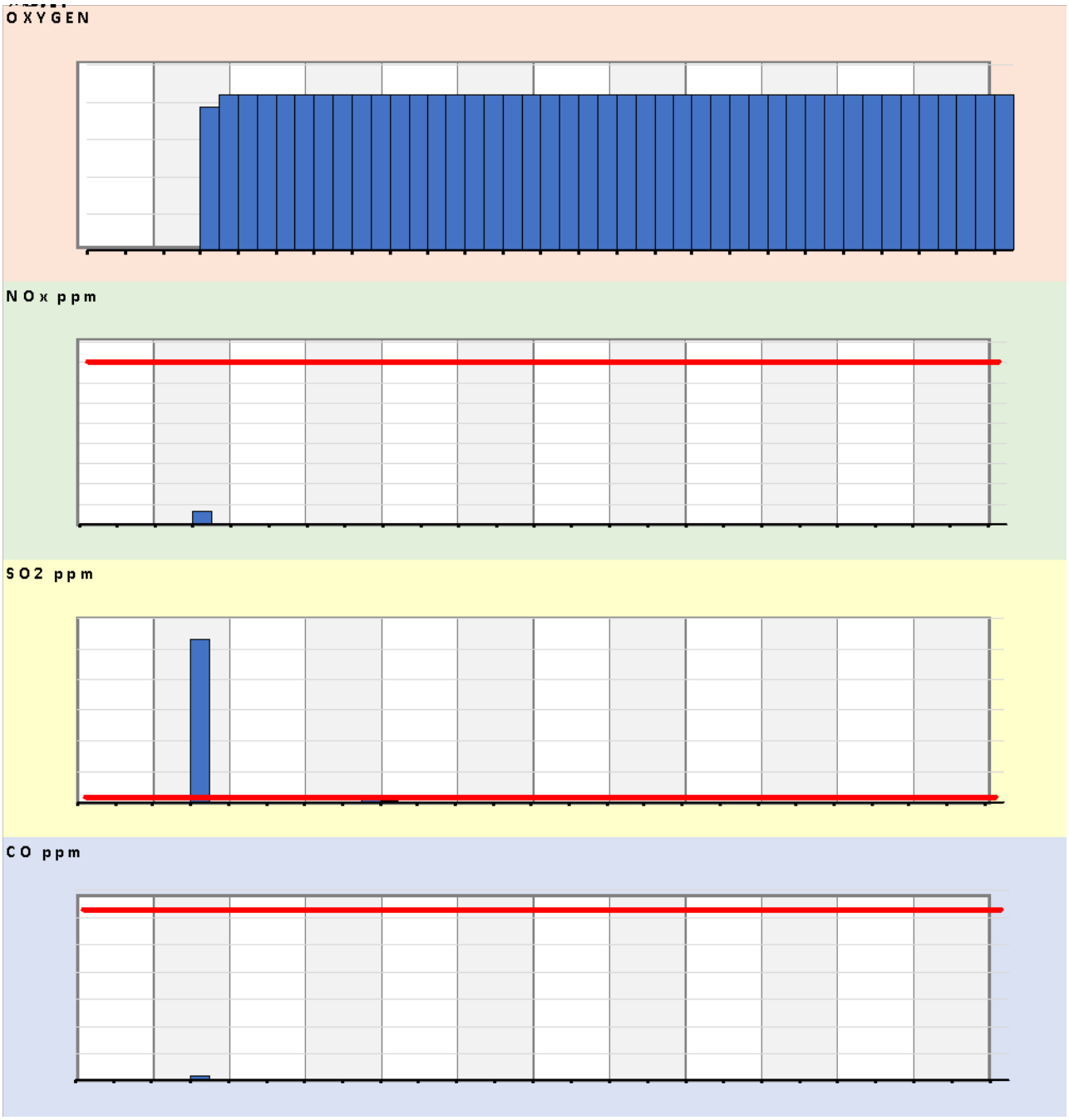


Figure 4-6: Run 6: 6-hour run (11/06/2020)

Run 7 (Figure 4-7) was completed using the Source 2 biosolids. It shows the presence of NOx, due to the source material being fresher because it had not been stored or stockpiled for the same length of time as the Source 1 material. Therefore, data was available for NOx mitigation and chemical dosage rates that appear to be like the rates estimated for the Loganholme WWTP. In Run 7, the SOx chemical mitigation agent was changed from lime to MgOH. Figure 4-7 shows that using MgOH resulted in no spikes, even during the start-up and shutdown periods.

The oxidisers were changed so that they could maintain increased temperature and retention times due to source material having a higher calorific value (more heat being produced) and the addition of insulating cladding previously installed to mitigate the low calorific value of the Source 1 biosolids material.

The issues with CO become apparent on this run and due to the difference in the biosolids, an increase in dust, and an increase in the temperature of the oxidisers that caused 'ashing out'. These issues became apparent in the full-scale air emission and particulates analysis, discussed in Section 4.7.

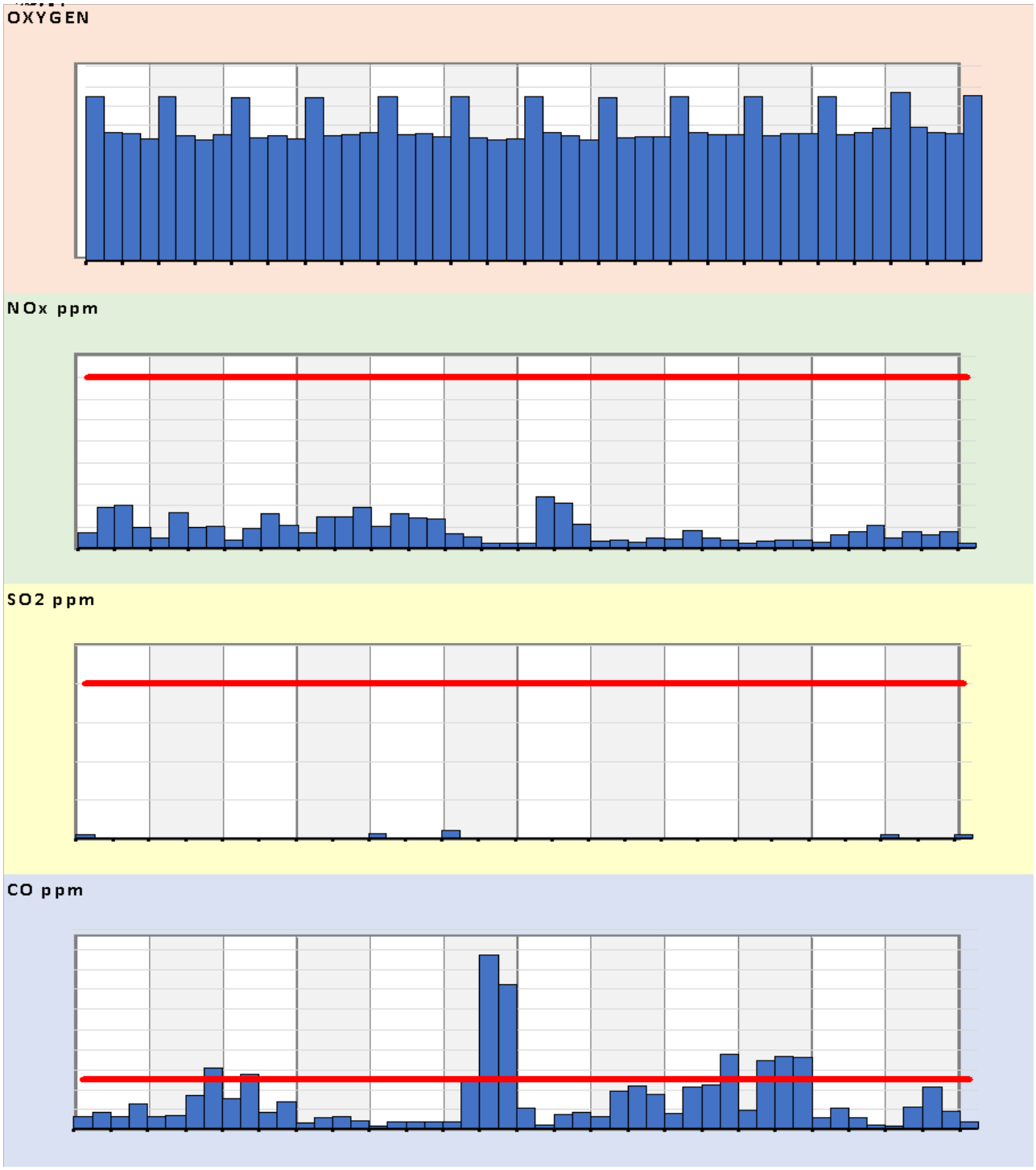


Figure 4-7: Run 7: 6-hour run (11/08//2020)

Figure 4-8, Figure 4-9, and Figure 4-10 show the data for the 24-hour run completed supplied by Assured Environmental. Because the CEMS unit was supplying incorrect data, it was considered prudent to obtain results for the 24-hour run by accredited samplers using their online unit to allow for the CEMS unit to be fixed and calibrated.

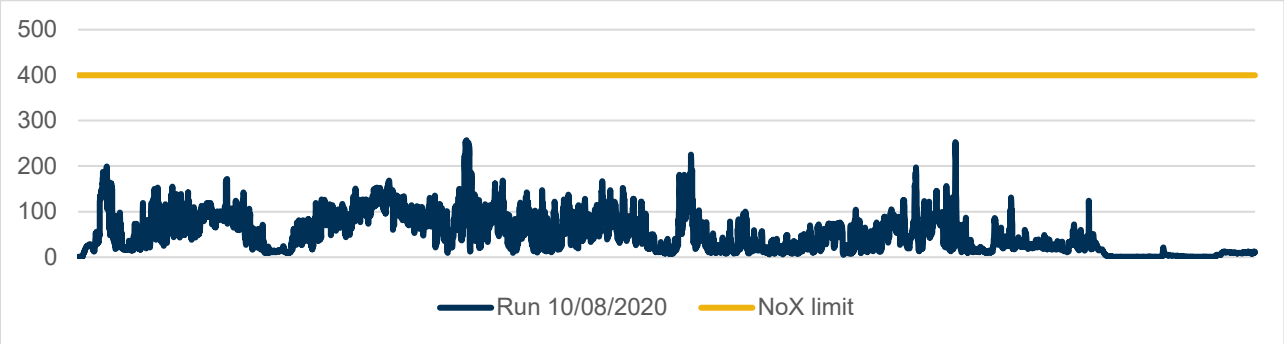


Figure 4-8: Run 8: 24-hour run NOx results (10/08/2020)

Figure 4-8 shows the NOx results over the first 24-hour run were within the license limits for the entire run.

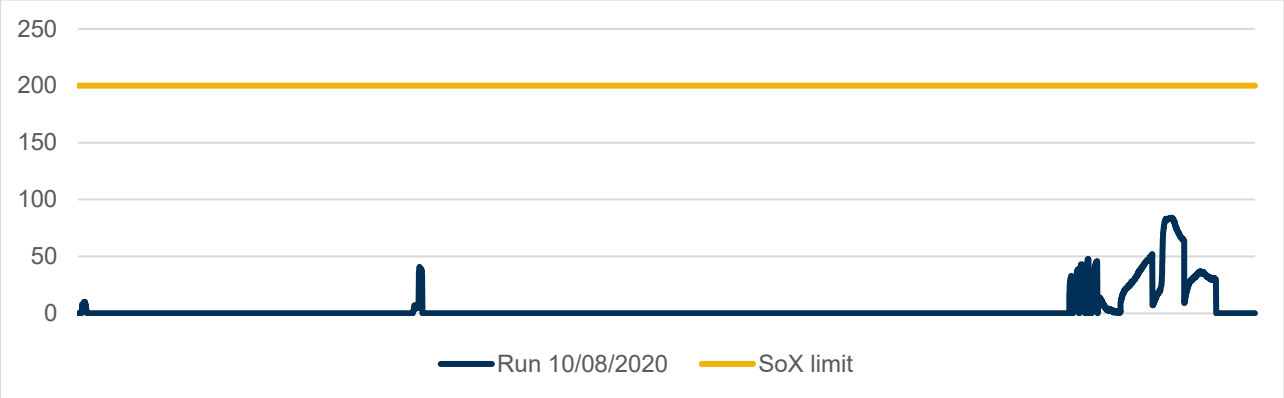


Figure 4-9: Run 8: 24-hour run SOx results (10/08/2020)

Figure 4-9 shows the SOx results over the first 24-hour run were within the license limits for the entire run.

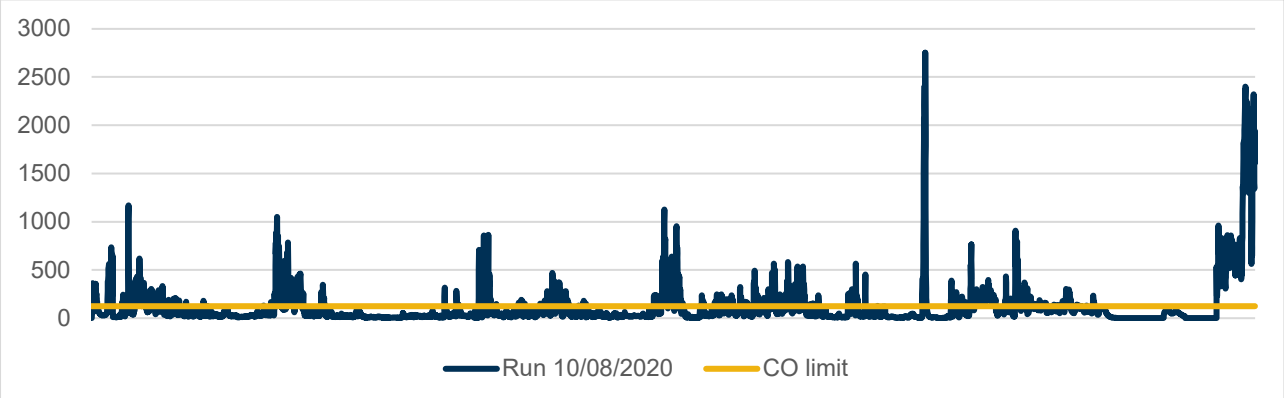


Figure 4-10: Run 8: 24-hour run CO results (10/08/2020)

Figure 4-10 shows that the issues with the current unit is the mitigation of CO. Modifications were made to the oxidisers for the next runs. These high CO results may have been carried over from the particulates with the change from Source 1 to Source 2.

CO, hydrocarbons (HC) and particulate matter are associated with incomplete combustion, which occurs in gasification processes compared to incineration. However, this issue can be engineered out by increasing the temperature in the oxidisers to start the process that will control the CO, and then the addition of an ESP to control the particulates. This strategy is supported by data obtained from the demonstration plant about particulates such as ionic characterisation (cation or anions) and sizing. This data has been used to implement the correct strategy for removing the particulates in the design. Installing an ESP in the demonstration plant was not cost or time efficient, but Assured Environment was able to sample and determine the particulates sizing.

Figure 4-11 shows that during Run 9, NOx and SOx had no exceedances. Although the CO limit appears to exceed a significant amount, these intervals were read every minute and the rolling average was 115 mg/m^3 within the limit of the 125 mg/m^3.



Figure 4-11: Run 9: 24-hour run (11/08/2020)

Run 11 (Figure 4-12) illustrates that NOx and SOx were controlled. The two exceedances for SOx were during start-up, which attributed to a CEMS blockage from previous runs. Although the CO limit was exceeded during a short period due to the maximum throughput being tested, which increased the particulates going through the oxidisers, the CO was again increased. This issue is being engineered out and will not be an issue with the full-scale facility.

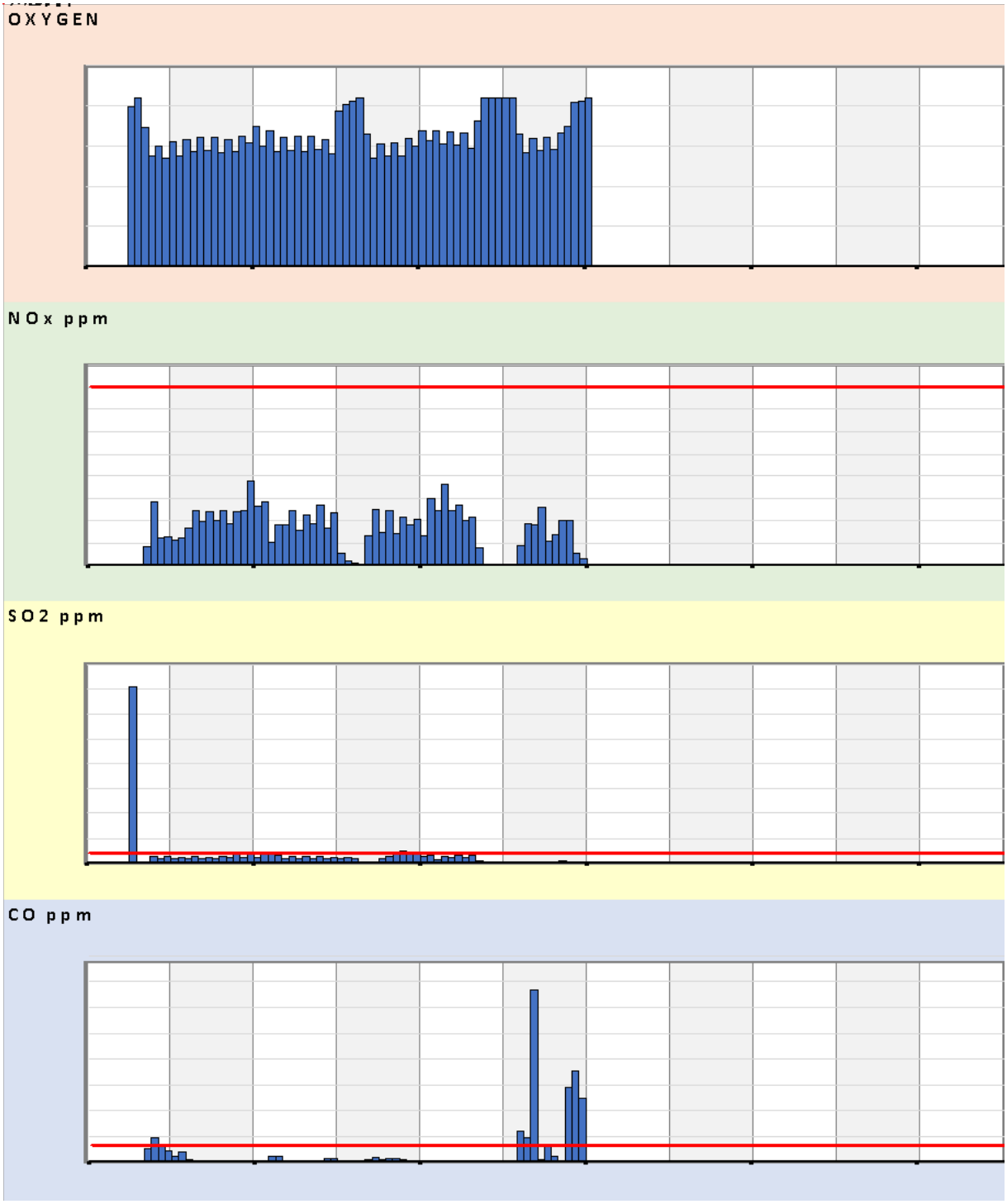


Figure 4-12: Run 11: 48-hour run (21/07/2020)

Run 12 (Figure 4-13) shows the NOx concentrations where within the limits; however, there were a few exceedances. These exceedances where due to dosing issues (blockages) and issues within the actual CEMS unit. This would not be an issue with the full-scale facility as there would also be limits and alerts set in place to assure this did not occur. The demonstration plant is much more manual then what is to be expected with the full-scale facility design. However, what the above results show is that urea can successful be used to mitigate NOx in a gasification facility.

SOx results demonstrate that MgOH is the best agent for mitigation in the air stream. There were two short exceedances, but this was again to do with manual error and would not be an issue with the full-scale facility.

There were issues with keeping within the limit of the CO. This demonstrates that there will be a requirement to engineer improved particulates capture in the full-scale facility, but it also could be due to the pelletizing of the dried biosolids through the respective dryers, which would not be an issue in the full-scale facility using belt dryers. The drum dryer handling techniques do produce a lot of dust and a high dried pellet density meaning that it is hard to get full combustion. Both factors do contribute to the CO and particulates; therefore, it is believed that by using the belt dryers this will reduce the risk.

The following is the data from the 100-hour run on the 27/07/2020.

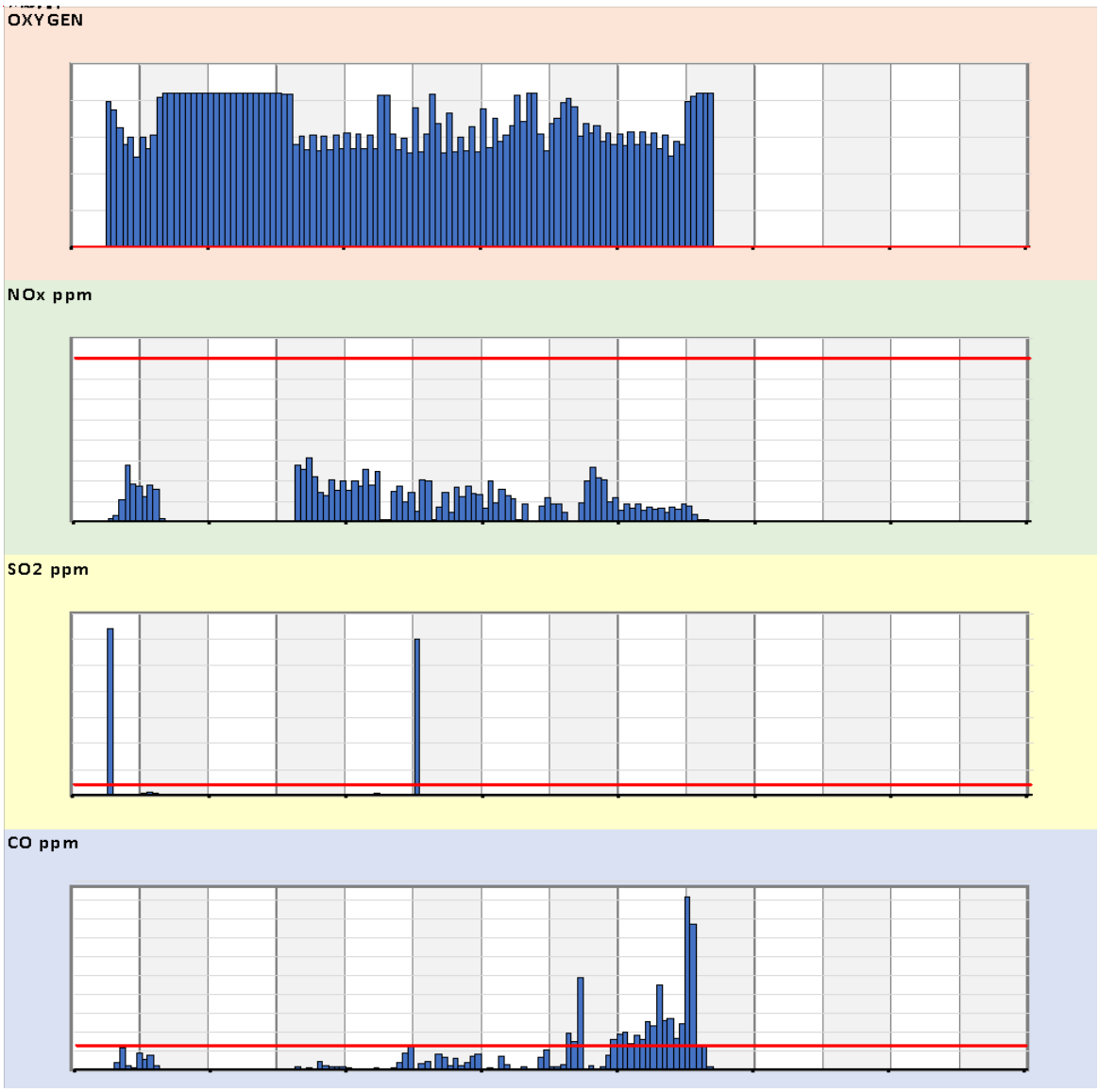


Figure 4-13: Run 12: 100-hour run (27/07/2020)

4.7 Full air emission results

This section provides a summary of the full air emissions results for three runs: 31/03/2020, 02/04/2020 and 22/07/2020.

As shown in Table 4-1 for the run on the 31 March 2020 there were no exceedances to the target limits set by the conditions of the EA.

Table 4-1: Air emission results—24-hour run (31/03/2020)

Parameter	< >	Result	Permit	Unit	Reference
Sample date		31/03/2020	-		-
Average source temperature		66	-	°C	-
Flue gas water vapour content		23	-	vol-%	-
Carbon dioxide concentration		7.0	-	vol-%	Sewage Treatment Plant (STP)
Oxygen concentration		12.2	-	vol-%	STP
Flue gas molecular weight—dry		1.3	-	kg/Nm^3	STP
Flue gas velocity		22.8	15 (min)	m/sec	-
Flue gas volume flow		66.8	-	Nm^3/min	STP
Particulate matter		17	-	mg/Nm^3	STP
- at 11% O_2		23	30	mg/Nm^3	
- emission rate		1.1	-	g/min	
Carbon monoxide		55	-	mg/Nm^3	STP
- at 11% O_2		69	125	mg/Nm^3	
- emission rate		3.5	-	g/min	
Oxides of nitrogen (as NO_2)		12	-	mg/Nm^3	STP
- at 11% O_2		16	400	mg/Nm^3	
- emission rate		0.7	-	g/min	
Sulfur dioxide (SO_2)		130	-	mg/Nm^3	STP
- at 11% O_2		160	200	mg/Nm^3	
- emission rate		8.3	-	g/min	
Hydrogen chloride		4.2	-	mg/Nm^3	STP
- at 11% O_2		5.1	60	mg/Nm^3	
- emission rate		0.27	-	g/min	
Hydrogen fluoride	<	0.1	-	mg/Nm^3	STP
- at 11% O_2	<	0.2	4	mg/Nm^3	
- emission rate	<	0.01	-	g/min	
Total VOCs (as n-propane)	<	0.9	-	mg/Nm^3	STP
- at 11% O_2	<	1.1	20	mg/Nm^3	
- emission rate	<	0.059	-	g/min	
Cadmium		0.0075	-	mg/Nm^3	STP
- at 11% O_2		0.010	0.05	mg/Nm^3	

Parameter	< >	Result	Permit	Unit	Reference
- emission rate		0.00048	-	g/min	
Mercury		0.015	-	mg/Nm^3	STP
- at 11% O_2		0.017	0.05	mg/Nm^3	
- emission rate		0.00094	-	g/min	
Total heavy metals (medium bound)		0.25	-	mg/Nm^3	STP
- at 11% O_2		0.29	0.50	mg/Nm^3	
- emission rate		0.016	-	g/min	
PCDD/F — i-TEQ (medium bound)		0.014	-	ng/Nm^3	STP
- at 11% O_2		0.018	0.10	ng/Nm^3	
- emission rate		1.1E-09	-	g/min	
PAHs—BaP (medium bound)		0.51	-	µg/Nm^3	STP
- at 11% O_2		0.64	-	µg/Nm^3	
- emission rate		4.0E-05	-	g/min	
Total PFAS (medium bound)		33.7	-	ng/Nm^3	STP
- at 11% O_2		38.5	-	ng/Nm^3	
- emission rate		2.6E-06	-	g/min	
Total TOPA (medium bound)		47.2	-	ng/Nm^3	STP
- at 11% O_2		53.9	-	ng/Nm^3	
- emission rate		3.6E-06	-	g/min	
Average odour		1,628	-	ou	STP
- emission rate		132,027	-	ou-m^3/min	
Hydrogen sulfide	<	0.8	-	mg/Nm^3	STP
- at 11% O_2	<	0.9	5	mg/Nm^3	
- emission rate	<	0.05	-	g/min	
Sulfur trioxide (as H2SO4)		38.7	-	mg/Nm^3	STP
- at 11% O_2		44.1	-	mg/Nm^3	
- emission rate		2.434	-	g/min	
Hexavelant chromium	<	0.0072	-	mg/Nm^3	STP
- at 11% O_2	<	0.0089	-	mg/Nm^3	
- emission rate	<	0.00046	-	g/min	

As shown in Table 4-2, the particulate matter failed the set limits when the oxygen correction factor was calculated. To mitigate this failure, the easiest and most cost-effective solution was the addition of the venturi as a way of mitigating dust particles. As a precaution, there was a break in runs and extra analysis was added to the schedule to investigate the exceedance and provide a corrective action.

Table 4-2 provides results of the particulate analysis after the addition of the venturi.

Table 4-2: Particulate analysis after the addition of a venturi—24-hour run (02/04/2020)

Parameter	< >	Result	Permit	Unit	Reference
Sample date		2/04/2020	-	dd.mm.yyyy	-
Average source temperature		66	-	°C	-
Flue gas water vapour content		23	-	vol-%	-
Carbon dioxide concentration		6.7	-	vol-%	STP
Oxygen concentration		12.9	-	vol-%	STP
Flue gas molecular weight—dry		1.3	-	kg/Nm^3	STP
Flue gas velocity		21.2	15 (min)	m/sec	-
Flue gas volume flow		61.8	-	Nm^3/min	STP
Particulate matter		28	-	mg/Nm^3	STP
- at 11% O_2		33	30	mg/Nm^3	
- emission rate		1.7	-	g/min	
Carbon monoxide		6	-	mg/Nm^3	STP
- at 11% O_2		7	125	mg/Nm^3	
- emission rate		0.3	-	g/min	
Oxides of nitrogen (as NO_2)		7	-	mg/Nm^3	STP
- at 11% O_2		9	400	mg/Nm^3	
- emission rate		0.5	-	g/min	
Sulfur dioxide (SO_2)		100	-	mg/Nm^3	STP
- at 11% O_2		124	200	mg/Nm^3	
- emission rate		6.1	-	g/min	
Hydrogen chloride		7	-	mg/Nm^3	STP
- at 11% O_2		9	60	mg/Nm^3	
- emission rate		0.4	-	g/min	
Hydrogen fluoride	<	0.1	-	mg/Nm^3	STP
- at 11% O_2	<	0.1	4	mg/Nm^3	
- emission rate	<	0.01	-	g/min	
Total VOCs (as n-propane)	<	1.0	-	mg/Nm^3	STP
- at 11% O_2	<	1.1	20	mg/Nm^3	
- emission rate	<	0.059	-	g/min	
Cadmium		0.0078	-	mg/Nm^3	STP
- at 11% O_2		0.0089	0.05	mg/Nm^3	
- emission rate		0.00049	-	g/min	
Mercury		0.010	-	mg/Nm^3	STP
- at 11% O_2		0.012	0.05	mg/Nm^3	
- emission rate		0.00063	-	g/min	
Total heavy metals (medium bound)		0.160	-	mg/Nm^3	STP

Parameter	< >	Result	Permit	Unit	Reference
- at 11% O_2		0.183	0.50	mg/Nm^3	
- emission rate		0.010	-	g/min	
PCDD/F—i-TEQ (medium bound)		0.000	-	ng/Nm^3	STP
- at 11% O_2		0.0	0.10	ng/Nm^3	
- emission rate		0.000	-	g/min	
PAHs—BaP (medium bound)		0.000	-	ng/Nm^3	STP
- at 11% O_2		0.0	-	ng/Nm^3	
- emission rate		0.000	-	g/min	
Total PFAS (medium bound)		15.9	-	ng/Nm^3	STP
- at 11% O_2		19.5	-	ng/Nm^3	
- emission rate		9.8E-07	-	g/min	
Total TOPA (medium bound)		19.482	-	ng/Nm^3	STP
- at 11% O_2		24.0	-	ng/Nm^3	
- emission rate		0.000	-	g/min	
Average odour		926	-	ou	STP
- emission rate		76,384	-	ou-m^3/min	
Hydrogen sulfide	<	0.8	-	mg/Nm^3	STP
- at 11% O_2	<	0.9	5	mg/Nm^3	
- emission rate	<	0.05	-	g/min	
Sulfur trioxide (as H2SO4)		37.2	-	mg/Nm^3	STP
- at 11% O_2		45.4	-	mg/Nm^3	
- emission rate		2.373	-	g/min	
Hexavelant chromium	<	0.0052	-	mg/Nm^3	STP
- at 11% O_2	<	0.0064	-	mg/Nm^3	
- emission rate	<	0.00033	-	g/min	

As illustrated in Table 4-3. the corrective action of the addition of the venturi significantly reduced the particulate concentration in the air emissions and therefore the results are all within the set limits

Table 4-3: Particulate analysis with inclusion of venturi—Run on 26/05/ 2020

Parameter	Result	Permit	Unit	Reference
Sample date	26/05/2020	-		-
Average source temperature	53	-	°C	-
Flue gas water vapour content	17	-	vol-%	-
Carbon dioxide concentration	2.7	-	vol-%	STP
Oxygen concentration	17.1	-	vol-%	STP
Flue gas molecular weight— dry	1.3	-	kg/Nm^3	STP
Flue gas velocity	19.8	15 (min)	m/sec	-
Flue gas volume flow	64.7	-	Nm^3/min	STP
TEST 1				
Particulate matter	7.6	-	mg/Nm^3	STP
- at 11% O_2	16	30	mg/Nm^3	
- emission rate	0.5	-	g/min	
Sulfur dioxide (SO_2)	4.1	-	mg/Nm^3	STP
- at 11% O_2	8.6	200	mg/Nm^3	
- emission rate	0.25	-	g/min	
Sulfur trioxide (as H2SO4)	0.34	-	mg/Nm^3	STP
- at 11% O_2	0.71	-	mg/Nm^3	
- emission rate	0.021	-	g/min	
Particulate matter	11	-	mg/Nm^3	STP
- at 11% O_2	25	30	mg/Nm^3	
- emission rate	0.71	-	g/min	
Sulfur dioxide (SO_2)	3.3	-	mg/Nm^3	STP
- at 11% O_2	7.3	200	mg/Nm^3	
- emission rate	0.21	-	g/min	
Sulfur trioxide (as H2SO4)	0.33	-	mg/Nm^3	STP
- at 11% O_2	0.75	-	mg/Nm^3	
- emission rate	0.021	-	g/min	

As shown in Table 4-4, the final full air emissions results show that the Loganholme demonstration plant could meet all the conditions set out in the EA. The 100-hour was the ideal run and showed that with the optimisation scheduled in the full-scale facility would be below the targets set in the EA. Table 4-4 also shows that the known issues, such as CO and particulates, can be optimised for the full-scale facility. Although the current EA can be met with current design, the addition of more analysis and determining the particulate sizing and characterisation has been prudent and efficient for making sure that tithe full-scale designs out these known risks.

Table 4-4: 100-hour run (22/07/2020 and 23/07/2020)

Parameter	< >	Result	Permit	Unit	Reference
Sample date		22/07/2020 & 23/07/2020	-	dd.mm.yyyy	-
Average source temperature		55	-	°C	-
Flue gas water vapour content		15	-	vol-%	-
Carbon dioxide concentration		4.9	-	vol-%	STP
Oxygen concentration		14.4	-	vol-%	STP
Flue gas molecular weight—dry		1.3	-	kg/Nm^3	STP
Flue gas velocity		21.8	15 (min)	m/sec	-
Flue gas volume flow		72.8	-	Nm^3/min	STP
Particulate matter		20	-	mg/Nm^3	STP
- at 11% O_2		26	30	mg/Nm^3	
- emission rate		1.4	-	g/min	
Carbon monoxide		71	-	mg/Nm^3	STP
- at 11% O_2		109	125	mg/Nm^3	
- emission rate		4.5	-	g/min	
Oxides of nitrogen (as NO_2)		140	-	mg/Nm^3	STP
- at 11% O_2		219	400	mg/Nm^3	
- emission rate		10.2	-	g/min	
Sulfur dioxide (SO_2)		119	-	mg/Nm^3	STP
- at 11% O_2		188	200	mg/Nm^3	
- emission rate		0.0	-	g/min	
Hydrogen chloride		2.2	-	mg/Nm^3	STP
- at 11% O_2		3.3	60	mg/Nm^3	
- emission rate		0.15	-	g/min	
Hydrogen fluoride		0.3	-	mg/Nm^3	STP
- at 11% O_2		0.5	4	mg/Nm^3	
- emission rate		0.02	-	g/min	
Total VOCs (as n-propane)	<	0.9	-	mg/Nm^3	STP
- at 11% O_2	<	1.4	20	mg/Nm^3	
- emission rate	<	0.066	-	g/min	
Cadmium		0.0027	-	mg/Nm^3	STP
- at 11% O_2		0.0045	0.05	mg/Nm^3	
- emission rate		0.00019	-	g/min	
Mercury		0.025	-	mg/Nm^3	STP
- at 11% O_2		0.041	0.05	mg/Nm^3	
- emission rate		0.0017	-	g/min	

Parameter	< >	Result	Permit	Unit	Reference
Total heavy metals (medium bound)		0.19	-	mg/Nm^3	STP
- at 11% O_2		0.31	0.50	mg/Nm^3	
- emission rate		0.013	-	g/min	
PCDD/F—i-TEQ (medium bound)		0.0013	-	ng/Nm^3	STP
- at 11% O_2		0.0021	0.10	ng/Nm^3	
- emission rate		1.5E-10	-	g/min	
PAHs—BaP (medium bound)		0.30	-	$\mu g/Nm^3$	STP
- at 11% O_2		0.51	-	$\mu g/Nm^3$	
- emission rate		3.6E-05	-	g/min	
Total PFAS (medium bound)		47	-	ng/Nm^3	STP
- at 11% O_2		71	-	ng/Nm^3	
- emission rate		5.1E-06	-	g/min	
Total TOPA (medium bound)		74.0	-	ng/Nm^3	STP
- at 11% O_2		112.0	-	ng/Nm^3	
- emission rate		8.2E-06	-	g/min	
Average odour		801	-	ou	STP
- emission rate		68,606	-	$ou\text{-}m^3/min$	
Hydrogen sulfide	<	0.8	-	mg/Nm^3	STP
- at 11% O_2	<	1.2	5	mg/Nm^3	
- emission rate	<	0.06	-	g/min	
Sulfur trioxide (as H2SO4)		3.6	-	mg/Nm^3	STP
- at 11% O_2		4.8	-	mg/Nm^3	
- emission rate		0.253	-	g/min	
Hexavelant chromium	<	0.0063	-	mg/Nm^3	STP
- at 11% O_2	<	0.012	-	mg/Nm^3	
- emission rate	<	0.00040	-	g/min	

Design modifications were made to the full-scale facility as part of the value engineering process. Extra analyses were conducted on 03/09/2020 to test these changes. The results show that the carbon monoxide and particulate concentrations were negatively affected by the changes to the oxidisers. The process can be engineered out in the full-scale facility with the addition of an ESP.

5. Biosolids and Biochar Characterisation

5.1 Biosolids analysis

This section is an in-depth analysis of the biosolids sourced from Victoria, as well as those from Loganholme and Beenleigh WWTPs. For reference, an independent review was undertaken on the Loganholme WWTP and the potential market for biochar using the analytical data from this report, titled *Char characteristics, potential value, and reuse options (Bridle, 2020)* (refer to Appendix A).

Drying concentrates the energy in sludge by removing water and increasing the gross calorific value, transforming the sludge into a combustible material. Several factors such as humidity, air velocity, sludge origin and the physical transformation of the material affect the drying operation, but temperature is the most influential parameter. Temperature affects the drying time, rheological properties, thermal stability, and the physicochemical characteristics of the biosolids, such as calorific value and nutrient content.

Thermal drying removes the moisture from sludge obtained from the Loganholme EETP (14% w/w solids), drying it through the belt filter press, which will change the content to >99% w/w solids. The belt dryers significantly reduce the moisture to below 10% w/w, reducing the cost for handling and transport. Drying biosolids at ≥80 °C also stabilises the material and destroys pathogens.

The potential for energy recovery from biosolids is a function of their composition, which is a mixture of organic (volatile) matter, inorganic matter (inert material) and associated water. The composition of biosolids may vary, and the energy recovery method and corresponding energy products must be compatible with the characteristics of the biosolids. Therefore, the volatile solids are important when considering the heat energy recovery.

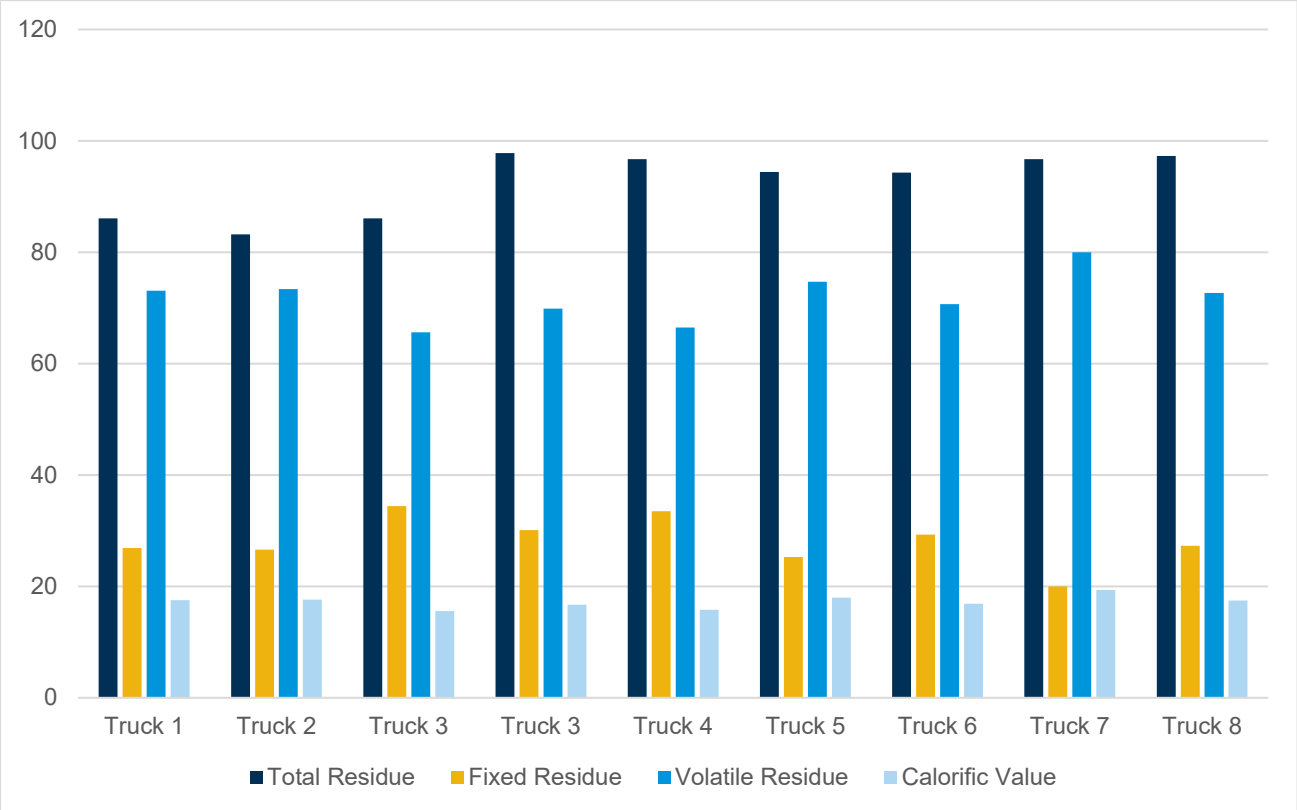


Figure 5-1: Biosolids chemical parameters: total residue (%), fixed residue (%), volatile residue (%) and calculated calorific value

Figure 5-1 shows that the Source 2 biosolids had a higher percentage of total solids and a higher overall percentage of volatile residue, which in turn correlates to a higher calorific value. The Source 1 biosolids had

an average calorific value of 19.26 MJ/kg. Source 2 biosolids had lower calorific values, which is to be expected due to the biosolids degrading while being stockpiled at a third-party storage for many months. This would not be an issue for the Loganholme biosolids gasification facility because it will process all biosolids as they are dried, and no storage will be involved. This is explained in detail in Section 7 Heat balance.

Biosolids are mainly a mix of water and organic matter and may contain:

- Macronutrients, such as nitrogen, phosphorus, potassium, and sulfur
- Micronutrients, such as copper, zinc, calcium, magnesium, iron, boron, molybdenum, and manganese.

Biosolids may also contain traces of synthetic organic compounds and metals, including arsenic, cadmium, chromium, lead, mercury, nickel, and selenium. These trace compounds can limit the uses for biosolids, with all potential uses regulated in Queensland by the *End of Waste Code for Biosolids* (DES,2020).

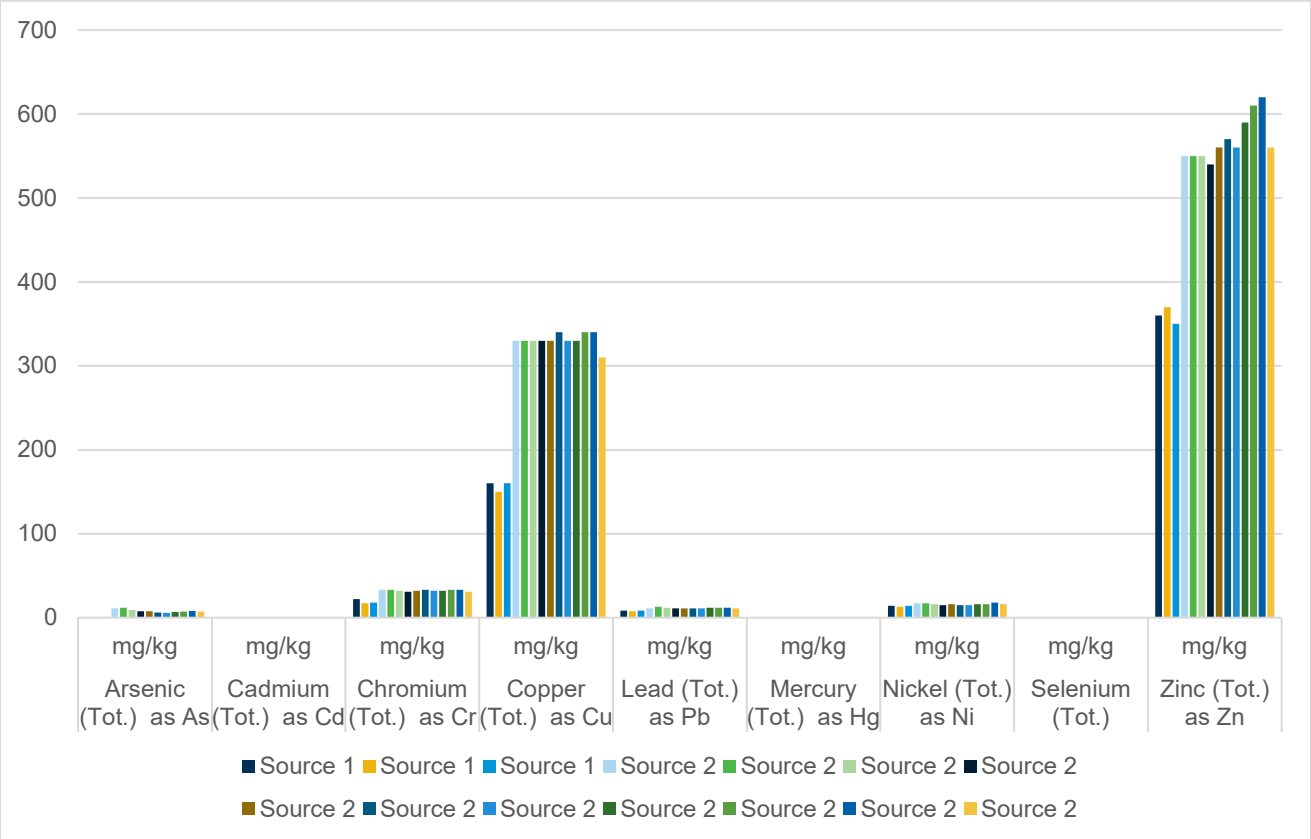


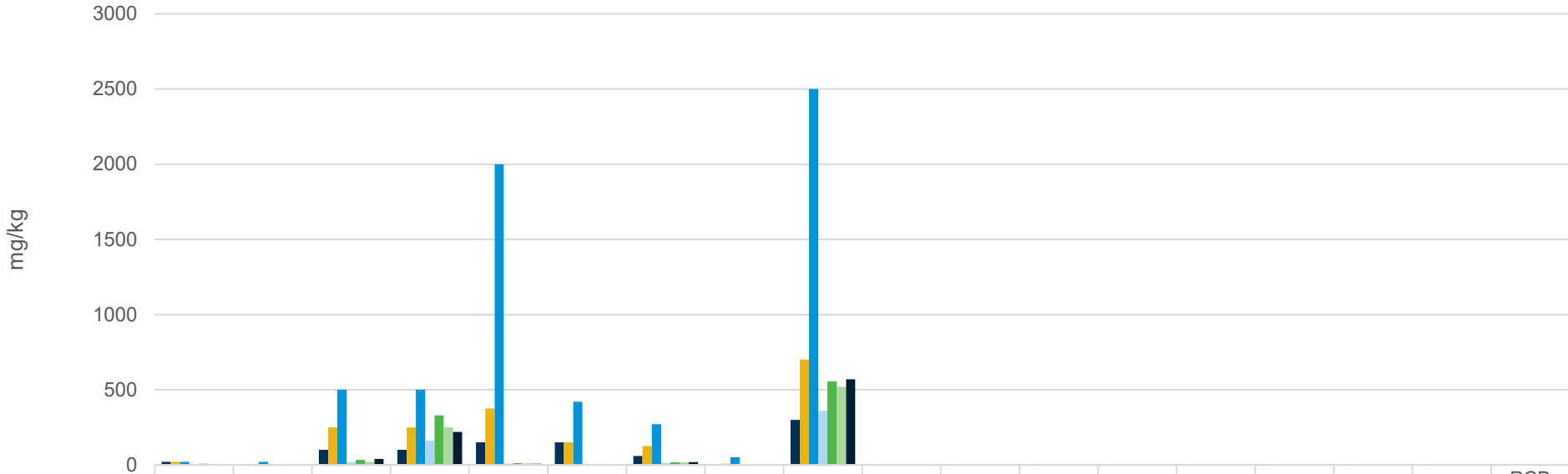
Figure 5-2: Comparison of trace metals in biosolids sources

Figure 5-2 shows the trace metals in the two sources of biosolids. Biosolids from any WWTP will usually have micronutrients such as copper and zinc. These trace metals are of high value in Australia for farmers; this benefit is undervalued in LCC's Biosolids Management Strategy.

The main difference between the two sources of biosolids for detectable heavy metal is that Source 2 has a higher concentration than Source 1, including arsenic, chromium, copper, lead, nickel, mercury, and zinc.

The heavy metal content of biosolids should be taken into consideration, but Source 2 is in a significantly more industrial catchment, which would explain the higher metal concentrations.

Figure 5-2 shows the average of the trace metals in each biosolids source, compared to the average trace metals found in the Loganholme WWTP biosolids.



	Arsenic (Tot.) as As	Cadmium (Tot.) as Cd	Chromium (Tot.) as Cr	Copper (Tot.) as Cu	Lead (Tot.) as Pb	Mercury (Tot.) as Hg	Nickel (Tot.) as Ni	Selenium (Tot.)	Zinc (Tot.) as Zn	DDT/DDD/DDE (sum)	Aldrin	Dieldrin	Chlordane (sum)	Heptachlor (sum)	HCB	Lindane	BHC (sum)	PCB total (sum of Aroclors)
Grade A	20	3	100	100	150	150	60	5	300	0.5	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0
Grade B	20	5	250	250	375	150	125	8	700	0.5	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.3
Grade C	20	20	500	500	2000	420	270	50	2500	1	0.5	0.5	0.5	0.5	0.5	0.5	0.5	1
Source 1	0	0	18	160	8.4	0.1	14	0	360	0	0	0	0	0	0	0	0	0
Source 2	7.5	0	32.5	330	11.5	0.4	16	0	555	0	0	0	0	0	0	0	0	0
Loganholme	0	0	21	250	10	0.3	16	0	520	0	0	0	0	0	0	0	0	0
Beenleigh	0	0	40	220	8.2	0.2	19	0	570	0	0	0	0	0	0	0	0	0

Figure 5-3: Comparison of heavy metals in biosolids

Figure 5-3 compares the biosolids from interstate sources to samples from Loganholme and Beenleigh WWTPs and the grades in the *End of Waste Code for Biosolids* (DES,2020). Figure 5-3 illustrates that all the sources of biosolids comfortably fall within in the category of Grade B Biosolids.

The contaminate grading for all sources of biosolids is Grade B (the rules for Zn and Cu from the *NSW Biosolids Guideline* is still in place in the *End of Waste Code for Biosolids* (DES,2020). Loganholme WWTP has several unusually high heavy metal results for As, Cr, Ni and Zn due trade-waste contamination.

The organic contaminates such as aldrin, dieldrin, chlordane, heptachlor, HCB, Lindane, BHC, DDT, DDE, DDD, PCB where analysed for the biosolids sourced from Victoria, but the limits were all below the detectable level. One organic contaminant found in biosolids from Beenleigh WWTP is dieldrin. Dieldrin was popular in the 1950s through to the 1970s as a pesticide, but it was banned in Australia in the late 1990s. Therefore, its presence at Beenleigh WWTP, although in low concentrations, should be monitored by the LCC Trade Waste Team.

5.2 Biochar analysis

Biochar is a kind of charcoal created when biomass or organic material is combusted at temperatures of 300–600 °C with limited oxygen. The biochar produced from 100% biosolids source is currently an unverified medium, which means a significant amount of analysis is required to prove the beneficial reuse of this product. However, overall, biochar contains high levels of carbon and nutrients, and a significant cation-exchange capacity. Most of the carbon in the biochar is sequestered (Lehmann, Gaunt, & Rondon, 2006) meaning that it is highly recalcitrant, with carbon turnover on a timescale of millennium, reducing greenhouse gas emissions from soils or from waste management practices. Biochar has significant demonstrated benefits for improving soil properties. The benefits of adding biochar to soil depends on the material used to produce the biochar (feedstock) and the temperatures achieved during gasification (carbonisation).

One study conducted by Bridle (Bridle, Cote, Constable, & Fraser, 1987) determined that biochar produced from biosolids contains low amounts of plant-available N, despite containing a low C:N ratio value of 7 and an elevated amount of total N (6.4%). This study reported that 45% of the initial N was not present in the final biochar, whereas losses of P during pyrolysis were negligible. Lower amounts of N were present, compared to the original biosolids, corroborated by other studies (Dokht, Naeini, Dordipour, De Jong, & Hezarjanbi, 2017). Biochar from biosolids contributes to soil retention of N in the long-term, acting as a slow release fertiliser. Ammonium and nitrate leaching are reduced following biochar amendment.

As part of the demonstration plant, the biochar produced from the two sources has been analysed. Figure 5-4 illustrates the biosolids and biochar analyses from Source 1.

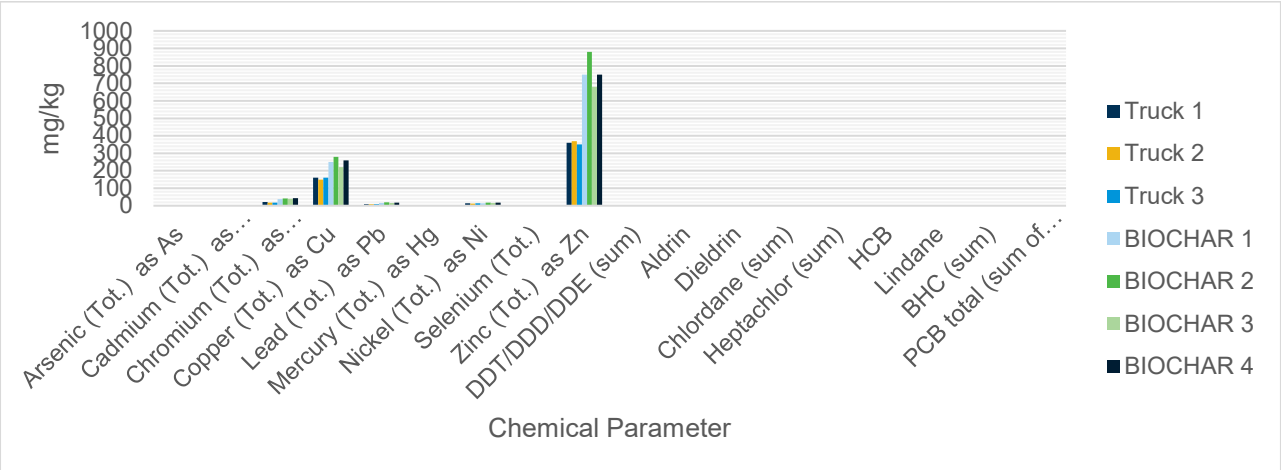


Figure 5-4: Source 1 biosolids and biochar characterization

Figure 5-4 shows that, as expected, once the biosolids are processed through the gasification unit, the concentrations of trace metals increase. Table 5-1 shows the difference in the material after it was treated through the gasification facility and how the biochar compares to the contaminant grading under the *End of Waste Code for Biosolids (*DES, 2020).

Table 5-1: Source 1 analysis of biosolids and biochar processed through the demonstration plant

	Biosolids	Biochar	Difference	Limit set in the EoW Code for Biosolids (Queensland)		
				GRADE A	GRADE B	GRADE C
Arsenic (Tot.) as	<5.0	<5.0		20	20	20
Cadmium (Tot.) as Cd	<0.50	<0.50		3	5	20
Chromium (Tot.) as Cr	19	40	53	100	250	500
Copper (Tot.) as Cu	157	253	38	100	250	500
Lead (Tot.) as Pb	8.2	17	53	150	375	2000
Mercury (Tot.) as Hg	0.1	<0.1		150	150	420
Nickel (Tot.) as Ni	14	16	15	60	125	270
Selenium (Tot.)	<5.0	<5.0		5	8	50
Zinc (Tot.) as Zn	360	765	53	300	700	2500
DDT/DDD/DDE (sum)	<0.02	<0.02		0.5	0.5	1
Aldrin	<0.02	<0.02		0.02	0.2	0.5
Dieldrin	<0.02	<0.02		0.02	0.2	0.5
Chlordane (sum)	<0.02	<0.02		0.02	0.2	0.5
Heptachlor (sum)	<0.02	<0.02		0.02	0.2	0.5
HCB	<0.02	<0.02		0.02	0.2	0.5
Lindane	<0.02	<0.02		0.02	0.2	0.5
BHC (sum)	<0.02	<0.02		0.02	0.2	0.5
PCB total (sum of Aroclors)	<0.1	<0.1		<0.1	0.3	1

Table 5-1 also shows the differences in trace metal concentration. As expected, the metals present in the biosolids, such as Cr, Pb and Zn, increased by ~50%. The other contaminant to note is mercury, which was present in the biosolids, but not in the biochar. Metals such as mercury, cadmium and arsenic have low boiling points; when they are processed through the hearth, they become volatile and become part of the biogas.

Under the *End of Waste Code for Biosolids* (DES,2020), the biochar produced from the Source 1 biosolids would be classified as a Grade C rather than a Grade B contaminate, because of the Zn and Cu levels. The biochar would be classified as Restricted Use 2, as the pathogen reduction is by thermal treatment and the contaminate limits would be graded Grade C. This would mean that the biochar could be used for:

- Agriculture
- Forestry
- Soil and site rehabilitation.

These applications fit the current view that biochar would make a useful soil ameliorant (Hossain, Strezov, Chan, & Nelson, 2010) especially as the increased levels of Zn and Cu, which are typically low in Australian soils, would be sought after by farmers, making the biochar more valuable as a product.

Figure 5-5 shows the biosolids and biochar analysis for Source 2.

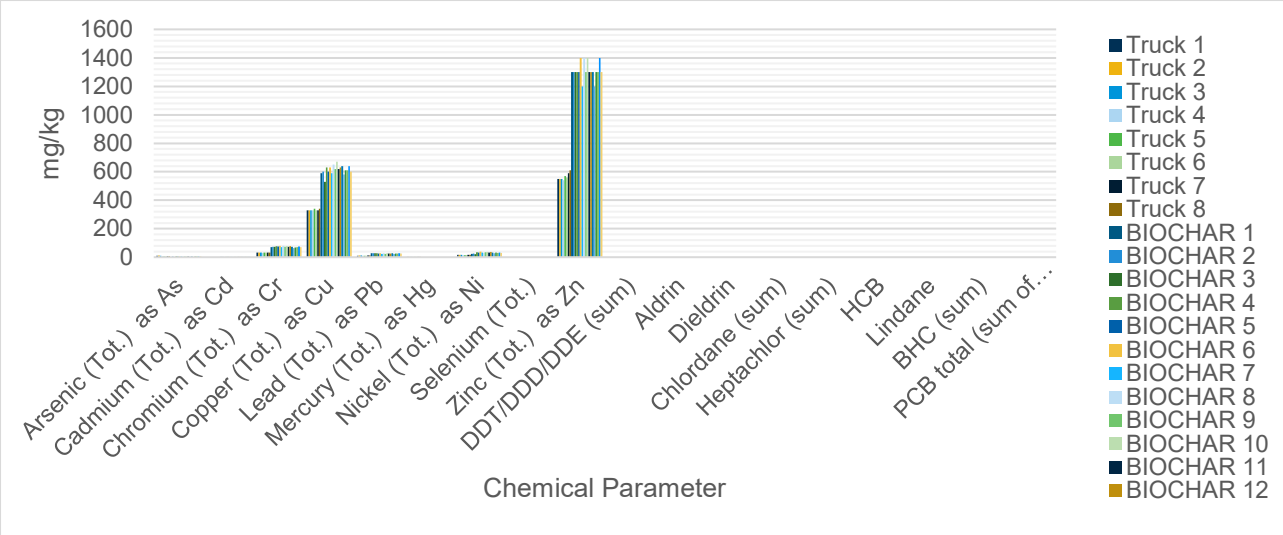


Figure 5-5: Source 2 biosolids and biochar characterisation

Like the biosolids from Source 1, when the biosolids from Source 2 were processed through gasification, the trace metals present were more concentrated in the biochar.

Table 5-2 shows the differences in the Source 2 after it was treated through the gasification facility and how the biochar compares to the contaminant grading under the *End of Waste Code for Biosolids* (DES,2020).

Table 5-2: Source 2 analysis of biosolids and biochar processed through the demonstration plant

	Biosolids	Biochar	Difference	Limit set in the EoW code for Biosolids (QLD)		
				GRADE A	GRADE B	GRADE C
Arsenic (Tot.) as	8.21	4.4		20	20	20
Cadmium (Tot.) as Cd	<1.0	1.4		3	5	20
Chromium (Tot.) as Cr	32.	73	56	100	250	500
Copper (Tot.) as Cu	333	613	46	100	250	500
Lead (Tot.) as Pb	12	26	56	150	375	2000
Mercury (Tot.) as Hg	0.39	<0.1		150	150	420
Nickel (Tot.) as Ni	16	31.	50	60	125	270
Selenium (Tot.)	<5.0	<5.0		5	8	50
Zinc (Tot.) as Zn	565	1312	57	300	700	2500
DDT/DDD/DDE (sum)	<0.02	<0.02		0.5	0.5	1
Aldrin	<0.02	<0.02		0.02	0.2	0.5
Dieldrin	<0.02	<0.02		0.02	0.2	0.5
Chlordane (sum)	<0.02	<0.02		0.02	0.2	0.5
Heptachlor (sum)	<0.02	<0.02		0.02	0.2	0.5
HCB	<0.02	<0.02		0.02	0.2	0.5
Lindane	<0.02	<0.02		0.02	0.2	0.5
BHC (sum)	<0.02	<0.02		0.02	0.2	0.5
PCB total (sum of Aroclors)	<0.1	<0.1		<0.1	0.3	1

Table 5-2 shows that after the biosolids are treated through gasification process, the trace metals present are more concentrated with an ~50% increase in the biochar, except for As and Hg (Yanjun, Guanyi, Wenchao, Mi, & Long, 2016). This result is expected because metals such as and Hg will decrease due to their boiling point because, after being processed through the gasification unit, they become volatile and part of the biogas stream. Therefore, it is recommended that when monitoring it would be prudent to characterise the biosolids before they are processed by the gasifier annually.

Under the *End of Waste Code for Biosolids* (DES,2020), the biochar produced from the Source 2 biosolids would be classified a Grade C rather than Grade B contaminate because of the Zn and Cu levels. The levels increase the value of the product as a soil ameliorant for farmers in the agricultural sector. The bioavailability of Cu and Zn are not usually valued; however, farmers use CU and zinc sulfate as well as a range of other trace metals to aid plant growth. Based on the main micronutrients present in biosolids, that is copper and zinc, the value of biosolids can be derived from the market price for CU and zinc sulfate, given they are the most used metals applied to land.

Comparing the Loganholme WWTP biosolids to those from Source 1 and Source 2, although useful, cannot be a direct comparison because the Source 1 and Source 2 biosolids have already been through a dewatering and drying process; therefore, the biosolids characterisation would be different, although there should not be a change in the metal concentration as mg/kg dry. This illustrates that because of the Zn and Cu values, the same results are expected from the Loganholme biosolids; therefore, they would also be classified as a Grade C contaminant, requiring thermal treatment to be classified as a Restricted Use 2.

Therefore, in a worst-case scenario, biochar from the Loganholme WWTP would be classified as Restricted Use 2 under the *End of Waste Code for Biosolids* (DES,2020) (no change to current classification), which means it can be used for:

- Agriculture
- Forestry
- Soil and site rehabilitation.

Provided that the biosolids meet the contaminant and pathogen reduction requirements specified in the *End of Waste Code for Biosolids* (DES, 2020), the biosolids are not considered to be waste, but rather a resource that can be reused in agriculture. However, to open other areas of marketability for biochar (Martin & Craggs, 2010) would be a key driver to develop an End of Waste Code for Biochar.

6. Emerging Contaminates of Concern

This section is an overview of how the demonstration plant addresses its objective of the quality of the biochar product and confirming destruction of Persistent Organic Pollutants (POPs) and contaminates of concern.

6.1 Perfluoroalkyl substances

PFAS is a POP found in biosolids (Latimer, 2016). PFAS are manufactured chemicals that have been used in everyday household items. PFAS make products non-stick; water repellent; and fire, weather, and stain resistant and are used in a range of consumer products, such as carpets, clothes, and paper, and have also been used in firefighting foams, pesticides, and stain repellents.

The chemical structure of PFAS, including variations in chemical structure between different types of PFAS, is an important consideration for understanding the behaviour of PFAS in the environment. The high solubility of PFAS in water means that PFAS may readily leach from soil to surface water and groundwater, where they can move long distances to enter creeks, rivers and lakes and become part of the food chain, being transferred from organism to organism (Rutludge, Cole, & Prasad) (USEPA, 2017).

In Queensland, PFAS monitoring has been included in the *End of Waste Code for Biosolids* (DES, 2020). This is the only regulatory standard for biosolids in Australia to date and the methodology for monitoring for PFAS is still being developed. As of January 2020, the application of biosolids to agricultural land is subject to the following PFAS-specific trigger values (Table 6-1), which apply after the application of biosolids to land.

Table 6-1: Trigger values for PFAS in soil after the application to land

Sum of PFOS and PFHxS	PFOA	Land use
0.01 mg/kg	0.1 mg/kg	Residential with garden/accessible soil (HIL A)
2 mg/kg	20 mg/kg	Residential with minimal opportunities for soil access (HIL B)
1 mg/kg	10 mg/kg	Public open space (HIL C)
20 mg/kg	50 mg/kg	Industrial/ commercial (HIL D)

Table 6-1 shows the trigger limits are in addition to other maximum allowable soil contaminant concentrations specified in the code. Sampling of the biosolids for PFOS, PFHxS and PFOA must be undertaken for every 120 tonnes of biosolids applied, in addition to post-application sampling to assess against the trigger values (Taylor & Yamada, 2003). TOPA analysis should be used to determine the PFAS concentrations in the soil prior to application of biosolids.

As PFAS analysis is a rapidly evolving field, there are recent advances in analytical systems and techniques. Therefore, PFAS were monitored by three different methods in the demonstration plant: TOP ASSAY, USEPA Method, and TOP Assay EWi (HPLC/MS). Table 6-2 is an extract from the PFAS NEMP 2.0 (Department of Agriculture, Water, and the Environment, 2020), which explains the different analytical methods and the limitations of these methods.

Table 6-2: PFAS standard methods of analysis

Method	Use	Sample matrices	Limitations	Analytes
USEPA Method EPA-821-R-11-007 Draft Procedure for Analysis of Perfluorinated Carboxylic Acids and Sulfonic Acids in Sewage Sludge and Biosolids by HPLC/MS/MS December 2011	To analyse for specific analytes in sewage sludge and biosolids	Sewage sludge and biosolids	**Only analyses for specific PFAS** Further details in the reference	PFBA, PFPA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnDA, PFDoDA, PFTriDA, PFTeDA, PFBS, PFHxS, PFHpS, PFOS, PFOSA, NMeFOSA, NEtFOSA, NMeFOSE, NEtFOSE

Method	Use	Sample matrices	Limitations	Analytes
Total Oxidisable Precursor Assay (TOP Assay)	**Can be used in conjunction with a USEPA method to estimate the total PFAS, and sometimes the approximate end point PFAS.** Can help inform risk assessment	Water samples and extracts including soil, biota, firefighting foam products and wastes	**Cannot be used to target exact PFAS precursors, as it is a semi-quantitiveP69FP69F1PP method. Allows for some inferences as to precursor chain length**	Total PFAS chains (C4-C14)
Total Organic Fluorine Assay (TOF Assay) as combustion ion chromatography (the most common method available)	Can be used in conjunction with a USEPA method to understand the total presence of organic fluorine in a sample and compare this to the organic fluorine equivalent detected by the USEPA method	Water samples and extracts including soil, biota, firefighting foam products and wastes	**Cannot be used to target exact PFAS precursor compounds**	Total organic fluoride corrected to remove inorganic forms

All of the biosolids data analysed in the following section used the accredited method TOP assay, originally published by Houtz and Sedlak (Hossain, Strezov, Chan, & Nelson, 2010), which is widely used as an estimate of the total perfluoro alkyl acids (PFAA) content of a sample, particularly in wastewater and biosolid matrices.

The laboratory contracted to undertake the PFAS analysis is developing an experimental method, which is still TOP assay, but uses a different set of analysis parameters that provide for a more robust oxidation of the pre-cursor substances that yield a higher extraction value. This method is still under development and peer review; however, the current parameters for extraction of samples via this method are consistent. It is believed that:

> '…the Houtz and Sedlak (Houtz, Sutton, Park, & Sedlak, 2016) method significantly underestimated the levels of PFAS compared with the modified method, which showed a 10-fold increase in the measured PFAS after digestion'. (Hutchinson, Rieck, & Wu, 2020).

Each biosolid or biochar sample was analysed via the complementary methods to gain a wholistic understanding of the biosolids and biochar characteristics. However, only data from the accredited method was used in statistical analysis.

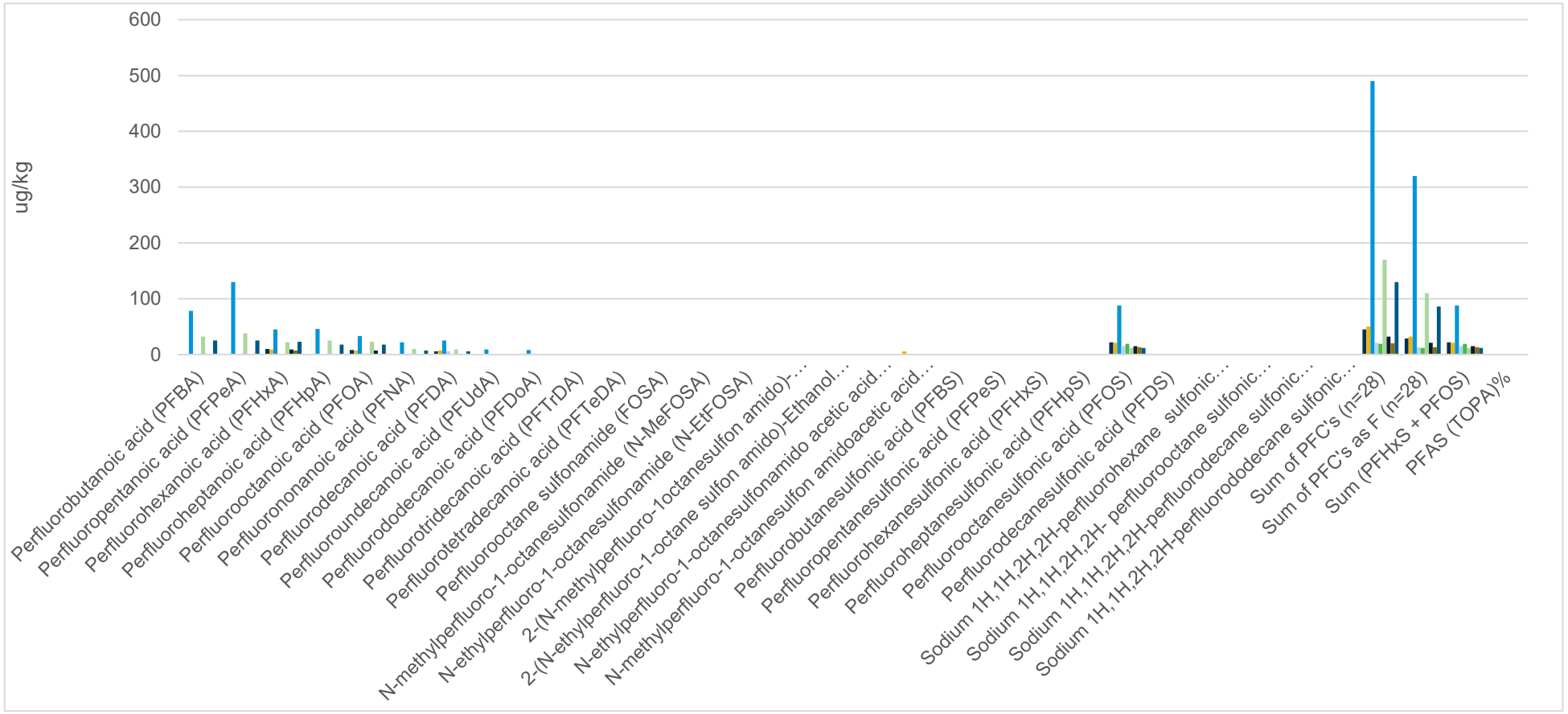


Figure 6-1: Source 1 PFAS concentrations

Figure 6-1 is an overview of the PFAS concentrations found in the biosolids from Source 1. The PFAS concentration found in these biosolids would not meet the criteria to apply to residential land with garden-accessible soil. The Source 1 biosolids average sum of PFHxS + PFOS was 17ug/kg and PFOA was 5ug/kg. Therefore, the PFAS concentration exceeds the trigger values for the use in residential areas and would need to be closely regulated.

Figure 6-2 is an overview of the PFAS concentrations found in the biosolids from Source 2.

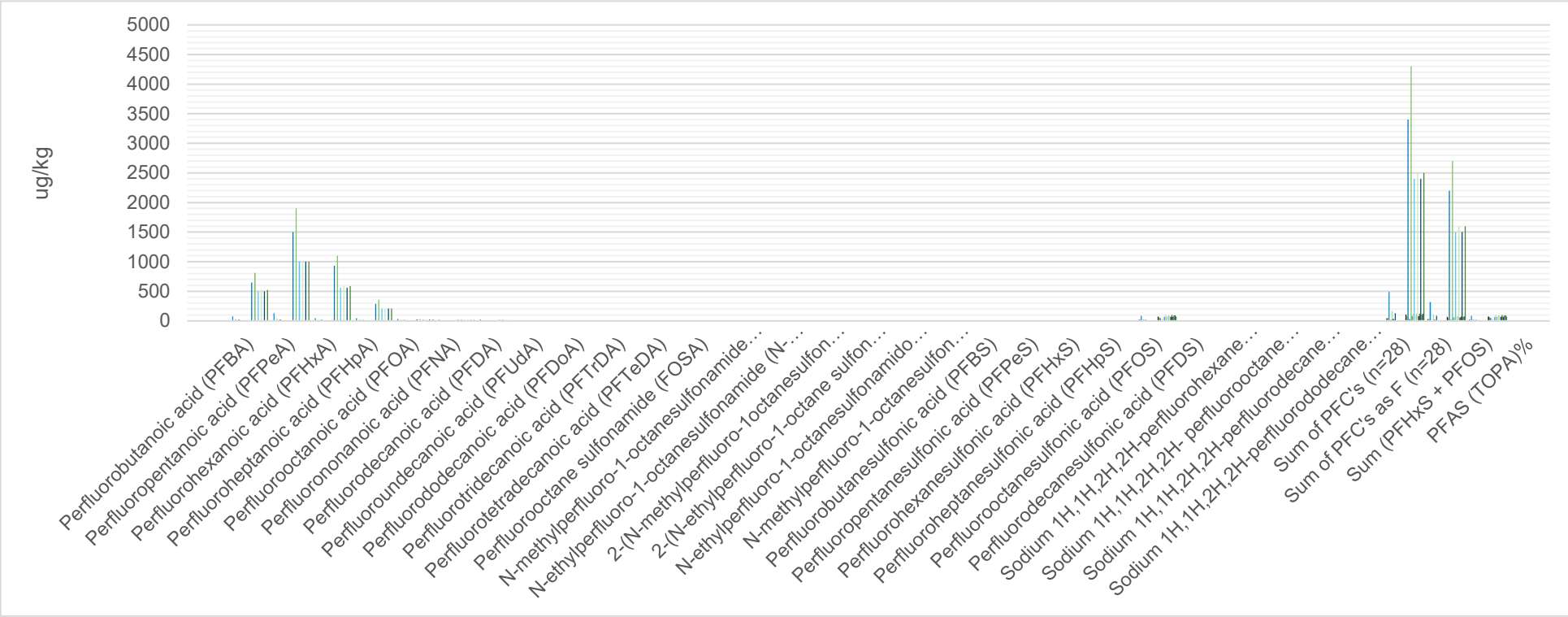


Figure 6-2: Source 2 PFAS concentration

Figure 6-2 is an overview of the PFAS concentrations found in the biosolids from Source 2. The PFAS concentration found in these biosolids would not meet the criteria to apply to residential land with garden-accessible soil. The PFAS concentrations for Source 2 biosolids are even higher than Source 1 and would not be meet the criteria to apply to public open spaces. Source 2 biosolids average sum of PFHxS + PFOS was 38ug/kg and PFOA the was <5ug/kg. Therefore, the PFAS concentration exceeds the trigger values for the use in residential areas and public open spaces and would need to be closely regulated.

6.2 Loganholme and Beenleigh WWTP PFAS concentrations

Figure 6-3 illustrates a direct comparison of biosolid samples from Loganholme and Beenleigh WWTPs.

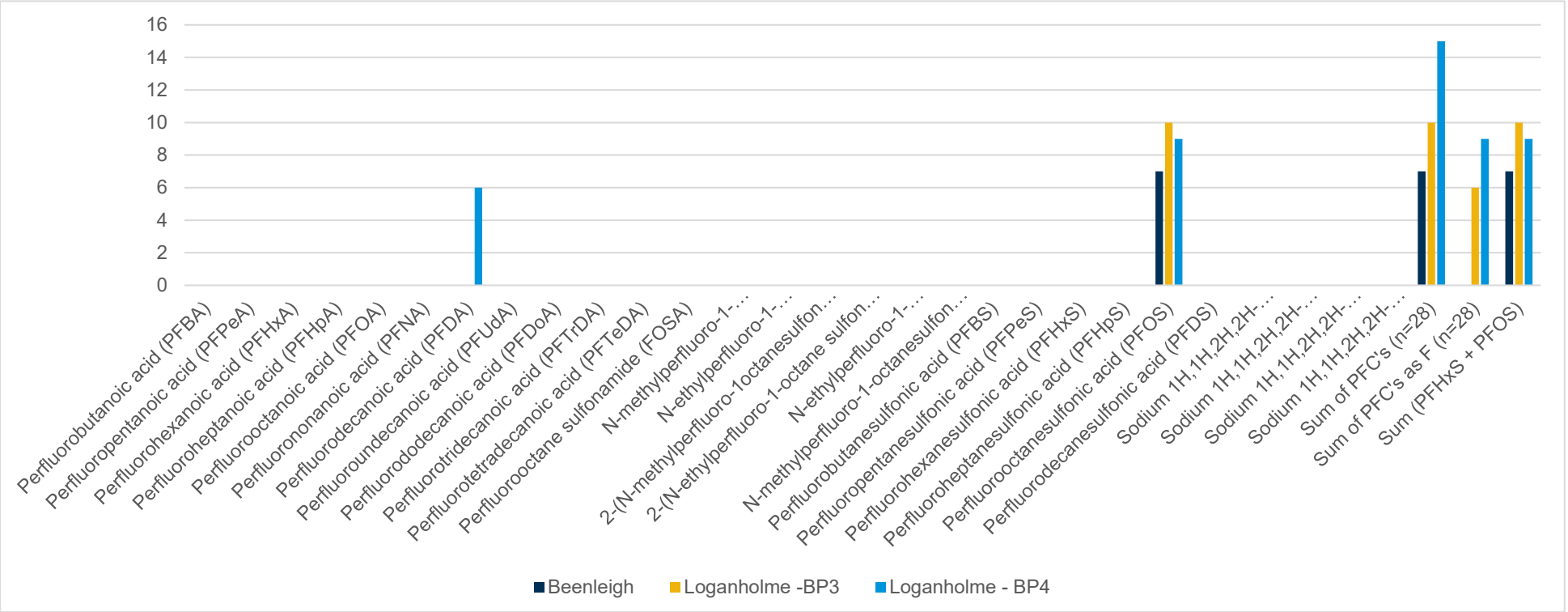


Figure 6-3: Loganholme and Beenleigh WWTP PFAS concentrations

Figure 6-3 is an overview of the PFAS concentrations found in the Loganholme and Beenleigh WWTP biosolids. The sum of PFHxS + PFOS for Loganholme WWTP belt press 3 (BP3) was 10 ug/kg and for belt press 4 (BP4) 9 ug/kg and PFOA was <5ug/kg for both sets of samples. For Beenleigh WWTP, the sum of PFHxS + PFOS was 7 ug/kg and PFOA was also <5 ug/kg. Therefore, the PFAS concentration, although within the detection levels, does not exceed the trigger values for soil application and, in its current form, is within the range for residential use.

6.3 Biochar and PFAS

Once the biochar was processed through the demonstration plant, the sum of all (PFHxS + PFOS) or PFAS TOPA% for Source 1 biosolids was <0.5ug/kg or not detectable (in four samples in total).

For Source 2, 24 biochar samples were analysed for the TOP assay method and PFHxS + PFOS was 2ug/kg and PFOA was also <0.5ug/kg. It is assumed that one of the reasons for the detection of PFAS in the biochar samples is that characteristics of the Source 2. Being processed thorough a 'pelletiser' dryer means that the biosolids are produced as spherical pellets, which are quite dense and, in some cases, have quite large particle sizes. This size is not conducive to being able to be processed and fully charred through the hearth. Sampling from the system was also difficult; however, Figure 6-4 are photos of the samples were received by the laboratory.



Figure 6-4: Source 2 pellets sizes produce biochar that is not fully charred

This is not the process that would occur in the full-scale facility due the use of the belt dryers, which produces more of a crumb rather than a pellet. All particle sizes would be within the range of 3–10mm. For corrective action, the bags of biochar were resampled two weeks later, which is out of holding time. However, but all these samples produced lower results and the average of sum was 1 ug/kg, which illustrates the importance of particle size consistency.

During the gasification process, all the PFAS compounds are made volatile in the hearth and then they progress through to the oxidiser. Therefore, the biochar does not contain PFAS at concentrations that would trigger the soil application limits and is a better overall product that will stop the bioaccumulation process in the environment.

6.4 Mass valance for PFAS

Table 6-3 is a mass balance that incorporates all data from all the sources: biosolids samples from both Source 1 and Source 2; the scrubber water (in the full-scale facility this water will return to the inlet works or be used as quenching water); and the stack (three full air emissions analyses). The oxidisers were incorporated in the air emissions analysis to give guidance on whether one or two oxidisers were required in the full-scale facility. The results show that only one oxidiser is required for each gasifier.

Table 6-3: PFAS mass balance

Perfluoroalkyl substances (PFAS) mass balance											
Analytes	**Biosolids**		**Biochar**		**Oxidisers**		**Scrubber**		**Stack**		**% Destruction**
	µg/kg	**µg/hour**	**µg/kg**	**µg/hour**	**g/min**	**µg/hour**	**µg/L**	**µg/hour**	**g/min**	**µg/hour**	
Perfluorobutanesulfonic acid	0	0	0	0	0.00000008	0.001	0	0	0.000000019	1.1259	
Perfluoropentane sulfonic acid (PFPeS)	0	0	0	0	0.00000008	0.001	0	0	0.000000011	0.6803	
Perfluorohexane sulfonate (PFHxS)	9	3654	0	0	0.00000008	0.001	0	0	0.000000021	1.2324	**100**
Perfluoroheptane sulfonate (PFHpS)	0	0	0	0	0.00000008	0.001	0	0	0.000000011	0.6803	
Perfluorooctane sulfonate (PFOS)	32.1	13482	2.8	1176	0.00000012	0.002	0	0	0.000000064	3.8578	**91**
Perfluorodecanesulfonic acid (PFDS)	1.2	504	0	0	0.00000016	0.003	0	0	0.000000023	1.3606	**100**
Perfluorobutanoic acid	1.3	546	0	0	0.00000048	0.008	0	0	0.000000284	17.0355	**97**
Perfluoropentanoic acid (PFPeA)	0	0	0.2	84	0.00000038	0.006	0.0022	2.42	0.000000384	23.0351	
Perfluorohexanoic acid (PFHxA)	8.7	3654	0	0	0.00000044	0.007	0.0008	0.88	0.000000077	4.6219	**100**
Perfluoroheptanoic acid (PFHpA)	0	0	0	0	0.00000027	0.005	0	0	0.000000104	6.2117	
Perfluorooctanoic acid (PFOA)	1.5	630	0	0	0.00000122	0.020	0	0	0.000000957	57.4321	**91**
Perfluorononanoic acid (PFNA)	0	0	0	0	0.00000024	0.004	0	0	0.000000060	3.6136	
Perfluorodecanoic acid	5.3	2226	0	0	0.00000049	0.008	0	0	0.000000071	4.2316	**100**
Perfluoroundecanoic acid (PFUnA)	0	0	0	0	0.00000052	0.009	0	0	0.000000071	4.2316	
Perfluorododecanoic acid (PFDoA)	0	0	0	0	0.00000047	0.008	0	0	0.000000057	3.4016	

Perfluorotridecanoic acid (PFTrDA)	0	0	0	0	0.00000044	0.007	0	0	0.000000057	3.4016	
Perfluorotetradecanoic acid (PFTeDA)	0	0	0	0	0.00000203	0.034	0	0	0.000000283	17.0079	
4:2 Flurotelomersulphonate	0	0	0	0	0.00000008	0.001	0	0	0.000000011	0.6803	
6:2 Fluorotelomersulphonate	0	0	0	0	0.00000011	0.002	0	0	0.000000180	10.7836	
8:2 Fluorotelomersulphonate	0	0	0	0	0.00000010	0.002	0.0056	6.16	0.000000134	8.0422	
10:2 Fluorotelomersulphonate	0	0	0	0	0.00000008	0.001	0	0	0.000000043	2.5596	
Perfluorooctane sulfonamide	0	0	0	0	0.00000039	0.007	0	0	0.000000057	3.4016	
N-Methyl-heptadecafluorooctane sulphonamide	0	0	0	0	0.00000039	0.007	0	0	0.000000057	3.4016	
N-Ethyl-heptadecafluorooctane sulphonamide	0	0	0	0	0.00000039	0.007	0	0	0.000000057	3.4016	
N-Me perfluorooctanesulfonamid oethanol	0	0	0	0	0.00000039	0.007	0	0	0.000000057	3.4016	
	0	0	0	0	0.00000197	0.033	0	0	0.000000283	17.0079	**94**

Table 6-3 shows that the overall mass balance for PFAS destruction through the process of gasification facility, which is 94% destruction of this persistent organic pollutant. This verifies that the gasification process when carbonisation occurs of the biosolids PFAS becomes volatile and is transferred to the off gas and is then destroyed in the thermal oxidisers. There was cross contamination seen when the biosolids particles sizes were too large and dense to fully carbonise and this carry over is not expected when processed through the belt dryers in the full-scale facility.

6.5 Microplastics

Microplastics are plastics smaller than 5 mm and they fall into two broad types:

- Primary-intentionally produced plastics, for example microbeads from toothpaste or glitter from cosmetics
- Secondary-resulting plastics from the weathering of larger plastics such as microfibres, that may cause adverse effects in organisms and ecosystems.

In Australia, between 2800 and 19,000 tonnes of microplastics are applied to agro-ecosystems each year through biosolids. Figure 6-5 shows examples of microplastics found in biosolids.

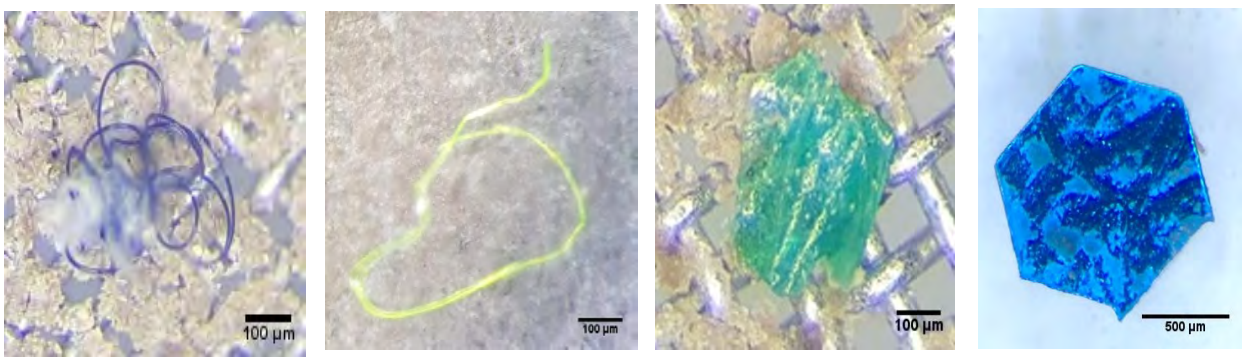


Figure 6-5: Micro and nano-plastics: microfibers, fragment (100µm), glitter (500µm)

The Source 1 biosolids contained a high proportion of microplastics. On average, the biosolids contained 8.8 particles/gram. Figure 6-6 shows the size of microplastics found in the biosolids and after they had been treated through the gasification process.

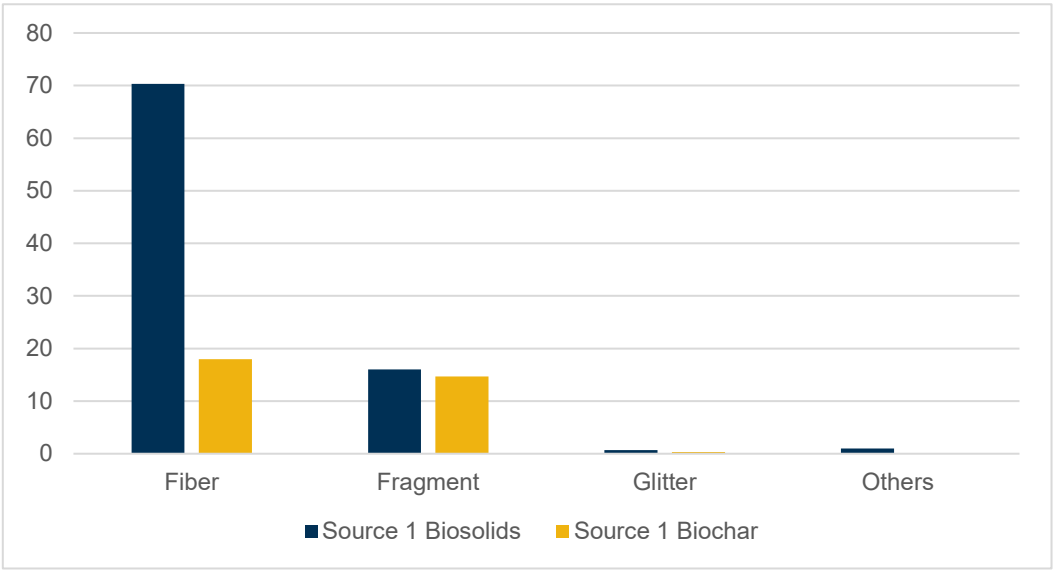


Figure 6-6: Source 1 types of microplastics in the biosolids and biochar

Figure 6-7 illustrates the particle types found in the Source 1 WWTP samples and after the treatment through the gasification system. It shows the gasification process reduces the microplastic plastic concentration; 1mm (81% less), 250µm (72% less), 150µm (60% less) and 63µm (35% less).

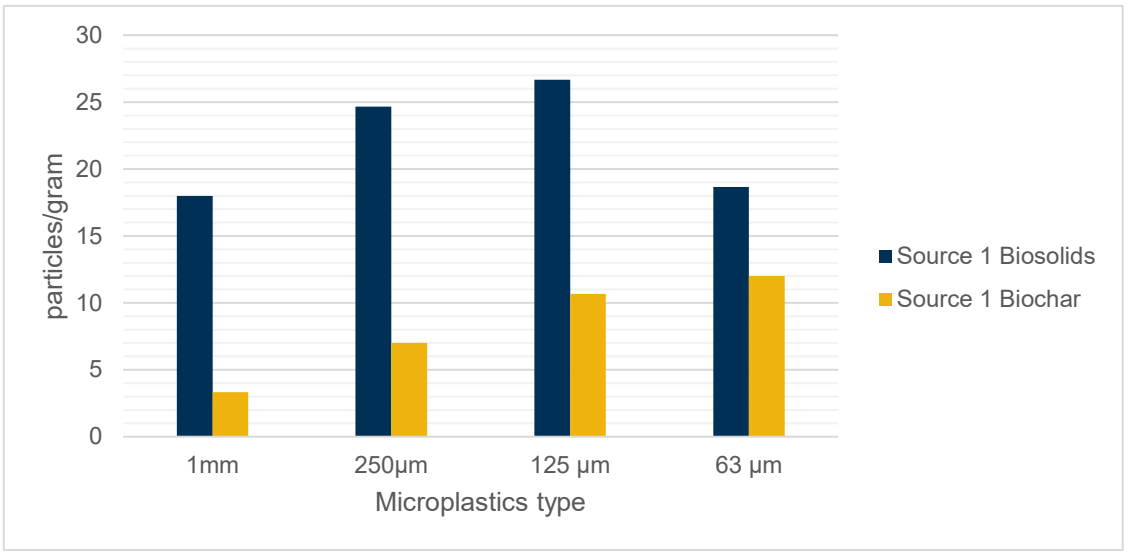


Figure 6-7: Source 1 microplastic types in the biosolids and biochar

Figure 6-7 shows that the gasification process destroys more of the fibre type microplastics with a 74% destruction rate compared with fragment (8%), glitter (50%) and others (100%). This emerging contaminate of concern can be significantly reduced through the gasification process.

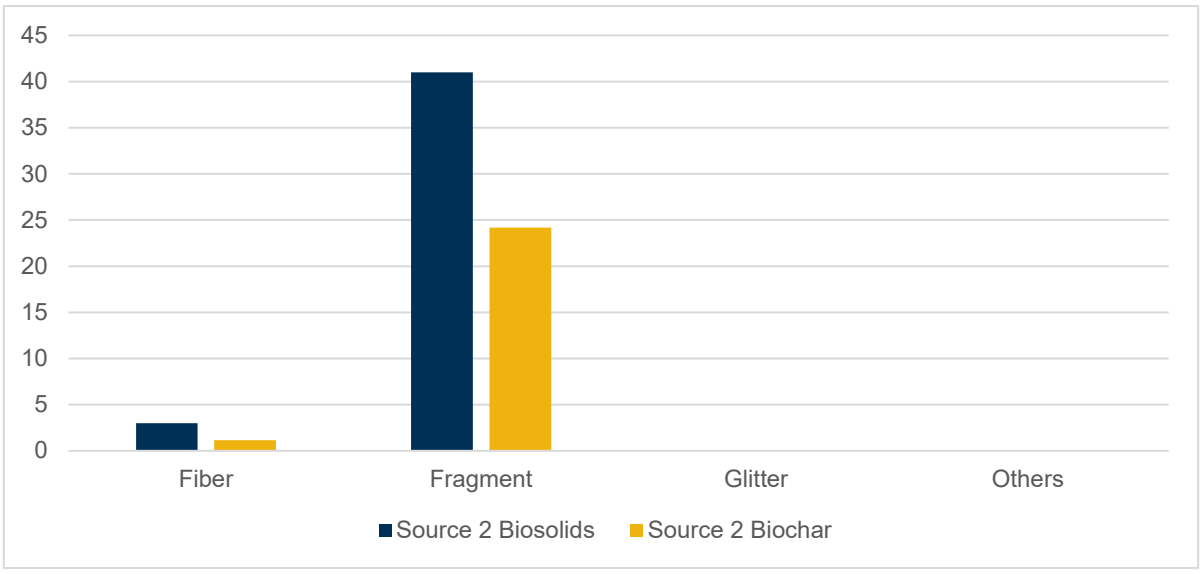


Figure 6-8: Source 2 particle size of microplastics in the biosolids and biochar

Figure 6-8 illustrates the particle types found in the Source 2 biosolid samples and after treatment through the gasification process. It shows the gasification process reduces the microplastic plastic concentration: 1mm (39% less); 250µm (48% less); 150µm (29% less); and 63µm (36% less). These destruction limits are less then Source 1, but these results are to be expected given the larger, denser particle size of Source 2 biosolids.

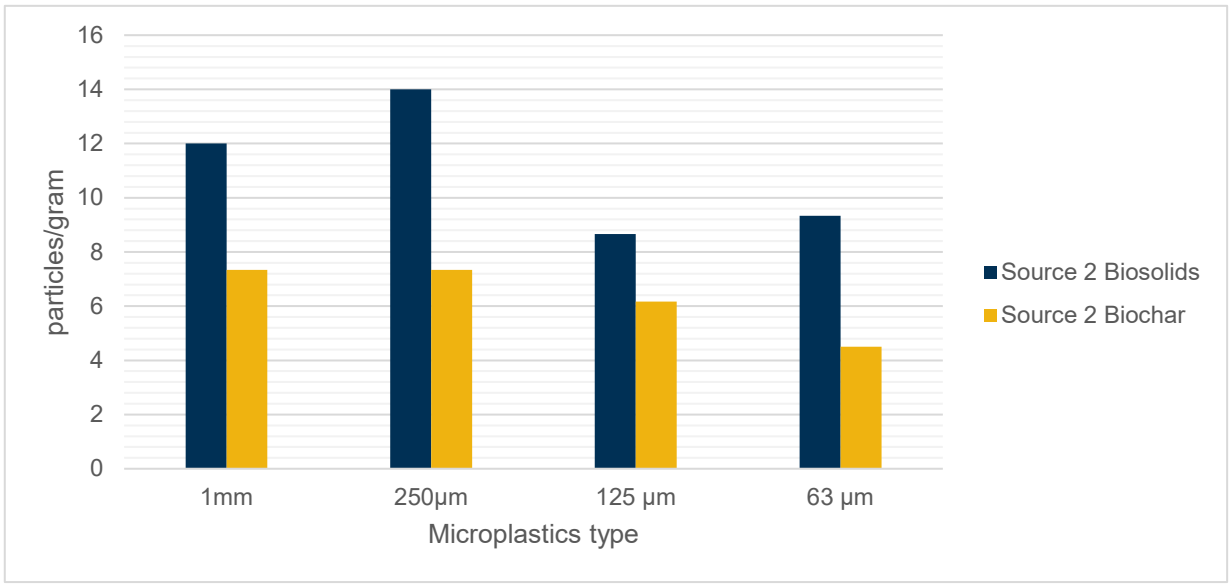


Figure 6-9: Source 2 microplastic types in the biosolids and biochar

Source 2 biosolids was small and dense because it is pelletised and not what is expected out of full-scale facility design of the belt dryers, which is more of a uniformed crumb. Also, even though the Source 2 biosolids is pelletised, they are not as dense and tightly bound and far easier to be crushed and more representative of what would be expected in the Loganholme WWTP full-scale facility. Therefore, it is expected that the microplastic destruction would be greater when processed through the belt dryer than what is illustrated in the Source 1 and 2 biosolids. Figure 6-9 shows that the gasification process does seem to destroy more of the fibre type microplastics with a 61% destruction rate compared with 41% fragment types.

The Loganholme WWTP biosolids have microplastics of around 3.3 particles/gram. Figure 6-10 shows the size and type of microplastics found in the Loganholme WWTP sludge.

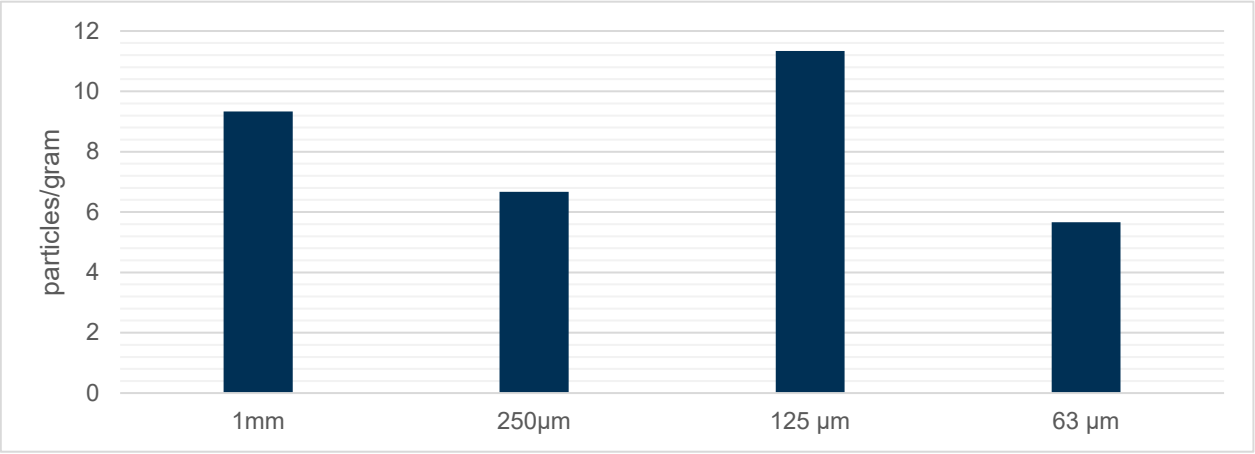


Figure 6-10: Loganholme WWTP particle size of microplastics in the biosolids and biochar

Figure 6-11 show a representative sample of biosolids from the Loganholme WWTP illustrating the types of microplastics found.

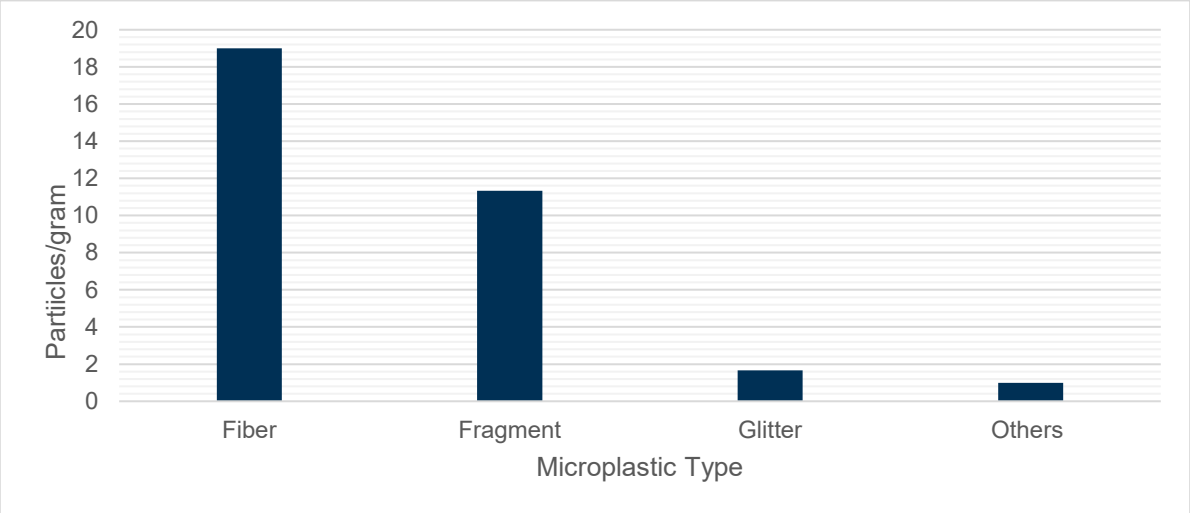


Figure 6-11: Loganholme WWTP microplastic types in the biosolids and biochar

As shown in Figure 6-11, Loganholme WWTP biosolids, like Source 1 biosolids, mostly contained fibre and fragment types of microplastic, as well as glitter. Overall, Loganholme WWTP biosolids had 63% less plastics than Source 1. This could be due to the dry concentration of the Source 1 biosolids.

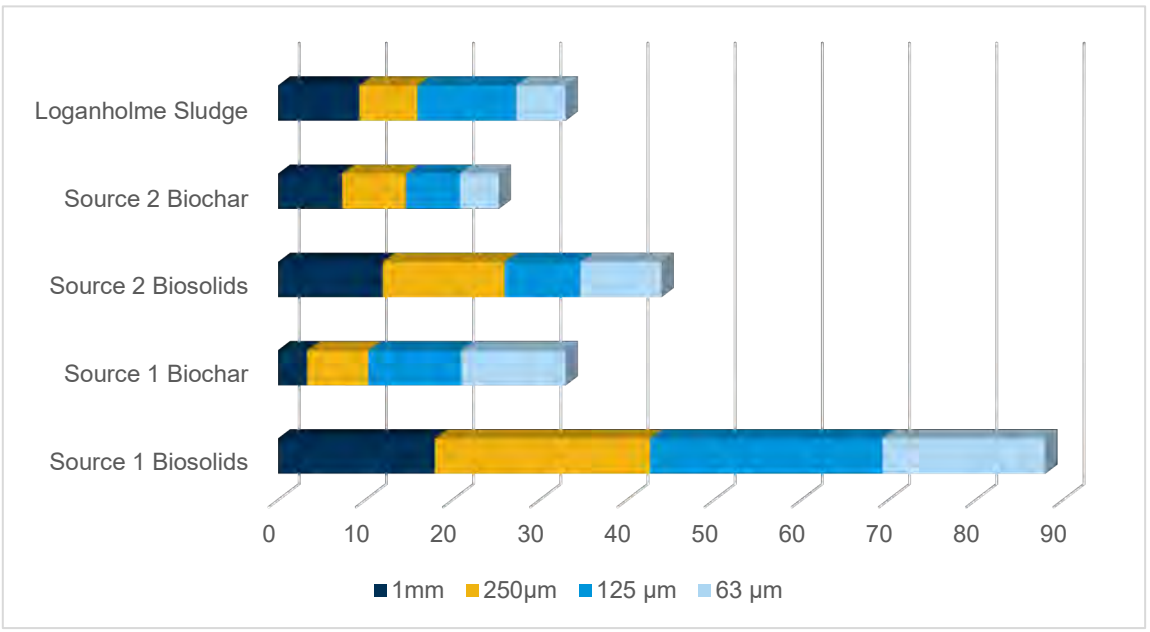


Figure 6-12: Microplastics particle size comparison across all sources of biosolids

Figure 6-12 shows that microplastics are present in the biosolids from all WWTPs; however, it also shows that Source 1 has a significant proportion compared to Loganholme WWTP and Source 2. Overall, the destruction of microplastics through the gasification process is around 60% when the source material has been through a pelletising drying process. Therefore, processing biosolids through a belt dryer that produces a less condensed source material would potentially mean a higher destruction rate of microplastics. This is an area to investigate once the full-scale facility is implemented in the Loganholme WWTP to determine the destruction rates.

7. Heat Balance

This section is an overview of how the demonstration plant addresses its objective for the heat balance for an integrated drying facility.

7.1 Background

The unit supplied for the demonstration plant incorporated a steam boiler and condensing unit to capture and measure heat generation and recovery (Pipatmonomai, Kaewluan, & Vitidsant, 2009) (Solarte-Toro, Chacon-Perez, & Cardona-Alzate, 2018). Hot flue gases from the thermal oxidiser passed through a steam boiler before being discharged to a wet scrubber.

For the demonstration plant, the steam was re-condensed via a heating coil submerged in a water bath to allow the recovered heat to be measured. Condensate was returned to the steam boiler. Plant service water used for the water bath drained back to the treatment plant. This was a controlled exercise and the scrubber water. In the full-scale facility, the flue gas will be used to heat a water circuit in a hot water generator rather than a steam boiler. The hot water set at 90–95 °C will exchange heat into a separate dryer water circuit that returns at 70–75 °C.

A key objective of the integration of dewatering, drying and gasification is that the overall process is heat-energy neutral. This effectively means that sufficient heat needs to be recovered from that produced in the gasification process to operate the dryer without the need for an additional fuel source during normal operation. The heat balance of the overall integrated drying–gasification process is mostly influenced by four parameters:

1. **Calorific value of the biosolids:** This is related to the volatile solids content of the biosolids and is inherent to the treatment plant and, therefore, not able to be adjusted in design or operation.
2. **Biosolids dewatering performance:** Mechanical removal of water via centrifugal dewatering is more efficient than evaporation and significantly impacts the heat required for drying. The initial dewatering step is designed to achieve 20% w/w dry solids (target) with 18% w/w minimum.
3. **Thermal efficiency of the dryer:** Measured as kW of heat input per tonne of water evaporated, a lower figure represents higher efficiency. This is a fundamental characteristic of the type of dryer.
4. **Operating temperature of the dryer:** A lower operating temperature allows for greater heat recovery from the gasification process flue gas as the final flue gas temperature can be reduced. The operating temperature range is inherent to the type of dryer.

Table 7-1: Calorific values for Source 2 and Loganholme and Beenleigh WWTPs

Calorific value	Unit	Loganholme WWTP	Beenleigh WWTP	Source 2
Net	MJ/kg DM	18.80	18.4	19.26
Gross	MJ/kg (calc)	2.25	2.20	2.23

Table 7-1 compares the calorific value of Source 2 biosolids to those from Loganholme WWTP and Beenleigh WWTP. Although Beenleigh WWTP is lower than Loganholme WWTP, once the sewage diversion is in place Beenleigh WWTP will be closer in value to the Loganholme WWTP values as calorific value is determined by the process stream. This shows that the Loganholme WWTP biosolids calorific value is 98% to that of Source 2 and therefore very similar results in heat energy recovery are expected. Table 7-2 is the calculated energy recovery based on the Source 2 biosolids passing through the gasification facility.

The heat balance on the Source 1 biosolids was inadequate to the extent that the radiant heat exchanger on the thermal oxidiser had to be partially disabled to allow adequate oxidiser temperatures to be maintained. The heat balance on Source 2 biosolids is outlined in Table 7-2.

Table 7-2: Summary of unit operations performance of the demonstration plant

Parameter	Value	Notes
Biosolids feed rate	480 kg/hr	85% of design feed rate (565 kg/hr) 74% of maximum continuous feed rate (650 kg/hr)
Heat recovered at radiant heat exchanger (integrated with the Thermal Oxidiser)	350 kW	Median value at 800–820 °C oxidiser temperature. Scales upwards with oxidiser temperature.
(a) Heat recovered by radiant heat exchanger scaled from 85% feed rate to 100%	0.4 MW	350 kW x 100/85
Heat recovered from flue gas heat recovery steam generator	780 kW declining to 600 kW	Median value at 540 °C inlet temperature. Declining heat transfer efficiency over 48 hours.
Flue gas heat exchanger outlet temperature (T_{out})	185 °C to 265 °C	Higher temperature reflects loss of heat transfer efficiency. Much of the loss of heat transfer efficiency occurred over a period of 24–48 hours, with a slow ongoing decline after that.
Heat recovery steam generator efficiency	66% declining to 51%	Calculated from (Tin-Tout)/Tin. Tin = 540 °C.
Heat recovered from flue gas scaled to 80% heat recovery.	941 kW	Heat recovered @ 80% = HR @ 51% efficiency x 80/51 This is a prediction based on implementation of a flue gas to hot water heat exchanger in the permanent plant with on-line soot blowing provisions to maintain performance.
(b) Heat recovered from flue gas scaled from 85% rate to 100%	1.1 MW	941 kW x 100/85
Forecast heat recovery in permanent system at design rate (565 kg/hr)	1.5MW	(a) + (b). The nominal requirement is 1.4MW per processing train. The expected performance exceeds the requirement; however, the design margin is minimal (less than 10%) and sensitive to the dewatering performance. Therefore, Pyrocal recommends the implementation of a condensing heat exchanger, located downstream of the main flue gas heat exchanger. The heat/temperature available at this point should be suitable for 150-200 kW of pre-heating for the water to the main flue gas heat exchanger. This would raise the design margin for the heat balance to 20%.

8. Operating Costs

The operating costs of the plant are dominated by the consumption of process additives for equipment protection and emissions control, and electrical consumption. The chemical additions required are an alkali such as magnesium hydroxide ($(Mg(OH)_2)$) for hot corrosion control and SOx/metals capture and an ammonia source such as urea for NOx reduction.

Electrical consumption was measured at 42–45 kW, at a power factor of -0.9 and a rate of 480 kg/hr, scaling directly with throughput at rates exceeding 33% of design throughput. This equates to 94kWh per tonne of biosolids feed.

Initially, hydrated lime ($Ca(OH)_2$) was used as the alkaline chemical additive. While this solution worked, the reliability of handling was poor in a weather-exposed environment. Hold-ups in the metering hopper were experienced. Lime powders are well known for this behaviour. In addition, accumulation of insoluble calcium compounds was observed in the scrubber and while the accumulation did not cause an operational issue, the issue could be foreseen. Therefore, a high concentration sodium hydroxide solution was trialed and then a magnesium hydroxide ($Mg(OH)_2$) slurry. Of the two, magnesium hydroxide was considered the best option because it caused no accumulation of precipitates in the wet scrubber and is safer to handle than sodium hydroxide.

A 50% urea solution was injected into the gas stream as a source of ammonia for NOx reduction. Two different addition points were trialed, with addition after the oxidiser providing the best performance.

The demonstration plant not only allowed optimisation of the choice of chemicals but also the ability to determine the final consumption rates.

8.1 Urea

When starting the demonstration plant, the urea dosing rates were around 40 L/tonne of dry biosolids of 50% w/w urea solution, subject to increases up to 180 L/tonne of biosolids if high carbonisation temperatures were used (greater than 700 °C). This was significantly optimised throughout the demonstration and Table 8-1 shows dosing rates expected for the full-scale facility.

Table 8-1: Urea dosing rates

	1 unit	2 units
Throughput	575 kg/h	1150 kg/h
Urea concentration	0.4%	0.4%
Dosing rate	2.3 kg/h	4.6kg/h
	12L/h	24 L/h
Dosing location	Spear of the inlet of the oxidisers	

Table 8-1 shows that the dosing rate has been optimised from 40–180 L/dry tonne to around 20L/dry tonne. This is a significant decrease in the amount of urea required for mitigation of NOx. Table 8-1 also shows the calculated dose rates for ultimate design capacity.

8.2 Magnesium hydroxide

Initially, the demonstration plant was consuming up to 30 litres per tonne of dry biosolids of $Mg(OH)_2$ 55% w/w slurry. This was primarily influenced by dust release rate (system throughput), i.e. higher dust rates resulted in lower alkali requirement, because the carbon dust captured in the scrubber was mildly alkaline with a pH in water of approximately 8.5. Table 8-2 calculates the dosing for ultimate design capacity.

Table 8-2: Magnesium hydroxide dosing rates

	1 unit	2 units
Throughput	575 kg/h	1150 kg/h
$Mg(OH)_2$ concentration	1.20%	1.20%
Dosing rate	6.9 kg/h	13.8kg/h
	4.5 L/h	9L/h
Dosing location	Suction of wet scrubber recirc pump (75%) Screw feeding the Hearth (25%)	

Table 8-2 illustrates that the dosing rate has been optimised from 30 L/dry tonne to around 4.5L per dry tonne. This is a significant decrease in the amount of urea required for mitigation of NOx.

8.3 Diesel

Diesel should also be accounted for in the operational. At start-up, each hearth requires around 100L per train—two gasifiers would require 200L of diesel each start-up. Overall, the full-scale facility requires continuous running for two weeks with two days shutdown. Therefore, the diesel consumption would be around 400L per month.

8.4 Boiler shield

The boiler shield is a required chemical that is used in a closed loop. However, every two weeks, 25L would have to be dosed as a top-up as part of the maintenance scheduled shutdown periods.

9. Conclusion

The Loganholme WWTP Biosolids Gasification Demonstration Plant has proven to be an innovative solution to a problem facing WWTPs that are managing biosolids: increasing energy costs, haulages rates and emerging contaminants of concern. The demonstration plant confirmed that gasification of biosolids is a reliable process that can be incorporated into a WWTP.

Each of the objectives set for the demonstration plant in terms of reliability, air emissions, persistent organic pollutants, heat balance and operating costs were achieved.

Therefore, it is recommended to proceed with the full-scale Biosolids Gasification Facility at Loganholme WWTP.

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Appendix A: Char Characteristics, Potential Value and Reuse Options

TO WHOM IT MAY CONCERN

I Trevor Bridle, of Bridle Consulting, confirm that I am the author of the report, "Task 2, Char Characteristics, Potential Value and Reuse Options", dated October 2020, prepared to Logan City Council.

Signed:

Trevor Bridle

Date: 8th December, 2020

TASK 2: CHAR CHARACTERISTICS, POTENTIAL VALUE AND REUSE OPTIONS

VALUE AND REUSE OPTIONS

Prepared for

Water Infrastructure
Logan City Council

Prepared by

BRIDLE CONSULTING

October 2020

LIST OF ACRONYMS

BoD	Basis of Design
DS	Dry Solids
Dry tpa	Dry tonnes per annum
Dry tpd	Dry tonnes per day
GCV	Gross Calorific Value
HM	Heavy Metal
K	Potassium
N	Nitrogen
P	Phosphorous
PFAS	Poly Fluoro Active Substance
PFC	Per Fluoro Compounds
TS	Total Solids
VS	Volatile Solids
Wet tpd	Wet tonnes per day
WWTP	Wastewater Treatment Plant

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1. Introduction

After the successful full-scale demonstration of the Pyrocal gasification process earlier this year Logan City Council is now proceeding with a commercial gasification facility at their Loganholme WWTP. The Basis of Design (BoD) for this facility, which includes Mass and Energy Balances, has been developed by Logan Water. The full-scale demonstration work was done using dried sewage sludge from Source 1 and Source 2 WWTPs since no dried sludge was available from any of the Logan Water WWTPs. Bridle Consulting was contracted by Logan City Council to estimate char characteristics that would be generated from Loganholme biosolids, based on the data generated from the demonstration trials and comment on the potential value and uses of the char.

2. Information Provided

Logan City Water provided the following documents to Bridle Consulting to conduct this Char Reuse Review:

1. DT 007 Loganholme Biosolids Treatment Process Mass Balance, Rev 3 xlsx.
2. Excel Spreadsheet named “Copy of Merge”. This spreadsheet included analytical data of the Loganholme, Source 1, and Source 2 biosolids as well as char analytical results from the Source 1 and Source 2 gasification trials.

3. Estimation of Loganholme Char Quality

The quality of the char that will be produced from gasification of Loganholme biosolids has been estimated based on the following information:

- Loganholme biosolids analytical data provided by Logan Water,
- The design char yield of 35% as reported by Logan Water,
- The classification of NPK from biosolids to char, based on published data,
- The classification of heavy metals from biosolids to char, based on results reported by Logan Water from the gasification trials, and,
- The destruction of PFCs as reported via the gasification trials. Estimates of PFAS destruction is by Bridle Consulting.

A summary of the average gasification trial results for the four runs using Source 1 biosolids and the eighteen runs using Source 2 biosolids are shown in Table 3-1.

Table 3-1: Average gasification trial results

Parameter	Units	Source 1		Source 2	
		Biosolids	Char	Biosolids	Char
VS	%	70.7	36.3	72.3	38.3
Ash	%	29.3	63.7	27.7	61.7
Calculated char yield	%		46.00		44.9
As	mg/kg	<5	<5	8.1	4.4
Cd	mg/kg	<1	<1	<1	1.4
Cr	mg/kg	19	40.3	32.3	73.1
Cu	mg/kg	157	252.5	331	613
Pb	mg/kg	8.2	17.3	11.5	26.4
Hg	mg/kg	0.1	<0.1	0.4	<0.1
Ni	mg/kg	13.7	16	16.1	31.3
Se	mg/kg	<5	<5	<5	<5
Zn	mg/kg	360	765	569	1311
PFAS	ug/kg	22			
Total PFCs	ug/kg	32.7	<5	57	<5

The average char yield for the Source 1 and Source 2 runs are calculated because all the ash in the biosolids is conservative and is classified (transferred) to the char.

A wealth of information on the fate of heavy metals in biosolids during gasification and pyrolysis has indicated that volatile and semi-volatile metals such as mercury, arsenic and cadmium are vaporised during the process. All the mercury is vaporised and much of the cadmium and a lesser amount of arsenic are also vaporised and transferred to the syngas. All the other heavy metals are completely retained in the char.[1]

Based on this fact the theoretical char concentrations of the non-volatile heavy metals have been calculated, based on measured char yields for the Source 1 and Source 2 gasification trials and these are reported, together with measured metal values, in Table 3-2.

Table 3-2: Calculated and measured char heavy metal values

Heavy Metal	Units	Measured/Theoretical Char HM Value			
		Source 1		Source 2	
		Measured	Theoretical	Measured	Theoretical
Cr	mg/kg	40.3	41.3	73.1	71.9
Cu	mg/kg	252.5	341.3	613	737.3
Pb	mg/kg	17.3	17.8	26.4	25.6
Ni	mg/kg	16	29.8	31.3	35.9
Zn	mg/kg	765.0	782.7	1311	1267.4

[1] Bridle, T.R, Hammerton, I.F and Hertle, C.K, "Control of Heavy Metals and Organochlorines using the Oil-from-Sludge Process", Proceedings of the IAWPRC Sludge Management Conference, Los Angeles, January 1990.

As can be seen there is very good agreement between the measured and theoretical char HM values. Consequently, to estimate the expected char heavy metal concentrations from the full-scale Loganholme gasification facility the theoretical approach has been adopted.

The predicted char characteristics from the full-scale Loganholme gasification facility are shown in Table 3. These predicted char characteristics are based on the Logan Water Loganholme biosolids characteristics provided to Bridle Consulting and the following assumptions:

- A char yield of 35%, as provided by Logan Water,
- That about 55% of the biosolids N and all the P and K is classified to the char[2], and,
- Metal and contaminant classification and PFC/PFAS destruction as noted above.

It is recommended that Logan Water use these char characteristics when discussing char reuse options with Regulatory Agencies and potential char users.

Table 3-3: Predicted Loganholme Gasification Facility char characteristics.

Parameter	Units	Loganholme	
		Biosolids	Char
VS	%	81	47.2
Ash	%	19	52.8
Nitrogen	%	5	7.9
Phosphorus	%	2.3	6.6
Potassium	%	0.67	1.9
Estimated char yield	%		35
As	mg/kg	2.6	1.5
Cd	mg/kg	0.7	2.0
Cr	mg/kg	48	137
Cu	mg/kg	260	743
Pb	mg/kg	13	37
Hg	mg/kg	0.5	<0.05
Ni	mg/kg	210	600
Se	mg/kg	4.2	12
Zn	mg/kg	626	1789
PFAS	ug/kg	22	<0.2
Total PFCs	ug/kg	44.9	<0.45

[2] Bridle, T.R and Prichard, D., "Energy and Nutrient Recovery from Sewage Sludge via Pyrolysis", Proceedings of the IWA Conference, Resources from Sludge, Forging New Frontiers, Singapore, March 2004.

4. Potential Value of the Char

The char from biosolids gasification contains many valuable components, the major valuable constituents being the following:

- Phosphorus and Potassium (P and K). These macro plant nutrients are 100% bio-available and have been speciated in a slow-release form by the thermal gasification process[3]
- Fixed Carbonised Carbon that significantly enhances soil fertility. Research by the NSW Department of Agriculture has demonstrated up to a 30% increase in broad acre crop yields when char is applied at a rate of 10 tonnes per hectare on agricultural soil
- Minor nutrients and Trace Elements such as Sulphur, Boron, Iron, Molybdenum, Zinc, Cobalt and Copper that are vital for crop growth, and,
- Energy.

The Nitrogen in the char is not plant available and thus has no agronomic value.

The most valuable components in the char are the nutrients P and K. Based on the current prices for the fertiliser's urea ($450/t), mono-ammonium-phosphate ($800/t) and potassium chloride ($380/t) the current value of P is $2,900/t and the value of K is $199/t. Based on the predicted concentrations of P and K in the Loganholme char, the value of these two nutrients is $211/t char. The estimated Fixed Carbon content of the char is 36%. It is difficult to put a value on this fixed carbon, but it is likely to be in the range of $100 to $200/t. At a value of $150/t the value of fixed carbon in the char is thus $54/t char. **Thus, the overall value of the char, for reuse in agriculture, is likely to be, at minimum, $265/t.**

The other major valuable component in char is energy. It is estimated that the Loganholme char will have and energy content of 20 GJ/t. Based on the current Australian price for coal ($56/t), with an energy content of 28GJ/t, the energy value of coal is $2/GJ. **Thus, the overall value of the char, for reuse as an energy source, is likely to be $40/t.**

This analysis strongly indicates that maximum returns from char sales will be in the agricultural sector. This reuse option also provides the best environmental outcomes.

[3] Pritchard, D, Curtin University of Technology, "Nutrient Properties of Char", Research Report prepared for Environmental Solutions International Ltd, Perth, WA, September 2003.

5. Potential Uses for the Char

A number of potential uses for char from gasification and pyrolysis of biosolids have been previously identified and these are summarised below[4]:

1. Use in agriculture as a fertiliser supplement
2. Use as a fuel in boilers, power plants and other industrial combustors
3. Use as a fuel and feedstock in cement kilns
4. Use as an ingredient to produce lightweight/insulating bricks and pavers
5. Use as an industrial adsorbent in place of Activated Carbon
6. Use as a low-grade Activated Carbon for odour control, and
7. Use as a metallurgical reductant.

Use in Agriculture as a Fertiliser/Soil Supplement

This reuse option has the highest potential economic return for Logan City Council with the best environmental outcomes. There is sufficient scientific evidence of the significant benefit that is achieved by applying char as a fertiliser supplement to agricultural soils.

The major issue for Logan City Council will be in obtaining the necessary regulatory approvals for this reuse option. The current Queensland “End of Waste Code – Biosolids” (ENEW 07359617, December 2019) stipulates the quality of biosolids that can be applied to agricultural land. Provided that the biosolids meet contaminant and pathogen reduction requirements specified in the code the biosolids are not considered as waste but rather a resource which can be reused in agriculture. There are 3 grades of quality prescribed for the 3 reuse options, namely Unrestricted Use, Restricted Use 1, and Restricted Use 2. Based on the predicted char quality as shown in Table 3, the char would not meet the Nickel quality requirement of 270 mg/kg to allow Restricted Use 2 category. Consequently, broad acre agricultural reuse for the char would NOT be permitted in Queensland. It must however be noted that the Code only applies to biosolids and thus char is unlikely to be managed by the Code. However, this should be confirmed by Logan City Council.

Thus, the most likely option for agricultural reuse would be to blend the char with other components to produce a blended or compounded fertiliser/soil supplement, like the many commercially produced and bagged products currently available at gardening centers and hardware stores. This option should be explored by Logan City Council.

Use as a Boiler/Combustor Fuel

Many industrial boilers/combustors in Queensland use fuels other than coal, oil, or gas as their primary fuel. The char would be a very suitable alternate fuel for many of these industrial boiler/combustors. As mentioned previously, the potential value of this fuel would be $40/t char. The only issue regarding this reuse option would be obtaining environmental approval from the EPA. The issue would likely be the controls required to meet heavy metal, SOx, and NOx emission limits for the facility.

Use as a Fuel/Feedstock in Cement Kilns

The char has significant inorganic constituents (Si, Al, Ca etc.) that would add to clinker formation in the kiln. This would be an added benefit of using the char to supplement the kiln energy requirements. The value of char to cement manufacturing would be at least $40/t char. Due to the likely small contribution the char would make to kiln fuel requirements it is unlikely that any emission issues would be raised. Char has been used as a kiln feedstock in Europe.

[4] Bridle, T.R, “Sludge Pyrolysis: Has its Time Arrived”. Presented at the Bioenergy Australia Conference, Fremantle, December 2006.

Feedstock for Lightweight/Insulating Bricks and Pavers

This reuse option has been demonstrated and is in use in Europe where light weight/insulating bricks are routinely used in the construction industry. The char is mixed with clay and then fired in the kilns. The organics in the char are combusted leaving small pores in the finished bricks, providing the lightweight and insulating properties. Traditionally Styrofoam pellets are used, but they are now being replaced by char.

A full-scale production trial run of light-weight brick manufacture was undertaken in Germany using 1 tonne of char produced from a demonstration sludge pyrolysis unit[4]. This trail produced bricks of essentially the same quality as when using Styrofoam pellets. A picture of a brick from this manufacturing run is shown in Figure 1.

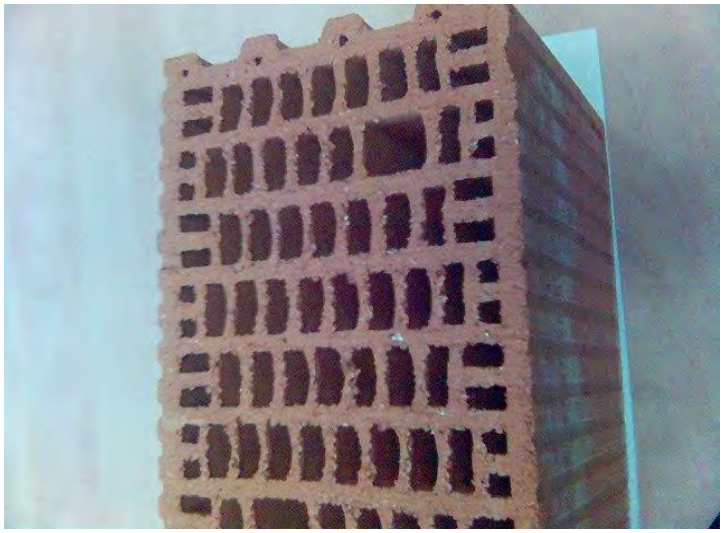


Figure 5-1: Light-weight clay brick made with char addition

Dried sludge has also been used in South Africa for the production of light-weight bricks. There is, as yet, no market for this in Australia but this reuse option is worthy of consideration by Logan City Council.

Use as a Low-grade Adsorbent to Replace Activated Carbon

Char from biosolids pyrolysis or gasification has significant adsorptive capacity and is equivalent to a low-grade activated carbon. Test-work in Germany has shown that char has a very good adsorptive capacity for heavy metals[5]. When the char was activated it had much superior adsorptive capacity for heavy metals than conventional Activate Carbon. This may be a very good use for the char, especially for treating heavy metal contaminated ground water and mining/industrial effluents.

Use as a Low-grade Activate Carbon for Odour Control

Due to its high adsorptive capacity the char would be a good alternative to conventional activated carbon for odour control applications. It would also me a good media to use in biological odour control filters commonly used in the sewage and industrial wastewater treatment industries.

Use as a Metallurgical Reductant

Char, with its relatively high carbon content could be used to replace commercial reductants used in the metallurgical processing industry. To Bridle Consulting's knowledge this, reuse option has not yet been evaluated.

[5] Skrypski-Maentele, S and T.R Bridle, "Environmentally Sound Disposal of Tannery Sludge", Wat. Res. Volume 29, No 4, 1995

6. Recommendations

Bridle Consulting recommends that Logan City Council evaluates, in more detail, the potential char reuse options identified in this report.

Reuse in the agricultural sector, as a blended soil supplement offers the maximum financial returns and also the best environmental outcomes. It is thus recommended that Logan City Council engages with the relevant Queensland Government Departments to identify any constraints on this reuse option. In addition, Logan City Council should contact the many commercial companies that operate in the blended fertiliser/soil markets to assess their interest in using char as one of the ingredients in their blended products.

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Drying reduces the total PFAS concentration in biosolids and alters the PFAS profile†

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While per- and polyfluoroalkyl substances (PFAS) are not actually generated at water resource recovery facilities (WRRFs), utilities are being forced to consider PFAS in biosolids management plans due to mounting political pressure and pending regulations. Emerging thermal technologies including pyrolysis, gasification, and super critical water oxidation have garnered recent attention for PFAS destruction. Drying, however, is a conventional technology that might also be a tool for utilities to manage PFAS in biosolids, but research on the impacts of drying on PFAS in biosolids is scarce. The objective of this research was to determine how drying affected the fate of PFAS in biosolids. Full-scale sampling was paired with lab-scale oven drying experiments to understand the impact of drying on measurable PFAS in biosolids. Overall, drying substantially reduced the total PFAS concentration in biosolids. PFAS removal during a full-scale facility's drying process matched the removal achieved when solids were taken from that facility and dried in a lab-scale oven instead, with average PFAS removal being approximately 80%. Precursors to perfluoroalkyl acids (PFAAs), primarily 5 : 3 fluorotelomer carboxylic acid (FTCA) and 6 : 2 FTCA, as well as perfluorooctane sulfonic acid (PFOS) were substantially reduced between pre-drying and post-drying triplicate samples. Additional lab-scale oven drying experiments corroborated that measurable PFAS were removed from biosolids collected from three different utilities. Drying experiments at 30 °C and 105 °C revealed that the PFAS profiles were similar, but PFAS concentrations were lower in the 105 °C samples compared to 30 °C samples. While more research is necessary to determine and validate the removal mechanism, drying could be a viable technology to reduce measurable PFAS levels in biosolids to concentrations below guidelines for land application.

Water impact

PFAS have substantially altered the biosolids management industry. Emerging technologies offer a means to reduce PFAS to non-detect levels in solid products such as biochar. Drying, however, is a widely employed technology that could sufficiently reduce PFAS levels to meet guidelines for land application.

1. Introduction

Per- and polyfluoroalkyl substances (PFAS) have altered the biosolids management landscape. Though water resource recovery facilities (WRRFs) are not actually a source of PFAS, they receive and convey PFAS because of the widespread use of PFAS in society and consumer products.[1] PFAS are abundant in a range of products including cosmetics, water-proof clothing, carpets, and fast-food wrappers.[2–5] PFAS are widely detected in people, including breastmilk, blood, and urine samples, and therefore municipal sewage also contains PFAS.[6–8] Moreover, consumer products laden with PFAS are sent to landfills where products degrade over time, and PFAS are released into leachate that is often sent to the head of a WRRF.[3,9,10] The concentration in biosolids depends on any nearby industrial sources of PFAS discharged to the WRRF as well as the unit operations used at the WRRF.[2,11–13] Some European countries, Australia, Canada, and states in the United States have set limits for PFAS concentrations in soil, and some states in the United States have set PFAS concentration limits for biosolids that can be land applied to minimize human health impacts.[7,14–20]

Biosolids management requires an understanding of how biosolids handling technologies impact the fate of PFAS. Emerging technologies, in particular thermal processes, have

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garnered attention for their potential to reduce PFAS concentrations in solids.[21,22] Indeed, pyrolysis has been shown to remove and transform PFAS at the lab and pilot scale.[23–29] Incineration, a more established technology, also removes the majority of PFAS from the solid phase.[30] Pyrolysis and gasification are still emerging technologies in the biosolids marketplace, and both of these technologies require drying first.[23,31] Drying, however, is already a widely used and established solids handling process. Aside from being useful when paired with emerging thermal technologies, drying offers the benefit of substantial mass reduction *via* removal of water. As numerous utilities already dry and land apply biosolids, it is important to understand the impacts of drying on the fate of PFAS in biosolids.

Limited studies have been conducted thus far regarding the potential impacts of drying on PFAS fate, and results have been mixed.[32–34] Indeed, heat treatment of wastewater solids at 480–650 °C led to an increase in total perfluoroalkyl acids (PFAAs) by 53%, mainly due to perfluorohexanoic acid (PFHxA) increases.[12] In another study, heat drying at 90–120 °C was found to increase PFAS concentrations, in particular the concentration of fluorotelomer phosphate diesthers (diPAPs).[32] A lab study on drying at 115 °C at 2 hours found that 6:2 diPAP approximately doubled; perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS) were similar before and after heat treatment; and perfluoropentanoic acid (PFPeA) decreased by approximately 50%.[32] One study on a full-scale dryer with a regenerative thermal oxidizer (RTO) found that 50–90% of PFAS were transferred from the biosolids to the gas phase, and 18 PFAS were detected in the gas phase headed for treatment in the RTO.[34] Despite widespread use of drying in the industry, limited studies are available that document the impacts of drying on the fate of PFAS in biosolids.

The objective of this research was to determine the impact of drying on PFAS in biosolids. As some government regulatory agencies move towards implementing concentration-based limits for PFAS in biosolids and soils, it is particularly important to know how drying processes impact measurable PFAS concentrations. This research combined sampling at a full-scale dryer with lab-scale oven drying experiments on the same full-scale pre-drying solids samples. Additionally, pre-drying solids samples provided by three different WRRFs were used for lab-scale oven drying experiments. Finally, lab-scale oven drying experiments were conducted on high moisture content (MC) samples at 30 °C and 105 °C.

2. Methods and materials

2.1. Comparison of full-scale drying to lab-scale oven drying on PFAS concentrations in biosolids

A WRRF agreed to provide pre-drying and post-drying samples from their full-scale dryer under conditions of anonymity. The average influent flow rate to this WRRF is over 50 million gallons per day (MGD). Liquid treatment steps include grit removal, primary clarification, conventional activated sludge, and chlorination. Solids undergo thickening and dewatering prior to drying. The total solids (TS) content for the pre-drying solids fed to the dryer was approximately 16% TS, and the TS content for the post-drying solids taken from the effluent of the dryer was approximately 90% TS. Triplicate pre-drying samples and triplicate post-drying samples were collected on-site from the full-scale dryer. In addition to full-scale sampling, pre-drying solids samples were dried in triplicate in a lab-scale oven at 105 °C for 5 hours to compare lab-scale oven drying to full-scale drying. All samples were sent to Eurofins Environment Testing USA (Eurofins) for PFAS extraction and analysis as described in section 2.4.

2.2. Lab-scale oven drying of solids from three different WRRFs

Solids from three different WRRFs were dried in a lab-scale oven to assess if PFAS removal was observed when drying different solids. The three WRRFs tested for this experiment were from different states than the WRRF described in section 2.1. Each WRRF requested to be kept anonymous and provided the following general information to provide context on their biosolids without revealing their identity. WRRF A receives less than 25 MGD of wastewater influent. Liquid processes include primary treatment and secondary treatment that has full nitrification and one train with anoxic zones for incidental denitrification. Primary sludge and thickened waste activated sludge (WAS) are sent to a mesophilic anaerobic digester. A portion of digested solids are sent to a screw press, and effluent samples from the screw press were collected for this work. The TS content was 18.3%. WRRF B is over 50 MGD and utilizes a two-stage anaerobic digestion process. The first stage is fed a mixture of primary sludge and WAS and has a solids retention time (SRT) of 2 days or less. The second stage has an SRT of 15–20 days. The digestate is processed through a centrifuge. Effluent samples from the centrifuge were collected for this work. The TS content was 20.8%. WRRF C is less than 10 MGD. The WRRF employs primary clarification, and solids from the primary clarifiers are pumped directly to anaerobic digesters. The WRRF employs activated sludge, and the WAS is thickened and then pumped directly to the anaerobic digesters as well. After anaerobic digestion, centrifuges are used for dewatering. Effluent samples from the centrifuges were collected for this work. The TS content was 24.2% TS.

The collected pre-drying solids samples were shipped to a laboratory at California State University-Bakersfield (CSU-B) for lab-scale oven drying. Samples were stored at 4 °C until used for the experiment. Storage was less than 7 days. "Pre-drying" refers to solids that were received from the WRRFs before undergoing any drying. "Post-drying" refers to solids after they were dried in the lab-scale oven. Approximately 20 g of pre-drying solids were placed in a drying tin. The samples were dried overnight (24 hours) at 105 °C in a conventional lab-scale oven with the fan mode on. After drying,

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samples were transferred to containers that were provided by Eurofins for PFAS analysis. Dried samples were stored in the dark at room temperature until shipped to Eurofins. Pre-drying and post-drying samples were shipped to Eurofins for targeted analysis of PFAS as described in section 2.4.

2.3. Lab-scale oven drying experiments on high moisture content samples at 30 °C and 105 °C

High moisture content samples (99.8% MC, 0.2% TS) were dried to assess PFAS profiles in biosolids dried at two different temperatures. Triplicate biosolids drying experiments were conducted at 105 °C at the CSU-B laboratory and at the lower temperature of 30 °C. The 105 °C test was conducted overnight and the 30 °C test lasted 5 days. The 30 °C test was conducted in a lab-scale oven with the temperature lowered to 30 °C. These experiments were also conducted to assess the possibility of PFAS sticking to the drying tins. After the first set of experiments conducted in section 2.1 with results shown in section 3.1, it seemed feasible that PFAS previously associated with water could stick to the drying tin (and not the solids) as the water evaporated. This possibility was tested by employing a 'wipe kit' provided by Eurofins. This kit includes a methanol-soaked cloth that is used to wipe the tin after biosolids are completely removed for sampling following drying.

2.4. PFAS analysis

Sixty targeted PFAS analytes were measured. They are all listed by chemical class in the ESI document (ESI†), Table S1. The associated Isotope Dilution Analytes (IDAs), also known as isotopically labeled analogs, used for each PFAS analyte are also listed in ESI† Table S1. PFAS analyses were performed on a SCIEC 5500+ triple quad LC/MS/MS system operated in multiple reaction monitoring (MRM) mode. Biosolid samples were manually mixed in their collection containers and then subsampled (nominally 1 g for both pre-drying and post-drying samples). Each subsample was fortified with IDAs of the native target analytes, then extracted *via* sonication with basic methanol for one hour. The resulting extracts were re-constituted in water and then cleaned up *via* solid phase extraction (SPE) using a WAX SPE cartridge (Strata X-AW or equivalent), which retains the desired PFAS. The IDAs and target analytes were then eluted from the SPE cartridge with basic methanol and adjusted to a final extract volume of 10 ml in 80 : 20 methanol/water, after additional fortification with 13C2-PFOA as the internal standard (ISTD). Identical sample extraction procedures were applied to pre-drying and post-drying biosolid samples.

Accuracy of measurements for the reported list of 60 target analytes is ensured *via* initial and ongoing assessment of accuracy and precision *via* laboratory control sample aliquots, control sample duplicates, laboratory method blanks, method detection limit (MDL) studies, MDL verification aliquots, and performance testing studies. Each of these is performed as described in the associated laboratory Standard Operating Procedures (SOPs), and the results are monitored and validated *via* 3rd party laboratory accreditation bodies for the corresponding regulatory programs including the National Environmental Laboratory Accreditation Program and the United States Department of Defense Environmental Laboratory Accreditation Program.

Within each sample, additional measures of method performance are monitored, including IDA recovery, ISTD response, analyte retention times, analyte ion ratios, and analyte signal-to-noise ratios. Each of these sample-specific quality controls or identification elements must meet the criteria specified in the corresponding laboratory SOPs. PFAS concentrations are reported on a dry weight basis whereby dry weight is determined when moisture has been removed at 105 °C and the sample has been cooled to room temperature in a desiccator.

2.5. Analysis & Statistics

To conduct conservative statistical analyses on the fate of PFAS during drying, the reporting limits (RLs) provided in the Eurofins PFAS analysis report were used to fill in the gaps of the non-detects in the triplicate data before percent removal or significance of species removal was determined, *i.e.*, "zero" values were not used for statistics. Note that these RL data are not reflected in the figures; only species concentrations higher than the reporting limits are shown.

Statistical analysis was conducted using GraphPad Prism v10.3.1 (GraphPad Software, La Jolla, CA). The tests conducted for each experiment are listed in the ESI† Tables S5, S8, & S16. P-values < 0.05 were considered statistically significant. Concentrations of PFAS were normalized and reported in micromoles (μmol) of F per kg dry solids, where the F content stemmed from the moles of F present in the species of PFAS that were measured through targeted analysis. These concentrations were used to calculate percent removals.

3. Results

3.1. Impacts of full-scale drying and lab-scale oven drying on PFAS in biosolids

3.1.1. Full-scale dryer results. The full-scale dryer reduced the mass of detectable PFAS and changed the measurable PFAS profiles between the pre-drying and post-drying samples (Fig. 1 and ESI† Tables S2 & S3). Ten PFAS were detected across all three pre-drying samples for the full-scale dryer. The most abundant species in the pre-drying samples was 5 : 3 fluorotelomer carboxylic acid (FTCA), and 6 : 2 FTCA was the third most abundant species. These FTCA compounds are common precursors to short-chain PFAAs; 'precursors' are chemical species known to degrade and transform into other species of PFAS.[35–37] PFOS was the second most abundant PFAS overall in the pre-drying samples, and the most abundant PFAA. Of the ten PFAS detected across all three pre-drying samples, seven were detected above RL in all three post-drying samples. 7 : 3 FTCA was detected in two of the three post-drying samples, perfluorodecanoic acid (PFDA) was detected in one of the three post-drying samples, and 6 : 2 FTCA was not detected in any of

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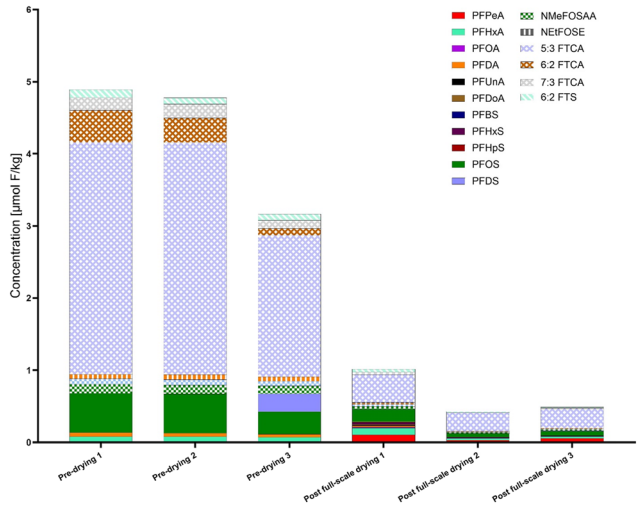


Fig. 1 Full-scale drying reduces measurable PFAS concentrations in biosolids. Samples were collected in triplicate (n = 3). Reprinted with permission. © The Water Research Foundation.[39]

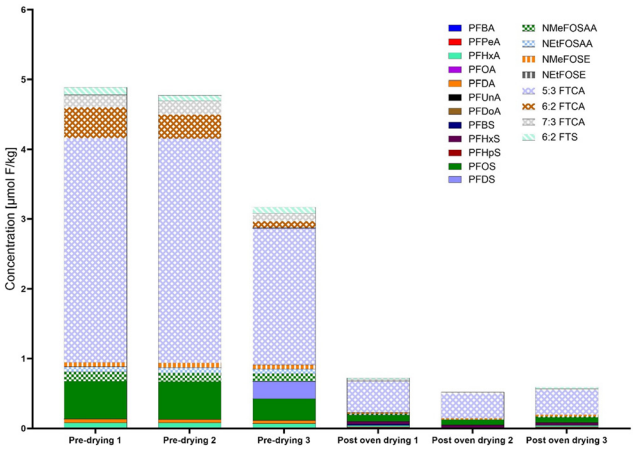


Fig. 2 Lab-scale oven drying at 105 °C reduces measurable PFAS concentrations in biosolids. Triplicate lab-scale oven drying experiments were conducted (n = 3). Reprinted with permission. © The Water Research Foundation.[39]

the post-drying samples (Fig. 1 and ESI† Tables S2 & S3). Two PFAAs, specifically perfluoropentanoic acid (PFPeA) and perfluorohexane sulfonic acid (PFHxS), were detected across all three post-drying samples, but not in any of the pre-drying samples. These two PFAAs were present at concentrations in the post-drying samples that were below the RLs for the pre-drying samples (ESI† Tables S3 & S4). Therefore, it is not possible to know if these two PFAAs appeared during the drying process due to a reaction or if they were already present in the pre-drying samples but could not be detected.[12,38] In general though, the PFAS fraction attributed to precursor compounds, *i.e.*, FTCA, fluorotelomer sulfonic acids (FTS), perfluorooctane sulfonamido acetic acids (FOSAA), and perfluorooctane sulfonamido ethanols (FOSE), decreased significantly during drying (ESI† Fig. S1, Tables S7 & S8).[35–37]

3.1.2. Lab-scale oven drying results. The solids samples collected before entering the full-scale dryer were also dried in a lab-scale oven at 105 °C to compare the impacts of lab-scale oven drying to full-scale drying on the PFAS profile in biosolids. The lab-scale oven data reflected similar trends as the full-scale dryer data. Total PFAS were reduced and the profile changed (Fig. 2). Of the ten PFAS detected across all three pre-drying samples, four were detected above RL in all three post-drying samples (ESI† Tables S3 & S4). One species, PFHxS, a terminal PFAA, appeared in all three lab-scale oven post-drying samples despite not being detected in the pre-drying samples. Similar to the full-scale dryer post-drying samples, PFOS was detected in all three lab-scale oven post-drying samples and was the second most abundant species detected after 5 : 3 FTCA. Together, 5 : 3 FTCA and PFOS accounted for, on average, more than half of the molar F as PFAS concentration in the lab-scale oven post-drying samples and full-scale dryer post-drying samples. There was no significant difference between the concentrations of these two species from the two drying methods (ESI† Table S5). Lab-scale oven drying also decreased the ratio of precursor species to total PFAS in biosolids (ESI† Fig. S1, Tables S7 & S8), which is consistent with the full-scale dryer sample data.

Overall, the full-scale dryer and lab-scale oven drying experiments revealed substantial reductions in measurable PFAS in the biosolids (Fig. 3). The average PFAS removal in the full-scale dryer was 84.9% and the average PFAS removal in the lab-scale oven was 81.9% (ESI† Table S9). Average removal of total PFAS was not significantly different between the full-scale dryer and the lab-scale oven (ESI† Table S8). These results indicate that drying biosolids could be a viable option to reduce the measurable PFAS concentration in biosolids, and, for these particular systems, the lab-scale oven setup can be used to help estimate removal percentage for the majority of detectable PFAS in biosolids in a full-scale system.

On a PFAS mass concentration basis, pre-drying total PFAS concentrations ranged from approximately 100–150 μg kg^{-1} (ESI† Table S3). The full-scale dryer post-drying samples had a wider range of concentrations than the lab-scale oven post-drying samples. The full-scale dryer post-drying samples total PFAS concentration ranged from 13–31 μg kg^{-1}, and the lab-scale oven post-drying samples ranged from 16–23 μg kg^{-1}. Some states have employed interim guidelines for land application of biosolids that set 20 μg kg^{-1} of PFOS and PFOA as a threshold wherein any concentrations below this level can be land applied.[40] The pre-drying biosolids samples had PFOS and PFOA concentrations already below this threshold.

3.2. Impact of lab-scale oven drying on PFAS in biosolids from three different utilities

Biosolids samples were collected anonymously from three WRRFs that were unique from the WRRF that provided samples for the results presented in section 3.1 above. The biosolids samples were collected from different WRRFs with varying

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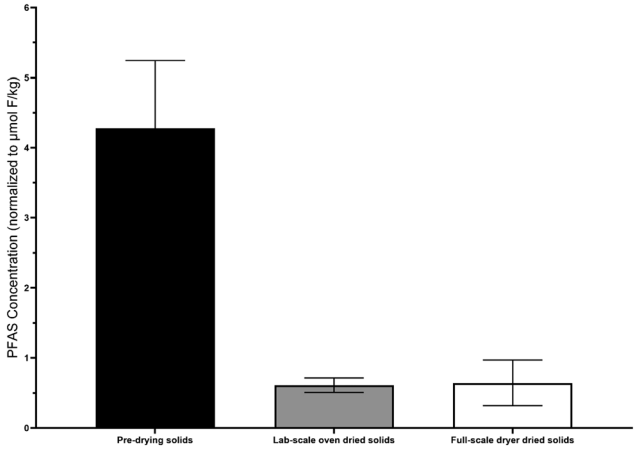


Fig. 3 Average total PFAS concentration (normalized to µmol F kg^{-1}) across pre-drying solids, lab-scale oven post-drying solids, and full-scale dryer post-drying solids. Samples taken in triplicate (n = 3) and error bars represent standard deviation. Reprinted with permission. © The Water Research Foundation.[39]

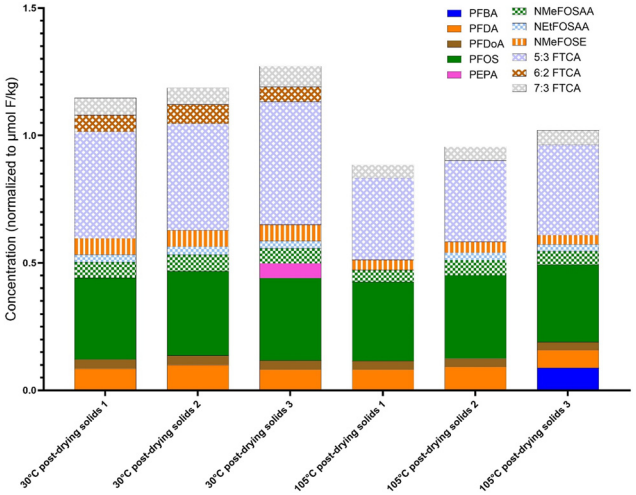


Fig. 5 Drying at 105 °C decreased total PFAS concentration by 16% compared to drying at 30 °C. Water content of pre-drying solids was 99.8%. 30 °C and 105 °C drying of samples were conducted in triplicate (n = 3). Reprinted with permission. © The Water Research Foundation.[39]

influent flow rates and unit operations as described in the methods section. Regardless of biosolids sample, the total sum of measurable PFAS decreased after drying (Fig. 4, ESI† Tables S10 & S11). For all three pre-drying samples, 5 : 3 FTCA was the first or second most abundant PFAS. PFOS was one of the three most abundant PFAS in each pre-drying sample as well. These two PFAS were still detected in post-drying samples, but their concentrations decreased. Average PFAS removal across the three samples was 76% (ESI† Table S13). These results indicate that drying can reduce measurable PFAS in biosolids, but drying does not completely remove PFAS from biosolids.

3.3. Impact of drying temperature on PFAS profiles in samples with high moisture content

High moisture content samples (99.8%) were dried at 30 °C and 105 °C. Overall, the PFAS fingerprint and concentration profiles were similar for samples dried at 30 °C and samples dried at 105 °C (Fig. 5), but temperature did have some effect on PFAS profiles in the dried biosolids. Four of the eight PFAS detected in both post-drying samples across all triplicates (5 : 3 FTCA, 7 : 3 FTCA, *N*-methyl perfluorooctane sulfonamido ethanol (NMeFOSE), and perfluorododecanoic acid (PFDoA)) were significantly lower in biosolids dried at 105 °C compared to biosolids dried at 30 °C (ESI† Tables S14–S16). Similar to the other data sets, 5 : 3 FTCA and PFOS were the first and second most abundant PFAS in every sample (Fig. 5 and ESI† Tables S14 & S15). Overall, the total PFAS concentration was 16% lower in the 105 °C post-drying sample than the 30 °C post-drying sample, with a statistically significant p-value of 0.0058. A wipe kit was provided by Eurofins to wipe the drying tins and test for residual PFAS. The reporting limit was 1 ng PFAS per wipe. No PFAS detections were observed for any of the six wipes tested (three tins at each temperature). Either there were no residual PFAS in the tins, or the wipes were not able to absorb residual PFAS that may have been left in the tins. This test was conducted to observe any major experimental artifacts of PFAS residual in tins that could be absorbed with a methanol wipe.

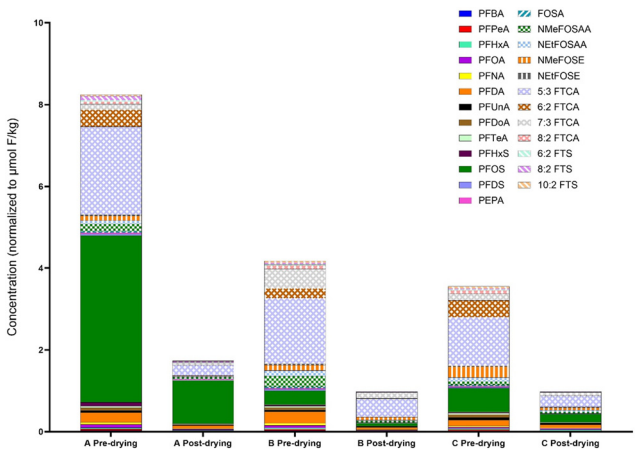


Fig. 4 Lab-scale oven drying reduced measurable PFAS in samples from three different WRRFs. Reprinted with permission. © The Water Research Foundation.[39]

3.4. Impact of moisture content on PFAS removal

Across the full-scale dryer and lab-scale oven data sets, moisture content was weakly correlated to total PFAS removal (Fig. 6, ESI† Table S18). The potential role of water in PFAS removal is discussed in more detail in the discussion section below. While certainly more data from a range of biosolids types and moisture contents are needed to validate this correlation, these data indicate that further study on the role of moisture content during drying of biosolids is warranted.

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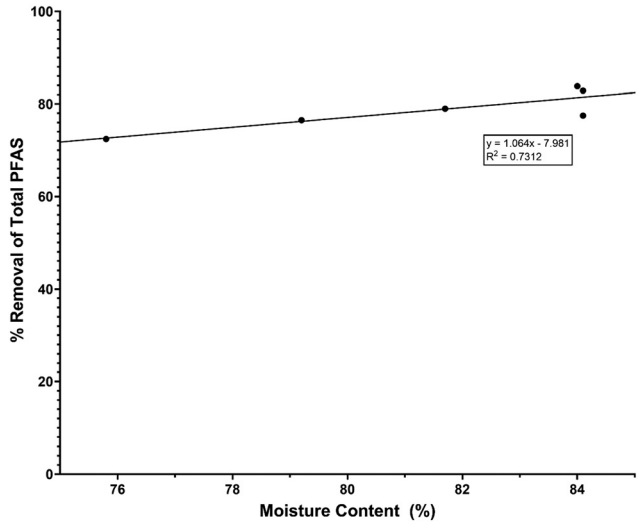


Fig. 6 Moisture content correlated to PFAS removal across sample sets. Reprinted with permission. © The Water Research Foundation.[39]

4. Discussion

The effect of drying on the PFAS profile of biosolids has been largely overlooked in literature.[34] Due to the recognized thermal stability of PFAS and their high boiling points as pure substances, it has been posited that temperatures around 100 °C would be insufficient to remove them from biosolids.[33] The following three sections discuss possibilities to explain the observed PFAS removal with recognition that more research is required to determine the removal mechanism.

4.1. Potential role of water and removal with aerosols

It has been established that PFAS readily partition to the air–water interface (AWI), and, in fact, a reduction in the aqueous concentration of PFAS increases this tendency of PFAS to partition to the AWI.[41–43] In the case of water-bearing biosolids, an accurate assessment of the AWI partitioning tendency of PFAS is complicated by the potential presence of air bubbles formed during the drying process as the temperature approaches 100 °C, at which point the water begins to boil.[44] As the boiling bubbles burst upon reaching the surrounding atmosphere of the biosolids, small water droplets may be flung into the air in what is referred to as the "bubble microtome effect"; these droplets, having existed as underwater pockets of air before bursting, may harbor significant quantities of PFAS that partitioned to their surface.[44] Furthermore, Hoff *et al.* suggest that the organic solutes most apt to partition to the interface of air bubbles have low vapor pressure and low solubility in water, and are typically polar; with polar functional groups and low Henry's constants, this characterization may apply to some PFAS, including PFAAs.[44,45] In this way, the drying of biosolids may provide an avenue by which non-volatile PFAS may escape wet biosolids, *i.e.*, *via* attachment to bubbles that form during drying and subsequent release (and removal from biosolids) attributed to the bubble microtome effect.

Additionally, recent research by Nguyen *et al.* revealed that PFAS can be removed from wastewater aeration basins *via* attachment to aerosols.[46] Indeed, PFOS removal was over 75%, and the concentration of PFOS associated with aerosols was 100 to 1000-fold greater than the PFOS associated with the wastewater.[46] PFOA also exhibited similar removal *via* association with aerosols.[46] This finding is important for two reasons. First, the removal of PFAS to aerosols occurred at 20 °C, a temperature well below the boiling point of water as well as lower than the temperatures reported for volatilization of PFAS, indicating that it is possible for PFAS to leave a water matrix without volatilizing.[46–50] Second, the PFAS that were removed *via* association to aerosols were not classically defined 'volatile' PFAS, *e.g.*, fluorotelomer alcohols (FTOH), that have been reported in landfill gas, for example.[45,51] Therefore, this aerosol removal mechanism does not require a phase change of PFAS. As the agitation of water due to aeration within aeration basins may catalyze the aerosolization of water, the tendency of PFAS to partition to the AWI may increase their susceptibility to removal with these water molecules.[43,52] This phenomenon of aerosolization of PFAS could also likely occur during tumbling of biosolids in rotary dryers.

In addition to these more nuanced potential mechanisms of removal, Hakeem *et al.* highlighted the need for more research to understand the impact of water vaporization on the fate of PFAS during the drying of biosolids; due to the complexity of interactions at the AWI, it may also be prudent to explore the impact of water content on removal.[33] As the PFAS concentration in the drying biosolids decreases, their tendency to partition to the AWI may increase, which may perpetuate the cycle of their removal during the above processes, despite the fact that they may occur at temperatures lower than the PFAS' boiling points.[43,47–50] In order to validate this claim, an analysis of the atmospheres of the drying environments (both lab-scale ovens and full-scale dryers), as well as of any water droplets generated and expelled during heating processes would be necessary.[45,53] Moreover, variations in moisture content may affect the consistency of PFAS extraction methods from solid samples due to the complex partitioning behavior of PFAS in multi-phase media. Further investigation on extraction methods and experimental artifacts is prudent for understanding the entire scope of the observed results.

4.2. Potential role of pure chemical volatilization

While PFAS are used in non-stick pans and fire-fighting foams because of their resistance to degradation, they still can volatilize as temperatures increase.[49] Sasi *et al.* observed PFOA weight loss at 100 °C *via* thermal gravimetric analysis (TGA), raising the possibility that some PFOA could be lost during drying *via* volatilization.[49] However, in the pure chemical studies by Sasi *et al.*, PFOS weight loss was not observed until temperatures were

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above 400 °C.[54] The drying temperatures in these studies were below 400 °C, yet PFOS removal was still observed, indicating that loss *via* volatilization was unlikely, or at the least not the major removal mechanism.[49,54]

4.3. Potential role of biotransformation

In addition to the concentration of PFAS changing during drying, the fingerprint, *i.e.* the specific PFAS present in each sample, changed after drying. FTCAs are a common class of precursors, and it is possible that biological enzymes converted FTCA precursors to terminal short-chain PFAAs such as perfluorobutanoic acid (PFBA), PFPeA, or PFHxA.[55] Research has been reported on the degradation of 5:3 FTCA to PFPeA and the more general theory that *n*:2 fluorotelomer carboxylic acids, *N*-alkyl perfluoroalkane sulfonamido acetic acids, and perfluoroalkane sulfonamido ethanols (*i.e.* 6:2 FTCA, NEtFOSAA, NMeFOSAA, and NMeFOSE) transform into perfluoroalkyl carboxylic acids (PFCAs) and perfluoroalkyl sulfonic acids (PFSAs).[35–37] The fraction of total PFAS attributed to precursors decreased after drying for the majority of experiments (ESI† Fig. S2). To confirm biotransformation, microbial activity assays and microbial community analyses would need to be conducted. If transformation were the only mechanism occurring, then total targeted PFAS measurements would increase as undetectable precursors were converted to detectable terminal species. The total sum of PFAS went down, implying that other mechanisms are at play even if transformation partially explained a change in PFAS profiles.

5. Conclusions

Biosolids management plans that incorporate PFAS management considerations are imperative while regulatory agencies weigh risk assessments for allowable PFAS concentrations that are safe for land application of biosolids. Source reduction is still the most important and effective way to mitigate PFAS in wastewater solids. It is essential to recognize the impact that all treatment processes may have on altering the PFAS profile of biosolids. Current treatment technologies could be leveraged to achieve compliance. Drying of biosolids may serve as a method by which PFAS contaminated biosolids are treated and made to achieve regulatory compliance for land-application. However, it is essential to recognize that the reduction in concentration of PFAS in biosolids during drying might result in air-borne PFAS associated with water droplets. Additional studies are needed to assess the fate of PFAS to assist the industry and regulators in establishing appropriate management and treatment protocols. Both lab-scale oven drying and full-scale drying processes had significant impacts on the measurable PFAS profile of biosolids, thereby suggesting that drying is indeed an effective process for altering the PFAS profile of biosolids. Drying is a technology already widely used in the industry. While drying does not completely remove PFAS to below reporting limits, it does substantially reduce measurable PFAS concentrations. Future research is needed to confirm if PFAS are emitted with the gas phase or if other mechanisms are responsible for these changes observed during drying.

Data availability

The data supporting this article have been included as part of the ESI.†

Conflicts of interest

There are no conflicts to declare.

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Incineration of PFBA with temperature: Reaction mechanisms, kinetics, and residence time from molecular level simulations

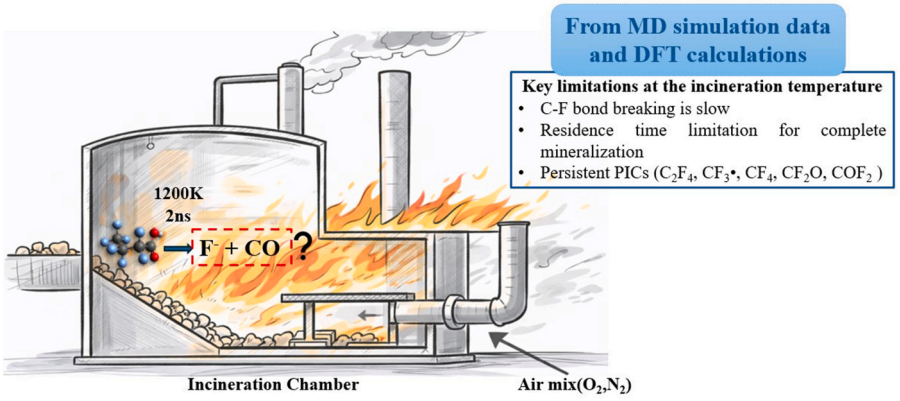
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HIGHLIGHTS

- PFBA degradation under incineration conditions were resolved by reactive MD and DFT.
- DFT shows defluorination of C2F4, CF2O & CF4 is kinetically inaccessible at 1200 K.
- Residence-time analysis shows, fluorocarbon intermediates outlast incinerator times.
- Molecular-level insights explain incomplete combustion & guide PFAS thermal treatment.

GRAPHICAL ABSTRACT



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ABSTRACT

Per- and polyfluoroalkyl substances (PFAS) are thermally stable organofluorine compounds whose complete destruction by incineration is difficult due to strong C–F bonds, radical stabilization, and formation of toxic intermediate products. In this research, atomistic degradation pathways of perfluorobutanoic acid (C_3F_7COOH) were investigated using a combined molecular dynamics (MD) and density functional theory (DFT) to quantify bond dissociation, intermediate formation, and kinetic limits under regular incineration temperature (1200 K). High-temperature MD simulations revealed that thermal cleavage of the C–C bond between the perfluoroalkyl chain and carboxyl group is the dominant initial reaction, generating $C_3F_7^{\bullet}$ radicals and CO_2. Subsequent pathways involve β-scission, $O\bullet/O_2$ addition, and radical recombination to produce intermediates such as C_2F_4, $CF_3^{\bullet}$, CF_2, $CF_3C(O)F$ and COF_2. The DFT transition-state calculations provide activation energies from 40 to 513 kJ mol^{-1}, enabling estimation of rate constants and residence times from transition-state theory. At 1200 K temperature, early fragmentation step occurs over micro-to millisecond timescales, whereas C_2F_4 defluorination, COF_2 decomposition, and CF_4 breakdown calculations show residence times exceeding seconds to hundreds of seconds well above typical incinerator gas-phase residence times. These findings provide quantitative evidence that PFAS incineration proceeds through complex multistep of degradation pathways and that kinetically

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persistent products of incomplete combustion result primarily from slow defluorination steps. The mechanistic and kinetic trends identified here provide molecular-level guidance for optimizing incineration conditions to improve PFAS mineralization.

1. Introduction

Per- and polyfluoroalkyl substances (PFAS) are a large class of synthetic organofluorine compounds widely used for their thermal stability, chemical resistance, and surfactant properties. These characteristics arise from strong C–F bonds and fully fluorinated carbon backbones, which also make PFAS highly persistent in natural and engineered environments (Perera and Meegoda, 2024; Sznajder-Katarzyńska et al., 2019; Xu et al., 2021; Buck et al., 2011; Jin et al., 2020; Lin et al., 2022; Murphy and Warner, 2022; Sunderland et al., 2019; Zuccaro et al., 2022). PFAS have been widely detected in groundwater, soils, biota, and even remote polar ecosystems, raising concerns about long-term exposure risks and the difficulty of achieving complete remediation (Roy et al., 2025; Sadia et al., 2023)

Incineration is currently the only full-scale technology used for PFAS destruction. Hazardous-waste incinerators typically operate above 1000 °C, where PFAS are expected to thermally decompose to CO_2 and HF. However, field and pilot-scale studies show that degradation is often incomplete, producing intermediates such as perfluoroolefins, COF_2, perfluoroacyl fluorides, and stable fluorocarbons (e.g., C_2F_4, CF_4, C_2F_6) through complex radical-mediated pathways and limited residence time or oxidizing conditions. (Baranovskii and Skorobogatov, 2016). Short-chain PFAS, such as perfluorobutanoic acid (PFBA), are particularly challenging during combustion because their high volatility and mobility can hinder complete destruction and promote the formation of ultrashort-chain perfluoroalkyl acids (PFAs)(Altmeyer Mendes et al., 2025; Bharti, 2025; Blotevogel et al., 2023; Kovacs et al., 2025; Yu et al., 2025).

Although macroscopic emission data show that temperature and residence time strongly influence PFAS destruction efficiency, the underlying atomistic mechanisms remain poorly understood. Thermal cleavage of C–C and C–F bonds, radical stabilization, recombination, and competing oxidation pathways determine whether incineration leads to complete mineralization or formation of fluorinated byproducts. Experimental studies confirm that PFAS decomposition depends on temperature, residence time, reactor material, and oxidizing conditions. For example, Weber et al. (2024) reported that PFOS decomposes to HF, CO_2, and SO_2 above 850 °C in the presence of water vapor, while pyrolysis under inert conditions forms intermediates such as CF_2 and C_2F_4(N. (2025) Weber et al., 2024). Rocchio et al. (2025) reported that incomplete combustion of PFOA in a pilot-scale incinerator can regenerate smaller PFAS (C_2–C_6) through competing α-lactone and C–C cleavage pathways (Rocchio et al., 2025). These results indicate that PFAS degradation proceeds through complex multistep reactions rather than direct oxidation. Although macroscale incineration studies provide temperature and emission data, molecular-level mechanisms remain poorly understood, highlighting the need for atomistic investigations of PFAS thermal.

Molecular dynamics (MD) and density functional theory (DFT) are powerful computational tools that provide complementary insights into PFAS degradation mechanisms during incineration. MD simulations capture the time-dependent atomic behavior of PFAS under reactive conditions, enabling the identification of bond cleavage, radical formation, and reaction pathways. Reactive force fields such as ReaxFF allow bond formation and dissociation, making it possible to simulate complex degradation networks. For example, Bezerra de Souza et al. (2025) used MD to investigate PFAS degradation under ultrasonic cavitation and showed that chain length and headgroup structure influence the onset of C–F bond cleavage and radical formation (de Souza and Meegoda, 2024). Similarly, Loganathan and Wilson (2022) used MD simulations to study PFAS adsorption and diffusion on kaolinite, showing that short-chain PFAS exhibit higher mobility and weaker interfacial binding than long-chain analogues. (Loganathan and Wilson, 2022).

In contrast, density functional theory (DFT) provides a quantum mechanical description of electronic structure and is widely used to determine reaction pathways, activation barriers, and transition states in PFAS decomposition. DFT calculations help identify charge transfer and bond weakening processes that initiate PFAS degradation (Mohamed et al., 2024). Moreover, DFT-based kinetic models have shown that PFAS pyrolysis proceeds through HF and SO_2 elimination, followed by fragmentation of perfluoroalkyl chains into smaller radicals such as CF_2 and CF_3 (Altarawneh, 2021). Integrating DFT with MD provides a comprehensive view of PFAS incineration, where DFT determines reaction energetics and MD captures the dynamic molecular evolution under high-temperature conditions.

This study employs a multiscale computational approach to investigate the thermal degradation of perfluorobutanoic acid (PFBA). High-temperature MD simulations identify dominant fragmentation pathways and radical intermediates, while DFT calculations determine transition states, activation barriers, and rate constants for key reactions. These atomistic insights help evaluate whether typical incineration conditions can achieve complete PFAS destruction or if kinetic limitations lead to persistent byproducts.

2. Materials and methods

2.1. MD simulation approach

As the initial step, the MD simulation was performed using the periodic simulation box was set to 383.57 Å × 383.57 Å × 383.57 Å (see Fig. S01). Perfluorobutanoic acid (PFBA or $C_4HF_7O_2$) was selected as a representative short-chain perfluorocarboxylic acid to investigate thermal degradation under incineration-relevant conditions utilizing the ReaxFF force field (Senftle et al., 2016; Van Duin et al., 2001). PFBA captures the key structural characteristics of perfluoroalkyl acids while reducing computational cost relative to longer-chain analogues. All simulations were carried out in the gas phase to mimic the high-temperature combustion zone of incinerators, where PFAS degradation occurs primarily through thermal excitation and radical reactions in an oxidative environment containing O_2 and N_2, with no water molecules present.

The simulation box containing 10 PFBA molecules, 750 N_2 molecules, and 250 O_2 molecules was constructed to an approximate oxidizing combustion atmosphere. Each system underwent an initial energy minimization, followed by equilibration in canonical (NVT) ensemble at 373.15 K and 1 atm. After equilibration, production runs were performed in a NVT ensemble spanning the temperature from 373.15 K to 1200 K. Simulations were performed at 1200 K, a representative high-temperature combustion condition within the range typically used in PFAS thermal decomposition and incineration studies (1000–1500 K) (Altarawneh et al., 2022a; Blotevogel et al., 2023).

All MD simulations were carried out using the LAMMPS package[17]. Molecular structures were constructed and geometry-optimized using Avogadro, and trajectory visualization and analysis were performed using OVITO (Stukowski, 2010).

2.2. Details of DFT calculation

All electronic structure calculations were performed using ORCA

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6.0.1 as the quantum chemistry software package (Neese et al., 2020) using the long-range corrected hybrid functional wB97X-D3BJ in combination with the def2-TZVP basis set for all atoms. Geometry optimizations of all reactants, intermediates, products, and transition states were carried out in the gas phase without symmetry constraints, using the TightSCF convergence criteria and the resolution-of-identity approximation (RIJCOSX) with the def2/J auxiliary basis to accelerate the Coulomb and exchange integrals. Empirical dispersion interactions were treated with the D3(BJ) correction as implemented in ORCA.

For each elementary step in the PFAS decomposition network, the reactant and product geometries were fully optimized to local minima and characterized by vibrational frequency analyses. Transition state (TS) structures were located either by constrained scans followed by OptTS optimizations or by NEB-TS searches using the optimized reactant and product structures as endpoints. All TS candidates were confirmed by a single imaginary frequency corresponding to motion along the desired reaction coordinate.

Temperature-dependent rate constants for unimolecular decomposition reactions were estimated using Arrhenius kinetics based on the activation energies obtained from DFT calculations. For unimolecular homolytic bond-cleavage reactions, the pre-exponential factor was approximated as 1.0×10^{15} s^{-1}, consistent with values predicted by transition-state theory for radical dissociation reactions and commonly reported in combustion-kinetics literature. The resulting k(T) values were then used to compute characteristic residence times at incineration temperatures, providing a direct link between atomistic reaction mechanisms and macroscopic PFAS destruction requirements in thermal treatment systems.

The expected uncertainty of activation energies at the wB97X-D3BJ/def2-TZVP level of theory is typically on the order of ±10–20 kJ mol^{-1} for fluorinated systems. While direct benchmarking with experimental rate constants was not performed, the predicted reaction intermediates and degradation pathways are consistent with products reported in laboratory and field-scale PFAS incineration studies. The reaction coordinates were visualized using Avogadro 1.2.0 software (Hanwell et al., 2012).

3. Results and discussion

3.1. Analyzing possible reaction pathways

3.1.1. Primary decomposition pathways of C_3F_7COOH

High-temperature molecular dynamics simulations were performed to identify the earliest bond-cleavage events, dominant dissociation pathways, and transient radical intermediates formed during thermal breakdown of PFBA under simulated incineration conditions. The simulation cell contained 10 PFBA molecules, 750 N_2 molecules, and 250 O_2 molecules, representing an oxidizing gas-phase combustion environment (Fig. S01). After equilibration at 373.15 K, the system was heated to 1200 K, where PFAS degradation is experimentally observed to begin within millisecond timescales.

Across independent trajectories, the first decomposition event consistently involved cleavage of the C–C bond between the perfluoroalkyl tail and the –COOH group, producing the highly stable $C_3F_7^{\bullet}$ radical and a carboxyl fragment $COOH^{\bullet}$. This is consistent with those reported in the literature suggesting that perfluoroacyl C–C bonds are thermally weaker than internal C–F bonds at incineration temperatures.

This observation is consistent with previous theoretical studies of PFAS thermal degradation. For example, Altarawneh et al. (2022) reported through kinetic modeling and quantum chemical calculations that PFCA decomposition is initiated by decarboxylation reactions that generate perfluoroalkyl radicals, followed by β-scission and fragmentation of the fluorinated carbon chain (Altarawneh et al., 2022a). Similarly, Krug et al. (2022) demonstrated through kinetic modeling and combustion experiments that PFAS decomposition proceeds through radical-mediated fragmentation producing intermediates such as $CF_3\bullet$, C_2F_4, and smaller fluorocarbon species. The agreement between these earlier studies and the MD trajectories observed here provides additional support for the proposed radical-driven degradation mechanism (Krug et al., 2022).

Following initial fragmentation, rapid decarboxylation of $COOH^{\bullet}$ to $CO_2 + H^{\bullet}$ was observed, in agreement with expected gas-phase thermolysis behavior. Fig. 1 shows results from the MD simulation for 55ns period. Detailed species information is recorded in Table S04 in the SI document. The $C_3F_7\bullet$ radical underwent several competing pathways, including.

- C–F bond homolysis yielding $C_3F_6 + F\bullet$,
- H-abstraction from $COOH^{\bullet}$ or O_2-derived radicals, producing C_3F_7H,
- Addition of $O^{\bullet}$ or O_2, leading to $C_3F_7O^{\bullet}$ and subsequently $CF_3C(O)F + {}^{\bullet}CF_3$

In addition to the radical C–C cleavage pathway, an alternative HF elimination pathway (Reaction 1D, However, this pathway likely represents a secondary or less favorable route under gas-phase thermal conditions) was also identified, producing C_3F_6, CO_2, and HF. This pathway is consistent with previously reported PFCA thermal decomposition mechanisms involving HF elimination and formation of fluorinated intermediates.

Importantly, MD trajectories demonstrated the formation of ultrashort-chain fluorocarbons (e.g., CF_4, C_2F_6, C_2F_4) and perfluoroacyl fluorides, which are experimentally recognized products of incomplete combustion. The MD simulations therefore provide mechanistic evidence that radical-driven cascades, not direct one-step fragmentation, dominate PFAS thermal breakdown. Fig. 2 illustrates the proposed reaction pathways.

The role of oxygen was also apparent in this simulation. Although the gas mixture contained 25% O_2, many of the first fragmentation events occurred before O_2 participation. Oxygen addition occurred later in the radical cascade, often stabilizing or oxidizing intermediates rather than driving primary bond scission. This implies that initial PFAS decomposition under incineration is governed largely by thermal excitation, while oxidation becomes dominant only after primary fragmentation has created reactive radicals.

3.1.2. Molecular geometries and transition state analysis

To validate and quantify the MD-identified pathways, key reaction steps were examined using DFT with the wB97X-D3BJ/def2-TZVP. All reactants, intermediates, products, and transition states were optimized using the wB97X-D3BJ/def2-TZVP. Transition states for bond-cleavage, β-scission, and O/O_2 addition steps were obtained either through NEB-TS searches or by scanning the relevant reaction coordinate to locate a maximum. All TS structures were verified by observing a single imaginary vibrational frequency, corresponding to the expected C–C, C–F, or O-insertion motion.

3.1.3. Fate of C_3F_7 radical

Once generated by the primary C–C bond cleavage of PFBA (Reaction 1A), the $C_3F_7\bullet$ radical becomes the central branching point of the entire thermal degradation network. Its subsequent reactions determine whether breakdown proceeds toward (i) oxidative fragmentation, (ii) short-chain PFAS formation, or (iii) stable fluorocarbon by-products. Under desired incineration conditions (1200 K), three major thermochemical pathways compete.

(I) **β-scission to form $CF_3\bullet$ and C_2F_4 (Reaction 2; E_a = 175 kJ/mol):** This β-scission pathway represents the most kinetically accessible C–C bond cleavage of the $C_3F_7^{\bullet}$ radical, producing $CF_3^{\bullet}$ and C_2F_4 (tetrafluoroethylene). The $CF_3^{\bullet}$ fragment subsequently participates in secondary reactions leading to CF_4, COF_2 (or CF_2O), while C_2F_4 an exceptionally stable short-chain fluorocarbon tends to persist because further C–F bond cleavage

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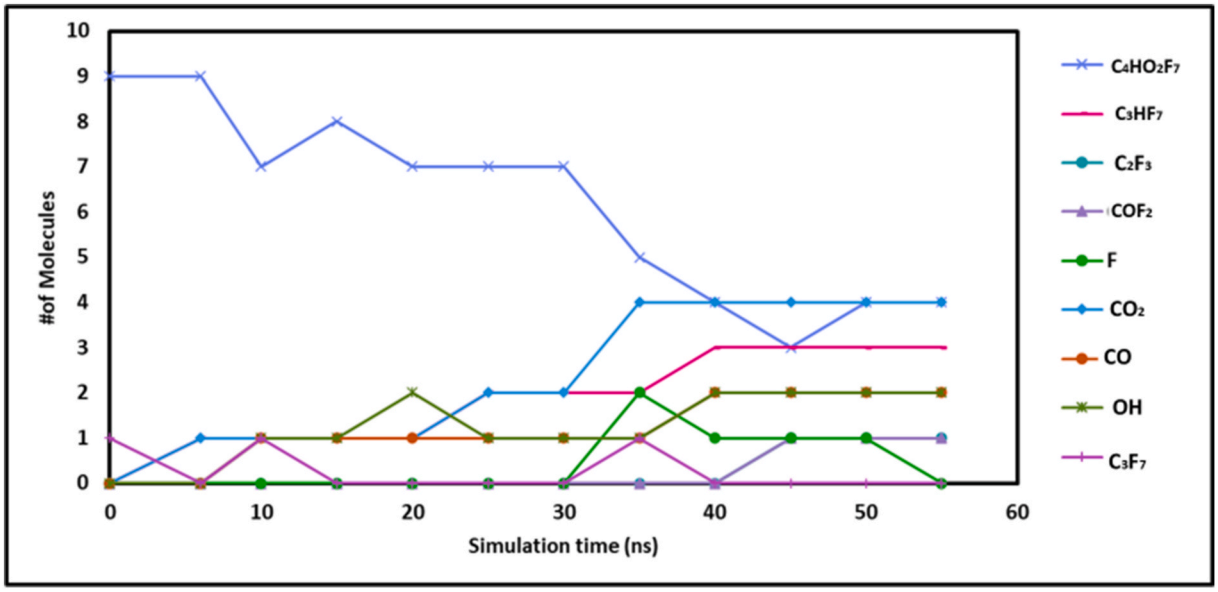


Fig. 1. Time evolution of PFAS fragments and radicals during reactive molecular dynamics (MD) simulation of PFBA thermal decomposition at 1200 K. Colored curves represent the number of molecules of each species (PFBA, C_3HF_7, C_2F_3, COF_2, F, CO_2, CO, OH, and C_3F_7) as a function of simulation time (ns). Additional fragment details are provided in Table S04 of the Supporting Information document.

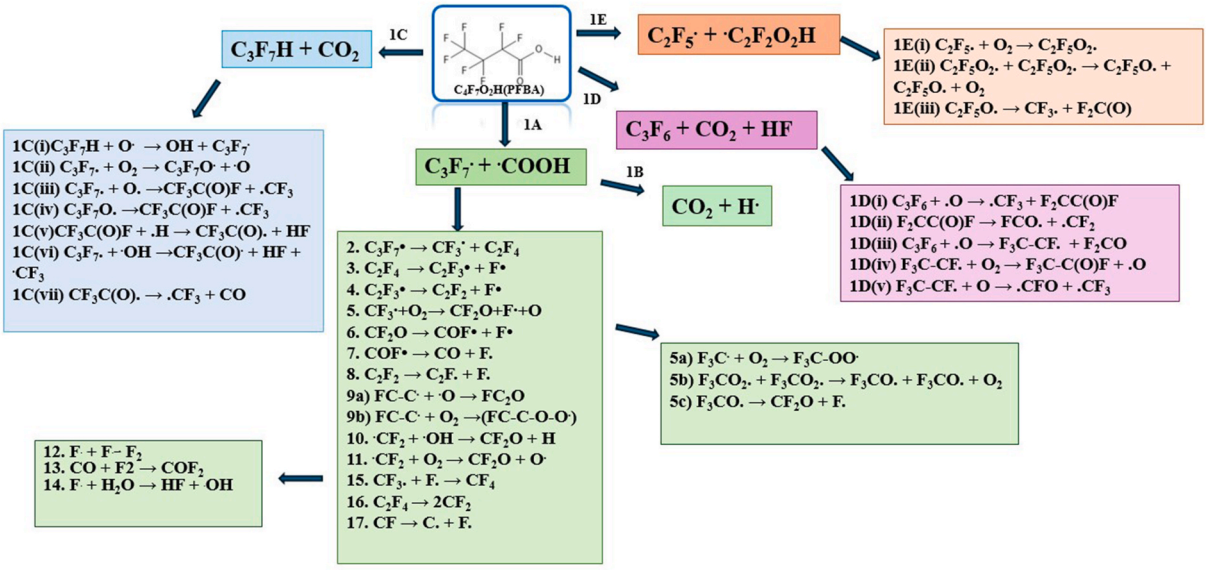


Fig. 2. Schematic illustration of proposed reaction mechanism pathways.

requires a much higher activation energy (E_a = 513 kJ/mol, Reaction 3). As a result, this pathway directs PFAS degradation toward stable, shorter-chain fluorocarbons and explains why C_2F_4 is frequently observed as an intermediate or byproduct during PFAS incineration.

(II) **Oxygen-driven degradation (Reactions 1C(ii)–1C(iv); Ea = 176–262 kJ/mol):** In oxidizing environments, the C_3F_7• radical readily undergoes additional reactions with O_2 to form $C_3F_7O_2$• or with atomic oxygen to generate C_3F_7O•, followed by β-scission of these fluorinated alkoxy intermediates to yield $CF_3C(O)F$ and •CF_3. These steps produce perfluoroacyl fluorides such as $CF_3C(O)F$, which are well-documented intermediates during PFAS combustion. The β-scission of C_3F_7O• (Ea = 262 kJ mol^{-1}) liberates CF_3•, further feeding the sequence of short-chain fluorocarbon fragments. Overall, this oxidative pathway steers the system toward formation of carbonyl species including COF_2, CO, and CO_2, marking the progression toward oxidative mineralization.

(III) **Hydrogen abstraction reactions (Reactions 1C(i) and 1C(v)–1C(vi)):** When H• or OH• radicals are present, C_3F_7• can participate in hydrogen-abstraction reactions or be regenerated through radical exchange. For instance, reaction of C_3F_7H with O• produces OH• and reforms C_3F_7•, while interaction of $CF_3C(O)F$ with H• yields $CF_3C(O)$• and HF, although this process requires a large activation barrier (~500 kJ mol^{-1}) and is therefore kinetically unfavorable at 1200 K. Additionally, reaction between C_3F_7• and OH• generates HF and perfluoroacyl radicals, and the absence of a stable transition state indicates that this step proceeds through a barrierless radical–radical capture mechanism. Collectively, these pathways account for the early formation of

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HF during incineration when hydrogen-containing species become available.

3.1.4. Formation and fate of toxic products of incomplete combustion (PIC)

Incomplete combustion of PFAS generates a suite of toxic and highly persistent intermediate species, commonly referred to as products of incomplete combustion (PICs). In this research, MD simulations and DFT results show that several PICs, most notably C_2F_4, CF_3•, CF_4, CF_2O, COF_2, and perfluoroacyl fluorides (e.g., $CF_3C(O)F$), emerge naturally along the thermal degradation pathway. These species form when primary PFBA fragmentation produces C_3F_7• radicals, which subsequently undergo β-scission to generate C_2F_4 and CF_3•, both of which are well-documented fluorocarbon PICs reported in experimental PFAS combustion and pyrolysis studies (Altarawneh et al., 2022a; Krug et al., 2022; N. H. Weber et al., 2024).

C_2F_4 is particularly problematic due to its high C–F bond strength and large activation barrier for further cleavage, allowing it to survive typical incineration residence times. CF_3• can also be oxidized or decomposed to COF_2 (carbonyl intermediate) or further react with F• and O• to yield COF_2 (carbonyl fluoride, also known as difluorophosgene), a highly reactive and toxic fluorinated gas. Perfluoroacyl fluorides such as $CF_3C(O)F$ are another important class of PICs; they form through oxygen-driven fragmentation pathways and can subsequently decompose to CF_3• and CO. Although many PICs are thermally stable, their fate ultimately depends on the availability of oxidizing radicals (O•, OH•, O_2), which convert them into final mineralization products CO_2, CO, HF, and inorganic fluoride at sufficiently high temperature and residence time. Altogether, these results highlight that PFAS incineration does not proceed directly to complete mineralization; instead, it passes through a complex network of PICs whose persistence and toxicity underscore the need for sufficiently high temperatures, long residence times, and optimized oxidation conditions to prevent their atmospheric release.

Fig. 3 summarizes the intrinsic C–F bond dissociation energetics for three representative fluorocarbon fragments formed during PFAS degradation. In all cases $C_2F_4 \rightarrow C_2F_3$• + F• (Fig. 4a), $F_2C_2 \rightarrow FC_2$• + F• (Fig. 4b), and CF → C• + F• (Fig. 4c) the potential energy scans reveal extremely high activation barriers (ΔEa ~536–762 kJ mol^{-1}), reflecting the exceptional thermodynamic stability of the C–F bond. The steep rise in energy prior to bond rupture indicates that unimolecular C–F cleavage is not kinetically favorable under typical incineration temperatures (1200 K), meaning that PFAS fragments such as C_2F_4 and CF_3 radicals can persist long enough to act as PICs(Altarawneh et al., 2022b; Krug et al., 2022).

These calculated barriers align with experimental reports showing that perfluoroolefins and fluorinated radicals resist further decomposition unless strong oxidizing species (O•, OH•, O_2) are present to drive alternative pathways such as HF elimination, oxidation, or radical recombination (N. H. Weber et al., 2024). Overall, Fig. 3 shows that PFAS do not decompose through direct C–F bond scission; instead, thermal treatment produces a network of radical intermediates whose fate is strongly governed by oxygen-assisted pathways that determine whether PICs accumulate or mineralize (Altarawneh et al., 2022b; N. H. Weber et al., 2024). These results are consistent with literature values reporting similarly large C–F bond dissociation energies in fluorocarbons (typically 500–750 kJ mol^{-1})(Giannetti, 2005).

Although DFT provides a computationally efficient way to explore PFAS fragmentation pathways, the extremely large dissociation barriers predicted for direct C–F bond dissociation barriers (ΔEa = 536–762 kJ mol^{-1}) require further validation using higher-level wavefunction methods. Coupled-cluster theory (CCSD and CCSD(T)) is the benchmark for thermochemistry and bond dissociation energies, particularly in fluorinated systems where electron correlation plays a critical role. CCSD(T) single-point energies on DFT-optimized structures will be employed in future research to refine the activation barriers and reaction energetics. Because reaction rate constants depend exponentially on activation energy, even moderate corrections (10–20 kJ mol^{-1}) can lead to differences of several orders of magnitude in predicted residence times at 1200 K. Accurate CCSD(T)-based energetics are therefore essential for determining whether specific PFAS fragmentation channels are kinetically accessible during incineration or whether the predicted residence times (hours to days) reflect methodological limitations of DFT-based scans rather than intrinsic chemical behavior.

The recombination of CF_3• + F• → CF_4 (Reaction#15) represents a kinetically favorable radical pathway during PFAS incineration. DFT calculations show that this reaction proceeds with an extremely low activation energy ($E_a \approx 0.13$ kJ mol^{-1}), making it effectively barrierless under thermal conditions. At incineration temperatures (1000–1400 °C), CF_3• and F• radicals generated from C–C and C–F bond scission can recombine rapidly to produce CF_4, a highly stable perfluorocarbon. However, in combustion environments containing hydrogen sources such as water vapor, CF_3• may also preferentially react with H•

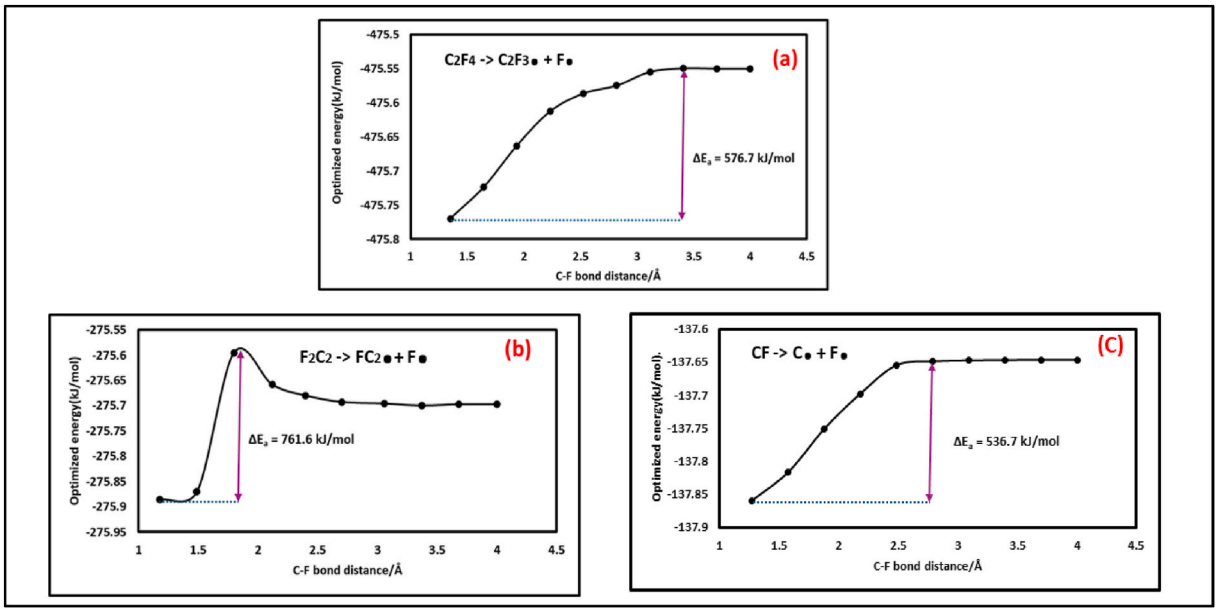


Fig. 3. Energy diagram of (a) $C_2F_4 \rightarrow C_2F_3$. + F., (b) $F_2C_2 \rightarrow FC_2$. + F. and (c) CF → C. + F. calculated using the wB97X-D3BJ/def2-TZVP level of theory.

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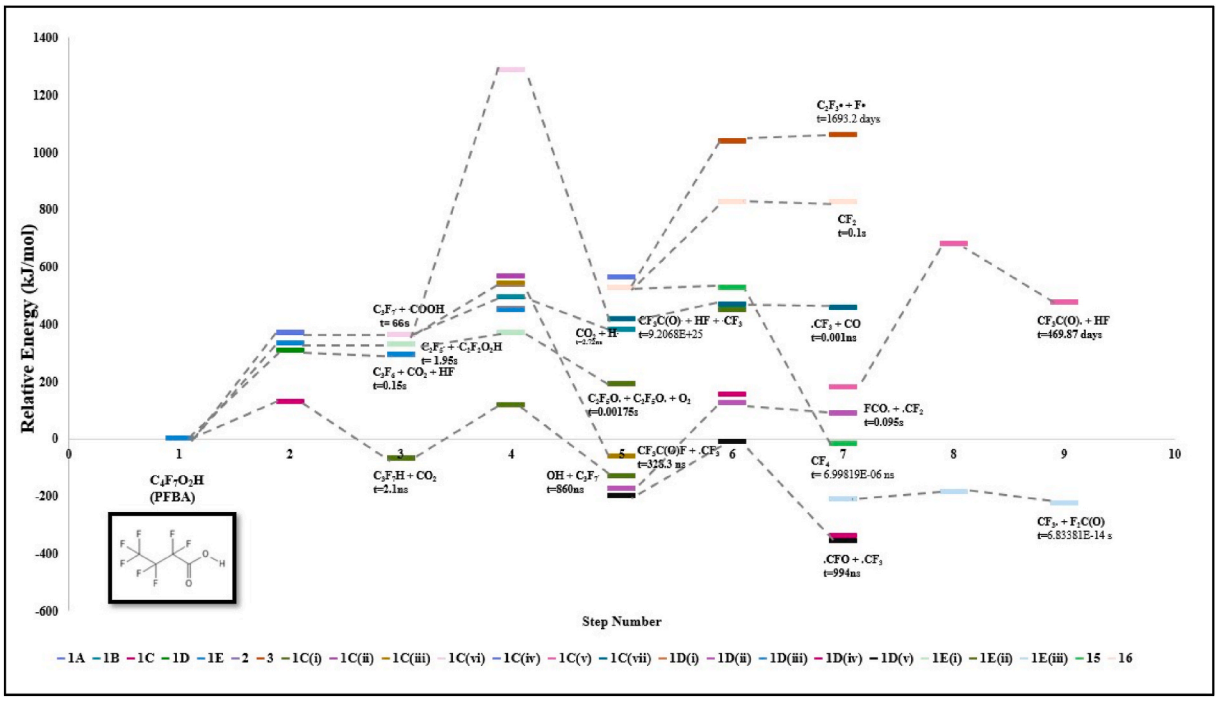


Fig. 4. Schematic diagram of the residence time profile calculated using the rate constant for each reaction step.

to form fluoroform (CHF_3). Therefore, the formation of CF_4 is not inevitable and depends on the availability of hydrogen and the overall combustion conditions.

Conversely, the reverse reaction, $CF_4 \rightarrow CF_3\bullet + F\bullet$, requires overcoming a large barrier (Ea ~542.35 kJ mol^{-1}), reflecting the exceptional thermodynamic stability of CF_4. Even at 1200 K, this barrier is far larger than the available thermal energy, making CF_4 essentially non-decomposable under typical incineration of residence times. Thus, once formed, CF_4 behaves as a terminal product rather than a reactive intermediate.

This large asymmetry between forward and reverse barriers has important implications for PFAS destruction. Any process generating $CF_3\bullet$ and $F\bullet$ radicals whether through β-scission, HF elimination, or fragmentation of perfluoroolefins inevitably channels a fraction of the reaction flux toward CF_4 formation. Because the conversion to CF_4 is kinetically favored and thermodynamically downhill, its breakdown is prohibitively slow and CF_4 accumulates as a product of incomplete combustion (PIC). This mechanistic insight helps explain why CF_4 is consistently detected in laboratory and pilot-scale incineration studies, even at temperatures exceeding 1000 °C, and why complete mineralization of PFAS requires not only high temperatures but also sufficiently long residence times and oxidizing conditions to suppress radical recombination.

3.2. Kinetic modelling, rate constant and residence time calculation

Activation energies (E_a) were calculated as the electronic energy difference between the optimized transition state and reactant structures obtained from DFT calculations. These E_a values (see Table S01 in the SI for details) were then used to estimate temperature-dependent rate constants, k(T), using the Arrhenius expression:

$$k(T) = A.\exp\left(\frac{-E_a}{RT}\right) \quad (1)$$

Where A is the pre-exponential factor estimated from unimolecular transition-state theory and R is the gas constant (0.008314 kJ/mol. K). Rate constants were calculated at the incineration temperature of 1200 K. All calculated rate constants for the individual reaction steps are provided in Table S02 in the SI document.

Assuming pseudo-first-order kinetics for high-temperature PFAS decomposition, degradation follows:

$$\frac{C}{C_0} = e^{-k(T)t} \quad (2)$$

A destruction efficiency of 99.9%, consistent with hazardous-waste combustion standards, corresponds to a remaining fraction:

$$\frac{C}{C_0} = 0.001 \quad (3)$$

Solving for t gives:

$$t_{99.9\%} = \frac{\ln(1000)}{k(T)} \quad (4)$$

Equation (4) was applied to each elementary reaction step to determine the minimum residence time required to achieve 99.9% conversion at incineration temperatures. Reactions with high activation energies (e.g., late-stage formation or destruction of CF_4, C_2F_6) exhibited extremely small k values at 1200 K, resulting in residence times that exceed to minutes, far longer than typical incinerator gas-phase residence times (0.5–2 s). In contrast, early PFBA fragmentation and oxygen-addition steps proceed rapidly (<μs), indicating that the major kinetic bottlenecks are the final defluorination and destruction of ultra-stable fluorocarbons, not the initial PFAS decomposition.

Table S02 summarizes the calculated activation energies (ΔEa), unimolecular rate constants k (1200 K), and corresponding residence times required to reach 99.9 percent conversion ($t_{99.9}$) for each elementary step in the proposed C_3F_7COOH degradation network calculated using the A value (Bao et al., 2016; Choi et al., 2023; Cobos et al., 2013; Confer et al., 2020; Harvey, 2013; Harvey et al., 2012) as $1.00*10^{15}$. The residence time is reported in seconds, nanoseconds, and days to highlight processes that are relevant on the incinerator timescale (milliseconds to a few seconds) versus those that are effectively frozen at 1200 K.

Overall, the data show that the initial decomposition of C_3F_7COOH is very fast at 1200 K. Decarboxylation to $C_3F_7\bullet$ + •COOH (Reaction 1A)

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has a moderate barrier (ΔE_a ~224 kJ mol^{-1}) but still proceeds with k ~ 1.7 × 10^5 s^{-1}, corresponding to $t_{99.9}$ ~ 4 × 10^{-5} s. Subsequent fragmentation of •COOH to CO_2 + H• (Reaction 1B) and the alternative decarboxylative hydrogen transfer channel $C_3F_7COOH \rightarrow C_3F_7H + CO_2$ (Reaction 1C) are even faster (ΔE_a ~126–129 kJ mol^{-1}, k ~ 10^9 s^{-1}, $t_{99.9}$ on the order of 10^{-9} s). Thus, the parent PFBA is essentially consumed instantaneously compared to typical gas-phase residence times in hazardous waste incinerators.

Once C_3F_7• is formed, its reactions with O, O_2 and OH proceed within microsecond to sub-millisecond timescales. Steps 1C(i)–1C(iii) and 2, which include $C_3F_7H + O$•, C_3F_7• + O_2, C_3F_7• + O• and C_3F_7• → CF_3• + C_2F_4, all have ΔE_a in the range of 176–203 kJ mol^{-1} and rate constants of 10^6–10^7 s^{-1}, giving $t_{99.9}$ values between about 10^{-7} and 10^{-6} s. Rearrangement of C_3F_7O• to $CF_3C(O)F + CF_3$• (Reaction 1C(iv)) is somewhat slower (ΔE_a ~262 kJ mol^{-1}, $t_{99.9}$ ~ 1.7 × 10^{-3} s), but still easily completed within a 2s incinerator residence time. Direct HF elimination to C_3F_6 (Reaction1D) and C2 fragment formation (Reaction 1E) are slower than the radical pathways but remain kinetically accessible at 1200 K, with $t_{99.9}$ of about 0.15s and 0.59s, respectively.

In contrast, several downstream fragmentation steps and PIC decomposition reactions are orders of magnitude slower. The hydrogen abstraction from $CF_3C(O)F$ by H• (Reaction 1C(v)) and the unimolecular decomposition of C_2F_4 to C_2F_3• + F• (step 3) and COF_2 to COF• + F• (step 6) have very high barriers (ΔE_a ~485–513 kJ mol^{-1}) and extremely small rate constants at 1200 K. The corresponding $t_{99.9}$ values are on the order of 10^7–10^{8}s, which translates from tens to thousands of days. C_2F_4 can undergo relatively rapid dissociation to CF_2 (16), its subsequent transformation depends on oxidation pathways not fully captured within the unimolecular framework considered here. In contrast, COF_2 exhibits intrinsic kinetic persistence due to its slow unimolecular decomposition. Additionally, OH-mediated oxidation, if available, may provide an alternative depletion pathway for CF_2O; however, it is not explicitly included in the present framework. Therefore, C_2F_4 is more appropriately described as a reactive intermediate reservoir, whereas COF_2 represents a kinetically persistent fluorinated product within the present reaction network. By comparison, many radical recombination or low barrier steps, such as C_2F_5• + O_2 (Reaction 1E(i)), FC–C• + O (Reaction 9A), CO + F_2 (Reaction 13), and F• + H_2O (Reaction 14), exhibit very small ΔE_a (0–40 kJ mol^{-1}), rate constants on the order of 10^{13}–10^{15}s^{-1}, and $t_{99.9}$ in the picosecond or sub-nanosecond regime, indicating that they are essentially diffusion controlled.

Table S02 shows a clear separation of timescales. Primary PFAS decomposition and most radical propagation steps are much faster than a 2 s (s) residence time at 1200 K, which explains efficient disappearance of the parent C_3F_7COOH. However, a small subset of high barrier steps that control the breakdown of C_2F_4, COF_2 and related fragments are far too slow to reach completion under typical incineration conditions. These slow steps constitute the kinetic bottlenecks that allow persistent fluorocarbon PICs to survive and be emitted even when PFAS destruction efficiencies appear high.

Fig. 4 is a schematic illustration of the energy and the residence time profile for selected reactions. The residence time data for all the proposed reactions are in Table S03 in the SI document.

Table 1 presents the calculated activation energy (ΔE_a = 512.9 kJ mol^{-1}), temperature-dependent rate constants, and corresponding residence times for the $C_2F_4 \rightarrow C_2F_3$• + F• dissociation. Because reaction rates follow the Arrhenius relationship, even small increases in temperature have an exponential effect on k(T). As a result, the residence time required for 99.9% conversion ($t_{99.9\%}$) decreases dramatically as temperature increases.

At low to moderate temperatures (500–1200 K), the rate constant remains exceedingly small (k ~ 10^{-39} –10^{-8}s^{-1}), resulting in residence times on the order of years to thousands of seconds. Such long timescales confirm that direct C–F bond dissociation is kinetically inaccessible under typical incineration conditions, where gas residence times rarely exceed 1–2s.

However, once temperatures exceed ~3000 K, the rate constant increases sharply (k ~ 10^6–10^{8}s^{-1}), and the corresponding residence times collapse into the nanosecond regime ($t_{99.9\%}$ ~10^{-6}–10^{-8}s). At 5000 K, $t_{99.9\%}$ is on the order of 1–2 s for 99.9% decomposition, with characteristic times of nanoseconds for single-molecule events. These extremely short times reflect the fact that, at such high temperatures, the thermal energy (RT) approaches the magnitude of the activation barrier, placing the system in a regime where bond dissociation becomes statistically probable within ultrashort time windows.

Residence times of minutes to days at these temperatures explain why C_2F_4 and related PFAS fragments persist and behave as PICs. Such temperatures are far above those reached in practical waste-incineration systems but can occur during shock heating, plasma treatment, or ultrasonic cavitation hot-spots, consistent with experimental observations of transient PFAS fragmentation under those conditions. Overall, kinetic calculations reported here demonstrate that thermal decomposition of PFAS cannot rely on unimolecular C–F cleavage, and must instead proceed through lower-energy radical, oxidative, or stepwise pathways. The $t_{99.9\%}$ values shifting from days to nanoseconds as temperature increases encapsulate this fundamental kinetic limitation.

Fig. 5 shows the temperature-dependent residence time required for 99.9% conversion of $C_2F_4 \rightarrow C_2F_3$• + F• based on transition-state-derived rate constants. Below ~1500 K, the predicted residence time exceeds practical incineration scales (>10^3–10^8 days), indicating that direct C–F scission is kinetically inaccessible. Only at temperatures above ~3500 K does the reaction occur on sub-second timescales, highlighting that unimolecular dissociation cannot drive PFAS destruction under conventional combustion conditions.

Fig. 6 illustrates the temperature dependence of PFBA decomposition obtained from reactive MD simulations over 10ns time limit. The detailed species list is shown in Table S04 ((a)-(f)) in the SI document. At 500 and 1000 K, the number of intact PFBA molecules remains nearly constant over the simulation time, indicating negligible thermal degradation. In contrast, at 2000 K and above, PFBA undergoes rapid fragmentation, with the intact molecule population declining sharply within the first few nanoseconds. At 3000 K and higher, complete PFBA decomposition occurs almost instantaneously on the MD timescale. This behavior demonstrates that increasing temperature significantly accelerates PFBA breakdown, reflecting the exponential temperature dependence of bond dissociation and radical-driven degradation

Table 1
Calculated ΔE_a values, rate constants and residence time in seconds, residence time in nanoseconds and residence time in days for reaction #3 ($C_2F_4 \rightarrow C_2F_3$. + F.) at different temperatures.

A	Ea (kJ/mol)	Temperature(K)	k (s^{-1})	$t_{99.9\%}$ (s)	$t_{99.9\%}$ (ns)	$t_{99.9\%}$(d)
1E+15	512.87	500	2.62E-39	2.63E+39	2.63E+48	3.05E+34
1E+15	512.87	1000	1.62E-12	4.26466E+12	4.26E+21	49359480.81
1E+15	512.87	1200	4.73E-08	146148073.5	1.46E+17	1691.528629
1E+15	512.87	1500	1.38E-03	5008.432958	5.01E+12	0.057967974
1E+15	512.87	2000	4.02E+01	0.171636889	1.72E+08	1.98654E-06
1E+15	512.87	3000	1.17E+06	5.88192E-06	5881.92	6.80778E-11
1E+15	512.87	4000	2.01E+08	3.44329E-08	34.43	3.98529E-13
1E+15	512.87	5000	4.38E+09	1.5756E-09	1.58	1.82361E-14

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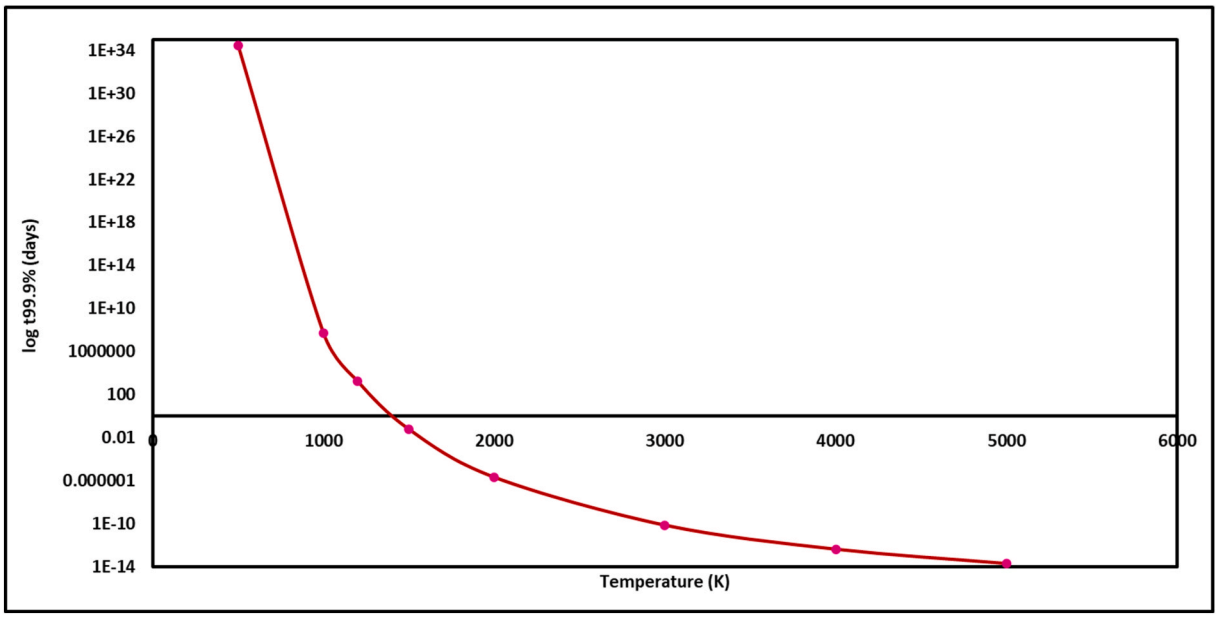


Fig. 5. Temperature dependence of residence time required to reach 99.9% conversion for the unimolecular dissociation of C_2F_4 into C_2F_3• and F• (Reaction 3).

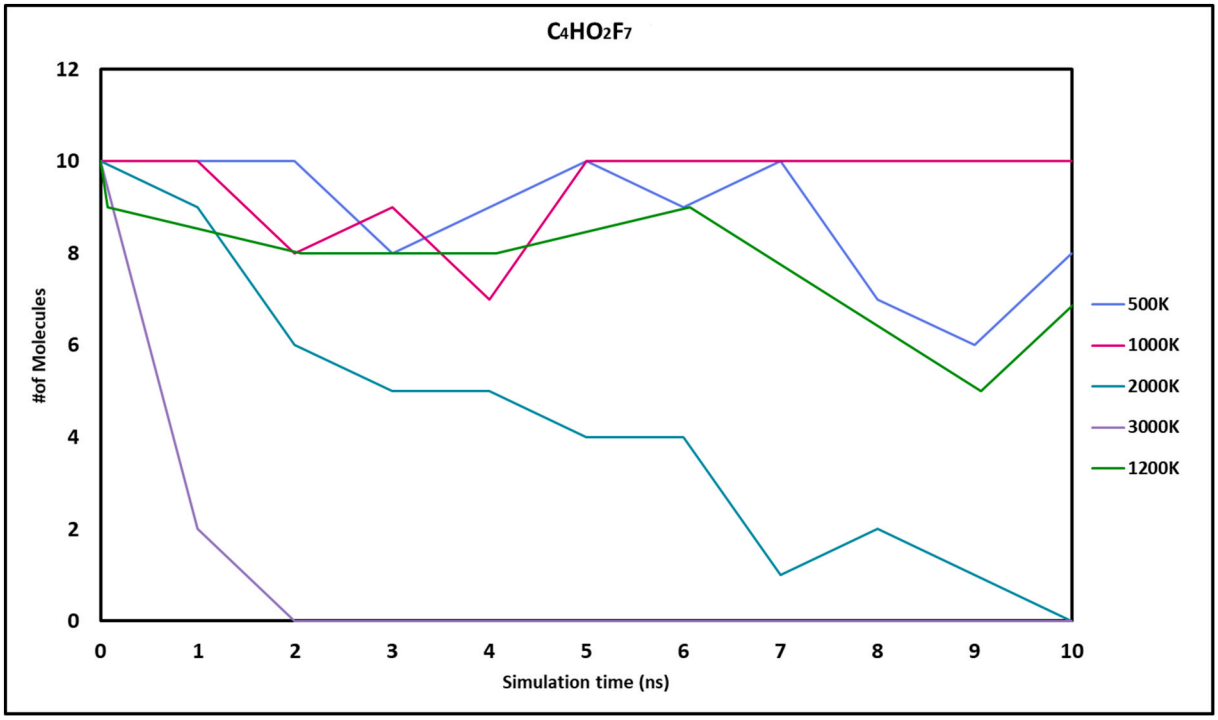


Fig. 6. Temperature-Dependent Decomposition of PFBA ($C_4HO_2F_7$) from Reactive MD Simulations at temperatures ranging from 500 to 3000 K.

pathways.

3.3. Comparison with the experiment and field scale data

The mechanistic and kinetic insights obtained from MD and DFT simulations align closely with observations from laboratory-scale thermal degradation and full-scale incineration studies. Experimental investigations have shown that PFAS degradation does not occur in a single step but proceeds through a sequence of intermediate products of incomplete combustion (PICs), including C_2F_4, CF_3•, CF_2O, COF_2, and perfluoroacyl fluorides, which matches the intermediate species identified in the MD trajectories and reaction pathways predicted in this study. Similar intermediates have been reported in experimental and kinetic studies of PFAS thermal decomposition and combustion chemistry (Altarawneh et al., 2022a; Krug et al., 2022; N. H. Weber et al., 2024).

Field-scale incinerator measurements have similarly detected short-chain fluorocarbons and acid fluorides under suboptimal operating conditions, supporting the conclusion that degradation pathways involving β-scission of perfluoroalkyl radicals and formation of perfluoroacyl fluorides are kinetically favored at high temperature (Rocchio et al., 2025).

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These qualitative agreements with experimental observations provide indirect validation of the computational reaction mechanisms and kinetic trends predicted in this study.

In addition, calculated activation barriers provide a mechanistic explanation for the persistence of certain fluorinated species in real systems. For example, the high Ea for C_2F_4 defluorination (~513 kJ/mol) is consistent with experimental findings that tetrafluoroethylene can survive typical residence times in rotary kilns and fluidized-bed incinerators. Similarly, the predicted formation of COF_2 follows established combustion chemistry, and both compounds have been reported in laboratory pyrolysis and smoldering tests (Krug et al., 2022; N. H. Weber et al., 2024).

Residence-time estimates derived from Arrhenius-based rate constants in this study likewise agree with engineering data: while early fragmentation steps proceed rapidly, complete destruction of stable PICs such as COF_2 and C_2F_4 requires residence times longer than those commonly used in conventional municipal or hazardous-waste incinerators. These findings are consistent with experimental combustion studies demonstrating that PFAS destruction efficiency depends strongly on temperature, residence time, oxidizing conditions, and reactor design (Altarawneh et al., 2022a; Krug et al., 2022; N. H. Weber et al., 2024).

Overall, computational results provide a mechanistic foundation that explains, and, in several cases, predicts the intermediates, byproducts, and destruction of efficiencies observed in experimental studies and full-scale thermal treatment systems.

4. Conclusions

This work provides molecular-level insight into the thermal breakdown of PFAS using combined MD simulations and DFT-based reaction kinetics. By systematically resolving elementary decomposition steps of PFBA and quantifying activation barriers, this study demonstrates that PFAS destruction during high-temperature treatment does not occur through direct C–F bond cleavage. Instead, early decomposition is controlled by rapid decarboxylation and β-scission steps that generate highly persistent intermediates such as C_2F_4, $CF_3\bullet$, and COF_2. These radicals and fluorinated fragments form within microsecond timescales, well within typical incineration residence times; however, their subsequent defluorination transitions exhibit activation energies more than 480–520 kJ mol^{-1} and residence times ranging from seconds to several days at 1200 K. Kinetic modeling clearly distinguishes fast, thermally accessible reactions from rate-limiting defluorination events. While initial PFBA dissociation and oxidative propagation reactions rapidly proceed toward intermediate fluorocarbon formation, the slow unimolecular breakdown of C_2F_4 and COF_2 acts as the dominant kinetic bottleneck. These findings provide a mechanistic basis for the recurrence of these species as products of incomplete combustion in laboratory and full-scale incineration studies. Moreover, the asymmetry in energetics such as essentially barrierless recombination of $CF_3\bullet + F\bullet \rightarrow CF_4$ contrasted with the very slow decomposition of CF_4 explains the accumulation of terminal perfluorocarbons even at elevated combustion temperatures.

Overall, the atomistic framework developed here based on the unimolecular intrinsic reaction kinetics quantitatively links reaction energetics to residence-time requirements and suggested that complete mineralization of fluorinated intermediates may require longer residence time than reported for conventional incineration.

Although PFBA was used as a representative short-chain PFCA in this study, the mechanistic framework developed here can be extended to other PFAS classes, since many PFAS share similar perfluoroalkyl backbones and radical-driven fragmentation pathways during thermal decomposition. The findings highlight the need for either extended high-temperature exposure, enhanced oxidizing environments, plasma/electrical heating zones, or catalytic interventions to accelerate terminal defluorination steps. The mechanistic insights and kinetic thresholds established in this research provide a scientific foundation for optimizing incineration design and preventing emission of persistent fluorocarbon combustion products. Unlike thermal treatment, which requires extended residence times to overcome large activation barriers, ultrasonication produces microsecond-duration hotspots exceeding 3000 K, enabling complete PFAS mineralization in a significantly shorter time.

CRediT authorship contribution statement

Jay N. Meegoda: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Targol Teymourian:** Writing – review & editing, Writing – original draft, Visualization, Validation, Investigation. **Duwage C. Perera:** Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare they have no known competing financial interests or personal relationships that could affect the work described in this article.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chemosphere.2026.144914.

Data availability

Data will be made available on request.

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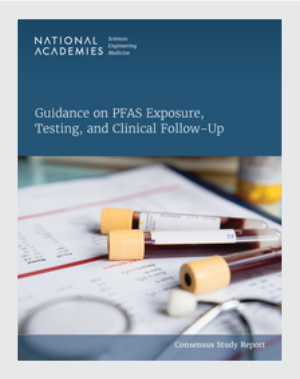
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[1] Deceased January 2022.

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Reviewers

This Consensus Study Report was reviewed in draft form by individuals chosen for their diverse perspectives and technical expertise. The purpose of this independent review is to provide candid and critical comments that will assist the National Academies of Sciences, Engineering, and Medicine in making each published report as sound as possible and to ensure that it meets the institutional standards for quality, objectivity, evidence, and responsiveness to the study charge. The review comments and draft manuscript remain confidential to protect the integrity of the deliberative process.

We thank the following individuals for their review of this report:

Andrea Amico, Testing for Pease
Dean Baker, University of California, Irvine
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Although the reviewers listed above provided many constructive comments and suggestions, they were not asked to endorse the conclusions or recommendations of this report, nor did they see the final draft before its release. The review of this report was overseen by **Joshua Sharfstein**, Johns Hopkins Bloomberg School of Public Health, and **Susan Brantley**, The Pennsylvania State University. They were responsible for making certain that an independent examination of this report was carried out in accordance with the standards of the National Academies and that all review comments were carefully considered. Responsibility for the final content rests entirely with the authoring committee and the National Academies.

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Many people were critical in helping the committee accomplish its charge. The committee gratefully acknowledges the work of the community liaisons who provided insight and viewpoints pertinent to planning for our public meetings. In particular, we thank Andrea Amico, Emily Donovan, Patrick Elder, Ayesha Khan, Kristen Mello, and Laura Olah, who provided helpful insights on the perspectives and experiences of their communities that informed our community engagement methods. This engagement ensured that our public meetings would include the relevant perspectives, which allowed us to learn about the social, environmental, public health, and medical challenges central to our charge. In addition, we found the information and perspectives provided by the presentations and discussions at our public meetings immensely helpful in informing our deliberations (see Appendix C).

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The committee thanks the staff of the National Academies of Sciences, Engineering, and Medicine who contributed to producing this report, especially the extraordinary, creative, and tireless study staff: Kaley Beins, Elizabeth Boyle, Clifford Duke, Kathryn Guyton, Rose Marie Martinez, Alexandra McKay, Marilee Shelton-Davenport, and Alexis Wojtowicz. Thanks as well go to other staff in the Division on Earth and Life Studies who provided additional support, including Tamara Dawson, Eric Edkin, Elizabeth Eide, Lauren Everett, Nancy Huddleston, Radiah Rose, and Maggie Walser. This project also received important assistance from Megan Lowry (Office of News and Public Information) and Matthew Anderson (Office of Financial Administration). Valuable research assistance was provided by Christopher Lao-Scott, senior research librarian in the National Academies Research Center. Finally, a thank you is extended to Rona Briere and Allison Boman, who assisted the committee with editing the report.

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Preface

The creation of the U.S. Environmental Protection Agency (EPA) in 1970 signaled public recognition of industrial impacts on the environment. The government subsequently acknowledged that three elements essential for life—air, water, and food—could be contaminated by industrial activity and threaten human health. In 1980, two steps were taken in response to the government's concern: passage of the Comprehensive Environmental Response, Compensation, and Liability Act and creation of the Agency for Toxic Substances and Disease Registry (ATSDR).

The mission of ATSDR is to prevent or mitigate the adverse impacts on human health and diminished quality of life resulting from exposure to hazardous substances in the environment. The earliest contaminants of concern included pesticides; heavy metals from mining; asbestos; munitions and their manufacturing by-products (including radioactive substances); petrochemicals, including solvents; and products and by-products associated with oil and gas extraction, refinement, and use. Over time, additional industrial products with significant potential to affect the population's health have been identified, including the class of chemicals known as per- and polyfluoroalkyl substances, or PFAS. PFAS have useful properties, such as oil and water repellency, temperature resistance, and friction reduction. For decades, they have been used in numerous applications and products, such as firefighting; chrome-plating; lubricants; insecticides; and coatings and treatments for such surfaces as carpeting, packaging, and cookware. As a result of the production and use of PFAS, many sites across the country are contaminated with PFAS, which in turn can result in contamination of soils and drinking water.

ATSDR faces a critical challenge in protecting people from the potential health impacts of PFAS exposures. Data from the National Health and Nutrition Examination Survey show that nearly 100 percent of people in the United States are exposed to at least one PFAS, but at what level of exposure do harms to human health occur? What PFAS-associated health outcomes might benefit from clinical follow-up or care? Would there be any benefit in testing people to know their PFAS exposure level? What clinical follow-up can help protect people from PFAS-associated harms?

Answering these questions requires bridging approaches used for chemical hazard assessments, such as those carried out by the EPA; public and community health benefits, such as those laid out in the Community Guide to Preventive Services; and medical care health benefit assessments, such as those of the U.S. Preventive Services Task Force. Chemical hazard assessments are conducted to determine whether a chemical exposure causes harm; public health assessments address the impact on and interventions to mitigate threats to the health of a population or community; and medical care health benefit assessments evaluate the effects of medical interventions, including their beneficial effects and potential adverse outcomes. One challenge with blending these different approaches is that in medical care health benefit assessments, the "gold standard" for informing clinical risk/benefit decisions for medical interventions is the randomized controlled trial (RCT). However, RCTs are typically impossible for chemical hazard assessments, for both ethical and practical reasons. For example, most environmental chemicals are not developed to improve human health, making intentional exposures in an RCT unethical. Chemical exposures also vary and may or may not be significant depending on the agent's toxicity, which sometimes makes controlled trials infeasible. And public health assessments, in which comparative studies are still required to support evidence-based recommendations, usually require many years of data and are challenged by the long-standing and often-lamented separation of health care and public health.

In 2010, researchers Stephen Rappaport and Martyn Smith reported that "70 to 90% of disease risks are probably due to differences in environments" and made the case for a more comprehensive approach to evaluating environmental exposures in order to understand the causes of and contributors to

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chronic disease.[2] Such an approach has, as in the case of lead, and will, for chemicals such as PFAS, ultimately depend on breaking down the barriers between environmental public health and the clinical care setting. These two health sectors have had some limited success in bridging the gap for infectious disease outbreaks and epidemics. Identifying environmental exposures, measuring exposure levels in patients, and providing indicated medical follow-up are elements of a critical frontier that could and should bring the two disciplines closer together to improve the health of those in the nation's communities.

Another challenge for the study committee was the critical need to include community voices in the study process as an important and credible source of evidence to inform guidance recommendations. To meet that challenge, this study included the testimony of more than 30 people who live in or work with a community impacted by PFAS contamination. Community members provided the committee with much needed data based on their lived experiences with PFAS contamination, and moved the committee's work from an academic exercise to a personal reality. The committee used the presentations of community members to inform frameworks within the report and to gain an understanding of the social context that the committee's recommendations will inform.

Atmospheric chemist Susan Solomon has suggested that successfully addressing environmental challenges requires making the problem personal, perceptible, and practical. The voices of affected individuals in contaminated communities make the PFAS issue personal, while the scientists researching the associations with human health make the impacts of PFAS exposure perceptible. In this report, the committee has endeavored to provide practical recommendations that can aid policy makers, state and federal environmental and public health agencies, clinicians, and concerned individuals in addressing this important health problem.

Bruce N. Calonge, *Chair*
Committee on the Guidance on PFAS Testing and Health Outcomes

[2] Rappaport, S., and M. Smith. 2010. *Science* 330(6003):460–461. https://doi.org/10.1126/science.1192603.

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Acronyms and Abbreviations

AAFP	American Academy of Family Physicians
AAP	American Academy of Pediatrics
ACOG	American College of Obstetricians and Gynecologists
AHRQ	Agency for Healthcare Research and Quality
ALT	alanine aminotransferase
ANHE	Alliance of Nurses for Health Environments
ASTHO	Association of State and Territorial Health Officials
ATSDR	Agency for Toxic Substances and Disease Registry
BE	biomonitoring equivalent
BLL	blood lead level
BMI	body mass index
CAS	Chemical Abstract Services
CDC	Centers for Disease Control and Prevention
CERCLA	Comprehensive Environmental Response, Compensation, and Liability Act
CI	confidence interval
CMS	Centers for Medicare & Medicaid Services
CPG	clinical practice guideline
CPT	Current Procedural Terminology
DNA	deoxyribonucleic acid
DOD	U.S. Department of Defense
EFSA	European Food Safety Authority
EPA	U.S. Environmental Protection Agency
EVIDEM	Evidence and Value in Decision Making
EWG	Environmental Working Group
FDA	U.S. Food and Drug Administration
FOSA	perfluorooctane sulfonamide
GRADE	Grading of Recommendations, Assessment, Development and Evaluations
HBM	human biomonitoring
HDL	high-density lipoprotein
HHS	U.S. Department of Health and Human Services
HMB	German Human Biomonitoring Commission
IARC	International Agency for Research on Cancer
IOM	Institute of Medicine
LDL	low-density lipoprotein
LOD	limit of detection

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MCL	maximum contaminant level
MeFOSAA	methylperfluorooctane sulfonamidoacetic acid
NAS	National Academy of Sciences
NHANES	National Health and Nutrition Examination Survey
NIEHS	National Institute of Environmental Health Sciences
NIOSH	National Institute for Occupational Safety and Health
NIST-SRM	National Institute of Standards and Technology Standard Reference Material
NRC	National Research Council
NTP	National Toxicology Program
OECD	Organisation for Economic Co-operation and Development
OR	odds ratio
OSHA	Occupational Safety and Health Administration
PBPK	physiologically based pharmacokinetic
PEHSU	Pediatric Environmental Health Specialty Unit
PFAS-REACH	PFAS Research, Education, and Action for Community Health
PFBA	perfluorobutanoic acid
PFBS	perfluorobutane sulfonic acid
PFCA	perfluorinated aliphatic carboxylic acid
PFDA	perfluorodecanoic acid
PFDoDA	perfluorododecanoic acid
PFHpA	perfluoroheptanoic acid
PFHpS	perfluoroheptanesulfonic acid
PFHxA	perfluorohexanoic acid
PFHxS	perfluorohexane sulfonic acid
PFNA	perfluorononanoic acid
PFOA	perfluorooctanoic acid
PFOS	perfluorooctane sulfonic acid
PFUnA	perfluoroundecanoic acid
PFUnDA	perfluoroundecanoic acid
QA	Quality Assurance
QC	Quality Control
RfD	reference dose
SD	standard deviation
SSEHRI	Social Science Environmental Health Research Institute
TSCA	Toxic Substances Control Act
USDA	U.S. Department of Agriculture
USPSTF	U.S. Preventive Services Task Force
VHA	Veterans Health Administration
WHO	World Health Organization

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Summary[1]

Perfluoroalkyl and polyfluoroalkyl substances (PFAS) are a class of chemicals that includes more than 12,000[2] different compounds with various chemical properties. PFAS are commonly used in thousands of products, from nonstick cookware to firefighting foams and protective gear, because they have desirable chemical properties that impart oil and water repellency, friction reduction, and temperature resistance. PFAS as a class have a wide variety of distinct chemical properties and toxicities; for example, some PFAS can bioaccumulate and persist in the human body and the environment, while others transform relatively quickly. The PFAS that do transform, however, will become one or more other PFAS because the carbon–fluorine bond they contain does not break naturally. It is for this reason that PFAS are termed "forever chemicals."

STUDY CONTEXT

Public concern about the impact of PFAS contamination on human health and the environment began in the late 1990s when perfluorooctanoic acid (PFOA) water contamination was identified in Parkersburg, West Virginia. As a result, 3M, a primary PFAS manufacturer initiated a voluntary phase-out of some PFAS (PFOA, perfluorooctanesulfonic acid [PFOS], and perfluorohexanesulfonic acid [PFHxS]). The contamination in Parkersburg also led to a class action lawsuit that identified several health effects related to PFAS exposure and led to the establishment of a medical monitoring program in 2013. Shortly thereafter, the U.S. Environmental Protection Agency (EPA) began requiring all community water systems serving more than 10,000 people to test for certain PFAS, which led to more communities learning that their water was contaminated. In 2016, researchers found that the drinking water supply for Wilmington, North Carolina, was contaminated with a chemical called "GenX," a PFOA replacement. This finding led to public concern about the potential health effects of replacement PFAS (see Figure S-1).

Organizations such as the International Agency for Research on Cancer (IARC), the Agency for Toxic Substances and Disease Registry (ATSDR), and the EPA have linked exposure to PFAS (particularly PFOA and PFOS) to multiple cancers, thyroid dysfunction, small changes in birthweight, and high cholesterol. Gaining a complete picture of the threat can be difficult, however, because of the chemical and toxicological differences among individual PFAS and uncertainty about the exposure level at which their adverse effects may occur. In addition, many of the chronic diseases associated with PFAS exposure have myriad causes.

An estimated 2,854 U.S. locations (in all 50 states and two territories) have some level of PFAS contamination (see Figure S-2). Although not all of the contamination represents exceedances of health advisories, the pervasiveness of the contamination is alarming. Furthermore, almost 100 percent of the U.S. population is exposed to at least one PFAS. Although exposures to the phased-out PFAS have been decreasing (see Figure S-3), people are still exposed to those PFAS from site contamination, occupational uses of stored products, and breakdown of PFAS polymer products that are found in homes. Carpeting, for example, is often treated with fluorotelomer-based polymers that can biodegrade to form phased-out PFAS, such as PFOA. Exposures also occur to the PFAS chemicals used to replace those that have been phased out. Although the harms of the replacement PFAS are less well understood, they may have

[1] No references are included in this Summary. References to the content herein are provided in the respective chapters of the main text.

[2] EPA Comptox Dashboard (https://comptox.epa.gov/dashboard/chemical-lists/pfasmaster [accessed May 25, 2022]).

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comparable or more serious toxicity than the PFAS they have replaced. The state of New Jersey, for example, recently set a groundwater standard for a replacement PFAS that is an order of magnitude lower than drinking water standards for other PFAS. The New Jersey maximum contaminant levels of perfluorononanoic acid (PFNA), PFOA, and PFOS in drinking water are 13, 14, and 13 nanograms per liter (ng/L), respectively,[3] while the groundwater standard for the replacement PFAS, chloroperfluoropolyether carboxylates, is 2 ng/L.[4]

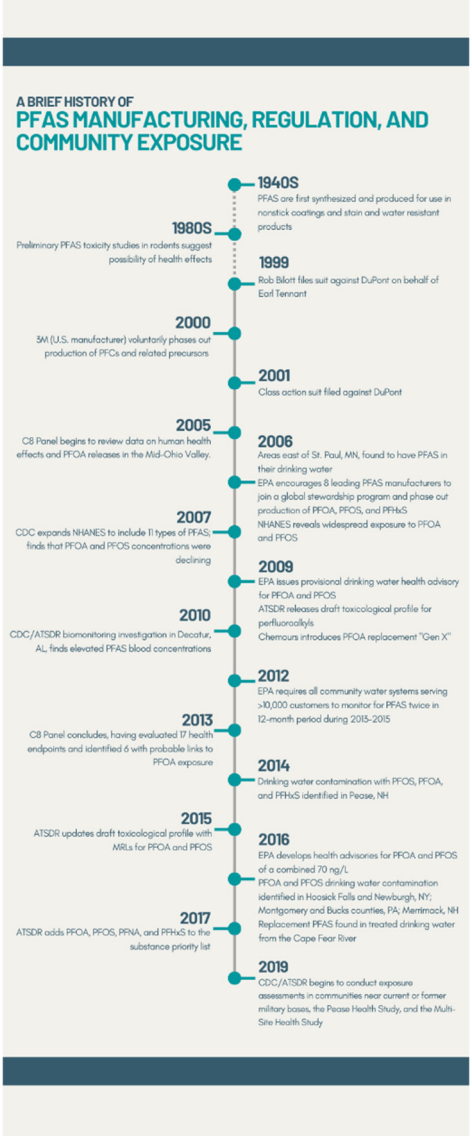


FIGURE S-1 Brief history of PFAS manufacturing, regulation, and community exposure.
NOTE: ATSDR = Agency for Toxic Substances and Disease Registry; CDC = Centers for Disease Control and Prevention; EPA = U.S. Environmental Protection Agency; MRL = minimal risk limit; ng/L = nanograms per liter; NHANES = National Health and Nutrition Examination Survey; PFAS = per- and polyfluoroalkyl substances; PFC = perfluorochemicals; PFHxS = perfluorohexane sulfonic acid; PFNA = perfluorononanoic acid; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid.
SOURCE: Committee generated based on slides included by Patrick N. Breysse in a presentation to the committee on February 4, 2021.

[3] See https://www.nj.gov/health/ceohs/documents/pfas_drinking%20water.pdf (accessed June 8, 2022).
[4] See https://www.nj.gov/dep/standards/ClPFPECA_Standard.pdf (accessed June 8, 2022).

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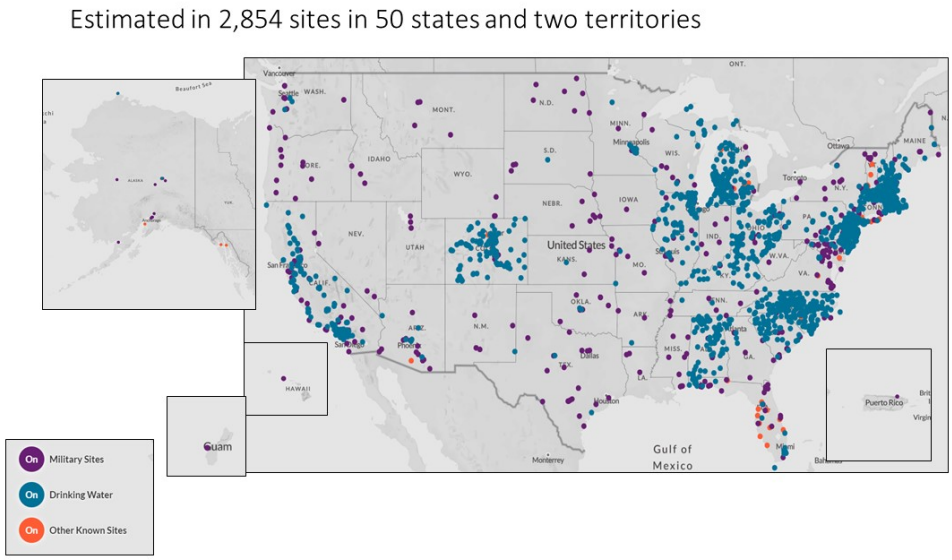


FIGURE S-2 PFAS contamination across the United States.
NOTES: According to the Environmental Working Group (EWG), "locations represented on the map are approximate and intended to portray the general area of a contamination site or a community water system. Locations were mapped using the best data available from official records, including data provided by tests of public drinking water systems, the Safe Drinking Water Information System and the Department of Defense report *Addressing Perfluorooctane Sulfonate (PFOS) and Perfluorooctanoic Acid (PFOA)*, and Department of Defense public records, among others. Data on contaminated industrial and military sites was current as of October 2021." Furthermore, "EWG has worked to ensure the accuracy of the information provided in this map. The map is dynamic. This contaminant site, results, suspected sources and other information in the database may change based on evolving science, new information or other factors. Please be advised that this information frequently relies on data obtained from many sources, and accordingly, EWG cannot guarantee the accuracy of the information provided or any analysis based thereon."
SOURCE: See https://www.ewg.org/interactive-maps/pfas_contamination/map (accessed May 11, 2022). Copyright © Environmental Working Group, www.ewg.org. Reproduced with permission.

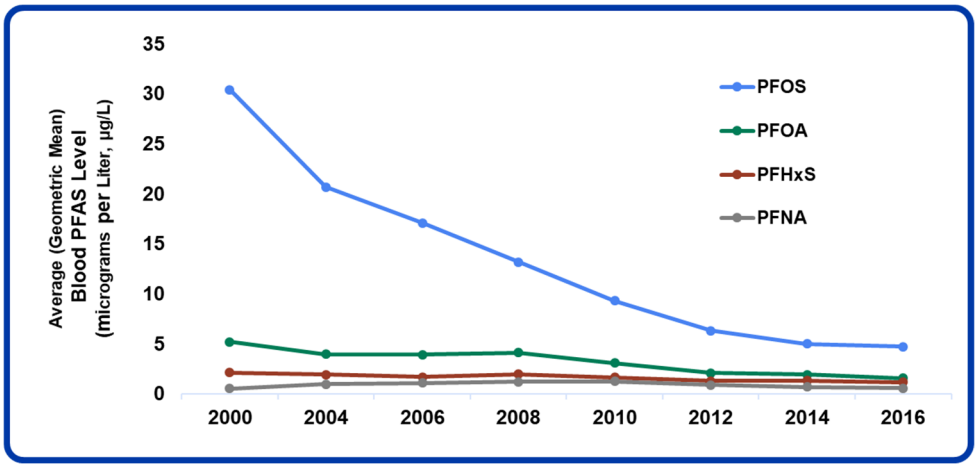


FIGURE S-3 Blood (serum) levels of PFAS, United States, 2000–2016.
NOTE: Average = geometric mean.
SOURCE: Patrick N. Breysse's presentation to the committee on February 4, 2021. DATA SOURCE: Centers for Disease Control and Prevention (2019, January). *Fourth Report on Human Exposure to Environmental Chemicals*, updated tables. Atlanta, GA: U.S. Department of Health and Human Services.

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The people who live, work, and play in environments where PFAS contamination exceeds standards do not know how to protect themselves from the health risks of exposure. Many have been exposed to PFAS for decades and may continue to be exposed in their homes or workplaces. Some members of communities that have discovered that their exposures exceed health advisory levels are calling for a medical program to prevent, lead to early detection of, or treat diseases related to the health risks they may face. Developing such a program is challenging, however, because of the uncertainties about the health effects of PFAS and potential harms from additional medical treatments; moreover, many of these diseases are not preventable or even treatable, and many clinicians lack information about what they can and should do for these patients.

To help clinicians[5] respond to patient concerns about PFAS exposure, ATSDR published *PFAS: An Overview of the Science and Guidance for Clinicians on Per- and Polyfluoroalkyl Substances.*[6] This guidance summarizes general information about PFAS and PFAS health studies and suggests answers to example patient questions. However, the ATSDR guidance does not provide specific recommendations on when to test for PFAS, how to order the tests, how to interpret the results, or what clinical follow-up based on PFAS exposure might look like. Interpretation of PFAS blood or urine results is challenging because the specific level of exposure at which harms may occur is unknown, and the science on the potential health effects of exposure to PFAS is advancing quickly, making it difficult to provide advice to clinicians about what follow-up tests might be helpful.

STUDY CHARGE AND APPROACH

ATSDR and the National Institute of Environmental Health Sciences (NIEHS) asked the National Academies of Sciences, Engineering, and Medicine to form an ad hoc committee to advise on PFAS testing and clinical care for patients exposed to PFAS. The Statement of Task asked the committee to

- develop principles for biological testing and clinical evaluation, given substantial scientific uncertainty about the health effects or the value of such measures in informing care,
- review the human health literature for the health effects of PFAS, and
- characterize human exposure pathways and develop principles for exposure reduction.

The Statement of Task asked the committee to recommend

- options and considerations to guide decision making for PFAS testing in a patient's blood or urine,
- PFAS concentrations that could inform clinical care of exposed patients, and
- appropriate patient follow-up and care specific to PFAS-associated health endpoints for those patients known or suspected to be exposed to PFAS.

The committee also was asked to provide advice on changes to ATSDR's clinical guidance. The committee was not asked for community prevention guidance or advice on policies that would reduce exposure to PFAS.

Figure S-4 summarizes the committee's approach to the Statement of Task. A critical component of the approach was community engagement because people who live with potentially harmful exposures have knowledge from experiential learning that precedes scientific findings. The study's community engagement consisted of a panel of community liaisons, three town halls (summarized in Appendix B),

[5] The committee uses "clinician" throughout this report to refer to "a healthcare professional qualified in the clinical practice of medicine. Clinicians may be physicians, nurses, pharmacists, or other allied health professionals" as defined by the Centers for Medicare & Medicaid Services (https://www.cms.gov/Medicare/Quality-Initiatives-Patient-Assessment-Instruments/MMS/QMY-Clinicians [accessed June 14, 2022]).

[6] See https://www.atsdr.cdc.gov/pfas/docs/clinical-guidance-12-20-2019.pdf (accessed June 14, 2022).

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community speakers at every public committee meeting, open sign-up for public testimony at every public meeting, and encouragement for written testimony throughout the data collection phase of the study. The following sections provide descriptions of the remaining components of the study approach and the committee's associated recommendations.

COMMITTEE'S PRINCIPLES FOR DECISION MAKING UNDER UNCERTAINTY

Providing clear advice to clinicians on clinical follow-up for PFAS-exposed patients is challenging. The questions in the Statement of Task all have some degree of uncertainty. First, clinicians need to know what the health consequences of PFAS exposure are. If everyone is exposed, what exposure level warrants follow-up? What actions can be taken to reduce exposure or prevent disease? What are the harms of those actions? Answering all of these questions requires making ethical judgments. Building on the work of other experts and evidence-to-decision frameworks, the committee developed five principles to guide decision making under uncertainty for use throughout this report and by ATSDR when updating the guidance for clinicians: proportionality, justice, autonomy, feasibility, and adaptability (see Box S-1). In the clinical setting, these principles converge under the principle of autonomy; thus, shared, informed decision making between clinician and patient is the practical way to incorporate the principles into a clinical encounter.

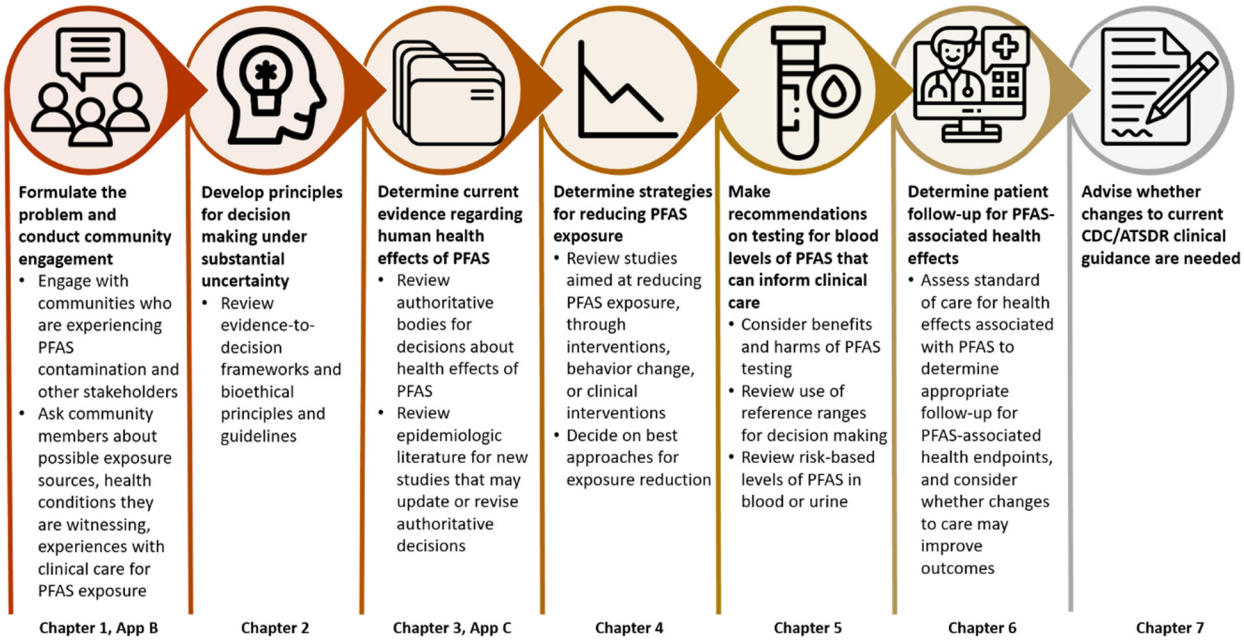


FIGURE S-4 The committee's approach to the Statement of Task and the chapters and appendixes where the topics are discussed.
NOTE: ATSDR = Agency of Toxic Substances and Disease Registry; CDC = Centers for Disease Control and Prevention; PFAS = per- and polyfluoroalkyl substances.

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BOX S-1
Principles for Decision Making Under Uncertainty Used in This Report

Proportionality: Decisions should balance plausible harms and benefits proportionally, meaning that the harms and benefits are considered together and weighed based on a qualitative assessment of their potential likelihoods and magnitudes.

Justice: Decisions should be informed by an emphasis on promoting justice, including by balancing benefits and harms fairly across the population of at-risk individuals, advancing health equity, and respecting human rights. In addition, justice requires consideration of sociohistorical context, stakeholders, existing structural inequalities, and issues of agency (the power a community has to advocate for itself in conflicts).

Autonomy: Decisions should be based on informed decision making by individuals and reflect respect for their values.

Feasibility: Decisions should take into account resource availability, including follow-up services.

Adaptability: Decisions should respond to new information about harms, benefits, and other relevant considerations (e.g., health equity and feasibility).

POTENTIAL HEALTH EFFECTS OF PFAS

To recommend PFAS levels that should inform clinical care, the committee conducted a literature review to determine health effects or conditions associated with PFAS. The Statement of Task limited this review to human studies of those PFAS included in the Centers for Disease Control and Prevention's (CDC's) *National Report on Human Exposure to Environmental Chemicals* (see Table S-1). Other PFAS may also cause harm, though they do not all have the same biological persistence and toxicity. Most people are exposed to mixtures of PFAS such that specific effects are difficult to disentangle. Considering these issues, and recognizing that some PFAS are infrequently measured, the committee provided one strength-of-evidence determination for all PFAS for each health effect, recognizing that providing one conclusion across PFAS may not account for the distinct physical, chemical, and toxicological properties of each type of PFAS.

TABLE S-1 PFAS Species Currently Included in the Centers for Disease Control and Prevention's *National Report on Human Exposure to Environmental Chemicals*

Abbreviated Name	Full Name	CAS Registry No.
MeFOSAA	Methylperfluorooctane sulfonamidoacetic acid	2355-31-9
PFHxS	Perfluorohexanesulfonic acid perfluorohexane sulfonic acid	355-46-4
n-PFOA (linear isomer), Sb-PFOA (branched isomers)	Perfluorooctanoic acid	335-67-1*
PFDA	Perfluorodecanoic acid	335-76-2
PFUnDA	Perfluoroundecanoic acid	2058-94-8
n-PFOS (linear isomer), Sm-PFOS (branched isomers)	Perfluorooctanesulfonic acid	1763-23-1*
PFNA	Perfluorononanoic acid	375-95-1

NOTES: CAS = Chemical Abstracts Service. * = CAS number refers to linear isomer only. Previous survey years have also included perfluorobutane sulfonic acid (PFBS), perfluoropentanoic acid (PFpA), perfluorododecanoic acid (PFDoDA), perfluorooctane sulfonamide (FOSA), and 2-(N-ethyl-perfluorooctane sulfonamido)acetate (EtFOSAA), according to Patrick N. Breysse's presentation to the committee on February 4, 2021.

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The committee began its review by identifying decisions of other authoritative bodies regarding PFAS exposure and any human health effects. The committee then considered more recent human studies, both systematic reviews and published epidemiologic research articles, that could inform updates to those decisions. This approach improved efficiency while minimizing the risk of excluding scientific findings that could inform the committee's conclusions.

The committee considered animal studies discussed in ATSDR's *Toxicological Profile for Perfluoroalkyls* and in systematic reviews to aid in its interpretation of the human studies. The committee synthesized the available evidence into four categories of association used by other National Academies committees: sufficient evidence of an association, limited or suggestive evidence of an association, inadequate or insufficient evidence of an association, and limited or suggestive evidence of no association (see Figure S-5). All associations between PFAS and a health outcome were considered inadequate or insufficient by default unless available evidence from all aspects of the committee's review warranted placing the evidence in another category of association.

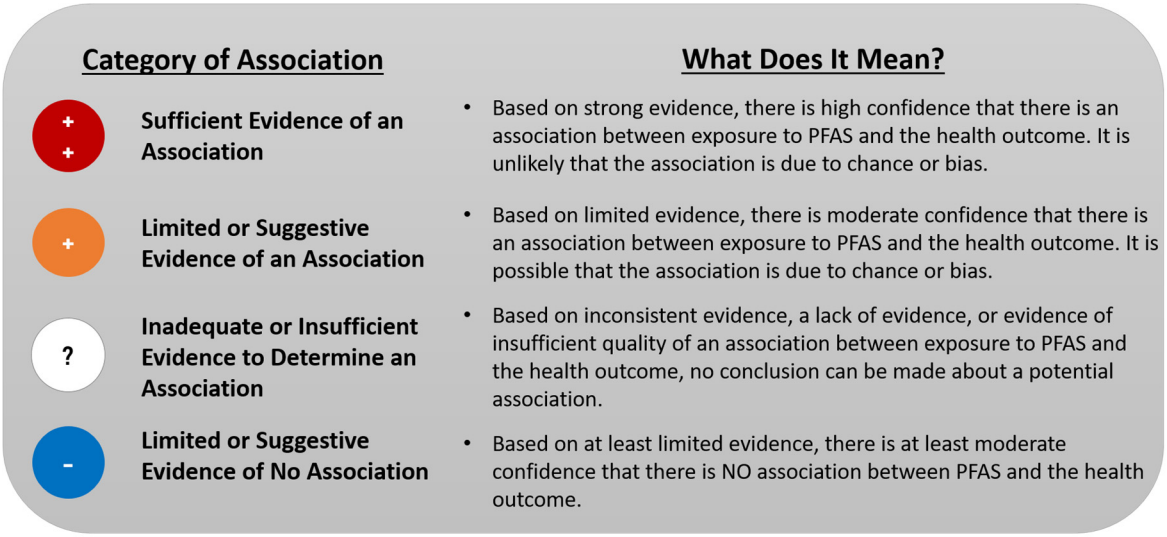


FIGURE S-5 Categories of association used in this report.
NOTES: The categories of association only describe how strong the evidence is between PFAS and the health outcome. The risk of developing an outcome from exposure to PFAS for things in the same category can vastly differ and are dependent on whether an individual has other risk factors for developing the outcome.

The committee found sufficient evidence of an association for the following diseases and health outcomes:

- **decreased antibody response (in adults and children),**
- **dyslipidemia (in adults and children),**
- **decreased infant and fetal growth, and**
- **increased risk of kidney cancer (in adults).**

The committee found limited or suggestive evidence of an association for the following diseases and health outcomes:

- **increased risk of breast cancer (in adults),**
- **liver enzyme alterations (in adults and children),**

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Guidance on PFAS Exposure, Testing, and Clinical Follow-Up

- **increased risk of pregnancy-induced hypertension (gestational hypertension and preeclampsia),**
- **increased risk of testicular cancer (in adults),**
- **thyroid disease and dysfunction (in adults), and**
- **increased risk of ulcerative colitis (in adults).**

The committee observed gaps in the evidence, rendering the evidence inadequate or insufficient, for many health effects including the following:

- immune effects other than reduced antibody response, and ulcerative colitis;
- cardiovascular outcomes other than dyslipidemia;
- developmental outcomes other than small reductions in birthweight;
- cancers other than kidney, breast, and testicular;
- reproductive effects other than hypertensive disorders of pregnancy;
- hepatic effects other than liver enzyme levels;
- endocrine disorders other than those involving thyroid hormone levels;
- respiratory effects;
- hematological effects;
- musculoskeletal effects, such as effects on bone mineral density;
- renal effects, such as renal disease; and
- neurological effects.

For some outcome categories, the research spanned many different tests or measures of effect, all of which assessed slightly different health outcomes, making the evidence difficult to synthesize and use to draw strong conclusions. The committee found this issue most apparent when reviewing the literature on neurodevelopmental effects, such as effects on learning and behavior. Another research issue is that most studies reviewed by the committee were not conducted among people known to have high exposures to PFAS, indicating a gap in understanding the effects of PFAS among those highly exposed.

PFAS EXPOSURE REDUCTION

Some people may be interested in reducing their exposure to PFAS. The primary route to PFAS in nonoccupational settings is likely ingestion, which may include drinking contaminated water; eating seafood from contaminated water; or consuming other contaminated foods, such as vegetables, game, or dairy products. PFAS are often used in materials that come in contact with food, such as microwave popcorn bags or packaging used for fast foods or processed foods. Exposure may also occur when dust containing PFAS is ingested. Inhalation is the most common route in occupational settings, and is a route of exposure for people living near fluorochemical plants or incinerators. PFAS transfer to the fetus during pregnancy and in early life during lactation. Dermal exposure is understudied.

If PFAS are in drinking water, switching to consumption of water lower in PFAS will reduce exposure. In general, however, it is difficult to reduce exposure to PFAS through personal behavior modifications.

For clinicians, based on its review of the evidence on PFAS exposure reduction, the committee makes the following recommendations:

Recommendation 4-1[7]: Clinicians advising patients on PFAS exposure reduction should begin with a conversation aimed at first determining how they might be exposed to PFAS

[7] The committee's recommendations are numbered according to the chapter of the main text in which they appear.

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(sometimes called an environmental exposure assessment) and what exposures they are interested in reducing. This exposure assessment should include questions about current occupational exposures to PFAS (such as work with fluorochemicals or firefighting) and exposures to PFAS through the environment. Known environmental exposures to PFAS include living in a community with PFAS-contaminated drinking water, living near industries that use fluorochemicals, serving in the military, and consuming fish and game from areas with known or potential contamination.

Recommendation 4-2: If patients may be exposed occupationally, such as by working with fluorochemicals or as a firefighter, clinicians should consult with occupational health and safety professionals knowledgeable about the workplace practices to determine the most feasible ways to reduce that exposure.

Recommendation 4-3: Clinicians should advise patients with elevated PFAS in their drinking water that they can filter their water to reduce their exposure. Drinking water filters are rated by NSF International, an independent organization that develops public health standards for products. The NSF database can be searched online for PFOA to find filters that reduce the PFAS in drinking water included in the committee's charge. Individuals who cannot filter their water can use another source of water for drinking.

Recommendation 4-4: In areas with known PFAS contamination, clinicians should advise patients that PFAS can be present in fish, wildlife, meat, and dairy products and direct them to any local consumption advisories.

There are fewer evidence-based, exposure-reduction recommendations for patients without known sources of exposure:

Recommendation 4-5: Clinicians should direct patients interested in learning more about PFAS to authoritative sources of information on how PFAS exposure occurs and what mitigating actions they can take. Authoritative sources include the Pediatric Environmental Health Specialty Units (PEHSUs), the Agency for Toxic Substances and Disease Registry (ATSDR), and the U.S. Environmental Protection Agency (EPA).

Recommendation 4-6: When clinicians are counseling parents of infants on PFAS exposure, they should discuss infant feeding and steps that can be taken to lower sources of PFAS exposure. The benefits of breastfeeding are well known; the American Academy of Pediatrics, the American Academy of Family Physicians, and the American College of Obstetricians and Gynecologists support and recommend breastfeeding for infants, with rare exceptions. Clinicians should explain that PFAS can pass through breast milk from a mother to her baby. PFAS may also be present in other foods, such as the water used to reconstitute formula and infant food, and potentially in packaged formula and baby food. It is not yet clear what types and levels of exposure to PFAS are of concern for child health and development.

Additionally, there is a critical need for more data to understand PFAS exposure among breastfed infants:

Recommendation 4-7: Federal environmental health agencies should conduct research to evaluate PFAS transfer to and concentrations in breast milk and formula to generate data that can help parents and clinicians make shared, informed decisions about breastfeeding.

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PFAS TESTING AND CONCENTRATIONS TO INFORM CLINICAL CARE OF EXPOSED PATIENTS

Decisions about PFAS testing require shared informed decision making between patient and clinician. Clinicians should explain that exposure biomonitoring may provide important information about an individual's exposure levels that might guide clinical follow-up. At the same time, this information cannot indicate or predict the likelihood that an individual will end up with a particular condition. Allowing people the opportunity to determine whether they will undergo PFAS testing shows respect for patient values. Discussions about PFAS testing should always include information about how PFAS exposure occurs, potential health effects of PFAS, limitations of PFAS testing, and the benefits and harms of the testing (see Box S-2).

BOX S-2
Potential Harms and Benefits of PFAS Testing

Potential Harms

- Fear of blood draw
- Small risk of injury or infection at draw site
- Difficulties in interpreting results
- Stress or concern about the health effects of exposure
- Decreased property values resulting from identifying property contamination
- Social isolation
- Clinical consequences from medical follow-up as a result of exposure

Potential Benefits

- Increased awareness of exposure so it can be reduced
- Empowerment of communities to respond to contamination
- Relief from the stress of not knowing one's exposure level
- Identification of the potential risk for health conditions associated with PFAS exposure, informing subsequent preventive care
- Help in monitoring whether efforts to reduce exposure are working through the conduct of baseline and follow-up tests

The German Human Biomonitoring Commission has risk-based guidance levels for two PFAS chemicals—PFOS and PFOA. The European Food Safety Authority has one guidance level for the sum of PFOA, PFOS, PFHxS, and PFNA. No individual values are available for PFHxS and PFNA, and the committee could find no values for methylperfluorooctane sulfonamidoacetic acid (MeFOSAA), PFDA, and perfluoroundecanoic acid (PFUnDA). Based on its review of the evidence for PFAS testing, the committee recommends:

Recommendation 5-1: As communities with PFAS exposure are identified, government entities (e.g., Centers for Disease Control and Prevention [CDC]/Agency for Toxic Substances and Disease Registry [ATSDR], public health departments) should support clinicians with educational materials about PFAS testing so that they can discuss testing with their patients. These educational materials should include the following information:

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- **How people can be exposed to PFAS: Exposure routes include occupational exposures and work with fluorochemicals or as a firefighter; consumption of contaminated drinking water in communities that obtain their water from sources near commercial airports, military bases, fluorochemical manufacturing plants, wastewater treatment plants, landfills, or incinerators where PFAS-containing waste may have been disposed of or farms where sewage sludge may have been used; and consumption of contaminated fish or game if fishing or hunting occurs in contaminated areas. Individuals living near fluorochemical plants may also be exposed via inhalation of air emissions.**
- **Potential health effects of PFAS exposure and strategies for reducing exposure.**
- **Limitations of PFAS blood testing: PFAS blood testing does not identify the sources of exposure or predict future health outcomes; it only assesses body burden at the time of sample collection. For example, a person with low blood levels today may have had higher levels in the past.**
- **The benefits and harms of PFAS testing.**

Recommendation 5-2: Clinicians should offer PFAS testing to patients likely to have a history of elevated exposure. In all discussions of PFAS testing, clinicians should describe the potential benefits and harms of the testing and the potential clinical consequences (such as additional follow-up), related social implications, and limitations of the testing so that patient and clinician can make a shared, informed decision. Patients who are likely to have a history of elevated exposure to PFAS include those who have

- **had occupational exposure to PFAS (such as those who have worked with fluorochemicals or served as a firefighter);**
- **lived in communities where environmental and public health authorities (Centers for Disease Control and Prevention [CDC], Agency for Toxic Substances and Disease Registry [ATSDR], U.S. Environmental Protection Agency [EPA], state and local environmental or health authorities), or academic researchers have documented PFAS contamination; or**
- **lived in areas where PFAS contamination may have occurred, such as near facilities that use or have used fluorochemicals, commercial airports, military bases, wastewater treatment plants, farms where sewage sludge may have been used, or landfills or incinerators that have received PFAS-containing waste.**

Recommendation 5-3: Clinicians should use serum or plasma concentrations of the sum of PFAS* to inform clinical care of exposed patients, using the following guidelines for interpretation:

- **Adverse health effects related to PFAS exposure are not expected at less than 2 nanograms per milliliter (ng/mL).**
- **There is a potential for adverse effects, especially in sensitive populations, between 2 and 20 ng/mL.**
- **There is an increased risk of adverse effects above 20 ng/mL.**

*** Simple additive sum of MeFOSAA, PFHxS, PFOA (linear and branched isomers), PFDA, PFUnDA, PFOS (linear and branched isomers), and PFNA in serum or plasma. Caution is warranted when using capillary blood measurements as levels may differ from serum or plasma levels.**

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The cutoff levels should be updated as additional information becomes available. The committee also noted that children younger than 12 years are not routinely included in the *National Report on Human Exposure to Environmental Chemicals*, nor are pregnant people included in large numbers. More reference- and risk-based values are needed, for other PFAS and other biological matrices, but given the expansiveness of the class, this gap can best be addressed with relative potency factor approaches rather than the development of risk-based levels for each PFAS.

Recommendation 5-4: The National Health and Nutrition Examination Survey should begin collecting and sharing more data on children younger than 12 years of age and pregnant people to generate reference populations for those groups.

Testing for PFAS, although expensive, offers an opportunity to identify people who may need to reduce their PFAS exposure and are at increased risk of certain health outcomes. It is important to recognize, however, that race, age, and other social and demographic characteristics already have disadvantaged many patients with respect to accessing clinical preventive services, such as PFAS testing. The disadvantage would be compounded as PFAS testing services should be linked to counseling on steps to mitigate exposure and its impacts. Therefore, encouraging testing primarily among people with relatively stable access to care could have the unintended effect of aggravating disparities in exposure to PFAS absent a funded, national PFAS testing program with a counseling component.

APPLICATION OF THE COMMITTEE'S PRINCIPLES TO PATIENT FOLLOW-UP FOR PFAS-ASSOCIATED HEALTH EFFECTS

Many health outcomes or conditions that the committee found to be associated with PFAS exposure are common in the general population. All have multiple known risk factors. The committee categorized the strength of the evidence for an association between PFAS and various health outcomes, and concluded that all conditions with an adequately supported association should be considered for patient follow-up. The committee then used its established cutoff levels to determine appropriate follow-up based on PFAS exposure level. Risks from PFAS likely increase with exposure, and PFAS levels of 3 ng/mL and 19 ng/mL do not represent the same risk even though they are listed in the same category. Clinical providers should use judgment and shared, informed decision making in making follow-up decisions based on PFAS exposure and other risk factors. Figure S-6 suggests that clinicians engage in shared decision making with their patients regarding follow-up care for PFAS-associated health endpoints.

Recommendation 6-1: Clinicians should treat patients with serum PFAS concentration below 2 nanograms per milliliter (ng/mL) with the usual standard of care.

Recommendation 6-2: For patients with serum PFAS concentration of 2 nanograms per milliliter (2 ng/mL) or higher and less than 20 ng/mL, clinicians should encourage PFAS exposure reduction if a source of exposure is identified, especially for pregnant persons. Within the usual standard of care clinicians should:

- **Prioritize screening for dyslipidemia with a lipid panel (once between 9 and 11 years of age, and once every 4 to 6 years over age 20) as recommended by the American Academy of Pediatrics (AAP) and the American Heart Association (AHA).**
- **Screen for hypertensive disorders of pregnancy at all prenatal visits per the American College of Obstetricians and Gynecologists (ACOG).**

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- **Screen for breast cancer based on clinical practice guidelines based on age and other risk factors such as those recommended by the U.S. Preventive Services Task Force (USPSTF).**

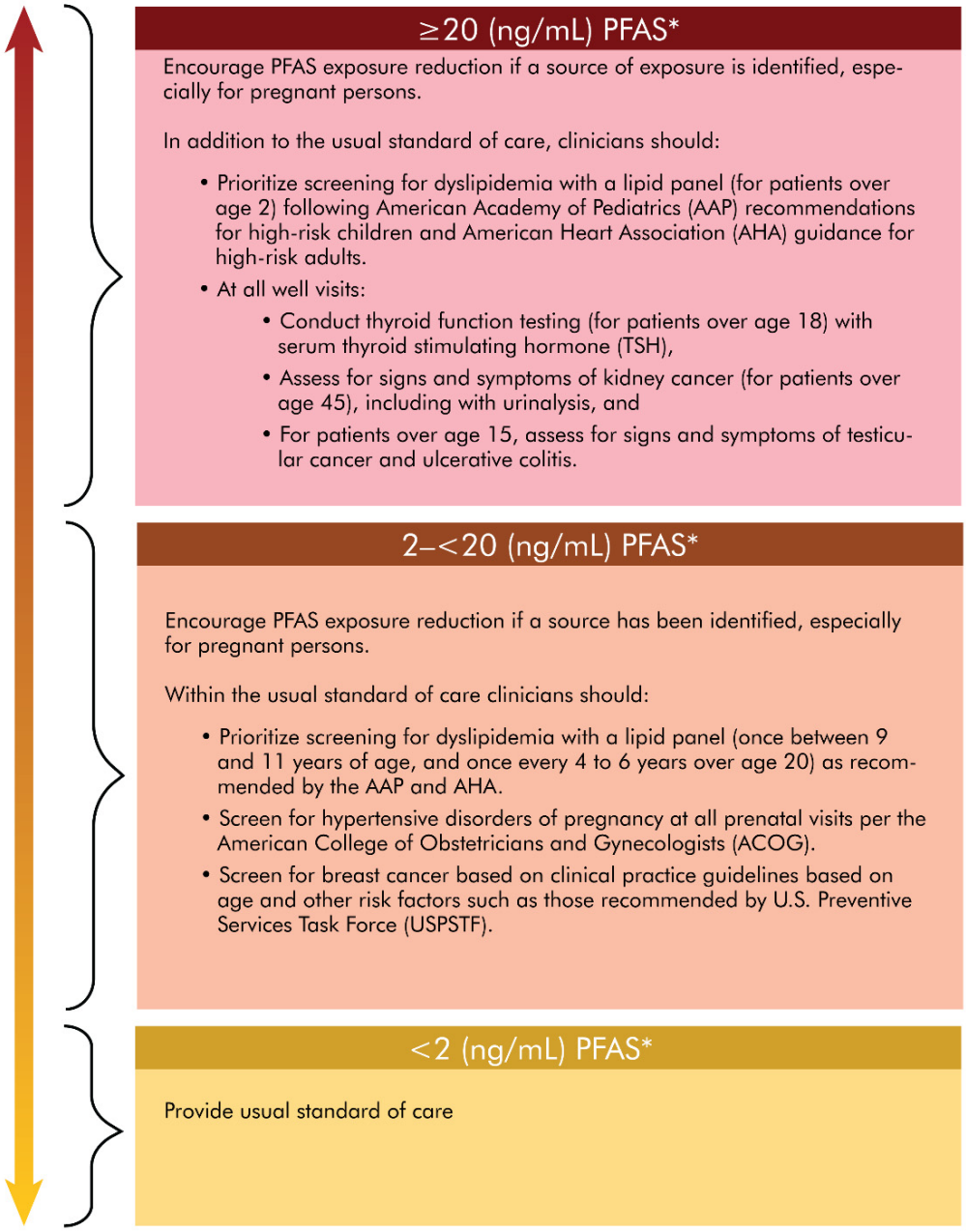


FIGURE S-6 Clinical guidance for follow-up with patients after PFAS testing.
NOTE: MeFOSAA = methylperfluorooctane sulfonamidoacetic acid; PFDA = perfluorodecanoic acid; PFHxS = perfluorohexane sulfonic acid, PFNA = perfluorononanoic acid; PFOA = perfluorooctanoic acid; PFOS = perfluorooctanesulfonic acid; PFUnDA = perfluoroundecanoic acid.

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Recommendation 6-3: For patients with serum PFAS concentration of 20 nanograms per milliliter (ng/mL) or higher, clinicians should encourage PFAS exposure reduction if a source of exposure is identified, especially for pregnant persons. In addition to the usual standard of care, clinicians should:

- **Prioritize screening for dyslipidemia with a lipid panel (for patients over age 2) following American Academy of Pediatrics (AAP) guidelines for high-risk children and American Heart Association (AHA) guidance for high-risk adults.**
- **At all well visits:**
  - **conduct thyroid function testing (for patients over age 18) with serum thyroid stimulating hormone (TSH),**
  - **assess for signs and symptoms of kidney cancer (for patients over 45), including with urinalysis, and**
  - **for patients over 15, assess for signs and symptoms of testicular cancer and ulcerative colitis.**

APPLYING THE COMMITTEE'S EXPOSURE, TESTING, AND CLINICAL FOLLOW-UP RECOMMENDATIONS

The committee created a flow chart summarizing PFAS education, exposure assessment, and clinical follow-up (see Figure S-7). In communities where PFAS exposure has been identified, ATSDR and other government entities should support local clinicians with educational materials about PFAS exposure and testing. Clinicians should then determine whether a particular patient is likely to have a history of elevated exposure to PFAS. If so, the clinician should offer PFAS testing and make a shared, informed decision on that testing. If testing is chosen, the labs should be ordered (Test Code 39307 Current Procedural Terminology [CPT] code 82542). Test results should be interpreted by summing the concentrations of MeFOSAA, PFHxS, PFOA (linear and branched isomers), PFDA, PFUnDA, PFOS (linear and branched isomers), and PFNA. The laboratory may not report results for all PFAS considered by the committee or may include different PFAS in its panel. In that case, the sum of PFAS should include only the PFAS in the analyte list that the committee considered. For example, if the lab tests for PFOA, PFOS, PFHxS, PFNA, and perfluorobutane sulfonic acid (PFBS), the summation should include PFOA, PFOS, PFHxS, and PFNA. Differing analytes lists may cause some variation in response. Still, as long as PFOA, PFOS, PFHxS, and PFNA are included in the analyte list, the results may not vary too greatly as these four analytes are most commonly detected in the United States. If any analyte is below the limit of detection, the clinician should calculate the analyte limit of detection divided by the square root of 2 and use this value in the summation. The sum thus derived should be compared against Figure S-6 to determine an appropriate clinical follow-up plan based on shared, informed decision making between patient and clinician.

REVISING ATSDR'S PFAS CLINICAL GUIDANCE

The committee recommends several changes to ATSDR's guidance to ensure consistency with the findings, conclusions, and recommendations presented in this report and improve the guidance's writing, design, dissemination, and implementation:

Recommendation 7-1: The Agency for Toxic Substances and Disease Registry (ATSDR) should update its PFAS clinical guidance to make it more succinct and accord with the review of PFAS-associated health effects, exposure reduction considerations, PFAS testing recommendations and interpretation, and recommendations for clinical follow-up presented in this report. When describing the health effects of PFAS, ATSDR should avoid using terms typically used to

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categorize toxicants, such as "endocrine disrupter" or "neurotoxin," because they are vague and not necessarily clinically meaningful. When discussing the strength of the association between PFAS and a health outcome, ATSDR should use standard categories of association (such as sufficient evidence of an association, limited or suggestive evidence of an association, inadequate or insufficient evidence of an association, and limited or suggestive evidence of no association).

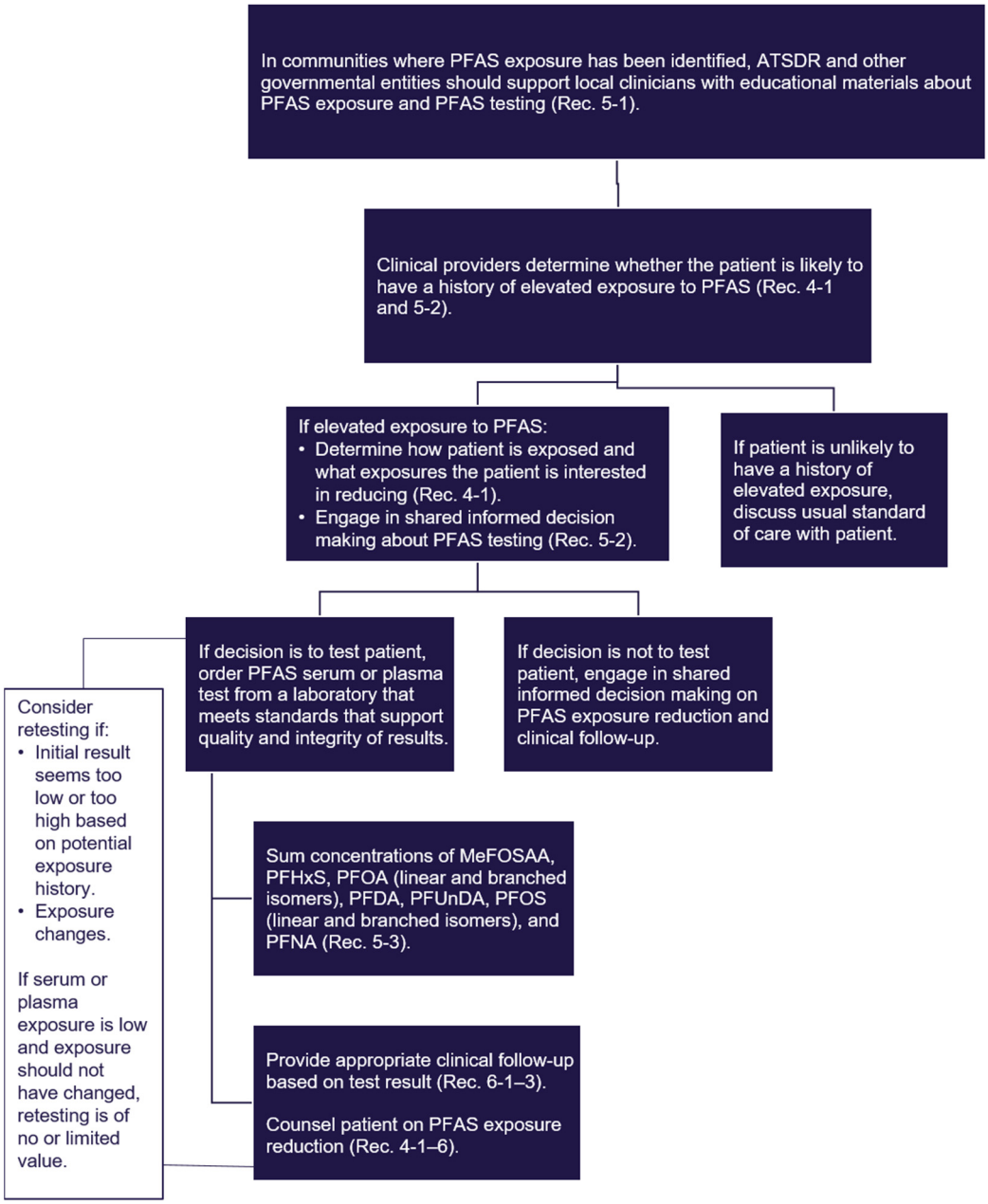


FIGURE S-7 Flow chart showing how the committee's recommendations work together in a clinical setting.
NOTE: ATSDR = Agency for Toxic Substances and Disease Registry; MeFOSAA = methylperfluorooctane sulfonamidoacetic acid; PFDA = perfluorodecanoic acid; PFHxS = perfluorohexane sulfonic acid, PFNA = perfluorononanoic acid; PFOA = perfluorooctanoic acid; PFOS = perfluorooctanesulfonic acid; PFUnDA = perfluoroundecanoic acid.

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Recommendation 7-2: The Agency for Toxic Substances and Disease Registry (ATSDR) should incorporate a reader-centered approach when developing its guidance, with the knowledge that many different audiences will turn to its clinical guidance document to prepare for discussions with their clinicians. ATSDR should also solicit feedback on the guidance from a variety of stakeholders, such as community groups, practicing clinicians, and medical associations. In addition, ATSDR should encourage clinicians to use evidence-based organizational health literacy strategies to support shared, informed decision making; patient-centered care; cultural humility; and accessible language when communicating with patients about potential health risks.

Evidence on the health effects of PFAS should be updated every 2 years, and the clinical guidance should be updated at least every 5 years. In that process, ATSDR should strive to ensure that its clinical guidance follows criteria for making guidelines trustworthy when possible. Figure S-8 provides an overview of how the process used by this committee could be applied by ATSDR to improve its clinical guidance.

Recommendation 7-3: The Agency for Toxic Substances and Disease Registry (ATSDR) should develop a process for updating its PFAS guidance that adheres to criteria for making guidelines trustworthy, such as being based on a thorough, transparent, unbiased review of the evidence and being developed by a knowledgeable panel of experts free from strong biases and conflicts of interest. A review of the evidence on the health effects of PFAS should be completed by an authoritative neutral party every 2 years, and the clinical guidance should be updated every 5 years or sooner if warranted by the evidence on the health effects of PFAS. Clinicians and members of communities with elevated PFAS exposure should be engaged to inform the problem and review updated guidance.



FIGURE S-8 Suggested framework for updating the Agency for Toxic Substances and Disease Registry's clinical guidance based on new evidence.

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IMPLEMENTING THE COMMITTEE'S RECOMMENDATIONS TO IMPROVE PUBLIC HEALTH

Public health requires the use of multifaceted approaches to emerging health issues. In environmental health—the subset of public health focused on environmental factors—mitigation of potential harms associated with chemical exposures is often complicated because there is no exposure surveillance system for most chemicals. The committee's recommendations will best protect the public health if they are part of a national effort focused on increased biomonitoring, exposure surveillance, and education of clinicians and public health professionals on environmental health issues:

> **Recommendation 8-1: Laboratories conducting PFAS testing of serum or plasma should report the results to state public health authorities, following the respective states' statutes and reporting regulations. This reporting would improve PFAS exposure surveillance; it could be linked with the Centers for Disease Control and Prevention's (CDC's) environmental public health tracking network and help build capacity for improvements in the state-based national biomonitoring network.**

The people and communities with high exposures to PFAS need to be identified. As the committee looks forward, it sees a pressing need for a robust environmental health infrastructure to continue to respond to PFAS and address other complex emerging and persistent environmental challenges.

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1

Introduction

More than a decade ago, the United Nations General Assembly passed Resolution 64/292, recognizing that safe and clean drinking water is a human right and is essential to realizing all human rights. Yet today, drinking water in thousands of communities across the United States is contaminated with chemicals known as per- and polyfluoroalkyl substances (PFAS) (Hu et al., 2016) (see Box 1-1). PFAS do not occur in nature and are manufactured for a wide range of purposes, from nonstick cookware, to stain-resistant fabrics and carpets, to firefighting foams and protective gear. PFAS constitute a large class of up to 12,000 different chemicals (EPA, 2020). They are commonly used in thousands of products because they have desirable chemical properties that impart oil and water repellency, friction reduction, and temperature resistance (ITRC, 2017). PFAS as a class have a wide variety of distinct chemical properties and toxicities; for example, some PFAS can accumulate and persist in the human body and the environment, while others transform relatively quickly. The PFAS that do transform, however, will become one or more other PFAS, because the carbon–fluorine bond they contain does not break naturally. It is for this reason that PFAS are termed "forever chemicals."

In the most rudimentary sense, PFAS can be thought of as either polymer or nonpolymer. Nonpolymer PFAS, such as perfluroalkyl acids (PFAAs), are more commonly detected in the environment and are often considered highly persistent and mobile (Blum et al., 2015; ITRC, 2021). By contrast, many polymer PFAS, such as polytetrafluoroethylene (PTFE), are insoluble, less bioavailable, and often considered less of a direct concern, with respect to human or ecological health (Lohmann et al., 2020; ITRC, 2021). Nonetheless, the production of fluoropolymers requires other PFAS chemicals, such as PFAAs. For example, the nonpolymer PFAS perfluorooctanoic acid (PFOA) was used as a surfactant in the emulsion polymerization of fluoropolymers.[1]

BOX 1-1
What Are PFAS?

What are PFAS? A consensus definition does not exist. Buck and colleagues (2011) define PFAS as fluorinated substances that "contain 1 or more C atoms on which all the H substituents (present in the nonfluorinated analogues from which they are notionally derived) have been replaced by F atoms, in such a manner that they contain the perfluoroalkyl moiety $C_nF_{2n+1}-$" (p. 513).

The Organisation for Economic Co-operation and Development (OECD) defines PFAS as "fluorinated substances that contain at least one fully fluorinated methyl or methylene carbon atom (without any H/Cl/Br/I atom attached to it), that is, with a few noted exceptions, any chemical with at least a perfluorinated methyl group (–CF3) or a perfluorinated methylene group (–CF2–) is a PFAS" (OECD, 2021, p. 7).

continued

[1] See https://www.acs.org/content/acs/en/molecule-of-the-week/archive/p/perfluorooctanoic-acid.html (accessed June 22, 2022).

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BOX 1-1 Continued

The U.S. Environmental Protection Agency's (EPA's) (2020) CompTox Chemicals Dashboard includes all substances that contain a specific set of substructural elements. The Dashboard says that "there is no precisely clear definition of what constitutes a PFAS substance" and PFAS lists include "partially fluorinated substances, polymers, and ill-defined reaction products" (EPA, 2020, para. 1).

What are PFAS used for? PFAS have desirable chemical properties such as oil and water repellency, temperature resistance, and friction reduction. Since the late 1940s, PFAS have been used in numerous applications, such as firefighting, chrome-plating, lubricants, and insecticides, as well as coating and treating of such surfaces as carpeting, packaging, and cookware (ATSDR, 2021).

How long do PFAS stay in the human body following exposure? PFAS levels in people's bodies will persist unless exposure ceases, and will continue to persist even after exposure ends. Half-life estimates based on repeated serum measurements range from days (e.g., PFBA) to years (e.g., PFOA, PFOS). It is generally assumed that it takes five half-lives to eliminate PFAS after exposure has ceased (ATSDR, 2021).

What are the potential hazards of PFAS? Health effects associated with PFAS include altered immune function, elevated cholesterol, thyroid disease, hypertension during pregnancy, testicular cancer, and kidney cancer (ATSDR, 2021; Fenton et al., 2021; Steenland et al., 2020).

SOCIOHISTORICAL TIMELINE OF PFAS

Public concern about the impact of PFAS contamination on human health and the environment began in the late 1990s when PFOA water contamination was identified in Parkersburg, West Virginia.[2] In response, 3M, a primary PFAS manufacturer, initiated a voluntary phase-out of some PFAS (PFOA, perfluorooctanesulfonic acid [PFOS], and perfluorohexanesulfonic acid [PFHxS)]) (ITRC, 2017). The contamination in Parkersburg also led to a class action lawsuit that identified several health effects related to PFAS exposure and subsequently to the establishment of a medical monitoring program (see Box 1-2). Shortly thereafter, the U.S. Environmental Protection Agency (EPA) began requiring all community water systems serving more than 10,000 people to test for certain PFAS, which led to more communities learning that their water was contaminated.[3] In 2016, researchers found that the drinking water supply for Wilmington, North Carolina, was contaminated with a chemical called "GenX," a PFOA replacement.[4] This finding led to public concern about the potential health effects of replacement PFAS (see Figure 1-1).

An estimated 2,854 U.S. locations (in all 50 states and two territories) have some level of PFAS contamination (see Figure 1-2). Although not all of the contamination represents exceedances of health advisories, the pervasiveness of the contamination is alarming. Furthermore, almost 100 percent of the U.S. population is exposed to at least one PFAS. Although exposures to the phased-out PFAS have been decreasing (see Figure 1-3), people are still exposed to those PFAS from site contamination, occupational uses of stored products, and breakdown of PFAS polymer products that are found in homes. Carpeting, for example, is often treated with fluorotelomer-based polymers that can biodegrade to form phased-out PFAS, such as PFOA (Washington and Jenkins, 2015). Exposures also occur to the PFAS chemicals used to replace those that have been phased out. Although the harms of the replacement PFAS are less well understood, they may have comparable or more serious toxicity than the PFAS they have replaced (Kwiatkowski et al., 2020). The state of New Jersey, for example, recently set a groundwater standard for

[2] See https://pfasproject.com/parkersburg-west-virginia (accessed June 16, 2022).

[3] See https://www.epa.gov/sites/default/files/2016-05/documents/ucmr3-factsheet-list1.pdf (accessed June 16, 2022).

[4] See https://news.ncsu.edu/2018/04/finding-genx (accessed June 16, 2022).

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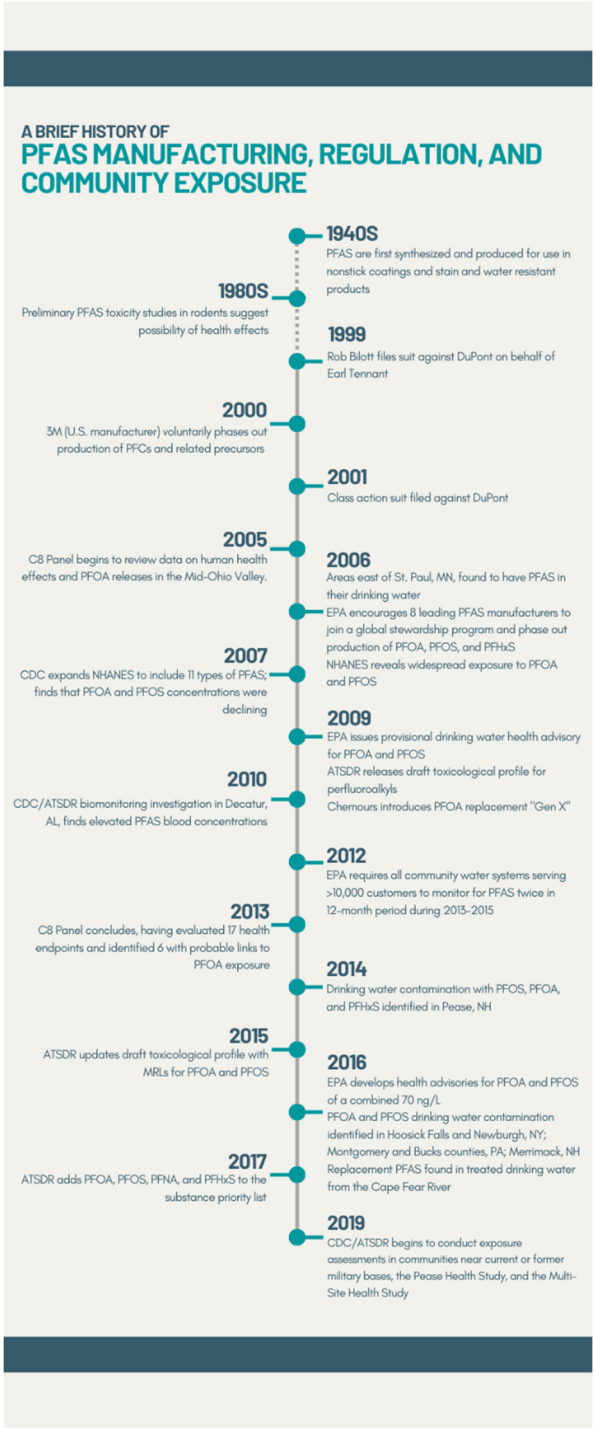


FIGURE 1-1 Brief history of PFAS manufacturing, regulation, and community exposure.
NOTE: ATSDR = Agency for Toxic Substances and Disease Registry; CDC = Centers for Disease Control and Prevention; EPA = U.S. Environmental Protection Agency; MRL = minimal risk limit; ng/L = nanograms per liter; NHANES = National Health and Nutrition Examination Survey; PFAS = per- and polyfluoroalkyl substances; PFC = perfluorochemicals; PFHxS = perfluorohexane sulfonic acid; PFNA = perfluorononanoic acid; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid.
SOURCE: Committee generated based on slides included by Patrick N. Breysse in a presentation to the committee on February 4, 2021.

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a replacement PFAS (chloroperfluoropolyether carboxylates) that is an order of magnitude lower than drinking water standards for other PFAS. The New Jersey maximum contaminant levels of perfluorononanoic acid (PFNA), PFOA, and PFOS in drinking water are 13, 14, and 13 nanograms per liter (ng/L), respectively,[5] while the groundwater standard for the replacement PFAS, chloroperfluoropolyether carboxylates, is 2 ng/L.[6]

A reason PFAS contamination became well known only recently, after decades of their use, is that they can be difficult to detect. The physical and chemical properties that make PFAS persistent and mobile in the environment also make them particularly challenging to analyze (Guelfo et al., 2021). Analytical methods sensitive enough to detect environmentally relevant concentrations became widely available in the early 2010s. Although analyte lists continue to expand, currently available methods still allow identification of only a small fraction of the thousands of PFAS that have reportedly been created and used since the 1950s. As existing analytical methods improve, additional PFAS and new release sites will likely be identified (De Silva et al., 2021; Guelfo et al., 2021).

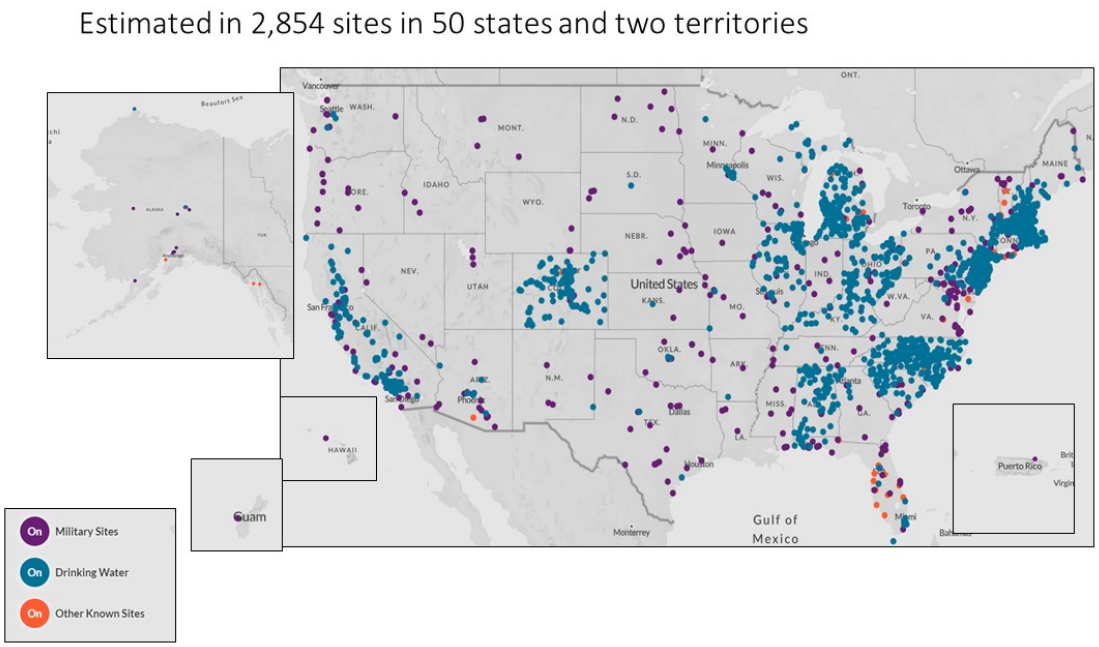


FIGURE 1-2 PFAS contamination across the United States.
NOTES: According to the Environmental Working Group (EWG), "locations represented on the map are approximate and intended to portray the general area of a contamination site or a community water system. Locations were mapped using the best data available from official records, including data provided by tests of public drinking water systems, the Safe Drinking Water Information System and the Department of Defense report *Addressing Perfluorooctane Sulfonate (PFOS) and Perfluorooctanoic Acid (PFOA)*, and Department of Defense public records, among others. Data on contaminated industrial and military sites was current as of October 2021." Furthermore, "EWG has worked to ensure the accuracy of the information provided in this map. The map is dynamic. This contaminant site, results, suspected sources and other information in the database may change based on evolving science, new information or other factors. Please be advised that this information frequently relies on data obtained from many sources, and accordingly, EWG cannot guarantee the accuracy of the information provided or any analysis based thereon."
SOURCE: See https://www.ewg.org/interactive-maps/pfas_contamination/map (accessed May 11, 2022). Copyright © Environmental Working Group, www.ewg.org. Reproduced with permission.

[5] See https://www.nj.gov/health/ceohs/documents/pfas_drinking%20water.pdf (accessed June 8, 2022).
[6] See https://www.nj.gov/dep/standards/ClPFPECA_Standard.pdf (accessed June 8, 2022).

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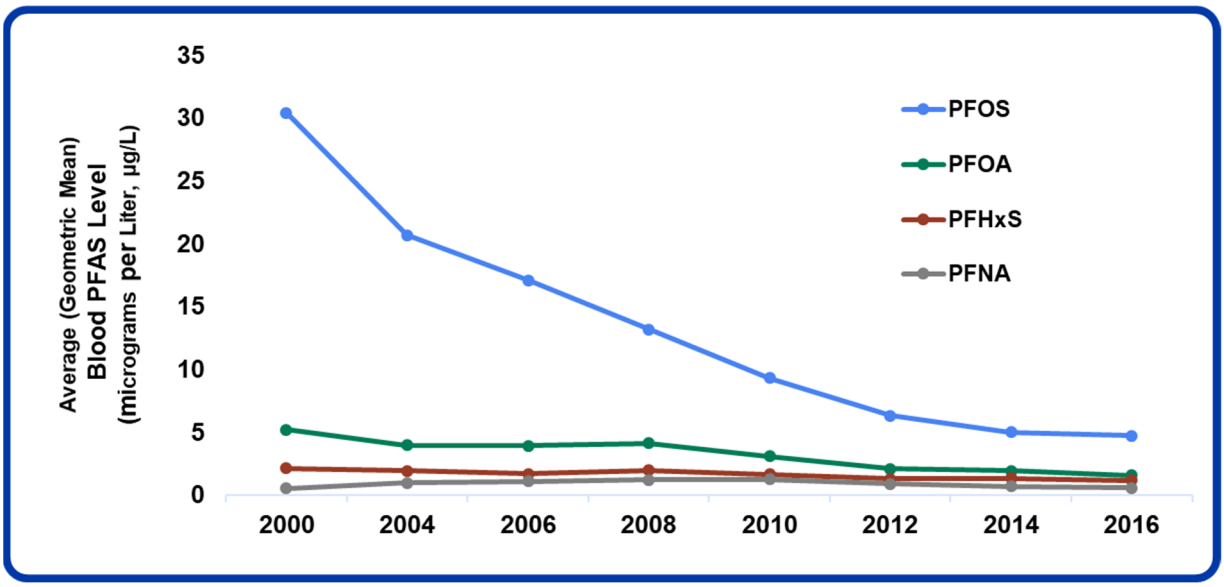


FIGURE 1-3 Blood (serum) levels of PFAS, United States, 2000–2016.
NOTE: Average = geometric mean.
SOURCE: Patrick N. Breysse's presentation to the committee on February 4, 2021. DATA SOURCE: Centers for Disease Control and Prevention (2019, January). *Fourth Report on Human Exposure to Environmental Chemicals*, updated tables. Atlanta, GA: U.S. Department of Health and Human Services.

The people who live, work, and play in environments where PFAS contamination exceeds standards often do not know how to protect themselves from the health risks of exposure. Many have been exposed to PFAS for decades and may continue to be exposed in their homes or workplaces. Some members of communities with documented exposure report feeling violated and concerned about the health and well-being of their families, friends, and loved ones (Raponi et al., 2021; Rizzuto, 2021). Many in these exposed communities who have discovered that their exposure exceeds health advisory levels are calling for a medical program to prevent, lead to early detection of, or treat any diseases related to the health risks they may face (Raponi et al., 2021; Rizzuto, 2021). Developing such a program is challenging, however, because of the uncertainties about the health effects of PFAS and potential harms from additional medical treatments; moreover, many of these diseases are not preventable or even treatable once exposures have occurred (Wones et al., 2009). Accordingly, clinicians generally do not know how to respond to patients' concerns about PFAS.

ENVIRONMENTAL JUSTICE

Compounding the challenge of responding clinically to PFAS contamination are issues of environmental justice and systemic racism in medical care. It is well established that environmental risks are not distributed uniformly across populations. Race, ethnicity, poverty, age, life stage, and other social factors can place people at disproportionately high risk for diseases with environmental causes as a result of hazardous exposures at increased levels compared to the general population (Gochfeld and Burger, 2011). While environmental justice research specific to PFAS contaminants has been limited, place-based factors that may put individuals at greater risk of exposure (siting of chemical companies, refineries, and industrial sites), coupled with insufficient access to environmental screening, information, and adequate health care, have disproportionate impacts on Black, Hispanic, and Indigenous communities, as well as low-income populations. A special report from *Scientific American* called this a "triple whammy of race, poverty, and environment converging nationwide to create communities near pollution sources where nobody else wants to live" (Kay and Katz, 2012, para. 12).

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According to a 2014 paper by the Environmental Justice Health Alliance for Chemical Policy Reform, more than 134 million Americans live within the "vulnerability zones" of industrial facilities that store or use highly hazardous chemicals (Orum et al., 2014). The companies themselves define these vulnerability zones, determined by guidance from the EPA,[7] as areas that could be affected by the release of toxic chemicals. The scope of the research on which this paper was based was broad, but included industries with known PFAS contamination, such as waste management and chemical manufacturing. The authors found that, relative to the U.S. average, communities within the vulnerability zones are disproportionately African American or Latinx; are more likely to live in poverty; and have lower housing values, incomes, and education levels. As the extent of contamination becomes known, housing values have been shown to decrease, and those with the means to move are more likely to do so, leaving behind those with the fewest resources and options (Harclerode et al., 2021).

When considering environmental justice, it is important to think beyond exposure disparities (Mohai et al., 2009); core environmental justice issues relevant to PFAS also include rural health, industrial siting, and access to environmental exposure reduction (Bullard, 1996) and clinical care. A framework is needed that allows consideration of structural factors beyond race, ethnicity, and socioeconomic status and accounts for how individuals and communities are impacted by decisions made at the local, state, and policy levels by government, industry, and health care professionals. Pellow's Environmental Justice Framework accounts for the complexity of relationships and decisions that impact PFAS exposure and associated health outcomes (Pellow, 2004) (see Figure 1-4). Sze and London (2008) advance this argument by incorporating negotiation at the stakeholder level, as well as problem identification at the level of sociohistorical and structural factors, and then solution-oriented approaches reflecting considerations of sustainability and safety.



FIGURE 1-4 Pellow's Environmental Justice Framework.

[7] See https://www.epa.gov/rmp/forms/vulnerable-zone-indicator-system (accessed June 16, 2022).

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Guidance on PFAS Exposure, Testing, and Clinical Follow-Up

A first consideration in addressing environmental justice with respect to PFAS is understanding the sociohistorical context rather than a particular discrete event that has contributed to the presence of PFAS in certain communities. For example, PFAS contamination did not just randomly occur in rural communities serviced by well water that happened to be near industrial sites. Rather, the locating of certain industrial sites and decisions to dispose of PFAS with limited regard for the surrounding community's access to safe water are rooted in the relationship and history of these industries and communities. The disposal of these chemicals did not occur as single events lacking context, but reflected a pattern of decisions made over time. Understanding the historical and social context influencing how and where PFAS are distributed is an essential part of identifying effective mitigation strategies.

Second, Pellow's Environmental Justice Framework emphasizes the complex roles of the stakeholders involved. In the case of PFAS, environmental inequality can affect many different stakeholders, ranging from industry, to workers, to community members and organizations, to government entities such as local departments of public health. The complexity of these roles means there can exist synergistic and contradictory allegiances that impact what is valued, how resources are accessed and distributed, who has power, and what is considered profitable. With PFAS, industry's power and profit motive may trump a community's access to information and ability to test for and reduce exposure. Likewise, key health information for clinicians is governed by boards that decide about the content of medical education and training. Clinicians' lack of knowledge about environmental exposures such as PFAS, particularly in highly contaminated communities, can be particularly deleterious for the least-advantaged stakeholder groups.

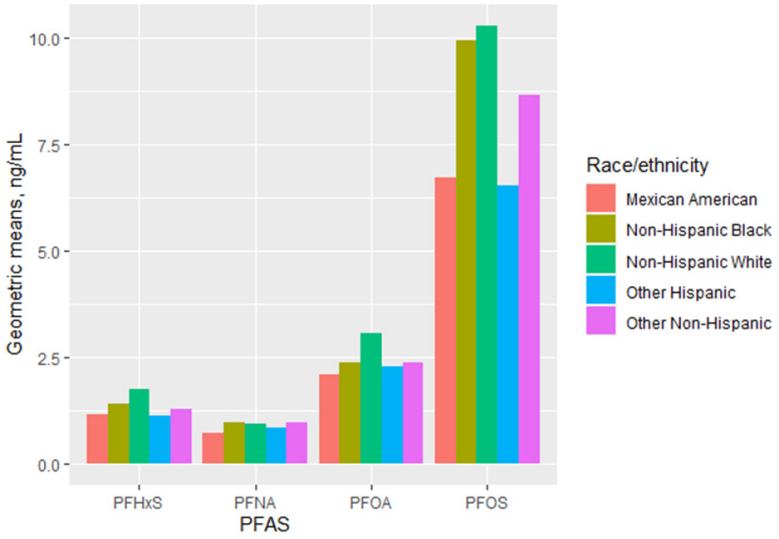
A third consideration is the effects of social inequalities on exposure to PFAS, as relevant social stratification that affects exposure is not always clear or easy to study. Rural and urban disparities, for example, can be analyzed using National Health and Nutrition Examination Study (NHANES) data (with appropriate clearance), and living in a rural versus an urban area can definitely affect PFAS exposure, often through exposure to well water that is common in rural areas. Other, less obviously measurable influences can also drive exposures, however. Food insecurity, for example, even if only temporary, increases subsistence fishing (Quimby et al., 2020), which may cause people to fish for food in contaminated lakes or rivers. These structural and social factors put people at risk of exposure and can contribute to inequality. In short, even though relatively affluent areas can be heavily contaminated with PFAS, and non-Hispanic Whites are exposed to some of the highest PFAS concentrations (see below), solving the larger problem of PFAS contamination will still require attention to persistent social inequities. Otherwise, action to mitigate PFAS exposure could aggravate existing (or create new) environmental health disparities.

Fourth is the need to consider the role of agency—specifically, the power a community has to advocate for itself in conflicts. PFAS provide a unique example. PFAS contamination rose to national prominence partly because of exposures among well-educated, high-income, and mainly White communities. With PFAS, as with many social problems, power matters. At the same time, PFAS exposure varies regionally. In some parts of the country, the highest exposures are borne by less advantaged communities, making their relative ability to advocate for testing and responsive health care or to demand effective mitigation from their government somewhat tenuous.

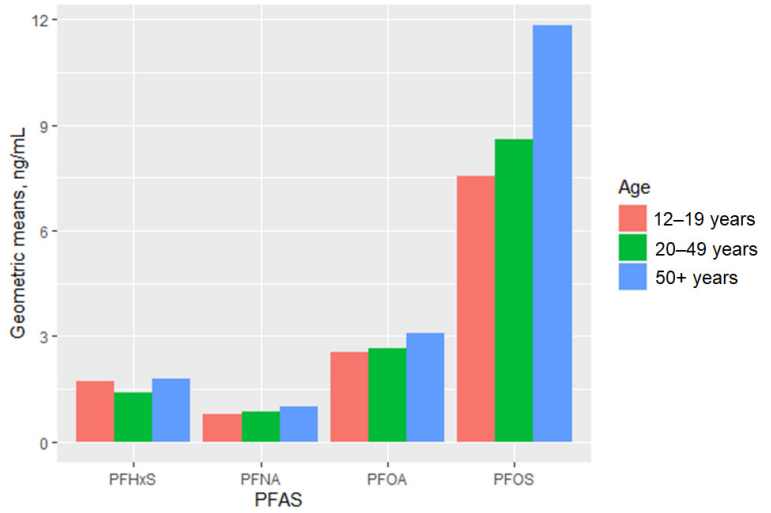
While research on disparities in PFAS exposure is limited, several studies suggest that no differences exist by race, ethnicity, or socioeconomic status (Buekers et al., 2018; Nguyen et al., 2020; Sagiv et al., 2015). If one looks at time-aggregated estimates of PFAS exposure for the U.S. population, non-Hispanic Whites, older adults, and people of higher socioeconomic status have higher concentrations of these chemicals (see Figure 1-5). However, time-aggregated exposures present an incomplete picture. Looking at PFAS exposure by year stratified by race orethnicity suggests that in 2000, before the voluntary phase-out of PFOS andrelated chemicals, those in the racial group other non-Hispanic had the highest exposure to PFOS and PFHxS (see Figure 1-6). The implication of this finding is that widespread policy changes—such as the phase out of certain types of PFAS—greatly reduced exposure disparities. Because there are no national estimates of biomonitoring data for replacement PFAS, however, it is unknown whether the disparities observed with PFOS, PFHxS, and PFNA exist today for other PFAS.

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(a)



(b)



(c)

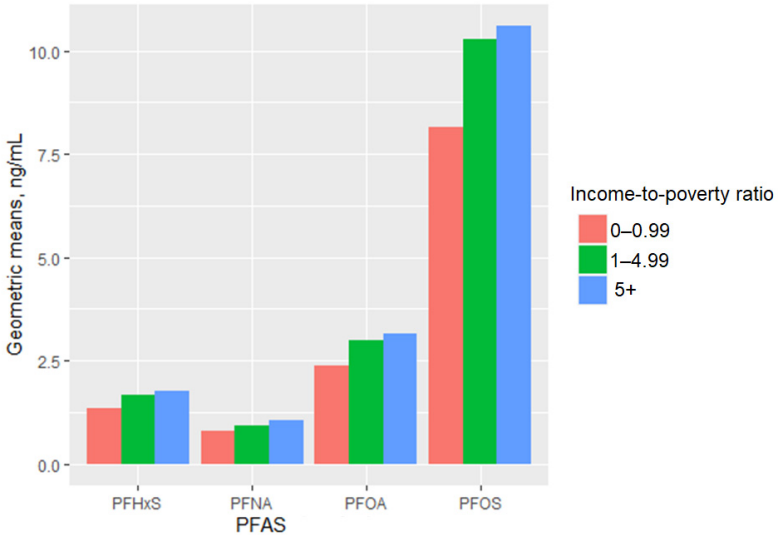


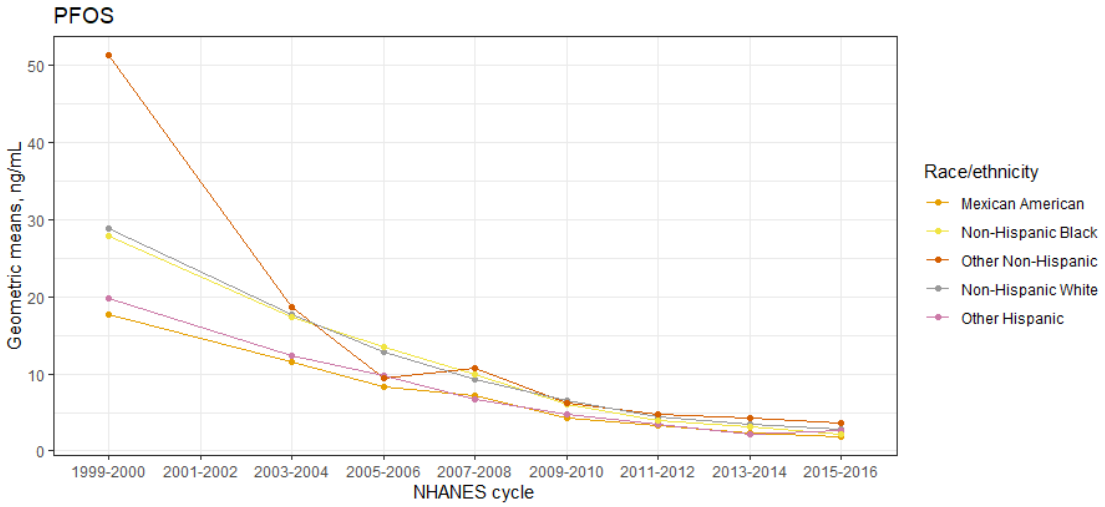
FIGURE 1-5 Serum PFAS concentrations (unadjusted geometric means) from the National Health and Nutrition Examination Survey, 1999–2016, by race/ethnicity (panel a), age (panel b), and income-to-poverty ratio (panel c).

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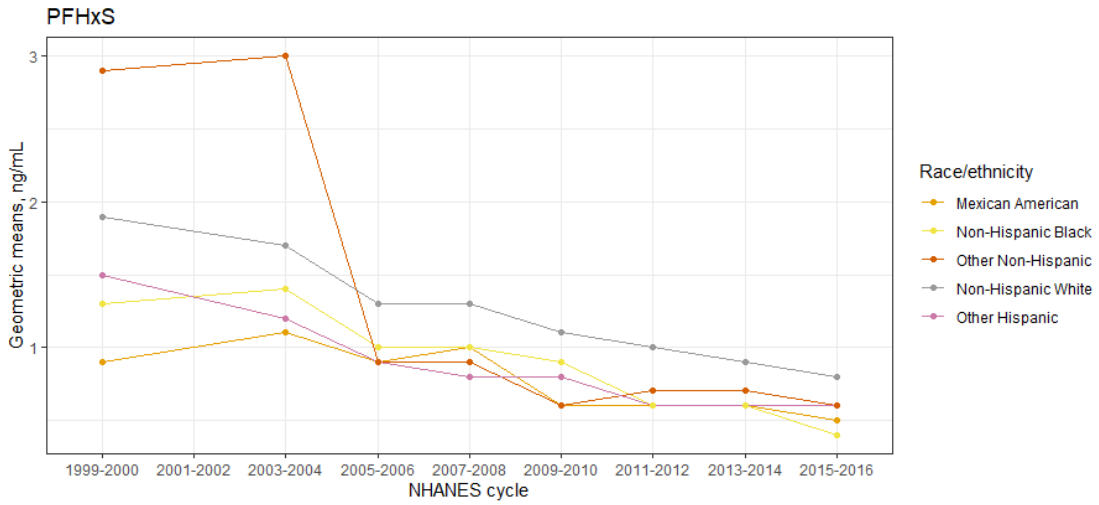
(a)



(b)



(c)



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(d)

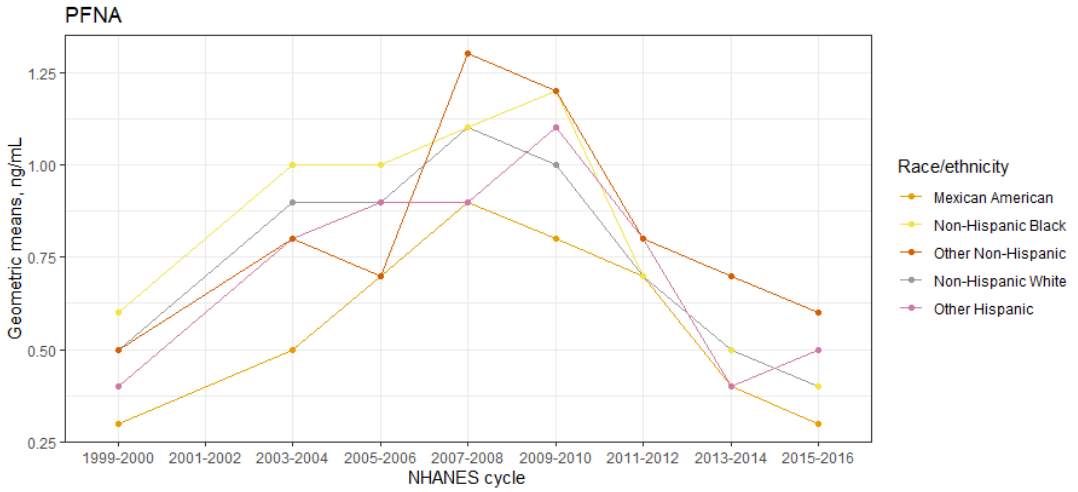


FIGURE 1-6 Serum PFAS concentrations (unadjusted geometric means) from the National Health and Nutrition Examination Survey, 1999–2016, by race/ethnicity, for PFOA (panel a), PFOS (panel b), PFHxS (panel c), and PFNA (panel d).

PFAS CONTAMINATION AND ROUTES OF EXPOSURE

PFAS contamination is global. PFAS have been detected in regions with little human activity, including the atmosphere of remote locations (Shoeib et al., 2010), the Arctic and Antarctic seas (Armitage et al., 2006), and remote soils of every continent (Rankin et al., 2016). Environmental contamination with PFAS occurs in countless ways. Fluorochemical manufacturing sites release PFAS into water as well as into the air, from which they can settle into both soil and water. At military sites and commercial airports PFAS-containing foams are used in training exercises for firefighting. PFAS also can leak from landfills where PFAS-containing wastes are disposed, and can be released into the environment by PFAS-containing wastewater from wastewater treatment plants (Evich et al., 2022; Gomez et al., 2021; Sunderland et al., 2019) (see Figure 1-7). PFAS-treated consumer products have been implicated as sources of exposure in indoor settings (Harrad et al., 2010), and fluorotelomer products used to treat a wide range of consumer household and occupational products have been shown to degrade to form PFAS (Washington and Jenkins, 2015).

PFAS in the environment can contaminate drinking water when the chemicals reach public drinking water systems and private wells (Hu et al., 2016). PFAS can bioaccumulate in fish, shellfish, livestock, dairy, and game animals that contact them through contaminated food or water (De Silva et al., 2021; Death et al., 2021; Domingo and Nadal, 2017). Produce also can be contaminated if it is grown with contaminated drinking water or PFAS-contaminated compost or biosolids (Blaine et al., 2013; Scher et al., 2018).

Certain occupations may lead to increased PFAS exposures. They include, for example, jobs in facilities used to manufacture fluorochemicals or to produce PFAS-containing products, such as textiles or food contact materials. Other jobs with a known increased risk of exposure to PFAS include electroplating, painting, carpet installation and treatment, and jobs that require prolonged work with ski wax; increased exposures also occur among military and civilian firefighters who use PFAS-containing foams in training exercises and wear PFAS-impregnated gear (ATSDR, 2021). In addition, food workers and others in the hospitality industry may have elevated exposure if they handle PFAS-containing food packaging as part of their job (Carnero et al., 2021; Curtzwiler et al., 2021; Schaider et al., 2017).

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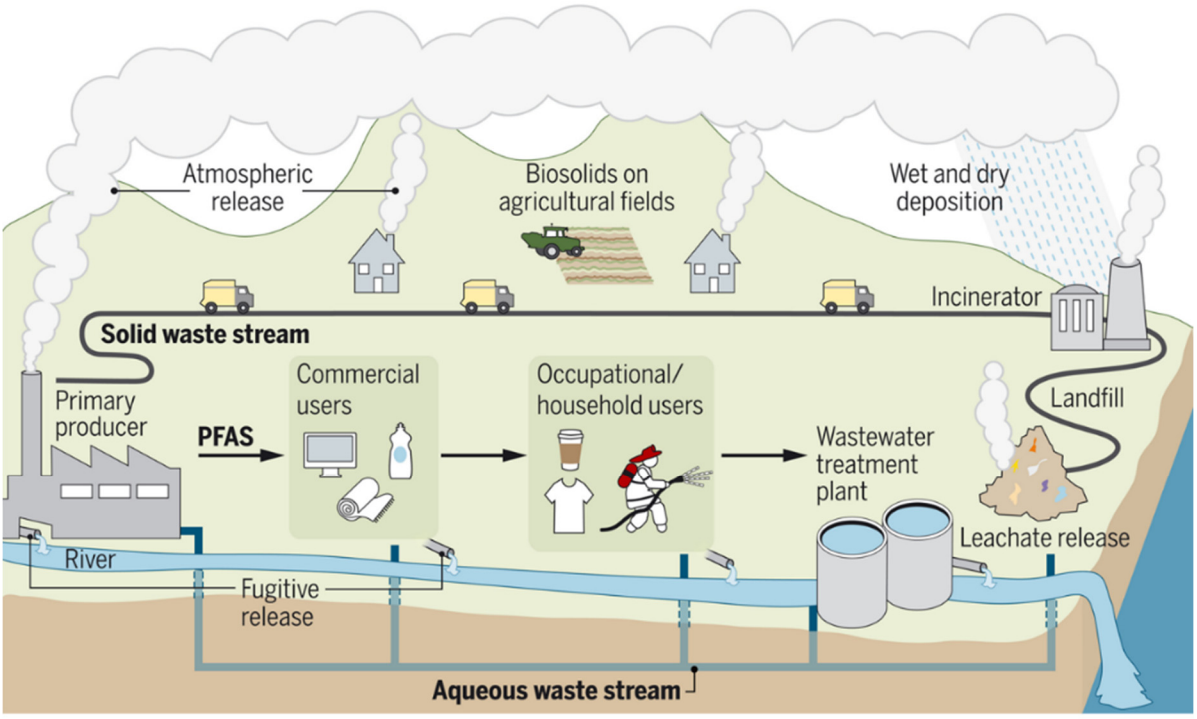


FIGURE 1-7 Examples of how PFAS enter the environment.
SOURCE: Evich et al., 2022.

Ingestion is the most well-studied route of exposure to PFAS in nonoccupational settings (Trudel et al., 2008). PFAS can be ingested by drinking contaminated water or by eating contaminated seafood or other contaminated foods, such as vegetables, game, or dairy products (Bao et al., 2019, 2020; Death et al., 2021; Domingo and Nadal, 2017; Herzke et al., 2013; Li et al., 2019). PFAS are often used in cookware and food contact materials, such as microwave popcorn bags and packaging of fast foods and processed foods (Carnero et al., 2021; Curtzwiler et al., 2021; Schaider et al., 2017). Exposure can also occur through accidental ingestion of PFAS-containing dusts (ATSDR, 2021[8]). PFAS cross the placenta, and PFAS from the mother's body burden can be passed on to her developing fetus (Gao et al., 2019; Manzano-Salgado et al., 2015); PFAS can also pass from mother to child through breast milk (Serrano et al., 2021; Zheng et al., 2021).

Inhalation and transdermal absorption are less well studied. Inhalation is well documented in occupational settings that use aerosolized PFAS (Gilliland, 1992). Volatile PFAS have been detected indoors (Fromme et al., 2015; Morales-McDevitt et al., 2021) and have been associated with persistent PFAS in serum (Fraser et al., 2013). Inhalation near factory emissions and incinerators contributes to exposures in nearby communities (ATSDR, 2021[9]). Inhalation from showering in contaminated water is an active area of research, but there are as yet no data formally evaluating this route.

PFAS are used in thousands of products (e.g., water- and stain-resistant clothing) and personal care products (e.g., sunscreen, makeup, dental floss). They also are used in such products as paint, textiles, firefighting foam, electroplating materials, ammunition, climbing ropes, guitar strings, artificial turf, and soil remediation substances (Glüge et al., 2020). The extent to which the use of such products contributes to human exposures remains unclear, however, because the relative contribution of PFAS exposures from sources other than food or water is not well characterized (DeLuca et al., 2021).

[8] This text was altered to correct the references after release of a pre-publication version of the report.
[9] This text was altered to correct the references after release of a pre-publication version of the report.

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Guidance on PFAS Exposure, Testing, and Clinical Follow-Up

POLICIES THAT COULD REDUCE EXPOSURE TO PFAS

The EPA's major policy levers for reducing harmful exposures to PFAS are the Toxic Substances Control Act (TSCA), which limits chemicals in commerce; the Safe Drinking Water Act; and the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA, commonly known as the "Superfund"), which designates hazardous sites for cleanup.

In 2000, the 3M Company (Maplewood, Minnesota), the primary U.S. manufacturer of PFOS, began a voluntary phase-out of perfluorooctanyl chemicals and related precursors, including PFOS, PFHxS, and PFOA[8] (ITRC, 2017). Between 2002 and 2013, the EPA issued four Significant New Use Rules under the TSCA that prevented others from manufacturing the chemicals 3M had voluntarily phased out (Richter et al., 2021) and required notifying the agency prior to any manufacture, use, or import of 271 other PFAS.[9] In 2006, the EPA encouraged eight leading PFAS manufacturers to join a global stewardship program focused on phasing out and ending the production of PFOA. At the same time, the TSCA allows some industries (including photographic imaging, semiconductors, etching, metal plating, and aviation) to continue using banned PFAS at low levels, and these industries are exempted from the provision requiring that the EPA be notified of any new use of banned PFAS (ITRC, 2017). Furthermore, these bans apply only to a handful of chemicals, most of which could easily be substituted for by other PFAS. And other PFAS not yet banned remain in use by industry while undergoing further biologic profiling and toxicity evaluation (Houck et al., 2021).

If PFAS were designated as hazardous substances, CERCLA could be used to lower exposures from contaminated sites, giving the EPA more authority to investigate and remediate those waste sites. The agency has not yet designated PFOS or PFAS as hazardous, however. And the Safe Drinking Water Act would be more protective if the standards were enforceable.[10]

Policy changes that could reduce PFAS exposures may be forthcoming. Recently, the EPA established an EPA Council on PFAS charged with building on the agency's ongoing work to understand better and ultimately reduce the potential risks posed by these chemicals. A PFAS Strategic Roadmap also was released, committing the agency to action. In addition, the EPA released for review by a science advisory board draft documents that will inform an enforceable National Primary Drinking Water Regulation for PFOA and PFOS.[11]

The EPA is not alone in its lack of action. The U.S. Food and Drug Administration (FDA) has delayed limiting PFAS in bottled water,[12] but has revoked regulations that allowed for long-chain PFAS (such as PFOA and PFOS) in food packaging in 2016,[13] and reached an agreement to phase out PFAS substances containing 6:2 fluorotelomer alcohol (Hahn, 2020). The Occupational Safety and Health Administration (OSHA) has set no occupational exposure limits.

The lack of federal standards has left states and communities responsible for creating policies to reduce exposures to these chemicals in the interest of protecting public health (Brennan et al., 2021). There is no consistency in these policies, and not all states have the authority to set standards more protective than those of the federal government (ECOS, 2020). Furthermore, leaving policy making to

[8] See https://archive.epa.gov/epapages/newsroom_archive/newsreleases/33aa946e6cb11f35852568e1005246b4.html (accessed June 16, 2022).

[9] See https://www.epa.gov/assessing-and-managing-chemicals-under-tsca/risk-management-and-polyfluoroalkyl-substances-pfas (accessed June 16, 2022).

[10] See https://www.epa.gov/pfas/epa-actions-address-pfas (accessed June 16, 2022).

[11] See https://www.epa.gov/pfas/pfas-strategic-roadmap-epas-commitments-action-2021-2024 (accessed June 16, 2022).

[12] See https://www.consumerreports.org/bottled-water/fda-delays-setting-limits-on-pfas-in-bottled-water-a8292013869 (accessed June 30, 2022).

[13] See https://www.fda.gov/food/chemical-contaminants-food/authorized-uses-pfas-food-contact-applications (accessed June 16, 2022).

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state governments encourages a confusing patchwork of rules and advice. Health inequities thrive in such chaotic circumstances.

PROVIDING CLINICAL ADVICE IN COMMUNITIES EXPOSED TO PFAS

To help clinicians[14] respond to patient concerns about PFAS exposure, the Agency for Toxic Substances and Disease Registry (ATSDR) published *PFAS: An Overview of the Science and Guidance for Clinicians on Per- and Polyfluoroalkyl Substances.*[15] This guidance summarizes general information about PFAS and PFAS health studies and suggests answers to example patient questions. However, the ATSDR guidance does not provide specific recommendations on when to test for PFAS, how to order the tests, how to interpret the results, or what clinical follow-up based on PFAS exposure might look like. Interpretation of PFAS blood or urine results is challenging because the specific level of exposure at which harms may occur is unknown, and the science on the potential health effects of exposure to PFAS is advancing quickly, making it difficult to advise clinicians about what follow-up tests might be helpful.

ATSDR has the legal authority to issue guidance to clinicians in its "Criteria for Determining the Appropriateness of a Medical Monitoring Program under Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA),"[16] promulgated in 1995 (42 USC 9604[i][9]). Under the criteria, the purpose of medical monitoring is to detect individuals with exposures to hazardous substances and refer them to medical care for further evaluation and treatment. The criteria recommends actions that clinicians can take, such as early detection, treatment, or other interventions that interrupt "the progress to symptomatic disease, improve the prognosis of disease, improve the quality of life of the affected individual, or address diseases that are amenable to primary prevention" (42 USC 9604[i][14], p. 38842). Medical monitoring under the criteria is not a research mechanism, and suggests that other epidemiologic studies be carried out to further investigate the cause–effect relationship between exposures and health outcomes. ATSDR's authority to issue guidance to clinicians comes from a different section in the same law (42 USC 9604[i][14]), which grants the agency the authority to develop education materials on medical surveillance, screening, and methods of diagnosing and treating injury or disease related to exposure to hazardous substances.

The first well-documented case of community water contamination with PFAS resulted in recommendations for medical monitoring (see Box 1-2). Releases of PFOA-contaminated drinking water from the DuPont Washington Works facility near Parkersburg, West Virginia, were detected in the local area and a few nearby communities across the Ohio River. A class action lawsuit was filed—*Jack W. Leach et al. v. E.I. du Pont de Nemours & Company* (no. 01-C-608 W.Va., Wood County Circuit Court, filed April 10, 2002). The lawsuit resulted in a medical monitoring program that included suggested screenings for high cholesterol, thyroid disease, ulcerative colitis, testicular cancer, kidney cancer, pregnancy-induced hypertension, and blood testing for PFOA (Frisbee et al., 2009). The medical monitoring guidance generated by this high-profile case has led some community members to believe that the ATSDR guidance should provide recommendations for clinical follow-up, and advocates in many PFAS-exposed communities are working to establish medical monitoring programs (Rizzuto, 2021).

The absence of recommendations on testing and follow-up in ATSDR's PFAS clinical guidance is aligned with standard medical practice, as clinicians are expected to order tests only when how to interpret and act on the results is known. In addition, reporting of levels of PFAS in blood and urine to patients raises ethical questions, such as whether to report results in the absence of established health guidelines.

[14] The committee uses "clinician" throughout this report to refer to "a healthcare professional qualified in the clinical practice of medicine. Clinicians may be physicians, nurses, pharmacists, or other allied health professionals" as defined by the Centers for Medicare & Medicaid Services (https://www.cms.gov/Medicare/Quality-Initiatives-Patient-Assessment-Instruments/MMS/QMY-Clinicians [accessed June 16, 2022], para 2).

[15] See https://www.atsdr.cdc.gov/pfas/docs/clinical-guidance-12-20-2019.pdf (accessed June 16, 2022).

[16] See https://www.govinfo.gov/content/pkg/FR-1995-07-28/pdf/FR-1995-07-28.pdf (accessed June 16, 2022).

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BOX 1-2
The C-8 Science Panel and the C-8 Medical Panel

Background: In the early 2000s, contamination of drinking water with PFOA (also known as C-8 for the eight carbons in its chemical structure) was discovered in six water districts in two states near the DuPont Washington Works facility near Parkersburg, West Virginia. As a result, a class action lawsuit, *Jack W. Leach et al. v. E.I. du Pont de Nemours & Company* (no. 01-C-608 W.Va., Wood County Circuit Court, filed April 10, 2002), was filed. The lawsuit resulted in the formation of the C-8 Science Panel to determine "probable link conditions" associated with PFOA-contaminated drinking water. The settlement agreement defined "probable link" to mean that, based on the weight of the available scientific evidence, it is more likely than not that there is a link between exposure to PFOA and a particular human disease (Frisbee et al., 2009). The settlement also called for the formation of a separate medical panel "to develop general guidelines for medical monitoring related to the human diseases for which the science panel delivered a probable link finding and is different from what would otherwise be prescribed" if exposure to PFOA were absent.

Science Panel Findings: The science panel identified pregnancy-induced hypertension (including preeclampsia), kidney cancer, testicular cancer, thyroid disease, ulcerative colitis, and high cholesterol (hypercholesterolemia) as probable linked conditions (C-8 Medical Panel, 2013).

Medical Panel Findings: The medical panel recommended screenings for high cholesterol, thyroid disease, ulcerative colitis, testicular cancer, kidney cancer, pregnancy-induced hypertension, and blood testing for PFOA (C-8 Medical Panel, 2013).

At a workshop on PFAS exposure that occurred more than 1 year before the present study began, however, Andrea Amico, a member of a PFAS-impacted community in Pease, New Hampshire, traveled to Washington, DC, to represent her community's views on PFAS testing. She said that people in her community

> were exposed … without their consent, and now they have to fight tooth and nail to get a blood test result to know how much exposure they had? It just seems incredibly wrong…. We don't have all the answers yet, but not testing them is not the right answer.

Amico also argued for more access to testing despite the knowledge gaps regarding the interpretation of test results. She believes PFAS exposure testing would allow people to compare their levels with those of others in highly exposed communities, which could help them understand their potential health risks (NASEM, 2020). As it stands now, clinicians in communities with known PFAS contamination are left to make their own decisions about whether to test patients for PFAS exposure, which PFAS to test for, how to interpret the results, what health effects are associated with PFAS, and how to determine appropriate follow-up care for exposed patients. Pressed for time in clinic visits, clinicians may dismiss interest in PFAS testing or avoid recommending specific medical follow-up, and they may not even be clear on when or how to order a test.

COMMITTEE'S TASK AND APPROACH

Prompted by the tension between people in PFAS-exposed communities wanting preventive care for their exposure and clinicians not knowing what care should be provided, ATSDR and the National Institute of Environmental Health Sciences (NIEHS) asked the National Academies of Sciences, Engineering, and Medicine to form an ad hoc committee to provide advice on clinical care for patients exposed to PFAS. The committee included experts in epidemiology, toxicology, preventive medicine, pediatrics, nursing, public health, environmental medicine, philosophy, ethics, exposure science, and risk communication (see Appendix A for biographical information on the committee members). The committee's Statement of Task is provided in Box 1-3.

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BOX 1-3
Statement of Task

An ad hoc committee appointed by the National Academies of Sciences, Engineering, and Medicine (the National Academies) will consider current evidence regarding human health effects of the most widely studied per- and polyfluoroalkyl substances (PFAS). The National Academies will provide the Centers for Disease Control and Prevention and the Agency for Toxic Substances and Disease Registry (CDC/ATSDR) and the National Institute of Environmental Health Sciences (NIEHS) an objective and authoritative review of current evidence regarding human health effects of those PFAS being monitored in the CDC's National Report on Human Exposure to Environmental Chemicals. The National Academies will also provide recommendations regarding potential changes to CDC/ATSDR PFAS clinical guidance including:

- Options and considerations to guide decision making for PFAS testing in a patient's blood or urine.
- PFAS concentrations that could inform clinical care of exposed patients.
- Appropriate patient follow-up and care specific to PFAS-associated health endpoints for those patients known or suspected to be exposed to PFAS.

This information will be used to inform how communities and individuals exposed to PFAS could be best served by clinicians. Specifically, the committee will undertake the following tasks:

1. Assess the strength of evidence for the spectrum of putative health effects suggested by human studies (including immune response, lipid metabolism, kidney function, thyroid disease, liver disease, glycemic parameters and diabetes, cancer, and fetal and child development) to establish a basis for prioritized clinical surveillance or monitoring of PFAS health effects. This assessment should characterize the likelihood of those health effects occurring (qualitative probability) given real world human exposures and identify the human populations at most risk (consider life stage, health status, exposure level). Data/evidence gaps that contribute to uncertainty about health effects of most concern should be annotated.
2. Develop general principles for clinical evaluation or biological testing given substantial scientific uncertainty about health effects or the value of such measures in informing care. These principles should address reasons for testing (e.g., opportunities to reduce morbidity and mortality), when to test, who to test, how to test, what to test for, risks of testing, and the related social and ethical implications of testing.
3. Review current knowledge about the contribution of PFAS exposure sources (i.e., drinking water, diet, the indoor environment, etc.) to human exposure and develop principles clinicians can use to advise patients on exposure reduction.
4. Advise whether changes to current CDC/ATSDR clinical guidance/recommendations on PFAS blood or urine testing are needed given the committee's general principles and assessment of the associations between PFAS exposure and clinically relevant health outcomes. Ultimately, the goal is to provide guidance on how clinicians can advise patients on PFAS testing and health outcomes that may be associated with PFAS as well as what to advise patients regarding standard medical or preventive care and exposure reduction.
5. Outline a process by which the CDC/ATSDR PFAS clinical guidance can be effectively reviewed and revised over the next decade.

Figure 1-8 provides an overview of the steps in the committee's approach to its charge: problem formulation and engagement with communities, development of principles for decision making under substantial scientific uncertainty, determination of strategies for reducing exposure to PFAS, development of advice for PFAS testing and levels of PFAS in blood or urine that could inform clinical care of exposed patients, determination of the health effects of PFAS, appropriate follow-up care of exposed patients, and advice on whether changes are needed with respect to ATSDR's PFAS clinical guidance.

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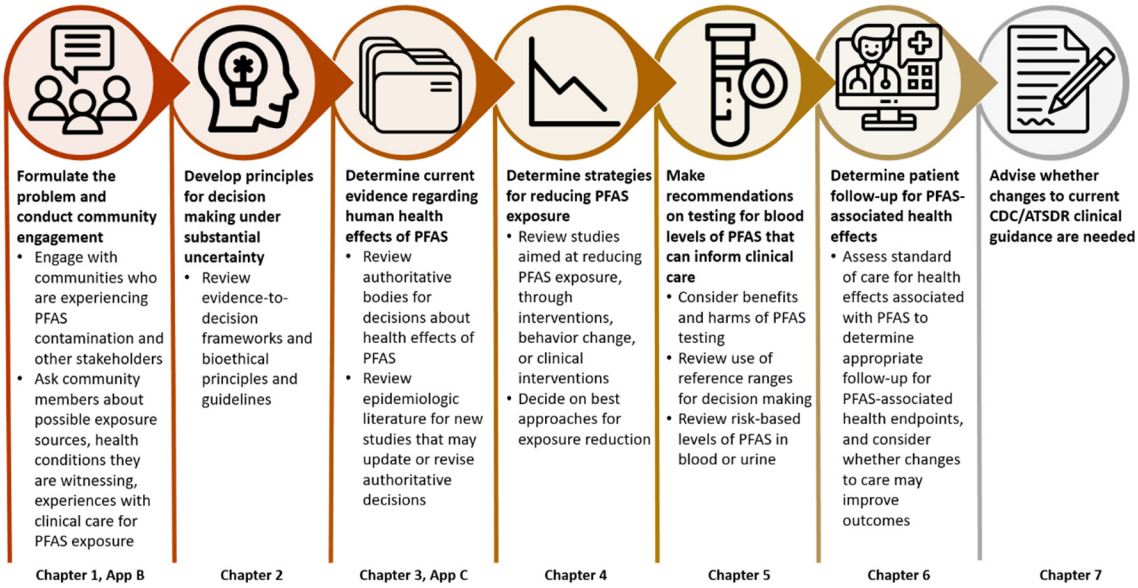


FIGURE 1-8 The committee's approach to the Statement of Task and the chapters and appendixes where the topics are discussed.
NOTE: ATSDR = Agency of Toxic Substances and Disease Registry; CDC = Centers for Disease Control and Prevention; PFAS = per- and polyfluoroalkyl substances.

Problem Formulation and Community Engagement

The committee held six public meetings between February 4 and August 12, 2021 (see Appendix C for the agendas of these public meetings). Leading up to its first meeting, the committee began to receive public input on the study. The testimony provided at the first public meeting revealed that many members of the public feel ignored or dismissed by clinicians and the medical community, and have assumed the role of community scientists, becoming experts in their communities' illnesses and experiences. People who live with potentially harmful exposures can have knowledge that is inaccessible to scientists as their experiential learning provides essential context for scientific findings (Brown, 1992). To incorporate this knowledge into the study, the committee, National Academies staff, and the study sponsors developed a community engagement plan. To aid in this process, the committee considered the Jemez Principles for Democratic Organizing, a set of recommendations developed to serve as a foundation on which diverse coalitions can together make justice-based decisions specifically weighing on inclusivity, as well as letting people speak for themselves (Solís and SPWU, 1997). Ultimately, the committee's community engagement plan consisted of several key elements (see Figure 1-9):

- a panel of community liaisons;
- three 2021 town halls (April 7, Eastern Town Hall; May 6, Middle Town Hall; May 25, Western Town Hall);
- community speakers from PFAS-impacted communities at every public meeting;
- open sign-up for public testimony to the committee at every public meeting; and
- encouragement for providing written testimony to the committee throughout the data-collection phase of the study.

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FIGURE 1-9 The committee's approach to community engagement.

The community liaisons were solicited through a nomination process similar to that used for National Academies committees. The community liaisons were intended to be an inclusive group; all nominees who appeared to be genuinely interested or had experience working with PFAS or similar environmental contamination issues were asked to join the group. National Academies staff held several meetings with the liaisons throughout the study process. The first call with the liaisons clearly described the mission of the National Academies, the goals of the study, what the study would not address, and the study timeline.

The role of the community liaisons was to provide input to the committee on behalf of PFAS-impacted communities. The liaisons aided the study process by suggesting speakers, topics, and discussion questions for the public meetings, and by answering questions to inform the report or the committee's work. They also prepared letters to the committee providing consensus opinions related to the Statement of Task and suggested reviewers for the report for consideration by National Academies staff.

Health Effects Associated with PFAS Exposure

The committee carefully considered the purpose of the literature review of putative health effects prescribed in its Statement of Task: to *establish a basis for prioritized clinical surveillance or monitoring of PFAS health effects.* Accordingly, the committee's review was focused on determining a set of health effects that may be associated with PFAS, which could then be used for preventive medicine recommendations and decisions. The Statement of Task limited the review to those PFAS included in the Centers for Disease Control and Prevention's (CDC's) *National Report on Human Exposure to Environmental Chemicals* (see Table 1-1).

Although the committee believes that studies on health effects in humans will most likely be limited to those PFAS in Table 1-1 because they are the most commonly studied in humans, other PFAS may cause harm because of similarities in biological persistence and toxicities (Kwiatkowski et al., 2020). Moreover, while different PFAS have distinct physical, chemical, and toxicological properties, people are exposed to more than a single PFAS. As a result, exposures are often to mixtures of PFAS such that specific effects are difficult to disentangle. Considering these issues and recognizing that some PFAS are less frequently measured than others, the committee ultimately decided to provide one strength-of-evidence determination for all PFAS for each health effect. Further description of the methods used for the committee's review is provided in Appendix D.

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TABLE 1-1 PFAS Species Currently Included in the Centers for Disease Control and Prevention's *National Report on Human Exposure to Environmental Chemicals*

Abbreviated Name	Full Name	CAS Registry No.
MeFOSAA	Methylperfluorooctane sulfonamidoacetic acid	2355-31-9
PFHxS	Perfluorohexanesulfonic acid perfluorohexane sulfonic acid	355-46-4
n-PFOA (linear isomer), Sb-PFOA (branched isomers)	Perfluorooctanoic acid	335-67-1*
PFDA	Perfluorodecanoic acid	335-76-2
PFUnDA	Perfluoroundecanoic acid	2058-94-8
n-PFOS (linear isomer), Sm-PFOS (branched isomers)	Perfluorooctanesulfonic acid	1763-23-1*
PFNA	Perfluorononanoic acid	375-95-1

NOTES: CAS = Chemical Abstracts Service. * = CAS number refers to linear isomer only. Previous survey years have also included perfluorobutane sulfonic acid (PFBS), perfluoropentanoic acid (PFpA), perfluorododecanoic acid (PFDoDA), perfluorooctane sulfonamide (FOSA), and 2-(N-ethyl-perfluorooctane sulfonamido)acetate (EtFOSAA), according to Patrick N. Breysse's presentation to the committee on February 4, 2021.

Reducing Exposure to PFAS

This report does not review EPA policy actions, which are beyond the scope of the committee's task. A review of individual-level exposure reduction strategies is presented in Appendix E. Chapter 4 provides an overview of that literature and medical interventions to reduce PFAS body burden. The report reviews these individual-level strategies because the task calls for them; however, the committee does not believe that people should be responsible for protecting themselves from harmful chemicals. Well-established exposure prevention frameworks, such as the hierarchy of controls and the health impact pyramid, dictate that individuals should not be responsible for making such decisions (Frieden, 2010). Systems approaches to exposure reduction, such as setting an enforceable water standard, regulating PFAS from nonessential uses, and cleaning up hazardous waste sites, will be far more effective at reducing population-level exposures (see Figure 1-10).

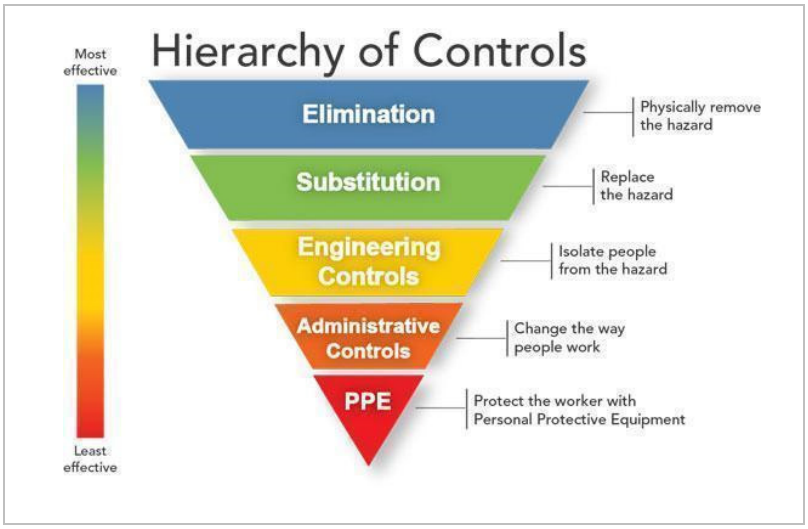


FIGURE 1-10 Hierarchy of controls.
SOURCE: See https://www.cdc.gov/niosh/topics/hierarchy/default.html (accessed June 16, 2022).

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Advice on PFAS Testing and Levels That Could Inform Clinical Care

The committee's approach to providing advice on PFAS testing and clinical care included a review of the harms and benefits of testing. To determine the levels of PFAS exposure that could inform clinical care of exposed patients, the committee did not complete dose-response modeling to determine a tolerable risk level. Instead, the committee reviewed various strategies for interpreting PFAS concentrations, such as use of reference ranges for comparison, and levels of risk determined by other agencies.

Follow-Up Care for Patients Known or Suspected to Be Exposed to PFAS

The committee considered relevant for patient follow-up recommendations for each condition that its review found had suggestive or limited suggestive evidence of an association with PFAS exposure. The committee believed that while it was important to categorize the strength of the evidence, all conditions with an association should be considered for patient follow-up since acknowledging the potential risk may make clinicians and patients more likely to prioritize screenings.

ORGANIZATION OF THE REPORT

This report is organized into eight chapters and five appendixes. Chapter 2 presents the committee's general principles for decision making under substantial scientific uncertainty. Chapter 3 presents the committee's assessment of the health effects of PFAS. Chapter 4 reviews strategies for reducing exposure to PFAS. Chapter 5 provides recommendations for PFAS testing and concentrations in blood or urine that could inform clinical care. Chapter 6 presents the committee's specific recommendations for patient follow-up. Chapter 7 addresses suggested changes to the ATSDR PFAS clinical guidance. Finally, Chapter 8 considers the implementation of the committee's recommendations. Appendix A provides biographical information on the committee members, National Academies staff, and the community liaisons; Appendix B provides a summary of the town halls; Appendix C contains the agendas of the committee's public meetings; Appendix D is a summary of the methods used in the committee's literature review; and Appendix E is a white paper describing a review of the PFAS personal intervention literature.

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2

Principles for Decision Making Under Uncertainty

Given that there is some degree of uncertainty in all science, science-based decision making also involves uncertainty (Fischhoff and Davis, 2014). This uncertainty can arise from many different factors, including lack of available evidence, statistical variability, model uncertainty, and "deep" uncertainty about the fundamental scientific processes relevant to a decision (IOM, 2013). Yet, despite this uncertainty, when evidence exists, it helps predict what may happen when a decision is made (Fischhoff and Davis, 2014). Values also influence how scientific evidence is gathered and interpreted, thereby influencing how uncertainty is characterized and what decisions are made on the basis of that particular interpretation of the evidence (Douglas, 2009; Elliott, 2017; IOM et al., 1995). Thus, it is important to consider uncertainty in the broader context of the decision to be made and the values underlying the decision-making process (IOM, 2013).

In response to uncertainty, some public health professionals may wish to avoid acting as soon as science determines the risks of an exposure for fear that the association is not a true one and that acting too quickly could lead to inappropriate and costly public health measures (Boffetta et al., 2008). On the other hand, some may fear that if society waits for more certain evidence, meaningful action will be delayed (Blair et al., 2009). As a group of scientists with different backgrounds and perspectives, the committee grappled with both of these potential outcomes of its decisions. The committee established the principles in this chapter to help accomplish its charge, but also believes they may be useful to support the Agency for Toxic Substances and Disease Registry (ATSDR) in making decisions about medical follow-up and advice to communities exposed to per- and polyfluoroalkyl substances (PFAS) or other chemicals with uncertain effects.

DEVELOPMENT OF THE COMMITTEE'S PRINCIPLES

In its effort to identify principles for decision making under uncertainty, the committee solicited input from members of PFAS-impacted communities through its virtual town halls (see Chapter 1). The committee also reviewed decision-making frameworks, including a review paper (Norris et al., 2021), the criteria used by the U.S. Preventive Services Task Force (USPSTF) (Krist et al., 2018; Sawaya et al., 2007), GRADE (Grading of Recommendations Assessment, Development and Evaluation) (Moberg et al., 2018), a National Academies evidence framework for decisions regarding genetic testing (NASEM, 2017), the Evidence and Value: Impact on DEcision-Making (EVIDEM) framework (Goetghebeur et al., 2008), the principles used by the C-8 Medical Panel (C-8 Medical Panel, 2013), the ethical principles from the Belmont Report (HHS, 1979), and principles for precautionary reasoning (Resnik, 2021). A brief overview of these sources is included below.

The existing principles and frameworks the committee reviewed were not entirely satisfactory for the purposes of this study. Most of the existing frameworks were not designed to address situations characterized by limited evidence and substantial scientific uncertainty—the contexts the committee was charged to address. The committee understood that when evidence is incomplete, dangers can be associated both with taking action and with failing to act (Douglas, 2009; Elliott and Richards, 2017). Therefore, the committee adapted the principles and frameworks it reviewed to develop a set of principles appropriate for decision making under substantial scientific uncertainty.

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PRINCIPLES PUT FORWARD BY THE COMMITTEE

The committee decided to use the ethical principles proposed in the Belmont Report (HHS, 1979) and developed by Beauchamp and Childress (2001) as a foundation for its approach. Although these principles (nonmaleficence, beneficence, autonomy, and justice) were not initially proposed for decision making under uncertainty, they provide an ethical starting point for addressing such situations. In addition, they encompass many of the criteria and concepts included in other frameworks, such as considerations of benefits, harms, health equity, and human rights. The committee combined the principles of nonmaleficence and beneficence into a principle of proportionality—drawing on the Resnik (2021) framework for precautionary reasoning in response to uncertainty. The committee also added two other principles included in other decision-making frameworks but not captured by the principles from the Belmont Report. One is the principle of feasibility, found in several of the frameworks reviewed by Norris and colleagues (2021), including GRADE. It captures the importance of considering the capabilities of the current medical system and the ways it might need to develop to respond to decision needs. The other is the principle of adaptability, which builds on the call for review and revision in the National Academies framework for genetic testing and the emphasis in the USPSTF framework on revising recommendations in response to emerging scientific information. Box 2-1 briefly describes the committee's resulting five principles for exposure biomonitoring and patient follow-up under substantial scientific uncertainty about the health effects of PFAS exposure. Each of these principles is discussed in turn below.

Proportionality

The committee's first principle, proportionality, is adapted from Resnik's recommendations for precautionary decision making under uncertainty (Resnik, 2021, p. 81):

> **Proportionality:** Decisions should balance plausible harms and benefits proportionally, meaning that the harms and benefits are considered together and weighed based on a qualitative assessment of their potential likelihoods and magnitudes.

BOX 2-1
Principles for Decision Making Under Uncertainty Used in This Report

Proportionality: Decisions should balance plausible harms and benefits proportionally, meaning that the harms and benefits are considered together and weighed based on a qualitative assessment of their potential likelihoods and magnitudes.

Justice: Decisions should be informed by an emphasis on promoting justice, including by balancing benefits and harms fairly across the population of at-risk individuals, advancing health equity, and respecting human rights. In addition, justice requires consideration of sociohistorical context, stakeholders, existing structural inequalities, and issues of agency (the power a community has to advocate for itself in conflicts).

Autonomy: Decisions should be based on informed decision making by individuals and reflect respect for their values.

Feasibility: Decisions should take into account resource availability, including follow-up services.

Adaptability: Decisions should respond to new information about harms, benefits, and other relevant considerations (e.g., health equity and feasibility).

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This principle draws on a common theme that cuts across most frameworks the committee considered: balancing harms and benefits. For example, the USPSTF states that in determining the benefits of a preventive services framework, one should consider evidence about the accuracy of screening, harms of early-intervention treatment and not treating, and the treatment benefits and harms of a particular preventive service. The USPSTF framework also considers the accuracy of a screening test and its relationship to the clinical health effect, and the harms associated with the screening for the health condition and treatment of the condition.

The principle of proportionality addresses harms and benefits in situations of substantial scientific uncertainty. To balance harms and benefits proportionally is to consider them together such that, all else being equal, policies with fewer or less severe harms (assessed in terms of their likelihood and magnitude) can be justified based on lesser benefits relative to policies with increased harms. This principle is particularly relevant for decision making under uncertainty because it can justify taking actions to realize potentially significant benefits even when the evidence for those benefits is limited, as long as the potential harms associated with the actions are minimal.

Proportionality provides an alternative to probability-based decision principles that require numerical ranking of the probabilities or utilities associated with effects. Although applying the principle of proportionality calls for considering the likelihood and magnitude of benefits and harms in some form, it does not require assigning numerical rankings to their likelihoods or magnitudes. Because of the qualitative nature of this principle, reasonable decision makers can potentially disagree about how to apply it in specific situations because judgments are required to weigh harms and benefits. Especially when the benefits associated with two policies are similar, and the likelihood and the magnitude of their harms cannot be estimated precisely, the principle of proportionality may not offer decisive guidance. Instead, this principle provides a general way of reasoning compatible with a range of more specific strategies for balancing harms and benefits under different forms of uncertainty (Douglas, 2009; Workman et al., 2020). It affirms that both harms and benefits need to be considered and that they need to be weighed against one another when making decisions (Resnik, 2021).

For this report, the harms of a decision could include physical effects, psychological effects, and opportunity costs (Harris et al., 2014). The committee does not consider the financial costs of paying for tests or treatment a harm. Costs could be considered under the principle of feasibility, but the committee excluded costs from feasibility as well. The reason for excluding costs is that, although a certain test or treatment may be expensive today, costs typically decrease over time if demand for the test or treatment increases. Many other organizations, such as the USPSTF, do not consider financial costs when formulating recommendations based on assessment of health benefits and harms, and the committee followed this approach. The committee did consider as potential harm the loss of income or financial damage incurred as a result of decisions (e.g., decreased property values caused by a community's learning about chemical contamination).

The principle of proportionality calls for considering benefits and harms that are plausible. The concept of plausibility provides a minimal threshold for determining which benefits and harms should even be considered. Resnik (2021, pp. 80–81) defines a scientific statement as plausible "if it is consistent with well-established scientific facts, hypotheses, laws, models, or theories"—a lower standard of evidence than is typically required for even weak scientific confirmation. Instead, this definition is designed to rule out "armchair speculation" about potential harms and benefits. If a harm or benefit is judged to be plausible, it can be considered in the overall assessment of proportionality. Nevertheless, harms and benefits deemed to have a reasonably low level of evidential support might still be given relatively little weight in proportionality assessment unless their potential magnitudes were estimated to be exceptionally high.

In situations of substantial scientific uncertainty, it is generally unrealistic to expect more than a qualitative assessment of the likelihoods and magnitudes of harms and benefits. This assessment may be informed by evidence, including epidemiological studies, toxicological studies (both animal models and mechanistic studies), modeling, and support from analogy. It should be guided by norms for the responsible acquisition and utilization of evidence, knowledge, and expertise. Still, the appropriate

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standards of evidence for drawing tentative conclusions about potential likelihoods and magnitudes may be much lower than would apply in other scientific contexts (Douglas, 2009; Elliott and Richards, 2017; Ginsberg et al., 2019).

Justice

Justice is the second principle for decision making in situations of substantial scientific uncertainty:

> **Justice:** Decisions should be informed by an emphasis on promoting justice, including by balancing benefits and harms fairly across the population of at-risk individuals, advancing health equity, and respecting human rights. In addition, justice requires consideration of sociohistorical context, stakeholders, existing structural inequalities, and issues of agency (the power a community has to advocate for itself in conflicts).

Justice is central to the Belmont Report and Beauchamp and Childress's framework for biomedical ethics (Beauchamp and Childress, 2001; HHS, 1979), and elements of justice are included in many of the frameworks discussed in the review by Norris and colleagues (2021). Beauchamp and Childress (2001, p. 226) describe justice as "fair, equitable, and appropriate treatment in light of what is due or owed to persons." Thus, an injustice involves "a wrongful act or omission that denies people benefits to which they have a right or distributes burdens unfairly."

In this report, the focus is on three elements of justice: fairness, equity, and human rights. Resnik (2001) emphasizes that decisions under uncertainty should be handled in a manner that is procedurally and distributively fair: the *process* for making decisions should be fair in the sense of incorporating all relevant stakeholders and employing transparent and accountable procedures, and the *endpoints* of the decisions should also be fair.

In the health care setting, the second element of justice—equity—calls for promoting health equity. Health equity is "the state in which everyone has the opportunity to attain full health potential, and no one is disadvantaged from achieving this potential because of social position or any other socially defined circumstance" (NASEM, 2017, p. 32). Promoting health equity is not the same as achieving similar health outcomes for everyone. Instead, promoting health equity in the context of this report means designing approaches that enable people to lead full, healthy lives regardless of their social circumstances, such as race, socioeconomic status, and geographic location. Health equity is a particularly important element of justice because it establishes the positive responsibility to provide the conditions necessary for people to lead healthy lives regardless of their social circumstances; without this positive responsibility, some ethicists have argued that the principle of justice does not provide significant guidance beyond what is already found in other bioethical principles, such as proportionality or autonomy (London, 2022).

Finally, justice involves respect for human rights. The committee's conception of human rights is drawn from the World Health Organization (WHO)-Integrate evidence-to-decision framework (Rehfuess et al., 2019), which emphasizes rights related to the availability and accessibility of health care and such general rights as nondiscrimination.

In cases of environmental pollution such as PFAS contamination, it is crucial to incorporate the concept of environmental justice into efforts to promote fairness, health equity, and human rights. According to the U.S. Environmental Protection Agency (EPA), "Environmental justice is the fair treatment and meaningful involvement of all people regardless of race, color, national origin, or income, concerning the development, implementation, and enforcement of environmental laws, regulations, and policies. This goal will be achieved when everyone enjoys:

- The same degree of protection from environmental and health hazards, and
- Equal access to the decision-making process to have a healthy environment."[1]

[1] See https://www.epa.gov/environmentaljustice (accessed September 17, 2021).

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The environmental justice movement in the United States arose in recent decades out of the realization that communities of color, which already faced other social disadvantages, were also exposed to disproportionately high levels of environmental pollution (Bullard, 2018; Shrader-Frechette, 2002). Many places where PFAS contamination has been identified are rural and served by private well water, and they face other environmental and social challenges, such as co-occurrences of other contaminants; occupational exposures; and health inequities, such as less access to health care and a lack of economic resources with which to mitigate exposures. These less-advantaged rural communities may also have structural and agency-related factors that can impact their ability to minimize their exposure, as well as to seek and access adequate health care linked to exposures. Promoting environmental justice requires addressing these inequities, such as by taking steps to eliminate disproportionate lack of access to health care, exposure biomonitoring, patient follow-up for PFAS-associated health effects, and environmental remediation and mitigation. Required as well is addressing the needs of vulnerable populations, such as pregnant and nursing women, young children, and those who are immunocompromised.

Autonomy

The committee's third principle is autonomy:

> **Autonomy:** Decisions should be based on informed decision making by individuals and reflect respect for their values.

The Belmont Report emphasizes the importance of respecting individuals' autonomy and ability to make judgments; it also warns against withholding information that would enable them to judge. Beauchamp and Childress (2001) likewise emphasize that respect for autonomy requires eliminating constraints on individuals' decision making and fostering their self-determination. In practice, this principle has often been operationalized by focusing on obtaining individuals' informed consent to medical treatments or research experiments, but its implications are much broader. In the context of this report, the principle of autonomy calls for health care services to provide individuals with information and collaborate with them to facilitate decisions that accord with their values. This approach is fundamental in situations of substantial scientific uncertainty, when difficult decisions need to be made about how to weigh harms and benefits given limited information about their likelihoods and magnitudes. Different individuals can reasonably approach such situations in different ways. Therefore, the principle of autonomy affirms that patients should play a role in making these decisions.

In addition to fostering decisions that accord with patient values, the principle of autonomy supports other steps to empower patients and their communities. To facilitate patients' self-determination, health care providers and researchers should take steps to report information back to patients in ways that facilitate their future informed decision making. When patients are tested for exposure to potentially toxic substances, it is essential to provide them with (Brody et al., 2014; Morello-Frosch et al., 2009)

- information about their exposure level;
- a description of the potential health impacts, as well as the related signs and symptoms;
- how their exposure level compares with those of others in their area and across the country;
- the potential significance of their exposure level; and
- how they can reduce future risk, either of the exposure itself or its potential health impact(s).

The PFAS Research, Education, and Action for Community Health (PFAS-REACH) project is an example of an effort to provide accessible information to community members about how to interpret exposure biomonitoring results and what follow-up activities could be warranted to address PFAS-associated health effects (Boronow et al., 2017). It is also essential to respect the decision of some

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individuals not to be tested. Some individuals may not want to have information that increases their uncertainty or could lead to future medical monitoring that might not improve their health outcomes.

The principle of autonomy can also guide actions taken at the community level to help foster decision making by individuals. For example, there is growing recognition that community organizations and advocacy groups can generate important information about public health threats and develop actionable solutions (Corburn, 2005; Elliott, 2017; Wandersman, 2003). Thus, health care providers and public health institutions can foster individuals' autonomy by collaborating with these organizations that can help facilitate informed patient decision making.

Feasibility

The committee's fourth principle for making decisions under substantial scientific uncertainty is feasibility:

> **Feasibility:** Decisions should into take account resource availability, including follow-up services.

Feasibility, included in many evidence-to-decision frameworks reviewed by Norris and colleagues (2021), denotes the ability to conduct testing, clinical evaluation, and follow-up activities. An assessment of feasibility encompasses the infrastructure and resources currently available and whether they are sufficient to achieve the goals of a decision. In the context of PFAS exposure biomonitoring, for example, feasibility could include such considerations as the time and knowledge providers have available to facilitate environmental exposure assessments or their access to environmental occupational health physicians. Feasibility assessments may also include consideration of whether an action or policy is sustainable over time and it entails important legal, ethical, or bureaucratic barriers (Moberg et al., 2018). At the same time, however, it is crucial to recognize that feasibility assessments are relative to a particular point in time, and inputs and outputs can change. In some cases, a particular policy may not be feasible immediately because of a lack of resources or other institutional barriers, but those barriers may reflect past or present injustices that need to be remedied. Thus, for example, a feasibility assessment can guide policy makers to allocate additional resources when exposure biomonitoring or follow-up services are likely to have benefits or are needed to promote a just health care system. Therefore, the principle of feasibility needs to be considered alongside the principle of justice so it can guide future investments and institutional changes instead of detracting from efforts to address inequities and promote just outcomes. Unfortunately, the lack of a coordinated health system in the United States complicates any assessment of feasibility that goes beyond the requirements imposed on public health agencies. Individuals must rely on insurance or self-pay for many follow-up services, and access to high-quality primary care and specialty care services can be fraught with challenges.

Adaptability

The fifth and final principle is adaptability:

> **Adaptability:** Decisions should respond to new information about harms, benefits, and other relevant considerations (e.g., health equity and feasibility).

The National Academies evidence framework for decisions regarding genetic testing (NASEM, 2017) emphasizes that adaptability is important in decision contexts in which scientific information changes rapidly, as is the case with genetic testing. The USPSTF also emphasizes that its evaluation of the benefits and harms of preventive services is a process that needs to be revisited in light of new advances in research, testing, and treatment capabilities (Sawaya et al., 2007). This responsiveness to new information is especially important in situations of substantial scientific uncertainty, such as the decision

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context surrounding PFAS. In such situations, it is often necessary to develop plans for acting even without decisive information about what course of action is best, and the harms associated with failing to act when warranted can often be more severe than the harms associated with taking actions that ultimately turn out to be unwarranted. When actions are taken without compelling information, however, it is crucial to reexamine regularly the state of the evidence and the consequences of the actions taken to reassess the best course of action. The adaptive management movement in environmental policy exemplifies this commitment to an ongoing process of learning and reevaluation. According to a National Research Council report on managing water resources, "Adaptive management promotes flexible decision-making that can be adjusted in the face of uncertainties as endpoints from management actions and other events become better understood" (NRC, 2004, pp. 1–2).

COMMITTEE'S CONSIDERATIONS IN DEVELOPING ITS PRINCIPLES

Among the committee's considerations in developing its principles for decision making under substantial scientific uncertainty were input from contaminated communities and a number of previously published evidence-to-decision frameworks.

Community Input from Town Halls

During its three town halls, the committee obtained perspectives from people living in or working with communities with PFAS contamination during its three Town Halls (see Appendix B). The committee recognizes that the views expressed at the town halls do not necessarily represent the views of all affected communities or all individuals within those communities; nonetheless, they provided input for the committee to consider. Important themes relevant to the development of principles for decision making under substantial scientific uncertainty included the following:

- Community members shared that they had to educate their clinicians about the exposures in their communities and the potential health effects that could result. They stated further that clinicians do not know how to provide advice on patient follow-up following PFAS exposure, whether PFAS exposure biomonitoring should be provided, or how to interpret PFAS exposure biomonitoring results if shared by the patient.
- Community members expressed the view that PFAS exposure biomonitoring should be available through traditional clinical care to all individuals. In addition, given that PFAS are ubiquitous in the environment and that many of the contamination sites have not been identified, exposure biomonitoring should be more widely available.
- Significant harms were not associated with exposure biomonitoring for PFAS. Community members understand that PFAS exposure biomonitoring may not directly inform their health, but they wish to have knowledge of their exposure levels as a first step.
- Speakers stated that PFAS exposure information could inform health care decisions and patient follow-up related to potential adverse health effects associated with PFAS exposure.

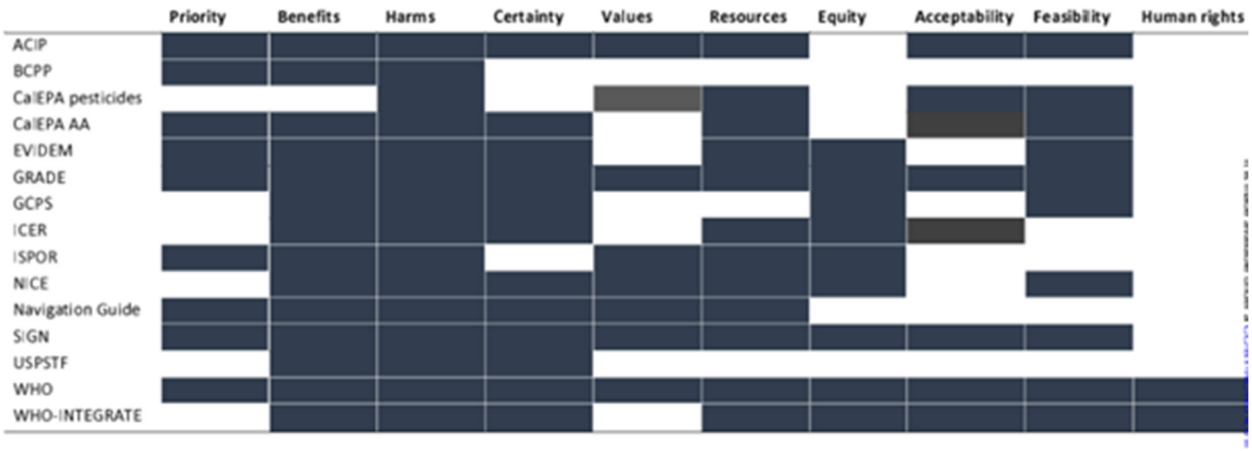
Evidence-to-Decision Frameworks Considered by the Committee

Norris and colleagues recently conducted a review of evidence-to-decision frameworks to inform the development of a new framework for recommending interventions to prevent or mitigate the harmful effects of adverse environmental exposures (Norris et al., 2021). The authors reviewed 18 frameworks and compared the criteria they used to justify decisions. Those criteria included the priority of the problem and several considerations related to benefits and harms, including desirable effects, undesirable effects, the certainty of evidence regarding desirable and undesirable effects, and the balance of effects. Other criteria included values, resources, equity, acceptability, feasibility, and human rights. Briefly, the frameworks reviewed by Norris and colleagues are summarized in Table 2-1.

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TABLE 2-1 Evidence-to-Decision Frameworks Reviewed by Norris and Colleagues (2021)



NOTES: ACP = Advisory Committee on Immunization Practices; BCPP = Breast Cancer Prevention Partners; CalEPA = California Environmental Protection Agency; CalEPA AA = California Environmental Protection Agency, Alternative Assessments; EVIDEM = Evidence and Value: Impact on DEcision-Making; GRADE = Grading of Recommendations Assessment, Development and Evaluations; GCPS = Guide to Community Preventive Services; ICER = Institute for Clinical and Economic Review; ISPOR = International Society for Pharmacoeconomics and Outcomes Research; NICE = United Kingdom National Institute for Health and Care Excellence; SIGN = Scottish Intercollegiate Guidelines Network; USPSTF = U.S. Preventive Services Task Force; WHO = World Health Organization.
SOURCE: Norris et al., 2021. Creative Commons CC BY.

GRADE (Grading of Recommendations Assessment, Development, and Evaluations)

GRADE has become the dominant evidence-to-decision framework over the past 15 years, including for clinical decision making and public health (Guyatt et al., 2008; Moberg et al., 2018; Norris et al., 2021). It includes evidence frameworks for several different decision contexts including clinical recommendations, individual perspective; clinical recommendations, population perspective; coverage decisions; and health system and public health recommendations or decisions. According to Norris and colleagues (2021), all applications of GRADE include roughly the same considerations:

- Is the problem a priority?
- How substantial are the desirable anticipated effects?
- How substantial are the undesirable anticipated effects?
- What is the overall certainty of the evidence of effects?
- Does the balance between desirable and undesirable effects favor the intervention or the comparison?
- Is there important uncertainty about, or variability in, how much people value the main outcomes?
- How large are the resource requirements (costs)?
- What is the certainty of the evidence of resource requirements (costs)?
- Does the cost-effectiveness of the intervention (the out-of-pocket cost relative to the net desirable effect) favor the intervention or the comparison?

It is important to recognize that GRADE emphasizes financial considerations, equity, acceptability, and feasibility more for health systems and public health decisions than for individual decisions (Norris et al., 2021).

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U.S. Preventive Services Task Force

The USPSTF is an independent panel of primary care, prevention, and evidence-based medicine experts. It uses an analytic framework (see Figure 2-1) to evaluate systematically the benefits and harms of a particular preventive service. In evaluating evidence concerning the benefits and harms of widespread implementation, both the evidence's certainty and the magnitude of the benefits and harms are assessed. As shown in Table 2-2, the USPSTF assigns a letter grade to each preventive service signifying its recommendation about the service's provision (Sawaya et al., 2007). It is important to note that the USPSTF does not consider cost when determining a letter grade.[2] If evidence is not available, the USPSTF considers the "chain of indirect evidence, including evidence about the accuracy of screening tests, the efficiency and harms of early treatment, and the association between changes in intermediate endpoints due to treatment and changes in health endpoints" (Mabry-Hernandez et al., 2018). As Table 2-2 indicates, when the certainty, based on the evidence, for the net benefit of screening is rated low, the USPSTF considers the evidence insufficient to make a recommendation. The USPSTF views its evaluation of the benefits and harms of a preventive service as a process, and continually revisits and reevaluates based on new advances in research, testing, and treatment capabilities (Sawaya et al., 2007).

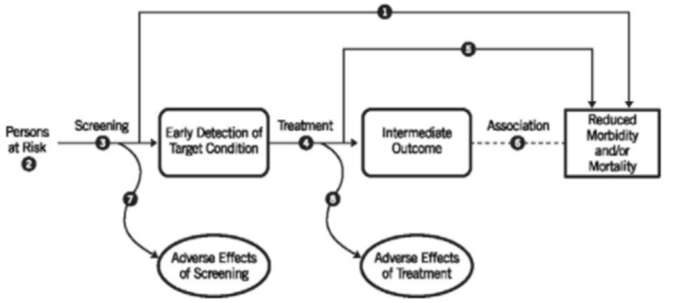


Figure 1. Generic analytic framework for a screening preventive service.

Note: Numbers correspond to the following key questions:

1. Is there evidence that randomizing a cohort of persons to screening or not screening results in greater reduced morbidity and mortality than the observed harms from screening and treatment?
2. Who are the persons at risk for the condition?
3. What are the performance characteristics of the screening test for the condition?
4. Does treatment of the screen detected condition result in improved intermediate outcomes?
5. Does treatment of the screen detected condition result in reduced morbidity and mortality?
6. Does an improvement in intermediate outcomes lead to improved health outcomes?
7. What are the harms associated with screening and diagnostic testing for the condition?
8. What are the harms associated with treatment of the condition?

FIGURE 2-1 U.S. Preventive Services Task Force's generic analytic framework for a screening preventive service. SOURCE: Mabry-Hernandez et al., 2018. CC BY-NC-ND 4.0.

[2] See https://www.uspreventiveservicestaskforce.org/uspstf/about-uspstf/task-force-resources/uspstf-and-cost-considerations (accessed September 16, 2021).

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TABLE 2-2 U.S. Preventive Services Task Force's (USPSTF's) Recommendation Grid[a]

Certainty of Net Benefit	Magnitude of Net Benefit			
	Substantial	Moderate	Small	Zero/ Negative
High	A	B	C	D
Moderate	B	B	C	D
Low	Insufficient			

[a] *A, B, C, D*, and *Insufficient* represent the letter grades of recommendation or statement of insufficient evidence assigned by the U.S. Preventive Services Task Force after assessing certainty and magnitude of the net benefit of the service.
SOURCE: Sawaya et al., 2007.

Evidence and Value: Impact on DEcision-Making (EVIDEM) Framework

Goetghebeur and colleagues (2008) developed this framework to facilitate health care decision making. They conducted an extensive review and analysis of the literature and determined that a framework for addressing the value judgments related to assessing health care interventions should be able to do the following: "1) disentangle intrinsic and extrinsic value components; 2) develop a simple and rigorous system that applies multiple criteria decision analysis (MCDA) from a pragmatic standpoint based on actual thought processes; 3) provide practical access to the evidence on which value judgments are based; and 4) provide a practical method for decision-makers to provide feedback to data producers and all other stakeholders" (Goetghebeur et al., 2008, p. 273). Based on these needs, the authors built the EVIDEM framework, tailored to provide a "comprehensive, transparent structure grounded in global standards and local needs" (Goetghebeur et al., 2008, p. 283). The framework aims to consider all perspectives, values, and rationales related to the decision at hand.

National Academies Evidence Framework for Genetic Testing

In 2017, a National Academies committee developed an evidence framework for genetic testing that calls for a clear definition of the genetic scenario being considered and a triage process for evaluating whether the purpose of a test is worthwhile and an expedited provisional decision can be made (see Figure 2-2). In addition, given the rapidly advancing nature of the field of genetic testing, the framework includes the opportunity for review and revisions (NASEM, 2017).

C-8 Medical Panel

In February 2005, the West Virginia Circuit Court approved a class action settlement agreement in a lawsuit resulting from contamination of drinking water with perfluorooctanoic acid (PFOA, also known as C-8) from DuPont's Washington Works facility in Wood County, West Virginia (see Box 1-2 in Chapter 1). The agreement included establishing a science panel that conducted epidemiologic evaluations to determine probable link conditions associated with exposure to PFOA, and a separate medical panel that defined medical monitoring for members of the class (C-8 Medical Panel, 2013). The settlement agreement stated that the medical panel needed to consider the following factors in developing the medical monitoring protocol:

1. Increased risk … that the Class Member has a significantly increased risk of contracting the particular diseases relative to the risk in the absence of exposure;
2. Necessity of Diagnostic Testing … that the Class Member should undergo specific periodic diagnostic testing that would not be required in the absence of exposure to

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C-8. The Settlement further specified that the desires of Class Members for reassurance that they did not have a probable link condition was a sufficient rationale for testing, and factors such as financial cost and the frequency of testing need not be given significant weight in assessing the need for testing; and

3. Existence of Monitoring Procedures … that testing procedures must exist that it is not necessary to show that detection and treatment in a pre-symptomatic state reduces the burden of the probable link condition. (C-8 Medical Panel, 2013, p. 1)

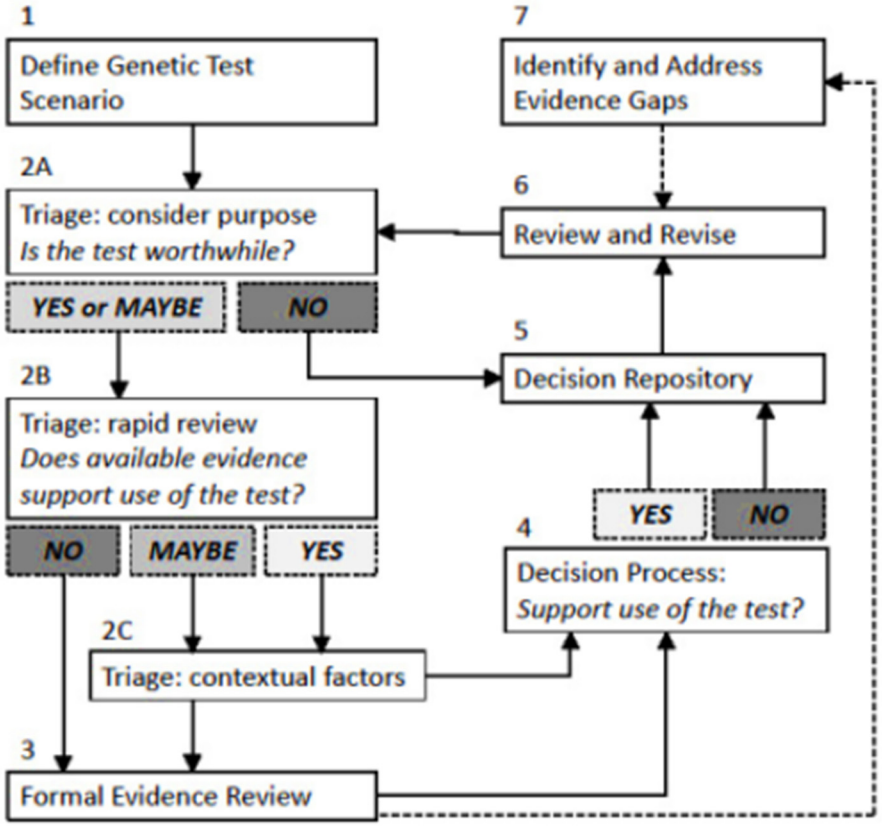


FIGURE 2-2 Visual representation of the seven components of the evidence framework for genetic testing developed by the National Academies of Sciences, Engineering, and Medicine.
SOURCE: NASEM, 2017.

Belmont Report

The Belmont Report (HHS, 1979) was developed as a set of guidelines for protecting research participants. This seminal report on ethics and health care research outlines basic ethical principles and guidelines meant to assist in resolving ethical problems surrounding the conduct of research with human subjects. The three main principles are respect for persons, beneficence, and justice.

- *Respect for persons* requires that people be considered autonomous. Autonomous individuals can consider their personal goals and make their own decisions. To respect persons is to give weight to their opinions and choices while refraining from obstructing their actions or choices unless detrimental to others. Conversely, a lack of respect for persons or autonomous agents is to refuse to accept or validate their judgments, deny them the freedom to act on their judgments, or withhold information necessary to make a judgment.

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- *Beneficence* is an obligation to respect individuals and their decisions and protect them from harm. Two general rules guide the committee's understanding of beneficence: (1) do no harm, and (2) maximize possible benefits and minimize harms. The Hippocratic Oath requires physicians to make medical recommendations and decisions that benefit their patients "according to their best judgment." The committee recognizes that zero harm or risk may not be possible, but medical decisions need to consider when the benefits outweigh the risks.
- *Justice* refers to "fairness in distribution." In the present context, it means that PFAS research should have a fair distribution such that a certain group of people is not selected for the research to the exclusion of others. An injustice occurs when some individuals benefit from the research or intervention or are denied that benefit without good, ethical reason.

Beauchamp and Childress (2001) have developed these principles further in the established textbook *Principles of Biomedical Ethics* to guide biomedical decision making beyond the context of research ethics. For example, these authors refer to respect for autonomy instead of respect for persons and divide the principle of beneficence into nonmaleficence (avoiding harm) and beneficence (providing benefits).

Precautionary Reasoning

An extensive body of scholarship in medical and environmental decision making is devoted to precautionary reasoning and the precautionary principle. This work can inform decision making under substantial scientific uncertainty. Resnik (2021) proposes four criteria for assessing the reasonableness of precautionary measures, as shown in Table 2-3.

CONCLUSION

Building on the work of other experts and evidence-to-decision frameworks, the committee developed five principles to guide decision making under uncertainty for use throughout this report: proportionality, justice, autonomy, feasibility, and adaptability. The principles of proportionality, justice, and autonomy build on the ethical principles outlined in the Belmont Report (HHS, 1979) and developed further by Beauchamp and Childress (2001). These principles affirm that decision making in response to PFAS should weigh plausible benefits and harms proportionally while treating all individuals fairly, promoting health equity, respecting human rights, and supporting informed decision making by individuals in accordance with their values. The principles of feasibility and adaptability affirm that decisions should be responsive to emerging information about harms, benefits, and other considerations.

TABLE 2-3 Criteria for Reasonableness of Precautionary Measures

Proportionality	Reasonable measures balance plausible risks and possible benefits proportionally
Fairness	Reasonable measures are based on a fair balancing of risks and benefits; fairness includes distributive and procedural fairness
Epistemic Responsibility	Reasonable measures comply with norms for the responsible acquisition and utilization of evidence, knowledge, and expertise
Consistency	Reasonable measures are based on a consistent rationale for decision making

SOURCE: Resnik, 2021.

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In the clinical setting, the committee's five principles converge under the principle of autonomy and shared decision making[3] between patient and clinician. For this reason, shared decision making is likely to be the practical way a clinician can incorporate the principles into the clinical encounter.

The committee's principles may provide a framework that ATSDR can use when writing clinical guidance for environmental exposures. Despite findings of associations between adverse health effects and chemical exposures, substantial scientific uncertainty exists about the causal role of environmental chemicals in many health outcomes. Regulatory agencies, public health agencies, and clinicians need to make thoughtful decisions about how to act in response to this uncertainty. The principles of proportionality, justice (particularly environmental justice), autonomy, feasibility, and adaptability can play a central role in these decisions.

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[3] Barry and Edgman-Levitan (2012) builds on concepts in Charles (1997) and the Institute of Medicine's (2001) *Crossing the Quality Chasm: a New Health System for the 21st Century* and describes shared decision making as the pinnacle of patient-centered care: "The process by which the optimal decision may be reached ... is called shared decision-making and involves, at minimum, a clinician and the patient.... In shared decision-making, both parties share information: the clinician offers options and describes their risks and benefits, and the patient expresses his or her preferences and values. Each participant is thus armed with a better understanding of the relevant factors and shares responsibility in the decision about how to proceed" (p. 780).

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3

Potential Health Effects of PFAS

This chapter summarizes results of the committee's review of the potential health effects of per- and polyfluoroalkyl substances (PFAS) in response to the following element of the committee's Statement of Task: "to establish a basis for prioritized clinical surveillance or monitoring of PFAS health effects." The aim of this review was to identify a set of health effects that may be associated with PFAS to support preventive medicine recommendations and decisions. Based on the review, the committee developed strength-of-evidence conclusions for the various health effects associated with PFAS.

The committee's Statement of Task limited this review to those PFAS included in the Center for Disease Control and Prevention's (CDC's) *National Report on Human Exposure to Environmental Chemicals* (see Table 3-1). The PFAS in Table 3-1 are those most commonly studied in epidemiological research, although other PFAS may also cause harm given some similarities with those in Table 3-1 with respect to biological persistence and toxicities (Kwiatkowski et al., 2020). It is important to note as well that while different PFAS have distinct physical, chemical, and toxicological properties, people are exposed to more than a single PFAS. As a result, exposures are often to mixtures of PFAS such that specific effects are difficult to disentangle. Considering these issues, and recognizing that some PFAS are less frequently measured, the committee ultimately decided to provide one strength-of-evidence determination for all PFAS for each health effect.

The Statement of Task did not limit the health effects included in the committee's review. Speakers at the committee's town halls described a variety of health effects of concern that they had observed in their communities and that may be associated with PFAS exposure. Cancers, endocrine effects, immune function, and fertility were the most frequently mentioned health effects (see Table 3-2). The committee considered this input to be valuable and paid special attention to the health effects of concern observed in communities when describing the evidence.

TABLE 3-1 PFAS Species Currently Included in the Centers for Disease Control and Prevention's (CDC's) *National Report on Human Exposure to Environmental Chemicals*

Abbreviated Name	Full Name	CAS Registry No.
MeFOSAA	Methylperfluorooctane sulfonamidoacetic acid	2355-31-9
PFHxS	Perfluorohexanesulfonic acid perfluorohexane sulfonic acid	355-46-4
n-PFOA (linear isomer), Sb-PFOA (branched isomers)	Perfluorooctanoic acid	335-67-1*
PFDA	Perfluorodecanoic acid	335-76-2
PFUnDA	Perfluoroundecanoic acid	2058-94-8
n-PFOS (linear isomer), Sm-PFOS (branched isomers)	Perfluorooctanesulfonic acid	1763-23-1*
PFNA	Perfluorononanoic acid	375-95-1

NOTES: CAS = Chemical Abstracts Service. * = CAS number refers to linear isomer only. Previous survey years have also included perfluorobutane sulfonic acid (PFBS), perfluoropentanoic acid (PFpA), perfluorododecanoic acid (PFDoDA), perfluorooctane sulfonamide (FOSA), and 2-(N-ethyl-perfluorooctane sulfonamido)acetate (EtFOSAA), according to Patrick N. Breysse's presentation to the committee on February 4, 2021.

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TABLE 3-2 Categories of Health Effects Mentioned by Speakers at the Committee's Town Halls

Health Effect Category	No. of Speakers Mentioned
Cancers, including bladder, urinary tract, liver, breast, testicular, thyroid, bone, kidney, pancreatic, and ovarian, as well as melanoma, leukemia, multiple myeloma, and lymphoma	16
Disruption of the endocrine system, including impaired thyroid and disease	10
Impaired immune function	9
Fertility and reproductive issues, including menstruation and lactation concerns	9
Diabetes, other metabolic concerns, and obesity	8
Liver disease and impairment, including nonalcoholic fatty liver disease	7
Disease and impairment of the digestive system, including ulcerative colitis, gallbladder dysfunction, irritable bowel syndrome, and other colon impairment	6
High cholesterol	6
Autoimmune disease	5
Birth defects	5
Developmental and neurological impacts, including learning disorders and autism	5
High blood pressure, gestational hypertension, and preeclampsia	5
Asthma, pulmonary disease, and cardiovascular disease	5
Premature and underweight births	4
Skin rashes, hair loss, and other skin concerns	3
Chronic inflammation and allergic reactions	3
Arthritis, osteoporosis, and other impacts to the skeletal system	2
Multiple sclerosis	1

OVERVIEW OF EVIDENCE REVIEW APPROACH

Appendix D provides full details of the methods used for the committee's review. Briefly, the committee aimed to build upon existing decisions from other authoritative bodies. The focus was on more recent studies, both high-quality systematic reviews and published epidemiologic research articles, which could inform updates to authoritative conclusions regarding PFAS exposure and any human health effects. The review approach improved efficiency while minimizing the risk of excluding scientific findings that could inform the committee's recommendations.

Consideration of Authoritative Reviews

The committee focused on authoritative reviews produced by government agencies or other bodies that publish strength-of-evidence determinations through a process that includes peer review. As detailed in Appendix D, the committee focused on the findings from the following organizations, presented in chronological order by year the literature search was completed:

- C-8 Science Panel reports (last report published 2012)
- European Food Safety Authority (EFSA) (search complete: 2013)
- Organisation for Economic Co-operation and Development (OECD) (published 2013)

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- International Agency for Research on Cancer (IARC) (search complete: 2014)
- U.S. Environmental Protection Agency (EPA) (search complete: 2015)
- National Toxicology Program (NTP) (search complete: 2016)
- Agency for Toxic Substances and Disease Registry (ATSDR) (search complete: 2018)

Among the authoritative reviews, ATSDR's *Toxicological Profile for Perfluoroalkyls* included the greatest number of PFAS that were included in the committee's Statement of Task (Methylperfluorooctane sulfonamidoacetic acid [MeFOSAA] was not included), was the most recent (literature search completed in September 2018), and was not limited in terms of health effects covered (ATSDR, 2021). Therefore, the committee used this source as the primary basis for the next stages of the review process.

The committee did not formally critique the quality of the any authoritative reviews, as each authoritative body has its own procedures for public comment and peer review to ensure that biases are limited in its reviews. Nonetheless, the committee noted several areas in which ATSDR's *Toxicological Profile for Perfluoroalkyls* could be strengthened. First, the toxicological profile does not provide a detailed description of the evidence identification methods or document its reasons for excluding specific studies. Second, the study quality assessment does not appear to follow a standard approach, and in some cases, it is difficult to identify the study designs that were included in the review. Third, the process for assessing the strength of the evidence is not well described. Despite these limitations, however, the committee concluded that the ATSDR review provided a basis for assessing evidence of association between PFAS and health effects.

Review of Systematic Reviews

As detailed in Appendix D, the committee's review of systematic reviews consisted of the following steps: a literature search, screening of abstracts, a full-text review of studies identified in the abstract screening, and evaluation of a final set of relevant studies. The literature search identified 639 potentially relevant articles, of which 26 systematic reviews met the committee's inclusion criteria and were evaluated using AMSTAR-2 (A MeaSurement Tool to Assess systematic Reviews) (Shea et al., 2017). The committee conducted a critical appraisal of the systematic reviews because such reviews can be subject to a range of biases. All high-quality systematic reviews included studies that were also reviewed in ATSDR's *Toxicological Profile for Perfluoroalkyls*. Thus, the systematic reviews were used as sources for reference in the committee's assessment, but they were not formally included as part of the final strength-of-evidence determination.

Review of Recent Epidemiologic Studies

As detailed in Appendix D, the committee's review of original literature consisted of the following steps: a literature search, screening of abstracts, a full-text review of studies identified in the abstract screening, evaluation of a final set of studies identified as relevant after the full-text review, data abstraction, and an evidence synthesis step.

The literature search identified 5,172 potentially relevant studies. After removal of duplicates (112 articles), 5,060 articles were subject to title and abstract screening by two independent reviewers. After 4,434 articles had been excluded because the titles and abstracts did not meet the committee's inclusion criteria, 626 articles were subjected to full-text review, during which additional articles were excluded if they had been published before 2018 or were listed among the references in ATSDR's *Toxicological Profile for Perfluoroalkyls* (n = 320); were cross-sectional in design (n = 160); were not published in English (n = 1); did not provide risk estimates associated with PFAS exposure (n = 3); or documented studies not conducted in humans (n = 3). Cross-sectional studies were excluded largely because this study design measures exposure and disease at the same time, and so cannot determine cause

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and effect. The remaining 139 articles were categorized according to the human health outcomes studied, as shown in Figure 3-1.

The committee's review of the recent literature focused mainly on those health effect categories for which additional evidence might have changed the committee's understanding of the association between PFAS exposure and health outcomes. The committee formally evaluated the individual studies for internal validity or "risk of bias," using a tool adapted from the Navigation Guide of Woodruff and Sutton (2014), assigning to each an overall assessment of its risk of bias (low, probably low, probably high, or high risk of bias). Effect estimates from the individual studies included in the review were extracted into a database and uploaded to a public website (Tableau Public) to allow for visualizations such as evidence maps and forest plots.[1] The effect estimates in the Tableau represent those from the model most adjusted for confounders. Appendix D provides the critical domains used by the committee to assess risk of bias, as well as the data abstraction procedure.

Evidence Synthesis

To assess the strength of evidence regarding the potential for PFAS to cause a particular health effect, the committee integrated the evidence reviewed in ATSDR's *Toxicological Profile for Perfluoroalkyls* with the evidence from its review of the original epidemiologic studies. A framework based on the Hill considerations, which help determine whether associations are causal, guided the synthesis of available evidence (Fedak et al., 2015; Hill, 1965; NASEM, 2018a). The committee considered the animal studies discussed in ATSDR's *Toxicological Profile for Perfluoroalkyls* and in the systematic reviews examined by the committee in making its determinations, as an aid to interpretation of the human studies. Toxicologic evidence, whether it supports or conflicts with evidence from epidemiologic studies, provides insights about biologic processes and informs how an observed association might be interpreted (NASEM, 2018a).

The committee's strength-of-evidence conclusions reflect one of the four categories described below (see Figure 3-2).

Sufficient Evidence of an Association

For effects in this category, a positive association between PFAS and the outcome must be observed in studies in which chance, bias, and confounding can be ruled out with reasonable confidence. For example, the committee might regard as sufficient evidence of an association evidence from several small studies that is unlikely to be due to confounding or to otherwise be biased and that shows an association that is consistent in magnitude and direction. Experimental data supporting biologic plausibility strengthen the evidence of an association but are not a prerequisite, nor are they sufficient to establish an association without corresponding epidemiologic findings.

Limited or Suggestive Evidence of an Association

In this category, the evidence must suggest an association between exposure to PFAS and the outcome in studies of humans, but the evidence can be limited by an inability to rule out chance, bias, or confounding with confidence. One high-quality study may indicate a positive association, but the results of other studies of lower quality may be inconsistent.

[1] The committee's public Tableau can be accessed at the following link: https://public.tableau.com/app/profile/nationalacademies/viz/NASEMPFASEvidenceMaps/PFASEvidenceMap. The information may be viewed as an evidence map or as a forest plot. Within forest plots, filters can be accessed using the "toggle filters" function in order to restrict the view to data on specific health effect categories and other factors (reference, chemical, study design, study population, etc.).

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	Chemical								
Health Effect Category	MeFOSAA	PFDA	PFHxS	PFNA	PFOA	PFOS	PFUnDA	sumPFAS	Grand Total
Bodyweight	4	4	10	10	13	12	2	0	13
Cancer	2	3	5	4	7	7	4	0	7
Cardiometabolic	7	9	21	20	19	20	6	3	21
Developmental	6	25	36	38	44	46	16	4	47
Endocrine	3	8	12	12	11	12	6	2	13
Hepatic	1	2	3	2	4	3	1	0	4
Immunological	0	8	13	13	14	14	10	2	14
Musculoskeletal	0	1	2	2	2	2	1	1	2
Neurological	1	5	9	10	10	11	3	2	11
Other	0	3	3	3	4	4	2	0	4
Renal	3	1	3	3	4	3	0	1	4
Reproductive	3	16	25	24	25	27	11	1	29
Respiratory	0	6	9	10	10	10	8	0	10
Grand Total	20	72	118	118	130	133	56	13	139

FIGURE 3-1 Evidence map describing the number of studies found, by PFAS, for each health outcome category.
NOTES: MeFOSAA = methylperfluorooctane sulfonamidoacetic acid; PFDA = perfluorodecanoic acid; PFHxS = perfluorohexanesulfonic acid; PFNA = perfluorononanoic acid; PFOA = perfluorooctanoic acid; PFOS = perfluorooctanesulfonic acid; PFUnDA = perfluoroundecanoic acid; sumPFAS = the additive sum of PFAS.

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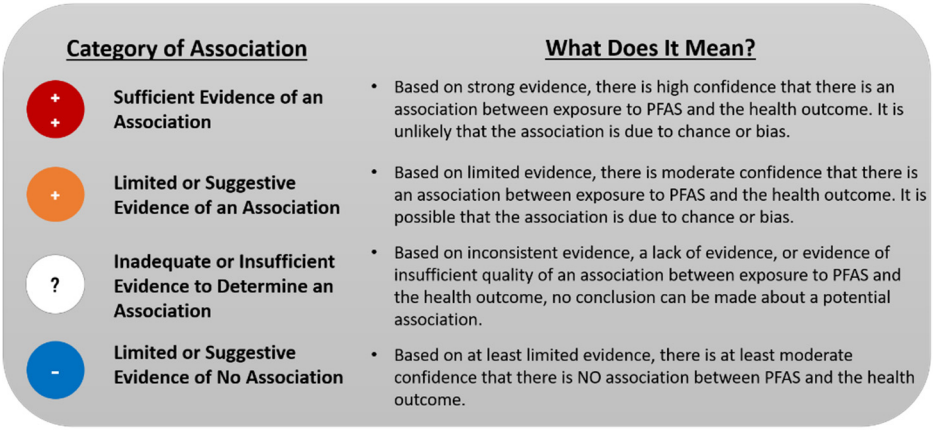


FIGURE 3-2 Categories of association used in this report.
NOTES: The categories of association only describe how strong the evidence is between PFAS and the health outcome. The risk of developing an outcome from exposure to PFAS for things in the same category can vastly differ and are dependent on whether an individual has other risk factors for developing the outcome.

Inadequate or Insufficient Evidence to Determine an Association

If there was not enough reliable scientific data to categorize the potential association with a health effect as "sufficient evidence of an association," "limited or suggestive evidence of an association," or on the other end of the spectrum, "limited or suggestive evidence of no association," the health outcome was placed in the category of "inadequate or insufficient evidence to determine an association" by default. In this category, the available human studies may have inconsistent findings or be of insufficient quality, validity, consistency, or statistical power to support a conclusion regarding the presence of an association. Such studies may have failed to control for confounding factors or may have had inadequate assessment of exposure.

Limited or Suggestive Evidence of No Association

A conclusion of "no association" is inevitably limited to the conditions, exposures, and observation periods covered by the available studies, and the possibility of a small increase in risk related to the magnitude of exposure studied can never be excluded. However, a change in classification from inadequate or insufficient evidence of an association to limited or suggestive evidence of *no* association would require new studies that corrected for the methodologic problems of previous studies and that had samples large enough to limit the possible study results attributable to chance.

COMMITTEE'S CONCLUSIONS

Annex Table 3-1 at the end of the chapter summarizes the health outcomes considered by the committee, the relevant conclusions from authoritative reviews, and the committee's overall conclusions for endpoints relevant to each outcome. The committee's conclusions reflect its integration of evidence reviewed in ATSDR's *Toxicological Profile for Perfluoroalkyls* and other authoritative reviews with the evidence garnered from the review of recent epidemiologic studies.

The committee found *sufficient* evidence of an association for the following diseases and health outcomes:

- decreased antibody response (in adults and children),
- dyslipidemia (in adults and children),

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- decreased infant and fetal growth, and
- increased risk of kidney cancer (in adults).

The committee found *limited* or *suggestive* evidence of an association for the following diseases and health outcomes:

- increased risk of breast cancer (in adults),
- liver enzyme alterations (in adults and children),
- increased risk of pregnancy-induced hypertension (gestational hypertension and preeclampsia),
- increased risk of testicular cancer (in adults),
- increased risk of thyroid disease and dysfunction (in adults), and
- increased risk of ulcerative colitis (in adults).

For a range of other health effects, the evidence was *inadequate* or *insufficient*. These include type 1 and gestational diabetes; cardiovascular disease; metabolic syndrome; obesity; infertility; male and female reproductive effects; reproductive hormone levels; and cancers other than kidney, breast, and testicular.

The committee's rationale for these conclusions is provided in the sections that follow, organized by human health outcomes. For effects with *limited* or *sufficient* evidence, the range of effect size estimates considered by the committee is indicated. Additional information, including an evidence map of recent studies, information about the quality of individual studies, and forest plots showing effect size estimates in a searchable format, can also be accessed from the committee's public Tableau.

With one exception (decreased infant and fetal growth), the committee provided forest plots for all outcomes with *sufficient* evidence of an association to display effect estimates from the studies with low or probably low risk of bias. Specifically, forest plots based on the data in the Tableau are displayed in this chapter for the following outcomes: changes in antibody response (a measure of immune function), total cholesterol (a marker of dyslipidemia), and hypertensive disorders of pregnancy. For these outcomes, the committee chose to display the effect estimate that was the most common across studies if more than one was available in the Tableau. For cancer, forest plots were created based on studies included in ATSDR's *Toxicological Profile and Polyfluoroalkyls* and the more recent epidemiologic studies. The committee handled cancer in this way because it was upgrading the previously observed association between PFAS and kidney cancer and drawing a new conclusion on breast cancer. Each cancer figure displays the PFAS with the strongest effect. For birthweight, there were many studies with probably or definitely low risk of bias, and those with definitely low risk of bias are displayed in a table rather than a forest plot. In addition to the outcomes with *sufficient* evidence of an association, one outcome with *limited* or *suggestive* evidence, hypertensive disorders of pregnancy, is displayed in a forest plot. This was an important outcome category to speakers at the committee's town halls.

SUMMARY AND RATIONALE FOR THE COMMITTEE'S CONCLUSIONS BY HUMAN HEALTH OUTCOMES

Immune System Effects

The committee's evaluation of the impact of PFAS on immune function considered evidence relevant to the three basic functions of the immune system: response to infection, response to foreign substances (allergy), and response to self (autoimmunity). The committee found *sufficient* evidence for an association of PFAS exposure with decreased antibody response to vaccination or infection, and *limited* or *suggestive* evidence of an association with ulcerative colitis (relevant to autoimmunity). There was *inadequate* evidence for other immune system endpoints, including infectious disease (response to infection) and response to allergens.

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Response to Infection

ATSDR concluded that there is suggestive evidence for an association between serum levels of perfluorooctanoic acid (PFOA), perfluorooctanesulfonic acid (PFOS), perfluorohexanesulfonic acid (PFHxS), and perfluorodecanoic acid (PFDA) and decreased antibody response to vaccines, and limited evidence of an association for perfluorononanoic acid (PFNA), perfluoroundecanoic acid (PFUnDA), and perfluorododecanoic acid (PFDoDA). Other authoritative reviews (including those of EFSA, EPA, NTP, and OECD) also found associations between PFAS and decreased antibody response to vaccines. Three more recent papers focus on antibody response early in life. Huang and colleagues (2020) conducted a study, with probably low risk of bias, which found that immunoglobulin G (IgG) levels were slightly lower among children with higher PFAS serum levels. The regression coefficient per 1-log10 nanograms per milliliter (ng /mL) increase in PFAS ranged from −0.04 (95% confidence interval [CI]: −0.11–0.04) for PFHxS to 0.00 for PFUnDA (95% CI: −0.06–0.05) (see Figure 3-3). In another study with probably low risk of bias, Timmermann and colleagues (2020) evaluated measles antibodies at three time points: before first vaccination (measuring antibodies passed from the mother) and after first and second vaccination; for most PFAS, no strong associations with antibody levels were found. For PFOS, there was an inverse association between PFAS levels in blood and antibody levels before first vaccination in boys (see Figure 3-4). In a study of bias of antibodies to hand, foot, and mouth disease (CA 16 and EV71 antibody), with probably high risk of bias, Zeng and colleagues (2019) observed that 3-month-old infants with higher PFAS cord blood levels were two to four times more likely to have levels of antibodies to hand, foot, and mouth disease that were below the clinically protective level (see Figure 3-5). This finding suggests that higher PFAS blood levels may contribute to lower antibody levels over time. Taken together, these three recent studies add support for the conclusion of *sufficient* evidence of an association between PFAS exposure and decreased antibody response.

The committee reviewed four papers focused on specific infectious diseases (chicken pox, common cold, otitis media, pneumonia, and respiratory tract infection) in children (Ait Bamai et al., 2020; Huang et al., 2020; Kvalem et al., 2020; Manzano-Salgado et al., 2019). All four studies were rated as having a probably low risk of bias; however, three of the studies used parental reports of infection to ascertain outcomes, which could result in information bias, leading in turn to null findings. The fourth study, by Huang and colleagues (2020), collected data on infections from medical records. These four studies did not provide strong evidence of an association of PFAS with these common illnesses, although there was some suggestion that for children without siblings, PFAS may be associated with respiratory syncytial virus (Ait Bamai et al., 2020). There was no evidence for fever among children with higher PFAS blood levels (Timmermann et al., 2020). The committee concluded there is *inadequate* or *insufficient* evidence of an association between PFAS exposure and risk of infection, although this is an area worthy of future research, including the relationship of PFAS to novel infections such as SARS-CoV-2 (see Box 3-1).

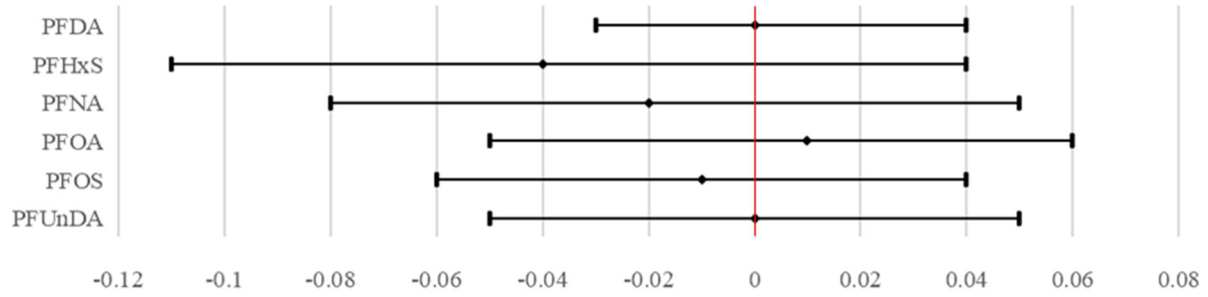


FIGURE 3-3 Regression coefficients for changes in immunoglobulin (IgG) concentrations per 1-log10 nanograms per milliliter (ng /mL) increase in PFAS serum level.
DATA SOURCE: Huang et al., 2020.

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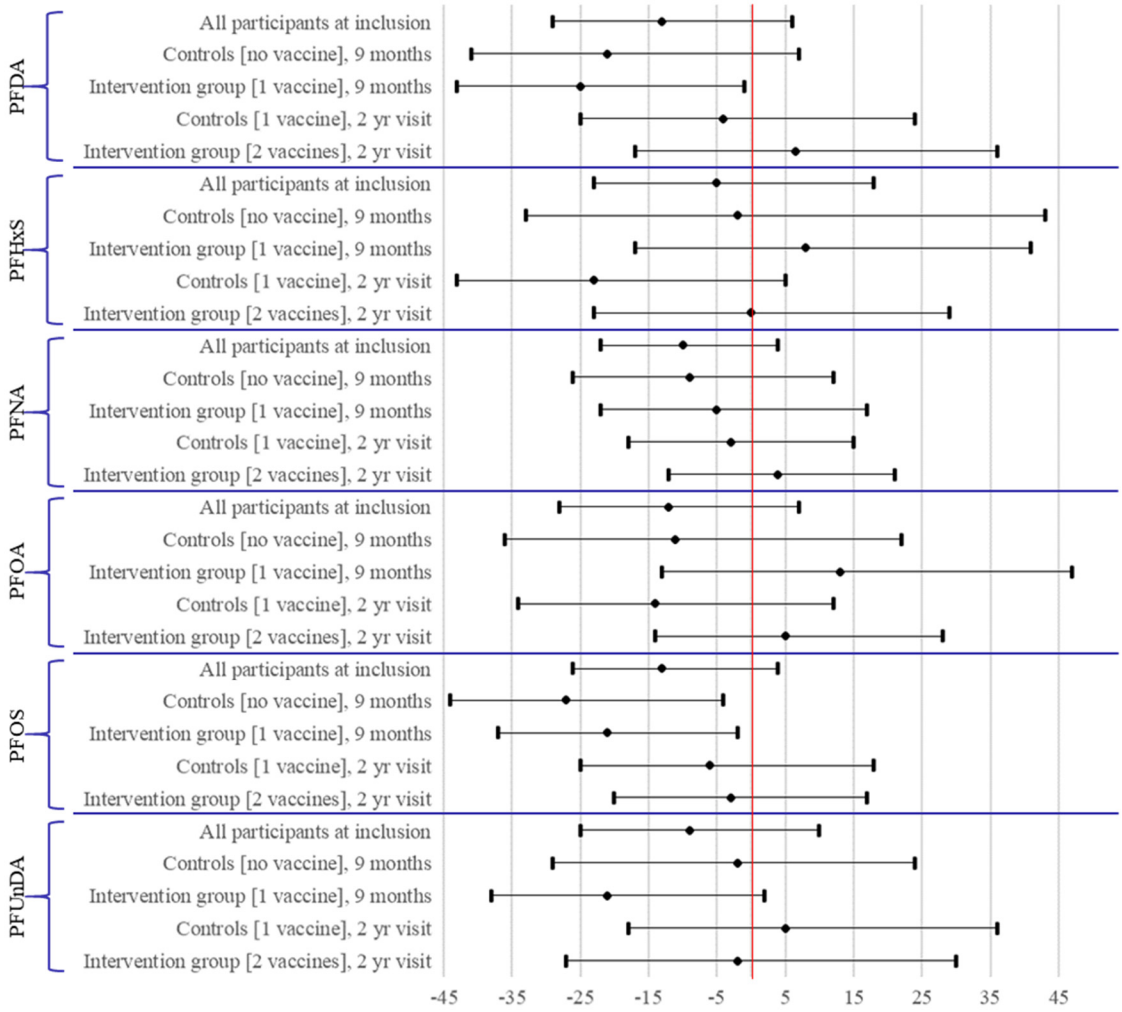


FIGURE 3-4 Regression coefficients for percent difference in measles antibody response per doubling of log10 nanograms per milliliter (ng /mL) serum PFOS.
DATA SOURCE: Timmerman et al., 2020.

Response to Foreign Substances (Allergy)

The committee reviewed several studies evaluating the impact of PFAS exposure on allergic symptoms and disease, all with a probably low risk of bias. The specific outcomes studied included allergies to food and inhaled substances, atopic dermatitis, dermatitis, changes in serum immunoglobulin E (IgE) levels, rhinitis, rhinoconjunctivitis, and results of skin prick tests. Six studies (Ait Bamai et al., 2020; Chen et al., 2018; Huang et al., 2020; Impinen et al., 2019; Kvalem et al., 2020; Wen et al., 2019) evaluated some aspect of allergic response, with most not providing strong evidence of an association with PFAS. The one exception is the study by Wen and colleagues (2019), which showed increased atopic dermatitis among those in the highest tertile of PFOA levels, but not for other PFAS. One study included in ATSDR's *Toxicological Profile for Perfluoroalkyls* found that PFAS exposure was associated with increased odds of asthma diagnosis among children at ages 5 and 13, but only those children who had not received measles, mumps, and rubella vaccination (Timmerman et al., 2020). Overall, the evidence for an association between PFAS and allergy response is *inadequate* or *insufficient*, a finding consistent with the authoritative review by EFSA.

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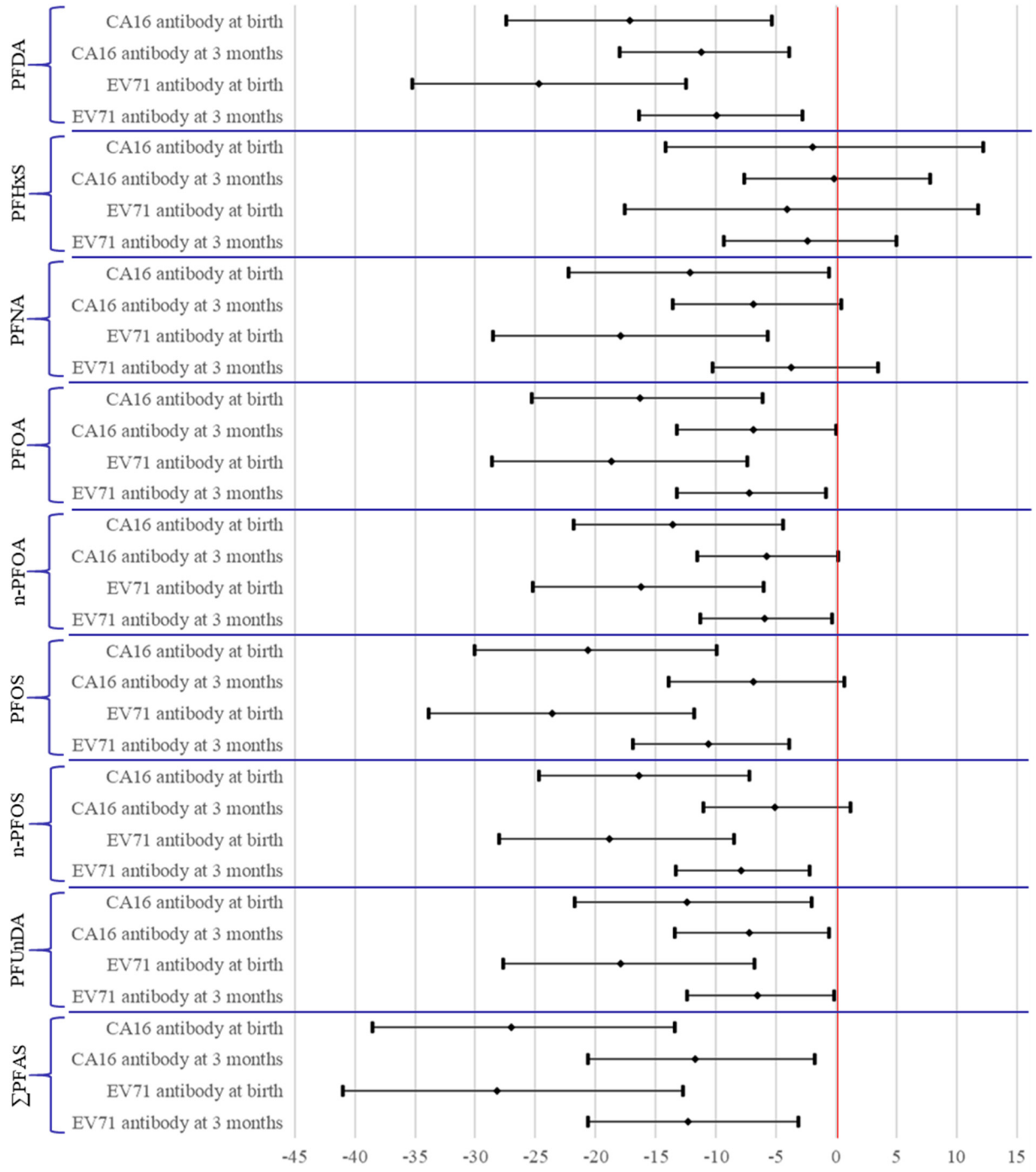


FIGURE 3-5 Regression coefficients for percent change in hand, foot, and mouth disease antibody response per doubling of natural logarithm (ln)-nanograms per milliliter (ng /mL) sum of PFAS.
DATA SOURCE: Zeng et al., 2019.

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BOX 3-1
PFAS Exposure and Risk of SARS-CoV-2 Infection

SARS-CoV-2 is a novel virus with low immunity worldwide prior to the pandemic, which provides an opportunity to evaluate the impact of PFAS exposure on response to infection.

What types of studies have been conducted? Available studies evaluate SARS-CoV-2 infection, severity, and mortality.

What have the results shown?

- *Studies on SARS-CoV-2 infection*. The results of two studies show a slightly increased risk of SARS-CoV-2 infection associated with PFAS exposure, but the designs of both preclude drawing causal conclusions. One study, conducted in China, used a case-control format to compare PFAS levels in urine in cases of SARS-CoV-2 infection versus noninfected controls. PFOS, PFOA, and total PFAS levels in urine were higher in those with infection than those without. The observed odds ratios, adjusted for age, sex, body mass index (BMI), comorbidities, and urine albumin-to-creatinine ratio, were 1.94 (95% confidence interval [CI]: 1.39–2.96) for PFOS, 2.73 (95% CI: 1.71–4.55) for PFOA, and 2.82 (95% CI: 1.97–3.51) for the sum of 12 PFAS (Ji et al., 2021). Another study, of ecologic design, calculated sex- and age-standardized incidence ratios (SIRs) for SARS-CoV-2 infection among adults in the Ronneby, Sweden, area, where drinking water was highly contaminated with PFAS, and in a neighboring reference town with similar demographic characteristics but background levels of PFAS exposure (Nielsen and Jöud, 2021). The authors found a slight, but significant, elevated risk of SARS-CoV-2 infection in the former group (SIR = 1.19; 95% CI: 1.12–1.27).
- *SARS-CoV-2 severity*. The results of one study indicate that PFAS may influence the severity of COVID-19 disease, but the study design does not allow for causal determinations. Researchers in Denmark analyzed plasma samples from 323 people with COVID-19 (Grandjean et al., 2021). The authors observed that perfluorobutanoic acid (PFBA) showed an unadjusted odds ratio of 2.19 (95% CI; 1.39–3.46) for increasing the severity of the disease.
- *Studies on SARS-CoV-2 mortality*. One study showed a slight but not statistically significant increased risk of mortality following SARS-CoV-2 infection among individuals exposed to high levels of PFAS contamination; however, its design does not allow for causal determinations. Investigators compared SARS-CoV-2 infection in a community in Venetto, Italy, where drinking water was highly contaminated with PFAS, and in a similar Italian community without contaminated water, using a Bayesian ecological regression model adjusted for education level and baseline all-cause mortality. They found that the rate ratio for COVID-19 mortality for the area of high exposure versus the area of low exposure was 1.60 (90% CI: 0.94–2.51) (Catelan et al., 2021).

Conclusion: Taken as a whole, the extant research does not allow conclusions to be drawn on whether PFAS exposures may influence COVID-19 infection, severity, and mortality. Given that most of the available studies have been ecological, more evidence is necessary to address this question.

Response to Self (Autoimmunity)

As noted in authoritative reviews by the EPA and OECD, the C-8 Science Panel identified an association between PFAS and ulcerative colitis, a rare autoimmune condition of the gastrointestinal (GI) tract. In a follow-up to that study, Steenland and colleagues (2018) further evaluated the exposure–response relationship between PFAS and ulcerative colitis, examining Crohn's disease as well. They found that ulcerative colitis was positively associated with PFOA but not with other PFAS. The odds ratio for ulcerative colitis per 1 unit of log PFOA was 1.60 (95% CI: 1.14–2.24), but the trend by quintiles was not monotonic (1, 0.84, 40.98, 33.36, 2.86) (Steenland et al., 2018). The only other analysis of the association of PFAS with ulcerative colitis is a 2022 case-control study from the Nurses' Health Study using blood specimens collected between 1989 and 1999 (Lochead et al., 2022), identified after the committee had completed its review. This study did not find an association between ulcerative

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colitis and any PFAS measured; the median concentration of PFOA among the 80 cases was 3.97 ng/mL, higher than the median value of 2.93 ng/mL for the 114 ulcerative colitis cases in the Steenland study. Given the low incidence of ulcerative colitis, it will be difficult to replicate the findings from either of these studies in other populations. The Nurses' Health Study also observed a statistically significant decreased risk of Crohn's disease with PFAS; the Steenland study found no association with Crohn's disease. To assess the relationship between PFAS and inflammatory bowel disease, Xu and colleagues (2020b) measured two proteins (calprotectin and Zonulin) in feces and saw no association with PFAS.

Given the difficulty of evaluating these diseases, future studies are needed to characterize the impact of PFAS on autoimmunity. Although there is more recent inconsistent evidence, it is not strong enough to override the previous conclusion from the C-8 study. Overall, the committee concluded that there is *limited* or *suggestive* evidence of an association of PFAS with ulcerative colitis. The committee did not review studies that considered other autoimmune endpoints.

Cardiometabolic Outcomes

The committee's evaluation of the impact of PFAS on cardiometabolic outcomes considered evidence relevant to four disorders of the three basic functions of the cardiometabolic system: dyslipidemia, high blood pressure or hypertension, metabolic syndrome, and elevated body mass index (BMI) or obesity. The committee did not identify any studies that evaluated the association between PFAS and cardiovascular disease, a group of disorders involving the heart and blood vessels (coronary [ischemic] heart disease, cerebrovascular disease [stroke], peripheral arterial disease, rheumatic heart disease, congenital heart disease, and deep vein thrombosis and pulmonary embolism).[2] The committee concluded that there is *sufficient* evidence of an association of PFAS exposure with dyslipidemia in adults and children. This conclusion builds on those of the authoritative reviews considered by the committee, all of which (including those of ATSDR, EFSA, EPA, OECD, and the C-8 Science Panel) found associations between PFAS and dyslipidemia. The committee concluded that the evidence is *insufficient* for other findings related to cardiovascular risk factors and nonpregnancy clinical cardiovascular diseases. This conclusion is consistent with those of the authoritative reviews, which concluded that there was mixed to limited evidence supporting associations between PFAS and cardiovascular risk factors and diseases other than the four discussed above because of inconsistencies in measurement, differences in study designs and populations, and differences in adjustment for potential confounding factors.

Dyslipidemia

The studies identified by the committee that evaluated dyslipidemia used several types of study designs, including cohort and nested case-control approaches, and the studies encompassed both children and adults. Some of the major challenges to interpretation of their findings were that outcome definitions were inconsistent across studies (total triglycerides, total cholesterol, and low-density lipoprotein [LDL], and high-density lipoprotein [HDL], sometimes measured in a variety of different units). In addition, the study populations included were very broad with respect to age range, sex, and race or ethnicity representation, making it difficult to interpret and generalize the results across studies. In addition, the timing of exposure to PFAS was often unclear, particularly in the studies of adults, in which confounding could be an issue. The study designs and sample sizes varied; there were six cohort studies and one nested case-control study, most of which were rated as having probably low risk of bias. Figures 3-6 and 3-7 display effect estimates from those studies of low or probably low risk of bias that evaluated the impact of PFAS exposure on total cholesterol (the most consistent effect measured across

[2] See https://www.heart.org/en/health-topics/consumer-healthcare/what-is-cardiovascular-disease (accessed June 28, 2022).

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studies) in adults (Donat-Vargas et al., 2019b; Lin et al., 2019; Tian et al., 2021) and children (Mora et al., 2018). The effects documented across studies were heterogenous, possibly because of the timing of the exposures and outcome measurements. Overall, the committee concluded that there is *sufficient* evidence of an association between PFAS and dyslipidemia, as the recent epidemiologic literature provides additional confidence in the conclusions of authoritative reviews regarding this association.

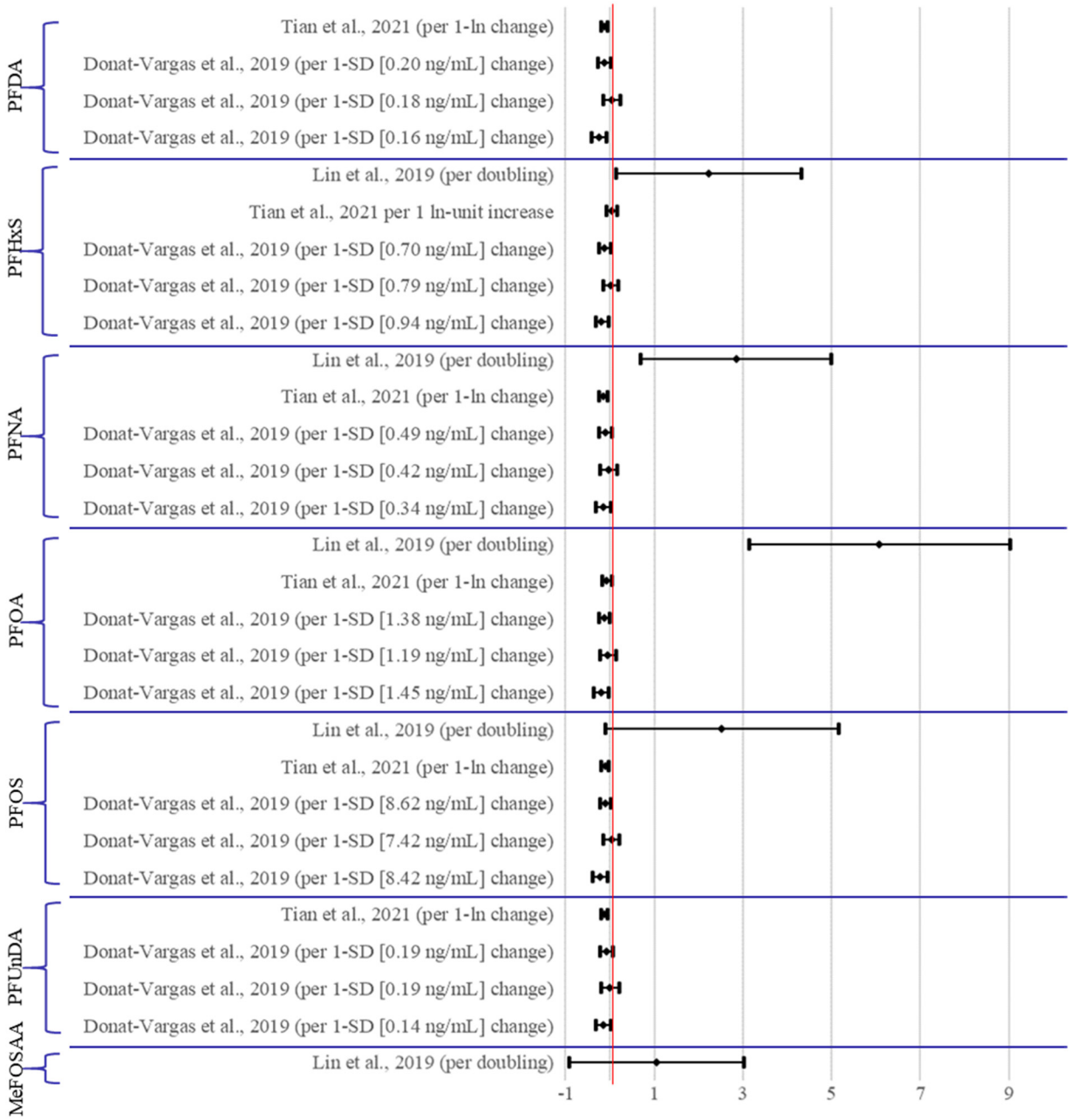


FIGURE 3-6 Regression coefficients for changes in total cholesterol in adults.
DATA SOURCES: Donat-Vargas et al., 2019b; Lin et al., 2019; Tian et al., 2021.

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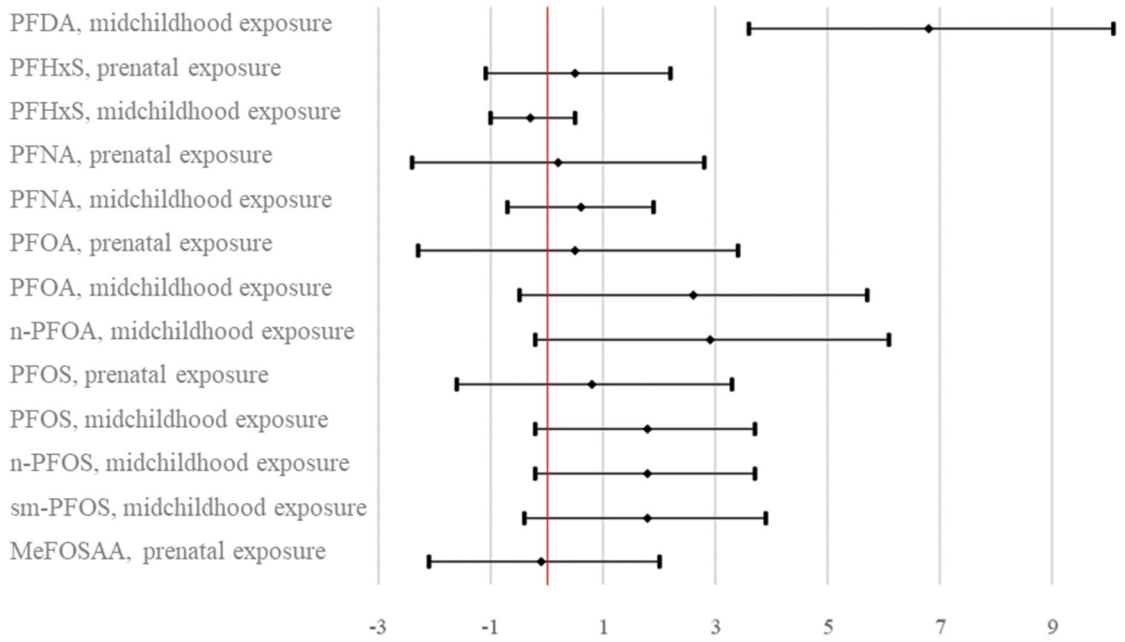


FIGURE 3-7 Regression coefficients for total cholesterol per interquartile range (IQR) increase in PFAS exposure in children.
DATA SOURCE: Mora et al., 2018.

High Blood Pressure or Hypertension

The authoritative reviews do not identify an association between PFAS and high blood pressure or hypertension. The committee identified four studies rated as having probably low or definitely low risk of bias that evaluated the impact of PFAS exposure on hypertension, blood pressure, systolic blood pressure, and diastolic blood pressure (Donat-Vargas et al., 2019b; Lin et al., 2020b; Mitro et al., 2020a). The populations and designs varied greatly across these studies: Donat-Vargus and colleagues (2019b) was a nested case-control study of middle-aged women and men; Lin and colleagues (2020b) was a randomized, controlled clinical trial conducted at 27 clinical centers around the United States from 1996 to 2001; and Mitro and colleagues (2020a) was a cohort study among postpartum females. Donat-Vargus and colleagues (2019b observed that the effect estimates for the impact of exposure to PFAS and hypertension were inconsistent. Lin and colleagues (2020b) observed modest and mostly null associations of plasma PFAS concentrations with high blood pressure and hypertension. Mitro and colleagues (2020a) observed higher systolic blood pressure (e.g., 1.2 mm Hg [95% CI: 0.3, 2.2] per doubling of PFOS) at 3 years postpartum. Given the inconsistency of the evidence, the committee concluded that the evidence is *inadequate* or *insufficient* to determine an association between PFAS and high blood pressure or hypertension.

Metabolic Syndrome

The authoritative reviews do not identify an association between PFAS and metabolic syndrome. Metabolic syndrome is a group of risk factors that increases the risk of heart disease and stroke. Diagnosis requires that an individual have three of the following risk factors: (1) a large waist circumference (males: >102 cm, females: >88 cm); (2) high triglyceride levels (≥1.7 mmol/L); (3) low HDL cholesterol (males <1.04 mmol/L, females <1.30 mmol/L); (4) high blood pressure (≥130 over ≥85 mm Hg); and (5) high fasting glucose levels (≥6.1 mmol/L) (Beilby, 2004). The committee did not identify any new

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epidemiologic studies reporting associations between exposure to PFAS and a diagnosis of metabolic syndrome. The committee concluded that the evidence is *inadequate* or *insufficient* to determine such an association, although the relationship is plausible given the association between PFAS and dyslipidemia.

Elevated Body Mass Index or Obesity

The authoritative reviews do not identify an association between PFAS and elevated BMI or obesity. Epidemiologic studies have assessed associations between exposure to PFAS and anthropometric outcomes because some PFAS may activate peroxisome proliferator-activated receptor (PPAR) gamma, which promotes adipogenesis (Liu et al., 2018; Takacs and Abbott, 2007). Sex differences in obesity, coupled with differences in exposures within certain subpopulations, may place certain groups at increased risk of overweight and obesity (Fenton et al., 2021; Mitro et al., 2020b; Starling et al., 2019). In addition, exposure during certain periods of growth and development may have short- and long-term consequences for overweight and obesity among children and adolescents, with later-life health consequences (Araújo and Ramos, 2017; Fenton et al., 2021; Gross et al., 2020; Hruby and Hu, 2015; Yeung et al., 2019). Co-exposures to psychosocial factors and other chemicals that also promote fat cell development may work synergistically with PFAS chemicals to impact fat growth and development, with impacts on body weight and growth measures (Araújo and Ramos, 2017; Braun et al., 2021; Chen et al., 2019; Fenton et al., 2021; Jensen et al., 2020a; Liu et al., 2018, 2020; Mitro et al., 2020b; Rahman et al., 2019; Romano et al., 2021; Shoaff et al., 2018; Starling et al., 2019, 2020).

The committee identified several studies that evaluated the impact of exposure to PFAS on obesity, four being rated as having a definitely low risk of bias (Braun et al., 2021; Chen et al., 2019; Mitro et al., 2020a,b; Shoaff et al., 2018). These four studies varied greatly in the ages of the populations assessed. Two of the studies used data from the Health Outcomes and Measures of the Environment (HOME) study cohort (Braun et al., 2021; Shoaff et al., 2018), and one was a study of children in Shanghai, China (Chen et al., 2019). The fourth investigated the impact of PFAS exposure on adiposity among postpartum women (Mitro et al., 2020b). The study by Braun and colleagues (2020) (which largely updates Shoaff et al., 2018)—assessed exposure to PFAS (PFOA, PFOS, and PFHxS) at 16 weeks' gestation and delivery, and measured weight and length or height and calculated child BMI at several time points across the life course (4 weeks to 12 years). The authors observed some suggestive evidence of an impact on BMI trajectory (age × PFOA interaction p value = 0.03) for PFOA, but not for PFOS and PFHxS. The study in Shanghai, China, was a prospective birth cohort study that measured 10 PFAS in cord blood plasma and assessed child adiposity measures at 5 years of age. The authors observed no association for the PFAS considered in this review, but did observe that, among girls, perfluorobutane sulfonic acid (PFBS) exposure had a significant positive association with waist circumference and waist-to-height ratio (p values <0.05). Mitro and colleagues (2020b) observed that PFOS and PFOA were associated with greater adiposity at 3 years' postpartum. The heterogeneity of the effects found across studies is the reason the committee concluded that the evidence for an association between PFAS exposure and elevated BMI or obesity is *inadequate* or *insufficient* in adults and children, although this is an area worthy of future study.

Developmental Outcomes

The committee's evaluation of the impact of PFAS on developmental outcomes considered evidence relevant to fetal growth, development of genitalia, and neurodevelopment. The committee concluded that there is *sufficient* evidence of an association between PFAS exposure and reductions in birthweight. This conclusion builds on those of the authoritative reviews (including ATSDR, EFSA, and OECD). ATSDR concluded that the evidence is suggestive of association between serum PFOA and PFOS and small decreases in birthweight. The committee concluded that there is *insufficient* evidence of an association between PFAS exposure and either development of the external genitalia or neurodevelopmental outcomes.

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Birthweight

The committee identified numerous studies with low or probably low risk of bias that examined the relationship between exposure to PFAS and birthweight (Buck Louis et al., 2018; Chu et al., 2020; Gao et al., 2019; Kashino et al., 2020; Marks et al., 2019; Wikstrom et al., 2020; Workman et al., 2019). The magnitude and precision of the estimates of the impacts of PFAS exposure on birthweight varied across and within studies, but the direction of the effect was consistent. None of the studies found a statistically significant effect of PFAS exposure on an increase in birthweight. Table 3-3 presents the estimated impact of PFAS on birthweight from the two studies reviewed by the committee that were rated as having low risk of bias. Overall, these studies strengthened conclusions and supported the committee's assessment that there is *sufficient* evidence of an association of PFAS exposure with small reductions in birthweight.

TABLE 3-3 Effect Estimates Change in Birthweight Per Change in PFAS, from Studies Rated as Having Low Risk of Bias[3]

PFAS	Short Citation	Population	Estimated Change in Birthweight (g) (95% CI)	Units
PFOA	Buck Louis et al., 2018	infants	−5.9 (−28.75 to 16.94)[a]	standard deviation (SD) increase in log-PFOA
	Chu et al., 2020	infants	−73.64 (−126.39 to − 20.88)[b]	1 natural logarithm (ln) change in PFOA
		female infants	−56.04 (−129.32 to 17.24)[c]	1 ln change in PFOA
		male infants	−71.8 (−148.61 to 5)[c]	1 ln change in PFOA
PFOS	Chu et al., 2020	infants	−83.28 (−133.2 to − 33.36)[b]	1 ln change in PFOS
		female infants	−71.91 (−143.86 to 0.05)[c]	1 ln change in PFOS
		male infants	−71.52 (−142.44 to − 0.61)[c]	1 ln change in PFOS

[a] Adjusted for maternal age, education, prepregnant body mass index, serum cotinine, infant sex, and a chemical–maternal race/ethnicity interaction term.
[b] Adjusted for gestational age, maternal age, maternal occupation, maternal education, family income, parity, and infant sex.
[c] Adjusted for gestational age, maternal age, maternal occupation, maternal education, family income, and parity.

Development of Genitalia

Authoritative reviews have yet to associate PFAS exposure with the development of genitalia, a possible indicator of reproductive disorders (Bonde et al., 2016). The committee identified one study that evaluated PFAS exposure and hypospadias and cryptorchidism, potential manifestations of testicular dysgenesis syndrome at birth (Anand-Ivell et al., 2018). Two studies (Arbuckle et al., 2020; Tian et al., 2019) evaluated PFAS and measures of anogenital distance (distance from the anus to the penis or scrotum in males or to the clitoris in females).

Anand-Ivell and colleagues (2018) conducted a case-control study within a large national biobank of amniotic fluid samples, which was rated as having a probably high risk of bias due to the high potential for confounding. The study found no influence of PFOS on cryptorchid or hypospadias (comparison of mean PFOS in amniotic fluid: control versus cryptorchid versus hypospadias). Arbuckle and colleagues (2020) conducted a cohort study with probably low risk of bias and reported inconsistent findings regarding PFAS and anogenital distance. Although the authors observed an association between PFOA (measured in first-trimester maternal plasma) and increased anoscrotal distance (adjusted for active smoking status during pregnancy and gestational age), when they examined the data by quartiles, they found no consistent patterns of association, and the effect estimates were imprecise with wide confidence intervals. Tian and colleagues (2019) conducted a cohort study with probably low risk of

[3] This table was altered after release of the pre-publication version of the report to correct data entry errors.

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bias to evaluate the impacts of a PFAS on anogenital distance measures. They observed that maternal plasma concentrations (ln-transformed) of PFOS, PFDA, and PFUnDA were inversely associated with anoscrotal distance and anopenile distance measures at birth. For anoscrotal distance, they found per ln unit increase in PFAS concentrations −0.65 (−1.27 to −0.02) mm for PFOS; −0.58 (−1.11 to −0.06) mm for PFDA; and −0.57 (−1.09 to −0.06) mm for PFUnDA. For anopenile distance, they found per ln unit increase in PFAS concentrations −0.63 (−1.24 to −0.01) mm for PFDA and −0.76 (−1.36 to −0.16) mm for PFUnDA. The committee determined that, taken together, the evidence is *inadequate* or *insufficient* to determine an association between PFAS exposure and the development of external genitalia, largely because effects were inconsistent across studies.

Neurodevelopmental Effects

The committee divided the literature on neurodevelopmental effects of PFAS into studies of learning and behavior and of autism spectrum disorder.

Learning and behavior The committee identified 12 studies with low or possibly low risk of bias that examined learning and behavior using psychometrically valid tools to evaluate the impact of PFAS on neurodevelopment in children. The ages of the children varied greatly across studies, as did the timing of the exposure measurement used in the analysis, making it difficult to generalize the findings. For example, Hoyer and colleagues (2018) observed weak effects on child behavior of prenatal exposure to some PFAS. In analysis that combined results from birth cohorts in Greenland and Ukraine, the odds ratio (OR) for hyperactivity was 1.8 (CI: 1.0–3.2) for one nanoliter-unit (nL-unit) increase in prenatal PFNA and 1.7 (CI: 1.0–3.1) for one nL-unit increase in prenatal PFDA exposure. Using the Ages and Stages Questionnaire, Niu and colleagues (2019) found that prenatal plasma concentrations of most PFAS, including PFHxS, PFOS, PFOA, PFNA, and PFDA tended to be associated with an increased risk of developmental problems in personal/social skills, and the associations for PFNA and PFDA were significant (per nL-unit increase). The committee concluded that the evidence is *inadequate* or *insufficient* to determine an association of PFAS exposure with neurodevelopmental effects, largely because of the heterogeneity of both the effects measured and the results observed.

Autism spectrum disorder The committee identified four studies with low or probably low risk of bias evaluating the impacts of PFAS exposure on autism spectrum disorder (Long et al., 2019; Lyall et al., 2018; Oh et al., 2021; Shin et al., 2020). Lyall and colleagues (2018) conducted a population-based, nested case-control study of children born from 2000 to 2003 in southern California and did not observe an association between exposure to PFAS and autism. Long and colleagues (2019) conducted a case-control study that compared exposure to PFAS in amniotic fluid between cases and controls, and observed a negative association between PFAS in amniotic fluid and autism spectrum disorder diagnosis (OR: 0.410; 95% CI: 0.174–0.967). Shin and colleagues (2020) conducted a case-control study of autism spectrum disorder and observed that PFHxS and PFOS were borderline associated with increased odds of child diagnosis of autism spectrum disorder (per ng/mL increase: OR = 1.46; 95% CI: 0.98–2.18 for PFHxS, OR = 1.03; 95% CI: 0.99–1.08 for PFOS). Oh and colleagues (2021) conducted an analysis of the impacts of PFAS exposure on the risk of developing autism spectrum disorder and found that increased PFOA exposures were associated with negative trend Early Learning Composite scores and all four subscales. When they compared trajectories of the scores between low- and high-scoring groups, PFOA was associated with having lower or decreasing Early Learning Composite scores (risk ratio [RR] = 1.49; 95% CI: 1.09–2.03). The committee determined that the evidence is *inadequate* or *insufficient* to determine an association between exposure to PFAS and neurodevelopment, largely because effects were inconsistent across studies.

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Cancers

Authoritative reviews have considered the carcinogenic potential of PFAS, and IARC has classified PFOA as possibly carcinogenic to humans (Benbrahim-Tallaa et al., 2014). The committee concluded that there is *sufficient* evidence for an association between PFAS and kidney cancer. This conclusion builds on those of the authoritative reviews (C-8 Science Panel, EPA, ATSDR), which concluded that the evidence for an association between PFAS and cancer in humans is limited, and takes into account robust findings from more recent epidemiological studies. The committee concluded that there is *limited* or *suggestive* evidence for cancers of the testis and breast. The conclusion on testicular cancer is consistent with the authoritative reviews, and the finding on breast cancer is based on the more recent epidemiological studies considered by the committee. The committee found that the existing body of literature on other cancers constituted *inadequate* or *insufficient* evidence to determine an association with PFAS.

Kidney Cancer

The committee's assessment that there is *sufficient* evidence of an association between PFAS exposure and kidney cancer was motivated in large part by the study with low risk of bias conducted by Shearer and colleagues (2021). These investigators conducted a nested case-control study within the Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial with a large sample size, appropriate controls, and validated endpoints (renal cell carcinoma diagnosis [C64.9 in the *International Classification of Diseases for Oncology*, Second Edition]). The statistical analyses conducted by the authors were robust and adjusted for relevant confounders, and sensitivity analysis was performed to assess whether effects were still observed regardless of kidney function. The study clearly showed that ORs for kidney cancer were significantly elevated among individuals in the highest PFOA exposure category, with a strong exposure-response trend. This study and earlier studies (Barry et al., 2013; Vieira et al., 2013) demonstrating a consistency in the direction and magnitude of this effect among those with the highest exposure form a body of literature that the committee concluded constitutes *sufficient* evidence of an association. Effect estimates for associations between PFOA exposure and kidney cancer are summarized by study in Figure 3-8.

Testicular Cancer

The committee's conclusion of *limited* or *suggestive* evidence of an association between PFAS exposure and testicular cancer is consistent with the conclusions of the authoritative reviews. ATSDR does not draw a clear conclusion with respect to PFAS and testicular cancer, but the C-8 Science Panel identified a probable link with incident testicular cancer based on evidence from studies by Barry and colleagues (2013) and Vieira and colleagues (2013). These two studies were also included in the EPA review (2016), which noted the positive associations they found with PFOA and their overlap in cases. IARC (2016) stated that the evidence for an association with testicular cancer was credible and unlikely to be explained by bias and confounding, but limited by small sample numbers. EFSA (2020) found that there was insufficient support for the carcinogenicity of PFOA and PFOS in humans. Given a lack of new studies on this association, the committee found the existing evidence to be supportive of a conclusion of *limited* or *suggestive* evidence of an association between PFAS exposure and testicular cancer (see Figure 3-9).

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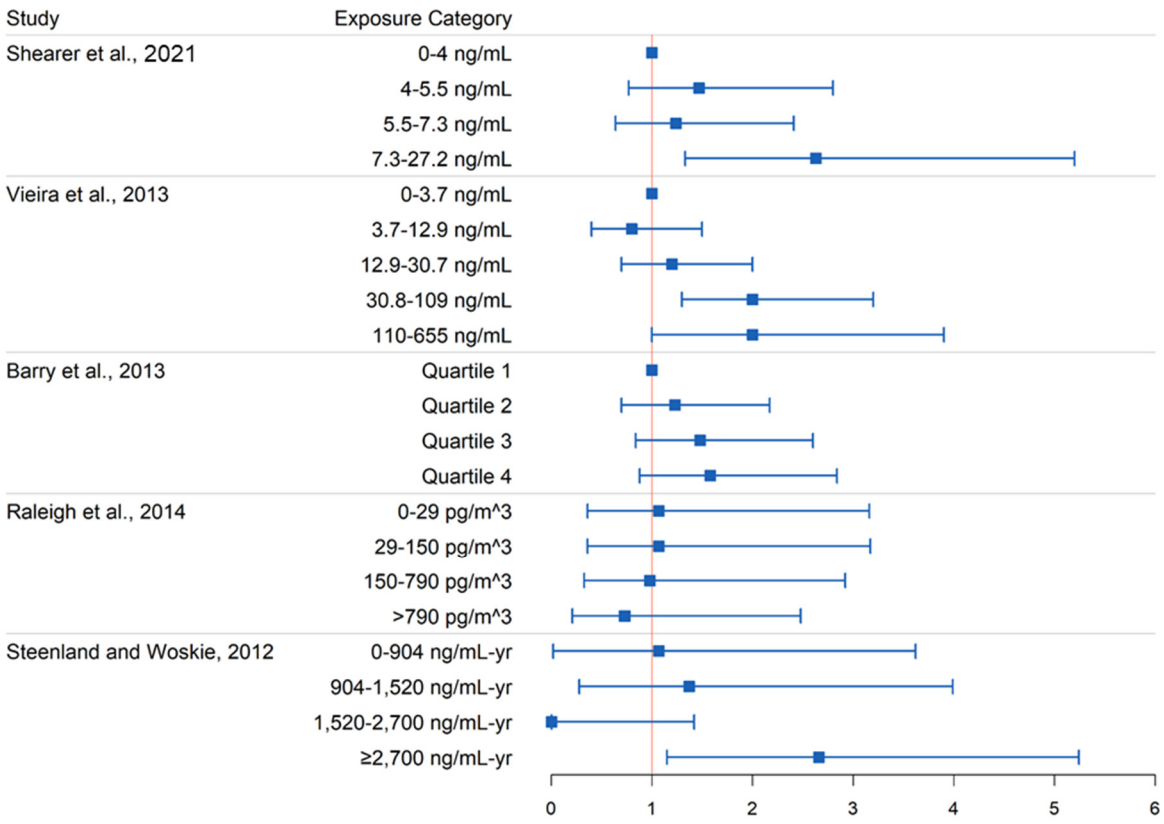


FIGURE 3-8 Kidney cancer adjusted rate ratios and 95% confidence intervals by study and PFOA exposure category.
DATA SOURCES: Barry et al., 2013; Raleigh et al., 2014; Shearer et al., 2021; Steenland and Woskie, 2012; Vieira et al., 2013.

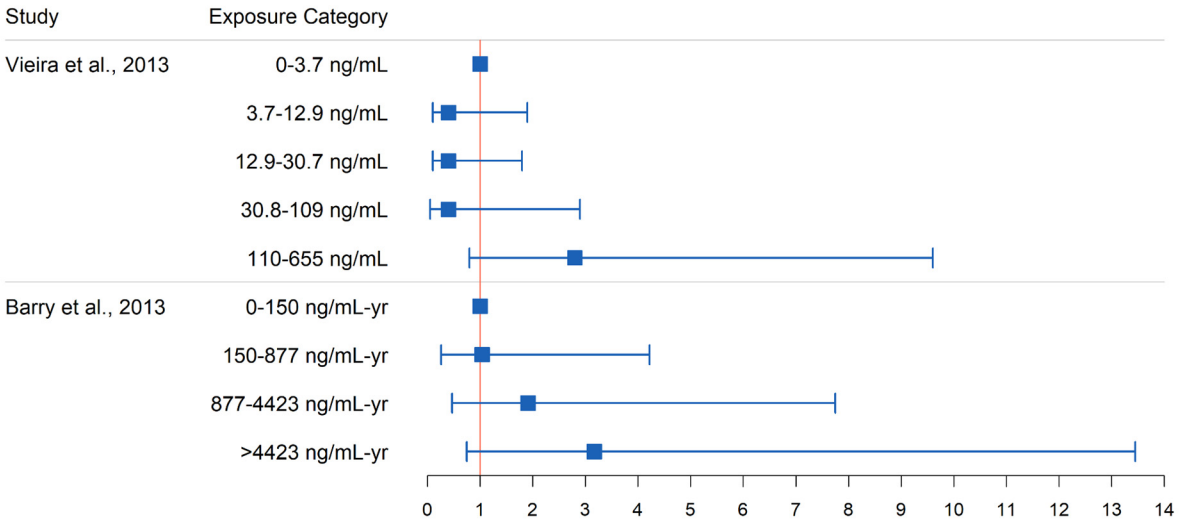


FIGURE 3-9 Testicular cancer adjusted rate ratios and 95% confidence intervals by study and PFOA exposure category.
DATA SOURCES: Barry et al., 2013; Vieira et al., 2013.

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Breast Cancer

ATSDR did not draw a clear conclusion with respect to PFAS and breast cancer. Studies included in the ATSDR review found inconsistent associations, with Bonefeld-Jørgensen and colleagues (2014) finding an inverse association with PFHxS and null association for PFOS and PFNA, while Wielsøe and colleagues (2017) found positive associations with all these chemicals. Wielsøe and colleagues (2017) also found positive associations between PFDA, PFUnDA, and PFHpA (not statistically significant), and no association for PFDoDA. Bonefeld-Jorgensen and colleagues (2014) found a positive association with FOSA. IARC (2016) also reviewed the null study of Bonefeld-Jorgensen and colleagues (2014), as did EPA (2016), which stated that no associations were found in the general community. Recent studies, however, found more associations suggestive of a relationship between PFAS and breast cancer. A nested case-control study with low risk of bias found an association between estrogen receptor–positive breast cancer and PFOS (Mancini et al., 2020). An additional study with probably low risk of bias found no evidence of associations between PFAS and breast cancer (Cohn et al., 2020). Two additional recent studies had high risk of bias because the exposure was measured after the cancer diagnosis had been made (Hurley et al., 2018; Tsai et al., 2020). Hurley and colleagues (2018) found no evidence of associations between PFAS exposure and breast cancer, whereas Tsai and colleagues (2020) found evidence of associations among women aged ≤50. Those associations were stronger among women with estrogen receptor–positive tumors. Figure 3-10 summarizes the effect estimates for associations between PFOS exposure and breast cancer by study. The committee found that this body of literature constitutes *limited* or *suggestive* evidence of an association of PFAS exposure with breast cancer.

Reproductive Outcomes

In its review of reproductive outcomes, the committee considered evidence on hypertensive disorders of pregnancy, female reproductive effects, reproductive hormone levels, infertility and subfecundity, and gestational diabetes. The committee concluded that there is *limited* or *suggestive* evidence of an association between PFAS exposure and hypertensive disorders of pregnancy, preeclampsia, and gestational hypertension without preeclampsia. This conclusion is consistent with that of the C-8 Science Panel and subsequent authoritative reviews by ATSDR, EPA, and OECD. Consistent with ATSDR, the committee concluded that there is *insufficient* evidence of an association between PFAS exposure and other reproductive outcomes.

Hypertensive Disorders of Pregnancy (Gestational Hypertension and Preeclampsia)

The committee identified five recent studies examining PFAS exposure and preeclampsia, all having probably low risk of bias (see Figures 3-11–3-15). The four cohort studies (Birukov et al., 2021; Borghese et al., 2020; Huang et al., 2019; Wikstrom et al., 2019) supported the conclusions from the authoritative reviews. Wikstrom and colleagues (2019) observed modestly elevated risk for preeclampsia, but not necessarily a consistent exposure-response trend, and Borghese and colleagues (2020) observed no association with gestational hypertension without preeclampsia. Birukov and colleagues (2021) evaluated exposure to PFAS in early pregnancy and maternal blood pressure trajectories in pregnancy, gestational hypertension, and preeclampsia. No clear associations were observed with gestational hypertension (de novo blood pressure >140/90 mm Hg after 20 weeks' gestation on two or more episodes with at least 4 h in between or significant aggravation of preexisting hypertension) or preeclampsia (gestational hypertension with proteinuria [>0.3 g/24 h or at least +1 on sterile urine dipstick]). Birukov and colleagues (2021) did observe modest but not statistically significant increases in blood pressure for PFOS and PFOA. Another cohort study measuring PFAS in cord blood found an association with preeclampsia but not gestational hypertension (Huang et al., 2019).

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A case-control study of preeclampsia and PFAS measured in maternal serum categorized PFAS into quartiles; the women in the highest quartiles had no significant increased risks of developing preeclampsia compared with the women in the lowest quartile in adjusted analyses (Rylander et al., 2020). Given that the studies showed a tendency toward an association between PFAS and hypertensive disorders of pregnancy, the committee concluded that there is *limited* or *suggestive* evidence of an association.

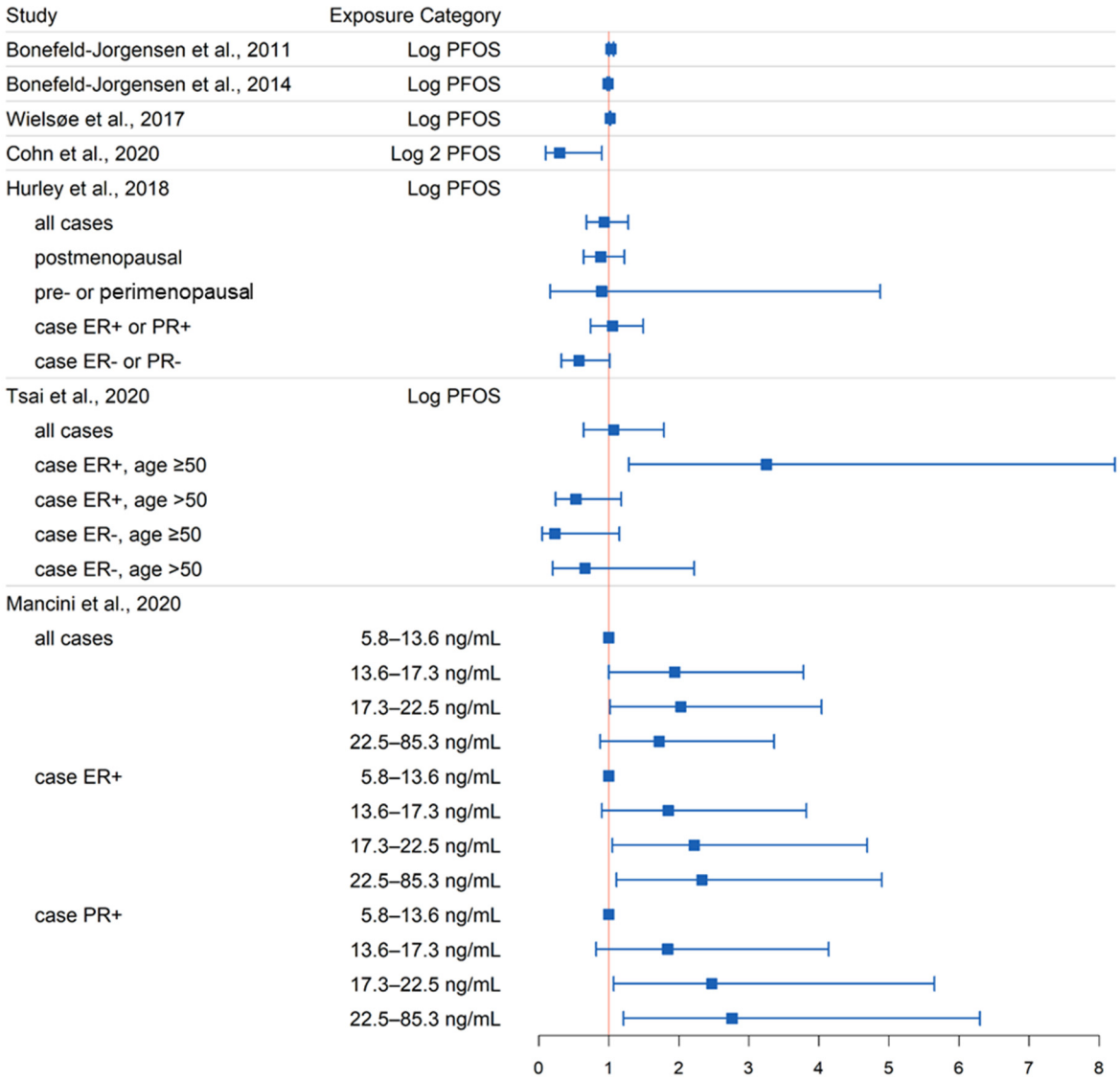


FIGURE 3-10 Breast cancer adjusted rate ratios and 95% confidence intervals by study and PFOS exposure category.
NOTE: ER = estrogen receptor; PR = progesterone receptor.
DATA SOURCES: Bonefeld-Jorgensen et al., 2011; Cohn et al., 2020; Hurley et al., 2018; Mancini et al., 2020; Tsai et al., 2020; Wielsøe et al., 2017.

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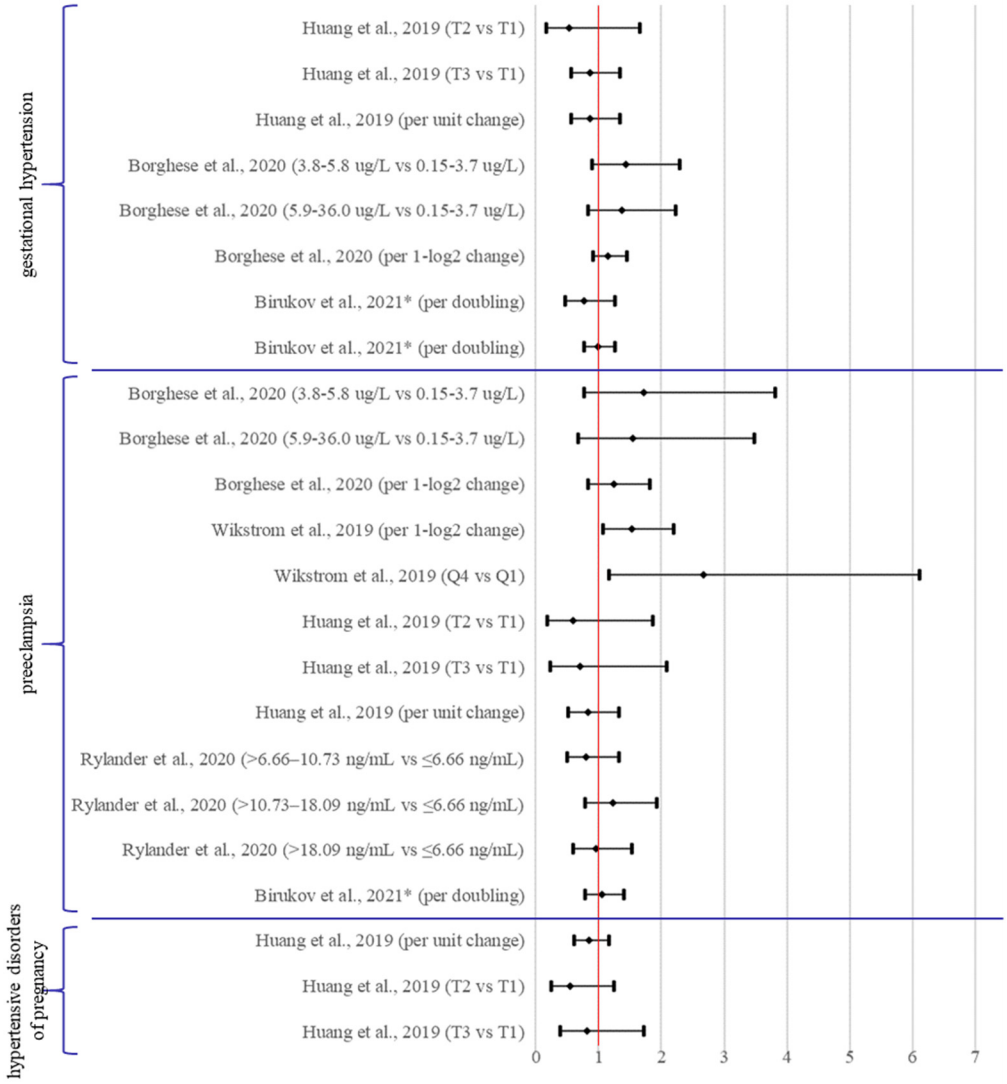


FIGURE 3-11 Adjusted risk estimates for preeclampsia, gestational hypertension (hypertension without preeclampsia), and hypertensive disorders of pregnancy and 95% confidence intervals by study and PFOS exposure category.
NOTE: All studies present odds ratios except Birukov et al., 2021, which presents a hazard ratio.
DATA SOURCES: Birukov et al., 2021; Borghese et al., 2020; Huang et al., 2019; Rylander et al., 2020; Wikstrom et al., 2019.

Fertility and Fecundity

A conclusion in ATSDR's *Toxicological Profile for Perfluoroalkyls* is that "epidemiological studies provided mixed evidence of impaired fertility (increased risks of longer time to pregnancy and infertility) for PFOA, PFOS, PFHxS PFNA, PFHpA, and PFBS: the results are not consistent across studies or were only based on a single study. The small number of studies evaluating fertility for PFDA, PFUnDA, PFDoDA, and FOSA did not find associations and no study has evaluated reproductive outcomes and PFBA" (ATSDR, 2021, p. 359). The committee identified a few more recent studies to update that authoritative review.

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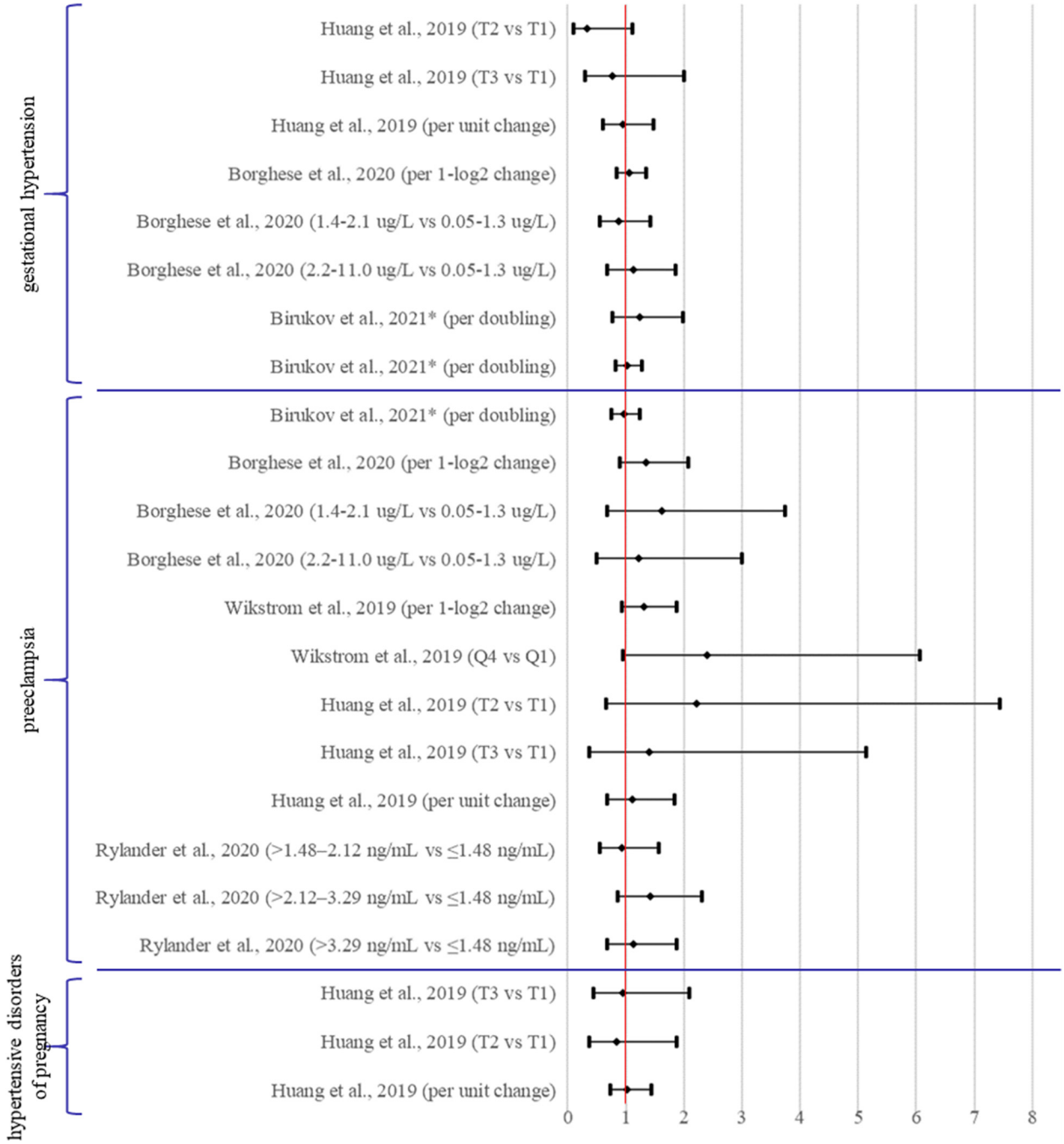


FIGURE 3-12 Adjusted risk estimates for preeclampsia, gestational hypertension (hypertension without preeclampsia), and hypertensive disorders of pregnancy and 95% confidence intervals by study and PFOA exposure category.
NOTE: All studies present odds ratios except Birukov et al., 2021, which presents a hazard ratio.
DATA SOURCES: Birukov et al., 2021; Borghese et al., 2020; Huang et al., 2019; Rylander et al., 2020; Wikstrom et al., 2019.

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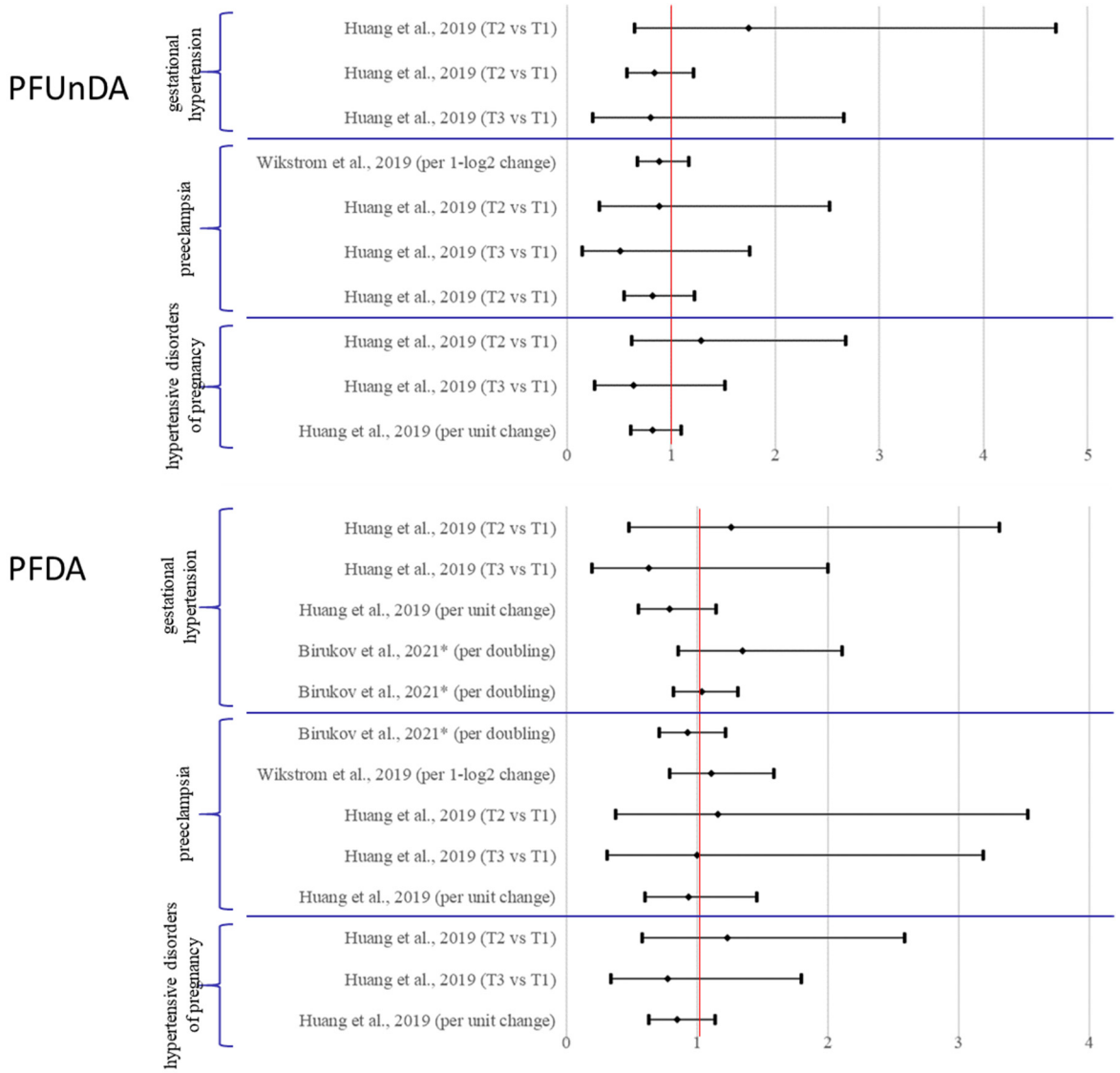


FIGURE 3-13 Adjusted risk estimates for preeclampsia, gestational hypertension (hypertension without preeclampsia), and hypertensive disorders of pregnancy and 95% confidence intervals by study and PFuDA and PFDA exposure category.
NOTE: All studies present odds ratios except Birukov et al., 2021, which presents a hazard ratio.
DATA SOURCES: Birukov et al., 2021; Borghese et al., 2020; Huang et al., 2019; Rylander et al., 2020; Wikstrom et al., 2019.

Zhang and colleagues (2018) conducted a case-control study to evaluate the impact of PFAS on risks of premature ovarian insufficiency, and observed positive associations with PFOA, PFOS, and PFHxS (highest versus lowest tertile, PFOA: OR, 3.80; 95% CI: 1.92–7.49; PFOS: OR, 2.81; 95% CI: 1.46–5.41; PFHxS: OR, 6.63; 95% CI: 3.22–13.65). The study was rated as having high risk of bias for a potential for reverse causality (Zhang et al., 2018). Ma and colleagues (2021) conducted a small cohort study in a fertility clinic in Zhejiang, China, that evaluated the association between PFAS exposure and fertility measures (numbers of retrieved oocytes, mature oocytes, two-pronuclei (2 PN) zygotes, good-quality embryos, and semen parameters). The authors found that maternal plasma concentrations of PFOA were negatively associated with the numbers of retrieved oocytes (p-trend 0.023), mature oocytes

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(p-trend 0.015), 2 PN zygotes (p-trend 0.014), and good-quality embryos (p-trend 0.012). Higher paternal plasma PFOA concentrations were found to be significantly associated with reduced numbers of 2 PN zygotes (p-trend 0.047), but no associations were found between maternal or paternal PFAS levels and the probability of implantation, clinical pregnancy, or live birth. Given the mixed evidence, the committee concluded that there is *insufficient* evidence of an association between PFAS exposure and fertility or fecundity.

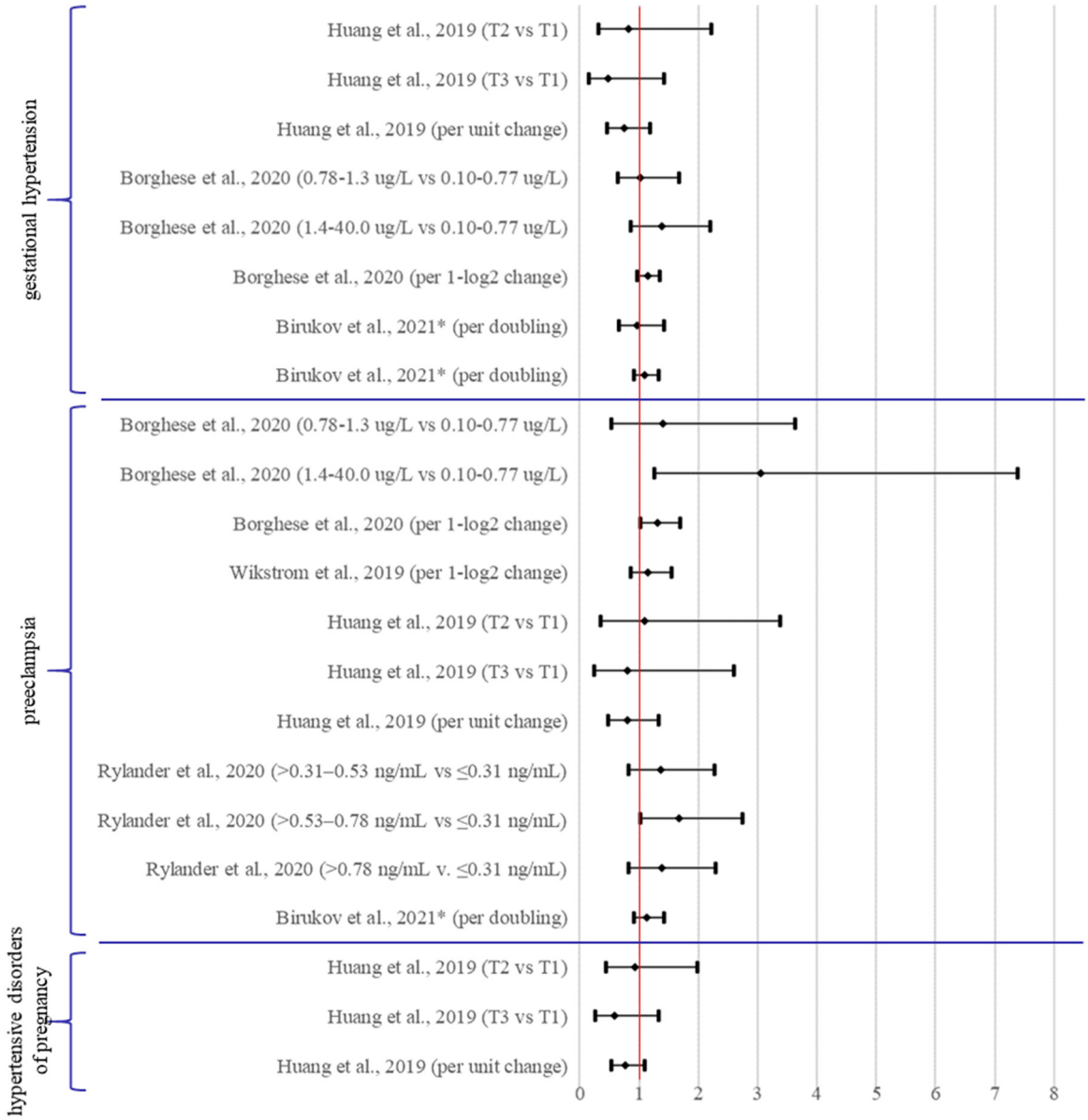


FIGURE 3-14 Adjusted risk estimates for preeclampsia, gestational hypertension (hypertension without preeclampsia), and hypertensive disorders of pregnancy and 95% confidence intervals by study and PFHxS exposure category.
NOTE: All studies present odds ratios except Birukov et al., 2021, which presents a hazard ratio.
DATA SOURCES: Birukov et al., 2021; Borghese et al., 2020; Huang et al., 2019; Rylander et al., 2020; Wikstrom et al., 2019.

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Male Reproductive Effects

For its authoritative review, ATSDR looked at articles examining the relationship between PFAS and sperm quality and concluded that while some associations with serum perfluoroalkyl levels were observed for some markers of sperm quality, the markers measured were not consistent across studies. The committee identified only one new study evaluating the effect on PFAS on male reproduction (Ma et al., 2021). This study found no significant association between PFAS and sperm progressive motility rate, but did find associations of some PFAS with decreased sperm concentration. Given that this study has some potential for bias, the committee concluded that the evidence is *inadequate* or *insufficient* to determine an association.

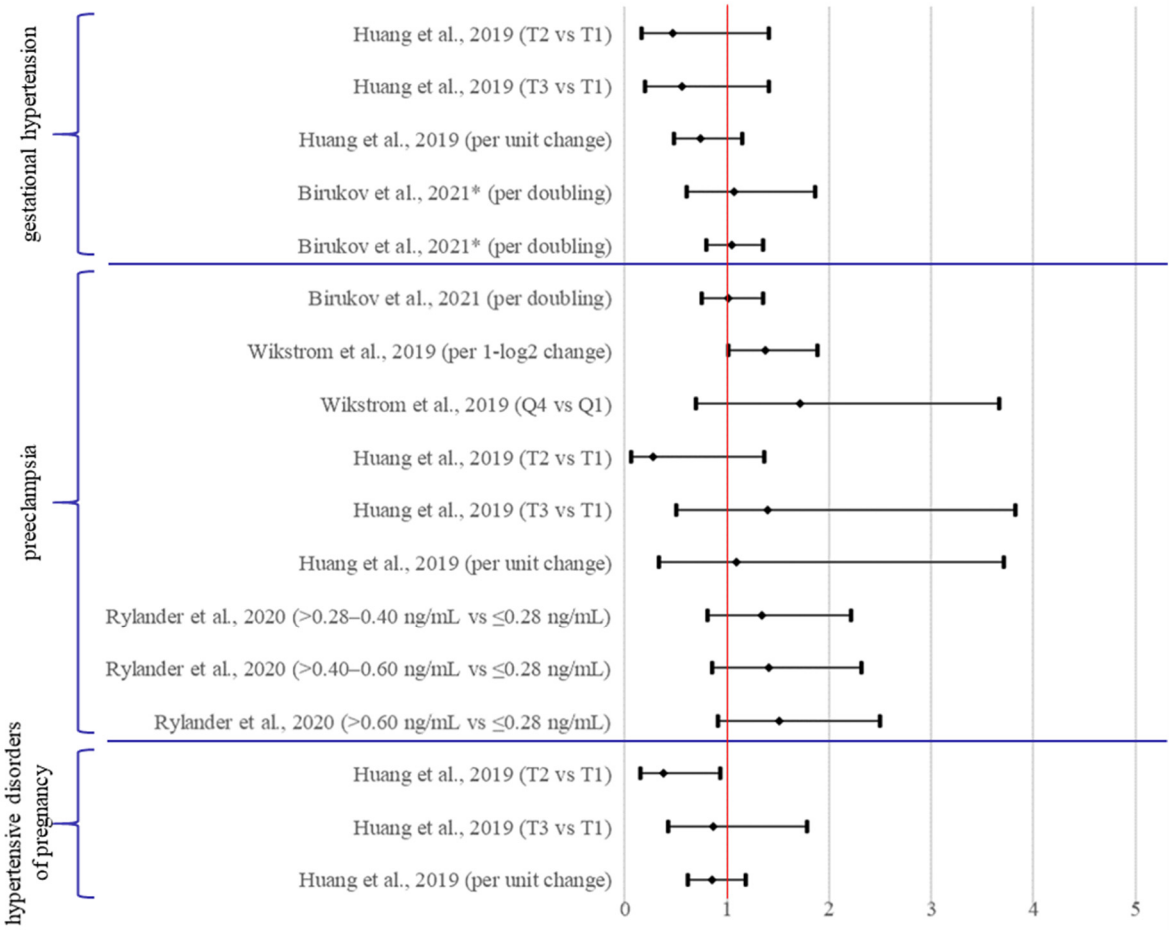


FIGURE 3-15 Adjusted risk estimates for preeclampsia, gestational hypertension (hypertension without preeclampsia), and hypertensive disorders of pregnancy and 95% confidence intervals by study and PFNA exposure category.
NOTE: All studies present odds ratios except Birukov et al., 2021, which presents a hazard ratio.
DATA SOURCES: Birukov et al., 2021; Borghese et al., 2020; Huang et al., 2019; Rylander et al., 2020; Wikstrom et al., 2019.

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Female Reproductive Effects

Female reproductive effects discussed here include menopause, age at menarche, and duration of breastfeeding. The authoritative reviews did not find associations for other female reproductive outcomes (polycystic ovary syndrome, endometriosis), and the committee identified no new studies of these effects.

In its *Toxicological Profile for Perfluoroalkyls*, ATSDR concludes that there is some suggestive evidence of an association between serum PFAS levels and an increased risk of early menopause; however, this finding may be due to reverse causation since an earlier onset of menopause would result in a decrease in the removal of perfluoroalkyls in menstrual blood. One more recent cohort study (Ding et al., 2020), with low risk of bias, found an association of PFAS with earlier onset of menopause. Mixed results were observed in a study of age at menarche (Ernst et al., 2019) and a study of cycle irregularity (Singer et al., 2018) (rated as probably having and having low risk of bias, respectively).

ATDSR's *Toxicological Profile* does not offer conclusions on the impact of PFAS exposure on duration of breastfeeding. The committee identified one more recent study, with probably low risk of bias, that observed mostly null associations but also a decreased hazard of breastfeeding cessation by 3 and 6 months with increasing maternal serum concentrations of PFNA, PFDA, and PFUnDA during pregnancy (Rosen et al., 2018). The committee concluded that there is *insufficient* human evidence of an association of PFAS with female reproductive effects, including breastfeeding duration.

Reproductive Hormone Levels

ATSDR's *Toxicology Profile* reviews the literature on associations between PFAS concentrations and reproductive hormones. The conclusion of this review is that while some studies examining reproductive hormone levels have observed associations with PFAS, the findings are inconsistent across studies, and there are too few studies to enable interpretation of the results.

The committee found several more recent studies evaluating the relationship between PFAS and reproductive hormone levels, but these studies varied in the populations they included and the hormones measured, among other factors, making it difficult to synthesize the evidence. For example, three studies evaluated the impact of PFAS on estradiol levels (Ma et al., 2021; Yao et al., 2019; Zhang et al., 2018). The study by Ma and colleagues (2021) was conducted among couples visiting a fertility clinic in China; the study by Zhang and colleagues (2018) was a case-control study of adult women in China; and Yao and colleagues (2019) analyzed PFAS and hormone levels in infant cord blood. The studies that measured testosterone were also distinct; two were analyses of data from birth cohorts (Jensen et al., 2020b; Nian et al., 2020); two were studies in pregnant women (Anand-Ivell et al., 2018; Yao et al., 2019); and two were studies in Chinese adults (Ma et al., 2021; Zhang et al., 2018). The committee concluded that the evidence is too heterogeneous to support drawing conclusions and therefore *inadequate* or *insufficient* to determine an association.

Gestational Diabetes

ATSDR's *Toxicology Profile* reviews the evidence for an association between PFAS and gestational diabetes and concludes that the results of the studies reviewed do not suggest an association. The committee identified four more recent studies evaluating the impact of PFAS on gestational diabetes. These studies had varying designs, including case-control, cohort, and nested case-control (Preston et al., 2020a; Rahman et al., 2019; Wang et al., 2018; Xu et al., 2020a) and had somewhat inconsistent results. Xu and colleagues (2020a) and Rahman and colleagues (2019) observed an effect on gestational diabetes, whereas Preston and colleagues (2020a) and Wang and colleagues (2018) observed an effect on glucose homeostasis. Given the inconsistent effects reported, the committee concluded the evidence is *inadequate* or *insufficient* to determine an association.

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Endocrine Outcomes

The committee's evaluation of the impact of PFAS on endocrine outcomes considered evidence in the following subcategories: thyroid disease, hyper- and hypothyroidism and thyroid hormones, and diabetes. The committee found *limited* or *suggestive* evidence of an association with thyroid hormones and disease, and *inadequate* evidence for diabetes.

Thyroid Hormones and Disease

The authoritative review of the C-8 Science Panel and subsequent authoritative reviews completed by ATSDR, EPA, and OECD found associations between PFAS exposure and thyroid hormones and disease. Among the more recent cohort studies with probably low or low risk of bias, the majority observed weak to no association with thyroid hormone levels or subclinical hypothyroidism in children and adults (Blake et al., 2018; Itoh et al., 2019; Jansen et al., 2020; Kim et al., 2020; Lebeaux et al., 2020; Liang et al., 2020; Preston et al., 2020b; Reardon et al., 2019; Xiao et al., 2020). Timing of exposure, life stage, and dietary factors all are likely to modify the relationship between PFAS and thyroid hormones, which could account for the weak observations observed in recent studies. For example, in a cohort study with low risk of bias that measured maternal and cord sera for PFAS and thyroid hormones, Lebeaux and colleagues (2020) found that individual PFAS or mixtures of PFAS were generally not associated with any thyroid hormones, although they did observe a slight association between PFAS and cord serum thyroid-stimulating hormone, with PFOS being the major contributor of the mixture (Bayesian kernel machine regression model estimate per doubling of PFOS [$\beta = 0.09$; 95% credible interval: −0.08–0.27]). They also observed some indication of effect measure modification by maternal thyroid peroxidase antibody status for the associations of PFAS with cord free thyroxine. The committee concluded that there is *limited* or *suggestive* evidence of an association between PFAS and thyroid hormones and thyroid disease.

Diabetes

Authoritative reviews have not found evidence of an association between PFAS and diabetes. ATSDR concluded that, while one prospective cohort study (Sun et al., 2018) suggested an association of PFOA and PFOS with risk of diabetes, overall the epidemiological studies did not provide support for an association between serum PFAS levels and increased risk of diabetes or related outcomes (e.g., increases in blood glucose, glucose tolerance). The committee identified three more recent studies with a probably low risk of bias examining type 2 diabetes. Their results were mixed, with elevated associations observed by Charles and colleagues (2020), an inverse association by Donat-Vargas and colleagues (2019a), and weak or null associations observed by Cardenas and colleagues (2019). One study identified after the committee had completed its literature review (Valvi et al., 2021) observed a modest association with decreased insulin sensitivity and increased pancreatic beta cell function in a cohort study of young adults, but associations with type 2 diabetes were not examined. No new studies examined type 1 diabetes. Given the mixed effects observed, the committee concluded that evidence is *inadequate* or *insufficient* to determine an association between PFAS exposure and diabetes.

Hepatic Outcomes

Authoritative reviews, including those by ATSDR, EFSA, and EPA, have consistently found associations between PFAS and liver effects. ATSDR noted that decreases in serum bilirubin were observed in studies of PFOA, PFOS, and PFHxS, suggestive of liver alterations. The committee identified four more recent studies on PFAS and liver effects with probably low or definitely low risk of bias, including a prebirth cohort study in Boston, a cohort study in Sweden, a study of pooled data from longitudinal birth cohorts across Europe, and a study based on a liver registry in Atlanta (Jin et al., 2020;

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Mora et al., 2018; Salihovic et al., 2018; Stratakis et al., 2020). All studies observed some association between PFAS and the liver, but the effects observed were slightly heterogeneous. Mora and colleagues (2018) observed an inverse association between PFAS exposure and alanine transaminase in the prenatal period and in childhood. Salihovic and colleagues (2018) observed that changes in levels of many measured PFAS were positively associated with alanine transaminase and alkaline phosphatase levels and negatively associated with bilirubin. Stratakis and colleagues (2020) observed that higher prenatal exposure to a PFAS mixture was associated with increased risk of liver injury during childhood, as indicated by enzyme levels exceeding the 90th percentile for the study population. And Jin and colleagues (2020) observed that PFAS exposure was associated with more severe disease in children with nonalcoholic fatty liver disease. Taken together, the committee concludes that the available studies provide *limited* or *suggestive* evidence of an association between PFAS exposure and liver enzyme levels.

Respiratory Outcomes

Authoritative reviews, including those of ATSDR, EFSA, and EPA, have not yet drawn conclusions about PFAS exposure and respiratory effects. Respiratory outcomes considered by the committee include pulmonary function tests (objective measures of how well the respiratory system is working); respiratory diseases, including obstructive airway diseases, such as asthma and chronic obstructive pulmonary disease; restrictive diseases, such as pulmonary fibrosis; and respiratory symptoms, such as wheeze, cough, phlegm, and dyspnea (shortness of breath). The committee identified 10 recent studies evaluating the association between PFAS exposure and respiratory outcomes. Three studies with probably low risk of bias evaluated PFAS and pulmonary function in cohorts of children (Agier et al., 2019; Kung et al., 2021; Manzano-Salgado et al., 2019); results were mixed within each study. Several studies evaluated the impact of PFAS exposure on asthma and respiratory symptoms, such as cough and wheeze; results were mixed both between and within studies (Ait Bamai et al., 2020; Beck et al., 2019; Impinen et al., 2019; Kvalem et al., 2020; Manzano-Salgado et al., 2019; Timmermann et al., 2020; Zeng et al., 2019). Taken together, the committee concludes that the available studies provide *inadequate* or *insufficient* evidence of an association between PFAS exposure and liver enzyme levels.

Hematological Outcomes

ATSDR evaluated the impact of PFAS on hematological parameters and concluded that PFAS are associated with no consistent alteration in hematological parameters. The committee did not identify any more recent studies on hematological effects. Thus, the committee concludes that the evidence is *inadequate* or *insufficient* to determine an association between PFAS exposure and hematological effects.

Musculoskeletal Outcomes

The authoritative reviews identify several studies evaluating possible PFAS-associated risk of osteoarthritis, osteoporosis, and reduced bone mineral density (BMD). The committee identified two more recent studies evaluating the relationship between PFAS exposure and bone health (Banjabi et al., 2020; Hu et al., 2019). A case-control study with high risk of bias found that serum PFAS concentrations increased the odds of diagnosis of osteoporosis among adults in Saudi Arabia (Banjabi et al., 2020). A study with probably low risk of bias evaluated BMD within a weight-loss trial of U.S. adults aged 30–70 and found associations between higher plasma PFAS concentrations and lower BMD at baseline, as well as a faster decline in BMD (Hu et al., 2019). The study by Hu and colleagues (2019) provides longitudinal evidence. However, the study was designed to measure weight loss, and its conclusions may not be generalizable to the broader population. In addition, the study is at risk of

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selection bias because only a small percentage of the study participants had both measures of BMD. There is also a risk of information bias from residual confounding because the analysis did not account for nutritional and menopausal status. Because of the small sample size, moreover, the authors did not adjust for multiple comparisons. Taken together with the evidence presented in ATSDR's *Toxicological Profile*, these findings are intriguing and merit further study. The committee concluded that the evidence is *inadequate* or *insufficient* to determine an association between PFAS and bone health. Nevertheless, the available evidence does raise concerns about the potential adverse effects of PFAS on bone health in both children and adults that warrant further investigation.

Renal Outcomes

ATSDR evaluated the impact of PFAS on kidney disease and biomarkers of renal function, but did not draw any conclusions because results were mixed across studies for most outcomes, and most studies were cross-sectional, so causal determinations could not be made. The committee identified three more recent studies evaluating impacts of PFAS on glomular filtration rate. A cohort study of participants in a medical surveillance program for residents near a former U.S. Department of Energy uranium-processing site assessed serum PFAS and measures of glomular filtration rate at repeated time points from 1990 to 2008 and found decreased glomular filtration rate to be associated with serum PFAS (Blake et al., 2018). The study had a probably high risk of bias because of a small potential for selection bias into the cohort and a slight risk of reverse causality. Two studies analyzing a diabetes prevention trial (Lin et al., 2021, which updates Cardenas et al., 2019) found that plasma PFAS concentrations during the diabetes prevention program were inversely associated with glomular filtration. Each quartile increase in baseline plasma measures of six PFAS was associated with 2.26 ml/min/1.73 m^2 lower glomular filtration (95% CI: −4.12, −0.39) at years 5 and 9. The study's strengths included tests of reverse causation and a lengthy follow-up period, although there is potential for residual confounding. The study's principal limitation is related to the generalizability of the findings to the general population given the inclusion criteria of overweight or obesity and prediabetes. Overall, the committee concluded that the evidence is *inadequate* or *insufficient* to draw a conclusion regarding an association between PFAS exposure and renal function. Nevertheless, the available evidence does raise concerns about the potential adverse effects of PFAS on renal function that warrant further investigation.

Neurological Outcomes

The authoritative reviews do not draw conclusions about the impact of PFAS on neurological outcomes, such as changes in motor function; behavioral changes; mood disorders; sensory disorders; cognitive disorders; and changes in neurochemistry, neurophysiology, or neuropathology. One recent case-control study nested within the diabetes prevention trial mentioned above found no association between any PFAS measured in serum and neuropathy in either the diabetes cases or the controls (Cardenas et al., 2019). The committee concluded that the evidence is *inadequate* or *insufficient* to draw a conclusion regarding an association between PFAS exposure and neurological outcomes.

EVIDENCE GAPS

The committee found several conditions to be associated with exposure to PFAS. The effects of PFAS span many different organ systems and disease states. The human populations most at risk of these health effects include those with a family history of or other risk factors for associated health effects and those who are in vulnerable life stages, including pregnancy, fetal development or early childhood, and the elderly. The committee did not complete a meta-analysis of the impact of each individual PFAS on each health outcome or provide an overall estimate of risk because the data from the studies are highly heterogeneous, limiting the applicability of meta-analytic techniques. The committee

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also observed gaps in the evidence for many health effects, whereby the evidence was *inadequate* or *insufficient* to determine associations. These gaps include

- immune effects other than reduced antibody response, and ulcerative colitis;
- cardiovascular outcomes other than dyslipidemia;
- developmental outcomes other than small reductions in birthweight;
- cancers other than kidney, breast, and testicular;
- reproductive effects other than hypertensive disorders of pregnancy;
- endocrine disorders other than thyroid hormone levels;
- hepatic effects other than liver enzyme levels;
- respiratory effects;
- hematological effects;
- musculoskeletal effects, such as effects on bone mineral density;
- renal effects, such as renal disease; and
- neurological effects.

It is critical to recognize that an assessment of *inadequate* or *insufficient* evidence does not mean there is no significant and important association between PFAS exposure and the outcome under consideration. It is quite possible that further research into the association of PFAS exposure with these outcomes would provide the evidence necessary to change the assessment to the category of either *limited* or *suggestive* or *sufficient* evidence. The committee believes that ongoing research on and review of these associations will be important in updating its clinical recommendations. A gap also remains in determining which developmental effects are the most clinically meaningful. For some outcome categories, the available research spans many different tests, all of which assessed slightly different effects, making the evidence difficult to synthesize and support strong conclusions. An authoritative organization needs to determine which endpoints are the most critical to evaluate to support clinical follow-up recommendations.

Additionally, most studies reviewed by the committee were not conducted among people known to have high exposures to PFAS. As a result, there is a gap in understanding of the effects of PFAS among those highly exposed, and the evidence presented in this report may therefore underestimate the effects of PFAS.

Although the committee aimed to assess the available scientific evidence as carefully and systematically as possible, it was sensitive to the fact that value judgments are unavoidable when performing an assessment of this kind (Elliott, 2017; Elliott and Richards, 2017; Jasanoff, 1998). These judgments include decisions about what forms of evidence to include, how to weigh and categorize different pieces of evidence, and what standards of evidence to demand before drawing conclusions. Literature from the sociology of science and medicine emphasizes not only that different expert communities may disagree about how to make these decisions (Cetina, 1999) but also that lay communities may make these decisions in ways that differ from those of expert communities (Epstein, 1996; Ottinger, 2010; Suryanarayanan and Kleinman, 2016). For example, because lay communities are often particularly concerned about addressing urgent health issues or informing time-sensitive policy decisions, they may accept lower standards of evidence than experts typically do (Brown, 1992). These differing evidential approaches across different communities raise the potential for differing rates of positive and negative errors (Douglas, 2009; Elliott, 2017; Elliott and Richards, 2017). Different communities also have varying background disease risk, which may lead to differing associations with PFAS and differing needs for risk assessment as it relates to these associations.

With these observations in mind, the committee acknowledges that other expert and lay communities might draw different conclusions about PFAS health risks, either by including different lines of evidence or by making alternative judgments when assessing the available evidence. This is one of the reasons that the committee emphasizes the importance of patient autonomy and shared decision making in

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subsequent chapters. Although the committee's evaluation of the evidence can provide an important starting point for decision making by clinicians and their patients, some individuals and groups could employ different evidential standards. Therefore, the committee encourages ongoing efforts to make scientific information about PFAS publicly available and understandable so that patients and clinicians can make informed decisions that respect individual patient values.

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ANNEX TABLE 3-1 Health Effects of PFAS by Category

Health Effect Category	Specific Health Effects Included	Authoritative Strength-of-Evidence Conclusion	Committee's Strength-of-Evidence Conclusion
Immunological	Response to infection Response to allergens Response to self: autoimmunity	ATSDR: Evidence is suggestive of an association between serum levels of PFOA, PFOS, PFHxS, and PFDA and decreased antibody responses to vaccines; there is also limited evidence of this effect for PFNA, PFUnDA, and PFDoDA. EFSA: PFOS and PFOA are associated with reduced antibody response to vaccination, observed in several studies. Some of the studies suggest that serum levels of PFOS and PFOA are associated with increased propensity for infection. Epidemiological studies provide insufficient evidence to draw conclusions on associations between exposure to PFASs and asthma and allergies. EPA: Human epidemiology data show associations between PFOA exposure and decreased vaccination response. Increased risk of ulcerative colitis was found in the PFOA high-exposure community study, as well as in a study limited to workers in that population. NTP: The evidence indicating that PFOA affects multiple aspects of the immune system supports the overall conclusion that PFOA alters immune function in humans. However, the mechanism(s) of PFOA-associated immunotoxicity is not clearly understood, and effects on diverse endpoints, such as suppression of the antibody response and increased hypersensitivity, may be unrelated. The NTP concludes that PFOS is presumed to be an immune hazard to humans based on a high level of evidence from animal studies that PFOS suppressed the antibody response and a moderate level of evidence from studies in humans. Although the mechanism(s) of PFOS-associated immunotoxicity is not clearly understood, potential mechanisms by which PFOS may reduce disease resistance include suppression of the antibody response and of NK cell function. OECD: A study with 656 children demonstrated that elevated exposures to PFOA and PFOS are associated with reduced humoral immune response to routine childhood immunizations in children aged 5 and 7. A large epidemiological study of 69,000 persons conducted by the C-8 Science Panel found probable links between elevated PFOA blood levels and ulcerative colitis.	Sufficient: decreased antibody response (in adults and children) Limited or suggestive: ulcerative colitis in adults Inadequate or insufficient: response to allergens, all other immune outcomes

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Cardiometabolic	Cardiovascular disease, dyslipidemia, metabolic syndrome, obesity	ATSDR: The results of epidemiological studies of PFOA, PFOS, PFNA, and PFDA suggest an association between perfluoroalkyl exposure and increases in serum lipid levels, particularly total cholesterol and low-density lipoprotein (LDL) cholesterol. EFSA: Epidemiological studies provide clear evidence of an association between exposure to PFOS, PFOA, and PFNA and increased serum levels of cholesterol. There is insufficient evidence of associations with diabetes, obesity, and metabolic syndrome. EPA: Human epidemiological data show associations between PFOA exposure and high cholesterol. These epidemiological studies have generally found positive associations between serum PFOA concentration and total cholesterol in PFOA-exposed workers and high-exposure communities (i.e., increasing lipid level with increasing PFOA); similar patterns are seen with LDL cholesterol but not with high-density lipoprotein (HDL) cholesterol. OECD: The C-8 Science Panel's epidemiological study of 69,000 persons found probable links between elevated PFOA blood levels and high cholesterol (hypercholesterolemia). C-8 Science Panel: There is a probable link between exposure to C-8 and high cholesterol (hypercholesterolemia).	Sufficient: dyslipidemia in adults and children Inadequate or insufficient: other outcomes
Developmental	Infant and fetal growth, neurodevelopment, development of the reproductive system	ATSDR: Evidence is suggestive of an association between serum levels of PFOA and PFOS and small decreases in birthweight. EFSA: There may well be a causal association between PFOS and PFOA and birthweight. Maternal serum levels in studies reporting results on other PFASs were generally much lower, and those studies provide no evidence of an adverse association between other PFASs and birthweight. EPA: The epidemiological studies found no association of PFOA with neurodevelopmental effects or with preterm birth and other complications of pregnancy. OECD: High levels of PFOS and PFOA are toxic for reproduction and development of the fetus (such as reducing birthweight and lowering semen quality).	Sufficient: small reductions in birthweight Inadequate or insufficient: all other outcomes, such as development of the reproductive system, neurodevelopment
Cancer	Kidney cancer, testicular cancer, breast cancer	EPA: Under EPA's *Guidelines for Carcinogen Risk Assessment* (EPA, 2005), there is "suggestive evidence of carcinogenic potential" for PFOA. Epidemiology studies demonstrate an association of serum PFOA with kidney and testicular tumors among highly exposed members of the general population. There is suggestive evidence of carcinogenic potential for PFOS. Human epidemiology studies found no direct correlation between PFOS exposure and the incidence of carcinogenicity in worker-based populations. Although one worker cohort showed an increase in bladder cancer, smoking was a major confounding factor, and the standardized incidence ratios were not significantly different from those for the general population. Other worker and general population studies found no statistically significant trends for any cancer type.	Sufficient: kidney cancer in adults Limited or suggestive: testicular cancer, breast cancer in adults Inadequate or insufficient: all other cancers

continued

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ANNEX TABLE 3-1 *continued*

Health Effect Category	Specific Health Effects Included	Authoritative Strength-of-Evidence Conclusion	Committee's Strength-of-Evidence Conclusion
		IARC: There is "limited" evidence in humans for the carcinogenicity of PFOA. A positive association was observed for cancers of the testes and kidney. Overall, PFOA is possibly carcinogenic to humans (Group 2B). OECD: High levels of PFOS and PFOA have been found to be potentially carcinogenic in animal tests. The C-8 Science Panel's epidemiological study of 69,000 persons found probable links between elevated PFOA blood levels and testicular and kidney cancer. C-8 Science Panel: There is a probable link between exposure to C-8 and kidney and testicular cancer.	
Reproductive	Infertility, male reproductive effects, female reproductive effects, hormone levels, hypertension during pregnancy, gestational diabetes	ATSDR: There is suggestive epidemiological evidence of an association between serum PFOA and PFOS and pregnancy-induced hypertension and/or preeclampsia. EPA: Human epidemiological data show associations between PFOA exposure and pregnancy-induced hypertension and preeclampsia. OECD: High levels of PFOS and PFOA are toxic for reproduction and development of the fetus (e.g., reducing birthweight and lowering semen quality). In addition, 8:2 fluorotelomer phosphate diesters (8:2 PAPs), 8:2 FTOH, and PFOA show endocrine effects in different in vitro and in vivo tests. The C-8 Science Panel's epidemiological study of 69,000 persons found probable links between elevated PFOA blood levels and preeclampsia and elevated blood pressure during pregnancy. C-8 Science Panel: There is a probable link between exposure to C-8 and pregnancy-induced hypertension (including preeclampsia).	Limited or suggestive: pregnancy-induced hypertension (gestational hypertension and preeclampsia) Inadequate or insufficient: all other effects, such as fecundity, infertility, male reproductive effects, female reproductive effects, reproductive hormone levels, gestational diabetes
Endocrine	Thyroid disease, hypothyroidism, hyperthyroidism, thyroid hormone levels, type 1 and 2 diabetes	EPA: Human epidemiology data show associations between PFOA exposure and thyroid disorders. The epidemiological studies found no associations between PFOA and diabetes. OECD: 8:2 fluorotelomer phosphate diesters (8:2 PAPs), 8:2 FTOH, and PFOA show endocrine effects in different in vitro and in vivo tests. The C-8 Science Panel's epidemiological study of 69,000 persons found probable links between elevated PFOA blood levels and thyroid disease. C-8 Science Panel: There is a probable link between exposure to C-8 and thyroid disease.	Limited or suggestive: thyroid disease or dysfunction in adults Inadequate or insufficient: all other outcomes, including type 1 and 2 diabetes

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Hepatic	Liver disease, altered serum liver enzymes and bilirubin	ATSDR: Increases in serum enzymes and decreases in serum bilirubin, observed in studies of PFOA, PFOS, and PFHxS, are suggestive of liver alterations. EFSA: Epidemiological studies provide evidence for an association between exposure to PFAS and increased serum levels of the liver enzyme alanine transferase (ALT). The magnitude of the association was small, however, and few studies found an association with ALT outside the reference range. There were no associations with liver disease. EPA: Human epidemiology data show associations between PFOA exposure and increased liver enzymes.	Limited or suggestive: liver enzyme alterations in children and adults
Respiratory	Respiratory symptoms, pulmonary function, respiratory diseases	None	Insufficient
Hematological	Blood disorders or impacts on blood-forming organs	None	Insufficient
Musculoskeletal	Loss of muscle tone or strength, muscular rigidity, muscular atrophy, arthritis, altered bone density, arthralgia (joint pain)	None	Insufficient
Renal	Kidney disease and biomarkers of renal function	None	Insufficient
Neurological	Parkinson's disease, memory loss, neuropsychological function	None	Insufficient

NOTE: ATSDR = Agency for Toxic Substances and Disease Registry; EFSA = European Food Safety Authority; EPA = U.S. Environmental Protection Agency; IARC = International Agency for Research on Cancer; NK = natural killer; NTP = National Toxicology Program; OECD = Organisation for Economic Co-operation and Development.

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PFAS Exposure Reduction

It is difficult to provide clear advice on how to reduce exposure to per- and polyfluoroalkyl substances (PFAS) because there are many potential exposure sources. Importantly, even if the source of a person's exposure is completely removed, it will take years for the internal body burden (levels in the body) of many PFAS to be fully eliminated. Biological half-lives (i.e., the time it takes for plasma concentration to decrease by 50 percent after exposure) vary depending on the PFAS; half-life estimates for the four most studied PFAS (perfluorooctanoic acid [PFOA], perfluorooctane sulfonic acid [PFOS], perfluorohexane sulfonate [PFHxS], and perfluorononanoic acid [PFNA]) range from around 2 to 8 years (Li et al., 2018; Olsen et al., 2007). These long biological half-lives are due to reabsorption in the kidney and enterohepatic recirculation, both processes greatly reducing the capacity to eliminate PFAS (Harada et al., 2007).

In developing this chapter, the committee first created a conceptual model for exposure reduction (see Figure 4-1). The premise of this model is that if any exposure pathway is interrupted, exposure should be reduced. For example, if PFAS were no longer used in industries, they would not be in consumer products or waste streams, so exposures would be reduced. Similarly, if PFAS in the environment are cleaned up, exposures will decrease.

Support for the committee's conceptual model is found in the changes in exposure at the population level due to industry-wide changes in PFAS production. For example, serum PFAS concentrations in the United States declined over time following the 3M company's (Maplewood, Minnesota) voluntary phase-out of perfluorooctanyl chemicals and related precursors, including PFOS and PFOA, in 2000 (ITRC, 2017) (see Figure 4-2). Therefore, there is evidence that removing these chemicals from products on a large scale can result in lower levels in a population.

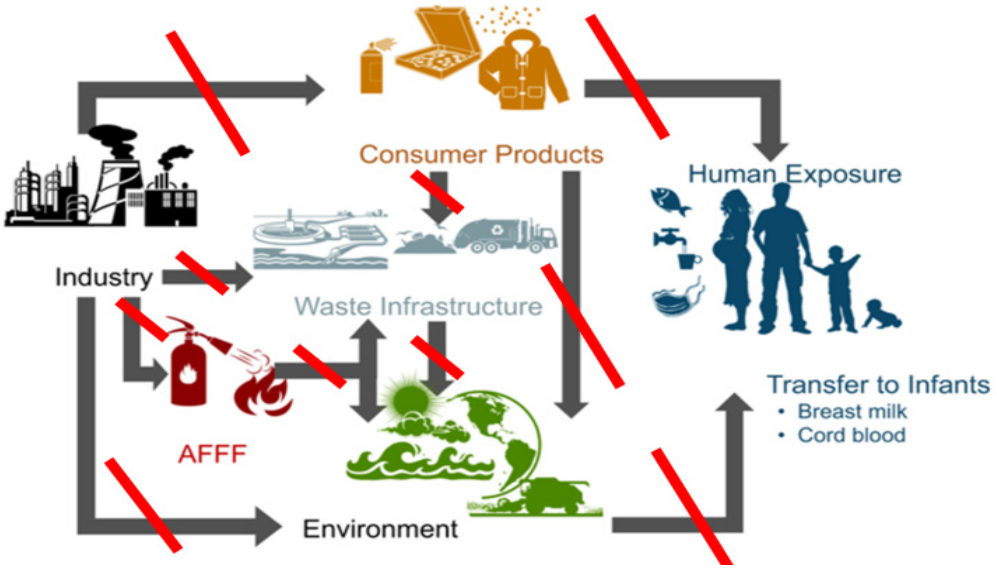


FIGURE 4-1 Conceptual model for PFAS exposure reduction.
NOTE: Red lines indicate a break in the pathway that could reduce human exposure.
SOURCE: Adapted from Sunderland et al., 2019.

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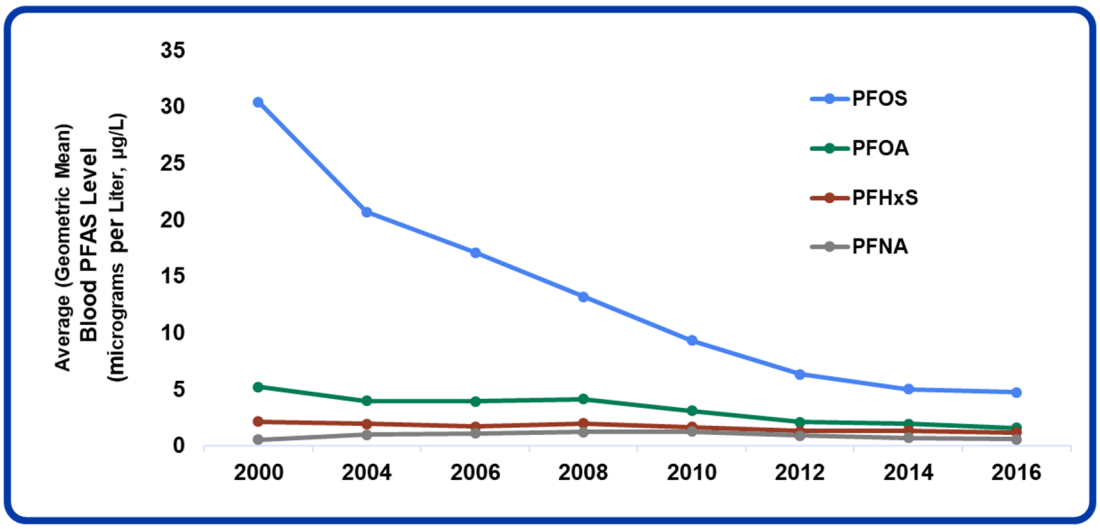


FIGURE 4-2 Blood (serum) levels of PFAS, United States, 2000–2016.
NOTE: Average = geometric mean.
SOURCE: Patrick N. Breysse's presentation to the committee on February 4, 2021. DATA SOURCE: Centers for Disease Control and Prevention. Fourth Report on Human Exposure to Environmental Chemicals, Updated Tables, (January, 2019). Atlanta, GA: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention.

SOURCES AND ROUTES OF EXPOSURE TO PFAS

The sources of and routes of exposure to PFAS are an active area of investigation. What is well known is that PFAS exposure is highly complex, with pathways that include occupational exposures, environmental contamination, consumer product use, and food exposures. Specific sources of exposure include, for example, jobs in fluorochemical manufacturing facilities or where PFAS-containing products, such as textiles or food contact materials, are made. Other jobs with a known increased risk of exposure to PFAS include electroplating; painting; carpet installation and treatment; serving as a military or civilian firefighter, which entails using PFAS-containing foams in training exercises and wearing PFAS-impregnated gear; and jobs that require prolonged work with ski wax (ATSDR, 2021). In addition, food workers and others in the hospitality industry may have elevated exposure since they handle PFAS-containing food packaging as part of their job duties (Carnero et al., 2021; Curtzwiler et al., 2021; Schaider et al., 2017).

Ingestion is the most well-studied route of exposure in nonoccupational settings (Trudel et al., 2008). Ingestion of PFAS can occur through drinking contaminated water; eating contaminated seafood; or consuming other contaminated foods, such as vegetables, game, or dairy products (Bao et al., 2019, 2020; Death et al., 2021; Domingo and Nadal, 2017; Herzke et al., 2013; Li et al., 2019). PFAS are often used in cookware and in materials that came in contact with food, such as microwave popcorn bags or packaging used for fast foods or processed foods (Carnero et al., 2021; Curtzwiler et al., 2021; Schaider et al., 2017). Exposure can also occur through accidental ingestion of PFAS-containing dusts (Fraser et al., 2013). PFAS cross the placenta, and PFAS from the mother's body burden can be passed on to her developing fetus (Gao et al., 2019; Manzano-Salgado et al., 2015). Maternal transfer of PFAS can also occur through breastfeeding (Serrano et al., 2021; Zheng et al., 2021).

Inhalation and transdermal exposures are less well studied. Inhalation of PFAS is well documented in occupational settings that use aerosolized PFAS (Gilliland, 1992). Volatile PFAS have been detected indoors (Fromme et al., 2015; Morales-McDevitt et al., 2021), and inhalation near factory emissions and incinerators contributes to exposures in nearby communities (Fenton et al., 2021). There

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are as yet no data formally evaluating inhalation from showering in contaminated water, but this is an active area of research.

PFAS are used in thousands of products (e.g., products containing water; stain-resistant clothing; and personal care products, such as sunscreen, makeup, and dental floss). PFAS are also used in such products as paint, textiles, firefighting foam, electroplating material, ammunition, climbing ropes, guitar strings, artificial turf, and soil remediation products (Glüge et al., 2020). The extent to which use of products contributes to human exposures is unkown, however, because the relative contribution of PFAS exposures from sources other than food or water is not well characterized (DeLuca et al., 2021).

The presence of PFAS in everyday consumer products may be an important source of exposure for the general population, but this likely varies greatly by individual (Rodgers et al., 2022). Consumer products are often treated with fluoropolymers to impart water and stain resistance, and fluoropolymers can result in exposure to the nonpolymer PFAS discussed in this report in several ways. Nonpolymer PFAS can be present as impurities in fluoropolymer-containing products because these PFAS are used as processing aids in chemical production or are degradation products of the fluoropolymers (Rodgers et al., 2022; Schellenberger et al., 2019). Moreover, fluoropolymer PFAS can degrade (biodegradation) slowly when exposed to water and release fluorotelomer alcohols, which are precursors to nonpolymer PFAS. Once the precursor PFAS are in the human body, they can transform to such PFAS as PFOA, PFOS, and PFHxS (Washington and Jenkins, 2015).

APPROACH TO DETERMINING ADVICE ON PFAS EXPOSURE REDUCTION

To inform its recommendations on how clinicians can advise patients on PFAS exposure reduction, the committee looked at several sources of evidence. The committee contracted with a consultant to review the literature evaluating the effectiveness of behavioral interventions in reducing exposure to PFAS. The consultant also reviewed several studies that model estimates of PFAS intake, which could inform predicted changes in serum PFAS levels if exposure routes were modified (see Appendix E). The committee reviewed studies on PFAS exposure through breastfeeding since this route impacts a vulnerable population and is of particular interest to people in PFAS-impacted communities, as voiced by speakers at the committee's town halls (see Appendix B). The committee also evaluated studies of medical interventions for reducing internal levels of PFAS (e.g., phlebotomy or taking of prescription drugs). PFAS exposure pathways discussed in Chapter 1 were used to outline strategies clinicians can use to determine whether a patient may be at risk of PFAS exposure based on residential and work history. In addition, the committee reviewed advice from other entities on PFAS exposure reduction, such as that provided by nongovernmental organizations, state and local governments, and other countries.

CONTRIBUTION OF INDIVIDUAL EXPOSURE SOURCES TO HUMAN EXPOSURE

Behavioral Intervention Studies

Studies evaluating the impact of behavior change on reducing exposures would help determine the impact of behavior on human exposure. This section, based on a literature review conducted by LaKind Associates (see Appendix E), provides an overview of the literature available to inform what individuals can do to reduce their serum PFAS levels. The literature review used a three-step approach to identify relevant publications in PubMed, EMBASE, and Google Scholar (see Appendix E for keywords and the search strategy). Studies were selected that entailed interventions designed to reduce human exposure to PFAS, specifically interventions that could be carried out by individuals. Secondary references of retrieved articles were reviewed to identify publications not found through the electronic search. An additional literature search was then conducted to identify reviews containing estimates of human PFAS intakes. The final search date was March 5, 2021.

The reviewed intervention studies are presented here by exposure route, with the primary focus being on drinking water and diet. Literature on interventions for other exposure sources, such as dust and

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consumer products, is more limited. Lastly, breastfeeding is an important potential source of exposure for infants, and lactating could reduce parents' PFAS levels. After reviewing the relevant studies, this section concludes by assessing the effectiveness of behavior modifications based on the current literature.

Drinking water has been identified as a substantial source of PFAS exposure for many populations (Andrews and Naidenko, 2020; Domingo and Nadal, 2019). Studies of interventions focused on drinking water are consistent in demonstrating the effectiveness of water filtration at reducing levels of certain PFAS. Consumers have a variety of options for filtering PFAS from drinking water, including whole-house, under-sink, and filtering-pitcher devices. The committee identified seven publications and one agency report evaluating possible drinking water interventions. Of these, five (Ao et al., 2019; Herkert et al., 2020; Iwabuchi and Sato, 2021; MDH, 2008; Patterson et al., 2019) evaluate use of whole-house, under-sink, and filtering-pitcher devices, and three (Ao et al., 2019; Gellrich et al., 2013; Heo et al., 2014) evaluate differences in PFAS concentrations between tap water and bottled water.

In a study of real-world uses of water filtration options, Herkert and colleagues (2020) tested municipal, well, and filtered (n = 89) and unfiltered (n = 87) tap water in residences (N = 73) in North Carolina for 11 PFAS. The filters tested varied in both type and filtration method (reverse osmosis, granulated activated carbon, single-stage, dual-stage). Notably, reverse osmosis and dual-stage filters were found consistently to remove most measured compounds at an average of ≥90 percent efficiency; some short-chain replacement PFAS are difficult to remove with carbon filtration (Herkert et al., 2020). Use of bottled water can also be a way to reduce PFAS exposure from drinking water, although some bottled waters contain detectable levels of C-3–C-10 perfluorocarboxylic acids (PFCAs) and C-3–C-6 and C-8 perfluorosulfonic acids (PFSAs) (Chow et al., 2021). In Parkersburg, West Virginia, for example, the use of bottled water resulted in a dramatic reduction in serum PFOA levels among a contaminated community living near a Teflon-manufacturing plant (Emmett et al., 2006).

Changes in diet may potentially reduce PFAS exposure, given that PFAS can be present in a number of food products, including wild-caught fish and game, livestock, and produce, as well as prepared foods. Fish and seafood have been identified as sources of PFAS, but the levels of PFAS vary by fish type and water body (Sunderland et al., 2019). PFAS-contaminated drinking water can also impact home-grown vegetables (Brown et al., 2020; Emmett et al., 2006). The majority of ingestion-based intervention studies focused on seafood preparation, but there was no strong evidence that fish preparation methods influenced PFAS levels (Alves et al., 2017; Barbosa et al., 2018; Bhavsar et al., 2014; Del Gobbo et al., 2008; Hu et al., 2020; Kim et al., 2020; Luo et al., 2019; Taylor et al., 2019).

A study on PFAS exposure from indoor dust found significantly lower PFAS levels in vacuumed dust from rooms with PFAS-free furnishings relative to control rooms (78 percent reduction, 95% CI: 38–92). Results suggest that modifying personal behavior to be capable of identifying and purchasing PFAS-free furnishings can decrease exposure levels from indoor dust (Scher et al., 2019).

Literature on PFAS in consumer products is available only for nonstick pans and dental floss, and these studies have several weaknesses, including recall bias, small sample sizes, and lack of replication. Their results do not provide enough evidence to suggest that modifications in behavior relative to those products would decrease PFAS exposure, but in the absence of such evidence, consumers should be aware of which products contain PFAS (Scher et al., 2019; Young et al., 2021).

Another factor that complicates consumer choices aimed at avoiding PFAS is the lack of consistent labeling of products. A recent study (identified after Appendix E of this report had been completed) that screened 93 market items across three different product types (furnishings, apparel, and bedding) found that PFAS were present in many items that were labeled as green or nontoxic (Rodgers et al., 2022).

To summarize, the available literature is limited in presenting recommendations for effective behavior modifications to reduce internal levels of PFAS. In places with water contamination, individuals can reduce their exposure through use of water filtration. In places without PFAS water contamination or workplace exposure, diet is believed to be the primary exposure route, but there is limited information with which to recommend dietary interventions. No intervention study has examined exposure reduction and its impact on serum concentrations, likely in part because to fully show effectiveness for an

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intervention, it would have to be conducted over a long enough time to account for the long half-lives of PFAS.

Modeled Estimates of PFAS Intakes

In the absence of studies demonstrating the impact of interventions on reducing PFAS exposure, exposure models may help inform predicted changes in serum PFAS levels if exposure routes are modified. Pharmacokinetic modeling is useful for estimating the body burden from different exposure routes, but such models are often limited by the many assumptions made about intake factors (e.g., food contamination). High-quality data on the distribution of PFAS in different food types and consumer products are sparse. Nonetheless, pharmacokinetic models can be used to estimate the variability possible in exposure reduction and the impact of changes with different parameters. Although imperfect, exposure modeling can provide, at a minimum, an estimate of the change in internal PFAS body burden if PFAS levels in diet or water were decreased.

Studies that have estimated intake of PFOS and PFOA have been used to determine the dominant routes of exposure in communities without contaminated drinking water (see Appendix E). Estimates of how much PFAS exposure comes from diet in adults vary widely, from 16–99 percent for PFOA to 66–100 percent for PFOS (Egeghy and Lorber, 2011; Haug et al., 2011; Lorber and Egeghy, 2011; Sunderland et al., 2019; Vestergren and Cousins, 2009); no estimates are available for individual food products. For dust, the estimates are 1–11 percent for PFOA and 1–15 percent for PFOS (Sunderland et al., 2019). For PFOA, the dominant routes are thought to be oral exposure resulting from consumption of fish and seafood, drinking water, and ingestion of dust. For PFOS, the dominant routes are thought to be ingestion of food and water, ingestion of dust, and hand-to-mouth transfer from treated carpets (Trudel et al., 2008). Residual PFOA in food packaging (used to greaseproof food-containing paper products) is another potential route of exposure (Trudel et al., 2008); polyfluoroalkyl phosphoric acids in food packaging can also be metabolized in the body to PFOA (Begley et al., 2005; Carnero et al., 2021; Curtzwiler et al., 2021; Schaider et al., 2017).

Interpretation of the estimated intake studies is challenged by several factors. First, while diet appears to be a major pathway of exposure, there is little information on PFAS in commercial foods commonly consumed in the United States. The U.S. Food and Drug Administration (FDA) has released PFAS data for certain foods that could be used to model source contributions to PFAS intake in future studies. However, the FDA data for produce, meat, dairy, and grain products are based on a small sample size, and the results "cannot be used to draw definitive conclusions about the levels of PFAS in the general food supply."[1]

The relative importance of different PFAS sources varies by study, population, and time period of exposure (Sunderland et al., 2019). The production and use of individual PFAS have changed over time and will continue to do so. Serum PFAS levels in the United States dropped following the phase-out of production of PFOS and PFOA; however, exposures to C9–C11 PFCAs have not followed the same trend. Thus, it is important to use recent environmental, consumer product, and dietary data to develop robust estimates of current dominant pathways of PFAS exposure. In a recent review evaluating nonoccupational intakes via background PFAS exposures, De Silva and colleagues (2021) observed that the inconsistency among studies in the relative importance of different exposure sources may be due to differing concentrations of PFAS in sources, as well as the assignment of differing values for exposure intake factors (e.g., exposure frequency and duration). The authors conclude, "Without rigorously conducted exposure studies it is challenging to rank order the most important human exposure pathways and without these data, our ability to design evidence-based exposure intervention strategies will be limited."

[1] See https://www.fda.gov/food/chemicals/analytical-results-testing-food-pfas-environmental-contamination (accessed May 12, 2021).

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Exposure to PFAS Through Breastfeeding

Breastfeeding is a route of exposure of great interest to people who spoke at the committee's town halls. Breast milk is the only food many infants receive in their first 6 months of life, and if the breast milk they receive is contaminated with PFAS, it may take years for their body burden to be reduced, given the long half-lives of some PFAS.

In her testimony at the committee's first town hall (April 7, 2021), Loreen Hackett (PFOA Project New York) stated that in her view, health care mantras such as "breast milk is best" need to be thoroughly reevaluated in exposed communities, noting that breastfeeding "may double or triple PFAS levels in an infant compared to the mothers thereby increasing risks to their developing systems." She stressed that families in exposed communities cannot make informed reproductive choices or other family decisions without improved information tailored to their situation, and she relayed concerns among community members expressing guilt for unknowingly poisoning their child over the course of pregnancy and breastfeeding.

Nonetheless, data on PFAS in breast milk are very limited. A few studies measuring PFAS in breast milk in North America (Kubwabo et al., 2013; Tao et al., 2008; Zheng et al., 2021) indicate transfer from the parent to the child during the first months of life. And although the concentrations in breast milk are generally much lower than the concentrations in maternal serum (Cariou et al., 2015; Kärrman et al., 2007; Kim et al., 2011; Liu et al., 2011), breastfeeding has been shown to contribute significantly to children's serum levels of some PFAS (Gyllenhammar et al., 2018, 2019; Koponen et al., 2018). In a cohort of 2- to 4-month-old infants in Sweden, for example, bottle-fed infants had mean serum concentrations twice as low as those of their exclusively breastfed counterparts, and serum levels of PFOA, PFNA, and PFHxS increased 8−11 percent per week of exclusive breastfeeding (Gyllenhammar et al., 2018). Where measured and estimated PFAS concentrations in breast milk in the United States have been compared with drinking water screening values of the Agency for Toxic Substances and Disease Registry (ATSDR), some exceedances have been observed, especially in communities impacted by PFAS contamination (LaKind et al., 2022) (see Figure 4-3).

Whether lactational exposure to PFAS can have adverse health effects in children has not been well studied to date. Formula feeding can also lead to PFAS exposure through either contaminated formula or formula reconstituted with contaminated drinking water. Given the increased exposures observed in breastfed versus formula-fed infants, it is not clear whether the benefits of breastfeeding outweigh the risks to the child among lactating persons with very high levels of PFAS exposure.

Guidance to breastfeed remains the best feeding advice for most infants given the many benefits of breastfeeding for both mothers and babies.[2] Even though PFAS exposures have been occurring for many years, research has consistently shown benefits of breastfeeding, providing confidence in the traditional guidance, although a more in-depth understanding of this exposure route is warranted to inform protection of such a vulnerable population.

MEDICAL INTERVENTIONS FOR POTENTIALLY REDUCING PFAS BODY BURDEN

There have been few studies overall and no clinical trials evaluating treatments to reduce PFAS body burden, even in cases of very high exposure. The few evaluations available have focused on the use of cholesterol-lowering medications and phlebotomy.

PFAS are secreted in the bile and have enterohepatic recirculation; therefore, researchers have been interested in medications that enhance bile sequestration as potential approaches for reducing PFAS body burden. Cholestyramine is a bile-sequestering agent that is used mainly to reduce low-density lipoprotein (LDL) cholesterol. In a cross-sectional study of C-8 Health Project participants, 36 of 56,175 adults were being treated with cholestyramine and were found to have lower levels of PFAS compared with those not taking this medication (Ducatman et al., 2021). Another medication, probenecid, was not

[2] See https://www.cdc.gov/breastfeeding/about-breastfeeding/why-it-matters.html (accessed May 23, 2022).

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significantly associated with serum PFAS levels in this study. Cholestyramine has also been evaluated in a few small case studies of 1 to 20 individuals (Genius et al., 2010, 2013). Results of these studies suggest that cholestyramine may be an effective treatment to accelerate PFAS fecal excretion, but replication in studies with more participants is needed. No studies have assessed whether PFAS levels rebound when treatment with cholestyramine is discontinued.

Phlebotomy has been discussed as a way to reduce the body burden of PFAS. Genuis and colleagues (2014) asked six patients aged 16–53 years from a highly PFAS-exposed family to submit to routine blood draws (500 mL) for up to 5 years, resulting in a cumulative 2–12 L of blood drawn. The levels of PFOA, PFOS, and PFHxS decreased in these individuals over that time at a faster rate than expected according to first-order excretion kinetics. In a recent randomized controlled trial of 285 firefighters, serum PFOS and PFHxS concentrations were significantly reduced in subjects who regularly donated blood or plasma over 12 months compared with the control group; the decline was more pronounced in the plasma donation group (Gasiorowski et al., 2022). While these studies indicate that phlebotomy can be effective at reducing PFAS levels in blood, there are no established serum concentrations of PFAS at which the benefits of this intervention reasonably outweigh the harms, and the safety and utility of this approach are uncertain.

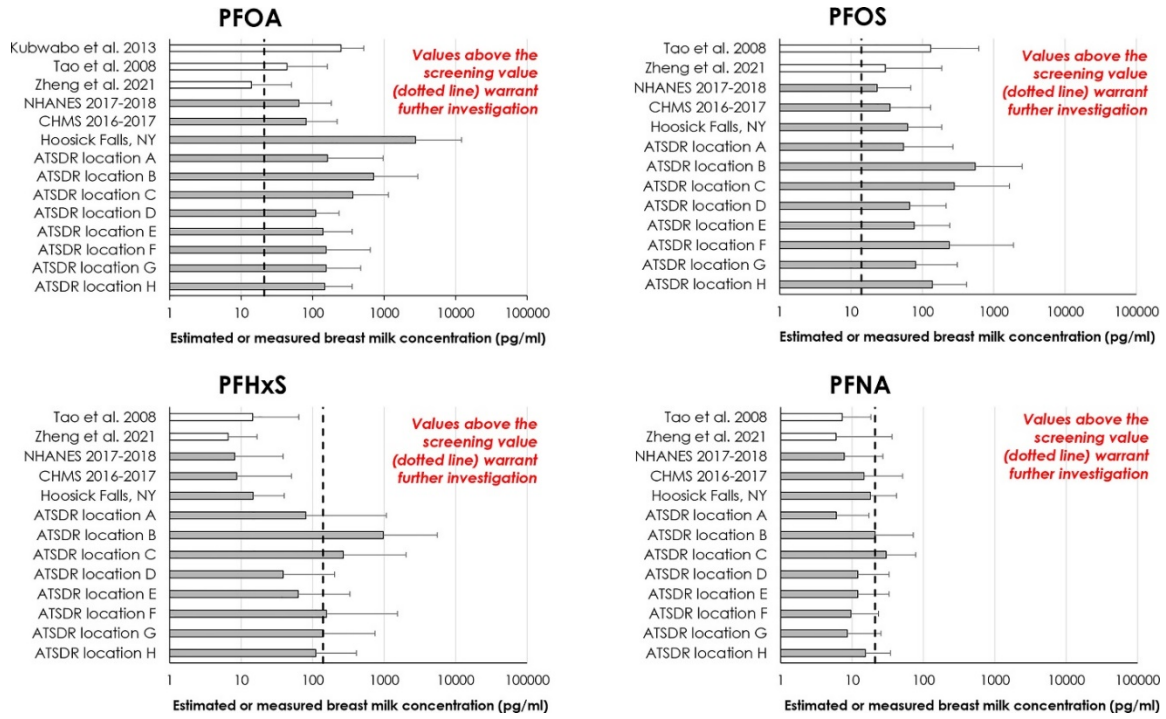


FIGURE 4-3 Measured (white bars) and estimated (gray bars) breast milk concentrations of per- and polyfluoroalkyl substances (PFAS) in the United States and Canada, in comparison with children's drinking water screening values (dotted line).
NOTES: Bars represent the mean measured breast milk levels for the data from Kubwabo and colleagues (2013) and Tao and colleagues (2008); the median measured breast milk levels from Zheng et al. (2021); and the geometric mean estimated breast milk levels for the National Health and Nutrition Examination Survey (NHANES), the Canadian Health Measures Survey (CHMS), and PFAS-contaminated communities. Error bars represent maximum measured concentrations in Kubwabo and colleagues (2013) and Tao and colleagues (2008), and 95th percentile estimated concentrations for NHANES, CHMS, and PFAS-contaminated sites. Of note, PFOS, PFHxS, and PFNA were not detected in breast milk samples from Kubwabo and colleagues (2013). The xx-axis is log-scale. Agency for Toxic Substances and Disease Registry children's drinking water screening values: PFOA (21 ppt), PFOS (14 ppt), PFHxS (140 ppt), and PFNA (21 ppt) (ATSDR, 2018).
SOURCE: LaKind et al., 2022.

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EXISTING ADVICE ON PFAS EXPOSURE REDUCTION

Several federally funded academic projects and nonprofit organizations provide information about PFAS, including how to identify potential community exposure and reduce personal exposure. Because concerned individuals look to online resources for data and information, a few of the resources are summarized here, with the caveat that they have not been tested empirically, and the data they present may be incomplete, or the sources on which the data are based may be missing. Furthermore, in discussing these online resources, the committee is not endorsing them, nor do their content and conclusions necessarily represent the committee's views. In addition to the sources discussed below, many state health departments have state-level resources on PFAS that may be trusted sources of information.

PFAS-REACH

PFAS-REACH (Research, Education, and Action for Community Health) is a project funded by the National Institute of Environmental Health Sciences to develop guidance materials and data interpretation tools for use by communities impacted by PFAS-contaminated drinking water. The project is led by Silent Spring, Northeastern University, and Michigan State University, with collaboration from community partner organizations that include Testing for Pease, Massachusetts Breast Cancer Coalition, and Community Action Works. The project's online resource center, PFAS Exchange, provides factsheets and interactive maps; a factsheet on how to reduce one's exposure is most relevant to the discussion in this chapter.[3] Figure 4-4 shows the PFAS Exchange recommendations.



FIGURE 4-4 Recommendations for reducing PFAS exposure available through the PFAS Exchange. SOURCE: PFAS-REACH project.

[3] See https://pfas-exchange.org/how-to-reduce-your-exposure-to-pfas (accessed June 17, 2022).

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PFAS Project Lab

Northeastern University, one of the academic partners for the PFAS-REACH project, operates the PFAS Project Lab within its Social Science Environmental Health Research Institute (SSEHRI). Among the Lab's publicly available resources is the PFAS Sites and Community Resources Map.[4] The map interface provides the locations of known and suspected PFAS contamination sites (see Figure 4-5), as well as community resources and state action. The data were collected from government websites, news articles, and publicly available sources. The map began as a collaborative effort with the Environmental Working Group (EWG), an advocacy group (see below and Chapter 1, Figure 1-2). The SSEHRI map "aims to help affected residents and community groups to access information about data in their states and learn how to connect with other activists working on PFAS issues."[5]

Environmental Working Group

The EWG is included as a data source in the PFAS Project Lab. The EWG site also provides an interactive map that "serves to show the extent of PFAS water contamination as documented by states, the department of defense and EWG's testing," providing the locations of industrial and military sites with known PFAS contamination.[6] Additionally, the EWG provides a guide for avoiding exposure to PFAS chemicals. The EWG's recommendations are similar to those in the PFAS-REACH factsheet (see Figure 4-4) regarding consumer choices. The EWG has developed several consumer guides providing information on the chemicals (not just PFAS) present in a variety of commercial products, including sunscreen, cosmetics, personal care and beauty products, bug repellants, and household cleaners, among others.

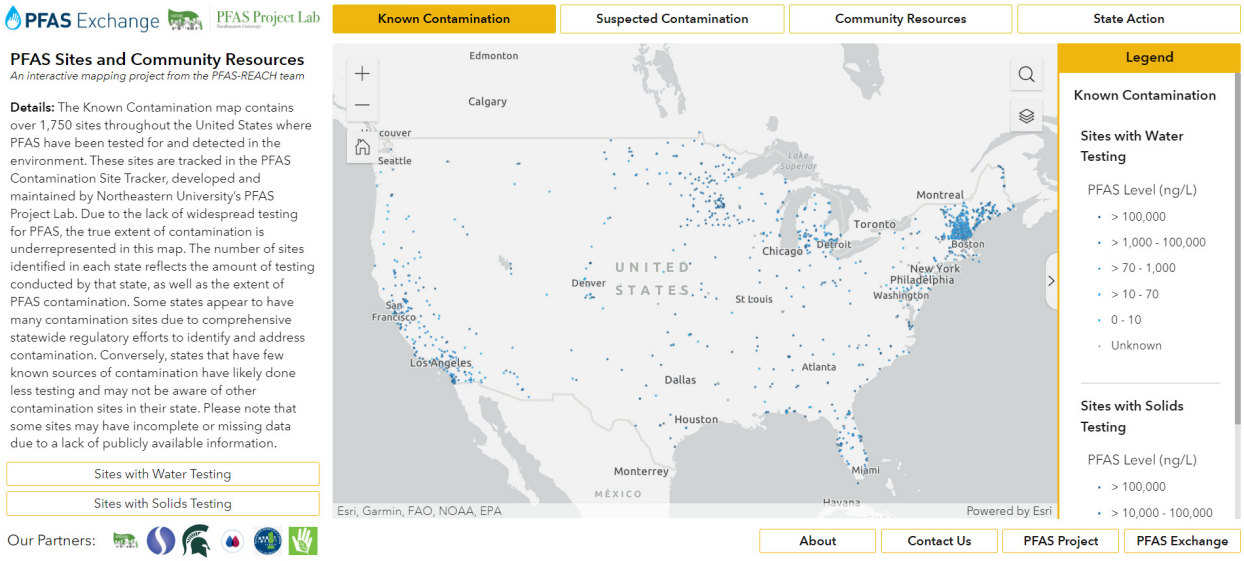


FIGURE 4-5 PFAS Project Lab map showing PFAS contamination sites in the continental United States. SOURCE: Social Science Environmental Health Research Institute (SSEHRI). https://experience.arcgis.com/experience/12412ab41b3141598e0bb48523a7c940 (accessed May 25, 2022).

[4] See https://experience.arcgis.com/experience/12412ab41b3141598e0bb48523a7c940 (accessed May 25, 2022).
[5] See https://www.ewg.org/news-insights/news/mapping-pfas-contamination-crisis (accessed June 15, 2022).
[6] See https://www.ewg.org/interactive-maps/pfas_contamination/#about (accessed June 17, 2022).

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FINDINGS AND RECOMMENDATIONS

The committee found that few evidence-based recommendations can be made for reducing exposure to PFAS on an individual level.

Occupational exposures to PFAS may be much higher than community exposures. In accordance with the hierarchy of controls, methods for reducing workplace exposure can include replacing the chemical with a less hazardous one; engineering controls, such as ventilation to reduce inhalation of the chemical; administrative controls, such as rotating operations to reduce the amount of time an individual worker is around a chemical; or personal-level controls, such as personal protective equipment, including gloves and masks.

Ingestion is an important route of exposure to PFAS in the general population; thus it is important to reduce consumption of PFAS in drinking water and foods. Contamination of drinking water with PFAS is a widespread problem in the United States, and the extent of the contamination has not been completely characterized. Both municipal and private sources of drinking water (e.g., private wells) can be contaminated with PFAS as a result of fluorochemical manufacturing, use of firefighting foams, or discharge of landfill leachate to drinking water sources. If PFAS are in drinking water, switching to another source of water with lower PFAS concentrations will reduce exposure.

Consumption of game may also cause to exposure to PFAS. To date, 11 states have developed or are in the process of developing advisory guidelines for fish, wildlife, and other food products to protect human health from exposure to PFAS. These advisories offer guidance on limiting the quantity of consumption of these foods. These advisories are state-specific and range from do not eat (e.g., fish or deer in Michigan with PFOS concentrations over 300 parts per billion [ppb]) to no need to limit consumption (e.g., New Jersey fish with more than 0.56 nanograms per gram [ng/g] of PFOS). The Environmental Council of the States has compiled information from participating states on state PFAS standards, advisories, and guidance values (ECOS, 2020).

For clinicians, based on its review of the evidence on PFAS exposure reduction, the committee makes the following recommendations:

Recommendation 4-1[7]: Clinicians advising patients on PFAS exposure reduction should begin with a conversation aimed at first determining how they might be exposed to PFAS (sometimes called an environmental exposure assessment) and what exposures they are interested in reducing. This exposure assessment should include questions about current occupational exposures to PFAS (such as work with fluorochemicals or firefighting) and exposures to PFAS through the environment. Known environmental exposures to PFAS include living in a community with PFAS-contaminated drinking water, living near industries that use fluorochemicals, serving in the military, and consuming fish and game from areas with known or potential contamination.

Recommendation 4-2: If patients may be exposed occupationally, such as by working with fluorochemicals or as a firefighter, clinicians should consult with occupational health and safety professionals knowledgeable about the workplace practices to determine the most feasible ways to reduce that exposure.

Recommendation 4-3: Clinicians should advise patients with elevated PFAS in their drinking water that they can filter their water to reduce their exposure. Drinking water filters are rated by NSF International, an independent organization that develops public health standards for products. The NSF database can be searched online for PFOA to find

[7] The committee's recommendations are numbered according to the chapter of the main text in which they appear.

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filters that reduce the PFAS in drinking water included in the committee's charge. Individuals who cannot filter their water can use another source of water for drinking.

Recommendation 4-4: In areas with known PFAS contamination, clinicians should advise patients that PFAS can be present in fish, wildlife, meat, and dairy products and direct them to any local consumption advisories.

There are fewer evidence-based exposure-reduction recommendations for patients without known sources of exposure:

Recommendation 4-5: Clinicians should direct patients interested in learning more about PFAS to authoritative sources of information on how PFAS exposure occurs and what mitigating actions they can take. Authoritative sources include the Pediatric Environmental Health Specialty Units (PEHSUs), the Agency for Toxic Substances and Disease Registry (ATSDR), and the U.S. Environmental Protection Agency (EPA).

Recommendation 4-6: When clinicians are counseling parents of infants on PFAS exposure, they should discuss infant feeding and steps that can be taken to lower sources of PFAS exposure. The benefits of breastfeeding are well known; the American Academy of Pediatrics, the American Academy of Family Physicians, and the American College of Obstetricians and Gynecologists support and recommend breastfeeding for infants, with rare exceptions. Clinicians should explain that PFAS can pass through breast milk from a mother to her baby. PFAS may also be present in other foods, such as the water used to reconstitute formula and infant food, and potentially in packaged formula and baby food. It is not yet clear what types and levels of exposure to PFAS are of concern for child health and development.

Additionally, there is a critical need for more data to understand PFAS exposure among breastfed infants:

Recommendation 4-7: Federal environmental health agencies should conduct research to evaluate PFAS transfer to and concentrations in breast milk and formula to generate data that can help parents and clinicians make shared, informed decisions about breastfeeding.

At this time, it not possible to eliminate all sources of PFAS exposure. There are some sources people can try to limit if they desire and have the resources to do so. If patients are resource-limited, it is most important that if PFAS contamination of their water is known or suspected, they use water filtration or another source of water for drinking that is lower in PFAS. In keeping with the principle of adaptability, it is also important to direct patients to reliable sources of information on PFAS, such as ATSDR, the U.S. Environmental Protection Agency, and state and local departments of public health so they can obtain accurate and up-to-date information.

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5

PFAS Testing and Concentrations to Inform Clinical Care of Exposed Patients

The Centers for Disease Control and Prevention (CDC) and various other health agencies routinely conduct biomonitoring, including measuring of exposure biomarkers in human tissues, as an important part of environmental public health surveillance (Latshaw et al., 2017). With the exception of a few environmental exposures, however, such as children's blood lead measurements, biomonitoring is rarely included in routine clinical care. Biomonitoring integrates all sources and routes of exposure into measurements of internal exposure. In this regard it is advantageous because it is more reflective of aggregate exposure than are measures of external exposure, but as a consequence, it does not identify the specific source of an exposure. Nonetheless, when properly interpreted, biomonitoring data can be used to monitor exposure levels and trends, evaluate potential health risks connected to specific sites or populations, and inform public health decisions (Latshaw et al., 2017).

Biomonitoring can be based on the direct measurement of environmental chemicals or their reaction or breakdown products (metabolites) in human tissues and fluids, such as blood (serum, plasma, and whole blood), urine, hair, nails, and breast milk (CDC, 2021; NRC, 1987). Traditionally, biomarkers have been classified as biomarkers of exposure, effect, or susceptibility (see Figure 5-1).

This chapter addresses options and considerations to guide decision making for per- and polyfluoroalkyl substances (PFAS) testing in a patient's biological samples, strategies for interpreting biomonitoring data, and PFAS concentrations that could inform clinical care of exposed patients.

OPTIONS AND CONSIDERATIONS TO GUIDE DECISION MAKING FOR PFAS TESTING

PFAS Laboratory Methods

There are no standard methods for PFAS exposure biomonitoring; some, but not all, laboratories use methods similar to those used by the CDC. Unlike most clinical laboratories, laboratories that offer PFAS testing are not subject to measurement standardization through external proficiency testing programs that evaluate laboratory performance against preestablished criteria. Laboratories that offer PFAS testing also need not comply with clinical certification, such as Clinical Laboratory Improvement Amendments (CLIA) certification, for reporting of results to patients. To support the quality and integrity of results, PFAS testing should be conducted in laboratories that meet the following criteria:

- have an extensive quality assurance/quality control (QA/QC) program (Kannan et al., 2021);
- report National Institute of Standards and Technology Standard Reference Material (NIST-SRM)–traceable data (Kannan et al., 2021); and
- employ laboratory methods with relative standard deviations of less than 15 percent and with limits of detection (LODs) in the picogram/mL region, consistent with the LODs of the CDC and academic laboratories (FDA, 2018; SWGTOX, 2013).

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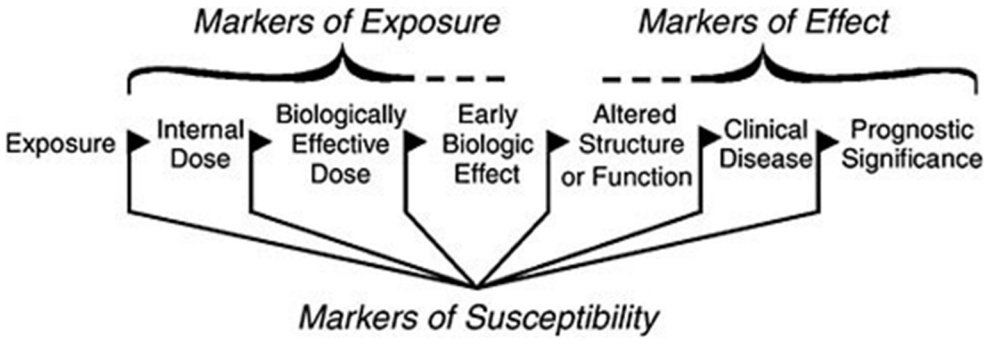


FIGURE 5-1 Simplified flow chart of classes of biomarkers.
SOURCE: NRC, 1987.

Which PFAS are most important to measure depends on the reasons for testing. A method comparable to that of the CDC that reports the linear and branched isomers of PFAS will allow comparison of individual results with those in the *National Report on Human Exposure to Environmental Chemicals* (CDC, 2015, 2021)—an informative method for individuals who wish to understand whether their exposure is high or low compared with background exposures in the U.S. population. For people with a past history of exposure to PFAS not included in the CDC panel, laboratory tests need to detect the compounds to which the person is suspected to have been exposed. However, adequate targeted exposure biomonitoring approaches may not currently exist for measuring many PFAS—especially those that are emerging or newly studied—that may be of concern in some communities. Most laboratories can test only for a limited number of PFAS; a typical PFAS panel will include fewer than 25 specific PFAS. SRMs exist for some but not all PFAS. NIST 1957 has reference values for 7 PFAS, and NIST 1958 includes 5 PFAS. The availability of SRMs for a broader range of PFAS would support higher-quality data for more PFAS.

Interpretation of PFAS biomonitoring data depends on the analytical methods being used, the matrix (blood, serum, plasma, urine, breast milk, etc.) in which the PFAS are measured, and the pharmacokinetics of the PFAS being measured.

Analytical Methods

Biological samples are generally analyzed using targeted analytical methods (i.e., for specific PFAS for which methods and standards are available), leaving a wide array of PFAS unaccounted for. Methods for measuring total extractable or adsorbable organofluorine can circumvent the limitations of targeted analytics, but these methods are also plagued by important challenges. Some non-PFAS are merged into the total organofluorine load. Moreover, clear health guidance values for total organofluorine have not been—and may very well never be—derived (see Box 5-1) because toxicological testing and risk assessments are typically conducted on a chemical-by-chemical basis so regulations can be derived for specific chemicals, although it is well known that single-chemical exposures are not reflective of how exposures occur in reality (NRC, 2009). Untargeted testing can also be used to assess exposure to PFAS, but is less standardized and precise than current targeted testing. Untargeted approaches, especially those that employ multiplexing techniques to account for high levels of endogenous chemicals, may provide a better overall picture of total PFAS exposure; as of now, however, those data would not be quantifiable and would provide only a qualitative (presence or absence) estimate of exposure (Guo et al., 2022).

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BOX 5-1
Total Organofluorine Testing

In communities that could be exposed to many different PFAS, total organofluorine testing could be considered. This method measures all the organic compounds containing fluorine in a sample. In so doing, it addresses the challenge of the lack of a standard reference for so many PFAS. In a study of blood samples from the Swedish population, for example, 70 percent of organofluorine compounds measured in samples from women aged 16–44 using extractable organofluorine measurements were not accounted for by the 63 PFAS measured in targeted analyses (Aro et al., 2021). However, these measures of total organic fluorine include compounds that have a fluorine atom but are not typically considered PFAS (e.g., pesticides such as trifluralin, fluometuron, and benefin; or drugs such as Prozac). With untargeted mass spectroscopy techniques, then, it may be possible to detect many more PFAS, but confirming their identity and quantifying them can be challenging if authentic standards are not available for them, which is often the case. At this point, moreover, methods for measuring total organofluorine are not standardized and are used primarily in academic and research laboratories (Aro et al., 2022), and there are no reference- or risk-based concentrations with which to compare measurements, making interpretation difficult. Still, the committee believes total organofluorine measurements hold promise for addressing the vast number of PFAS compounds as a class and notes that at least one commercial laboratory has started using this testing.

Biological Matrix

The specific PFAS to which a patient is at risk of exposure may also dictate the biological samples (e.g., blood, urine) that should be collected. Most studies measure PFAS in serum, which is likely the best matrix for measuring PFAS with long biological half-lives (Calafat et al., 2019). Other matrices, such as whole blood or urine, may be better suited to the detection of PFAS with short biological half-lives or those whose distribution in the body differs from that of the most commonly studied PFAS, such as perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic (PFOS) (Calafat et al., 2019; Poothong et al., 2017).

PFAS can be measured in multiple biological matrices, including but not limited to whole blood, serum, plasma, urine, breast milk, and hair (Alves et al., 2015). Overall, most studies have used either serum or plasma to evaluate PFAS exposure, and the measured concentrations in serum and plasma are comparable for PFOA, PFOS, and perfluorohexane sulfonate (PFHxS) (Ehresman et al., 2007). PFAS concentrations in other matrices can provide information on exposure during specific periods of life; for example, PFAS levels in segments of hair can help reconstruct historical exposures, and PFAS levels in breast milk make it possible to estimate infants' intake through breastfeeding (Zheng et al., 2021). On multiple occasions, this study's town hall participants indicated that they wanted to know not only their serum PFAS levels but also their breast milk levels, as these translate into early-life exposure for their children.

While urine, breast milk, and hair samples can be obtained relatively noninvasively, biomonitoring efforts in the United States have focused largely on serum, which is one of the most important biological compartments for the distribution of many PFAS in the body. Consequently, reference ranges and risk-based biomonitoring levels are limited or unavailable for other matrices. To date, the published literature provides measurements in breast milk for fewer than 200 people in the United States. If breast milk levels are measured, they can be interpreted by comparison with breast milk levels estimated using data from the National Health and Nutrition Examination Survey (NHANES), a series of surveys on the health status, health-related behaviors, and nutrition of the U.S. population (Lakind et al., 2022). Finally, much remains unknown about the distribution of the wide array of PFAS; for some of them, such traditional matrices as serum may not be optimal for estimating exposure to those PFAS whose distribution into these matrices is not significant.

Micro-samples are samples in which blood, usually from a pricked finger or heel stick, is collected into a small cuvette or spotted onto specially prepared filter paper and dried in open air under

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ambient conditions. Micro-samples have several advantages: they are minimally invasive, do not have to be collected by a phlebotomist, reduce biohazard risks, and in some cases do not require refrigeration or freezing (Freeman et al., 2018). On the other hand, the blood collected from a pricked finger is capillary blood, which, unlike venous blood, contains interstitial fluid, and research is needed to determine whether capillary blood concentrations are comparable to serum measurements. Capillary blood also requires validation, particularly if collected as dried blood spots because volume is difficult to estimate as a result of hematocrit and chromatographic effects, although K+ standardization is showing promise (Barr et al., 2021). Newborn blood spots have, however, been used successfully in research studies to evaluate exposures to PFAS and assess changes in exposures over time (Gross et al., 2020; Ma et al., 2013; Yeung et al., 2019).

Pharmacokinetics

Some PFAS, including PFOA and PFOS, have a relatively long biological half-life, and measurement of these PFAS in serum may represent chemical exposures that occurred in past years. For some other PFAS, half-lives are on the order of days. For example, GenX is estimated to have a biological half-life of 81 hours (Clark and TCS, 2021); as a result, its measurement in serum or urine likely represents exposure that occurred much more recently, such as in the last few weeks. In such cases, samples taken at different times may be needed to derive an adequate representation of long-term exposure. Alternative measures for estimating long-term exposure include exposure reconstruction of drinking water levels. Also, some physiological events may influence biological levels of PFAS. Pregnancy and breastfeeding can increase PFAS excretion, for example, so levels in mothers may not be reflective of levels prior to pregnancy (Kato et al., 2014).

Sample Contamination

The concentrations of PFAS measured in most human tissues can be considered trace concentrations, making sample contamination an issue. Contamination occurring during sample collection, transport, storage, or preparation can impact the ability to interpret a laboratory result. For example, PFAS are commonly used in medical collection equipment, such as Teflon-capped blood test tubes. Teflon should therefore be avoided in sample collection equipment intended for PFAS measurement (NASEM, 2018a), and field blanks may need to be performed to ensure that contamination is negligible.

Commercially Available PFAS Testing Laboratories

A few laboratories currently offer PFAS testing. NMS labs (Horsham, Pennsylvania) offers PFAS testing through a clinician request for out-of-pocket payment (Test Code 39307, CPT Code[s] 82542; reported by town hall speakers to cost more than $600) or reimbursement from insurance. NMS can measure six PFAS in serum: perfluorobutanesulfonic acid (as the linear isomer) (PFBS), perfluoroheptanoic acid (as the linear isomer) (PFHpA), perfluorohexanesulfonic acid (as the linear isomer) (PFHxS), perfluorononanoic acid (as the linear isomer) (PFNA), perfluorooctanesulfonic acid (as the linear isomer) (PFOS), and perfluorooctanoic acid (as the linear isomer) (PFOA).[1] It is important to note that although the NMS panel does not completely overlap with the specific PFAS the committee was charged to evaluate, it does include PFOA, PFOS, and PFNA, three of the PFAS that are commonly reported as detected in NHANES and therefore typically contribute the most to exposure. The NMS panel would be more comparable to NHANES if it included the branched isomers of PFOS and PFOA, and PFHxS.

[1] See https://www.nmslabs.com/tests/3427SP (accessed June 16, 2022).

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EMPower DX (Framingham, Massachusetts), a subsidiary of Eurofins Scientific (Luxembourg City, Luxembourg), recently began offering direct-to-consumer testing for more than 40 PFAS via self-collected finger prick sample at a cost of $399.[2] The website for this lab reports that the test will "quantitatively determine PFAS concentrations … and positive or negative detections are reported with 99% confidence" (para. 4). In an email communication, company representatives reported that NIST reference standards are used, and that coefficients of variation are being developed for all PFAS in the panel.[3] As discussed above, method validation is needed for PFAS measurements from capillary blood to know how comparable they are to measurements from other PFAS tests.

Harms and Benefits of PFAS Testing

Based on the committee's principle of *proportionality* (see Chapter 2), it is necessary to consider the plausible harms and benefits associated with PFAS testing, although the weight of each will depend on the individual. Harms of PFAS testing include fear induced by blood draw, a small risk of injury or infection at the draw site, difficulties in interpreting results, and psychological stress that may occur when people who are tested learn that they or their family members have high levels of PFAS exposure. On the other hand, biomonitoring for PFAS blood levels may also alleviate fears associated with not knowing one's PFAS levels. Another potential benefit is increasing awareness of exposure so that exposures can be reduced. If sources of exposure are identified, actions taken to reduce these exposures, such as using a water filter, may also benefit family members in addition to the person who was tested. Community-level benefits may be associated with PFAS testing as well, such as empowering communities to respond to contamination and providing a baseline with which to evaluate the impact of community-level interventions to reduce exposure. Additionally, biomonitoring for PFAS in the context of epidemiologic studies could provide more information about PFAS-associated health effects.

To evaluate these potential harms and benefits associated with biomonitoring, the committee considered research studies evaluating the harms and benefits of reporting biomonitoring results to study participants (report-back). Report-back has been an active area of research by multidisciplinary teams, focused on whether and how to report study results to participants. These studies have varied in design, but methods used generally include interviews with study participants, investigators, and/or institutional review boards. They may also include focus groups; advisory councils; stakeholder workshops; observations at community meetings; one-on-one user testing of reports; and digital analytics, such as page views or time spent interacting with digital results pages. Investigators in these studies have identified harms associated with reporting of biomonitoring results, such as stress or concerns about the health effects associated with exposures, but the levels of stress reported have not been high enough to be considered extreme worry or panic (Emmett et al., 2009; Ohayon et al., 2017; Ramirez-Andreotta et al., 2016). Report-back studies have found further that when biomonitoring results are returned with information about how study participants could be exposed to the chemicals, what health effects are potentially associated with exposure, and what strategies could reduce exposure, participants express appreciation for receiving the results and feel empowered by the information (Adams et al., 2011; Altman et al., 2008; Brody et al., 2014; Giannini et al., 2018; Hernick et al., 2011; Perovich et al., 2018; Ramirez-Andreotta et al., 2016; Tomsho et al., 2019). Moreover, research studies have found that participants appreciate receiving test results even when the health implications of those results are unclear (Adams et al., 2011; Hernick et al., 2011; NASEM, 2018b). One study that compared the benefits of receiving individual versus aggregate biomonitoring results found that the former motivated participants to access information about environmental sources of chemical exposure and their health effects. The study also found that personal report-back increased engagement with exposure reports among Black participants.

[2] See https://empowerdxlab.com/products/product/pfas-exposure-test (accessed June 16, 2022).
[3] Personal communication, R. Mitzel, President Eurofins Air Toxics, LLC SVP Eurofins Specialty Services, December 6, 2021.

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Report-back was associated with a small increase in psychological stress, but the authors suggest that this stress could motivate appropriate behavior change to reduce exposure (Brody et al., 2021).

In many cases, community members who provided testimony at the committee's town halls were strongly in favor of PFAS testing and described being frustrated with the numerous difficulties encountered in accessing the testing (see Appendix B). Kristen Mello of Westfield Residents Advocating for Themselves (WRAFT) said to the committee:

> You don't have a problem getting an insurance assessor when your car is hit, you don't have a problem getting an insurance assessor when you have a tornado, but this slow motion unfolding environmental and public health disaster … is intentionally keeping the information from us so that we cannot take action.

Furthermore, Emily Donovan of Clean Cape Fear, said:

> Sadly it feels like guinea pigs are treated better, because at least their exposures are thoroughly studied for the betterment of humanity.

And Cathy Wusterbarth of Need Our Water said:

> We've tested the fish; we've tested the deer; we've tested the groundwater, the waterways, and the foam. When are we going to test the people?… The only risk [of testing] is to the polluters who do not want us to link them to our exposure.

Community members stated that they want access to PFAS testing so that they can understand their personal level of exposure and, as suggested by Ayesha Khan of Nantucket PFAS Action Group, "help those who are exposed to be proactive in reducing exposure and managing risk." An example of the how PFAS testing can help people manage health risks was presented by Sandy Wynn-Stelt, who learned that the drinking water in her home was contaminated with PFAS at a level more than 1,000 times the current U.S. Environmental Protection Agency (EPA) health advisory and paid out of pocket for PFAS testing. She shared her results with her clinician, and they decided to conduct clinical follow-up for any of the conditions associated with PFAS, including impacts on thyroid hormone levels. The results of her thyroid tests led to other follow-up and ultimately to her diagnosis of thyroid cancer. Wynn-Stelt believes her PFAS testing led to an earlier diagnosis and better outcomes for her cancer.

Social and Ethical Implications of PFAS Testing

Many town hall speakers voiced frustration about not having access to testing for PFAS. Speakers mentioned the injustices related to having been exposed to PFAS without their consent or knowledge and their frustration with being unable to access testing. They shared that PFAS testing, when available, is expensive. Town hall speakers also suggested that discussion of PFAS testing should be conducted in a culturally sensitive manner, be available in a variety of languages, and be at a reading level that the average American can understand (e.g., 4th-grade level).

Another important social consideration is that people who are tested for PFAS may experience social and economic conflicts related to their testing. If a PFAS blood test identifies community contamination, business revenue and property values may be adversely affected (Harclerode et al., 2021). People who draw attention to the contamination may experience anger and social isolation from other community members. Indeed, one town hall speaker asked that their name be removed from the town hall agendas and the committee's website because they were worried about angering neighbors by talking about their contaminated well.

Art Schaap, a dairy farmer in New Mexico, discovered that his farm was unknowingly subjected to severe PFAS contamination due to use of military aqueous film-forming foam (AFFF). After learning

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of the contamination, Schaap voluntarily tested his herd for PFAS and discovered that his cows were highly exposed. The PFAS contamination on Schaap's farm devastated his livelihood because he had limited options available for getting rid of the contaminated animals in any profitable way. The dairy, beef, and rendering industries do not want PFAS-contaminated animals or products. The result for Schaap was the stranding of at least 4,000 cows, the death of 1,200, and the dumping of 1,500 loads of milk (see Appendix B for Schaap's complete testimony).

PFAS Testing: Findings and Recommendations

Applying the principle of *proportionality*, the committee believes that the benefits of PFAS testing for those who request it typically outweigh the harms (see Box 5-2). The harms reported in studies of report-back of biomonitoring results are worry about the harms of exposure, decreased property values, and potential social isolation or ostracism. In many cases, people who request testing are already worried about their exposure, which is why they are requesting the testing, and they may already be at risk of decreased property values associated with contamination. People in exposed communities have been "contaminated without consent" and "poisoned without permission."[4]

BOX 5-2
Potential Harms and Benefits of PFAS Testing

Potential Harms

- Fear of blood draw
- Small risk of injury or infection at draw site
- Difficulties in interpreting results
- Stress or concern about the health effects from exposure
- Decreased property values
- Social isolation
- Clinical consequences from medical follow-up as a result of exposure

Potential Benefits

- Increased awareness of exposure so that exposure can be reduced
- Empowerment of communities to respond to contamination
- Relief from the stress of not knowing one's exposure level
- Identification of the potential risk for health conditions associated with PFAS exposure to inform subsequent preventive care
- Help in monitoring whether efforts to reduce exposure are working through the conduct of baseline and follow-up tests.

An important element of the principle of *justice* is making PFAS testing easily accessible and readily available to all regardless of ability to pay, race, ethnicity, age, occupation, or location. Some populations are at increased risk of PFAS exposure and therefore may be at increased risk for a wide range of health conditions. These factors favor making exposure biomonitoring available to all who desire it. The principle of *autonomy* (i.e., respect for the ability of people to make their own health decisions) also favors allowing people who are likely to have a history of elevated exposure to PFAS and want PFAS testing to receive it and those who do not, to refuse it. These decisions require *shared decision making*[5] between patient and clinician and should include clarifying that exposure biomonitoring results

[4] Quotes from Andrea Amico, speaker at the committee's meeting on July 13, 2021.

[5] Barry and colleagues (2012) build on concepts in Charles (1997) and the Institute of Medicine's (2001) *Crossing the Quality Chasm: A New Health System for the 21st Century*, describing shared decision making as the

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do not predict future health conditions and can only indicate the potential for an increased risk for certain conditions associated with exposures. Allowing people the opportunity to discuss with their clinicians whether they should undergo PFAS testing shows respect for patient values and is particularly important for people who have experienced environmental injustice as a result of PFAS contamination in their community.

The committee acknowledges that important factors need to be addressed. There are deficiencies in the current cost payment model, and the availability of PFAS testing may need to be addressed. Clinicians and health care facilities will also need to be made aware of reimbursement policies and laboratory codes (e.g., CPT; Reference Lab Order Code). New Hampshire recently required that health insurance cover PFAS blood testing.[6] PFAS testing can currently be ordered online without a provider, and the committee believes the testing and interpretation of its results are most beneficial if done with the guidance of a clinician.

The committee makes the following recommendations:

Recommendation 5-1: As communities with PFAS exposure are identified, government entities (e.g., Centers for Disease Control and Prevention [CDC]/Agency for Toxic Substances and Disease Registry [ATSDR], public health departments) should support clinicians with educational materials about PFAS testing so they can discuss testing with their patients. These educational materials should include the following information:

- **How people can be exposed to PFAS: Exposure routes include occupational exposures and work with fluorochemicals or as a firefighter; consumption of contaminated drinking water in communities that obtain their water from sources near commercial airports, military bases, fluorochemical manufacturing plants, wastewater treatment plants, landfills, or incinerators where PFAS-containing waste may have been disposed of or farms where sewage sludge may have been used; and consumption of contaminated fish or game if fishing or hunting occurs in contaminated areas. Individuals living near fluorochemical plants may also be exposed via inhalation of air emissions.**
- **Potential health effects of PFAS exposure and strategies for reducing exposure.**
- **Limitations of PFAS blood testing: PFAS blood testing does not identify the sources of exposure or predict future health outcomes; it only assesses body burden at the time of sample collection. For example, a person with low blood levels today may have had higher levels in the past.**
- **The benefits and harms of PFAS testing.**

Recommendation 5-2: Clinicians should offer PFAS testing to patients likely to have a history of elevated exposure. In all discussions of PFAS testing, clinicians should describe the potential benefits and harms of the testing and the potential clinical consequences (such as additional follow-up), related social implications, and limitations of the testing so that patient and clinician can make a shared, informed decision. Patients who are likely to have a history of elevated exposure to PFAS include those who have

pinnacle of patient-centered care: "The process by which the optimal decision may be reached ... is called shared decision-making and involves, at minimum, a clinician and the patient.... In shared decision-making, both parties share information: the clinician offers options and describes their risks and benefits, and the patient expresses his or her preferences and values. Each participant is thus armed with a better understanding of the relevant factors and shares responsibility in the decision about how to proceed" (p. 780).

[6] See https://providernews.anthem.com/new-hampshire/article/coverage-for-pfas-and-pfc-blood-tests-for-new-hampshire-residents (accessed June 17, 2022).

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- **had occupational exposure to PFAS (such as those who have worked with fluorochemicals or served as a firefighter);**
- **lived in communities where environmental and public health authorities (Centers for Disease Control and Prevention [CDC], Agency for Toxic Substances and Disease Registry [ATSDR], U.S. Environmental Protection Agency [EPA], state and local environmental or health authorities), or academic researchers have documented PFAS contamination; or**
- **lived in areas where PFAS contamination may have occurred, such as near facilities that use or have used fluorochemicals, commercial airports, military bases, wastewater treatment plants, farms where sewage sludge may have been used, or landfills or incinerators that have received PFAS-containing waste.**

When clinicians discuss results of PFAS with patients, the results will be most useful if accompanied by information on how exposure occurs, the potential associated health effects, and strategies that may reduce exposure—the same contextual information included in the report-back literature reviewed by the committee. Clinicians may also want to consider the frequency of PFAS testing based on the considerations in Box 5-3.

BOX 5-3
Considerations for Frequency of PFAS Testing

- Consider confirmatory retesting when the result is much higher or lower than anticipated given exposure history.
- Consider retesting if exposure changes because
  - public health actions (such as drinking water treatment programs or site cleanup are taken to reduce exposure);
  - the patient takes action(s) to reduce exposure (such as installing water filters, moving from a community with known high levels of PFAS in drinking water, or modifying occupational exposures); or
  - the patient moves into a community with known high levels of PFAS or otherwise has a suspected increase in exposure risk.
- For follow-up testing of PFAS with a long half-life, allow at least a year before retesting.
- Retesting is of no or limited value if initial serum levels are low and exposure does not change.

STRATEGIES FOR INTERPRETING BIOMONITORING DATA

There are two general strategies for interpreting chemical concentrations for exposure biomonitoring: reference-based (or descriptive) and risk-based (NRC, 2006). Both approaches can be useful to inform clinical care of exposed patients. Reference-based approaches can allow a clinician to understand whether the concentration of a chemical in the patient's biological sample is within or outside of the normal background range, while risk-based approaches can allow a clinician to determine whether a patient's biological concentration is below or above a value associated with tolerable, negligible, or minimal risk. Note that comparing concentrations of individual PFAS against reference- or risk-based levels may underestimate overall exposure to a complex mixture of PFAS and associated risk, such as in areas contaminated with PFAS used to replace other PFAS that have been phased out.

Figure 5-2 illustrates the distribution of biomarker concentrations in a generic reference population, expressed as cumulative frequency. As is commonly done in a clinical test, the 95th percentile of the distribution can be used to determine the upper-limit value of the test result. However, a different percentile may be chosen, depending on the circumstances, the characteristics of the reference population, the distribution of the results, and the purpose of the study. It is important to be aware that a particular

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cutpoint does not represent a level that separates the population into typical versus highly exposed (NRC, 2006). Box 5-4 describes the use of reference ranges to interpret other environmental exposures, such as exposures to lead and arsenic (see Box 5-4).

Reference-Based Approaches

Use of a reference-based approach to interpret biomonitoring data requires descriptive statistics from a reference population against which to compare the data. Typically, an individual's result would be compared with a statistical review of the reference data, typically in the form of a data distribution, such as 10th, 25th, 50th, 75th, and 95th percentiles within the reference population. The reference range offers a point of comparison, although some people or subgroups within that range may be subject to more or less exposure. This section describes a number of interpretive issues that arise with this approach. The validity and utility of biomonitoring values for use as reference ranges depend on the design of the studies from which those values were derived and the quality of the data, with special attention to the availability and comparability of data on the reference population in relation to the study population (NRC, 2006).

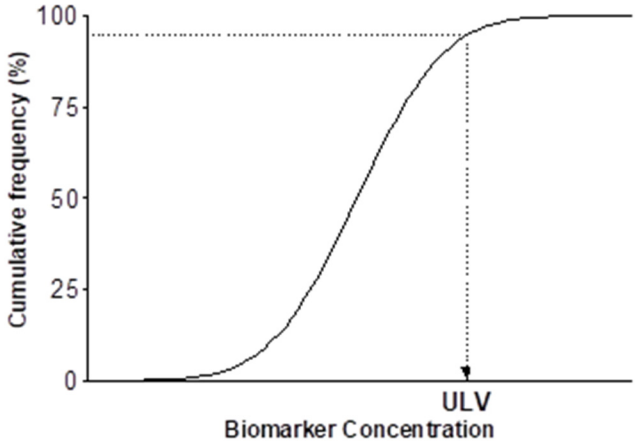


FIGURE 5-2 Distribution of biomarker concentrations in a generic reference population.
NOTE: ULV = upper limit value.
SOURCE: NRC, 2006.

BOX 5-4
Clinical Use of Reference Ranges

The examples of lead and arsenic may serve to illustrate the clinical use of biomonitoring results and reference ranges. In 2009, an elevated blood lead level (BLL) was defined by the Council of State and Territorial Epidemiologists as 10 micrograms per deciliter (µg/dL) or greater for children and adults. Subsequently, additional research documented health effects at BLLs below 10 µg/dL. In 2012, the Centers for Disease Control and Prevention (CDC) and the Advisory Committee on Childhood Lead Poisoning recommended that a BLL of 5 µg/dL or greater be defined as elevated. This reference range value was based on the 97th percentile of the BLL distribution for children aged 1–5 years, using 2007–2010 National Health and Nutrition Examination Survey (NHANES) data. This reference range value can be updated every 4 years using the two most recent NHANES surveys.[a] In May 2021, a BLL reference value of 3.5 µg/dL was set using the two most recent NHANES surveys to identify children with BLLs higher than those of most children. The adult elevated level was set at 5 µg/dL in 2012 on the basis of evidence of adverse health effects with no BLL threshold identified. The mean BLL at that

continued

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BOX 5-4 *continued*

time was 1.09 µg/dL for U.S. adults older than age 20, with a 95th percentile value of 3.36 µg/dL. When a patient presents with a BLL above this reference value, the clinician, often in conjunction with public health officials, will initiate medical surveillance that includes repeated testing at intervals based on the level, history taking as to potential sources of the lead exposure, evaluation for signs and symptoms of lead toxicity, and treatment if necessary.[b]

Reference ranges are not used as commonly to interpret urinary arsenic levels. NHANES reports urinary total arsenic results (inorganic arsenic and methylated metabolites) to survey participants based on the following categories: ≤50 micrograms per liter (µg/l) is considered normal; >50 to <200 µg/L is considered high normal; and ≥200 µg/L is considered high. NHANES recommends that participants with a "high" result consult their medical provider. In a clinical setting, when the level of urinary arsenic is high normal (above 50 µg/dL or 100 µg/dL depending on the laboratory), the clinician will search for sources of exposure to arsenic and evaluate for signs and symptoms of arsenic toxicity. Diagnosis of arsenic toxicity is based on integration of exposure history, clinical findings, and laboratory results. In acute arsenic toxicity, total urine arsenic is typically greater than 1000 µg/L. In chronic arsenic toxicity, simple comparison with the "normal range" is not sufficient. It is important to evaluate for signs of arsenic toxicity and compare urine levels with a toxicity threshold from the literature (Baker et al., 2018).

[a] See https://www.cdc.gov/niosh/topics/ables/ReferenceBloodLevelsforAdults.html#_ftn3 (accessed June 17, 2022).
[b] See https://cdn.ymaws.com/www.cste.org/resource/resmgr/PS1/15-EH-01_revised_12.4.15.pdf (accessed June 17, 2022).

The reference range approach depends on data availability and data comparability for both the method used to measure PFAS and the population with which the results are being compared. Ideally, the reference population includes people similar in age, race or ethnicity, sex, and other demographic factors to the person whose PFAS testing result is being interpreted. Additionally, it is important that the PFAS be analyzed in the same tissues or fluids (blood, serum, breast milk, urine, etc.) and that the chemical analysis methods used are comparable and measure the same PFAS (NRC, 2006).

Note that interindividual variability in PFAS testing results may be a function of differences not only in exposure but also in pharmacokinetics with respect to excretory clearance. Such host factors as parity, breastfeeding status, menstrual status, age, genetic polymorphisms, concurrent acute or chronic disease, and medication use can affect pharmacokinetics.

Because NHANES is conducted in only a few communities each year, regional estimates or even urban versus rural comparisons cannot be made. Table 5-1 provides the most recent years of NHANES data (in serum ng/mL) for the total population sampled (ages 12 and older) for the four most predominant PFAS: PFOA, PFOS, PFHxS, and PFNA. These PFAS have been detected in almost all NHANES participants. Since the production and use of PFOS and PFOA were phased out, their levels have been declining (Brennan et al., 2021). Important limitations of NHANES data are that data for vulnerable populations, such as children younger than 12 years old and pregnant persons, are not always available. Children younger than 12 also are not included in the *National Report on Human Exposure to Environmental Chemicals*, although there are published estimates of PFAS exposure for children aged 3–11 years for 2013 and 2014 (Ye et al., 2018). Pregnant people are also not included in large numbers in NHANES, so multiple years of NHANES data will need to be combined to obtain a large enough sample for comparison (Watson et al., 2020); it may be more appropriate to compare pregnant people with the data for females in the same age range and time period. NHANES also does not specifically enroll participants in PFAS-exposed communities.

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Distribution of PFAS Concentrations in Exposed Communities

While NHANES provides descriptive statistics for the general U.S. population, it is noteworthy that clinicians in exposed communities will likely encounter higher PFAS levels in their patients. Discussed below are examples from some of the contaminated communities throughout the United States. The first community identified as having known PFOA (C-8) exposure was residents living near the DuPont Teflon-manufacturing plant in Parkersburg, West Virginia. Contamination of six nearby public water districts and hundreds of private drinking water wells in West Virginia and Ohio was discovered, leading to public health concerns. As part of a settlement for a large class action lawsuit against DuPont, the C-8 Science Panel was established to determine potential health effects of PFOA exposure, and a 1-year cross-sectional survey (2005–2006), known as the C-8 Health Project, was conducted among approximately 70,000 residents with contaminated drinking water (Frisbee et al., 2009). The average measured serum PFOA level among residents in Little Hocking, Ohio, with the highest PFOA drinking water contamination was 227.6 ng/mL; for the entire C-8 Health Project survey, the average value for PFOA was 82.9 ng/mL. For comparison, the average PFOA serum level in the general U.S. population was 4.2 (ng/mL) in 2005 (CDC, 2015).

The 3M Company (Maplewood, Minnesota) produced PFAS at its Cottage Grove facility from the late 1940s until 2002. PFOA was a prominent PFAS made at this site. In late 2003, the Minnesota Pollution Control Agency discovered groundwater contamination near Cottage Grove and several other sites in the suburbs east of St. Paul, Minnesota (the 3M Chemolite site, 3M Woodbury site, and 3M Oakdale site, as well as the Washington County Landfill).[7] In 2006, water filtration systems for polluted public and private wells were installed to reduce PFAS exposure. The Minnesota Department of Health completed three projects to test blood levels of PFAS in people living in the east metro area of St. Paul (see Figure 5-3).

TABLE 5-1 Distributions of Serum PFAS Concentration (nanograms per milliliter [ng/mL]) in Four Cycles of the National Health and Nutrition Examination Survey (NHANES), 2011–2018

PFAS Chemical	NHANES Survey Years	Geometric Mean (95% confidence interval [CI])	50th Percentile (95% CI)	95th Percentile (95% CI)
PFOA	2011–2012	2.08 (1.95–2.22)	2.08 (1.96–2.26)	5.68 (5.02–6.49)
	2013–2014	1.94 (1.76–2.14)	2.07 (1.87–2.20)	5.57 (4.60–6.27)
	2015–2016	1.56 (1.47–1.66)	1.57 (1.47–1.77)	4.17 (3.87–4.67)
	2017–2018	1.42 (1.33–1.52)	1.47 (1.37–1.57)	3.77 (3.17–5.07)
PFOS	2011–2012	6.31 (5.84–6.82)	6.53 (5.99–7.13)	21.7 (19.3–23.9)
	2013–2014	4.99 (4.50–5.52)	5.20 (4.80–5.70)	18.5 (15.4–22.0)
	2015–2016	4.72 (4.40–5.07)	4.80 (4.40–5.30)	18.3 (15.5–22.7)
	2017–2018	4.25 (3.90–4.62)	4.30 (3.80–4.90)	14.6 (13.1–16.5)
PFNA	2011–2012	0.881 (0.801–0.968)	0.860 (0.750–0.960)	2.54 (2.28–2.89)
	2013–2014	0.675 (0.613–0.742)	0.700 (0.600–0.800)	2.00 (1.80–2.30)
	2015–2016	0.577 (0.535–0.623)	0.600 (0.500–0.600)	1.90 (1.50–2.20)
	2017–2018	0.411 (0.364–0.464)	0.400 (0.400–0.500)	1.40 (1.10–1.80)
PFHxS	2011–2012	1.28 (1.15–1.43)	1.27 (1.11–1.45)	5.44 (4.61–6.82)
	2013–2014	1.35 (1.20–1.52)	1.40 (1.20–1.60)	5.60 (4.70–7.10)
	2015–2016	1.18 (1.08–1.30)	1.20 (1.10–1.40)	4.90 (4.10–5.80)
	2017–2018	1.08 (0.996–1.18)	1.10 (1.00–1.20)	3.70 (3.30–5.60)

[7] See https://www.pca.state.mn.us/waste/pfas-investigation-and-clean-up (accessed June 17, 2022).

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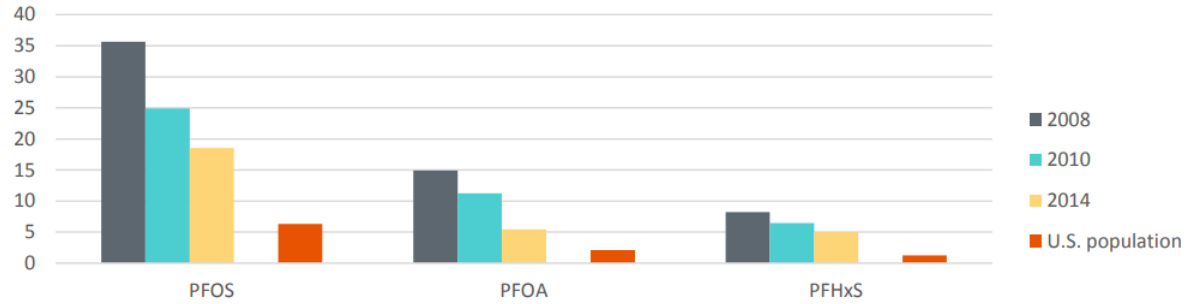


FIGURE 5-3 Geometric means of PFAS in blood from east metro St. Paul biomonitoring, in nanograms per milliliter (ng/mL).
SOURCE: U.S. population levels are from the 2011–2012 National Health and Nutrition Examination Survey (NHANES).

In Hoosick Falls, New York, high PFOA levels in water led to a public outcry, and a federal class action lawsuit was filed against Saint-Gobain Performance Plastics and Honeywell International. In 2016, the geometric mean PFOA serum level among participants in the Hoosick Falls Biomonitoring Study who used village water was 43.5 ng/mL (N = 1,640).[8] In the aftermath of Hoosick Falls, impacted community members voiced significant concern that the EPA's recommended health advisory level for drinking water at the time (400 ng/L[9]) provided insufficient protection. In January 2016, the EPA recommended that the community not drink water with PFOA in excess of 100 ng/L,[10] and in May 2016, after reviewing the existing body of PFOA and PFOS data,[11] it revised its health advisory level for drinking water to 70 ng/L for the sum of PFOA and PFOS. In New Hampshire, the state conducted biomonitoring in Merrimack Village District, where another Saint-Gobain plant had contaminated local drinking water. The average PFOA serum levels among participants was 3.9 ng/mL (N = 217).

In 2014, it was discovered that a public water supply well in Pease, New Hampshire, was contaminated with PFOS, PFOA, and PFHxS as a result of the use of firefighting foams at Pease Tradeport, a former Air Force Base. Beginning in April 2015, the New Hampshire Department of Health and Human Services conducted blood testing for people in communities where PFAS had been found in drinking water above lifetime health advisory levels. Between April and October 2015, 1,578 members of the Pease Tradeport community had their blood tested for PFAS. Results of biomonitoring from all individuals in the study showed that the geometric mean of PFAS exceeded that of the general U.S. population for PFOS (Pease: 8.59 ng/mL, NHANES: 6.31 ng/mL), PFOA (Pease: 3.09 ng/mL, NHANES: 2.08 ng/mL), and PFHxS (Pease: 4.12 ng/mL, NHANES: 1.28 ng/mL).[12]

Communities in Bucks and Montgomery counties in Pennsylvania have detected PFOS in their drinking water from use of firefighting foams during military activities. In response to this water contamination, the state health department conducted biomonitoring for PFOS exposure. The average PFOS serum level in this community was 10.2 ng/mL (N = 235).[13] A military base in Newburgh, New

[8] See https://www.health.ny.gov/environmental/investigations/hoosick/docs/infosheetgrouplong.pdf (accessed June 17, 2022).
[9] See https://www.epa.gov/sites/default/files/2015-12/documents/hoosickfallsmayorpfoa.pdf (accessed June 17, 2022).
[10] See https://www.epa.gov/sites/default/files/2016-01/documents/epa_statement_on_private_wells_in_the_town_of_hoosick.pdf (accessed June 16, 2022).
[11] See https://www.epa.gov/sites/default/files/2016-06/documents/drinkingwaterhealthadvisories_pfoa_pfos_updated_5.31.16.pdf (accessed June 16, 2022).
[12] See https://www.dhhs.nh.gov/dphs/documents/pease-pfc-blood-testing.pdf (accessed June 16, 2022).
[13] See https://www.health.pa.gov/topics/Documents/Environmental%20Health/PEATT%20Pilot%20Project%20Final%20Report%20April%2029%202019.pdf (accessed June 16, 2022).

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York, also contaminated drinking water in that community, resulting in a median PFOS serum level of 16.3 ng/mL (N = 1,917).[14]

The primary manufacturer of PFOS, 3M, has also been implicated in drinking water contamination, in Decatur, Alabama. In a community study there, the median level was 39.3 ng/mL (N = 121) among participants with contaminated drinking water.[15]

Risk-Based Approaches

The most complex approaches for interpreting biomonitoring data are those that evaluate the risk associated with a biomonitoring result. Evaluation of risk may be desirable given the importance of the question of how risky a blood concentration is, as well as the fact that the descriptive approaches provide only relative information without assessing the risk to human health (NRC, 2006). Some exposure disease processes are thought to have a "threshold" or exposure level that needs to be exceeded before a response occurs, while others are thought to have a "nonthreshold" response such that there is no level at which no harm occurs. Examples of nonthreshold dose responses at a population level include lead, particulate matter, radon, and secondhand smoke. To set standards for nonthreshold effects, an acceptable risk needs to be determined (NRC, 2009). The standard reflects the expected daily intake associated with an acceptable risk for one or more adverse outcomes. Drinking water health advisories are risk-based standards that use dose estimates associated with daily consumption to predict long-term health effects. As a result, they should not be used to interpret biomonitoring results that represent an integration of multiple exposure sources over different periods depending on PFAS-specific half-lives (see Box 5-5).

BOX 5-5
PFAS Serum Levels Are Not Directly Comparable to PFAS Drinking Water Levels

In an effort to interpret PFAS testing results, members of the lay public sometimes compare PFAS levels in serum with PFAS drinking water advisory levels. This comparison may involve converting the units of the PFAS serum levels (usually given in nanograms per milliliter [ng/mL] of blood) to align with how PFAS drinking water advisory levels are generally reported (as nanograms per liter [ng/L] of water). The committee strongly discourages this comparison. Drinking water advisory levels reflect the levels of PFAS (in ng/L of water) that are assumed to represent a tolerable, negligible, or minimal risk for daily consumption in addition to other sources of exposure. In contrast, PFAS serum (ng/mL) levels are an integration of multiple exposure sources representing different periods of exposure, depending on PFAS-specific half-lives.

The committee also strongly recommends against translating a drinking water advisory level into a "safe" serum PFAS level. Using an online resource (https://www.ics.uci.edu/~sbartell/pfascalc.html [accessed June 16, 2022]; Bartell et al., 2017; Lu and Bartell, 2020), calculations can be made to answer such questions as "How long will it take for my serum PFOA levels to return to normal now that there's no more PFOA in my water?" or "My water has PFOA in it, but my blood hasn't yet been tested; do you expect my serum PFOA levels to be elevated?" The online calculator includes four PFAS chemicals (PFOA, PFOS, PFHxS, and PFNA) and an optional physiological adjustment to the serum half-life for menstruation. Although informative for some questions, the calculator does not allow users to include exposure sources other than drinking water. In contrast, agencies typically account for the contribution of drinking water to total exposure (e.g., 20 percent) when determining drinking water advisory levels. Serum PFAS levels reflect an integration of multiple exposure sources at a single time and are distinct from recommended levels in water for daily consumption.

Instead of comparing PFAS serum levels with PFAS drinking water levels, the committee recommends that individuals refer to health-based and reference-based serum levels.

[14] See https://www.health.ny.gov/environmental/investigations/newburgh/docs/infosheetgroupresults.pdf (accessed June 16, 2022).

[15] See https://www.atsdr.cdc.gov/hac/pha/Decatur/Perfluorochemical_Serum%20Sampling.pdf (accessed June 16, 2022).

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There are several options for deriving serum or plasma concentrations that are consistent with health-based guidance values. In the most straightforward risk-based approach, epidemiologic studies with exposure-response relationships could be used to conduct biomonitoring-based risk assessment. As presented below, the German Human Biomonitoring Commission (HBM Commission) reviewed the epidemiologic literature to compile serum or plasma levels with and without observable effects. Contrasting patient serum PFAS levels with levels with or without effects in an epidemiologic study can facilitate understanding risk for the given response endpoint (Apel et al., 2020).

Another option is to derive serum or plasma concentrations that are consistent with health-based guidance values such as reference doses (RfDs) and tolerable daily intakes (TDIs), commonly referred to as biomonitoring equivalents (BEs) (Hays et al., 2008). Because these values are frequently based on animal experiments, some with no measure of serum concentrations, pharmacokinetic modeling is often used to translate a point of departure (e.g., the benchmark dose) into animal serum levels, which can be converted into BEs by adjusting for uncertainty factors. For this report, the committee focused on the human data to evaluate risk levels.

The committee reviewed the International Human Biomonitoring (i-HBM) Working Group dashboard to search for biomonitoring guidance values for PFAS and found that the only risk-based standards were the German HBM values for PFOS and PFOA. The committee also searched for RfDs based on human data in the tables in the *Environmental Council of the States White Paper on Setting State PFAS Standards* and in the authoritative reviews considered in the committee's review of PFAS health effects (see Chapter 3). The committee found that only the European Food Safety Authority (EFSA) scientific opinion contained a risk-based value estimated in humans.

German HBM Values

The German HBM Commission develops human biomonitoring (HBM) values for interpretation of the concentrations of environmental chemicals measured in biological samples. These values, which are derived for the general population (including vulnerable subgroups) with chronic exposure, represent concentrations below which no observed risk of adverse health effects is expected (HBM-I) or above which adverse health effects are possible (HBM-II) (Schulz et al., 2011).

In 2021, the HBM Commission published HBM-I values for PFOS and PFOA in plasma, which is assumed to have concentrations similar to those of serum (Ehresman et al., 2007; Hölzer et al., 2021). To derive HBM-I values, the HBM Commission conducted a literature review for studies of the health effects of PFOS and PFOA. It then selected epidemiological studies and identified points of departure for various health effects for use in quantifying an association between exposure and critical effects. The determination of these points of departure varied depending on the methods of each study. Where epidemiological analyses were based on quantiles of exposure, the point of departure represented the lower limit of the quantile for which significantly increased risk was observed. For studies using continuous measures of exposure, points of departure were based on either benchmark dose-response modeling or a qualitative assessment of the effects and dose-response relationship. Health outcomes in the reviewed studies included fertility (time to pregnancy), pregnancy-induced hypertension and diabetes, reduced birthweight, serum cholesterol concentrations, serum uric acid concentrations, reduced antibody response to vaccination, pubertal development, thyroid metabolism, and onset of menopause. Once points of departure for each compound, study, and outcome had been selected, HBM-I values were selected in the low range of points of departure for both chemicals. The points of departure identified represented a wide range of exposure values. It was determined that the HBM-1 values were 2 ng/mL for plasma PFOA and 5 ng/mL for plasma PFOS. The HBM Commission considers these HBM-I values to be precautionary. Exceedance of these levels should not be interpreted as increasing risk, but may warrant efforts to reduce exposure (Hölzer et al., 2021).

The HBM Commission also established HBM-II values in 2021, based on epidemiological studies on PFOA or PFOS and adverse health outcomes, including reduced birthweight, developmental effects, reduced fertility, reduced antibody response to vaccination, increased cholesterol concentrations

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(low-density lipoprotein [LDL] and total cholesterol), and type 2 diabetes. Because HBM-II values represent levels above which adverse health effects are possible, the points of departure for those values differed from those identified for HBM-I values. Where appropriate, benchmark dose-response modeling was used to determine a level for a given effect level. For studies with continuous exposure data in which benchmark dose-response modeling could not be performed, risk estimates from adjusted regression analyses were used. In the case of studies in which analyses were based on quantiles of exposure, the median value of the lowest quantile with a significant association with an adverse health outcome was used as the point of departure.

The points of departure chosen represent quantitatively defined changes (such as 5–10 percent, calculated with a confidence interval for a population) in certain target parameters (e.g., morbidity, laboratory values). In the evaluation of reduced birthweight, for example, the points of departure of 10 ng PFOA/mL and 15 ng PFOS/mL were determined from a meta-analysis that observed a reduction in birthweights by approximately 20 g per ng PFOA/mL and 20 g per ng PFOS/mL. Compiled points of departure were 3–10 ng/mL for PFOA and 1–30 ng/mL for PFOS. HBM-II values for plasma PFOA (10 ng/mL) and PFOS (20 ng/mL) were established as the mid- to high values in these ranges for the general population, excluding women of childbearing age. Lower values were derived for plasma PFOA (5 ng/mL) and PFOS (10 ng/mL) in women of childbearing age, mainly because studies indicated associations with developmental toxicity, reduced fertility, and increased incidence of gestational diabetes. However, the HBM Commission indicated that there is ample uncertainty around HBM-II values for PFOA and PFOS (Schümann et al., 2021).

European Food Safety Authority's Human Point of Departure

In 2020, EFSA published a scientific opinion on the derivation of a tolerable weekly intake for the sum of four predominant PFAS: PFOA, PFOS, PFHxS, and PFNA. Following a review of the animal and epidemiological literature on adverse health effects of PFAS, immunological endpoints were considered the most robust and sensitive for risk assessment. An epidemiological study in German children was used as the critical study for deriving the health-based guidance value. In this study, children's serum PFAS levels at 1 year of age were associated with lower antibody titers against diphtheria at a $BMDL_{10}$ (the lower one-sided confidence limit of the benchmark dose for a 10 percent response) value of 17.5 ng/mL for the sum of PFOS, PFOA, PFHxS, and PFNA (Abraham et al., 2020). Physiologically based pharmacokinetic (PBPK) modeling was used to translate this value in children's serum into a daily dose in their mothers, assuming that levels at 1 year of age in breastfed children reflect primarily the body burden acquired through placental and lactational transfer. To reach this $BMDL_{10}$ value in children's serum at the end of 12 months of breastfeeding, EFSA estimated that the maternal level at 35 years of age would need to be 6.9 ng/mL for the sum of the four PFAS, a level associated with an estimated maternal intake of 0.631 ng/kg body weight/day. Although EFSA did not specifically aim to determine acceptable serum PFAS levels, the 6.9 ng/mL serum concentration could be considered a serum level for women of reproductive age below which risk is negligible.

PFAS Concentrations That Could Inform Clinical Care: Findings and Recommendations

The HBM Commission has identified risk-based levels for two PFAS chemicals—PFOS and PFOA—while EFSA has established such values for the sum of PFOA, PFOS, PFHxS, and PFNA. No individual values are available for PFHxS and PFNA, and no values could be found for methyl-perfluorooctane sulfonamide (MeFOSAA), perfluorodecanoic acid (PFDA), and perfluoroundecanoic acid (PFUnDA). The lowest PFAS risk-based level is 2 ng/mL (HBM-I for PFOA), and the highest PFAS risk level is 20 ng/mL (HBM-II for PFOS in the general population), demonstrating that risks are unexpected below 2 ng/mL and that the risk of PFAS-associated effects at the population level is increased at 20 ng/mL. The risk-based levels for sensitive populations fall between 2 ng/mL and 20 ng/mL (see Figure 5-4).

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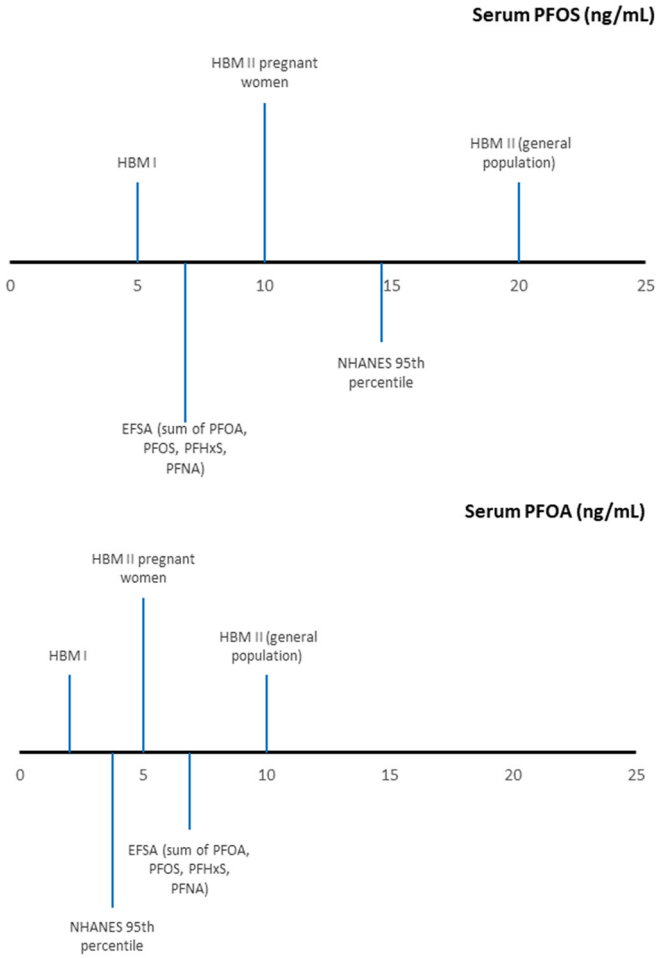


FIGURE 5-4 Reference- and risk-based serum PFOS and PFOA concentrations that could inform clinical assessments.
NOTES: Human biomonitoring (HBM) values are risk-based values derived from a compilation of epidemiological studies and represent levels at which no effect is expected (HBM-I) and above which effects are possible (HBM-II). The European Food Safety Authority (EFSA) risk-based value is a maternal serum PFAS concentration derived from an epidemiological study of children's prenatal and postnatal PFAS exposure and decreased antibody response to vaccines; maternal levels below this value are expected to have negligible impact on children's response to vaccines. The National Health and Nutrition Examination Survey (NHANES) reference-based serum concentration represents the 95th percentile of serum levels in a representative sample of the population aged 12 and older. PFHxS = perfluorohexanesulfonic acid; PFNA = perfluorononanoic acid; PFOA = perfluorooctanoic acid; PFOS = perfluorooctanesulfonic acid.

The committee used weighted data from NHANES to estimate PFAS exposures in the U.S. population. The HBM-I value, or the value below which effects are not expected, for PFOA (2 ng/mL) is the 73rd percentile, and the corresponding value for PFOS (5 ng/mL) is the 57th percentile. The HBM-II value, or the value above which effects may be expected for PFOA (10 ng/mL) is the 99th percentile and for PFOS (20 ng/mL) is the 98th percentile. For women of childbearing age (15–49), the HBM-II values for pregnant women for PFOS and PFOA are the 99th and 98th percentile, respectively. The committee observed that 25 percent of the U.S. population is exposed to PFAS above the EFSA point of departure (6.9 ng/mL sum of PFOA, PFOS, PFHxS, and PFNA).

Given the large number of PFAS and the fact that all humans are exposed to mixtures of PFAS, an approach that accounts for mixtures of PFAS would better inform clinical care than do single-chemical

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exposure values. Because the toxicities of different PFAS may not be equal, an approach using potency factors (e.g., dioxins) may be optimal for determining how a mixture of PFAS may exert its toxic effects. However, EFSA did not identify studies comparing the dose-response curves for different PFAS that would allow derivation of their potencies, and interspecies and sex differences also would complicate that effort. Thus EFSA assumed equal potencies for the four selected PFAS, which in humans share half-lives on the order of years. To facilitate easier comparison with estimated exposure, this calculation was performed on a weight rather than a molar basis. Bil and colleagues (2021) recently developed relative potency factors for several PFAS, which when applied result in the sum of PFOA equivalents in a mixture. Overall, the approach entails uncertainties, as the potency factors are derived from animal studies using mainly liver endpoints, which may correlate with effects in humans but to what degree is unknown. The additive approach used by EFSA has advantages. It is simple to apply and has been used in other efforts to regulate exposures to mixtures.[16] Also, there is evidence that many PFAS have similar toxic effects (Kwiatkowski et al., 2020). The committee believes the additive approach could be applied to the PFAS currently measured in the NHANES (MeFOSAA, PFHxS, PFOA [linear and branched isomers], PFDA, PFUnDA, PFOS [linear and branched isomers], and PFNA). Applying this approach of weight-based dose additivity, and using the HBM Commission's values, the committee makes the following recommendations (see Figure 5-5):

Recommendation 5-3: Clinicians should use serum or plasma concentrations of the sum of PFAS* to inform clinical care of exposed patients, using the following guidelines for interpretation:

- **Adverse health effects related to PFAS exposure are not expected at less than 2 nanograms per milliliter (ng/mL).**
- **There is a potential for adverse effects, especially in sensitive populations, between 2 and 20 ng/mL.**
- **There is an increased risk of adverse effects above 20 ng/mL.**

*** Simple additive sum of MeFOSAA, PFHxS, PFOA (linear and branched isomers), PFDA, PFUnDA, PFOS (linear and branched isomers) and PFNA in serum or plasma. Caution is warranted when using capillary blood measurements as levels may differ from serum or plasma levels.**

The committee estimated that for the sum of PFAS in the NHANES, 2 ng/mL corresponds to the 2nd percentile, and 20 ng/mL corresponds to the 91st percentile, indicating that 89 percent of the U.S. population falls in the orange area in Figure 5-5, and 9 percent in the red area. Choosing cutoffs, as the committee has done, reflects ethical decisions and risks overstating or understating the risk of PFAS exposure given the uncertainty of the available information. Values suggested herein were derived from epidemiological studies evaluating associations at the population level, and the relevance of these values to interpret risk in individuals is uncertain. Also, these values were derived in part from studies in vulnerable populations (e.g., children, pregnant women), and so should account for many sensitive populations, but they may not protect all populations. Moreover, the assumption of weight-based dose additivity is likely an oversimplification. The molar sum may be more appropriate if the equal potency of all PFAS is assumed, and future research to produce toxic equivalency data or identify relevant potency factors could help refine the calculation (Bil et al., 2021). Furthermore, regardless of how the dose is calculated, there may not even be a level of PFAS exposure without some biological effect. Still, the increased risk from low levels of exposure is better addressed through population-health efforts than through individual action. The cutoff levels should be updated as more information becomes available.

[16] See https://www.epa.gov/sites/default/files/2014-11/documents/chem_mix_1986.pdf (accessed June 8, 2022).

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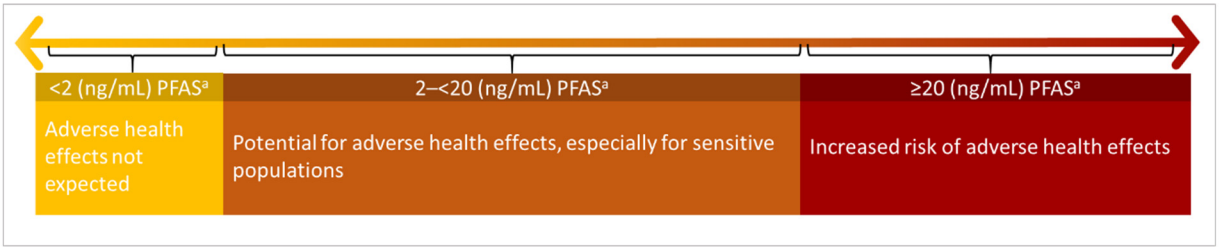


FIGURE 5-5 Graphical display of levels of PFAS to inform clinical care for the sum of MeFOSAA, PFHxS, PFOA (linear and branched isomers), PFDA, PFUnDA, PFOS (linear and branched isomers), and PFNA in serum or plasma.

The committee acknowledges that, in many communities, a large percentage of the population may have exposures to PFAS that would be considered high using the cutoffs presented here. This does not mean that health effects will occur, but likely relates to increased risk. More reference- and risk-based values are needed for other PFAS and other biological matrices, but given the expansiveness of the class, approaches based on relative potency factors may be more successful than developing risk-based levels for each PFAS in addressing this gap.

The committee also acknowledges the existence of data gaps with respect to reference populations. Appropriate PFAS reference populations may not be available for vulnerable populations such as pregnant people and young children, as pregnant people are included in low numbers in NHANES, and children younger than 12 are not routinely included in the publicly available data. Therefore, the committee makes the following recommendation:

Recommendation 5-4: The National Health and Nutrition Examination Survey should begin collecting and sharing more data on children younger than 12 years of age and pregnant people to generate reference populations for those groups.

CONCLUSION

Determining options and considerations to guide decision making for PFAS testing and PFAS concentrations that could inform clinical care of exposed patients will be beneficial in helping communities identify those who have elevated exposure to those chemicals. Testing for PFAS offers an opportunity to identify those who may be at increased risk of certain health outcomes. The recommendations in this chapter could expand PFAS testing among people already integrated into the health system (i.e., those with reliable insurance or other health care coverage). The committee acknowledges, however, that PFAS testing is expensive. Race, age, and other social and demographic characteristics already have disadvantaged many patients from accessing clinical preventive services. That disadvantage would be compounded by the expansion of PFAS testing services, as those services should be linked to counseling on steps for mitigating exposure and its impacts. Therefore, encouraging testing primarily among people with relatively stable access to care could have the unintended effect of aggravating disparities in exposure to PFAS, a severe disadvantage of encouraging testing without a funded, national PFAS testing program.

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6

Guidance for Clinicians on Exposure Determination, PFAS Testing, and Clinical Follow-Up

Despite continued uncertainty about the exact nature of risks from PFAS exposure, clinicians will need to advise and make decisions with patients regarding their exposure. While there is evidence of an association of PFAS with several health outcomes, the likelihood that a particular individual will have any specific adverse health outcome following exposure to PFAS cannot currently be determined with great specificity. There are also gaps in knowledge about how individuals can reduce any potential risks related to PFAS exposure. Despite these gaps, however, many individuals and communities expect clinicians to address PFAS-associated risks as part of routine health care delivery. Although close monitoring and exposure mitigation might prevent or lessen the severity of health effects for those exposed to PFAS, aggressive clinical follow-up could lead to unnecessary treatment, with attendant risk of treatment-related adverse effects; increase patients' anxiety; and even provide false assurance. For these reasons, trust and clear communication between clinicians and patients are of the utmost importance as they face the task of making decisions that consider all options and incorporate informed preferences, although how best to include children, especially adolescents, in shared decision making is a complicated matter and an active focus of research (Boland et al., 2019). Ongoing and future research should eventually guide clinicians in predicting patient risk and provide an understanding of the benefits and harms of interventions designed to avoid adverse health outcomes.

CRITERIA FOR SCREENING

Screening is the process of testing to identify individuals at high risk for developing a clinical condition or those who have a condition for which signs or symptoms may not be evident. Population screenings are one type of clinical preventive services recommended by health and medical professional agencies and organizations such as the American Academy of Family Physicians (AAFP), the American College of Obstetricians and Gynecologists (ACOG), the American Academy of Pediatrics (AAP), the American Heart Association (AHA), the Health Resources and Services Administration (HRSA), and the U.S. Preventive Services Task Force (USPSTF). These organizations usually recommend population-level screenings as part of routine clinical care when it is clear that they offer a net benefit (compared with potential harms of the screening itself and any subsequent treatment). Although there are different frameworks for determining when to adopt population-level screening, the criteria articulated by Wilson and Jungner (1968) are a common platform for these frameworks:

- The condition should be an important public health concern.
- There should be a treatment for the condition.
- Facilities for diagnosis and treatment should be available.
- There should be a latent stage of the condition.
- There should be a test or examination for the condition.
- The test should be acceptable to the population.
- The natural history of the disease should be adequately understood.
- There should be an agreed-upon policy on whom to treat.

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- The total cost of finding a case should be economically balanced in relationship to medical expenditure as a whole.
- Case finding should be a continuous process.

The degree to which these criteria are applied and how they are weighted must be tailored to the particular clinical issue and the perspective of those developing the preventive service. For example, the degree to which the evidence base must be clear can vary based on the urgency of the clinical context, and cost is often not explicitly considered because cost/benefit data from the societal perspective are rarely available. Despite this variation, a key common theme across all frameworks is the need to assess whether the expected benefits of population screening exceed the potential harms. This assessment can be challenging when significant scientific uncertainty exists, as is the case for population screening for PFAS exposure. The committee faced challenges in making population-level recommendations related to the following issues:

- PFAS testing could identify risks for many diverse health outcomes, as opposed to the usual case in which screening identifies one condition or a group of related conditions. Assessment of benefit and harm is difficult if the various potential health outcomes differ in this regard. Furthermore, developing recommendations for clinical follow-up after PFAS exposure is challenging because of the heterogeneity of potential health outcomes.
- The benefits of screening might accrue to individuals other than those who would be screened. For example, determining that an individual had a harmful environmental exposure might not help that person but could lead to broader public health measures that could protect the community.

When there are gaps in knowledge about the benefits and harms of screening or when benefits and harms are closely matched, clinicians should assess individual patient preferences. Informed decision making can be challenging given the above gaps and the limited time clinicians and patients have together. Because standard screening criteria have important limitations in settings of substantial scientific uncertainty, such as PFAS-related health effects, this chapter offers recommendations for basing screening decisions on the principles the committee proposes in Chapter 2.

PFAS-ASSOCIATED HEALTH OUTCOMES

The committee identified several health outcomes associated with PFAS exposure (see Chapter 5). Many are common diseases in the general population, and all have multiple known risk factors (Schrager, 2018). The committee believed it was important to categorize the strength of the evidence for each outcome, but concluded that all conditions with an association should be considered for patient follow-up, as acknowledging the potential risk may make doctors and patients more likely to prioritize screenings. If a patient has a known or suspected exposure to PFAS, the committee encourages clinicians to prioritize screenings for those conditions related to PFAS when relevant and possible. The committee encourages clinicians to use evidence-based best practices and strategies when speaking with patients to support shared decision making and clear health risk communication. Resources such as the Consumer Assessment of Healthcare Providers and Systems (CAHPS) Ambulatory Care Improvement Guide can provide evidence-based trainings, tools, and strategies for shared decision making and clear communication (AHRQ, 2020). The committee did not conduct a meta-analysis to determine the level of increased risk posed by PFAS exposure for each health outcome. Risks vary by exposure level, life stage, and whether patients have other risk factors for developing a health effect. These uncertainties make it infeasible to determine the optimal screening tests and their frequency.

The committee found sufficient evidence of increased risk for the following health outcomes with exposure to PFAS:

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- reductions in birthweight;
- dyslipidemia in children and adults;
- kidney cancer in adults; and
- decreased antibody response in children and adults, but with insufficient evidence of an increase in risk or severity of infection or differences in vaccine effectiveness.

The committee found limited suggestive evidence of increased risk for the following health outcomes with exposure to PFAS:

- breast cancer in adults,
- pregnancy-induced hypertension (gestational hypertension and preeclampsia),
- liver enzyme elevations (in children and adults),
- testicular cancer (in adults),
- thyroid dysfunction (in adults), and
- ulcerative colitis (in adults).

For many of these adverse health effects, however, including cancers, it is unclear what clinicians and individuals can do to monitor for their development and intervene to lower the risk related to PFAS exposure. Guidance for clinicians' engagement in sharing decision making with their patients regarding follow-up care for PFAS-associated health outcomes is included later in this chapter.

Clinical Practice Guidelines for PFAS-Associated Health Outcomes

The committee reviewed clinical practice guidelines for the health effects associated with PFAS exposure, as well as the recommendations from the C-8 Medical Panel (see Table 6-1). The C-8 Medical Panel developed a medical monitoring protocol for a community with high PFAS exposure surrounding Parkersburg, West Virginia. The protocol specifies follow-up for human diseases for which the C-8 Science Panel found a probable link: dyslipidemia, kidney cancer, pregnancy-induced hypertension (gestational hypertension and preeclampsia), testicular cancer, thyroid disfunction, and ulcerative colitis.

Outcomes with Sufficient Evidence of Association

Dyslipidemia in Children and Adults

In the United States, blood testing for lipid and cholesterol levels is recommended throughout the life course based on age and risk factors. AAP recommends that all children be screened once between ages 9 and 11 years and again between 17 and 21 years, with selective screening for children over 2 years of age with a family history of lipid and cholesterol disorders or heart disease (Richerson et al., 2017). AHA recommends screening every 4–6 years for people aged 20 or older who are at low risk for cardiovascular disease (Grundy et al., 2019). The C-8 Medical Panel provided cholesterol screening starting as early as 2 years of age, which is similar to the age recommended for cholesterol screening for children with a family history of lipid disorders (C-8 Medical Panel, 2013).

Reductions in Birthweight

Birthweight is an important and well-established pregnancy outcome. Clinical prevention guidance for reductions in birthweight is to screen for risk factors that lead to reductions in birthweight. Common prenatal exposures associated with a risk of reduced birthweight in full-term newborns include use of tobacco, alcohol, or other drugs.

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TABLE 6-1 An Overview of Screening Recommendations for the Health Effects Associated with Exposure to PFAS

Recommendations for the General Population[a]	C-8 Medical Panel Recommendation[b,c] for Class Members
Lower birthweight	
Screen for risk factors for low birthweight in pregnant persons during prenatal well visit[d]	No screening recommended for C-8 class members[e]
Dyslipidemia (in adults and children)	
Screen all children once between ages 9 and 11 years and again between ages 17 and 21; among those with a familial history, begin screening at age 2 years and follow up yearly[f]	Screen children for cholesterol levels at age 2 years and older unless already screened during the prior 5 years, already diagnosed, or receiving treatment
Screen adults aged 40–75 with no history of cardiovascular disease (CVD), one or more CVD risk factors, and a calculated 10-year CVD event risk of 10% or greater[g]	
American Heart Association recommends all adults aged 20 or older have their cholesterol and other traditional risk factors checked every 4–6 years as long as their risk remains low[h]	
Kidney cancer (in adults)	
No routine screening recommended	Screen individuals aged 20–39 years with a symptom questionnaire; follow up with abdominal exam and urine test if symptoms present
	Screen individuals aged 40 or older with a symptom questionnaire, abdominal exam, and urine testing
Decreased antibody response (in adults and children)	
No routine screening recommended	No screening recommended for C-8 class members[e]
Breast cancer in adults	
U.S. Preventive Services Task Force (USPSTF): Screen average-risk women aged 50–74 with mammography every 2 years[i]	No screening recommended for C-8 class members[e]
Women's Preventive Services Initiative (WPSI): Screen average-risk women with mammography; mammography should be initiated no earlier than age 40 and no later than age 50 and occur annually or biennially until age 74[j]	
Pregnancy-induced hypertension (gestational hypertension and preeclampsia)	
Screen pregnant persons for hypertension and preeclampsia throughout pregnancy[k,l]	Screen pregnant persons for hypertension and proteinuria throughout pregnancy
Liver enzyme alterations in children and adults	
No routine screening recommended	No screening recommended for C-8 class members[e]
Testicular cancer in adults	
American Academy of Family Physicians (AAFP) and USPSTF recommend against screening asymptomatic adolescents and adults for testicular cancer[m]	Screen for testicular cancer with a physical exam and questionnaire
Thyroid dysfunction in adults	
Screen newborns as part of recommended uniform screening panel[n]	Screen adults' serum thyroid-stimulating hormone; otherwise test based on signs and symptoms of thyroid dysfunction
No routine screening recommended[o]	
Ulcerative colitis in adults	
No routine screening recommended	Screen for symptoms with questionnaire starting at age 15 years

[a] In cases in which conflicting or differing recommendations for population-level screenings have been issued, the clinical practice guideline developers have been identified for clarity.

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[b] C-8 Medical Panel, 2013.
[c] The C-8 Medical Panel recommended that members of the class action lawsuit, *Jack W. Leach et al. v. E.I. du Pont de Nemours & Company* (no. 01-C-608 W.Va., Wood County Circuit Court, filed April 10, 2002), be screened once in 2013; the panel has since updated its guidance, and now recommends that class members be screened three times, 3 years apart (C-8 Medical Panel, 2022). See Chapter 1 for more details about the lawsuit.
[d] Hagan et al., 2017.
[e] The C-8 Medical Panel was allowed to develop recommendations only for the six conditions identified by the C-8 Science Panel in 2013: pregnancy-induced hypertension, kidney cancer, testicular cancer, thyroid disease, ulcerative colitis, and hypercholesterolemia. The C-8 Medical Panel did not deliberate about screenings for lower birthweight, decreased antibody response, breast cancer, or liver enzyme functions (C-8 Medical Panel, 2013).
[f] Richerson et al., 2017.
[g] USPSTF, 2016b.
[h] Grundy et al., 2019.
[i] USPSTF, 2016a.
[j] WPSI, 2019.
[k] USPSTF, 2021.
[l] USPSTF, 2017.
[m] USPSTF, 2011.
[n] HRSA, 2018.
[o] USPSTF, 2015.

Kidney Cancer in Adults

There are no effective screening approaches for identifying kidney cancer early in its course, and therefore no authoritative clinical screening recommendations. Urinalysis is effective at finding blood in the urine, which can be a sign of advanced kidney cancer but is also indicative of other disorders, such as infections and kidney stones. The C-8 Medical Panel recommends that clinicians ask about family history, symptoms of kidney cancer (gross hematuria, chronic abdominal pain, recent involuntary weight loss, unexplained fever for 1 week), conduct a physical exam for abdominal mass, and check a urine dipstick for blood (C-8 Medical Panel 2013, 2022). Although palpation for an abdominal mass is not harmful, it is unlikely to lead to early detection and may provide false reassurance.

Decreased Antibody Response in Children and Adults

The predictive value of antibody titers after vaccination or infection is usually unclear, and the evidence for an association between PFAS exposure and infection risk or severity is insufficient. Nor are there any evidence-based recommendations or other clinical guidance for addressing decreased antibody response. This is an important area for additional research.

Outcomes with Limited Suggestive Evidence of Association

Breast Cancer in Adults

The USPSTF recommends biennial mammographic screening for all average-risk women aged 50–74 (USPSTF, 2016a). For women aged 40–49, the USPSTF states that the decision to start screening mammography should be an individual one, and that women who place a higher value on the potential benefit than on the potential harms may choose to begin biennial screening. For women aged 75 and older, the USPSTF makes no recommendation because of insufficient evidence (USPSTF, 2016a). The HRSA-supported Women's Preventive Services Initiative recommends that average-risk women initiate "mammography screening no earlier than age 40 and no later than age 50," that "screening mammography should occur at least biennially and as frequently as annually," and that it "should continue through at least age 74 and age alone should not be the basis to discontinue screening" (WPSI, 2019).

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Pregnancy-Induced Hypertension

Pregnancy-induced hypertension, including gestational hypertension and preeclampsia, affects about 1 in every 12–17 pregnant persons aged 20–44 in the United States, and poses serious health risks for pregnant persons and fetuses during pregnancy (Bateman et al., 2012). As part of standard prenatal care, pregnant persons seeing a clinician will have their blood pressure monitored routinely throughout their pregnancy to reduce various risks to both parent and fetus associated with high blood pressure (Kilpatrick, 2017). This standard measurement is feasible, and its benefits outweigh its harms. The C-8 Medical Panel reinforces the standard blood pressure monitoring recommendations (C-8 Medical Panel 2013, 2022).

Elevated Liver Enzymes in Children and Adults

Elevated liver enzymes do not represent a health outcome by themselves, although they generally indicate a level of liver inflammation. Elevations are often found incidentally with multiphasic blood test panels administered as part of routine medical care or as part of an assessment of patient symptoms or concerns. Elevated liver enzymes have many causes, the most common of which include use of over-the-counter pain medications (particularly acetaminophen) and certain prescription medications (including statin drugs), alcohol consumption, heart failure, viral hepatitis, fatty liver disease, and obesity. There are no authoritative recommendations for screening for liver enzyme alterations in otherwise healthy patients who are not being monitored for liver dysfunction, and follow-up recommendations for elevations depend on which enzymes are affected, the degree of elevation, and characteristics of the individual patient.

Testicular Cancer in Adults

Testicular cancer, though rare, is the most common cancer in American males aged 15–25 (NCI, n.d.). Most cases of testicular cancer are discovered incidentally by patients or their partners. The USPSTF found that there is inadequate evidence that screening by clinician examination or patient self-examination has a higher yield or greater accuracy for detecting testicular cancer at earlier stages. It is also not known whether earlier detection would lead to better health outcomes. The USPSTF concludes that there is no benefit of screening for testicular cancer in the general population and recommends against it (USPSTF, 2011). The C-8 Medical Panel recommends a risk questionnaire and clinical testicular exam, considering ultrasound if additional risk factors are identified (C-8 Medical Panel, 2013, 2022).

Thyroid Disease and Dysfunction in Adults

There are well-defined approaches for identifying and treating thyroid hormone dysfunction, especially among older adults and women (CDC, 2014). The standard clinical practice for identifying thyroid dysfunction is to order blood testing for levels of thyroid-stimulating hormone (TSH) when there are signs or symptoms that could be attributable to hypo- or hyperthyroidism (NIDDK, 2017). Standard approaches exist for diagnosis and treatment of thyroid disorders. In its review, however, the USPSTF concluded that the current evidence is insufficient to assess the balance of benefits and harms of screening for thyroid dysfunction in nonpregnant, asymptomatic adults. The C-8 Medical Panel recommends that adults in PFAS-contaminated communities receive TSH-level screening in addition to testing based on signs and symptoms (C-8 Medical Panel 2013, 2022).

Ulcerative Colitis in Adults

Ulcerative colitis is a chronic bowel disease with a prevalence of 1.3 percent among U.S. adults that results in inflammation and sores in the lining of the large intestine (colon) and rectum (Dahlhamer et

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al., 2016). It can be debilitating and lead to life-threatening complications. In addition to such generalized symptoms as fatigue, fever, and weight loss, affected individuals experience abdominal pain; blood or pus in stool; rectal bleeding; and frequent, recurring diarrhea. Diagnosis requires endoscopy and laboratory testing (Hanauer, 2004). There are no authoritative recommendations for screening for ulcerative colitis. The C-8 Medical Panel recommends that clinicians administer a questionnaire to elicit symptoms of the condition beginning at age 15 (C-8 Medical Panel, 2013, 2022); however, no information is available regarding the accuracy of the questionnaire.

RECOMMENDATIONS FOR PATIENT FOLLOW-UP

Where appropriate, the committee developed recommendations for patient follow-up for PFAS-associated health outcomes that should be offered to patients based on shared, informed decision making between patient and clinician. The clinical practice guidelines for standard medical care and the C-8 Medical Panel recommendations served as the basis of these recommendations. Some conditions associated with PFAS exposure have no established clinical prevention guidance, while other clinical prevention recommendations are beneficial when applied in cases of increased risk. There is value in specifying follow-up that is part of the standard of care as defined by authoritative clinical professional groups because clinicians often are unable to deliver all recommended preventive services during primary care visits (Privett and Guerrier, 2021) and must decide with patients which services to focus on considering both evidence and patient values and preferences (USPSTF et al., 2022).

The committee used its three established cutoff levels for PFAS in serum or plasma (detailed in Chapter 5) to determine follow-up based on PFAS exposure level, although the risks are not the same within each of these three categories. The risks from PFAS likely increase with increased exposure thus PFAS levels of 3 nanograms per milliliter (ng/mL) and 19 ng/mL do not represent the same risk. PFAS blood testing measures burden at the time of sample collection. For example, a person with low blood levels today may have had higher levels in the past. Clinicians should use judgment and shared decision making in making follow-up decisions based on PFAS exposure and other risk factors. Figure 6-1 suggests that clinicians engage in shared, informed decision making with their patients regarding follow-up care for PFAS-associated health outcomes.

Recommendation 6-1: Clinicians should treat patients with serum PFAS concentration below 2 nanograms per milliliter (ng/mL) with the usual standard of care.

Recommendation 6-2: For patients with serum PFAS concentration of 2 nanograms per milliliter (2 ng/mL) or higher and less than 20 ng/mL, clinicians should encourage PFAS exposure reduction if a source of exposure is identified, especially for pregnant persons. Within the usual standard of care clinicians should:

- **Prioritize screening for dyslipidemia with a lipid panel (once between 9 and 11 years of age, and once every 4 to 6 years over age 20) as recommended by the American Academy of Pediatrics (AAP) and the American Heart Association (AHA).**
- **Screen for hypertensive disorders of pregnancy at all prenatal visits per the American College of Obstetricians and Gynecologists (ACOG).**
- **Screen for breast cancer based on clinical practice guidelines based on age and other risk factors such as those recommended by the U.S. Preventive Services Task Force (USPSTF).**

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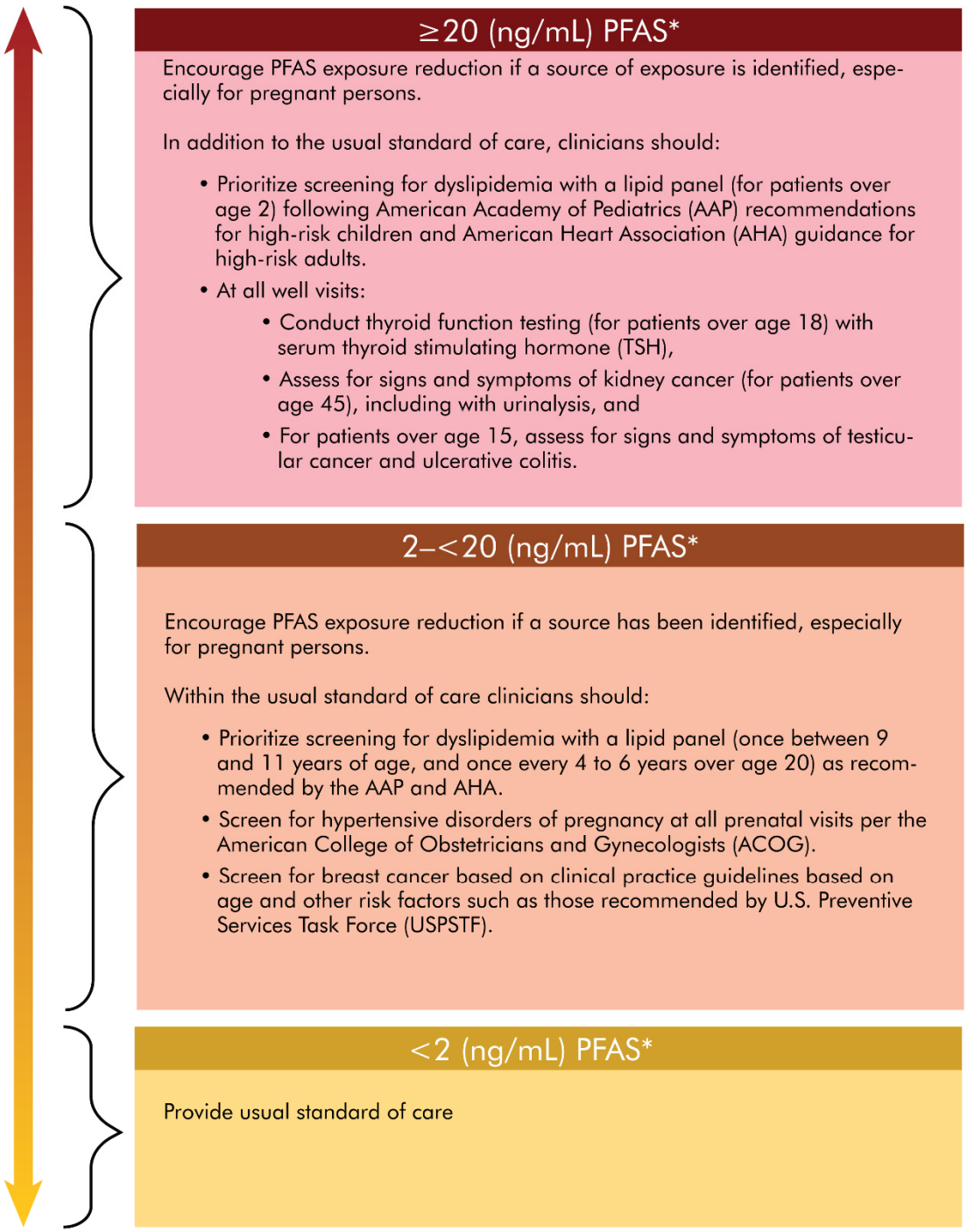


FIGURE 6-1 Clinical guidance for follow-up with patients after PFAS testing.
NOTE: MeFOSAA = methylperfluorooctane sulfonamidoacetic acid; PFDA = perfluorodecanoic acid; PFHxS = perfluorohexane sulfonic acid, PFNA = perfluorononanoic acid; PFOA = perfluorooctanoic acid; PFOS = perfluorooctanesulfonic acid; PFUnDA = perfluoroundecanoic acid.

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Recommendation 6-3: For patients with serum PFAS concentration of 20 nanograms per milliliter (ng/mL) or higher, clinicians should encourage PFAS exposure reduction if a source of exposure is identified, especially for pregnant persons. In addition to the usual standard of care, clinicians should:

- **Prioritize screening for dyslipidemia with a lipid panel (for patients over age 2) following American Academy of Pediatrics (AAP) guidelines for high-risk children and American Heart Association (AHA) guidance for high-risk adults.**
- **At all well visits:**
  - **conduct thyroid function testing (for patients over age 18) with serum thyroid stimulating hormone (TSH),**
  - **assess for signs and symptoms of kidney cancer (for patients over 45), including with urinalysis, and**
  - **for patients over 15, assess for signs and symptoms of testicular cancer and ulcerative colitis.**

APPLYING THE COMMITTEE'S EXPOSURE, TESTING, AND CLINICAL FOLLOW-UP RECOMMENDATIONS

The Committee created a flow chart summarizing PFAS education, exposure assessment, and clinical follow-up (see Figure 6-2). In communities where PFAS exposure has been identified, the Agency of Toxic Substances and Disease Registry (ATSDR) and other government entities should support local clinicians with educational materials about PFAS exposure and testing. Clinicians should then determine whether a particular patient is likely to have a history of elevated exposure to PFAS. If so, the clinician should offer PFAS testing and make a shared, informed decision on that testing. If testing is chosen, the labs should be ordered (Test Code 39307, Current Procedural Terminology [CPT] Code 82542). Test results should be interpreted by summing the concentrations of MeFOSAA, PFHxS, PFOA (linear and branched isomers), PFDA, PFUnDA, PFOS (linear and branched isomers), and PFNA. The laboratory may not report results for all PFAS considered by the committee or may include different PFAS in their panel. In that case, the sum of PFAS should include only the PFAS in the analyte list considered by the committee. For example, if the lab tests for PFOA, PFOS, PFHxS, PFNA, and PFBS, the summation should include PFOA, PFOS, PFHxS, and PFNA. Differing analyte lists may cause some variation in test results. Still, as long as PFOA, PFOS, PFHxS, and PFNA are included in the analyte list, the results may not vary too greatly, as these four analytes are the ones most commonly detected in the United States. If any analyte is below the limit of detection, the clinician should calculate the analyte limit of detection divided by the square root of 2 and use this value in the summation. The summation should then be compared against Figure 6-2 to determine an appropriate clinical follow-up plan based on shared, informed decision making between patient and clinician.

CONCLUSION

The committee believes its clinical follow-up recommendations may be helpful to clinicians who have been asked to address PFAS-associated risks as part of routine health care delivery. There are potential harms both from aggressive clinical follow-up and from ignoring the risks of PFAS exposure. Clinicians and patients should decide which screening practices and services to pursue through a process of shared, informed decision making, along with consideration of the patients' level of PFAS exposure and other risk factors they may have. For young children, these discussions will likely take place with the parents; for adolescents, shared, informed decision making is complicated and an active area of research (Boland et al., 2019). The committee's patient follow-up recommendations should be updated as clinical practice guidelines change and as more is learned about the health effects of PFAS.

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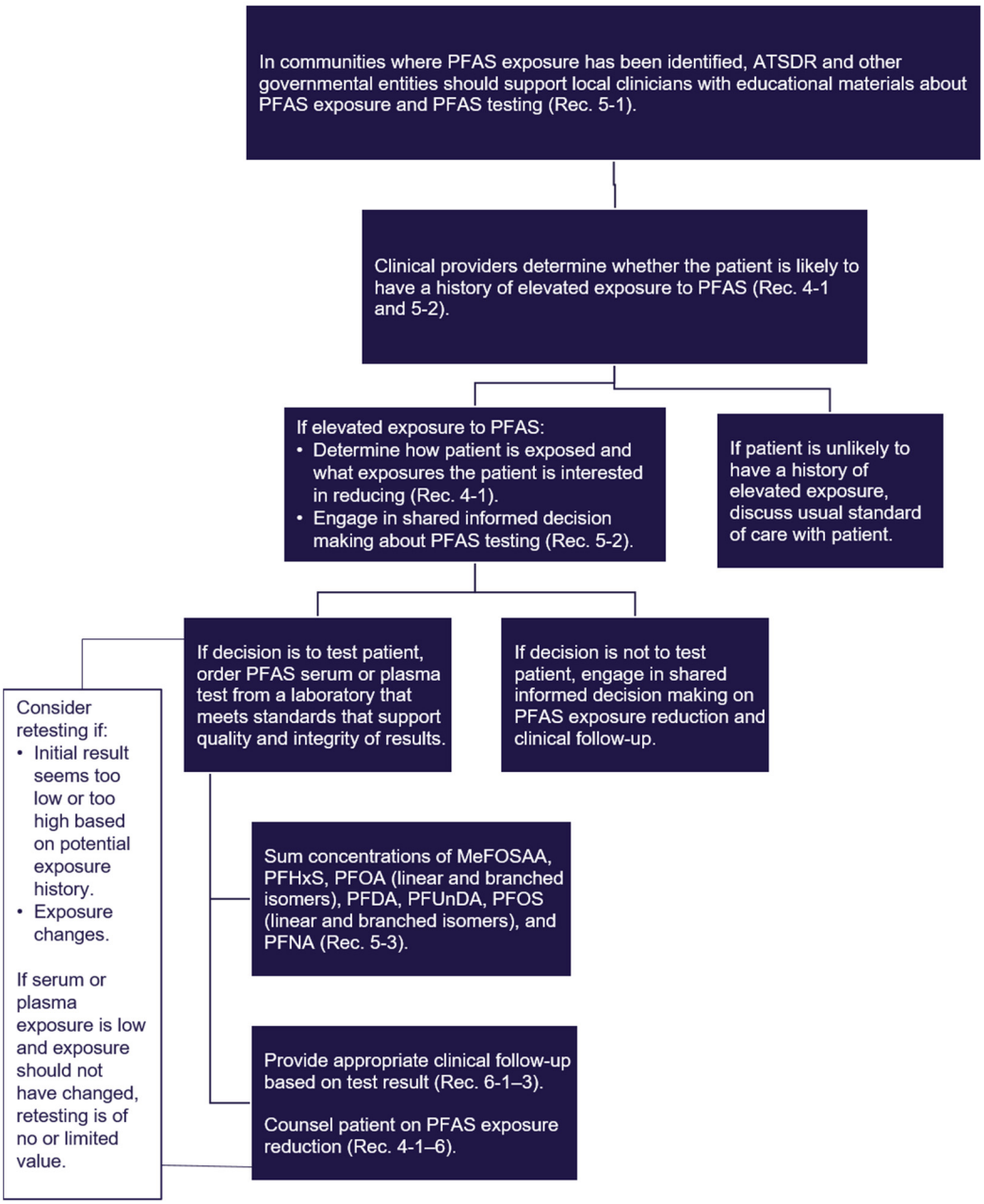


FIGURE 6-2 Flow chart on how the committee's recommendations work together in a clinical setting.
NOTE: ATSDR = Agency for Toxic Substances and Disease Registry; MeFOSAA = methylperfluorooctane sulfonamidoacetic acid; PFDA = perfluorodecanoic acid; PFHxS = perfluorohexane sulfonic acid, PFNA = perfluorononanoic acid; PFOA = perfluorooctanoic acid; PFOS = perfluorooctanesulfonic acid; PFUnDA = perfluoroundecanoic acid.

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7

Revising ATSDR's PFAS Clinical Guidance

Since the potential for harmful effects of per- and polyfluoroalkyl substances (PFAS) exposure were made known to the public in 2000, clinicians have increasingly needed guidance on advising their communities regarding sources, routes, and effects of exposure, even as significant uncertainty about the health effects of exposure remains (see Chapter 3). The C-8 Medical Panel was the first body to offer clinical guidance regarding PFAS exposure, but the guidance was limited to members of the class action lawsuit (C-8 Medical Panel, 2013).[1] In response to this increasing need from clinicians for advice about responding to this environmental hazard, the Agency for Toxic Substances and Disease Registry (ATSDR) published clinical guidance regarding PFAS exposure in December 2019 (ATSDR, 2019).

RECOMMENDATIONS FOR CHANGES TO ATSDR'S CLINICAL GUIDANCE

In accordance with its Statement of Task (see Chapter 1), the committee in this chapter recommends several changes to ATSDR's guidance to ensure consistency with the conclusions, findings, and recommendations in this report. The Statement of Task specifies three main considerations regarding changes to the guidance. The first two—decision making for PFAS testing and PFAS concentrations informing clinical care of exposed patients—are discussed in Chapter 5; the third—clinical follow-up care specific to PFAS exposure—is addressed in Chapter 6.

The following recommendations illustrate the potential use of the information presented in previous chapters of this report:

Recommendation 7-1: The Agency for Toxic Substances and Disease Registry (ATSDR) should update its PFAS clinical guidance to make it more succinct and accord with the review of PFAS-associated health effects, exposure reduction considerations, PFAS testing recommendations and interpretation, and recommendations for clinical follow-up presented in this report. When describing the health effects of PFAS, ATSDR should avoid using terms typically used to categorize toxicants, such as "endocrine disrupter" or "neurotoxin," because they are vague and not necessarily clinically meaningful. When discussing the strength of the association between PFAS and a health outcome, ATSDR should use standard categories of association (such as sufficient evidence of an association, limited or suggestive evidence of an association, inadequate or insufficient evidence of an association, and limited or suggestive evidence of no association).

Recommendation 7-2: The Agency for Toxic Substances and Disease Registry (ATSDR) should incorporate a reader-centered approach when developing its guidance, with the knowledge that many different audiences will turn to its clinical guidance document to prepare for discussions with their clinicians. ATSDR should also solicit feedback on the guidance from a variety of stakeholders, such as

[1] See Chapter 1 for a brief overview of the lawsuit, *Jack W. Leach et al. v. E.I. du Pont de Nemours & Company* (no. 01-C-608 W.Va., Wood County Circuit Court, filed April 10, 2002), and Chapter 6 for details regarding the clinical guidance for class members.

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community groups, practicing clinicians, and medical associations. In addition, ATSDR should encourage clinicians to use evidence-based organizational health literacy strategies to support shared, informed decision making; patient-centered care; cultural humility; and accessible language when communicating with patients about potential health risks.

Recommendation 7-3: The Agency for Toxic Substances and Disease Registry (ATSDR) should develop a process for updating its PFAS guidance that adheres to criteria for making guidelines trustworthy, such as being based on a thorough, transparent, unbiased review of the evidence and being developed by a knowledgeable panel of experts free from strong biases and conflicts of interest. A review of the evidence on the health effects of PFAS should be completed by an authoritative neutral party every 2 years, and the clinical guidance should be updated every 5 years or sooner if warranted by the evidence on the health effects of PFAS. Clinicians and members of communities with elevated PFAS exposure should be engaged to inform the problem and review updated guidance.

In addition to these considerations, the committee believed it would be useful to address some more technical aspects of the ATSDR guidance document, including its writing and design and its dissemination and implementation. To this end, the committee referred to existing clinical guidance on PFAS exposure, as well as materials on health literacy and health communication, including the Agency for Healthcare Research and Quality's (AHRQ's) Health Literacy Universal Precautions Toolkit (Brega, 2015) and the Centers for Medicare & Medicaid Services' (CMS's) Toolkit for Making Written Material Clear and Effective (McGee, 2010). Given the Centers for Disease Control and Prevention's (CDC's) long-standing commitment to effective health communication (Donovan, 1995; Gagen and Kreps, 2019; Roper, 1993; Tinker and Silberberg, 1997), the committee decided not to focus on the finer details of these considerations but on their high-level aspects.

The committee reviewed several other materials published by other organizations to aid clinicians in addressing exposure to PFAS and determining options for clinical follow-up. Table 7-1 describes the clinical guidance documents published by the Pediatric Environmental Health Specialty Units (PEHSUs), and PFAS Research, Education, and Action for Community Health (PFAS-REACH) and identifies the strengths of each.

TABLE 7-1 Description of PFAS Clinical Guidance Documents

Clinical Guidance Document	Description of Content	Strengths of Document
How to Conduct a Clinical Visit with Patients Concerned About PFAS (PEHSU, 2021)	Includes a numbered list of five strategies for clinicians to use in discussing PFAS exposure with concerned patients and provides some detail on how to apply them. Also provides some references to other materials with further detail.	Focuses on empathy and shared decision making and the potential need for exposure reduction. Offers advice only for overall approaches to navigating discussions with patients, and directs clinicians to supplementary Pediatric Environmental Health Specialty Units (PEHSUs) and Agency for Toxic Substances and Disease Registry (ATSDR) resources.
PFAS Exposure: Information for Patients and Guidance for Clinicians to Inform Patient and Clinician Decision Making: For Clinicians (PFAS-REACH, 2021)	Summarizes clinical services recommended by other trustworthy groups for adults and children with above-average PFAS exposure. Categorizes information by type of service—laboratory tests, exams, or counseling topics	Accessible summary of material produced by others. Does not claim to provide rigorous analysis of others' findings. Does provide more information than the PEHSU document in an accessible format.

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WRITING AND DESIGN OF ATSDR'S CLINICAL GUIDANCE

The CMS Toolkit for Making Written Material Clear and Effective provides advice about paying attention to content, organization, writing style, engagement, and motivation (McGee, 2010):

- "Content" relates to what readers want and need to know and whether the information provided is accurate and up to date.
- The "organization" of the written material involves how well the material paces readers by grouping information in meaningful sections.
- "Writing style" refers to whether the text is conversational and uses the active voice, is specific and concrete, and uses familiar and culturally appropriate terms.
- "Engagement and motivation" refers to whether the material has a positive and friendly tone, offers trustworthy information sources, provides relatable statistics, or offers information about how readers can learn more.

The CMS Toolkit also includes "design" principles related to such matters as

- overall design and page layout;
- font, size of print, and contrast;
- headings, lists, and blocks of text;
- use of color;
- photographs and illustrations; and
- tables, charts, and diagrams (McGee, 2010).

As noted above, the committee limited its attention to design intricacies and overall layout, other than noting that the ATSDR guidance document is likely too long for the target audience of clinicians. Best practices for guidance (AAFP, 2021) include providing recommendations that are specific, offer clear direction, and are succinct—principles consistent with comments made to the committee by community members and clinicians (see Appendix B). Incorporating such best practices and using a reader-centered approach will allow ATSDR's clinical guidance to reach and support as many clinicians (and other community members) as possible.

DISSEMINATING AND IMPLEMENTING ATSDR'S CLINICAL GUIDANCE

Process for Updating the Guidance

In addition to updates to the ATSDR guidance stemming from this report, the committee proposes the establishment of a process for regularly updating the guidance (see Figure 7-1). The first step in that process is engaging with impacted communities to inform understanding of the issue at hand. It will also be important for ATSDR to update its reviews regarding PFAS-associated health effects, as well as to catalyze future research by identifying gaps in the evidence. This process should include both a review of guidelines issued by other authoritative bodies reflecting decisions about the health effects of PFAS and a review of the epidemiologic literature to identify any new studies that may warrant updating or revising ATSDR's own authoritative guidance. These reviews should be conducted by a neutral party every 2 years, or sooner if a watershed study, such as a large cohort or nested case-control study, on PFAS exposure and health effects is published before the next review is scheduled. AHRQ, the U.S. Preventive Services Task Force (USPSTF), and other organizations use similar processes that may be informative. One such process includes conducting "living" systematic reviews to continually update an existing review as new evidence becomes available (Elliott et al., 2017). It will be important in this process to incorporate reviews conducted by the U.S. Environmental Protection Agency, the National

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Toxicology Program, and other authoritative bodies. The committee proposes that ATSDR revise its guidance within 5 years of its 2021 analysis, consistent with the timelines for updating of the National Guideline Clearinghouse (NGC) and the USPSTF recommendations. The process for updating the literature review should encompass studies on PFAS exposure reduction, including those evaluating behavior change, interventions, or clinical measures, as well as studies on risk-based levels of PFAS to inform clinical care. It will also be important to review recommendations on standard care, as clinical follow-up recommendations would change if, for example, a beneficial screening test existed for a PFAS-associated health outcome. Finally, as noted above, the updating process should incorporate approaches for assembling feedback from clinicians and community members prior to each review. It should also include processes for updated documents to be reviewed by clinicians, in consultation with community members impacted by PFAS (see Figure 7-1 for an overview of this proposed updating process).

Transparency and Trustworthiness

Transparency enhances the trust clinicians and others place in clinical guidance. To advise ATSDR in this regard, the committee turned to the Institute of Medicine's (IOM's) standards for developing trustworthy clinical practice guidelines (CPGs) (IOM, 2011). The 2011 IOM report *Clinical Practice Guidelines We Can Trust* defines CPGs as

> statements that include recommendations intended to optimize patient care. They are informed by a systematic review of evidence and an assessment of the benefits and harms of alternative care options. (p. 15)



FIGURE 7-1 Suggested framework for updating the Agency for Toxic Substances and Disease Registry's clinical guidance based on new evidence.

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The definition also includes six attributes required to make CPGs trustworthy, including that they

- are based on a systematic review of the existing evidence;
- are developed by a knowledgeable, multidisciplinary panel of experts and representatives from key affected groups;
- consider important patient subgroups and patient preferences, as appropriate;
- are based on an explicit and transparent process that minimizes distortions, biases, and conflicts of interest;
- provide a clear explanation of the logical relationships between alternative care options and health outcomes, and provide ratings of both the quality of evidence and the strength of recommendations; and
- are reconsidered and revised as appropriate when important new evidence warrants modifications of recommendations (p. 26).

The above definition of and standards for trustworthiness are intended to distinguish CPGs as transparent and methodologically rigorous, evidence-based guidelines as compared with other important forms of clinical guidance, such as expert advice, consensus statements, and practice bulletins. CPGs provide recommendations based on the balance of benefits and harms of different interventions for preventing or treating disease. Although ATSDR may be unable to issue CPGs regarding general PFAS exposure, as the complexity of the data may make a formal systematic review to inform such CPGs difficult, it should still strive to ensure that its clinical guidance reflects these criteria when possible.

CONCLUSION

The need for clear clinical guidance regarding PFAS exposure has only increased, despite the remaining uncertainties regarding the specific health risks of exposure. Yet it is difficult to distill the necessary information for effective clinical practice with respect to PFAS exposure, its possible health effects, and options for shared decision making once exposure has been confirmed. In addition to the distillation of the information itself, moreover, the writing, design, and general implementation of the clinical guidance are as important and as complex as the distillation of the information itself. This chapter offers recommendations that, if implemented, may assist ATSDR in its efforts to offer the guidance needed by clinicians. The committee believes ATSDR can continue to offer up-to-date, useful, and trustworthy clinical guidance by incorporating the updated data on the health effects of PFAS exposure presented in earlier chapters of this report, as well as adhering to the important risk communication and health literacy principles discussed above.

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8

Implementing the Committee's Recommendations to Improve Public Health

Improving public health requires the use of multifaceted approaches to emerging health issues. In environmental health—the subset of public health focused on environmental factors—mitigation of potential harms associated with chemical exposures is often complicated because there is no exposure surveillance system for most chemicals. To address per- and polyfluoroalkyl substances (PFAS) effectively as a public health issue, it will be important to identify the people and communities with high exposures to PFAS, improve environmental health education for both clinicians and the public, and prevent exposures. Thus, the committee's recommendations would be most effective if implemented as part of a larger national effort toward increased PFAS-focused biomonitoring, exposure surveillance, and education around environmental health.

To promote a coordinated response to PFAS exposures, the committee makes the following recommendations:

> **Recommendation 8-1: Laboratories conducting PFAS testing of serum or plasma should report the results to state public health authorities, following the respective states' statutes and reporting regulations. This reporting would improve PFAS exposure surveillance; it could be linked with the Centers for Disease Control and Prevention's environmental public health tracking network and help build capacity for improvements in the state-based national biomonitoring network.**

BIOMONITORING AND SURVEILLANCE

PFAS exposure is nearly ubiquitous, but individual levels of exposure are not consistently measured or documented (CDC, 2021; NASEM, 2020, 2021a). Given the persistence of most PFAS in the human body, biomonitoring will likely produce meaningful exposure data, especially for those PFAS with longer half-lives. Collecting these data is critical to identifying communities and other areas with elevated PFAS exposure. Failing to identify such communities magnifies existing disparities for those that already have limited access to health care, live in areas of high exposure, and do not know that they may be at elevated risk for PFAS-associated health outcomes. Using biomonitoring data to establish exposure surveillance programs while also ensuring that medical professionals and the public have access to clear, accurate information about the risks of PFAS exposure will improve public health overall while also empowering people and communities to safeguard their own health.

According to the 2006 report by the National Research Council *Human Biomonitoring for Environmental Chemicals*:

> Identifying, controlling, and preventing population exposures to potentially harmful environmental chemicals have been cornerstones of U.S. environmental health efforts. Biomonitoring has become a tool that is central to these efforts. (NRC, 2006, p. 1)

While aggregate PFAS biomonitoring data can be used to promote population health, individual PFAS biomonitoring can provide people with information about their personal exposures, which is

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important in informing clinical care (see Chapter 5). Therefore, access to PFAS blood tests for patients likely to have a history of elevated exposure as well as the results of those tests can help individuals better understand and, if possible, address their personal risk from PFAS exposure. Additionally, while biomonitoring studies are important to identify exposed communities and study potential health outcomes, PFAS testing intended to inform an individual's medical care should be coordinated by clinicians to ensure appropriate care and follow-up.

Improving access to PFAS blood testing for individuals could also allow for the collection of aggregate biomonitoring data if the results were compiled into a database. As discussed in Chapter 5, public health professionals can use those data to determine reference ranges of PFAS exposure in the general population. Understanding those ranges of exposure would support prioritization and allocation of funding and resources for preventive medicine or public health actions. Other high-profile cases demonstrate this powerful use of biomonitoring data to improve public health practice, as occurred in 2015 when Dr. Mona Hanna-Attisha began reviewing her patients' medical records and realized that the percentage of patients with elevated blood lead levels had increased after the city of Flint, Michigan, changed its water source.[1]

In addition to informing clinical care, biomonitoring data can be used in establishing exposure surveillance programs. Biomonitoring-based exposure surveillance enables public health practitioners and policy makers to identify exposure trends, mitigate ongoing exposures, and study associations between exposures and disease outcomes (Goldman et al., 1995; Thacker et al., 1996). In contrast with agencies' ongoing monitoring of environmental contaminants in air, soil, and water, there are few comprehensive databases or collection strategies for measuring and tracking levels in exposed people (Latshaw et al., 2017). The data that are currently collected, such as measurements from the National Health and Nutrition Examination Survey (NHANES),[2] often provide a snapshot of exposure that is useful in tracking nationwide trends in exposure over time, but cannot inform a localized public health response or help identify potential exposure "hot spots." Additionally, unlike the National Health Interview Survey,[3] the NHANES does not list urban versus rural as a publicly available variable, which limits researchers' ability to identify potential location-based disparities in PFAS exposure. Without collecting and compiling comprehensive PFAS biomonitoring data, federal agencies are less able to prioritize highly exposed communities in their mitigation strategies. Additionally, a lack of biomonitoring data limits studies on putative health effects, resulting in continued uncertainty that hinders public health response. As Kristen Mello stated during the committee's April 7, 2021, town hall, "Don't avoid taking our data and then complain about data gaps."

An effective strategy for PFAS exposure surveillance would involve multiple levels of public health response (Colles et al., 2021; Eatman and Strosnider, 2017). As with U.S. programs on pediatric monitoring for lead exposure, laboratories would report the biomonitoring results, zip code, and demographic information to state health departments, which in turn would report the data to the federal government. Data collected by states could be more geographically granular than the data typically accessible through NHANES, thereby enabling localized public health responses for highly contaminated areas (Nassif et al., 2021). Additionally, hierarchical data collection and reporting processes might include repeated stopgaps and promote oversight to ensure that clinicians, researchers, and public health agencies were using the data as effectively as possible.

ENVIRONMENTAL HEALTH EDUCATION

If the use of biomonitoring data and environmental surveillance programs is to be most effective at preventing or at least minimizing clinical disease associated with exposure, clinicians and the public

[1] See ww.npr.org/sections/health-shots/2018/06/25/623126968/pediatrician-who-exposed-flint-water-crisis-shares-her-story-of-resistance (accessed June 17, 2022).

[2] See https://www.cdc.gov/nchs/nhanes/index.htm (accessed June 17, 2022).

[3] See https://www.cdc.gov/nchs/nhis (accessed June 17, 2022).

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must have access to the information and know what to do with it. From a clinical perspective, providers need a better working knowledge of environmental health and chemical exposures. At the committee's town halls, speakers described their frustration with trying to obtain medical care from practitioners who were unfamiliar with PFAS and did not understand the environmental health contexts of the communities in which they practiced. At the town hall on April 7, 2021, Hope Grosse said:

> When I would go to the doctors and tell them about some of the exposures of over 50 chemicals that I was exposed to, the doctors would laugh and say no. Clearly, they didn't have any information about environmental components [of disease]. They made me feel small; they made me feel stupid and embarrassed even just asking the question.

The National Academies has previously acknowledged the issue of limited clinical education in environmental health. The 1991 Institute of Medicine (IOM) report *Addressing the Physician Shortage in Occupational and Environmental Medicine: Report of a Study* notes that all levels of medical education, from undergraduate to graduate and continuing education programs, provide limited, if any, training in occupational and environmental medicine. The report recommends that occupational and environmental medicine concepts be introduced early and continuously during medical education (IOM, 1991). Likewise, *Environmental Medicine: Integrating a Missing Element into Medical Education* notes a need for more environmentally literate physicians (IOM, 1995a). Decades later, however, Green-McKenzie and colleagues (2021) found that only 70 percent of medical students had heard of occupational and environmental medicine, and most of them had received only one lecture on the topic. After medical school, residency programs align with specialty requirements, which may not include further training in environmental and occupational medicine. In addition to the more formal medical training doctors receive in medical school and residency programs, professional associations such as the American Medical Association,[4] the American College of Preventive Medicine,[5] and the American College of Occupational and Environmental Medicine[6] offer continuing medical education modules on environmental health. However, these modules are optional, meaning that physicians may not learn about environmental and occupational medicine after medical school, either.

Physicians are not alone in their limitations with respect to environmental health education. According to the 1995 IOM report *Nursing, Health, and the Environment: Strengthening the Relationship to Improve the Public's Health*, "environmental health currently receives scant attention in nursing education and research" (IOM, 1995b, p. 14). The report calls for more nurses with education in environmental health, given that nurses represent the largest proportion of the health care workforce. Similarly, a recent commentary on the nursing profession notes that most nurses do not see themselves as environmental health practitioners or scientists (McCauley and Hayes, 2021). To address this shortcoming, environmental health is now a recommended domain for nursing education (NASEM, 2021b); in addition, nurses may need additional continuing education on environmental health topics.

Continuing education of clinicians is important to address knowledge gaps in environmental health. Increasing the opportunities for clinician education in environmental and occupational medicine; promoting collaborative, interdisciplinary environmental health networks; and framing environmental health as a tenet of preventive medicine and primary care are essential to ensure proper medical care for those facing environmental exposures and to assist families in reducing exposures when possible. One resource that can be leveraged to help respond to environmental exposures is the Pediatric Environmental Health Specialty Units (PEHSUs). ATSDR funds the PEHSUs with support from EPA. The PEHSUs are a "national network of experts in the prevention, diagnosis, management, and treatment of health issues that arise from environmental exposures from preconception through adolescence."[7] While the primary

[4] See https://www.ama-assn.org (accessed June 17, 2022).
[5] See https://www.acpm.org (accessed June 17, 2022).
[6] See https://acoem.org (accessed June 17, 2022).
[7] See https://pehsu.net (accessed July 27, 2022).

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focus is reproductive and children's health, clinicians will work with the whole family in determining exposure pathways and reducing these exposures. The PEHSU network includes nurses, physicians, medical toxicologists, and public health professionals and promotes collaboration among these diverse groups of practitioners. The network provides continuing education opportunities and information on PFAS for both health care professionals and community members. PEHSU-affiliated practitioners have proposed concrete environmental health competency areas for medical education programs (Goldman et al., 2021). Similarly, the Alliance of Nurses for Healthy Environments (ANHE)[8] addresses environmental exposures and health, including PFAS and related clinical care, in various online resources and engages with universities to integrate environmental health into their nursing education curricula. Other organizations, such as the Children's Environmental Health Network,[9] also provide educational materials in environmental health for health care providers.

Many public health practitioners have received training in environmental health because until recently, it was a required component of master of public health (M.P.H.) programs; however, the curriculum for public health is changing. In 2016, the Council on Education for Public Health (CEPH) released new accreditation criteria, aimed at offering schools of public health greater flexibility in course offerings and students a more applied, practice-based education.[10] With these more flexible coursework requirements, the CEPH removed the explicit requirement for education in environmental health sciences, replacing it and the other core disciplines with a series of competencies. This change may inadvertently lead to reduced environmental health training for public health professionals.[11] Indeed, by December 2019, just 91 percent of accredited M.P.H. programs continued to offer a concentration in environmental health sciences.[12] Addressing such challenges as PFAS requires a public health workforce with competency in environmental health sciences, toxicology, and epidemiology, which is why the Association for Prevention Teaching and Research calls on schools and programs in public health to maintain existing environmental health content and develop such content if previously eliminated or never offered (Levy et al., 2022).[13]

The public's environmental health literacy complements clinician education in environmental health by promoting individuals' personal health decision making while galvanizing communities to demand population-level public health responses. Historically, health literacy as a concept centered on individual decision making based on an understanding of health information. In Healthy People 2030, however, the U.S. Department of Health and Human Services (HHS) expanded the definition to encompass organizations' responsibility to ensure that individuals have access, understanding, and services to inform their health decisions, a concept HHS refers to as organizational health literacy.[14]

The term *health literacy* as used in this report focuses on Nutbeam's (2000) second and third levels of health literacy—interactive and critical literacy—which emphasize empowerment, autonomy, and action. More specifically, National Institute of Environmental Health Sciences (NIEHS) authors Finn and O'Fallon (2017) describe environmental health literacy as

> a philosophical perspective, a public health policy to improve literacy and health literacy in the general public, and a set of strategies to empower individuals and communities to exert control over the environmental exposures that may lead to adverse health outcomes. (p. 495)

[8] See https://envirn.org (accessed June 17, 2022).
[9] See https://cehn.org (accessed June 17, 2022).
[10] See https://media.ceph.org/documents/Environmental_Health.pdf (accessed June 17, 2022).
[11] See http://www.publichealthnewswire.org/?p=environmental-health-in-education (accessed June 17, 2022).
[12] See https://media.ceph.org/documents/Environmental_Health.pdf (accessed June 17, 2022).
[13] See https://www.aptrweb.org/page/ClimateChange (accessed June 17, 2022).
[14] See https://health.gov/healthypeople/priority-areas/health-literacy-healthy-people-2030/history-health-literacy-definitions (accessed June 17, 2022).

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Building on the concept of health literacy, environmental health literacy has three dimensions: (1) awareness and knowledge of an environmental exposure, (2) skills and self-efficacy that allow individuals to make decisions to protect their own health, and (3) community change based on actions that reduce environmental exposures and protect public health (Gray, 2018). The committee recognizes that many exposures to PFAS are beyond individual control, and placing the onus for action on individuals often imposes an undue burden. Nevertheless, environmental health literacy can empower people to understand their own risks and ask their providers informed questions. As demonstrated by the community liaisons and town hall speakers who contributed to this study, many individuals have already worked especially hard to access information and educate themselves on PFAS.

In keeping with the principle of reducing the burden on exposed community members, PFAS-related environmental health literacy should center on making PFAS research publicly available and understandable for general audiences. Open access publication platforms are more accessible than those that are behind paywalls. Summaries written in plain language for the lay public, as blogs, news articles, and podcasts would be even better for increasing awareness about PFAS. Such resources as ATSDR's ToxFAQs[15]; Northeastern University's PFAS-TOX Database[16]; the PFAS Research, Education, and Action for Community Health PFAS Exchange[17]; and the Environmental Working Group's PFAS Contamination in the U.S. map[18] distill key information for the public and may serve as models for other environmental health literacy work. Other online resources, such as Purdue University's Online Writing Lab, describe considerations for evaluating the credibility of online sources that can serve as a guide for both those developing and those using the resources.[19] People must have access to information that can help them make health decisions and advocate for the health of their communities.

BARRIERS TO IMPLEMENTATION

Barriers to the committee's proposed implementation approach include difficulties with PFAS testing, lack of data standardization, poor coordination among experts from different disciplines, and a lack of funding for and availability of education in environmental and occupational medicine and environmental health literacy activities. Few laboratories currently have the capability to test for PFAS. The testing methodology is complex, and not all PFAS can be detected in serum or plasma. Data standardization is also a challenge (Latshaw et al., 2017) given the variability in testing methods, participant demographics, and exposure sources. As noted in Chapter 5 of this report, PFAS testing is expensive, and limiting the availability of testing based on access to consistent clinical care and insurance coverage could exacerbate health disparities in exposed communities. Furthermore, comprehensive PFAS exposure surveillance requires collaboration among a range of experts across different fields (Nassif et al., 2021); training in environmental and occupational medicine and environmental health is lacking; and support for PFAS health literacy is limited.

Given widespread PFAS exposure and the putative health effects associated with these chemicals, public health authorities would do well to prioritize addressing these barriers. Laboratory capacity to test for PFAS could be increased through coordination with the Association of Public Health Laboratories. Biomonitoring data and exposure surveillance programs could be standardized as long as they were developed with structures in place to ensure that the data were collected with the intent of their being comparable. The Network Steering Committee of the National Biomonitoring Network provides a template for successful implementation (Nassif et al., 2021).

[15] See https://wwwn.cdc.gov/TSP/ToxFAQs/ToxFAQsLanding.aspx (accessed June 17, 2022).
[16] See https://pfasproject.com/pfas-toxic-database (accessed June 17, 2022).
[17] See https://pfas-exchange.org (accessed June 17, 2022).
[18] See https://www.ewg.org/interactive-maps/pfas_contamination/map (accessed June 17, 2022).
[19] See https://owl.purdue.edu/owl/research_and_citation/conducting_research/evaluating_sources_of_information/evaluating_digital_sources.html (accessed June 17, 2022).

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Finally, continuing education opportunities such as "train-in-place" programs allow clinicians to learn environmental and occupational medicine concepts during the course of their ongoing practice (Green-McKenzie et al., 2021). Optimizing these programs and other innovative education strategies, as well as planning communication efforts as part of biomonitoring studies (NRC, 2006), can increase environmental health knowledge without requiring unattainable funding allocations.

MOVING FORWARD

Based on the recommendations and conclusions of this report, ATSDR, NIEHS, environmental health organizations, and bodies responsible for clinical education can take specific actions to address issues of PFAS contamination and clinical care, which will ultimately lead to improved public health (see Figure 8-1). By updating its PFAS clinical guidance according to the recommendations in Chapter 7, ATSDR will lay the groundwork for clinicians to participate in shared decision making and provide informed care for individuals exposed to PFAS. Access to informed care will allow communities with elevated exposure to PFAS to receive relevant medical advice, testing, screening, and treatment. In addition to improved guidance on clinical care, coordination between ATSDR and the National Biomonitoring Network will allow for the expansion of testing and surveillance. Additional communities identified as having elevated PFAS exposures can then receive informed care.

Additionally, NIEHS can use the information in this report to translate the research gaps identified in Chapter 3 into research needs, prioritizing research on potential associations between PFAS exposure and adverse health outcomes where the evidence is limited. As the research gaps narrow, ATSDR can update its clinical guidance to ensure that clinicians' knowledge and monitoring of PFAS-associated health outcomes remain current.

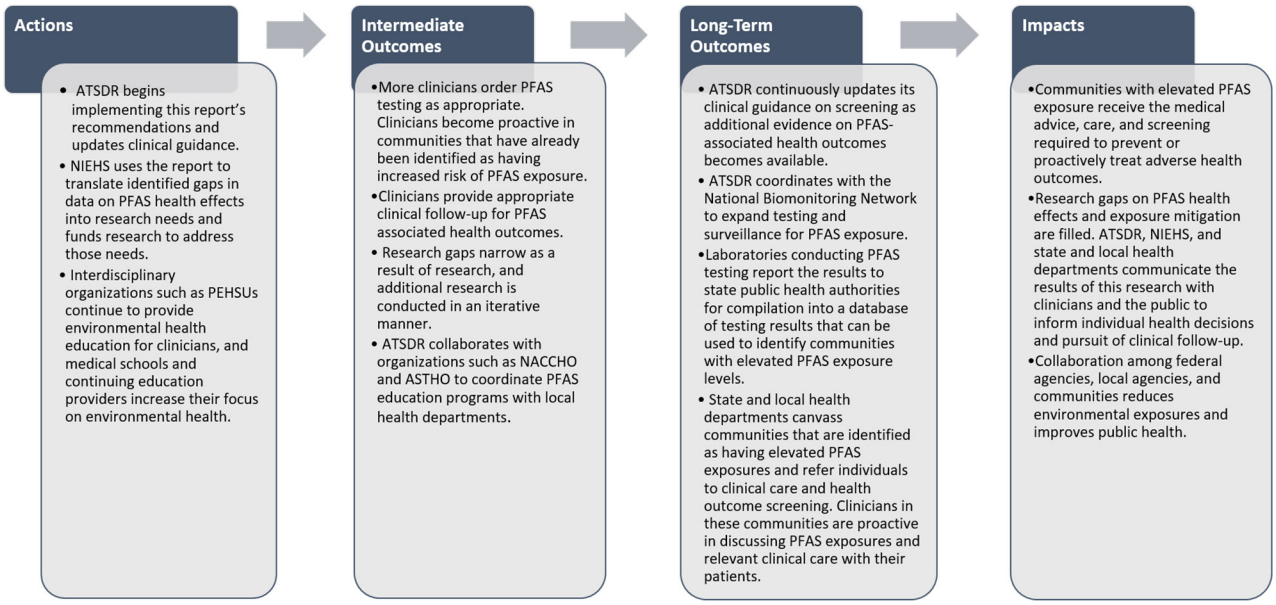


FIGURE 8-1 Recommended approach to mitigating PFAS exposure and adverse health outcomes.
NOTE: ASTHO = Association of State and Territorial Health Officials; ATSDR = Agency for Toxic Substances and Disease Registry; NACCHO = National Association of County and City Health Officials; NIEHS = National Institute of Environmental Health Sciences; PEHSU = Pediatric Environmental Health Specialty Unit.

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Environmental health education for clinicians and the public is key to reducing PFAS exposure and ensuring shared decision making and appropriate follow-up. Introductory environmental health training provides the foundation for PFAS-specific clinical education. Given this, the Liaison Committee on Medical Education,[20] the Accreditation Council for Graduate Medical Education,[21] the American Board of Medical Specialties,[22] the Accreditation Commission for Education in Nursing,[23] and other bodies responsible for curricula and accreditation of clinician education programs may consider incorporating environmental health into clinician training. Input from such interdisciplinary organizations as PEHSUs and ANHE can support these efforts. State and local health departments may serve as a link between the public and clinicians and proactively provide current information about environmental exposures to the communities they serve. Health departments could receive support for this work through liaison organizations funded by the Centers for Disease Control and Prevention, including the National Association of County and City Health Officials[24] and the Association of State and Territorial Health Officials.[25] Intentional collaboration at every level—from clinician education; to community partnerships; to local, state, and federal government initiatives—will begin closing the gaps in environmental health infrastructure and training, facilitating the overdue coordinated response and clinical care that people with elevated PFAS exposures deserve.

These efforts in environmental exposure surveillance, environmental health literacy, and improved environmental health tracking will aid in continuing to identify communities impacted by PFAS, which in turn will support exposure mitigation and effective preventive medicine responses. As the committee looks forward, it sees a pressing need for a robust environmental health infrastructure to continue to respond to PFAS, as well as to address other complex emerging and persistent environmental challenges.

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[22] See https://www.abms.org (accessed June 17, 2022).
[23] See https://www.acenursing.org (accessed June 17, 2022).
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Appendix A

Committee Member, Staff, and Community Liaison Biographies

COMMITTEE MEMBERS

Bruce N. Calonge, M.D., M.P.H. (NAM) (*Chair*), is an associate professor of family medicine at the Colorado School of Medicine, University of Colorado Denver, and an associate professor of epidemiology at the Colorado School of Public Health. He most recently served as the president and chief executive officer of The Colorado Trust, a private grant-making foundation dedicated to achieving health equity for all Coloradans. Nationally, Dr. Calonge chairs the Centers for Disease Control and Prevention's (CDC's) Task Force on Community Preventive Services; chairs the National Academies of Sciences, Engineering, and Medicine's Board on Population Health and Public Health Practice; and is a member of the National Academies' Roundtable on the Promotion of Health Equity. He is the past chair of the U.S. Preventive Services Task Force, the past chair of the CDC's Evaluation of Genomic Applications in Practice and Prevention Working Group, and a past member of the Secretary of Health and Human Services' Advisory Committee on Heritable Disorders in Newborns and Children. Prior to coming to The Colorado Trust, Dr. Calonge was the chief medical officer of the Colorado Department of Public Health and Environment. He received his B.A. in chemistry from Colorado College; his M.D. from the University of Colorado; and his M.P.H. from the University of Washington, where he also completed his preventive medicine residency. Additionally, Dr. Calonge completed his family medicine residency at the Oregon Health & Science University.

Laura Anderko, Ph.D., RN, is an environmental health nurse consultant and the co-director of the Federal Region 3 Pediatric Environmental Health Specialty Unit (PEHSU) of the Mid-Atlantic Center for Children's Health and the Environment at the M. Louise Fitzpatrick College of Nursing, Villanova University. Prior to joining Villanova University, she held the Robert and Kathleen Scanlon Chair in Values Based Health Care and was a professor at the School of Nursing & Health Studies at Georgetown University. Dr. Anderko is an educator, clinician, and scholar in public health, nursing, and environmental health. Over the past 8 years in her role with the Region 3 PEHSU, she has consulted with and educated local governments, communities, families, and health professionals about potential health impacts and risk-reduction strategies for PFAS. She has published extensively on PFAS, focusing on the clinicians' role in addressing health impacts and strategies for reducing risks. Dr. Anderko has served on several federal advisory committees, including the Children's Health Protection Advisory Committee for the U.S. Environmental Protection Agency, and is a founding member and steering committee member of the Alliance of Nurses for Healthy Environments. She currently serves as a special advisor for the National Environmental Health Partnership Council, coordinated by the American Public Health Association. Dr. Anderko was recognized by the Obama White House as a Champion of Change for her efforts in climate change and public health. She received her M.S. in nursing from Northern Illinois University and her Ph.D. in public health from the University of Illinois Chicago.

Erin M. Bell, Ph.D., is a professor in the Department of Epidemiology and Biostatistics and the Department of Environmental Health Sciences in the School of Public Health of the University at Albany. She joined the faculty at the University at Albany after completing her postdoctoral training at the National Cancer Institute's Occupational and Environmental Epidemiology Branch. Dr. Bell has taught

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epidemiology and environmental health to undergraduate and graduate students for almost 20 years, serving most recently as the faculty director of the undergraduate program in public health at the University at Albany. She is also an affiliate with the Center for Social and Demographic Analysis. Her research studies focus on environmental exposures as they relate to reproductive, immune, and cancer outcomes. Dr. Bell is currently the co–principal investigator of two cohort studies: the Upstate KIDS study, which follows more than 6,000 children to identify potential risk factors for developmental health effects, and the Health Study of New York State Communities Exposed to PFAS Contaminated Drinking Water, funded by the Agency for Toxic Substances and Disease Registry as one of seven sites participating in the Multi-site PFAS Health Study. She has served on several committees with the National Academies of Sciences, Engineering, and Medicine, including the Committee to Review the Health Effects in Vietnam Veterans of Exposure to Herbicides: Seventh, Eighth, and Tenth Biennial Updates. Dr. Bell received her M.S. in epidemiology and biostatistics from the University of Massachusetts Amherst and her Ph.D. in epidemiology from the University of North Carolina at Chapel Hill.

Dana Boyd Barr, Ph.D., is a research professor at the Emory University Rollins School of Public Health, where she co-directs the Laboratory for Exposure Assessment and Development in Environmental Research and leads research within the National Institutes of Health–funded Human Exposome Research Center: Understanding Lifetime Exposures (HERCULES). Her research focuses on using analytical chemistry techniques to assess exposure to toxicants, primarily related to maternal and child health. Prior to joining the faculty at Emory, Dr. Boyd Barr worked for 23 years at the Centers for Disease Control and Prevention (CDC), where she devoted much of her time to the development of methods for assessing human exposure to a variety of environmental toxicants. She is the past president of the International Society of Exposure Science (ISES), former editor-in-chief of the *Journal of Exposure Science and Environmental Epidemiology*, and the current deputy editor for *Environmental Health Perspectives*. Dr. Boyd Barr has received many awards for her research, including ISES's Daisy Award for Outstanding Investigator, two awards from the Secretary of Health and Human Services for exposure-health investigations involving diethylene glycol and methyl parathion poisoning, the 2004 Federal Scientific Employee of the Year award, the CDC's Mackel Award for outstanding collaboration among epidemiology and laboratory, and the U.S. Environmental Protection Agency's Silver Medal for outstanding service to environmental health. In 2014, 2015, and 2016, she was designated by Thomson Reuters as a Highly Cited Scientist in environment/ecology, representing the top 1 percent of scientists in her field. She recently became a member of the National Academies of Sciences, Engineering, and Medicine's Division on Earth and Life Studies' Board on Environmental Studies and Toxicology. Dr. Boyd Barr received her Ph.D. in analytical chemistry from Georgia State University.

Kevin C. Elliott, Ph.D., is a professor at Michigan State University (MSU) with joint appointments in the Lyman Briggs College, the Department of Fisheries and Wildlife, and the Department of Philosophy. His research lies at the intersection of the philosophy of science, research ethics, and environmental ethics, exploring the roles of ethical and social values in scientific research on environmental pollution, as well as ethical issues related to science communication, science policy, and team science. Dr. Elliott has authored a wide range of articles and book chapters and has published two books with Oxford University Press: *Is a Little Pollution Good for You?: Incorporating Societal Values in Environmental Research* (2011) and *A Tapestry of Values: An Introduction to Values in Science* (2017). He has also co-edited two books: *Exploring Inductive Risk: Case Studies of Values in Science* (with Ted Richards, Oxford University Press, 2017) and *Current Controversies in Values and Science* (with Dan Steel, Routledge Press, 2017). Dr. Elliott served as the program committee chair for the 2018 meeting of the Philosophy of Science Association and is the associate editor for the journal *Philosophy of Science*. He served as a member of the advisory council for the National Institute of Environmental Health Sciences from 2014 to 2018 and is currently a member of the MSU Center for PFAS Research. Dr. Elliott has served on organizing committees and workshops with the National Academies of Sciences, Engineering, and

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Medicine, including the Committee on the Use of Emerging Science for Environmental Health Decisions. He received his Ph.D. and M.A. in history and philosophy of science from the University of Notre Dame.

Melissa Gonzales, Ph.D., M.S., is a professor and the chair in the Department of Environmental Health Sciences at the Tulane University School of Public Health and Tropical Medicine. She formerly was a professor in the Division of Epidemiology, Biostatistics, and Preventive Medicine at the University of New Mexico (UNM) School of Medicine. At UNM she was the co-director of UNM's Center for Native Environmental Health Equity Research; director of evaluation for the vice chancellor's Office of Diversity; and associate vice chancellor for research and evaluation in the Office of Diversity, Equity and Inclusion at the UNM Health Sciences Center. As an environmental health scientist with expertise in exposure assessment and epidemiology, Dr. Gonzales's community-engaged, translational research focuses on understanding the contribution of environmental exposure to health disparities, rural and minority health, and the translation of research for improved health equity through informed policy. Her research and leadership roles at UNM include serving in the Metal Exposure Toxicity Assessment on Tribal Lands in the Southwest Superfund Center, and the Transdisciplinary Research, Equity, and Engagement Center for Advancing Behavioral Health. Her work includes the Albuquerque Hispanic Moms Study, the Zuni Exposure Study, the Colorectal Disease Prevention Study, and the UNM–University of Texas at El Paso ARCH study of asthma and air pollution among children living on the U.S.-Mexico border. Dr. Gonzales has previously served on two committees with the National Academies of Sciences, Engineering, and Medicine: the Committee to Review the Health Effects in Vietnam Veterans of Exposure to Herbicides: Tenth Biennial Update, and the Committee to Evaluate the Potential Exposure to Agent Orange/TCDD Residue and Level of Risk of Adverse Health Effects for Aircrew of Post-Vietnam C-123 Aircraft. She received her M.S. in toxicology/industrial hygiene at the University of Arizona College of Pharmacy and her Ph.D. in environmental health at the University of California, Berkeley, School of Public Health.

Erin N. Haynes, Ph.D., is the Kurt W. Deuschle Professor in Preventive Medicine and Environmental Health and the chair of the Departments of Epidemiology and Preventive Medicine and Environmental Health within the University of Kentucky (UK) College of Public Health. She is the deputy director of the National Institutes of Health's (NIH's) National Institute of Environmental Health Sciences' (NIEHS's) UK Center for Appalachian Research in Environmental Sciences; associate director of the UK Center for Clinical and Translational Science; co-chair of the NIEHS Disaster Research Response Committee; and senior associate editor of the *Journal of Appalachian Health*. Her current research, funded by NIEHS, focuses on investigating the effects of neurotoxicant exposure, particularly metals, on neurodevelopment in rural adolescents, and development and validation of a real-time, lab-on-a-chip sensor for blood metals detection. Dr. Haynes works with community members to address environmental health issues and develops citizen science tools to enable environmental health research. She also serves on the NIH/NIEHS Environmental Health Sciences study section and as an academic counselor for the International Society of Exposure Science. Dr. Haynes received her M.S. in environmental genetics and molecular toxicology from the University of Cincinnati, and her Dr.P.H. in environmental health sciences from the University of Michigan.

Jane Hoppin, S.M., Sc.D., is a professor of biological sciences and the deputy director of the Center for Human Health and the Environment at North Carolina State University. Her research focuses on how epidemiologic evidence can inform mechanisms of disease from environmental exposures. Dr. Hoppin is currently leading a National Institutes of Health–funded study of PFAS exposures in the Cape Fear River basin, as well as a study evaluating the impact of pesticide exposure to residents in the banana-growing region of Costa Rica. She has authored more than 200 publications related to exposure assessment for chemicals in the environment and health outcomes. Dr. Hoppin previously spent almost 15 years at the National Institute of Environmental Health Sciences, working on the Agricultural Health Study. She

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received her S.M. and Sc.D. in environmental health and epidemiology from the Harvard T.H. Chan School of Public Health.

Tamarra James-Todd, Ph.D., M.P.H., is the Mark and Catherine Winkler Assistant Professor of Environmental Reproductive and Perinatal Epidemiology at the Harvard T.H. Chan School of Public Health, an associate epidemiologist at Brigham and Women's Hospital, and an instructor in medicine at Harvard Medical School. Her research expertise is in reproductive and developmental outcomes following exposure to environmental risk factors. Specifically, she evaluates the impact of pregnancy as a sensitive period of environmental chemical exposure for women's cardiometabolic health across the reproductive life course. Dr. James-Todd is the principal investigator (PI) of the Environmental Reproductive and Glucose Outcomes (ERGO) Study, which evaluates the impact of environmental chemicals and glucose metabolism during the perinatal period. She is also the PI of a study evaluating the effects of PFAS exposure on maternal cardiometabolic health across the reproductive life course within the Project Viva pregnancy/birth cohort. Dr. James-Todd has a particular focus on racial and ethnic disparities in environmental exposures and women's reproductive health outcomes. She has served on the U.S. Environmental Protection Agency's Scientific Advisory Board for the Chemical Assessment Advisory Committee and on the National Academies of Sciences, Engineering, and Medicine's Committee for Gulf War and Health: Volume 11: Generational Health Effects of Serving in the Gulf War. Dr. James-Todd received her M.P.H. in international health from the Boston University School of Public Health and her Ph.D. in epidemiology from Columbia University.

Alex R. Kemper, M.D., is the division chief of primary care pediatrics at Nationwide Children's Hospital and a professor of pediatrics at The Ohio State University College of Medicine. The focus of his research is the evaluation of preventive services delivered in the primary care setting. Dr. Kemper currently serves as the chair of the Evidence Review Workgroup for the Secretary of Health and Human Services' Advisory Committee on Heritable Disorders in Newborns and Children and as the deputy editor of *Pediatrics*. He is a former member of the U.S. Preventive Services Task Force and has served on the National Academies of Sciences, Engineering, and Medicine's Planning Committee on Examining Special Nutritional Requirements for Disease States. Dr. Kemper received his M.D. from Duke University, where he also completed his pediatric residency training, followed by combined fellowship training in health services research and medical informatics, and residency training in preventive medicine at the University of North Carolina.

Brian Linde, M.D., M.P.H., is an assistant professor in the Yale Occupational and Environmental Medicine Program at the Yale School of Medicine, where he oversees medical education and training and has designed curricula for physicians in training. He is also the chief of occupational health services at the Veterans Affairs Connecticut Healthcare System, where he oversees more than 3,000 employees, offering services including medical surveillance, work injury evaluation and management, infection prevention, and employee health and well-being. As a board-certified occupational and environmental physician, Dr. Linde also provides patient consultations to evaluate health effects of occupational and environmental exposures. He received his M.P.H. in occupational and environmental medicine from the Yale School of Public Health and his M.D. from Albert Einstein College of Medicine, Yeshiva University.

Marc-André Verner, Ph.D., M.S., is an associate professor in the Department of Occupational and Environmental Health, School of Public Health, Université de Montréal (Canada). He is also a member of the Centre for Public Health Research. Dr. Verner's expertise is in human health risk assessment, toxicology, biological modeling, and environmental epidemiology. His current research projects focus primarily on developmental exposure to environmental chemicals in the womb and postnatally through breastfeeding in the context of epidemiologic studies and risk assessment, including estimating gestational and lactational exposure to PFAS. Namely, he participated in the Minnesota Department of Health reevaluation of water guidance values for perfluorooctanoic acid and perfluorooctane sulfonic

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acid. In 2016, Dr. Verner received the Joan M. Daisey Outstanding Young Scientist Award for his studies using pharmacokinetic modeling to assess exposures during hypothesized windows of susceptibility to contaminants. He received his M.S. and Ph.D. from the Université du Québec à Montréal.

Veronica M. Vieira, D.Sc., M.S., is a professor in and the chair of the Department of Environmental and Occupational Health at the University of California, Irvine (UC Irvine), Program in Public Health. Her current work includes evaluating birth defects and infant morbidity in relation to air pollution using generalized additive models, and she has worked with the Boston University Superfund Research Program. She works extensively with reconstructing historic environmental exposures using geographic information systems and has experience with groundwater modeling and perfluorooctanoic acid. Dr. Vieira collaborated on the C-8 Health Project, contributing to several health and exposure studies, and is currently an investigator on the UC Irvine PFAS Health Study, part of a multisite study sponsored by the Centers for Disease Control and Prevention and the Agency for Toxic Substances and Disease Registry. She has served on the National Academies of Sciences, Engineering, and Medicine's Committee to Review Possible Toxic Effects from Past Environmental Containment at Fort Detrick. Dr. Vieira received her M.S. in environmental engineering from Stanford University and her D.Sc. in environmental health from the Boston University School of Public Health.

Xiaobin Wang (NAM), M.D., M.P.H., is the Zanvyl Krieger Professor in Children's Health, the director of the Center on the Early Life Origins of Disease at the Johns Hopkins Bloomberg School of Public Health, a professor of pediatrics at the Johns Hopkins University School of Medicine, and an American Board of Pediatrics–certified pediatrician at Johns Hopkins Hospital. Her work unites biomarkers, clinical medicine, epidemiology, and disease prevention. Dr. Wang served as the principal investigator in a number of molecular epidemiological studies funded by the National Institutes of Health and led multi-institution, multidisciplinary teams to investigate environmental, nutritional, genetic, and epigenetic factors during critical developmental windows (preconception, in utero, infancy, and childhood). Her team has conducted a series of studies in three unique study cohorts (Boston Birth Cohort, Chicago Family Cohort, and Chinese Twin Cohort). Dr. Wang has served on two committees with the National Academies of Sciences, Engineering, and Medicine: the Committee on Food Allergies: Global Burden, Causes, Treatment, Prevention, and Public Policy, and the Committee on Understanding Premature Birth and Assuring Healthy Outcomes. She is also a member of the National Academy of Medicine. Dr. Wang received her M.D. from Beijing Medical University, her M.P.H. from the School of Public Health and Tropical Medicine at Tulane University, and her Sc.D. from the Johns Hopkins School of Hygiene and Public Health. She completed a 3-year research fellowship in environmental epidemiology at the Harvard T.H. Chan School of Public Health and a residency in pediatrics at the Boston University Medical Center.

Chris J. Wiant, Ph.D., M.P.H., is the founding president and the former chief executive officer of the Caring for Colorado Foundation, serving from 2000 until his retirement in 2020. Previously the executive director of Colorado's Tri-County Health Department, Dr. Wiant is experienced in risk assessment and communication, exposure science, and environmental policy, as well as in collaborating with communities dealing with environmental contamination. He also served as the chief of the Environmental Chemistry Section of the Illinois Department of Public Health. Dr. Wiant was appointed to five terms on the Colorado Water Quality Control Commission and has served on the U.S. Environmental Protection Agency's National Drinking Water Advisory Council, the federal advisory committee for development of regulations for disinfection by-products in drinking water (Stages I and II), and he was the chair of the National Science Foundation's International Council of Public Health Consultants. Dr. Wiant is also the past president of both the National Environmental Health Association and the Colorado Public Health Association, and was a key participant in the negotiation of the cleanup (Record of Decision) at the Rocky Mountain Arsenal Superfund site. He has been appointed to and recognized for his service on a variety of other local, state, and national advisory committees and boards, and as a facilitator of solutions to community public health challenges. Dr. Wiant received his M.S. in health services administration,

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M.P.H. from the University of Illinois, and Ph.D. in public policy with an emphasis in environmental policy from the University of Colorado.

STAFF

Elizabeth Barksdale Boyle, M.P.H., is a senior program officer in the National Academies of Sciences, Engineering, and Medicine's Health and Medicine Division's Board on Population Health and Public Health Practice. She previously served for several years as a program officer with the National Academies' Board on Environmental Studies and Toxicology. Formerly, she was an environmental health scientist at Westat, where she supported the U.S. Environmental Protection Agency, the National Institute of Child Health and Human Development, and the National Cancer Institute. Before her tenure at Westat, Ms. Boyle was a student epidemiologist at the Minnesota Department of Health and an industrial hygienist at a consulting firm in Cincinnati, Ohio. She is a fellow of the Bloomberg American Health Initiative at the Johns Hopkins Bloomberg School of Public Health, where she is pursuing a Dr.P.H. in environmental health. Ms. Boyle has an M.P.H. in environmental health from the University of Minnesota and a certificate in risk sciences and public policy from the Johns Hopkins Bloomberg School of Public Health, and is a certified industrial hygienist.

Marilee Shelton-Davenport, Ph.D., is a senior program officer at the National Academies of Sciences, Engineering, and Medicine, where she serves as the director of the innovative, cross-institutional Environmental Health Matters Initiative. Since 1999, Dr. Shelton-Davenport has worked to guide the country's best scientists and practitioners in providing authoritative advice to agencies and other organizations interested in biomedical research and regulatory issues. Specifically, she has experience developing and executing impactful activities that serve the National Institutes of Health, the U.S. Environmental Protection Agency, the U.S. Department of Defense, and the U.S. Food and Drug Administration. Her work with the National Academies' Board on Life Sciences and Board on Chemical Sciences and Technology has focused on emerging science related to the impact of environmental exposures on human health and defense. A leader of multidisciplinary and multisector teams, Dr. Shelton-Davenport is skilled at guiding experts in drilling down to the root of issues, generating strategies, and developing creative solutions. She has a B.S. in biochemistry from Clemson University and a Ph.D. in pharmacology from the University of North Carolina.

Kate Guyton, Ph.D., is a senior program officer with the Board on Environmental Studies and Toxicology within the Division on Earth and Life Studies at the National Academies of Sciences, Engineering, and Medicine. She has more than 20 years of experience applying her expert knowledge in mechanistic toxicology and carcinogenesis, and has been certified as a diplomate of the American Board of Toxicology since 1998. Her prior experience includes service as a senior toxicologist at the International Agency for Research on Cancer with the World Health Organization in Lyon, France (2014–2020). Previously, Dr. Guyton served as a toxicologist in the Office of Research and Development at the U.S. Environmental Protection Agency (2005–2014) and as the director of scientific affairs at CCS Associates (1998–2005), a woman-owned small business. She has authored more than 90 scientific articles in her areas of expertise with an overall h-index of 46. Dr. Guyton received her B.A. (cum laude) in biology from Johns Hopkins University, her Ph.D. in toxicological sciences from the Johns Hopkins Bloomberg School of Public Health, and her postdoctoral training at the National Institutes of Health.

Kaley Beins, M.P.H., is a program officer with the Board on Environmental Studies and Toxicology within the Division on Earth and Life Studies at the National Academies of Sciences, Engineering, and Medicine. Previously, she worked as a federal contractor for Abt Associates, where she supported the development of toxicological profiles for the Agency for Toxic Substances and Disease Registry, as well as green chemistry and product labeling programs for the U.S. Environmental Protection Agency. Ms. Beins focuses on the intersection of public health and toxicology, and has conducted research and led

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community engagement initiatives with nongovernmental organizations and local health departments, including service as a Fulbright Research Fellow. She is a board member for DC EcoWomen and the vice president of the Washington, DC, chapter of Sigma Xi Scientific Research Honor Society. Ms. Beins has a B.S. in environmental biology from Georgetown University and an M.P.H. in environmental health sciences from the University of Maryland.

Alexis Wojtowicz is an associate program officer who has supported the National Academies of Sciences, Engineering, and Medicine's Board on Population Health and Public Health Practice since 2016. She has also supported the Roundtable on Health Literacy, an action collaborative on preventing firearm violence, and consensus studies about Medicare payment and the public health effects of e-cigarettes. Before joining the National Academies, Ms. Wojtowicz conducted recruitment and intake at a culinary job training program in Washington, DC, and prior to that, coordinated an AmeriCorps VISTA program that placed summer associate members at anti-hunger nonprofit organizations across the United States. Ms. Wojtowicz has a B.A. in art history from the University of Maryland and is currently pursuing an M.P.H. at the Johns Hopkins Bloomberg School of Public Health, where she is a Bloomberg American Health Initiative fellow.

Alexandra McKay, M.A., is a senior program assistant in the Health and Medicine Division of the National Academies of Sciences, Engineering, and Medicine. During her graduate and undergraduate careers, she worked in museums and cultural heritage institutions, focusing on public education and assisting in database creation and exhibit curation. Ms. McKay also has experience working for the National Park Service as an interpretation ranger, concentrating on science education and public engagement. She received her M.A. in archaeological studies from Yale University.

COMMUNITY LIAISONS

Laurene Allen is a resident of Merrimack, New Hampshire, who co-founded the community group Merrimack Citizens for Clean Water after learning, in March 2016, that the water she and her family drank for decades was contaminated by PFAS. She has been an advocate for the needs of her PFAS-impacted community for the past 6 years, engaging local, state, and federal officials, and bringing together residents to work together on both a local and national level. After working to gather health data in her community, Ms. Allen co-authored an article in the journal *Environmental Health* titled "Making the Invisible Visible: Results of a Community-Led Health Survey following PFAS Contamination of Drinking Water in Merrimack, New Hampshire." Additionally, she co-founded and continues to serve in leadership of the National PFAS Contamination Coalition, composed of community members from across the nation working together to attain mutual federal needs.

Andrea Amico is the co-founder of the Testing for Pease community action group at the former Pease Air Force Base in Portsmouth, New Hampshire. In 2014, after learning her husband and two small children were impacted by drinking water highly contaminated by PFAS, she began advocating for her community and others, raising awareness and providing education, to achieve a common goal of reducing PFAS exposure to impacted communities. Ms. Amico testified at U.S. Senate hearings on PFAS in September 2018 and again in December 2021. She received the Citizen Excellence in Community Involvement Award from the U.S. Environmental Protection Agency Region 1 in November 2018. Ms. Amico attended the President's State of the Union Address as Senator Jeanne Shaheen's guest in February 2019 to raise awareness of PFAS contamination. She is the co-founder of the National PFAS Contamination Coalition, formed in June 2017 to bring PFAS advocates from around the country to organize for change at the federal level. Additionally, Ms. Amico helped organize the first two National PFAS Conferences held at Northeastern University in Boston, Massachusetts, in June 2017 and June 2019. She has given a TEDx talk on PFAS and presented at the Centers for Disease Control and Prevention's first Public Health Grand Rounds on PFAS in Atlanta, Georgia. Ms. Amico serves on the

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Pease Community Assistance Panel with the Agency for Toxic Substances and Disease Registry and is community co-chair of the Pease Restoration Advisory Board with the U.S. Air Force. She is also a community co-chair on the City of Portsmouth Safe Water Advisory Group. Ms. Amico has a master's degree in occupational therapy.

Stel Bailey is the chief executive director of Fight For Zero and the co-facilitator of the National PFAS Contamination Coalition, is certified in wildlife monitoring, and is a content administrator behind several online publications. Her life took a dramatic turn in 2013 when she and her father, brother, and uncle, as well as the family dog, were diagnosed with cancer. Her family's case was so unique that a genetic counselor invited them to do genetic testing, which showed no mutation genes, indicating they did not have an increased risk of developing the disease. Determined to find answers, Ms. Bailey began to crowdsource information about other cancer cases in her hometown on Florida's space coast. As she spoke out about her family's case, she connected with many others affected by diseases in unusual ways. As a cancer survivor and military dependent, Ms. Bailey is passionate about helping families and veterans prevent disease by sharing her insights about harmful toxins, including PFAS, and their health effects. Her broad knowledge in coordinating community engagement projects has resulted in various opportunities to share environmental health information, such as contributing to local publications and speaking at events. She has led additional efforts focused on harmful contaminants, such as assisting in getting a state health assessment, collecting water samples for countywide projects, and developing partnerships that will help improve understanding of the burdens of PFAS contamination and related chronic diseases.

Kyla Bennett, Ph.D., is the director of science at Public Employees for Environmental Responsibility (PEER), a national nonprofit service organization for environmental and public health professionals, land managers, scientists, enforcement officers, and other civil servants dedicated to upholding environmental laws and values. Prior to working at PEER, Dr. Bennett was an environmental scientist and attorney for the U.S. Environmental Protection Agency (EPA) for 10 years. Her work at PEER includes the investigation of sources of PFAS contamination in artificial turf and pesticides, and of impacts on endangered species, such as the North Atlantic right whale, manatee, and Louisiana black bear. Dr. Bennett is currently working on exposing the effects of aerial pesticide spraying and investigating faulty risk assessments of pesticides and other chemicals at EPA. She has a Ph.D. in ecology and evolutionary biology from the University of Connecticut, and a law degree with a certificate in environmental and natural resources law from Lewis and Clark Law School.

Karen Blondel is an environmental organizer/advocate for the Fifth Avenue Committee. She has conducted field trainings and has a background in health and engineering. A native of Brooklyn, Ms. Blondel has led the Gowanus Neighborhood Coalition for Justice activist group to demand the city include the neighborhood's New York City Housing Authority (NYCHA) houses in its development plan. During her tenure with AmeriCorps, she coordinated a women's history event at the Red Hook Library; started a volunteer program to escort seniors on their errands; and launched a summer Safe Streets program, facilitating a shutdown of local roads for recreational use for local residents. In 2018, Ms. Blondel began a "Know Your Rights" workshop for Red Hook public housing residents to inform them on bylaws, requirements, and deadlines. In her current role as an environmental organizer, she educates public housing residents on environmental burdens within and near NYCHA housing developments that are often overlooked by the city.

Phil Brown, Ph.D., is a university distinguished professor of sociology and health science at Northeastern University, where he directs the Social Science Environmental Health Research Institute and co-directs its PFAS Lab, which has grants from the National Science Foundation to study social policy and activism concerning PFAS, and from the National Institute of Environmental Health Sciences (NIEHS) to study children's immune responses and community's responses to PFAS contamination, and

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to develop a nationwide report-back and information exchange. He directs an NIEHS T-32 training program, "Transdisciplinary Training at the Intersection of Environmental Health Science and Social Science," heads the Community Outreach and Translation Core of Northeastern's Children's Environmental Health Center (Center for Research on Early Childhood Exposure and Development in Puerto Rico, orCRECE), and is the research translation director and the community engagement core co-director of Northeastern's Superfund Research Program (Puerto Rico Testsite to Explore Contamination Threats, or PROTECT). Dr. Brown is a past member of the NIEHS Council. His books include *No Safe Place: Toxic Waste, Leukemia, and Community Action*; *Toxic Exposures: Contested Illnesses and the Environmental Health Movement*; and *Contested Illnesses: Citizens, Science and Health Social Movements*. Dr. Brown earned his Ph.D. in sociology from Brandeis University.

Alberto J. Caban-Martinez, D.O., Ph.D., M.P.H., is a public health board-certified faculty member in the Department of Public Health Sciences at the University of Miami with an educational background in medicine, epidemiology, and public health and more than 10 years of domestic and international practice and research experience. He is the assistant provost for research standards and an associate professor of public health sciences and physical medicine and rehabilitation at the University of Miami Miller School of Medicine; the deputy director of the Firefighter Cancer Initiative at the Sylvester Comprehensive Cancer Center; and the co-director and principal investigator of the national Federal Emergency Management Agency–funded Fire Fighter Cancer Cohort Study. He is a former fellow of the National Academies of Sciences, Engineering, and Medicine's Gulf Research Program, and in 2014, he was appointed to the Institute of Medicine's Committee on Gulf War and Health for 2 years to provide scientific expertise on occupational exposures and work-related health conditions. He also served on the National Occupational Research Agenda committee of the National Institute for Occupational Safety and Health (NIOSH), helping to set the national research agenda on worker health and safety. He served 4 years as a standing member of the NIOSH study section and 1 year as the chair. He has published more than 120 peer-reviewed publications and shared more than 215 scientific presentations on a wide range of occupational health and safety topics.

Cheryl Cail is a small business owner in Myrtle Beach, South Carolina, the vice chief of the Waccamaw Indian People, and the chair for SC Idle No More, a committee within the South Carolina Indian Affairs Commission. In December 2018, after her 20-year-old son was diagnosed with testicular cancer, Ms. Cail became aware of PFAS contamination in the groundwater at the former Myrtle Beach Air Force Base, and later learned of the extent of contamination from the use of aqueous film-forming foam at other U.S. Department of Defense sites throughout the state. She joined the National PFAS Contamination Coalition in 2019, and is currently working to raise awareness of the impact of PFAS contamination of both the environment and people in South Carolina. Ms. Cail received associate degrees from Horry-Georgetown Technical College in both legal studies and human services and received the Phi Theta Kappa Society's All-State Academic Team award.

Courtney Carignan, Ph.D., is an exposure scientist and an environmental epidemiologist at Michigan State University (MSU). Her research helps protect reproductive and child health by investigating exposure to contaminants in food and water, and consumer and personal care products. She currently helps lead biomonitoring studies (PFAS UNITEDD and PFAS REACH) funded by the U.S. Environmental Protection Agency and the National Institutes of Health, investigating PFAS exposure and immunotoxicity for communities impacted by drinking water contamination. Dr. Carignan is on the organizing committee for the National PFAS Conference and helps lead MSU's Center for PFAS Research. She received her Ph.D. in environmental health from the Boston University School of Public Health and completed postdoctoral training at Dartmouth College and the Harvard T.H. Chan School of Public Health.

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Tracy Carluccio is the deputy director of the Delaware Riverkeeper Network (DRN), where she has worked as an environmental advocate since 1989. DRN is a nonprofit membership organization working throughout the entire length and breadth of the Delaware River Watershed to defend its outstanding values and restore them where needed. Ms. Carluccio works for the Watershed's protection in New Jersey, Pennsylvania, New York, and Delaware, addressing issues that include water quality; healthy habitats and communities; environmental regulation and policy; clean, efficient, and renewable energy; and biodiverse ecosystems. She has also worked on PFAS issues since 2005 and has commented and written extensively on community and regulatory matters related to these compounds in New Jersey, Pennsylvania, and Delaware. Ms. Carluccio serves on the New Jersey Department of Health Centers for Disease Control and Prevention Biomonitoring Advisory Committee on PFAS.

Jamie DeWitt, Ph.D., is an associate professor in the Department of Pharmacology & Toxicology of the Brody School of Medicine at East Carolina University. Her research focuses on the effects of environmental contaminants on the adult and developing immune systems, as well as on interactions between the immune and nervous systems. She is the principal investigator, co–principal investigator, and co-investigator on several funded studies concerning the immune effects of PFAS, especially those considered novel or understudied. Dr. DeWitt received her Ph.D.s in environmental science and neural science from Indiana University Bloomington and completed postdoctoral training in immunotoxicology at the U.S. Environmental Protection Agency under a cooperative training agreement with the University of North Carolina.

Emily Donovan is the co-founder of Clean Cape Fear. She is a tireless advocate for clean water, spending her free time educating the public on the dangers of PFAS and other toxins in drinking water and the environment. Ms. Donovan has testified before Congress twice regarding the influence of the DuPont/Chemours facility in Fayetteville, North Carolina, which is contaminating the downstream drinking water supply for 250,000 residents, including giving testimony during the first-ever congressional hearing on PFAS contamination. She participated in a *Washington Post Live* panel discussion with actor Mark Ruffalo and lawyer Rob Bilott. Most recently, Ms. Donovan worked to secure reverse osmosis filling stations in 49 public schools impacted by PFAS contamination in Brunswick and New Hanover counties. She regularly travels the country sharing her personal impact story, as well as those of her friends and neighbors. Ms. Donovan frequents Washington, DC, and Raleigh, North Carolina, pressuring lawmakers and regulators for swifter responses to the growing PFAS public health crisis.

Alan Ducatman, M.D., M.S., is a Mayo Clinic–trained internist and occupational physician, and a professor emeritus in the West Virginia University School of Public Health and School of Medicine. His clinical career has focused on medical screening related to environmental exposures, with research addressing environmental disease and disease prevention, including health communications to affected populations. He has designed community studies and actively published on population aspects of exposure to PFAS, and many of his more than 30 publications concerning PFAS are highly cited. As a clinical consultant to industry, labor, nonprofit organizations, and government organizations, Dr. Ducatman has also worked with a variety of community groups to provide information concerning PFAS clinical science. In addition, he has an active research program in quality assurance concerning clinician laboratory orders and interpretation, which informs his community service. His other public service has included serving as the chair of the external science advising committee to the National Center for Environmental Health and the Agency for Toxic Substances and Disease Registry of the Centers for Disease Control and Prevention, and as the chair of the Residency Review Committee in Preventive Medicine for the Accreditation Council for Graduate Medical Education.

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Patrick Elder is the director of Military Poisons, an organization that works to draw attention to the role of the military in the environmental contamination through the use of PFAS in firefighting foams and other applications. Two hundred of his articles on PFAS have been published in several dozen publications, including *Global Geneva*, *Truthout*, *Consortium News*, *Common Dreams*, and *LA Progressive*. Mr. Elder seeks to draw public attention to food, especially seafood, as the primary pathway of human exposure to PFAS. He receives support from the Women's International League for Peace and Freedom (WILPF); WILPF US Earth Democracy; the Patagonia Foundation; the Peace Development Fund; and the Center for Health, Environment & Justice. Mr. Elder is presently working on campaigns in several New England states and Maryland, as well as in Germany and Japan, to influence policy makers to take steps to protect public health from the scourge of PFAS. He has toured the United States and Europe several times, speaking on contamination at military bases. Mr. Elder holds an M.A. in government from the University of Maryland and a B.A. in political science/education from St. Mary's College of Maryland.

Teresa Gerade is an advisory member of a Vermont-Canadian volunteer organization in the Northeast Kingdom known as DUMP (Don't Undermine Memphremagog's Purity), which came into being in June 2018 to appeal the application for a 51-acre expansion of Vermont's only permitted, privately owned landfill. The group's goal is to restore and protect the waters and watershed of Lake Memphremagog, an international lake located in the northeast corner of Vermont. The appeal was unsuccessful because of a lack of funding for legal representation. However, the group participated in a mediation session with the owners of the landfill and achieved a 4-year moratorium on the discharge of landfill leachate (the garbage juice that is created in landfills) into the Lake Memphremagog watershed. DUMP is now actively pursuing a state legislative designation of "Lake in Crisis" to garner funding and additional support for restoring and protecting the waters of Lake Memphremagog.

Hope Grosse is the co-founder of Buxmont Coalition for Safer Water and serves on the National PFAS Contamination Coalition, working to establish enforceable federal and state drinking water standards for this chemical class. She acts as the community liaison for outreach and leads social media efforts, updating residents about PFAS water contamination and related issues. Ms. Grosse has been featured on WHYY's *Radio Times, Live with Marty Moss-Coane,* discussing PFAS chemicals health and regulations. She also serves as a community representative on the Warminster Naval Airbase Technical Review Committee, which is charged with oversight of the environmental cleanup under the Comprehensive Environmental Response, Compensation, and Liability Act. Ms. Grosse grew up directly across the street from Warminster Naval Base and worked on the base after high school graduation. Her father died of cancer at age 52, and she was diagnosed with stage 4 cancer at age 25, which quickly spread to her lymph system. Other unexplained tumors have also been removed from her body over the years. After learning that the Warminster Naval Base was named a Superfund National Priority site, she began to get involved in environmental advocacy and is committed to bringing awareness of PFAS and other contaminants to others in her community.

Loreen Hackett has been advocating for families in Hoosick Falls, New York, since the discovery of severe contamination more than 6 years ago led to issuance of the first National Priorities List Federal Superfund Site declarations for PFOA. Through biomonitoring, her family exhibited some of the highest levels of perfluorooctanoic acid tested in the area. In June 2016, she created #PfoaProjectNY, which has gone worldwide, and continues to share information on PFAS. Ms. Hackett is the co-chair of the Hoosick Falls Community Action Working Group established for the superfund sites, serves on the Community Action Partnership Committee for the Centers for Disease Control and Prevention–awarded site study, and is on the Committee of the National PFAS Contamination Coalition. She has attended and submitted testimony in two congressional hearings in Washington, DC, and continues working with elected officials on bills regulating PFAS, as well as with various environmental organizations. She survived cancer that may have been linked to PFAS exposure.

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Ayesha Khan worked at Vitamin Water in New York City for a decade before moving to the island of Nantucket, Massachusetts, where she worked for a local surgeon. Currently, she is a stay-at-home mom for her two young children. Her husband Nate, who works as a firefighter, was recently diagnosed with testicular cancer and is in remission. As Ms. Khan researched about her husband's cancer, she learned about PFAS and their connections to aqueous film-forming foam and firefighter gear. She co-founded Nantucket PFAS Action Group as an educational resource about the hazards of PFAS and as a space for local community members to openly discuss concerns, thoughts, and experiences. Ms. Khan holds a B.A. in applied mathematics and statistics from Boston University.

Rainer Lohmann, Ph.D., is a professor of oceanography and the director of the University of Rhode Island Superfund Research Center, where he and his group conduct research into the sources, transport, and bioaccumulation of anthropogenic pollutants, often relying on the use of passive samplers. In addition to PFAS, his research covers dioxins, polychlorinated biphenyls, legacy pesticides, and emerging contaminants. Dr. Lohmann is a scientific counselor for the U.S. Environmental Protection Agency's Sustainable and Healthy Communities Subcommittee and a speaker at the National Academies of Sciences, Engineering, and Medicine's Environmental Health Matters Initiative on PFAS. He was selected as an Alexander von Humboldt fellow, and as a Fulbright fellow for the 2021–2022 Arctic Initiative. Dr. Lohmann serves as the editor for *Environmental Toxicology and Chemistry* and is on the editorial boards of publications including *Environmental Science & Technology* and *Environmental Science & Technology Letters*.

Samraa Luqman works in social services with the federal government. A native of Dearborn, Michigan, she has experience working in public schools and serving in community hospitals and cultural centers, including the Arab Community Center for Economic and Social Services and the American Yemeni Women's Association, where she is the secretary and a board member. Ms. Luqman has observed a multitude of community members becoming ill with cancers, asthma, and other illnesses attributable to pollution, and has become involved in pursuing environmental justice, speaking at community meetings and town halls. In 2019, she joined the Environmental Health Research to Action Steering Committee, which educates high school students about the impacts of pollution, and the PFAS Alliance shortly thereafter. By 2020, Ms. Luqman also joined the Clean Air Council and presented to multiple audiences on the effects and existence of PFAS in both air and water. She has also worked with the Concerned Residents for South Dearborn, the University of Michigan's Environmental Interpretive Center, and Friends of the Rouge to raise awareness on the environmental issues of her community. Her most recent endeavors include providing input on the enactment and enforcement of a fugitive air dust ordinance; fighting for cumulative health impact studies; and partnering with organizations, universities, and other entities to add greenspace, trees, and rain gardens to the area. Ms. Luqman completed her bachelor's degree in psychology at the University of Michigan.

Beth Markesino is the founder of North Carolina Stop GenX in Our Water, advocating for regulations on GenX, PFAS, and perfluorooctane sulfonic acid in North Carolina. She is a participant in the first ever GenX human health study. Ms. Markesino has multiple endocrine issues associated with PFAS, including a thyroid tumor, an adrenal tumor, and placenta problems during her pregnancy that resulted in the death of her son. She actively advocates in her community for clean water and regulatory action addressing endocrine disruptor chemicals. With a grant from Hydroviv, she provided 120 filters for low-income residents in the Lower Cape Fear region. Additionally, she has lobbied against DuPont scientist Michael Dourson's appointment to the U.S. Environmental Protection Agency.

Aaron Maruzzo worked as a water lab analyst for a public utility company in the Northern Mariana Islands, serving the islands of Saipan, Tinian, and Rota. He is currently working toward an M.P.H. in environmental health science at the University of California (UC), Berkeley. In 2020, Mr. Maruzzo co-authored a report for the Berkeley Center for Green Chemistry to identify nontoxic alternatives to short-

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chain PFAS in molded fiber food packaging. His current interests are in community exposure to PFAS in drinking water, green technology, environmental epidemiology, and environmental justice, where he aims to leverage academic resources at UC Berkeley to benefit science and policy actions toward a PFAS-free future in the Western Pacific U.S. territories. Mr. Maruzzo received his B.A. in biology and comparative literature, with a concentration in public health, from Williams College, where he developed a strong interdisciplinary approach to diverse public health issues.

Tobyn McNaughton is a resident of Belmont, Michigan, who has been affected by PFAS. She is a former elementary teacher and now stays home with her two sons. Ms. McNaughton has become an activist and voice for people affected by PFAS.

Kristen Mello, M.S., is the director of Westfield Residents Advocating for Themselves (WRAFT), a community group formed in response to PFAS contamination of the drinking water in Westfield, Massachusetts, caused by the use of aqueous film-forming foam at the Barnes Air National Guard Base. With WRAFT, Ms. Mello has led efforts to get PFAS blood testing for Westfield residents and has served on the Massachusetts Department of Environmental Protection's PFAS MCL (Maximum Contaminant Level) Stakeholder Group. Her advocacy work on PFAS, in large part, led to her being elected a Westfield City Councilor At-Large in 2019 and 2021. Ms. Mello has a bachelor's degree in chemistry and a master's degree in analytical chemistry, specializing in chemometrics.

Pamela K. Miller is founder and Executive Director of Alaska Community Action on Toxics (ACAT). ACAT is an environmental health and justice research, policy, and advocacy organization. She brings more than 30 years of research, education, and advocacy experience to her present work. She serves as Principal Investigator for community-based participatory research projects funded by the National Institute of Environmental Health Sciences. Ms. Miller received a Meritorious Service Award from the University of Alaska and Alaska Conservation Foundation's Olaus Murie Award in recognition of her "long-term outstanding professional contributions to the conservation movement in Alaska." She holds a bachelor's degree in biology from Wittenberg University and a master's degree in environmental science from Miami University. Prior to coming to Alaska, she served as Ocean Issues Technical Coordinator for the Washington Department of Ecology and Director of a marine science education center at Nisqually Reach in southern Puget Sound. She received the Governor's Award for Environmental Excellence in Washington State.

Elizabeth Neary, M.D., is a pediatrician, as well as an educator and scholar in public and environmental health. Having practiced general pediatrics for 15 years, she has devoted herself to educating students, residents, legislators, and the general public about environmental health issues. Dr. Neary is the co-president of the Wisconsin Environmental Health Network, part of Physicians for Social Responsibility–Wisconsin. She is an adjunct assistant clinical professor of pediatrics at the University of Wisconsin–Madison School of Medicine and Public Health and the Wisconsin representative to the Region 3 Pediatric Environmental Health Specialty Unit. For the past 5 years, she has taught environmental health to pediatric residents. Dr. Neary has testified on the health effects of PFAS to the Wisconsin state legislature and to the Madison Water Utility Board.

Laura Olah is the executive director and the co-founder of Citizens for Safe Water Around Badger, a community-based group that organized in 1990 when the community learned that a plume of cancer-causing chemicals had poisoned private drinking water wells near Wisconsin's Badger Army Ammunition Plant. A member of the Sokaogon Ojibwa Community Mole Lake Band, Ms. Olah defines her role as part of a community "undefined by geography" that shares a deep concern for the human environment and the need to empower affected tribes and stakeholders in the decision-making process, ensuring powerful, long-term solutions to military and industrial toxins in our rural communities. She

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views the past 30 years of accomplishments not as hers alone but as shared with the many individuals who have and who continue to be at her side in the work for environmental justice.

Jacob Park, Ph.D., is an associate professor in the Castleton University College of Business. He specializes in innovation, entrepreneurship, and community health issues, with special focus and expertise in emerging and developing economies in Africa, Asia Pacific, and the Caribbean islands. Dr. Park is also a visiting professor at the University of Johannesburg (South Africa); a former Kevin Ruble fellow in conscious capitalism at the Rutgers University School of Management and Labor Relations; the Edmond J. Safra Network Fellow at Harvard University; and a research fellow in the Oxford University Smith School of Enterprise and the Environment. He has served as the coordinating lead author of the report for the United Nations (UN) Environment Programme's Global Environment Outlook (GEO-6); as lead author for the UN Millennium Ecosystem Assessment initiative; and as an expert reviewer for a number of Intergovernmental Panel on Climate Change publications, including the Sixth Assessment Report. Dr. Park is a former member of the Renewable Energy and Adaptation to Climate Technologies Investment Committee of the Africa Enterprise Challenge Fund, and the chair of the U.S. Sustainable and Responsible Investment Forum's International Working Group Steering Committee. He received his master's degree in city planning from the Massachusetts Institute of Technology and his Ph.D. in social sciences/public administration from the Erasmus University of Rotterdam.

Sue Phelan[1] was the co-founder and the volunteer director of GreenCAPE, a nonprofit community advocacy organization formed to educate Cape Cod residents about the hazards of pesticides and other harmful chemicals (including PFAS) being used above the Cape Cod's vulnerable sole source aquifer. The focus of GreenCAPE's work is the community's exposure to PFAS via the public drinking water system and other as-yet-unidentified sources. Its major concerns include that PFAS-exposed Hyannis residents have not been provided with blood testing and the lack of health-protective PFAS regulations nationwide. Ms. Phelan was a board member of the Alliance for a Healthy Tomorrow/Boston and Clean Water Action/Mass, as well as a member of the Cape Chapter of the Sierra Club, the Sierra Club Toxics Team, and the National PFAS Coalition. She served as a community project partner and a member of the Cape Cod Advisory Committee for the Sources, Transport, Exposure, and Effects of PFAS Research Center and was one of only two community representatives invited to be on the Massachusetts Department of Environmental Protection's PFAS MCL (Maximum Contaminant Level) Stakeholder Group. After working as a medical microbiologist/virologist, Ms. Phelan returned to the University of Connecticut College of Agriculture, graduated from the environmental horticulture program, and worked in research and development on insect pheromones and light-mediated changes in plant growth and development.

Andrea Rich is an active participant in fighting for clean water in Wisconsin. She works with S.O.H2O (Save Our Water) to bring the impacts of PFAS to light, and share relevant information with the community in Marinette. Marinette is the third-largest PFAS contamination site in the nation. Ms. Rich has been researching PFAS and its health impacts, as well as filtration methods, for several years. She has been an active participant in public hearings and Department of Natural Resources presentations, and has provided testimony to Senate and Assembly committees on multiple bills proposed to regulate PFAS. Ms. Rich was also instrumental in bringing into the spotlight the contamination of farm fields and dozens of contaminated wells in rural Marinette County, some of which may otherwise still be unidentified. Her current focus is trying to get testing for PFAS in the agricultural goods coming from the contaminated farm fields, and blood testing for the community to determine exposure levels, and possibly identify additional sources of exposure. She is an honors graduate of Lakeland College, with a double major in business administration and marketing.

[1] Deceased January 2022.

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Dana Sargent is the executive director of the environmental nonprofit Cape Fear River Watch (CFRW), based in Wilmington, North Carolina, where she works to protect and improve the water quality of the Cape Fear River basin for all people through education, advocacy, and action. Her work on PFAS began in 2017 when her community learned that DuPont and then Chemours had polluted their drinking water supply for more than 40 years. Her organization sued the polluter and the state regulatory body, which culminated in a Consent Order, parts of which she, along with her CFRW partners and their legal counsel, maintain oversight, ensuring the polluter and the regulatory agency are upholding the requirements of the Order. In addition, CFRW partners with academia on several PFAS research grants including the GenX exposure study, along with several ecological studies and outreach grants. She works among a coalition of advocates in North Carolina pushing for strong PFAS legislation and regulatory action and her organization is one of six that has sued the U.S. Environmental Protection Agency after it refused to hold a PFAS polluter accountable for funding health and toxicity studies. Ms. Sargent has published two websites and numerous articles and op-eds on PFAS, including an op-ed in which she questions whether PFAS exposure caused the brain cancer that took her brother's life in December 2019. He had been exposed for decades in the line of duty as a Chicago firefighter and a former U.S. Marine. She holds an M.S. in environmental sciences and policy from Johns Hopkins University and a B.A. in journalism with a music minor from San Diego State University.

Laurel Schaider, Ph.D., M.S., is a senior scientist in environmental chemistry and engineering at the Silent Spring Institute, where she leads water quality research on PFAS and other contaminants of emerging concern. Her research focuses on characterizing PFAS exposures from drinking water, diet, and consumer products; understanding health effects associated with PFAS; investigating socioeconomic disparities in exposures to drinking water contaminants; and working with communities to develop research studies and resources to address their concerns. Dr. Schaider is the principal investigator for the PFAS-REACH (Research, Education, and Action for Community Health) study, a researcher–community partnership that is evaluating PFAS exposures and immune system effects in children in communities with PFAS water contamination, and developing an online resource center for PFAS-affected communities. She is also the principal investigator of the Massachusetts PFAS and Your Health Study, one of seven projects within the Centers for Disease Control and Prevention PFAS Multi-site Health Study. Dr. Schaider co-leads the Community Engagement Core for the STEEP (Sources, Transport, Exposure, and Effects of PFAS) Superfund Research Program at the University of Rhode Island, including a study to evaluate PFAS levels in private wells on Cape Cod and identify contamination sources. Before joining the Silent Spring Institute, she was a research associate at the Harvard T.H. Chan School of Public Health, where she currently holds an appointment as a visiting scientist. Dr. Schaider served as a member of the planning committee for the 2020 National Academies of Sciences, Engineering, and Medicine's Workshop on Federal Government Human Health PFAS Research. She earned her master's degree and Ph.D. in environmental engineering at the University of California, Berkeley, and a bachelor's degree in environmental engineering science from the Massachusetts Institute of Technology.

Linda Shosie, a proud Latina woman, is a mother, grandmother, wife, and the founder and organizer of the grassroots community organization Mothers for Safe Air & Safe Water Force in Tucson, Arizona. In her fight for the human right to clean and safe water she has dedicated her entire life to protecting public health. Ms. Shosie has become one of the leading voices nationally regarding PFAS exposures in Latino barrios. In 2007, she lost her child to a rare disease she believes was caused by exposures to numerous toxic chemicals in the drinking water in the Tucson South-Side barrio. More recently Ms. Shosie has begun initiating a community-led PFAS health study in her community to assess for PFAS in human blood. She is also part of the National PFAS Contamination Coalition.

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Lenny Siegel is the executive director of the Center for Public Environmental Oversight, where he has been serving since 1994. He is one of the American environmental movement's leading experts on both military facility contamination and the vapor intrusion pathway. Mr. Siegel runs two Internet newsgroups for his organization: the Military Environmental Forum and the Brownfields Internet Forum, as well as activist discussion lists on trichloroethylene and PFAS. He has served on several Interstate Technology and Regulatory Council work teams on environmental remediation, as well as a dozen National Academies of Sciences, Engineering, and Medicine committees addressing military environmental issues. In July 2011, Mr. Siegel was awarded the U.S. Environmental Protection Agency's Superfund Citizen of the Year award. In 2018, he served as mayor of Mountain View, California.

Mike Watters is a community organizer in the Fayetteville, North Carolina, area. After learning his well was contaminated with more than 16 PFAS, Mr. Watters set up a community outreach group. He created a Facebook group with more than 2,700 members and actively provides information to the community, ensuring wells are tested in a rapidly growing area around the Fayetteville Works Facility in North Carolina. Mr. Watters provides input at all North Carolina Science Advisory committee meetings, leads teams in assisting in research with the NC PFAS Testing Network, and works actively with North Carolina State University research teams. Additionally, he engages with state and federal authorities to ensure that violations and spills are documented and action is taken; two notices of violation are directly tied to his actions. Mr. Watters also works with the North Carolina Department of Environmental Quality, having participated in the Granular Activated Carbon Pilot Test. He currently has a state PFAS air monitoring station on his property and assists the state in gathering information. While in the military, Mr. Watters was trained and certified as a U.S. Department of Defense hazardous materials handler. He has college degrees in information technology and firearms technology.

La'Meshia Whittington is a professor in the Division of Sociology at Meredith College. She is also the deputy director for Advance Carolina and the campaigns director for the North Carolina Black Alliance. She is the co-convener of the North Carolina Black and Brown Policy Network, a former national democracy campaigner for Friends of the Earth, the chair of the FRENC Fund Administration, a founding member of Democracy Green, a member of the Burke Women's Fund in Western North Carolina, and a former North Carolina spokesperson on fair courts for The Leadership Conference on Civil and Human Rights. A leader in intersectional democracy and environmental justice, Ms. Whittington was the co-author of North Carolina Senate Bill 673, prioritizing environmentally contaminated communities of color in voting rights, and the co-author in several Pro-Democracy North Star legislation bills. She is a member of the NC PFAS Testing Network, anchoring legislation on aqueous film-forming foam, and she is a convener of the Black Firefighters Fighting PFAS Collective. Ms. Whittington has created and co-convened national, regional, and statewide tours and workshops on environmental justice, focusing on chemical contaminants and dirty corporations. She works continually with the Brody School of Medicine at East Carolina University, the U.S. Department of Health and Human Services, and other government agencies to ground community needs and strategies in alleviating health disparities in Black and Brown communities. Ms. Whittington led the development of a statewide map to highlight the intersection of environmental justice contamination zones and the frequency in which they are located within gerrymandered Black majority voting districts. She is a petitioner in two active petitions to the U.S. Environmental Protection Agency. Ms. Whittington is an Afro-Indigenous woman from North Carolina, hailing from a former environmental justice settlement: The Kingdom of the Happy Land. She received her education at Western Piedmont Community College and Meredith College.

Alan Woolf, M.D., is a professor of pediatrics at Harvard Medical School, a board-certified pediatrician and medical toxicologist, and a senior attending physician at Boston Children's Hospital (BCH). He is the director of the Pediatric Environmental Health Center at BCH, directing its fellowship training program in pediatric environmental health and the Region 1 New England Pediatric Environmental Health Specialty Unit. Dr. Woolf is a member of the Executive Committee of the Council on Environmental Health of the

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American Academy of Pediatrics and a past president of both the American Association of Poison Control Centers and the American Academy of Clinical Toxicology. He is the editor and author of two books: *The Children's Hospital Guide to Your Child's Health and Development* (Perseus Publishers) and *History of Modern Clinical Toxicology* (Academic Press) and has authored or co-authored more than 300 scientific publications.

Cathy Wusterbarth is a registered dietitian nutritionist, and the co-leader and founder of NOW (Need Our Water) in her hometown, Oscoda, Michigan. NOW was created in 2017 to give Oscoda-AuSable and surrounding communities a voice. Its mission is to be a reliable resource and catalyst for education and communication while advocating for long-term health and environmental welfare plans on behalf of those affected by water contamination from the former Wurtsmith Air Force Base in Oscoda, Michigan. Ms. Wusterbarth and other NOW members have consistently demanded that the State of Michigan and the Air Force address the PFAS contamination that affects her community, as well as more than 700 military installations. She was invited to the 2019 State of the Union by Congressman Dan Kildee to represent communities affected by PFAS contamination and support the newly created Congressional PFAS Task Force. In addition to testing and monitoring the health of Oscoda residents, Ms. Wusterbarth believes the federal government should conduct the necessary epidemiological studies to correlate health outcomes among veterans and their families.

Sandy Wynn-Stelt is a clinical psychologist in private practice. In 2017, she learned that her groundwater had been contaminated by PFAS from a landfill that had been used by Wolverine Worldwide to dispose of Scotchgard-laden tannery waste. Since then, Ms. Wynn-Stelt has become an advocate for preventing similar contamination in other communities. She has spoken with several state and local legislators and the Michigan attorney general, and has testified in Washington, DC, at hearings on PFAS contamination. Ms. Wynn-Stelt currently participates on the leadership team of the local Wolverine Community Advisory Committee, as well as on the Michigan PFAS Action Response Team. She also participates in the Michigan Department of Health and Human Services' Health Study as a stakeholder for both the state PFAS study and the Agency for Toxic Substances and Disease Registry's Multisite PFAS Study. Ms. Wynn-Stelt participates in training with U.S. Environmental Protection Agency (EPA) staff on effective communications with community members. In 2020, she was awarded the Citizen Excellence in Community Involvement Award by the EPA for her work in the community and state in advocating for stronger regulation of this class of chemicals.

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Appendix B

Summary of the Committee's Town Halls

As noted in Chapter 1, the committee engaged with community members throughout the study process. A key component of the community engagement was the conduct of three town halls, held on April 7, May 6, and May 25, 2021. The town halls were conducted remotely, because of the COVID pandemic, and designated Eastern, Middle, and Western. In total, they featured 36 people affected by PFAS.[1]

This appendix summarizes the discussions at the town halls. It was prepared by Anna Ruth Robuck, Ph.D., as a factual summary of what occurred. The statements made are those of the rapporteur or individual meeting participants and do not necessarily represent the views of all town hall participants, the study committee, or the National Academies of Sciences, Engineering, and Medicine.

OVERVIEW

The town halls included presentations by invited community representatives detailing information about exposures, health effects, and health care needs; discussion sessions enabled exchange between committee members and participants. The presenters described frustrating and harrowing ordeals navigating PFAS exposure and related health issues and concerns. Several key themes were echoed by multiple presenters and in discussion sessions, including:

- an immediate need for accessible PFAS blood testing,
- continued assessment of PFAS health effects,
- the need for equitable action that best supports the people who are most vulnerable and disproportionately affected, and
- a continuing need for comprehensive exposure assessments.

Accessible Testing Multiple speakers highlighted the importance of readily available, affordable PFAS blood testing. Such testing would serve to establish baselines of exposure, provide agency to exposed communities and families, show respect for community concerns, and inform precautionary health care. They stressed that testing must be financially accessible and recognized by insurance coverage. Many recounted reluctance or refusal by health care providers to order such testing because of the inability to definitively relate blood concentrations to health effects. While acknowledging pervasive exposure to PFAS in the U.S. population, speakers suggested that exposed communities and vulnerable populations should be prioritized for PFAS blood testing, using equity as a guide to design testing protocols.

Continued Assessment of Health Effects Many speakers highlighted the lack of studies detailing health outcomes related to PFAS exposures, citing this as a glaring and troubling data gap, given the long list of health concerns and trends identified by and in exposed communities. Presenters pointed out that the lack of identified health effects often gets cited as a reason not to perform research or desired testing, leading to a circular situation in which health effects are not identified because of a lack of study, and studies are not conducted because of a lack of identified health effects.

[1] PFAS are chemicals known as perfluoroalkyl and polyfluoroalkyl substances; see Chapter 1.

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Equitable Action Numerous presenters acknowledged the importance of considering systemic inequities when designing health care interventions or protocols related to PFAS exposure. They noted that minority communities often bear disproportionate burdens of exposure, magnified by inequitable sociocultural and economic frameworks. Health care workers seeking to mitigate and address PFAS exposure must consider such compounding effects of inequity, relying on community participation and input to guide intervention. Some speakers pointed out that children, pregnant women, transient populations (such as service members and migrant workers), and people without health insurance or lacking accessible health care need to be considered given the demonstrated burden of PFAS on these populations. Moreover, information about PFAS and appropriate health care options must be framed and presented in culturally appropriate ways across the continuum of both formal and informal health care settings.

Comprehensive Exposure Assessment Throughout the town halls, speakers provided background information about PFAS exposure scenarios in their community or region. These descriptions revealed vast uncertainty about the scope and scale of exposure. Speakers suggested that exposure vectors beyond drinking water should be explored, including air, cooking and washwater, fish and seafood, wildlife products, garden vegetables, and other foods and agricultural products. Speakers also stated that a larger number of PFAS should be included in monitoring efforts considering the ever-expanding number of compounds included in the PFAS class.

EASTERN TOWN HALL

Laurene Allen (Merrimack Citizens for Clean Water)

Laurene Allen shared perspectives from the extensively exposed community of Merrimack, New Hampshire. Residents of this area learned about their exposure to PFAS, in 2016, related to local industrial activities. PFAS have since been found regionally in air, soil, groundwater, and drinking water across an area of more than 65 miles containing five towns. The community is therefore aware of their past exposure and frustratingly continues to grapple with current exposure from ongoing PTFE[2] used in fabric and film coating by a local manufacturing facility. Allen stated the community finds the continuing exposure to unregulated PFAS troubling, particularly in the absence of appropriate health care that considers both the history and current extent of PFAS exposure.

Allen described the inaccessibility of blood testing for PFAS and health screenings for PFAS-related ailments, with feedback from physicians that such information is expensive to obtain and may be unhelpful or difficult to frame. However, Allen asserted that such testing is important and validating to the community, and a key "piece of the puzzle" to establish baseline information about the evolving understanding of the nature of exposure. Allen also stated that the community sees patterns of disease and illness related to source proximity that are not currently acknowledged or understood by health care practitioners. Given the documented link between PFAS and specific health effects, including immune function and endocrine health, blood testing is vital for matched, relevant health care based on exposure history.

Allen noted a link between COVID-19 cases and PFAS exposure in New Hampshire, with the further suggestion that information about patient and community PFAS exposure should be incorporated into COVID-19 responses, vaccination protocols, and other public health considerations. Allen also underscored the importance of integrating environmental health and exposure history into health records to increase the capacity to monitor for PFAS-related health effects over time. Allen concluded by emphasizing the importance of improved guidance and support for physicians as to how to best incorporate PFAS exposure into clinical care and patient risk reduction to ensure support for highly exposed communities desperate for appropriate care.

[2] Polytetrafluoroethylene, a polymer PFAS; see Chapter 1.

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"It's been really difficult to get chronic PFAS exposure and risks posed from this chemical class acknowledged by health care providers." *Laurene Allen*

Teresa Gerade (Don't Undermine Memphremagog's Purity)

Teresa Gerade provided information about landfills as sources of PFAS contamination and her Vermont community's concern about an adjacent landfill. PFAS-enriched landfill leachate is often treated by nearby wastewater treatment plants. Wastewater treatment plants are typically not designed to remove PFAS, resulting in the generation of effluent and biosolids enriched in PFAS. PFAS are then reintroduced to the water cycle through effluent discharge or may enter plants grown in soils amended with sludge-derived biosolids.

Gerade focused on information about Vermont's landfill neighboring Lake Memphremagog, a lake spanning the border of the United States and Canada. The community surrounding Lake Memphremagog is particularly concerned about the local and regional PFAS exposure associated with the Vermont landfill and how it affects the health of both the lake and the community. Malignant melanoma has been found in a certain species of lake fish, raising questions about the health of fish populations. The surrounding community has related concerns about the safety of eating freshwater fish from the lake and crops grown in biosolid-amended soils. Gerade suggested the need for PFAS screening levels in human blood, like those in place for cholesterol or glucose, to allow for exposure monitoring over time and related risk mitigation.

Ayesha Khan (Nantucket PFAS Action Group)

Ayesha Khan provided insight about the experiences of the firefighting community and specifically the firefighting and AFFF-exposed[3] community on Nantucket, Massachusetts. Firefighters are routinely exposed to PFAS through both their gear and firefighting foams. Although the Centers for Disease Control and Prevention (CDC) recommends limiting exposure to PFAS, many firefighters are unaware of PFAS or their unique occupational exposure to the pollutants. As a result, firefighters often receive little or no training to mitigate the risk related to gear handling and foam use. This unawareness has led to previous practices that caused undue PFAS exposure, such as using AFFF to clean vehicles or allowing children to play in AFFF.

Khan emphasized the importance of medical monitoring and PFAS bloodwork for firefighters and other PFAS-exposed communities. Khan asserted that such access would establish exposure baselines, raise awareness, and empower people who are exposed to be proactive in reducing exposure and managing risk. Khan illustrated the limited and frustrating accessibility of bloodwork by recounting an experience with a physician, one who is aware of the PFAS crisis and lectures on PFAS exposures. During care for a child, the patient's parent requested PFAS bloodwork. The physician responded by trying to dissuade the parent from seeking the testing, saying if PFAS measurements in blood were carried out it would be difficult to frame the information, especially since the child was currently healthy. Khan also recounted that the doctor misquoted data from studies by the Agency for Toxic Substances and Disease Registry (ATSDR) that measured PFAS in residents of exposed communities throughout the United States, stating these studies "did not find much."

The physician went so far as to jokingly cite a study that found higher reading ability in children whose mothers were prenatally exposed to PFAS. Khan stated the doctor's response felt as though they were vastly minimizing exposure concerns to the detriment of informed health care for the patient and parent. Khan went on to assert the importance of improving CDC guidance about PFAS provided to clinicians, as the current guidance recommends limiting exposure with little or no information about how to do so in practice. Khan stated that the vagueness of the current guidance can result in situations in which clinicians become barriers to information or action, thereby imposing a burden on concerned

[3] AFFF are aqueous film-forming foams, used in firefighting, that often contain PFAS.

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patients to figure out how to reduce exposure and advocate for themselves in a health care setting. Khan reiterated the importance of providing candid information about PFAS to exposed communities, as well as to health care providers, lawmakers, union leaders, and other decision makers in order to help exposed communities and families rectify their burden of contamination.

> "We are not scientists or doctors. We assume our government is keeping us safe, and when there is a possible link to adverse health risks, we are led to believe that issue is promptly resolved and not with a regrettable substitution."

> "My hope is that [the committee] will educate physicians to be honest with those of us who have been exposed." *Ayesha Khan*

Kristen Mello (Westfield Residents Advocating for Themselves [WRAFT])

Kristen Mello offered perspectives from a PFAS-impacted community as a representative of Westfield, Massachusetts. Mello provided context about her experience with PFAS exposure by explaining she learned about her community's AFFF-contaminated drinking water in 2016 and founded WRAFT in response. An ATSDR study of 459 Westfield residents subsequently found that 92 percent of the city's residents had serum concentrations of at least one PFAS that exceeded the national average.

In considering the committee's task regarding challenges related to PFAS encountered in health care settings, Mello cited the frustrating lack of clinical guidance for health care providers as a major challenge. She noted that the current paucity of clinical guidance contrasts sharply with the availability of scientific information describing exposure assessment of specific human populations, animal studies, and toxicological models, and the rigorous collation of such scientific information provided by the ATSDR toxicological profile for PFAS.

Mello strongly stressed the need to collect data about PFAS exposure and associated health effects in exposed communities, citing the inherent disrespect of the plight of exposed communities when testing efforts are denied or discouraged. Without paired assessment of PFAS exposure and associated health outcomes, Mello suggested, the true scope of adverse health effects associated with specific PFAS thresholds cannot be identified, further limiting the advancement of clinical guidance for health care practitioners.

Mello highlighted health conditions of concern observed in her own community, including allergies, autoimmune and immune disorders, asthma and pulmonary disease, colon diseases, reproductive cancers, menstrual and fertility issues in women, diabetes and metabolic disorders, thyroid disease, cholesterol and liver disease, osteoarthritis and osteoporosis, cognitive and developmental disorders, neurological issues, brain cancer, kidney diseases, and bladder cancer. Mello further argued for the benefits of data collection in exposed communities by highlighting the value of PFAS exposure and health assessment in a tailored public health response. For example, Mello said, blood testing could facilitate identification of communities more likely to be immunocompromised due to PFAS exposure when designing COVID-19 intervention or protocols.

Even in the absence of information framing PFAS exposure levels in relation to specific health outcomes, however, Mello said exposure assessment is critical for affected communities. Mello described the contamination occurring in exposed communities as an intentional crime, without the same protections afforded to victims of equally degrading crimes. Mello argued that the accessibility and implementation of such testing validates and respects the plight of those contaminated without their knowledge or consent, akin to respecting the rights of a crime victim.

Mello also commented on the ubiquity of PFAS exposure in the general population, pointing out that scientists have identified thousands of PFAS while only nine are being considered as part of the committee's study. She further pointed out exposure vectors relevant to the general public, including the air and consumer products.

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Mello encouraged the committee to recommend amending guidance for health care practitioners to better support exposure reduction, ensure respect and support for those exposed, and enable equitable and voluntary data collection to assess PFAS levels and associated health effects. Additionally, Mello emphasized the importance of patient agency and fully informed consent during the care process. She also stressed the immediate need to use existing knowledge to revise clinician guidance and treatment plans for those currently exposed to PFAS, rather than waiting for further data. She also recommended the utility of future monitoring and a voluntary national database of exposure and health effects information so exposure and health data can be stored and further explored.

> "So we were asked as community liaisons what challenges we have had with our medical providers in dealing with PFAS exposure, and the challenge is that there's just no helpful information."

> "You don't have a problem getting an insurance assessor when your car is hit, you don't have a problem getting an insurance assessor when you have a tornado, but this slow-motion unfolding environmental and public health disaster … is intentionally keeping the information from us so that we cannot take action." *Kristen Mello*

Tracy Carluccio (Delaware Riverkeeper Network)

Tracy Carluccio summarized perspectives as a community activist and advocate based in New Jersey. Carluccio contextualized her perspectives by describing the scope of ongoing PFAS contamination and action in New Jersey. She acknowledged the state's heavy burden of legacy and novel environmental contamination, including prolific PFAS contamination, caused by several companies such as DuPont, 3M, and Solvay. In response, the state has taken ground-breaking regulatory steps to address PFAS, and it was the first to adopt maximum contaminant levels (MCLs) for drinking water that were lower than the health advisory guidelines proposed by the U.S. Environmental Protection Agency (EPA).

Despite New Jersey's progressive stance on PFAS, however, Carluccio said that the narrative in the state is primarily driven by affected communities, and large information gaps still exist. Some blood testing has been carried out in specific localities, but there has been limited blood testing across the state or near specific industrial sites. The PFAS crisis in New Jersey cannot be fully understood until the public has access to data claimed as confidential business information by companies producing or using PFAS, she said. Additionally, Carluccio argued, thorough exposure assessment, health studies, and medical monitoring must be carried out to fully characterize the scope of PFAS contamination and related effects for New Jersey residents.

Carluccio provided two specific examples to underscore the importance of community access to information and monitoring in pursuit of the most appropriate health care. She described legacy and emerging PFAS contamination originating from Solvay, an industrial user of PFAS located in West Deptford. Industrial activities by the company were found to contaminate the drinking water of 50,000 residents of the surrounding area with legacy PFNA,[4] first identified in 2013. Since that time, the company has shifted to new PFAS to replace legacy compounds, while providing little information to the community about the occurrence and health implications of these new compounds. Carluccio also stated that the company has thus far refused to comply with a state directive requiring PFAS users and producers to provide information about production activities to the state, prompting a lawsuit.

The lack of data describing community exposure around Solvay has left the community in a precarious position, unable to appropriately steer their own health care and family choices. Carluccio also detailed PFAS exposure in communities adjacent to military bases in the state. She emphasized that company and agency recalcitrance to share information about legacy and novel PFAS use and contamination actively thwarts the needs of surrounding communities. She suggested that "ignorance is not bliss," as it may lead to misinformed health decisions; instead, informed community members gain

[4] Perfluorononanoic acid, a PFAS; see Chapter 1.

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agency and can best decide whether they want to avoid tap water, install additional drinking water treatment, move out of a contaminated area, or consider exposure in making reproductive and family decisions.

Loreen Hackett (PFOA Project New York)

Loreen Hackett provided insight about her experiences as an organizer from Hoosick Falls, New York. In 2015 the town was found to be severely contaminated with PFAS, specifically PFOA,[5] and has since been designated as the first PFOA site on the EPA Superfund national priorities list in the United States. The town is now home to three federal sites, as well as several additional state Superfund site declarations, with more currently being investigated in the small community.

Hackett detailed her intimate experiences with PFOA contamination by citing the highly elevated levels of PFOA found in her own blood and the blood of her grandchildren, comparing these staggering figures to national averages that are hundreds of times lower. Hackett commented that the alarming levels found in her family's blood required guidance from health care professionals, yet thus far her family and community health care needs have been poorly met in a clinical setting. As an example, Hackett described a situation shortly after the community learned about exposure. The New York Department of Health organized a community meeting with a pediatrician who lacked PFAS expertise. The pediatrician responded to community questions about health concerns by stating "I don't believe any of your illnesses are caused by PFOA." Hackett described feeling mortified and frustrated at this response, given the community was familiar with existing research that decisively indicated the opposite.

Hackett also described results from a community health study that supported the community's concerns, listing cases of kidney cancer, testicular cancer, thyroid disease, pregnancy-induced hypertension, and ulcerative colitis in the community. Hackett stated the people had to do research for themselves, which was arduous and confusing at times. This fact-finding process took up valuable time that could have helped mitigate exposure-related effects in the community. Hackett indicated that state and local regulatory agencies are now taking PFAS contamination more seriously, with the state setting more protective drinking water MCLs and continued community blood testing.

Hackett stated that too few doctors are trained in the ramifications of environmental toxins and associated care protocols. As a result, Hackett described her habit of bringing research studies to appointments with specialists or unfamiliar doctors to illustrate relationships between PFAS exposure and health effects. She indicated that few listen, and she is reluctant or unwilling to follow up with those who do not listen, therefore missing out on further treatment and testing options, as well as any associated benefits.

Hackett also mentioned that Hoosick Falls residents often rely on the closest hospital, which is in Bennington, Vermont, which is itself a PFAS-contaminated community. Yet even there, Hackett stated, many health care providers are unaware of PFAS and associated health implications. She provided an example in which a provider suggested her breast cancer was genetic despite family history and medical history suggesting otherwise. She articulated that health care practitioners unfamiliar with the issue often made her feel intellectually inferior because of her lack of formal medical credentials, rather than acknowledging and validating her significant health concerns. She stated this dynamic continues to occur even with health care providers directly situated in currently contaminated communities.

Hackett also provided an example depicting the benefit of informed health care, by stating that a local general practitioner took time to learn about the medical ramifications of PFAS exposure; this awareness has resulted in concerted follow-up on PFAS-related health issues in the immediate community to keep pace with the emergence of health effects. Hackett suggested the implementation of continuing education credits to incentivize continued training on environmental health issues for health care practitioners.

[5] Perfluorooctanoic acid, a PFAS; see Chapter 1.

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Hackett also stressed the need to establish new health care paradigms and protocols specifically tailored to exposed communities. For example, she questioned whether blood donation is safe in highly exposed communities like Hoosick Falls. Additionally, she suggested that exposed women should be informed by obstetrical/gynecological care providers that PFAS will be passed to babies in utero and in breast milk. Health care mantras like "breast milk is best" need to be thoroughly reevaluated in exposed communities, given that breastfeeding may double or triple PFAS levels in infants in comparison with their mothers' levels. Families in exposed communities cannot make informed reproductive choices or other family decisions without information tailored to their situation. Hackett relayed concerns from community members now expressing guilt at unknowingly poisoning their child over the course of pregnancy and breastfeeding. Hackett also suggested that health care norms, such as visiting times, need to be adjusted to better fit environmental concerns because health care discussion about PFAS require more attention than a short office visit.

Hackett emphasized the need for health care professionals to trust their patients and their observations and knowledge on PFAS issues. By trusting local community members, practitioners and scientists can better use community data and experiences in building effective health care for people who have been exposed. Hackett described her participation on the Community Advisory Panel with University of Albany for their CDC-awarded multisite study, collecting and sharing published health studies related to PFAS for the study website. Working in this context, Hackett stated, the study design has shifted to include more tests beyond liver, kidney, and thyroid function, as immune suppression, endocrine disruption, neurological effects, reproductive issues, and breast cancer become increasingly salient community concerns.

Hackett also detailed ongoing contamination concerns in her community due to continued stack emissions and exposure to unregulated, short-chain compounds designed to replace PFOA that studies show to be as toxic as long-chain, legacy PFAAs.[6] As a result of regrettable substitution, Hackett advised that bioaccumulation and total body burden of numerous PFAS has to be considered for those requesting medical direction through continued blood testing beyond the limited number of PFAS currently under scrutiny.

Patrick Elder (Military Poisons)

Patrick Elder articulated concerns and insights about the understudied role of PFAS exposure from food. Elder stated that he believes there is too much emphasis on PFAS levels in drinking water, with too limited a focus on PFAS exposure from food, particularly seafood. Elder contextualized this position by detailing his experiences testing surface water and seafood items near his home in southern Maryland, adjacent to the Naval Air Station Patuxent River Webster Field Annex. Elder's efforts resulted in the detection of significant PFAS concentrations in surface water and seafood items. Elder published the results in the local press, leading to concern and outrage in the community. He indicated that a subsequent public meeting with Navy officials resulted in an unsatisfactory exchange of information with the local community, as Navy officials reiterated that the chemicals in question were no longer in use and there was no medical treatment to reduce PFAS in the human body. The community sought increased testing on seafood items and water, expressing disagreement with the Navy's assertion that not enough is known about PFAS in seafood and the human body to justify immediate intervention.

Elder further highlighted the untenable data gaps for PFAS in food in the United States through a comparison: the European Food Safety Authority recommendations suggest up to 86 percent of PFAS exposure stems from food intake while the U.S. Food and Drug Administration (FDA) issued a statement suggesting there is no evidence that dietary choices should consider PFAS contamination. Overall, Elder emphasized the importance of better limiting PFAS exposure from food and seafood items in the United States and incorporating this vector of exposure when considering health effects and health studies.

[6] Perfluoroalkyl acids, a class of PFAS; see Chapter 1.

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Hope Grosse (Buxmont Coalition for Safer Water)

Hope Grosse described her experiences in Bucks and Montgomery counties (BuxMont), Pennsylvania. Grosse described a lifetime of exposure due to drinking water contaminated by AFFF use at Warminster Naval Base and Willow Grove Naval Air Station.

Grosse reported that people in the BuxMont community have voiced concern regarding their PFAS exposure and a number of diseases and health conditions including thyroid cancer, non-Hodgkin's lymphoma, kidney cancer, testicular cancer, breast cancer, liver cancer, brain tumors, ovarian cancer, lung cancer, bladder cancer, melanoma, bone cancer, altered metabolism and obesity, fertility issues, birth defects, diabetes, cholesterol, high blood pressure, preeclampsia in pregnant women, decreased infant birthweight, autoimmune diseases, chronic inflammation, immune response, and alterations in liver enzyme levels. The implications of exposure are not limited to physical ailments: the community has also collectively experienced serious experiences with fear, anxiety, grief, emotional and physical stress, and a feeling of being forgotten.

Grosse said she personally wrestles with an unbelievable lack of trust, fear, and emotional scars accumulated related to the premature death of her father, her own early cancer diagnosis, and deaths of multiple cherished community members. Grosse further revealed that she struggles with shame and fear due to the feeling that she inadvertently poisoned children because she was unaware of her exposure. Grosse also described the financial stress imposed on exposed communities to pay for bottled water, increased public water rates, home filtration, diagnostic testing, medical fees, and loss of wages due to health issues and related loss of productivity.

Grosse stressed the importance of increased awareness and resources to better educate health care practitioners about the health effects of PFAS. She indicated that communities need to be able to trust that their caregivers and practitioners are knowledgeable on the issue and capable of advising action to address their concerns. She stated that the community feels they have no local medical resources or health care providers to answer their questions or advocate for them in the health care system. During her own health struggles, she said she felt belittled and embarrassed for asking questions about environmental health and its relation to her health maladies. Grosse recommended considering practical measures to improve the patient experience for exposed people, such as requiring questions about exposure history or concerns in health questionnaires and forms or designing better collaborations between insurance companies and physicians so practitioners can readily issue scripts for bloodwork and diagnostics that are covered by insurance.

Grosse stressed that such clinical guidance must particularly provide proper direction detailing how to best protect and treat children, given the particular risks of PFAS exposure during critical developmental periods. Mothers need awareness and guidance to test for PFAS in blood and breast milk and make effective parenting choices based on exposure results to best safeguard children in utero or during breastfeeding. Grosse mentioned she lacked such information and agency raising her own children and would have greatly valued the knowledge and associated opportunity to switch to filtered water or bottle-feed her children to reduce their early-life exposures.

Grosse also emphasized that blood testing remains imperative to characterize exposure and changes in PFAS blood levels over time. Grosse posited that the value of blood testing is not predicated on how well the derived information can be explained or compared to thresholds. Rather, documentation of the exposure can retrospectively serve communities, as health science progresses to more fully understand the health ramifications of exposure. Grosse also asserted that blood testing facilitates appropriate health planning, as exposed individuals have a right to preventive health measures and testing tailored to their exposure reality, such as titer testing to deduce PFAS-altered vaccine responses or tests for PSA and liver enzymes.

"When I would go to the doctor's and tell them about some of the exposures of over 50 chemicals that I was exposed to, the doctors would laugh and say no. Clearly they didn't have any information about

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environmental components [of disease]. They made me feel small, they made me feel stupid and embarrassed even just asking the question." *Hope Grosse*

Le'Meshia Whittington (North Carolina Black Alliance)

Le'Meshia Whittington discussed the perspectives and needs of Black and Brown communities in North Carolina. Whittington emphasized that Black and Brown communities face cumulative impacts in addition to environmental health concerns, resulting in disparate burdens and concerns for exposed minority communities well beyond drinking water. Whittington exemplified this reality as related to PFAS exposure in the Cape Fear River system in southeastern North Carolina. In 2017, the public learned about significant PFAS contamination in regional surface water and drinking water due to the industrial activities of Chemours in Fayetteville, North Carolina. Whittington highlighted that this news broke amid a history of climate-related disasters (e.g., hurricanes) that repeatedly damaged the regional water system over decades. As a result, with the occurrence of Hurricane Florence in 2018, regional communities were severely affected by floodwaters contaminated with coal ash, animal waste, and industrial wastes, as well as PFAS. However, the ramifications of PFAS-laden floodwaters were not addressed following Florence.

Whittington described the multifaceted and overlapping exposure vectors of concern for Black and Brown communities, highlighting that the interplay of these factors can cause undue cumulative exposure and subsequent harm. She highlighted that about 35 percent of North Carolinians are renters, leading to potential exposure from carpets, textiles, and building materials with no capacity to remove or replace these items. Whittington also pointed out 80 of the state's 100 counties are considered food deserts or contain food deserts, where residents face significant hurdles accessing fresh fruits, vegetables, and other sustainable foods. In these areas, people may unduly rely on packaged, processed, and fast foods prepared using PFAS-laden water, following storage or transport in food packaging materials that contain PFAS. This type of food sourcing leads to concentrated PFAS exposure. Whittington again highlighted the additive impact of many cumulative factors, noting many of these food desert areas in North Carolina are downstream from continued contamination from Chemours and are also routinely affected by climate-related flooding events.

Whittington also pointed out that occupational exposures require increased health monitoring, considering the cumulative risk imposed when different industrial activities collide in a given region. She stated that more information is needed describing how food service, essential, agricultural, and industrial workers may be exposed occupationally given inherent contact with potential PFAS sources in those jobs, as well as increased risk to those workers when their employment location is in a region rife with known contamination. For example, Whittington questioned how agricultural and abattoir workers who work in PFAS-contaminated zones may be routinely exposed through industrial air emissions and constant contact with PFAS-imbued agricultural products. Whittington suggested increased institutional study and support for workers through the Occupational Safety and Health Administration or other agencies, and the need for increased health care provisions for those closer in proximity and more consistently exposed to this group of chemicals.

Whittington also highlighted high-priority populations within wider Black and Brown communities. She identified pregnant women and children as two such priority populations given the demonstrated opportunity for maternal transmission of PFAS to their children. Whittington stressed the need for blood testing for expecting parents and children, and increased attention to fertility concerns. Whittington also highlighted veterans, people in law enforcement custody, people with disabilities, firefighters, residents of care and extended-stay facilities, and students as underserved priority populations. She stated that blood testing for PFAS should be made available and accessible to these and all demographic groups, with a critical need for exposure assessment over time.

Furthermore, Whittington stressed, it is important to have updated clinical guidance about PFAS blood testing, health concerns, and standards of care for health care professionals. This information must be disseminated among the wide range of practitioners, agencies, and institutions serving high-priority populations and wider Black and Brown communities. Whittington stressed that such guidance is also

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urgently needed to ensure health insurance claims related to PFAS exposure are viable and compensable, increasing access to exposure-informed health care.

> "This is what we are asking: our communities are willing to undergo testing, they will participate in studies, it just needs to be designed for our communities, alongside our communities."
> *Le'Meshia Whittington*

Mike Watters (Gray's Creek Residents United Against PFAS in Our Wells and Rivers)

Mike Watters provided perspective as a resident of a community affected by the industrial activities of Chemours in Cumberland and Bladen counties in North Carolina. Watters described concerted efforts to engage his community to ensure awareness and engagement. One such effort included a community health survey of 100 people that highlighted shared community health concerns related to PFAS exposure, including thyroid function and hyperthyroidism, weight gain, arthritis, asthma, autoimmune disorders, skin cancer, chronic inflammatory conditions, vitamin D deficiency, type 2 diabetes, prediabetes, hair loss, high blood pressure, high cholesterol, high triglycerides, irritable bowel syndrome, and itchy scalp. Watters emphasized that continued measurements of PFAS in blood are urgent to associate these health concerns with PFAS concentrations and to allow health studies to catch up with the state of exposure. Watters also stressed the value to the community of baseline exposure assessment and the importance of tracking exposure over time through continued blood testing given evolving exposure scenarios. This work is currently being done through the North Carolina State University GenX (PFAS) Exposure Study with participation from people in the community, as well as pets from their homes.

Watters also discussed understudied vectors of exposure, such as fish, air, rain, locally grown vegetables, cooking water, cleaning water, clothing washed in contaminated water, and showers. The contribution of these sources to PFAS exposure and associated health outcomes are poorly characterized. These understudied exposure sources therefore stand as sustained concerns for community members. Watters suggested the continued need for more monitoring to keep pace with the unique exposure scenario in North Carolina and to address these understudied routes of exposure, given growing evidence that air emissions of novel PFAS continue to affect a growing number of residents across the region.

Emily Donovan (Clean Cape Fear)

Emily Donovan provided insight about the community experience of residents in southeastern North Carolina subject to high levels of PFAS exposure. Donovan explained that regional communities have been overexposed to hundreds of novel and legacy PFAS for decades because of regional industrial activities and that contamination is continuing. Donovan noted that existing health guidance does not adequately address the amount or types of ongoing PFAS exposure in the state. She also described the frustrating inability of state and federal agencies to halt ongoing discharge of the complex PFAS mixture impacting the region. She noted the prevalence of PFAS occurrence in regional wildlife, sediment, rainwater, and consumer products, pointing out that communities in the region are concerned about PFAS exposure from these and other sources given the excessive PFAS exposure from their drinking water. Donovan said that clinical guidance tailored to ongoing exposure in the state must consider this and protect community members from routes of exposures beyond drinking water.

Donovan noted the community's limited capacity to obtain blood testing for the suite of PFAS found in North Carolina as a major and exasperating challenge. Analytical methods that screen for all relevant PFAS are uncommon, and those that do exist are expensive or possess detection limits that are too high to be useful. Donovan mentioned that she has heard commentary that the exposure profile experienced in southeast North Carolina is "too unique" to warrant development of rigorous testing; she countered this idea by highlighting the use of PFAS produced by Chemours at manufacturing facilities across the nation.

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Donovan cited the GenX Exposure Study as an example of blood testing that occurred regionally, which found a range of novel and legacy PFAS in 400 local residents. However, she asserted that a larger proportion of the more than 300,000 people exposed should be included in further testing, citing the opportunity for the population to be used in larger health effects studies, given the long history of unique exposure across the area and documented health concerns shared across the population. She asserted this overexposed, large population is the "statistical power" required to identify understudied health effects so far missed by institutional investigation.

Donovan presented specific and heart-wrenching examples of personal contacts who are currently uncertain and worried about links between serious health conditions and their history of exposure. She detailed a litany of regional health concerns, including pediatric bone cancers, osteosarcoma and brain tumors, pediatric kidney cancers and diseases, bladder cancer, gallbladder dysfunction, testicular cancer, pancreatic cancer, liver cancer, nonalcoholic fatty liver disease, leukemia, blood cancers, colon cancer, thyroid cancer and dysfunction, autoimmune diseases, digestive issues, multiple sclerosis, skin disorders, infertility, premature births, developmental delays, learning disabilities, autism, breast cancer, and non-Hodgkin's lymphoma.

Donovan stated that the currently available data indicate increased risk of negative health effects, including birth defects, kidney disease, and increased cholesterol related to PFOA exposure. However, many PFAS found in southeast North Carolina were designed as replacements for PFOA, yet no such thresholds exist for these next-generation PFAS. Donovan stated that North Carolina communities and beyond need relevant clinical guidance that adequately identifies, protects, and monitors those who have high levels of diverse PFAS exposure, as current clinician guidance falls well short of these goals. Donovan specified that the regional community needs information about relationships between specific health outcomes and cumulative serum levels for total PFAS in their blood. Health care guidance must consider the complex mixture of exposures experienced by communities and go beyond clinical guidance for legacy PFAS. Donovan cited her current experience as an example, where she often finds herself informing her children's pediatrician about the most current research rather than vice versa; she described this as counterintuitive and discouraging.

Donovan exhorted the committee to investigate information gaps confounding or limiting health studies, suggesting that underreporting of relevant toxicity and health data by corporate producers contributes to damaging data gaps. Donovan stated the committee and the public should have full access to industry data related to production, toxicity, and health outcomes to fully explore the range of linkages between human health and complex PFAS exposures in this region.

> "Sadly, it feels like guinea pigs are treated better, because at least their exposures are thoroughly studied for the betterment of humanity."

> "At the moment I'm informing my pediatrician about the latest toxicity studies related to PFAS placental transfer and breast milk contamination. This is backwards and depressing." *Emily Donovan*

Cheryl Cail (South Carolina Indian Affairs Commission/SC Idle No More)

Cheryl Cail discussed her experiences as a Native American community member and a member of a PFAS-impacted community in Horry County, South Carolina.

The path to PFAS advocacy started for Cail when her 20-year-old son Trevor was diagnosed with testicular cancer. She shared a photo of her son's back showing bright red spots indicative of unknown but acute dermatitis. Cail explained they went to the doctor to identify a cause and solution; the local doctor was not aware of environmental health concerns and did not ask the right questions to quickly arrive at a diagnosis. As a result, Trevor's cancer progressed unchecked for 9 months.

Cail related that Trevor was working and attending school adjacent to Myrtle Beach Air Force Base, though at the time they did not realize the importance of this location and their regional exposure. Cail explained they then watched a movie entitled "The Devil We Know" about PFAS contamination in

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West Virginia, and she started questioning whether PFAS were the cause of Trevor's testicular cancer given the demonstrated link between this type of cancer and PFOA exposure. The family also found a map detailing PFAS contaminated sites around the United States and realized their community's proximity to Myrtle Beach Air Force Base was problematic, as massive concentrations of PFAS had been found in the environment around the base despite remedial activities. Cail noted that right before the COVID-19 pandemic more state agencies began mobilizing to address the issue within her community and across the state, but this was only after years of inaction. She noted that she had seen this before, as institutions prioritize "wealth before health."

With more information about regional PFAS exposures, Cail and her family started trying to find answers from the medical community regarding how to get PFAS blood testing and how to get treatment for their exposure and related health effects. Cail mentioned that while her community is only beginning to understand the scope of PFAS contamination and related health effects, they have seen a concerning increase in cancers, autoimmune disorders, fertility issues, and birth-related defects. She stated that regional physicians were unaware of PFAS, its effects, and even the regional exposure event itself. Cail also described searching for PFAS blood testing options and the frustrating experience of trying to relay that information to a local physician. They found that regional testing was limited to people with occupational-related exposure, such as firefighters. Cail related that this left her son feeling defeated, but she saw a challenge that the committee and other PFAS-focused organizations could resolve.

Cail closed her statements by detailing specific asks from the committee, such as establishing PFAS as an environmental health issue and educating those working in the medical community. This education should involve establishing testing and treatment protocols for those in affected communities and those with diagnosed medical conditions linked to PFAS. She also petitioned the committee to seek expansive data collection to meaningfully assess the full scope of PFAS health effects in order to best design health-related services in exposed communities, including monitoring for children. Cail also relayed a statement from a local physician who has now treated thousands of exposed individuals in her community, who said that physicians need a protocol much like the protocol for lead poisoning; it is a nationwide issue, and so is PFAS poisoning.

Stel Bailey (Fight for Zero)

Stel Bailey offered perspectives as a cancer survivor and member of a military family from Florida's space coast. Bailey first recounted her personal journey with PFAS exposure to explain her path to advocacy. She explained that while her husband was deployed, multiple family members were diagnosed with various cancers in a span of a few months. Given this unusual frequency, she herself was told by multiple doctors that it was "impossible" for her to have cancer, and six different doctors provided care prior to reaching a cancer diagnosis despite continued breathing problems and swollen glands.

Within this harrowing process, Bailey was asked, "where did you grow up?" and subsequently made it her life's work to figure out how and why her background might be related to cancer outcomes. This endeavor took a major turn in 2018 with the release of a key report from the U.S. Department of Defense (DoD) explaining that the region around the facilities of the National Aeronautics and Space Administration, Cape Canaveral Air Force Base, and Patrick Air Force Base was highly contaminated with PFAS, with up to 4.3 million parts per trillion (ppt) of PFOS[7] and PFOA found in surrounding groundwater.

As a result of that report and prior community work investigating autoimmune and cancer cases in the surrounding population, Bailey connected with oncologist Julie Greenwalt. This physician went to high school near Patrick Air Force Base and was aware that dozens of high school classmates had since been diagnosed with cancer, starting at very young ages. Bailey stressed the importance of this advocate and resource for their community, stating "this support meant everything in our community." Bailey suggested that without Greenwalt's personal experience and concern, the surrounding community would

[7] Perfluorooctanesulfonic acid, a PFAS; see Chapter 1.

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still be woefully unaware of health issues related to their substantial PFAS exposure. Since 2018, Dr. Greenwalt has helped the community document 54 cases of various types of cancer in individuals under age 40, which served to bring awareness and resources to the issue. This community health information also helped frame relationships between health outcomes and PFAS levels in drinking water after it was discovered that regional drinking water was also grossly contaminated with PFAS. Bailey chronicled that a large group of people in the community helped crowdsource community health information and address this issue, yet many have been lost since the 2018 DoD report revealing the scope of contamination, including a girl who passed away from brain cancer just 3 days after her 17th birthday.

Community efforts have more recently focused on mapping autoimmune and cancer cases surrounding Patrick Air Force Base. Using this crowdsourced data, the community was able to push forward their own health assessment in the county. The assessment found an increased risk of certain cancers, including urinary, bladder, leukemia, liver, lymphoma, breast, and testicular cancer. Other concerns captured by the health assessment include liver damage, increased risk of thyroid disease, and birth defects. Bailey also described continued routes of exposure that concern the community, such as consumption of regional fish and wildlife and produce irrigated with contaminated water. Bailey mentioned that drinking water and groundwater contamination remain problematic, noting the emergence of short-chain, understudied compounds such as PFBA[8] in these sources.

Bailey emphasized that her primary message to the committee centers on the fact that early detection of cancer saves lives and reduces health care costs. Since learning about the PFAS problem in tandem with regional medical resources encouraging care, Bailey provided multiple examples of early interventions across the community that served to detect or treat cancer or other health problems before severe disease. Bailey concluded by outlining specific community needs from health care providers, including physician education, medical monitoring, PFAS blood testing, preventive health screening and assessments, documentation on medical records of PFAS exposure and environmental health attributes, and recognized guidance that ensures insurance coverage. Bailey particularly emphasized the need to normalize the inclusion of such questions as "Have you been exposed to any environmental toxins or chemicals in your home or workplace?" and "Where do you live?" on medical forms and records.

> "We are doing physicians a big disservice by not providing them the help or information. Lacking this guidance is only harming people. We need action to save lives now." *Stel Bailey*

Several other people at the Eastern Town Hall provided public testimony, which is available on YouTube[9]:

Andrea Amico, Testing for Pease
Katie L. Bryant, Clean Haw River
Jovita Lee, Democracy Green
Beth Markesino, North Carolina Stop GenX in Our Water
Meg Seymour, National Center for Health Research
Yolanda Taylor, Advance Carolina
Sanja Whittington, Democracy Green

[8] Perfluorobutanoic acid is a PFAS; see Chapter 1.

[9] See https://www.youtube.com/watch?v=YrYSj9BPbEQ&list=PLGTMA6QkejfimvAGwR7o_7hP9nXbfFfcr&index=24 (accessed June 29, 2022).

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MIDDLE TOWN HALL

Andi Rich (Save Our Water [S.O.H2O])

Andi Rich offered insight as a community member from Marinette, Wisconsin, home to the JCI Fire Technology Center. This site is considered the third-largest PFAS contamination site in the United States. Rich described the extent of contamination around the site, stating that PFAS levels in groundwater around the site reach 400,000 ppt, and the contamination plume has spread for miles around the site. Rich mentioned community concern about discharge of PFAS-laden effluent into regional surface waters, application of contaminated biosolids on agricultural lands, and potential air contamination related to continued onsite outdoor testing involving unknown PFAS.

Rich also described an ongoing class action lawsuit focused on PFOA and PFOS levels in well water, noting that the suit does not consider people exposed through other, more complex exposure pathways that affect the community. Rich indicated this leaves residents poorly informed and inadequately prepared to make decisions in the lawsuit, which in turn affects the fairness of payout claims. She said that lawsuit participants and community members have repeatedly requested blood testing and medical monitoring to further understand their exposure. These requests have been met with sustained resistance. Rich indicated that the community has been told that PFAS exposure does not equate to illness, that blood testing would not be useful, and that blood testing is not recommended. Rich pointed out that blood testing would be highly useful to ensure lawsuit payouts are based on internal exposure, as payouts are currently slated to be distributed arbitrarily.

Rich shared some personal health history to further highlight the utility of PFAS blood testing and care guidelines for practitioners. She related that a physician recommended bloodwork to check thyroid function, which overwhelmed and frustrated Rich given her awareness of the association between PFAS exposure and thyroid health. The doctor tried to comfort Rich, but Rich found out the doctor was visiting from Nashville and had no knowledge of local exposure issues. Rich stated that practices such as traveling practitioners makes blood testing, medical monitoring, and results-based guidelines that much more imperative to ensure environmental health factors are comprehensively and continuously considered by transient health care providers. Rich also stated that she has not yet sought the recommended thyroid bloodwork, due to logistical constraints, though she said she would be far more likely to prioritize follow-up if blood testing indicated PFAS exposure. Rich compared the utility of PFAS blood testing to the breast cancer gene (*BRCA*) test that detects a person's genetic proclivity for the disease. Each test serves as an indication of the increased potential for illness that can inform preventive care routines.

Rich went on to state that Marinette is an impoverished community where few can afford the cost of PFAS blood testing. The majority of community members have been denied covered testing through state agencies and insurance providers. Rich said that the committee's recommendations have the potential to improve care and community health outcomes by making sought-after care accessible and affordable, and she urged the committee to recommend blood testing and medical monitoring for residents in exposed communities, ensuring those exposed through complex pathways are included. Rich also emphasized that the committee should actively avoid "not doing anything because of lack of data, and not collecting data because of a lack of evidence of harm."

"How can above average incidence of PFAS-related illness be identified in a community where the doctors aren't in town long enough to identify the trend?"

"We need help putting a stop to the contamination, as the corporate polluters are far more powerful than our voices." *Andi Rich*

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Laura Olah (Citizens for Safe Water Around Badger)

Laura Olah commented as a community member affected by the Badger Army Ammunition Plant in Wisconsin. Olah's remarks focused on the major lack of exposure information available to the public and the urgent need for medical monitoring. Olah stated that minimizing PFAS exposure leads to reduced health risks, but communities and physicians trying to accomplish this run into challenges trying to obtain PFAS exposure information in individuals and in the environment. She provided several examples illustrating the pervasive institutional secrecy that prevents transparency about exposure and risk.

She described a public meeting hosted by the U.S. Army to discuss groundwater contamination that has migrated offsite, contaminating rural drinking water wells and discharging into the Wisconsin River. Hydrogeological experts recommended testing drinking wells beyond the currently monitored area, including on the other side of the Wisconsin River. Army personnel argued against this, saying there would be no way to ensure PFAS in well water was derived from the base, and they were not certain which wells should be tested. Olah highlighted this as "ridiculous," pointing out that all wells should be tested given uncertainty about which wells are most at risk as there are no offsite groundwater data. Olah also described several frustrating experiences seeking existing information about PFAS in drinking water. For example, a request for information about PFAS in drinking water around Volk Air National Guard base required a formal Freedom of Information Act request, which has now been pending for 3 years. Another request for a written report describing PFAS contamination around a military base in Tennessee was also unmet, and is now 2 years old. Olah also highlighted that this lack of transparency was not limited to the military, as private corporations refer to PFAS products like AFFF formulations as proprietary mixtures or confidential business information and do not disclose PFAS content in their products. This lack of information makes it impossible to assess and prevent exposure and possible health risks.

Olah asserted that we cannot predict the potential implications and future benefits of medical testing today, but without these data, exposed communities face battles akin to Vietnam War veterans who are still fighting for presumptive care based on exposure to Agent Orange and other toxicants. PFAS blood testing helps baseline exposures and raises awareness so people can take steps in reducing exposures. Baseline testing could help answer health questions in the future and help secure health studies in communities at risk

> "Given all these barriers [to PFAS exposure information], the public and the medical community cannot identify which patients are at greatest risk to and harm from PFAS; therefore, our care must be presumptive."

> "Without medical monitoring data now, presumptive care will be out of reach for civilians and service members and children exposed to PFAS now and in 50 years from now." *Laura Olah*

Samraa Luqman (Concerned Residents for South Dearborn)

Samraa Luqman offered insights as an environmental advocate and a Yemeni-American community member from the South End in Dearborn, Michigan. Luqman framed her remarks by providing context about her community, describing the South End of Dearborn as a community that grew around Ford Motor Company. The continued need for cheap labor attracted immigrant populations over time, with a more recent influx of Middle Eastern immigrants. A high percentage of the population possess limited English skills, are considered low income, rely on some kind of food or government assistance, and rent their homes. The surrounding area is also home to various industrial activities, in addition to the Ford Motor Company, that use diverse types of environmental pollution. PFAS contamination has recently been identified in the area, resulting in the inclusion of the town on Michigan's PFAS Action Response Team website.

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Luqman went on to highlight the importance of considering PFAS exposure beyond drinking water, including inhalation, dermal contact, and maternal offloading of PFAS from mother to child. These understudied exposure vectors are ongoing in the South End, along with continuous air quality issues, lead exposure, and other environmental health concerns. Luqman emphasized the importance of considering such cumulative multiple exposures, citing sustained community concern about deciphering causality or relationships between observed health effects and PFAS in what is clearly a complex exposure scenario. Luqman highlighted that PFAS blood testing can help clarify required follow-up care by identifying exposed individuals most prone to PFAS-related health effects. Luqman expressed particular concern about the relationship between PFAS exposure and immune impacts in the context of the ongoing COVID-19 pandemic. She stated that COVID-19 outcomes have been worse for her community compared with nonexposed communities. She also stated that a lack of PFAS exposure information for her community affects behavioral choices with real-world consequences. Luqman offered the example that someone from the South End exposed to PFAS may be more vulnerable to COVID-19 and require more diligent mask use compared with someone residing 10 miles away, but would never know this given the ongoing lack of exposure information.

Luqman also discussed the need for medical professionals to have awareness about environmental contamination and its potential implications, even decades after a person's leaving a contaminated area. She highlighted that health risks from environmental exposures must be assessed and considered by state agencies and health care practitioners; then this information must be provided to residents and patients to ensure full awareness of exposure. She provided an example of a community member who moved away from the South End area and is now dealing with cancer approximately 20 years later. Luqman stated that this person's doctor explained to them that despite moving away, the carcinogens and pollutants accrued while in the area do not go away. Luqman also underscored the need for health and exposure information to be communicated in culturally appropriate ways, acknowledging and overcoming language and sociocultural barriers.

Cathy Wusterbarth (Need Our Water)

Cathy Wusterbarth offered insights as a community member from Oscoda, Michigan. This community is adjacent to the Wurtsmith Air Force Base and is subject to major contamination of regional groundwater and surface water from AFFF use on the base. This widespread contamination has resulted in historical and ongoing PFAS exposure for service and community members, as well as the area's fish and wildlife.

Wusterbarth's remarks focused on several primary community needs: PFAS blood testing, improved guidance for health care practitioners, exposure assessment in the environment, and exposure mitigation. Wusterbarth highlighted the movie *No Defense* as a crucial example of the health effects of PFAS contamination. The film chronicles the serious lifelong health effects experienced by Mitchell Minor and his family, residents of Oscoda. Wusterbarth also introduced James Bussey, a service member in Oscoda. Wusterbarth explained that Bussey was too ill to present to the committee, but had asked her to provide his medical records for committee consideration. These records include a long list of ailments and physician recommendations based on his exposure to PFAS, which was documented through blood testing.

Wusterbarth emphasized that these real-world examples capture a crucial disconnect between exposure information and health outcomes, which can easily be rectified by improved access to PFAS blood testing. Wusterbarth declared testing should begin immediately to establish baseline exposure levels. Annual testing should occur thereafter, just as is done for other risk factor measurements, such as cholesterol. Wusterbath also identified that given the presence of PFAS in 98 percent of the U.S. population, everyone should have access to blood testing. However, priority should be given to communities and individuals with identified or hypothesized high-level PFAS exposure. Wusterbarth also suggested that testing should be implemented using simple, affordable labs, highlighting a recently opened laboratory in Oscoda as an example. Wusterbarth also recommended that all PFAS be included in

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blood testing, unless specific PFAS measurements are required to discern the role and liability of a specific polluter or product.

Wusterbarth further described a crisis of trust, stating that her community was experiencing the degradation of trust with the very institutions they believed would protect them, such as EPA, other government agencies, and the military. She detailed discouraging recent information from the DoD, noting that more than 700 military installations have now been recognized as PFAS hot spots, starting from just one in 2012. She also stated that health care providers have a duty to discern the causes of disease in their patients, but without guidance from the CDC or other medical authorities, practitioners are unaware of health risks associated with PFAS exposure to the detriment of patient care. Wusterbarth also emphasized the gravity of the committee's recommendations based on the town halls, stating that state agencies and communities are relying on the forthcoming guidance document to inform physicians and care. She shared a personal experience in which she provided PFAS information to her doctors, only to be told this information was too lengthy to review despite her history of cancer and immunological disease. Wusterbarth stressed the need for swift action by the committee to immediately curtail ongoing harm in exposed communities.

> "We've tested the fish; we've tested the deer; we've tested the groundwater, the waterways, and the foam. When are we going to test the people?"
>
> "The only risk [of testing] is to the polluters who do not want us to link them to our exposure."
> "This study is a result of the PFAS communities telling you changes are needed."
> *Cathy Wusterbarth*

Sandy Wynn-Stelt (Wolverine Community Advisory Committee, Michigan PFAS Action Response Team)

Sandy Wynn-Stelt lives in a PFAS-impacted community, Belmont, Michigan. Wynn-Stelt opened her remarks by sharing her own story of PFAS exposure. Wynn-Stelt and her husband moved to Belmont in 1992, seeking an idyllic and quiet home. They were not aware that the Christmas tree farm adjacent to the property was a dumping site for a major PFAS user, Wolverine Worldwide. In 2016 her husband's health rapidly deteriorated, and after only a few short weeks, he died of liver cancer, which Wynn-Stelt described as an unbelievable loss. Shortly thereafter, the Michigan Department of Environment, Great Lakes, and Energy tested her drinking water, and Wynn-Stelt learned the well water she and her husband had drank for more than 20 years was contaminated with PFAS at levels up to 80,000 ppt. On learning about her exposure, Wynn-Stelt sought blood testing. She described this process as challenging. She ultimately paid $800 for blood testing through a commercial laboratory in California. Her bloodwork indicated alarmingly high levels of PFHxS, PFOA, and PFOS, well above levels seen in blood studies as part of the National Health and Nutrition Examination Survey (NHANES) and in some other exposed communities.

Wynn-Stelt followed up with her community doctor regarding her bloodwork results; she described this physician as "very proactive and engaged." She stated that her doctor reviewed the ATSDR physician and patient guidance about PFAS that was available at the time. They concluded the guidance was not helpful and instead agreed on a plan to monitor those things listed as potential risks of PFAS exposure. As a result, when Wynn-Stelt experienced breathing problems several years later, this physician recommended thyroid testing based on their knowledge of Wynn-Stelt's PFAS exposure, although this diagnosis would normally be an unlikely candidate. This testing resulted in the identification of thyroid cancer. Wynn-Stelt credits this diagnosis and quick action to prior PFAS blood testing, adding this vital information to her medical history for consideration by her physician.

Wynn-Stelt provided an additional example of a neighbor exposed to PFAS from the same groundwater source. This neighbor's child received PFAS blood testing and was found to have elevated levels of PFOS. This information was provided to the child's pediatrician, who then monitored vaccine

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response given the child's exposure history. The child was found to have reduced vaccine responses and required some boosters not normally required. Wynn-Stelt reported similar examples of adverse health effects across the community, with cases of thyroid disease, cancer, kidney disease, liver disease, and cholesterol issues in children and adults. Wynn-Stelt also highlighted the mental and emotional toll of exposure and the lack of discussion of this in exposed communities and in care guidelines.

Wynn-Stelt also took time to address often-cited statements used to deny community access to PFAS blood testing. Wynn-Stelt countered the assertion "that it is not ethical to test for something we can't treat" by explaining knowledge of environmental health and exposure is just as medically relevant as asking any patient for a history of diabetes or heart disease. All these pieces of information are required for patients and physicians to monitor potential problems and reduce risk. Wynn-Stelt also addressed the concern that patients may panic given hard-to-frame PFAS information: she countered that exposed patients are not fragile, and knowledge is power. Wynn-Stelt labeled the assertion that PFAS levels in blood cannot be definitively linked to health effects, and therefore should not be monitored as circular logic, stating if testing is not occurring at various scales, links cannot be identified, further and erroneously justifying a lack of testing.

Wynn-Stelt closed her remarks by stating explicitly her community's needs, including accessible PFAS blood testing for people in various exposed communities. She also urged expansive testing for the entire class of PFAS, rather than a few targeted chemicals. Wynn-Stelt also requested research about possible mechanisms to lower PFAS body burdens in exposed individuals and further research to better understand how PFAS levels may impact health care choices, such as blood or organ donation.

Tom Johnson (Clean Water Action)

Tom Johnson shared his perspectives with the committee as an environmental advocate and organizer working with exposed communities across Minnesota and beyond. Johnson explained that much of his work involves public education, seeking to inform people about toxic chemicals overall. When conducting educational activities in the East Metro region of Minnesota, Johnson indicated that PFAS are a central topic of interest, as this area is rife with PFAS producers and users whose activities have massively contaminated the regional environment. This contamination resulted in an $850 million legacy settlement. Johnson suggested that communities are increasingly aware that, although this settlement sum will cover long-term drinking water treatment for all area residents, there will be little or no money left for regional remediation.

Johnson attested to high levels of frustration from exposed communities in the region, with unanswered questions about the likelihood of current or future health effects for families and their children. Despite the lack of citable research, the community possesses large amounts of anecdotal and experiential evidence observed over decades that suggests links to health effects and informs the community concerns. Johnson described the experience of a mother of a low-birthweight baby, who is now concerned about a similar outcome with her second pregnancy. Her doctor has no ability to comment on the likelihood of this outcome given vast uncertainties surrounding her exposure and risk due to a lack of needed exposure assessment and health effects research. Johnson also discussed the importance of accessible and expansive biomonitoring for exposed communities and more broadly. In the case of the East Metro contaminated area, biomonitoring was available for some people in this community, but it is not available for all Minnesotans, leaving data gaps for those who have moved from the area and for other exposed communities. Johnson also highlighted the utility of testing to assess the efficacy of interventions over time and the ability of testing to illuminate links between exposure and understudied health effects.

Vicki Quint (Foam Exposure Committee)

Vicki Quint is a firefighter advocate through her work as a co-chair with the Foam Exposure Committee. Following a massive tire fire in Watertown, Wisconsin, and her husband's death due to cancer, Quint learned about significant exposure to PFAS in the fire service. Firefighters are exposed to

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PFAS through AFFF use and the use of PFAS-containing protective gear. Quint provided further details about PFAS in AFFF and current activities, highlighting the current availability of fluorine-free alternatives to AFFF. She noted that there are several sites in Wisconsin contaminated with PFAS as a result of the use or product of AFFF.

Quint followed this information about firefighter exposure and AFFF use by describing the plight of exposed firefighters seeking PFAS blood testing. Military firefighters are now eligible for PFAS blood testing, but retired military firefighters and civilians are not. Quint related concerns from the fire service community regarding the ramifications of blood testing results and options to lower PFAS levels in blood. Quint emphasized the need for all fire departments to discontinue use of all fluorine-containing AFFFs, as there are no regulations requiring municipal fire departments to use AFFF.

Art Schaap (fourth-generation dairy farmer)

Art Schaap described his experience as a dairy farmer in New Mexico whose farm and family were unknowingly subjected to severe PFAS contamination due to military AFFF use.

Schaap stated he has been on his farm for more than 30 years and considered investments in his farm as his version of a 401(k). When he was approached about PFAS water testing, Schaap stated he readily agreed without prior knowledge about PFAS. Those tests revealed levels of PFAS up to 30,000 ppt in drinking well water from his property. These findings led Schaap to contact the New Mexico Department of Agriculture asking for PFAS testing in milk. The department informed Schaap that if PFAS were found in his milk, Schaap would need to dump the load. He insisted on the testing despite that risk, stating he did not want to distribute products with PFAS to customers around the nation. Schaap stated that PFAS testing in milk from his cows subsequently revealed PFAS concentrations ranging between approximately 800 and 2,500 ppt; this testing has been ongoing for more than 2.5 years.

The contamination has resulted in the devastation of his livelihood, as the cows can only be minorly rehabilitated, pose an economic burden, and reflect a major investment that will be devastating to lose without any possible profit. Schaap has limited options available to get rid of the contaminated animals in any profitable way. Dairy, beef, and rendering industries do not want PFAS-contaminated animals. Schaap described his efforts to filter water for his cows, only to find that it takes years for cows to eliminate PFAS from their body (also described by John Kern, below). PFAS have also been found in the soil on Schaap's farm, further exposing his herd beyond drinking water. This untenable situation has unfolded tragically for Schaap and has resulted in the stranding of at least 4,000 cows, the deaths of 1,200, and the dumping of 1,500 loads of milk.

Schaap enumerated a number of institutional failures that have left him with few resources to rectify his situation. He said that multiple government agencies continue to eschew responsibility for the situation and other PFAS exposure concerns, related to DoD pressure and the lack of actionable EPA standards. Schaap pointed out that if FDA provided standards for PFAS in food products, under the Comprehensive Environmental Response, Compensation, and Liability Act (Superfund), contamination on his farm would require action and remediation. He also mentioned the lack of discussion and accountability about this topic across the dairy industry at large.

Schaap concluded his remarks by recounting health effects he has observed over time in cattle, including decreased lactation, premature births, dwarf calves, reduced pregnancy rates, poor body condition, and increased mortality. He also described health concerns from his family, including high cholesterol, hypertension, kidney damage, kidney stones, diabetes concerns, and infertility.

John Kern (RuttenKern Policy Group)

John Kern offered comments to the committee from the perspective of a litigator and environmental advocate residing in New Mexico. Kern explained that in addition to concerns over drinking water, they also focus on PFAS exposure in the water relied on agriculture and a lack of

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associated safety standards needed to protect the food chain. Kern's testimony dovetailed with the testimony of the previous presenter, Art Schaap.

Kern framed his perspectives by providing information about Cannon Air Force Base near the city of Clovis. AFFF contamination at the military based resulted in groundwater contamination spreading 5–6 miles from the base. The region is home to multiple dairies and cheese plants. Water from this source is provided to livestock, which are then the source for milk, cheese, and meat. This water is also used to cultivate silage vegetation and other crops. PFAS-contaminated groundwater has resulted in PFAS contamination of regional livestock, leading to sundry economic and health questions and concerns unrelated to drinking water exposure.

Kern displayed data from a regional dairy with high levels of PFAS in groundwater; these data show a decrease in lactation in dairy cows over time. Kern also described increasing mortality and birth defects over time in cattle from the same farm. Additionally, Kern presented data about PFAS in milk, stating that the FDA cites 400 ppt as an acceptable level of PFAS in milk. Measurements in milk from the Highland Dairy Farm varied between approximately 900 and 4,600 ppt from November 2018 to August 2020; the data show that dairy milk slowly reflected reduced PFAS exposure. A shorter-term study conducted by the Food Safety Inspection Service showed increased variability and a faster rate of PFAS elimination in dairy milk from exposed cattle compared with data from a longer-term study conducted on the same farm. Kern pointed out these rates have serious implications regarding how quickly cattle are considered rehabilitated from PFAS exposure and allowed for market purchase or dairy use. The slow elimination rates in the longer-term study also pose severe economic ramifications for farmers, given the average lifespan of cattle is around 6 years.

Kern concluded his remarks by describing a frustrating current impasse with the DoD regarding standards. Federal legislation compelled the DoD to clean up agricultural waters in 2018, yet the DoD responded that they would take no action until a federal agency tasked with ensuring food safety (e.g., the FDA or the U.S. Department of Agriculture) set standards for PFAS in food products. Kern emphasized the need for agencies to set standards to allow effective action on PFAS at multiple scales.

Two other people at the Middle Town Hall provided public testimony, which is available on YouTube[10]:

Pam Ladds, Don't Undermine Memphremagog's Purity
Beth Markesino, North Carolina Stop Genx in Our Water

WESTERN TOWN HALL

Liz Rosenbaum (Fountain Valley Clean Water Coalition)

Liz Rosenbaum provided testimony as an AFFF-exposed community member in El Paso County, Colorado. Rosenbaum described hearing about regional drinking water contamination in a group setting based on *The New York Times* reporting. Following the initial news of exposure, the community focused on learning about PFAS and government agencies that could assist in an appropriate response. The community response also entailed building partnerships with water districts and assuaging community anger toward these entities, who learned about the contamination at the same time as the community with no prior knowledge of the issue. Since 2018, Rosenbaum stated, the community has been focused on navigating the regulatory and legislative system to reap meaningful action as soon as possible, turning their anger into action through state legislation. This has involved building connections to EPA, state and county health departments, and elected officials at city and state levels. Rosenbaum reported that, as a result of these organizing efforts, legislation has been adopted increasing fines for polluting corporations.

[10] See https://www.youtube.com/watch?v=mGeABDDTCuI&list=PLGTMA6Qkejfg1GIgAPeqkMCDncdx04qHN&index=16.

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Beyond detailing the exposure reality for her community, Rosenbaum described priority groups that require increased attention in further PFAS studies. She stated that military families move frequently and may be exposed at multiple sites; care must be provided to military families, as well as service members, from all branches. Rosenbaum also suggested that service members require assistance from the U.S. Department of Veterans Affairs (VA) like that provided to Vietnam veterans. Rosenbaum also indicated the need for equity in exposure assessment and mitigation for Black, Brown, and Indigenous families, as well as rural families, mentioning that rural families in her county are not being offered drinking water at the same nondetect level offered to households in PFAS-exposed municipalities. Rosenbaum stated that a recent ATSDR community-level exposure assessment revealed that this clean municipal water facilitated a decrease in PFHxS[11] levels in the blood of municipal residents.

Rosenbaum also pointed out the limited number of health care options in El Paso County, stating there are no hospitals in the southern half of the county. This lack of health care facilities exacerbates contamination issues because there is no capacity to monitor health outcomes, as families seek health care in the northern part of the county where doctors may not be able to identify localized health outcome patterns. Rosenbaum emphasized the immediate need for blood testing to establish a baseline that serves as evidence of contamination and an indicator of potential health effects. Rosenbaum concluded by emphasizing the need for continued patience and engagement between health care practitioners and researchers on this issue to find shared vocabulary. She explained that communities are living through frightening and unfamiliar exposures but may not have the appropriate technical vocabulary to describe their concerns or ailments.

> "Half the battle for the community has involved learning how to ask the right questions to get what we need to have clean and safe communities for our working families." *Liz Rosenbaum*

Martha Dina Argüello (Physicians for Social Responsibility)

Martha Dina Argüello provided perspectives as a Latina and an environmental advocate in California with experience connecting health care providers and communities. Argüello posited that guidance from the committee must include provisions for increased biomonitoring as many communities facing exposure are as yet unaware of the problem. She also discussed the distinct legacy of industrial contamination in Los Angeles. This history has resulted in community exposures to multiple pollutants, which means that approaches that focus on one chemical at a time do not address the lived reality of complex contamination. Argüello pointed out that these factors have led to distrust of water quality, particularly in the Latino community. She highlighted that Latino communities often pay more for water per capita than for fuel as a result of their reliance on bottled water. Comprehensive testing and proper health education for communities and practitioners is key to rebuild trust in water quality and address the comprehensive reality of complex environmental exposures.

Argüello also explained that communities often face the downplaying of anxieties and risks surrounding exposure; this reflects a lack of training that leads to an inability by clinicians to validate the lived experiences of exposed communities. This dynamic further compromises exposed communities. Argüello emphasized that it is essential for clinical guidance about PFAS exposure to include instruction about how to help concerned patients minimize exposure rather than brush off environmental health concerns.

Argüello cited the dynamics of PFAS regulation in California as an example illustrating how to avoid this paradigm of patient treatment. Argüello highlighted that helpful legislative efforts have been under way, yet early warning systems detecting PFAS in drinking water and through biomonitoring studies have not been appropriately heeded. She specifically flagged the response of a physician who is a member of the state legislature, who stated that exposure should not be quantified for patients as the worry is worse than the exposure. Argüello stated this exemplifies how physicians are trained to respond

[11] Perfluorohexanesulfonic acid, a PFAS; see Chapter 1.

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to environmental health concerns, and yet this lack of training and understanding of exposure often leads to minimizing people‘s experience with exposures.

Argüello also urged that clinical guidance should take an anticipatory approach to ensure health care practitioners are provided some education and literacy surrounding the PFAS issue and complex exposure scenarios: health care practitioners should be capable of some interpretation of water quality results and be able to comment on options for filtration. Argüello also specified that clinical guidance must provide intervention options accessible to different socioeconomic and cultural backgrounds. This stratification of intervention is critical to ensure appropriate guidance is provided for all types of exposed communities as many cannot "buy their way out of being exposed." Argüello also highlighted tools that allow tailored exposure assessment and risk reduction strategies, citing how doctors are now writing prescriptions for new carpeting or other household materials to aid rental tenants in substandard housing. Argüello also touted the use of a geospatial tool that allows physicians to assess cumulative exposure risk based on patient location overlaid with information about multiple ambient environmental health exposures.

"When scientists fear speaking truth to power, we know that truth dies."

"We need physicians to step forward because we have regulatory agencies that are actually not preventing exposure."

"We can't change this broken system without the partnership of science and physicians."
Martha Dina Argüello

Mark A. Favors (Fountain Valley Clean Water Coalition)

Mark A. Favors provided commentary as an Army veteran and community member from Colorado Springs, Colorado. Favors and his family learned of serious PFAS contamination affecting their region from activities at Peterson Air Force Base and other regional military bases, prompting Favors to assume an ardent advocacy role seeking safe water. Favors pointed out that while important state legislation regulating PFAS has been passed in Colorado (also discussed by Liz Rosenbaum, above), DoD is exempt from most state legislation. This exemption stands despite the fact the Air Force admitted to dumping AFFF into regional water resources three times per year for multiple decades.

Favors detailed that sustained contamination from AFFF exposure has led to high levels of PFHxS in the blood of people in the community: measured levels were the highest in the country except for individuals who directly manufactured the chemical. With this exposure in mind, Favors chronicled harrowing details about his family's struggle with health problems. For example, there have been many cases of kidney disease and cancer in family members of many ages in the contaminated zone, some requiring kidney transplants that were further complicated by development of cancer in the donated kidney. Favors stated that the abundance of serious kidney ailments in his family in Colorado Springs is particularly striking considering that no family members who live outside the contamination zone have encountered these issues.

Favors explained that these health issues assumed urgent relevance for his family in 2016 when the Air Force disclosed dumping PFAS into drinking water sources, given the known links between PFOA and kidney issues found at other locations. Favors reported that DoD also revealed the detection of PFAS in some community drinking water sources at concentrations up to 8,000 ppt, well above the EPA's health advisory limit of 70 ppt. Favors indicated that despite these levels, the state of Colorado has not provided expansive PFAS blood testing to all residents, even knowing the inherent value of blood testing as an indicator of exposure and potential health effects. Favors emphasized the particular importance of transparent information and access to testing when considering the transitory lifestyles of military members and other community residents. He pointed out that he did not find out about the contamination in his hometown until 2018 while visiting his mother in the region. Favors provided an additional

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example of a cousin who previously lived in the contaminated zone and has since moved, only to find unexplained liver tumors in one of their young children years later.

Favors also stressed the need for transparency and education in the health care community and shared his experience as a health professional in endoscopy. He stated that despite evidence linking PFAS to irritable bowel disease, gastroenterologists in his department were unaware of PFAS. Favors highlighted that such transparency and access to exposure information is key for both practitioners and community members, especially for exposed children and their families who are concerned about developing health problems in the future.

> "I have family members … buried at Fort Logan National Cemetery after surviving combat tours in Vietnam, Korea, and Afghanistan…. They're now buried in the National Cemetery from cancer after the military admitted dumping this toxic chemical into their water." *Mark A. Favors*

Andria Ventura (Clean Water Action)

Andria Ventura provided context about past and ongoing actions addressing PFAS in California from the vantage point of an environmental advocate. Ventura opened her remarks by describing her continued struggle engaging with the medical community about environmental health concerns as a long-time advocate, describing it as difficult for clinicians to make the connection between exposure and health outcomes. Ventura hypothesized that this stems from reluctance or avoidance by clinicians to assume an advocacy role. As a patient and a resident of a PFAS-affected city, she also stated she has never been asked about toxic exposure or environmental health background by her doctors or other medical caregivers, despite dealing with several chronic concerns over decades. She had to actively broach this topic with providers, likening this experience to the testimony of other presenters to the committee. However, it has been clear that doctors and nurses are not trained to take toxic exposure into consideration.

Ventura went on to provide abundant information about the PFAS problem overall. She highlighted the multifaceted ways humans can be exposed to PFAS, including through drinking water, surface waters, consumption of wildlife, or consumption of agricultural products exposed to PFAS in soil or water. She also reiterated the importance of considering PFAS as a class, stating thousands of PFAS have been identified and all are considered persistent, accumulative, mobile, and hazardous to some degree. Ventura also showed that multiple PFAAs have been linked to health effects that affect immunity, development and reproduction, fat and metabolism, liver function, endocrine function, and blood systems. She stressed the potential for additive and synergistic effects related to exposure to multiple PFAS, flagging this as a required point of awareness for health care practitioners. Ventura also emphasized that lack of research on novel PFAS should not hinder consideration of their health effects, stating that evidence is mounting that newer, short-chain PFAS have negative health effects. Ventura also stated that many novel PFAS degrade or transform into PFAAs.

Ventura went on to elaborate about specific actions and concerns in California, describing the recent position of the state to implement phased drinking water monitoring and site investigation. She added the caveat that this phased approach has failed to assess small water systems and private wells: this is a key data gap considering health care practitioners need detailed information about exposure to adequately consider environmental health concerns during care. Despite data collection limitations, data thus far indicate catastrophic PFAS contamination problems across the state that have so far not been addressed by sluggish regulatory efforts. Ventura also stressed key data gaps, such as uncertainties about PFAS levels in surface waters, PFAS in fish and wildlife consumed by humans, and implications for crops grown in PFAS-laden biosolids or irrigation water.

Ventura concluded her remarks by delineating key exposure assessment and health care needs in California. She emphasized the need for expanded understanding of the scope of the problem, including all PFAS in assessment efforts, as well as improved understanding about PFAS health effects. She suggested such fact-finding efforts should include expanded water monitoring; PFAS monitoring in

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diverse environmental media; assessment of diverse exposure vectors; identification of those most at risk of harm from exposure; expanded health studies, including mixture exposure scenarios; and communication of health risks in culturally appropriate ways.

Linda Shosie (Mothers for Safe Air & Safe Water)

Linda Shosie provided perspective as a Latina and exposed community member in the South Side neighborhood of Tucson, Arizona. Shosie provided information to the committee collected as part of community-based exposure and health assessment efforts, highlighted egregious environmental justice issues, and explained her own path to advocacy. Shosie explained that her community is affected by PFAS contamination from military sites and airport activities, resulting in PFAS concentrations in drinking water up to 13,000 ppt. This high level of PFAS exposure is plaguing a majority-Latino community across a 3-mile contamination plume; Shosie provided a number of maps providing geospatial context about the extent of contamination. She described leading health assessments within the community, which revealed high rates of cancer and immune system disease around the Tucson International Airport and Morris Air Force Base. These community-derived results led the county health director to conduct a more in-depth epidemiological study in 2017. Shosie stated this study indicated "significant invasive cancer incidence rates" compared with people living in other areas around Tucson.

Shosie expressed frustration, sadness, and anger at the lack of action addressing PFAS contamination in her community and at other Superfund sites, saying, "environmental justice provisions continue to fail meanwhile thousands of contaminated sites remain unclean for more than 40 years." She also stated that those sites that have been remediated are in White or upper-class neighborhoods, while sites like the one in her low-income community remain unresolved to the detriment of community health and trust in governing institutions. Shosie also shared emotional details about losing her daughter, prompting her own path to find answers about environmental health issues in her community.

"We cannot rely on state, CDC, and other local government officials who continue to turn their backs on the people who are affected."

"Many people asked me why I got involved in the fight for … clean water, and demand government transparency and accountability. I got involved because I witnessed the death of my daughter out of this devastation I knew that I needed to find out why my daughter got so sick. *Linda Shosie*

Aaron Maruzzo (University of California, Berkeley, School of Public Health)

Aaron Maruzzo commented as a voice representing PFAS-contaminated communities in Western U.S. territories like Saipan in the Northern Mariana Islands. Maruzzo was born in Saipan and returned to work in the territory as a water quality analyst. Maruzzo framed the PFAS contamination problem in Saipan by leveraging data collected as part of the EPA's Third Unregulated Contaminant Monitoring Rule program. These program data capture PFAS concentrations in select public water systems from around the United States. Maruzzo comparison revealed major contamination in Saipan, with an average concentration of 1,700 ppt in territory drinking water, well above EPA health advisory limits and the levels observed in other U.S. states and territories. The highest detectable concentration of PFOS was also found in Saipan. Maruzzo said these data have been hiding in plain sight for many years and illustrate an ongoing environmental injustice.

Maruzzo went further to explain that PFAS contamination across the small island is unevenly distributed, with the highest levels found in water resources adjacent to the only airport found on the island, home to firefighting training facilities that used AFFF. Sixteen villages along the south and southwest margins of the island were disproportionally exposed to PFAS from this drinking water source, including the village where Maruzzo grew up. The southern portion of the island tends to include more non-White, noncitizens who may be easily missed in health data collection efforts as a result of frequent

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immigration and emigration. Health effects in this population may be also missed because of reluctance to seek medical care due to costs and accessibility. Despite these data collection challenges, preliminary reports suggest deep impacts on morbidity and mortality in the region, including heart disease, cancer, abnormal birth outcomes, hypertension, obesity, and cholesterol issues.

Maruzzo indicated that environmental justice issues are a key concern for his community, as "toxic exposures to hazardous chemicals continue to be disproportionately placed on communities of color and the poor working class." Maruzzo also stressed the continued systemic exclusion of U.S. territories like Saipan from continued dialogue about issues like PFAS.

With all this context, Maruzzo explained that the community needs further information about PFAS exposure routes specific to a small island setting. He indicated the need for further information about PFAS in the water of neighboring islands in the Northern Mariana Islands and the levels in private water systems, bottled water, and wastewater. Maruzzo flagged information gaps surrounding AFFF, questioning when AFFF was first used on the island and the composition of AFFFs over time. Maruzzo also questioned how military testing, imperialism, and globalization affect the contamination crisis today. Additionally, Maruzzo pointed out a lack of information describing PFAS in many consumer products, such as textiles and food packaging. Maruzzo indicated data gaps surrounding PFAS in fish and highlighted this as a problem, considering the cultural and economic importance of fishing on the island.

Following discussion of the unique exposure scenario ongoing in Saipan, Maruzzo offered comments, recommendations, and questions for the committee to consider while drafting clinical guidance. He explained that monitoring in humans can help answer questions about the importance of all these exposure gaps, while establishing an important baseline perspective. Maruzzo also argued for the importance of biomonitoring for optimal health care, as it allows families to make informed health care and lifestyle choices while empowering individuals to take action in their communities and environments. Biomonitoring studies also help constrain health effects, even as the candidate list of health effects explicitly related to PFAS requires further study. Maruzzo suggested that despite many unknowns plaguing our understanding of health effects related to PFAS, biomonitoring should be prioritized as it enables a precautionary approach to allow identification of unknown PFAS and health effects. Maruzzo mentioned the highly persistent nature of PFAS, pointing out that it is unadvisable to allow highly persistent chemicals to remain in our body only to find out about health effects later. Maruzzo also said this characteristic should be emphasized to physicians to help them understand the complexity of PFAS given typically low awareness about the issue in health care settings and in communities.

Maruzzo echoed other participants by pointing out that highly exposed and vulnerable populations should be prioritized for PFAS blood testing, using equity as a guide to design testing protocols. He further suggested that inclusion in these categories should be constrained by occupation, location, and biosocial vulnerabilities. Maruzzo stressed the importance of considering who is in the 95th or 99th percentile of exposure, while questioning who is missing from the dataset, to maximize understanding of the issue while minimizing exposure harms. Maruzzo raised the issue that there is also a moral component to blood testing that must be considered by the committee, asking how physicians will be prepared to educate exposed community members who do not already know about issues related to PFAS exposure. He asked whether an established protocol, cost considerations, or health care access would drive such decisions. Maruzzo also discussed the need to develop guidelines describing how to care for transient populations like migrants and military personnel. He asked the committee to consider how testing programs and rigorous exposure assessment should account for high loss of follow-up and discontinuity of care for these populations. Maruzzo also highlighted the value of culturally appropriate guidance and communication, stating that general scripts about risk reduction are "useful for a broad audience but there should be a mechanism to specify what's known into a local context."

"If there's one takeaway today, I think it's important to consider the implications of what happens when you don't listen to the voices at the margins."

"The absence of evidence or the absence of consensus doesn't mean a PFAS compound is safe."

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"One of the most PFAS-polluted places in the United States is a U.S. territory, and this is a serious environmental injustice."

"Risk communication should prioritize not solely action but meaningful action which is guided through the lens of equity." *Aaron Maruzzo*

Pamela Miller (Alaska Community Action on Toxics)

Pamela Miller spoke as a community member and environmental health advocate in Alaska. Miller provided abundant context about the unique exposure and community dynamics present in Alaska, while offering clear recommendations to the committee about Alaskan health needs and concerns. Miller explained that Alaska's strategic military importance has resulted in the establishment of multiple military installations across Alaska that have used AFFF. Miller also explained that Alaska is a very aviation-dependent state, with AFFF-using airports situated directly adjacent to communities and their water resources. Miller stated that PFAS have been found in at least 100 different sites across 30 locations spanning the entirety of the state, with multiple communities exposed to unsafe amounts of numerous PFAS. A total of 11 current and former military installations are currently under investigation for PFAS contamination, with results to date indicating the environmental occurrence of PFAS above health advisory levels. Despite the abundance of known sites, and the likelihood of as-yet-unidentified sites, Miller explained that there is no cohesive state plan to measure PFAS in the environment, fish, and wildlife, or in people. Miller mentioned an overall failure of regulatory efforts to guide the state's response to PFAS contamination, despite the support of attempted legislative efforts by associations of health care professionals, such as the Alaska Nurses Association. Only a handful of the 33 communities relying on water likely contaminated by AFFF or other PFAS sources have been able to access drinking water testing, contributing to widespread unawareness of the problem across the state.

Miller stated that limited assessment of drinking water and other environmental factors is matched by a lack of health assessments probing the effects of PFAS in exposed communities; only two health assessments have been conducted. One of these studies found positive correlations between the PFAS found in drinking water and those compounds found in serum, indicating an influence of drinking water contamination on human body burdens. Miller highlighted the particular plight of Alaskan remote regions as a hemispheric sink for persistent organic pollutants like PFAS. Remote polar regions receive undue burdens of mobile pollutants as a result of global distillation processes that transport pollutants to remote areas, including PFAS. Marine mammals and fish from polar regions therefore contain some of the highest burdens of persistent organic contaminants in the world because of these transport mechanisms and the bioaccumulative nature of PFAS. She shared the results of a community-based study in an Indigenous community on the island of Sivuqaq (St. Lawrence Island) in the Northern Bering Sea. This study tested the blood of community members reliant on traditional diets incorporating polar fish and marine mammals. The assessment found 13 PFAS in the blood of 85 people, as well as correlated concentrations of select PFAS to thyroid disruption.

Miller urged the need for biomonitoring in Alaskan communities with known or suspected PFAS contamination, including remote communities exposed through water sources, traditional foods, firefighting workers, and other exposed workers. Miller stated that human biomonitoring should be paired with assessments investigating exposure from water, dust, produce, fish, and wild game. These data are vital to inform health care providers and the wider community about exposures, possible associations with adverse health effects, appropriate risk reduction interventions, and relevant health care options. These data are also vital to inform policy.

Miller provided examples that emphasized the need to ensure that clinical guidance about PFAS is made accessible to diverse health care providers through various communication and training avenues. She explained that in Alaska health care needs are often addressed by community health aides as many communities lack doctors or nurses. Health aides in Alaska require the same clinical guidance and education afforded physicians, given their central role in meeting community health needs. Current, clear

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synthesis of scientific information, as well as an ability to execute biomonitoring and medical monitoring, is needed for physicians, nurses, and community health aides to allow these health care providers to inform and protect their patients from PFAS. Miller suggested that provider education should occur through professional organizations, as well as continuing education credits offered through public health, medical, and community training programs tailored to different provider types, including the Indian Health Service and regional health care providers.

> "PFAS are contaminating groundwater, surface waters, fish, wild game, garden produce, and people throughout Alaska."

> "The burden of proof should not be on our communities, and this must change so that laws reflect current scientific understanding and are protective of public health."

> "Health care professionals must be informed to become even more effective advocates for their patients and for ending nonessential uses of PFAS." *Pamela Miller*

Randy Krause (Port of Seattle Fire Department)

Randy Krause, the port fire chief, provided commentary as a veteran firefighter and fire chief who previously used AFFF. Krause detailed his career as a firefighter, spanning experience with the DoD, private industry, and a public-serving fire department. Krause explained that fire training activities with the DoD involved regular training with military-grade AFFF or "mil-spec" foams. Training scenarios were enacted and AFFF was sprayed abundantly onto the training props and into the wider environment. Krause also related that, in 1985, these foams were used routinely to clean floors and wash trucks, and were thrown on other firefighters during training exercises.

When he moved to private industry, Krause found a similar approach to training, where an open pit was used to stage fire scenarios, and AFFF was thrown on training fires. Krause clarified that at the time firefighters were assured these mil-spec foams were safe, biodegradable, and did not pose a risk to the environment, which has seen been learned to be incorrect. Krause became fire chief at Seattle International Airport in 2010. While the department had at one time used a similar open pit training set-up with mil-spec foams for fire training activities, Krause indicated this approach was not in practice when he arrived. The department refrained from use of fluorine-containing AFFFs for training due to state bans.

Krause emphasized that the safety of his team is a top priority as fire chief and described an opportunity to contribute to the Firefighter Cancer Cohort Study. This study is a multicity, long-term national research effort focused on assessing cancer in firefighters across the nation. Participation in this study provided Krause and several other department members access to PFAS blood testing. Krause shared his blood testing data in graphical and chart format detailing concentrations of isomers of PFOA, isomers of PFOS, PFHxS,[12] PFDeA,[13] PFNA, PFUA,[14] and Me-PFOSA-AcOH.[15] His results varied in proximity to provided benchmarks, but showed levels of linear PFOS and PFHxS close to or surpassing the nationwide 95th percentile. Krause explained he does not know what these results mean at this time, but discussion with Dr. Jeff Burgess from the University of Arizona suggests high levels of PFOS and PFHxS are commonly elevated in other firefighters. Krause also highlighted that the reporting techniques used by the Firefighter Cancer Cohort Study were particularly helpful, providing his exposure data, average amounts in firefighters from his department, the 50th and 95th percentiles based on NHANES data, and the range of all amounts measured in the firefighters in his department.

[12] Perfluorohexanesulfonic acid or perfluorohexane sulfonic acid, PFAS; see Chapter 1.
[13] Perfluorodecanoic acid, a PFAS; see Chapter 1.
[14] Perfluoroundecanoic acid, a PFAS; see Chapter 1.
[15] 2-N-methyl-perfluorooctane sulfonamido acetate, a PFAS; see Chapter 1.

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Jean Mendoza (Friends of Toppenish Creek)

Jean Mendoza spoke as a community member from the Lower Yakima Valley in Washington. Mendoza stated her community and organization have concerns about PFAS because of the prolific application of PFAS-imbued biosolids in agricultural fields across the area. Washington state statutes require biosolid application to the fullest extent possible. This raises concern as the area is highly reliant on agricultural activities and is home to large dairy cow populations; no information has yet investigated crop and livestock safety in response to biosolid-driven PFAS exposure in this area.

Mendoza conveyed discontent and frustration about blatant disregard of environmental health concerns in her community. Mendoza cited efforts by state agencies to measure PFAS in some areas across the state, but noted that PFAS measurements in central Washington and across the Yakima watershed seem to be intentionally omitted. Mendoza also pointed out that Yakima County ranks poorly in the state with respect to health outcomes and health factors, reflecting poor environmental health in an area home to tribal communities and majority Latino populations. In light of these findings, Mendoza stated that the state seems to be blatantly neglecting the exposure of people of the Yakima Valley.

Mendoza listed community health issues, including asthma, myocardial infarction, and low birthweights, noting the region is home to multiple complex exposures including PFAS, air pollution, nitrates, and pesticides that are associated with a number of adverse health outcomes. Mendoza raised the point that it is challenging for Yakima Valley residents to attribute health effects to any one pollutant, given the cumulative exposure to so many pollutants in the region. Mendoza also stated that concern about social maladies often trumps concern about PFAS in the region, given limited awareness of the problem in the community and inaction on the issue by the Yakima Health District. Mendoza moreover detailed that some elected officials take an "ignorance is bliss" approach and opt to avoid investigating the regional PFAS problem to avoid taking action on the issue.

Mendoza enumerated several challenges observed in her community that should be considered by the committee when formulating clinical guidance about PFAS for her community and beyond. She noted that many patients do not understand public health and risk assessment, and clinicians in the area receive no support from local health districts about PFAS. She also highlighted that many of the most exposed are poor households that live paycheck to paycheck, with limited capacity to worry or plan for long-term illness. She also cited the intangible nature of the PFAS problem, with no ability for folks to see, taste, or smell the issue. Mendoza offered recommendations as well, asking the committee to consider educating and informing clinicians about PFAS and other environmental health risks through professional organizations, to support biomonitoring studies, to recommend PFAS testing in fish, and to encourage a moratorium on biosolid application.

"We pay lip service to scientific evidence over here, but very often in Yakima Valley science is suppressed." *Jean Mendoza*

Rebecca Patterson (Vietnam Veterans of America)

Rebecca Patterson presented commentary as a Navy veteran and veteran advocate, highlighting the importance of PFAS blood testing for veteran's health care. Patterson explained that one of the largest sources of PFAS exposure for service members and military communities is the use of AFFF. These firefighting products have been used since 1970 to fight petroleum fires; legacy AFFFs contain PFAAs like PFOS and PFOA. While these are no longer in service, the DoD continues to use AFFF formulations containing PFAS despite the availability of fluorine-free alternatives. Patterson pointed out that use of these firefighting foams readily introduced PFAS into the environment and the water cycle and has led to widespread environmental contamination now documented at hundreds of military bases around the United States.

Patterson stated that her presentation was intended to educate the committee regarding how blood testing could help PFAS-exposed veterans gain access to VA health care. To accomplish this, Patterson

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shared information about the Veterans Health Administration (VHA). The VHA is tasked with providing care to eligible veterans, though not all veterans access health care through the VHA. This means community health care practitioners may be serving veterans without knowledge of their service history and related exposure; this necessitates that health care providers ask patients about their service history. Patterson also explained that after basic eligibility criteria are met, service members qualify for VHA care based on several factors, including service-connected disability, income, and exposure to toxicants and environmental hazards.

Service-connected disability is of particular importance to the veteran community; this designation typically requires specific evidence substantiating the connection between the given disease or injury and military service. Since medical concerns can arise years after service, it can be difficult for a veteran to connect an ailment to military service or exposure incurred through service. Disability approval results in tiered compensation and priority access to VHA care. With this context, Patterson concluded that access to health care and disability compensation can have a tremendous impact on a veteran's quality of life. PFAS blood testing can provide evidence of exposure that enables veterans to access vital health care. Patterson also asserted that PFAS blood testing can lead to more informed health care, allowing service members and veterans to screen for and potentially prevent health conditions specifically associated with PFAS exposure.

Bucky Bailey (son of former DuPont Washington Works plant employee)

William "Bucky" Bailey III provided perspective as a community member from Parkersburg, West Virginia. Bailey was born with multiple birth defects, including only one nostril, a keyhole eyelid, a serrated eyelid, and breathing difficulties. The family had no idea what caused the defects, given the lack of similar issues in Bailey's siblings. However, Bailey's mother worked as a full-time employee at the DuPont Washington Works plant in Parkersburg, where she controlled the production of PFOA in a confined area. Upon returning to work from maternity leave, Bailey's mother found that other pregnant women were removed from the Teflon production process. She also discovered that studies had been previously conducted by 3M that showed the same birth defects in laboratory animals exposed to PFAS. Despite these lines of evidence, DuPont denied that Bailey's birth defects were a result of his mother's occupational exposure, and the Bailey family found litigation impossible to pursue given DuPont's stature in the community.

Years later, Bailey met Rob Bilott, the lawyer who uncovered DuPont's malfeasance and pursued settlements for exposed residents of Parkersburg. Bailey described feeling relief finding out about links between PFOA exposure and health effects, following years of surgeries and underlying uncertainty regarding the cause of his deformities. Yet Bailey explained that joy following these revelations was also met with disheartenment and discouragement, knowing that the contamination that likely caused his deformities was entirely out of his control and had caused other health problems and untimely deaths in his family and wider community.

Bailey indicated that the C-8 Health Project did not find concrete links between his specific deformities and PFOA exposure, despite admission by DuPont scientists that the compound can cause birth defects. Bailey was also told his children would have a 50 percent chance of inheriting his health issues, which Bailey described as a tough and deeply troubling finding considering his marriage and his love of children. Bailey stated he struggled deeply with the decision to have kids, not wishing to put his children through what he went through as a child. Bailey and his wife ultimately decided to have children after wrestling with the question for over 10 years, and he reported he is the happy father to a healthy son and daughter. Bailey pointed out that knowledge of his contamination and related health risks delayed their family's decision to have children, which ultimately occurred after his father passed away. This timing deprived his children a relationship with their grandfather and deprived Bailey's father the opportunity to meet his grandkids. Bailey stated that his concerns now center on potential health effects like kidney and testicular cancer that he may encounter in the future given his significant PFOA contamination.

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"It was joy for me to learn some of the things the scientific study found out."

"I hope we can all acknowledge that we need to move in the same direction at the same time and not point fingers and not fight and not quarrel but find out what we can do to stop this from happening because it is going to cost us our lives." *Bucky Bailey*

One other person at the Western Town Hall provided public testimony, which is available on YouTube[16]: Gina Solomon.

[16] See https://www.youtube.com/watch?v=WghqL6urt6w&list=PLGTMA6QkejfjVRybfjFf-vRkIQnEhOb0a&index=22.

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Appendix C

Public Meeting Agendas

COMMITTEE ON THE GUIDANCE ON PFAS TESTING AND HEALTH OUTCOMES

The Keck Center, 500 Fifth Street, NW
Washington, DC 20001

FEBRUARY 4, 2021

VIRTUAL MEETING

1:00	Purpose of Open Session and Introduction of Committee Members **Bruce N. Calonge**, *Committee Chair*
1:15–2:00	ATSDR Perspectives on Study Scope, Background, and Objectives **Patrick Breysse**, *Director, National Center for Environmental Health/Agency for Toxic Substances and Disease Registry*
2:00–2:45	NIEHS Perspectives on Study Scope, Background, and Objectives **Brian R. Berridge**, *Scientific Director, Division of the National Toxicology Program, Associate Director, National Toxicology Program*
2:45–3:35	Committee Discusses the Statement of Task with the Sponsor
3:35–3:40	Break
3:40–3:45	Instructions for Public Comment Session **Bruce N. Calonge**
3:45–4:45	Opportunity for Public Comment on Committee's Charge (must preregister, 1 person per organization, 3 minutes each)
4:45	ADJOURN

APRIL 7, 2021
EASTERN COMMUNITIES TOWN HALL (ATSDR REGIONS I–IV)
VIRTUAL MEETING

2:00–2:10	Welcome and Introductions **Bruce N. Calonge**, *Committee Chair*

SESSION A—Community Perspectives from ATSDR Region I

2:10–3:10	Community Perspectives from ATSDR Region I **Alan Woolf**, *Moderator*, Harvard Medical School/Boston Children's Hospital

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2:10–2:20 **Laurene Allen**, Merrimack Citizens for Clean Water

2:20–2:30 **Teresa Gerade**, Don't Undermine Memphremagog's Purity (DUMP)

2:30–2:40 **Ayesha Khan**, Nantucket PFAS Action Group

2:40–2:50 **Kristen Mello**, Westfield Residents Advocating For Themselves (WRAFT)

2:50–3:10 Discussion

SESSION B—Community Perspectives from ATSDR Region II

3:10–3:45 Community Perspectives from ATSDR Region II
Laurel Schaider, *Moderator*, Silent Spring Institute

3:10–3:20 **Tracy Carluccio**, Delaware Riverkeeper Network

3:20–3:30 **Loreen Hackett**, PFOA Project New York

3:30–3:45 Panel Discussion

3:45–3:55 Break

SESSION C—Community Perspectives from ATSDR Region III

3:55–4:30 Community Perspectives from ATSDR Region III
Maida Galvez, *Moderator*, Icahn School of Medicine at Mount Sinai

3:55–4:05 **Patrick Elder**, Military Poisons

4:05–4:15 **Hope Grosse**, Buxmont Coalition for Safe Water

4:15–4:30 Panel Discussion

SESSION D—Community Perspectives from ATSDR Region IV

4:30–5:40 Community Perspectives from ATSDR Region IV
Linda Birnbaum, *Moderator*, National Institute of Environmental Health Sciences, National Toxicology Program (retired)

4:30–4:40 **La'Meshia Whittington**, North Carolina Black Alliance

4:40–4:50 **Mike Watters**, Grays Creek Residents United Against PFAS in our Wells & Rivers

4:50–5:00 **Emily Donovan**, Clean Cape Fear

5:00–5:10 **Cheryl Sievers-Cail**, South Carolina Indian Affairs Commission/SC Idle No More

5:10–5:20 **Stel Bailey**, Fight For Zero

5:20–5:45 Panel Discussion

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5:40–5:45 Break

SESSION F—OPEN COMMENT PERIOD

5:45–6:45 Public Comments

6:45 ADJOURN

MAY 6, 2021
MIDDLE COMMUNITIES TOWN HALL (ATSDR REGIONS V–VII)
VIRTUAL MEETING

3:00–3:10 Welcome and Introductions
Bruce N. Calonge, *Committee Chair*

SESSION A—Community Perspectives from ATSDR Region V

3:10–3:55 Community Perspectives from ATSDR Region V
Phil Brown, *Moderator*, Northeastern University
Andi Rich, Save Our Water (S.O.H20)
Laura Olah, Citizens for Safe Water Around Badger (CSWAB)
Samraa Luqman, Concerned Residents for South Dearborn
Cathy Wusterbarth, Need Our Water (NOW)

3:55–4:15 Panel Discussion with Session B Speakers

SESSION B—Community Perspectives from ATSDR Region V (continued)

4:15–4:50 Community Perspectives from ATSDR Region V
Courtney Carignan, *Moderator*, Michigan State University
Sandy Wynn-Stelt, Belmont, Michigan, resident
Tom Johnson, Clean Water Action
Vicki Quint, Foam Exposure Committee/Code PFAS

4:50–5:10 Panel Discussion

5:10–5:20 Break

SESSION C—Community Perspectives from ATSDR Region VI and VII

5:20–5:55 Community Perspectives from ATSDR Regions VI and VII
Alan Ducatman, *Moderator*, West Virginia University
Art Schaap, Highland Dairy, Cedar Rapids, Iowa Resident
John Kern, Clean Water Partnership

5:55–6:15 Panel Discussion

6:15–6:20 Break

6:20–7:00 Public Comments

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7:00 ADJOURN

MAY 25, 2021
WESTERN COMMUNITIES TOWN HALL (ATSDR REGIONS VIII–X)
VIRTUAL MEETING

1:00–1:10 Welcome and Introductions
Bruce N. Calonge, *Committee Chair*

SESSION A—Community Perspectives from ATSDR Region VIII and IX

1:10–2:00 Community Perspectives from ATSDR Region VIII and IX
Elizabeth Neary, *Moderator*, Wisconsin Environmental Health Network
Liz Rosenbaum, Fountain Valley Clean Water Coalition
Martha Dina Argüello, Physicians for Social Responsibility, Los Angeles
Mark A. Favors, Army Veteran

1:40–2:00 Panel Discussion

SESSION B—Community Perspectives from ATSDR Region IX

2:00–2:30 Community Perspectives from ATSDR Region IX
Lenny Siegel, *Moderator*, Center for Public Environmental Oversight
Andria Ventura, Clean Water Action Fund
Linda Shosie, Environmental Justice Task Force, Tucson, Arizona
Aaron Maruzzo, University of California, Berkeley, School of Public Health

2:30–2:50 Panel Discussion

2:50–3:00 Break

SESSION C—Community Perspectives from ATSDR Region X

3:00–3:30 Community Perspectives from ATSDR Region X
Anna Reade, *Moderator*, Natural Resources Defense Council
Pamela Miller, Alaska Community Action on Toxics
Randy Krause, Port of Seattle/Washington State Association of Fire Chiefs
Jean Mendoza, Friends of Toppenish Creek

3:30–3:50 Panel Discussion

SESSION D—Additional Community Perspectives

3:50–4:20 Additional Community Perspectives
Celeste Anne Monforton, *Moderator*, Texas State University
Rebecca Patterson, Vietnam Veterans of America
Bucky Bailey, Son of former DuPont Washington Works plant employee

4:20–4:40 Panel Discussion with Session E Speakers

4:40–4:45 Break

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4:45–5:00 Public Comments

5:00 ADJOURN

JULY 13–14, 2021
INFORMATION-GATHERING SESSION, MEETING 5
VIRTUAL MEETING
Thursday, July 13, 2021

1:30–1:40 Welcome and Introductions
Bruce N. Calonge, *Committee Chair*

SESSION A—Patient Perspectives on PFAS Testing and Health Outcomes

1:40–1:55 Patient Perspectives on PFAS Testing and Health Outcomes
Andrea Amico, Testing for Pease

SESSION B—Human Exposure Sources

1:55–2:25 Current Knowledge About the Contribution of PFAS Exposure Sources to Human Exposure
Elsie M. Sunderland, Harvard University

2:25–2:55 Panel Reflection and Q&A from Committee
Chris Wiant, *Moderator*, Committee Member
Bruce H. Alexander, Colorado State University
Thomas F. Webster, Boston University School of Public Health
Laurel Schaider, Silent Spring Institute
Elsie M. Sunderland, Harvard University

2:55–3:05 Break

SESSION C—Human Exposure Reduction

3:05–3:35 Clinical Principles for Advising Patients to Reduce Exposure
Sheela Sathyanarayana, University of Washington

3:35–4:05 Panel Reflection and Q&A from Committee
Brian Linde, *Moderator*, Committee Member
Judy LaKind, LaKind Associates
Andrea Amico, Testing for Pease
Sheela Sathyanarayana, University of Washington

4:05–4:35 Open Comment Period

4:35 ADJOURN

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HEALTH EFFECTS
Wednesday, July 14, 2021

1:00–1:10 Welcome, Purpose of Open Session
Bruce N. Calonge, Committee Chair

SESSION D—Overview of Putative Health Effects

1:00–2:10 Epidemiology: **David Savitz**, Brown University
Toxicology: **Jamie DeWitt**, East Carolina University

2:10–2:40 Panel Reflection and Q&A from Committee
Jane Hoppin, *Moderator*, Committee Member
Linda Birnbaum, National Institute of Environmental Health Sciences, National Toxicology Program (retired)
Joseph M. Braun, Brown University
Matthew Longnecker, Ramboll
David Savitz, Brown University
Jamie Dewitt, East Carolina University

2:40–2:55 Break

SESSION E—Evidence Synthesis and Its Application

2:55–3:25 Methods for Evidence Synthesis
Jonathan Samet, Colorado School of Public Health

3:25–3:55 Making Useful Recommendations
Rebecca L. Morgan, McMaster University

3:55–4:25 Panel Reflection and Q&A from Committee
Bruce N. Calonge, *Moderator*, Committee Member
Ellen Chang, Exponent
Nicholas Chartres, University of California, San Francisco
Holger Schünemann, McMaster University
Rebecca L. Morgan, McMaster University
Jonathan Samet, Colorado School of Public Health

4:25–4:55 Open Comment Period

4:55 ADJOURN

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AUGUST 11–12, 2021
INFORMATION-GATHERING SESSION, MEETING 6
VIRTUAL MEETING
Wednesday, August 11, 2021

1:25–1:35 Welcome and Introductions
Bruce N. Calonge, *Committee Chair*

SESSION A—Frameworks for Making Decisions on Clinical Evaluation and Biomonitoring

1:35–2:05 Principles for Making Decisions on Clinical Evaluation and Biomonitoring
David Resnik, National Institute of Environmental Health Sciences

2:05–2:35 Application of Decision-Making Framework in the C-8 Medical Monitoring Panel
Dean Baker, University of California, Irvine

2:35–3:05 Panel Reflection and Q&A from Committee
Kevin Elliott, *Moderator*
Courtney Carignan, Michigan State University
Ayesha Khan, Nantucket PFAS Action Group
Jeffrey Brent, University of Colorado
Dean Baker, University of California, Irvine
David Resnik, National Institute of Environmental Health Sciences

SESSION B—Clinician Perspective on Advising Patients in PFAS-Exposed Communities

3:05–3:25 Clinician Perspective on Advising Patients in PFAS-Exposed Communities
Alan Ducatman, West Virginia University

3:25–3:55 Panel Discussion with Clinicians Who Have Advised Patients in PFAS-Exposed Communities
Laura Anderko, *Moderator*
Katie Huffling, Alliance of Nurses for Healthy Environments
Stewart Reed, University of California, Los Angeles
Maida Galvez, Icahn School of Medicine at Mount Sinai Hospital
Alan Ducatman, West Virginia University

3:55–4:25 Open Comment Period

4:25 ADJOURN

Thursday, August 12, 2021

1:00–1:10 Welcome, Purpose of Open Session
Bruce N. Calonge, *Committee Chair*

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Guidance on PFAS Exposure, Testing, and Clinical Follow-Up

SESSION C—Clinical Principles for Communicating Biomonitoring Results and Relationship with Clinical Care

1:10–1:40 Biomonitoring Results: Communication
Julia Brody, Silent Spring Institute

1:40–1:55 PFAS Clinical Guidance
Phil Brown, Northeastern University

1:55–2:25 Panel Reflection and Q&A from Committee
Erin Haynes, *Moderator*, Committee member
Marc A. Nascarella, Massachusetts Department of Public Health
Jessica Nelson, Minnesota Department of Health
Gary Ginsberg, New York Department of Health
Julia Brody, Silent Spring Institute
Phil Brown, Northeastern University

2:25–2:55 Open Comment Period

2:55 ADJOURN

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Appendix D

Evidence Review: Methods and Approach

This appendix describes the approach and methods that the committee used to address the portions of the Statement of Task that asked for "an objective and authoritative review of current evidence regarding human health effects of those PFAS [per- and polyfluoroalkyl substances] being monitored in the CDC's [Centers for Disease Control and Prevention's] National Report on Human Exposure to Environmental Chemicals" (see Box 1-3 in Chapter 1). The Statement of Task specifically asked the committee to:

> Assess the strength of evidence for the spectrum of putative health effects suggested by human studies (including immune response, lipid metabolism, kidney function, thyroid disease, liver disease, glycemic parameters and diabetes, cancer, and fetal and child development) to establish a basis for prioritized clinical surveillance or monitoring of PFAS health effects. This assessment should characterize the likelihood of those health effects occurring (qualitative probability) given real world human exposures and identify the human populations at most risk (consider life stage, health status, exposure level). Data/evidence gaps that contribute to uncertainty about health effects of most concern should be annotated.

The committee decided that this portion of the Statement of Task required three different determinations:

- qualitative categories that describe the strength of evidence of PFAS putative health effects that can be used to prioritize clinical surveillance or monitoring,
- identification of the human populations at most risk (considering life stage, health status, exposure level) from PFAS exposure, and
- a scoping review that maps the data or evidence gaps that contribute to uncertainty about health effects of most concern.

To produce these outputs, the committee developed a multistage process. The first stage was to catalog what is known about PFAS and their health effects. The committee identified all authoritative reviews of the PFAS identified by the CDC (see Table 1-1 in Chapter 1) and all human health outcomes. The second stage was to identify any recent, high-quality systematic reviews between PFAS and any human health outcome; as noted below, the results of this stage were uninformative for the committee's goals. The third stage was to review the published research articles describing the association between exposure to PFAS and human health outcomes, based on the authoritative and systematic reviews. The committee's review approach improved efficiency while minimizing the risk of excluding scientific findings that would inform the committee's recommendations.

The committee was charged with assessing

> the strength of evidence for the spectrum of putative health effects suggested by human studies (including immune response, lipid metabolism, kidney function, thyroid disease, liver disease, glycemic parameters and diabetes, cancer, and fetal and

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child development) to establish a basis for prioritized clinical surveillance or monitoring of PFAS health effects.

However, the committee did not restrict its evaluation to only the listed putative health effects.

The next section of the appendix covers the committee's analysis of the authoritative reviews; the following section covers its original literature review, and the final section covers the committee strength-of-evidence determination.

AUTHORITATIVE REVIEWS

The committee defined authoritative reviews to be reviews produced by government agencies or other bodies that publish strength-of-evidence determinations through a process that includes peer review. The committee focused on national or international organizations or agencies that influence other organizations. The following organizations met these criteria for authoritative reviews:

- C-8 Science Panel Reports
- European Food Safety Authority (EFSA)
- International Agency for Research on Cancer (IARC)
- Organisation for Economic Co-operation and Development (OECD)
- National Toxicology Program (NTP)
- Agency for Toxic Substances and Disease Registry (ATSDR)
- U.S. Environmental Protection Agency (EPA)

Table D-1 summarizes the authoritative reviews found by the committee. Among the authoritative reviews, the ATSDR's *Toxicological Profile for Perfluoroalkyls* included the greatest number of PFAS included in the committee's Statement of Task (MeFOSAA not included) and was the most recent (literature search conducted in September 2018). The other authoritative reviews were older and included chemicals that were also included in the ATSDR's *Toxicological Profile for Perfluoroalkyls.* Therefore, the ATSDR's *Toxicological Profile for Perfluoroalkyls* was used by the committee as the basis for the next stages of the review process.

TABLE D-1 Authoritative Reviews Found by the Committee

Review	PFAS Chemicals Covered in Review	Health Endpoints Covered in Review	Date of Last Literature Search by the Organization
ATSDR *Toxicological Profile for Perfluoroalkyls*	PFBA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnA, PFDoDA, PFBS, PFHxS, PFOS, FOSA	Not limited	September 2018
EFSA *Risk to Human Health Related to the Presence of Perfluoroalkyl Substances in Food*	PFOA, PFNA, PFHxS, PFOS	Fertility and pregnancy outcomes, development effects, neurotoxic outcomes, immune outcomes, endocrine effects, metabolic effects, kidney function, cardiovascular disease and mortality, bone mineral density	March 2013
EPA Health Effects Document (PFOA)	PFOA	Serum lipids, cardiovascular disease, liver disease, kidney disease, diabetes, developmental toxicity, thyroid effects, immunotoxicity, cancer: kidney and testicular cancer, neurotoxicity, steroid hormones	2015
EPA Health Effects Document (PFOS)	PFOS	Serum lipids, cardiovascular disease, liver disease, kidney disease, diabetes,	2015

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		developmental toxicity, thyroid effects, immunotoxicity, cancer: testicular and kidney, neurotoxicity, steroid hormones	
IARC Monograph	PFOA	Cancer	June 2014
NTP Monograph	PFOA, PFOS	Immunotoxicity	May 2016
OECD Synthesis Paper	PFOA, PFOS, PFHxS, PFNA, PFDA, PFBS, PFBA, PFHxA, PTFE, PVDF, PFBE	Developmental toxicity, hypocholesteremia, ulcerative colitis, thyroid diseases, testicular cancer, kidney cancer, preeclampsia	Not presented, published 2013
C-8 Science Panel Probable Link Reports[a]	PFOA	Heart disease, kidney disease, liver disease, osteoarthritis, Parkinson's disease, autoimmune disease, infectious disease, neurodevelopmental disorders in children, respiratory disease, evaluation of stroke, thyroid disease, cancer, diabetes, birth defects, pregnancy-induced hypertension, miscarriage and stillbirth, preterm birth and low birthweight	Last report published in 2012

[a] See http://www.c8sciencepanel.org/prob_link.html (accessed July 1, 2022).
NOTE: ATSDR = Agency for Toxic Substances and Disease Registry; EFSA = European Food Safety Authority; EPA = U.S. Environmental Protection Agency; IARC = International Agency for Research on Cancer; NTP = National Toxicology Program; OECD = Organisation for Economic Co-operation and Development.

The committee's review did not assess the quality of the authoritative reviews, but it notes several areas where the ATSDR's *Toxicological Profile for Perfluoroalkyls* could be strengthened. First, the toxicological profile does not provide a detailed description of the evidence identification methods and does not document decisions as to why specific studies may have been excluded. Second, the study quality assessment does not appear to follow a standard approach, and in some cases it is difficult to identify the study designs that were included in the review. Third, the process to assess the strength of the evidence is not always clear.

REVIEW OF SYSTEMATIC REVIEWS

The committee's review of systematic reviews consisted of the following steps: literature search, screening of abstracts, full text review of studies identified in the abstract screening, evaluation of a final set of relevant studies, evidence assessment, and synthesis.

Literature Search

Systematic reviews were identified through searches of the medical and scientific literature on three databases: Embase Update, Medline, and Scopus. These three searchable databases index biological, chemical, medical, and toxicological publications. Search terms included full and abbreviated chemical names, common and manufacturer trade names, Chemical Abstracts Service (CAS) numbers, and MeSH[1] descriptors for each of the PFAS species of interest. Systematic reviews were included regardless of when they were published or where they were conducted. Systematic reviews were considered if they reviewed human studies, were classified as review papers, and were published in English. The databases were searched on June 28, 2021.

[1] MeSH descriptors are sets of terms naming descriptors in a hierarchical structure that permits searching at various levels of specificity.

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Ovid Embase Update Search Terms

1	("335-76-2" or "335-67-1" or "375-92-8" or "375-95-1" or "355-46-4" or "1763-23-1" or "2058-94-8").rn.	3673
2	Limit 1 to (human and English language and "review")	154
3	exp perfluorooctanesulfonic acid/ or exp perfluorohexanesulfonic acid/ or exp perfluorononanoic acid/ or exp perfluorooctanesulfonic acid/ or exp perfluorodecanoic acid/ or exp perfluorooctanoic acid/ or exp perfluoroundecanoic acid/ or ("Methyl-perfluorooctane sulfonamide" or "Methylperfluorooctane sulfonamidoacetic acid" or "Perfluorodecanoic acid" or "Perfluoroheptanesulfonic acid" or "perfluorohexane sulfonic acid" or "Perfluorohexanesulfonic acid" or "Perfluorononanoic acid" or "Perfluorooctane sulfonic acid" or "Perfluorooctanesulfonic acid" or "Perfluorooctanoic acid" or "perfluoroundecanoic acid").mp. or ("MeFOSAA" or "PFHxS" or "n-PFOA" or "Sb-PFOA" or "PFOA" or "PFDA" or "PFUnDA" or "n-PFOS" or "Sm-PFOS" or "PFOS" or "PFNA" or "Perfluorinated chemical" or "perfluorinated compound" or "perfluorinated chemicals" or "perfluorinated compounds").mp.	7814
4	Limit 3 to (human and English language and "review")	352

MEDLINE Search Terms

1	("335-76-2" or "335-67-1" or "375-92-8" or "375-95-1" or "355-46-4" or "1763-23-1" or "2058-94-8").rn.	331
2	Limit 1 to (English language and "review articles" and humans)	1
3	Limit 1 to (English language humans and "review" or "scientific integrity review" or "systematic review")	1 (*same article*)
4	("Methyl-perfluorooctane sulfonamide" or "Methylperfluorooctane sulfonamidoacetic acid" or "Perfluorodecanoic acid" or "Perfluoroheptanesulfonic acid" or "perfluorohexane sulfonic acid" or "Perfluorohexanesulfonic acid" or "Perfluorononanoic acid" or "Perfluorooctane sulfonic acid" or "Perfluorooctanesulfonic acid" or "Perfluorooctanoic acid" or "perfluoroundecanoic acid").mp.	4159
5	Limit 4 to (English language and "review articles" and humans)	77
6	("MeFOSAA" or "PFHxS" or "n-PFOA" or "Sb-PFOA" or "PFOA" or "PFDA" or "PFUnDA" or "n-PFOS" or "Sm-PFOS" or "PFOS" or "PFNA" or "Perfluorinated chemical" or "perfluorinated compound" or "perfluorinated chemicals" or "perfluorinated compounds").mp.	5852
7	Limit 6 to (English language and "review articles" and humans)	192
8	Limit 4 to (English language humans and "review" or "scientific integrity review" or "systematic review")	81
9	Limit 6 to (English language humans and "review" or "scientific integrity review" or "systematic review")	203
10	("Methyl-perfluorooctane sulfonamide" or "Methylperfluorooctane sulfonamidoacetic acid" or "Perfluorodecanoic acid" or "Perfluoroheptanesulfonic acid" or "perfluorohexane sulfonic acid" or "Perfluorohexanesulfonic acid" or "Perfluorononanoic acid" or "Perfluorooctane sulfonic acid" or "Perfluorooctanesulfonic acid" or "Perfluorooctanoic acid" or "perfluoroundecanoic acid").rn.	2896
11	Limit 10 to (English language and "review articles" and humans)	49
12	Limit 10 to (English language humans and "review" or "scientific integrity review" or "systematic review")	51

Scopus Search Terms

(((TITLE-ABS-KEY (*"MeFOSAA"* OR *"PFHxS"* OR *"n-PFOA"* OR *"Sb-PFOA"* OR *"PFOA"* OR *"PFDA"* OR *"PFUnDA"* OR *"n-PFOS"* OR *"Sm-PFOS"* OR *"PFOS"* OR *"PFNA"* OR *"Perfluorinated chemical*"* OR *"perfluorinated compound*"*)) OR (TITLE-ABS-KEY (*"Methyl-perfluorooctane sulfonamide"* OR *"Methylperfluorooctane sulfonamidoacetic acid"* OR *"Perfluorodecanoic acid"* OR *"Perfluoroheptanesulfonic acid"* OR *"perfluorohexane sulfonic acid"*

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OR *"Perfluorohexanesulfonic acid"* OR *"Perfluorononanoic acid"* OR *"Perfluorooctane sulfonic acid"* OR *"Perfluorooctanesulfonic acid"* OR *"Perfluorooctanoic acid"* OR *"perfluoroundecanoic acid"*)) OR (CHEMNAME (*"Methyl-perfluorooctane sulfonamide"* OR *"Methylperfluorooctane sulfonamidoacetic acid"* OR *"Perfluorodecanoic acid"* OR *"Perfluoroheptanesulfonic acid"* OR *"perfluorohexane sulfonic acid"* OR *"Perfluorohexanesulfonic acid"* OR *"Perfluorononanoic acid"* OR *"Perfluorooctane sulfonic acid"* OR *"Perfluorooctanesulfonic acid"* OR *"Perfluorooctanoic acid"* OR *"perfluoroundecanoic acid"*)) OR (CASREGNUMBER (*"335-76-2"* OR *"335-67-1"* OR *"375-92-8"* OR *"375-95-1"* OR *"355-46-4"* OR *"1763-23-1"* OR *"2058-94-8"*))) AND (INDEXTERMS (*human**) OR TITLE-ABS-KEY (*human**))) AND NOT INDEX (*medline*) AND (LIMIT-TO (DOCTYPE , *"ar"*)) AND (LIMIT-TO (LANGUAGE , *"English"*))

Screening of Abstracts

The literature search identified 639 potentially relevant systematic reviews. The publications were imported into PICO Portal, a web-based tool for collaborative citation screening for systematic reviews.[2] After importing to PICO Portal, 119 articles were identified as duplicates, leaving 520 for title and abstract screening. The review used the following population exposure comparison and outcome (PECO) statement:

> **Population:** Systematic reviews of health effects of PFAS in humans
> **Exposure:** PFAS species measured in the CDC's *National Report on Human Exposure to Environmental Chemicals* (see Chapter 1, Table 1-3)
> **Comparison:** Any comparison groups, including internal controls
> **Outcome:** Any human health outcome

The inclusion and exclusion criteria related to the PECO statement were as follows:

> **Inclusion Criteria:** Includes human evidence; includes the PFAS species measured in the CDC's *National Report on Human Exposure to Environmental Chemicals*; assesses the evidence for an association of PFAS and a health outcome in humans; and has a methods section
> **Exclusion Criteria:** Did not review health effects of PFAS; reviewed only animal or mechanistic studies; reviewed chemicals other than the PFAS included in the Statement of Task; reviewed generic classes of chemicals such as "endocrine disruptors" or "persistent organic pollutants;" or reviewed the ecological effects of PFAS or PFAS exposure pathways

Title and abstract screening was completed by two screeners. Disagreements were resolved by an adjudicator who helped facilitate a consensus decision. Fifty-four articles were included for full-text review.

Full Text Review

For the full text review, articles were excluded because they did not include human studies (n = 2), did not include relevant PFAS (n = 5), did not have a methods section (n = 18), or did not evaluate the association of PFAS exposure with a human health outcome (n = 3) (see Figure D-1). Thus, the full text review covered 26 articles.

[2] See https://picoportal.net (accessed July 1, 2022).

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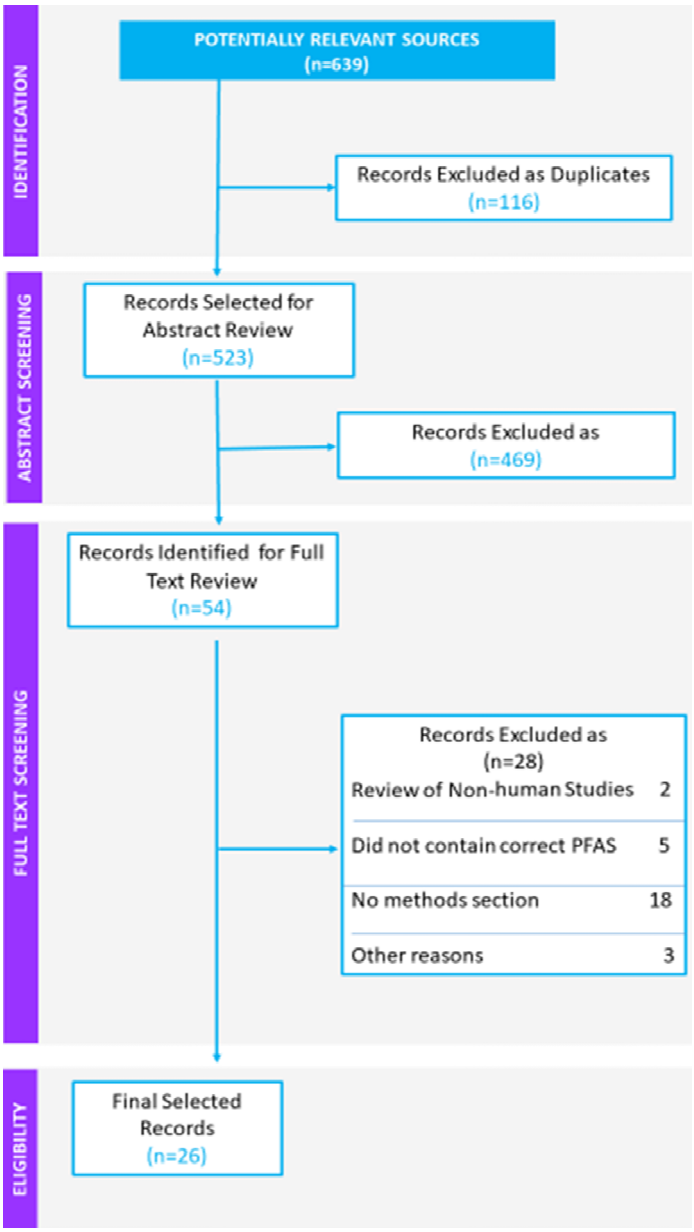


FIGURE D-1 Preferred reporting items for systematic reviews and meta-analyses (PRISMA) diagram for the committee's review of reviews on health effects of PFAS.

Evaluation and Evidence Assessment

The quality of the 26 included systematic reviews was evaluated using the AMSTAR-2 (A MeaSurement Tool to Assess systematic Reviews) (Shea et al., 2017), which has been used by several other committees of the National Academies of Sciences, Engineering, and Medicine (NASEM, 2019, 2021). AMSTAR-2 includes several critical appraisal domains (see Box D-1). The quality assessment was conducted by a staff member and was confirmed by a committee member. The committee conducted a critical appraisal of the systematic reviews because systematic reviews can be subject to a range of biases.

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BOX D-1
AMSTAR-2 Critical Domains and Overall Confidence in the Results

Critical Domains

- Protocol registered before commencement of the review (item 2)
- Adequacy of the literature search (item 4)
- Justification for excluding individual studies (item 7)
- Risk of bias from individual studies being included in the review (item 9)
- Appropriateness of meta-analytical methods (item 11)
- Consideration of risk of bias when interpreting the results of the review (item 13)
- Assessment of presence and likely impact of publication bias (item 15)

Rating: Overall Confidence in the Results of the Review

- *High:* No or one noncritical weakness: the systematic review provides an accurate and comprehensive summary of the results of the available studies that address the question of interest.
- *Moderate:* More than one noncritical weakness: the systematic review has more than one weakness but no critical flaws. It may provide an accurate summary of the results of the available studies that were included in the review.
- *Low:* One critical flaw with or without noncritical weaknesses: the review has a critical flaw and may not provide an accurate and comprehensive summary of the available studies that address the question of interest.
- *Critically Low:* More than one critical flaw with or without noncritical weaknesses: the review has more than one critical flaw and should not be relied on to provide an accurate and comprehensive summary of the available studies.

Synthesis: Results

Of the 26 systematic reviews identified by the committee, 9 included studies published after the ATSDR's *Toxicological Profile for Perfluoroalkyls* (Bartell and Vieira, 2021; Boesen et al., 2020; Deji et al., 2021; Dzierlenga et al., 2020; Ferrari et al., 2019; Luo et al., 2020; Petersen et al., 2020; Steenland and Winquist, 2020; Xie et al., 2020; Zare Jeddi et al., 2021). Although all were of moderate quality, some reviews covered the same original data and papers, and the committee found it challenging to synthesize across them. As a result, the systematic reviews were used as sources for reference in the committee's determination of the biologic plausibility between PFAS and a health effect, but they were not formally included as part of the final strength-of-evidence determination.

ORIGINAL LITERATURE REVIEW

The original literature review consisted of the following steps: literature search, screening of abstracts, full text review of studies identified in the abstract screening, evidence mapping and evaluation, and data abstraction.

Literature Search

As was done for the review of reviews, the literature for the original literature review was identified through searches of the medical and scientific literature on three databases: Ovid Embase, Ovid Medline, and Scopus. These three searchable databases index biological, chemical, medical, and toxicological publications. Search terms included full and abbreviated chemical names, common and

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manufacturer trade names, the CAS numbers, and MeSH[3] descriptors for each of the PFAS species of interest. There were no time constraints or geographic constraints included in the search. The only constraints were human studies and English language only. The databases were searched on March 30–31, 2021.

Ovid Embase Search Terms[4]

1	("335-76-2" or "335-67-1" or "375-92-8" or "375-95-1" or "355-46-4" or "1763-23-1" or "2058-94-8").rn.	3547
2	exp perfluorooctanesulfonic acid/	3114
3	exp perfluorohexanesulfonic acid/	625
4	exp perfluorononanoic acid/	890
5	exp perfluorooctanesulfonic acid/	3114
6	exp perfluorodecanoic acid/	713
7	exp perfluorooctanoic acid/	3493
8	exp perfluoroundecanoic acid/	386
9	("Methyl-perfluorooctane sulfonamide" or "Methylperfluorooctane sulfonamidoacetic acid" or "Perfluorodecanoic acid" or "Perfluoroheptanesulfonic acid" or "perfluorohexane sulfonic acid" or "Perfluorohexanesulfonic acid" or "Perfluorononanoic acid" or "Perfluorooctane sulfonic acid" or "Perfluorooctanesulfonic acid" or "Perfluorooctanoic acid" or "perfluoroundecanoic acid").mp.	5503
10	("MeFOSAA" or "PFHxS" or "n-PFOA" or "Sb-PFOA" or "PFOA" or "PFDA" or "PFUnDA" or "n-PFOS" or "Sm-PFOS" or "PFOS" or "PFNA" or "Perfluorinated chemical" or "perfluorinated compound" or "perfluorinated chemicals" or "perfluorinated compounds").mp.	6579
11	1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10	7610
12	(exp animal/ or nonhuman/) not exp human/	6205240
13	11 not 12	5435
14	Limit 13 to english language	5232
15	Limit 14 to "pubmed/medline"	540
16	14 not 15	4692
17	Limit 16 to article	3517

Scopus Search Terms[5]

(((TITLE-ABS-KEY (*"MEFOSAA"* OR *"PFHXS"* OR *"N-PFOA"* OR *"SB-PFOA"* OR *"PFOA"* OR *"PFDA"* OR *"PFUNDA"* OR *"N-PFOS"* OR *"SM-PFOS"* OR *"PFOS"* OR *"PFNA"* OR *"PERFLUORINATED CHEMICAL*"* OR *"PERFLUORINATED COMPOUND*"*)) OR (TITLE-ABS-KEY (*"METHYL-PERFLUOROOCTANE SULFONAMIDE"* OR *"METHYLPERFLUOROOCTANE SULFONAMIDOACETIC ACID"* OR *"PERFLUORODECANOIC ACID"* OR *"PERFLUOROHEPTANESULFONIC ACID"* OR *"PERFLUOROHEXANE SULFONIC ACID"* OR *"PERFLUOROHEXANESULFONIC ACID"* OR *"PERFLUORONONANOIC ACID"* OR *"PERFLUOROOCTANE SULFONIC ACID"* OR *"PERFLUOROOCTANESULFONIC ACID"* OR *"PERFLUOROOCTANOIC ACID"* OR *"PERFLUOROUNDECANOIC ACID"*)) OR (CHEMNAME (*"METHYL-PERFLUOROOCTANE SULFONAMIDE"* OR *"METHYLPERFLUOROOCTANE SULFONAMIDOACETIC ACID"* OR *"PERFLUORODECANOIC ACID"* OR *"PERFLUOROHEPTANESULFONIC ACID"* OR *"PERFLUOROHEXANE SULFONIC ACID"* OR *"PERFLUOROHEXANESULFONIC ACID"* OR *"PERFLUORONONANOIC ACID"* OR

[3] MeSH descriptors are sets of terms naming descriptors in a hierarchical structure that permits searching at various levels of specificity.

[4] Not all chemical names mapped to a heading.

[5] The search was limited to articles and English language.

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"PERFLUOROOCTANE SULFONIC ACID" OR *"PERFLUOROOCTANESULFONIC ACID"* OR *"PERFLUOROOCTANOIC ACID"* OR *"PERFLUOROUNDECANOIC ACID"*)) OR (CASREGNUMBER (*"335-76-2"* OR *"335-67-1"* OR *"375-92-8"* OR *"375-95-1"* OR *"355-46-4"* OR *"1763-23-1"* OR *"2058-94-8"*))) AND (INDEXTERMS (*HUMAN**) OR TITLE-ABS-KEY (*HUMAN**))) AND NOT INDEX (*MEDLINE*) AND (LIMIT-TO (DOCTYPE , *"AR"*)) AND (LIMIT-TO (LANGUAGE , *"ENGLISH"*))

Ovid Medline Search Terms

1	("335-76-2" or "335-67-1" or "375-92-8" or "375-95-1" or "355-46-4" or "1763-23-1" or "2058-94-8").rn.	330
2	("Methyl-perfluorooctane sulfonamide" or "Methylperfluorooctane sulfonamidoacetic acid" or "Perfluorodecanoic acid" or "Perfluoroheptanesulfonic acid" or "perfluorohexane sulfonic acid" or "Perfluorohexanesulfonic acid" or "Perfluorononanoic acid" or "Perfluorooctane sulfonic acid" or "Perfluorooctanesulfonic acid" or "Perfluorooctanoic acid" or "perfluoroundecanoic acid").mp.	4050
3	("MeFOSAA" or "PFHxS" or "n-PFOA" or "Sb-PFOA" or "PFOA" or "PFDA" or "PFUnDA" or "n-PFOS" or "Sm-PFOS" or "PFOS" or "PFNA" or "Perfluorinated chemical" or "perfluorinated compound" or "perfluorinated chemicals" or "perfluorinated compounds").mp.	5700
4	("Methyl-perfluorooctane sulfonamide" or "Methylperfluorooctane sulfonamidoacetic acid" or "Perfluorodecanoic acid" or "Perfluoroheptanesulfonic acid" or "perfluorohexane sulfonic acid" or "Perfluorohexanesulfonic acid" or "Perfluorononanoic acid" or "Perfluorooctane sulfonic acid" or "Perfluorooctanesulfonic acid" or "Perfluorooctanoic acid" or "perfluoroundecanoic acid").rn.	2837
5	1 or 2 or 3 or 4	6194
6	Animals/ not (Animals/ and Humans/)	4772259
7	5 not 6	4664
8	Journal Article/	30061095
9	7 and 8	4548
10	Limit 9 to English language	4379

Abstract Screening

The literature search identified 5,172 potentially relevant studies. The studies were imported into PICO Portal, a web-based tool for collaborative citation screening for systematic reviews.[6] After importing the studies to PICO Portal, 112 articles were identified as duplicates, leaving 5,060 articles to be screened. The titles and abstracts were screened for relevance to the research questions for the review. The review used the following PECO statement:

> **Population:** Studies of health effects of PFAS in humans
> **Exposure:** PFAS species measured in the CDC's *National Report on Human Exposure to Environmental Chemicals*
> **Comparison:** Any comparison groups, including internal comparisons
> **Outcome:** Any health outcome measured in humans

The inclusion and exclusion criteria related to the PECO statement were as follows:

> **Inclusion Criteria:** Is an epidemiologic or human study; includes a quantitative measure of the PFAS species measured in the CDC's *National Report on Human Exposure to Environmental Chemicals*; assesses the evidence for an association of PFAS and a health outcome in humans; and English language only

[6] See https://picoportal.net (accessed July 1, 2022).

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Exclusion Criteria: Did not review health effects of PFAS; reviewed only animal or mechanistic studies; was about chemicals other than the PFAS included in the Statement of Task; did not include a quantitative measure; or was on the ecological effects of PFAS or PFAS exposure pathways

The literature search identified 5,172 potentially relevant studies. After removal of duplicates (112 articles), 5,060 articles were subject to title and abstract screening by two independent reviewers.

Full Text Review

For the full text review, 4,434 of the articles identified in the literature search were excluded because the titles and abstracts did not meet the inclusion criteria, so 626 articles were subject to full text review. During that review, additional articles were excluded if they were published before 2018 or listed in the references to the ATSDR's *Toxicological Profile for Perfluoroalkyls* (n = 320); were cross-sectional in design (n = 160); were not published in English (n = 1); did not provide risk estimates associated with PFAS exposure (n = 3); or were not studies in humans (n = 3) (see Figure D-2). Cross-sectional studies were largely excluded because this study design measures exposure and disease at the same time so cannot determine cause and effect. Thus, the full text review covered 139 articles.

Evidence Mapping and Evaluation

The committee then categorized the 139 articles according to the human health outcomes studied. The committee mapped the evidence with the goal of determining evidence gaps and to inform strategies for the evidence evaluation and evidence synthesis (see Figure D-3).

The committee focused on those endpoints for which additional review might change the committee's understanding of the association between PFAS exposure and health outcomes.

The committee conducted a narrative evaluation of the study quality and considered factors that may contribute to the study's risk of bias (see Box D-2) using a tool adapted from the Navigation Guide (Woodruff and Sutton, 2014). Bias is a systematic error that leads to study results that differ from the actual results. Bias can lead to an observed effect when one does not exist or to no observed effect when there is a true effect. Risk of bias is the appropriate term, as a study may be unbiased despite a methodological flaw (Higgins et al., 2019). The risk-of-bias assessment in a systematic review is based on the quality of the individual component studies (Eick et al., 2020).

A trained reviewer from ICF International, the EPA, the National Academies, or Johns Hopkins University abstracted the critical domain information from each study that the committee used to support its judgment determinations regarding a study's risk of bias, and the committee made the final risk of bias judgments for each study.[7] Each paper was given an overall assessment of its risk of bias (low, probably low, probably high, or high risk of bias).

[7] For some studies included in the committee's review, the data had been previously abstracted by ICF or the EPA to support the EPA's ongoing assessments of PFAS; newer evidence was abstracted by ICF or consultants at Johns Hopkins.

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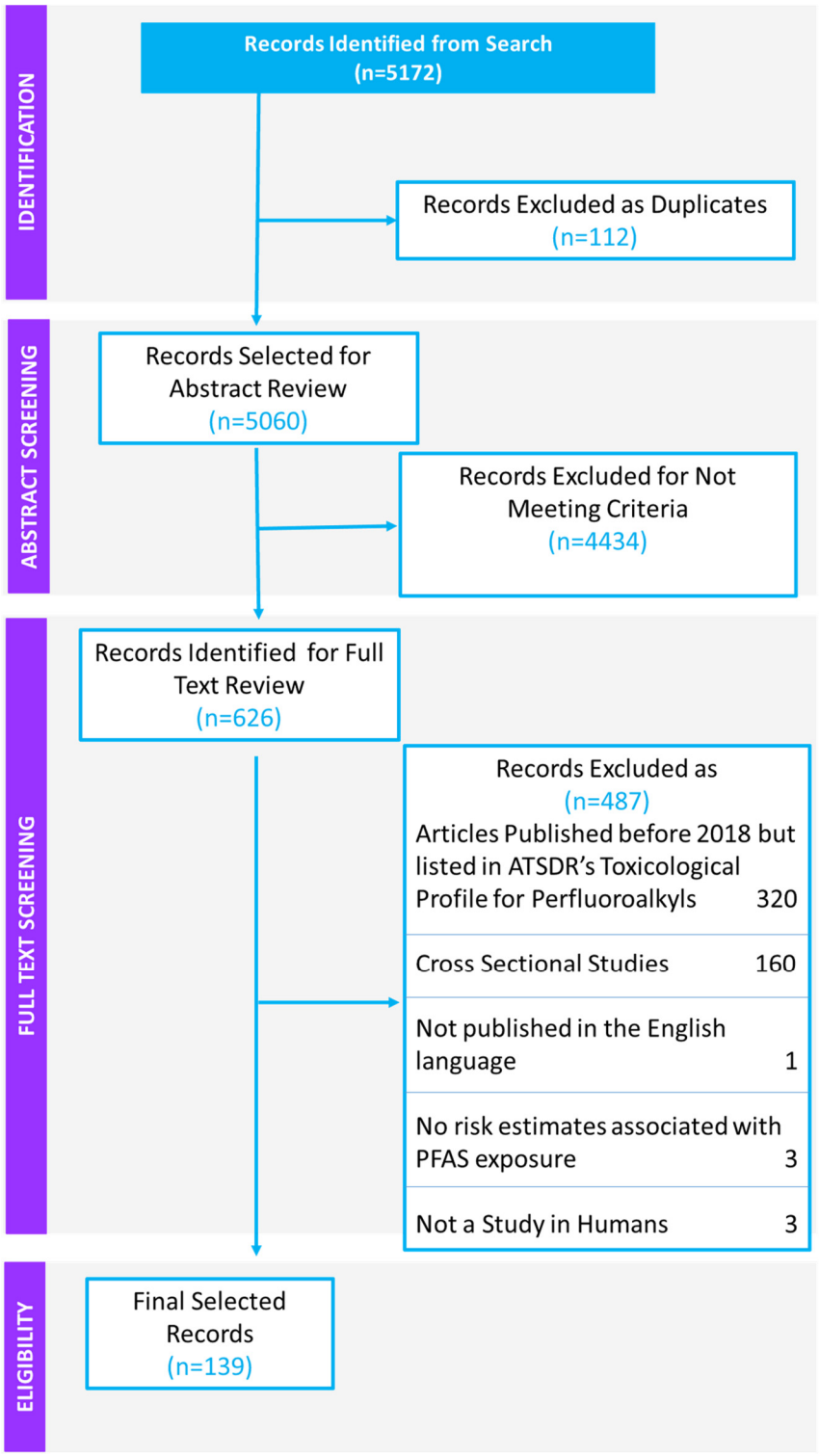


FIGURE D-2 Preferred reporting items for systematic reviews and meta-analyses (PRISMA) diagram for the committee's reviews on the health effects of PFAS.

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	Chemical								
Health Effect Category	MeFOSAA	PFDA	PFHxS	PFNA	PFOA	PFOS	PFUnDA	sumPFAS	Grand Total
Bodyweight	4	4	10	10	13	12	2	0	13
Cancer	2	3	5	4	7	7	4	0	7
Cardiometabolic	7	9	21	20	19	20	6	3	21
Developmental	6	25	36	38	44	46	16	4	47
Endocrine	3	8	12	12	11	12	6	2	13
Hepatic	1	2	3	2	4	3	1	0	4
Immunological	0	8	13	13	14	14	10	2	14
Musculoskeletal	0	1	2	2	2	2	1	1	2
Neurological	1	5	9	10	10	11	3	2	11
Other	0	3	3	3	4	4	2	0	4
Renal	3	1	3	3	4	3	0	1	4
Reproductive	3	16	25	24	25	27	11	1	29
Respiratory	0	6	9	10	10	10	8	0	10
Grand Total	20	72	118	118	130	133	56	13	139

FIGURE D-3 Evidence map describing the number of studies found by PFAS for each health outcome category.

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BOX D-2
Critical Domains Used by the Committee to Assess Risk of Bias

Exposure Measures: Assay information, quality control measures, repeat measures, validation studies

Outcome Measures: Sources of the effect measure, blinding to exposure status or level, methods of measurement or classification, incident versus prevalent disease, evidence from validation studies

Participant Selection: Study design, where and when was the study conducted, and who was included? Recruitment process, exclusion and inclusion criteria, type of controls, total eligible, comparison between participants and nonparticipants (or followed and not followed), final analysis group. Does the study include potential vulnerable/susceptible groups or life stages?

Potential Confounding: Potential confounders and outcome; degree of exposure to the confounder in the population

Analysis: Extent (and, if applicable, treatment) of missing data for exposure, outcome, and confounders, approach to modeling, classification of exposure and outcome variables (continuous versus categorical), testing of assumptions, sample size for specific analyses, and relevant sensitivity analyses

Selective Reporting: Are results presented with adequate detail for all of the endpoints of interest? Are results presented for the full sample as well as for specified subgroups? Were stratified analyses (effect modification) motivated by a specific hypothesis?

Sensitivity: What exposure range is spanned in this study? What are the ages of participants (e.g., not too young in studies of pubertal development)? What is the length of follow-up (for outcomes with long latency periods)? Choice of referent group and the level of exposure contrast between groups (i.e., the extent to which the "unexposed group" is truly unexposed, and the prevalence of exposure in the group designated as "exposed")

Source of Funding: Description of the disclosed sources of funding for the paper

Data Abstraction

Data abstraction was completed by a trained scientist from ICF, the EPA, the National Academies, or Johns Hopkins.[8] The data abstraction form included the following:

Reference: Author year and DOI number if relevant
Chemical: Acronym of specific PFAS
Endpoint: Name of the specific outcome for the risk estimate
Subpopulation: Description of the specific subpopulation for the risk estimate
N: Sample size that informs risk estimate
Exposure Levels: That apply to the risk estimate
Comparison: Description of type of risk estimate, such as "SMR for bladder cancer in the high exposure group compared to no workplace exposure" or "change in ln (TSH) per standard unit increase in serum PFOA."
Risk/Effect Estimate: Reported number
Lower Confidence Interval: Reported number
Upper Confidence Interval: Reported number

[8] For some studies included in the committee's review, the data had been previously abstracted by ICF or the EPA to support the EPA's ongoing assessments of PFAS; newer evidence was abstracted by ICF or consultants at Johns Hopkins University.

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Effect estimates from the individual studies included in the review were extracted into a database and uploaded to a public website (Tableau Public) to allow for visualizations, such as evidence maps and forest plots.[9] The effect estimates in the Tableau represent those from the model most adjusted for confounders.

STRENGTH-OF-EVIDENCE DETERMINATION

To assess the strength of evidence regarding the potential for PFAS to cause a particular health effect, the committee then integrated the evidence reviewed in the ATSDR's *Toxicological Profile for Perfluoroalkyls* and other authoritative reviews with the evidence from the original literature review from the epidemiologic studies.

The synthesis of available data was guided by a framework based on the Hill considerations (Hill, 1965), which help to determine whether associations are causal (see Box D-3). The committee did not consider the Hill considerations to be a heuristic for assessing causation in isolation, that is, as a checklist where each item must be met to establish causality. Rather, the committee considered them as a list of possible considerations meant to generate thoughtful discourse by the committee to help inform its determinations for the strength of evidence (Fedak, 2015; NASEM, 2018).

BOX D-3
Bradford Hill Considerations

Strength: A small association does not mean that there is not a causal effect, though the larger the association, the more likely that it is causal.

Consistency: Consistent findings observed by different people in different places with different samples strengthens the likelihood of an effect.

Specificity: Causation is likely if there is a very specific population, at a specific site, with a specific disease with no other likely explanation.

Temporality: The effect has to occur after the cause.

Biological Gradient: Greater exposure should generally lead to greater incidence of the effect. In other cases, greater exposure leads to lower incidence.

Plausibility: A plausible mechanism between cause and effect is helpful.

Coherence: Coherence between epidemiological and laboratory findings increases the likelihood of an effect but "lack of such [laboratory] evidence cannot nullify the epidemiological effect on associations" (Hill, 1965, p. 298).

Experiment: Occasionally it is possible to appeal to experimental evidence.

Analogy: The effect of similar factors may be considered.

SOURCE: Hill, 1965.

[9] The committee's public Tableau is available at https://public.tableau.com/app/profile/nationalacademies/viz/NASEMPFASEvidenceMaps/PFASEvidenceMap (accessed July 1, 2022). The information may be viewed as an evidence map or as a forest plot. Within forest plots, filters can be accessed using the "toggle filters" function in order to restrict the view to data on specific health effect categories and other factors (such as reference, chemical, study design, study population).

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Animal and Mechanistic Studies

The Statement of Task called for the committee to "assess the strength of evidence for the spectrum of putative health effects suggested by human studies." The committee focused on more recent epidemiological literature in line with its Statement of Task. However, the committee recognizes the importance of toxicologic evidence in making strength-of-evidence conclusions and relied on the toxicological data included in the ATSDR's *Toxicological Profile for Perfluoroalkyls*, other authoritative reviews, and systematic reviews that provide integrative conclusions based on multiple lines of evidence. In synthesizing evidence in this manner, the committee acknowledges that animal evidence greatly improves the interpretation of the human studies.

An observed association between PFAS exposure and a health effect does not necessarily mean that the exposure is the cause of that outcome. Toxicologic evidence, whether it supports or conflicts with evidence from epidemiologic studies, provides insights about biologic processes and informs how an observed association might be interpreted. The degree of biologic plausibility itself influences whether the committee perceives positive findings to be indicative of a pattern or the product of statistical fluctuations. Ultimately, the results of the toxicology studies should be consistent with what is known about the human disease process if they are to support a conclusion that the development of the disease was influenced by an exposure (NASEM, 2018).

Categories of Association

Sufficient Evidence of an Association

For effects in this category, a positive association between PFAS and the outcome must be observed in studies in which chance, bias, and confounding can be ruled out with reasonable confidence. For example, the committee might regard as sufficient evidence of an association evidence from several small studies that is unlikely to be due to confounding or to otherwise be biased and that shows an association that is consistent in magnitude and direction. Experimental data supporting biologic plausibility strengthen the evidence of an association but are not a prerequisite, nor are they sufficient to establish an association without corresponding epidemiologic findings.

Limited or Suggestive Evidence of an Association

In this category, the evidence must suggest an association between exposure to PFAS and the outcome in studies of humans, but the evidence can be limited by an inability to rule out chance, bias, or confounding with confidence. One high-quality study may indicate a positive association, but the results of other studies of lower quality may be inconsistent.

Inadequate or Insufficient Evidence to Determine an Association

If there was not enough reliable scientific data to categorize the potential association with a health effect as "sufficient evidence of an association," "limited or suggestive evidence of an association," or on the other end of the spectrum, "limited or suggestive evidence of no association," the health outcome was placed in the category of "inadequate or insufficient evidence to determine an association" by default. In this category, the available human studies may have inconsistent findings or be of insufficient quality, validity, consistency, or statistical power to support a conclusion regarding the presence of an association. Such studies may have failed to control for confounding factors or may have had inadequate assessment of exposure.

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Limited or Suggestive Evidence of No Association

A conclusion of "no association" is inevitably limited to the conditions, exposures, and observation periods covered by the available studies, and the possibility of a small increase in risk related to the magnitude of exposure studied can never be excluded. However, a change in classification from inadequate or insufficient evidence of an association to limited or suggestive evidence of *no* association would require new studies that corrected for the methodologic problems of previous studies and that had samples large enough to limit the possible study results attributable to chance.

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Appendix E

White Paper: Review of the PFAS Personal Intervention Literature

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Acronyms and Abbreviations

AC	activated carbon
ATDSR	Agency for Toxic Substances and Disease Registry
CDC	Centers for Disease Control and Prevention
CI	confidence interval
EPA	U.S. Environmental Protection Agency
Et-PFOSA-AcOH	2-(n-ethyl-perfluorooctane sulfonamido) acetic acid
EtFOSAA	2-(n-ethyl-perfluorooctane sulfonamido) acetic acid
FDA	U.S. Food and Drug Administration
GAC	granular activated carbon
H2PFDA	2H,2H-perfluorodecanoic acid
H4PFOS	1H,1H,2H,2H-perfluorooctane sulfonic acid
H4PFUnDA	2H,2H,3H,3H-perfluoroundecanoic acid
L-PFHpS	sodium perfluoro-1 heptanesulfonate
LOD	limit of detection
LOQ	limit of quantitation
MDH	Minnesota Department of Health
Me-PFOSA-AcOH	2-(n-methyl-perfluorooctane sulfonamido) acetic acid
MeFOSAA	2-(n-methyl-perfluorooctane sulfonamido) acetic acid
MRL	Minimum Reporting Level
n-EtPFOSAA	n-ethyl-perfluoro-1 octanesulfonamido acetic acid.
n-MePFOSAA	n-methylperfluoro-1 octanesulfonamido acetic acid
n-PFOA	n-perfluorooctanoic acid
n-PFOS	n-perfluorooctane sulfonic acid
NEtFOSE	N-ethyl perfluorooctane sulfonamidoethanol
NHANES	National Health and Nutrition Examination Survey
NIEHS	National Institute of Environmental Health Sciences
PBDE	polybrominated diphenyl ether
PCB	polychlorinated biphenyl
PFAA	perfluoroalkyl acid
PFAS	per- and polyfluoroalkyl substances
PFBA	perfluorobutanoate
PFBS	perfluorobutane sulfonic acid
PFC	perfluorinated compound
PFCA	perfluoroalkyl carboxylic acid
PFDA	perfluorodecanoic acid
PFDcA	perfluorodecanoate
PFDoA (PFDoDA)	perfluorododecanoic acid
PFDeA	perfluorodecanoic acid
PFDS	perfluorodecane sulfonate

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PFHpA	perfluoroheptanoic acid
PFHpS	perfluoroheptane sulfonate
PFHxA	perfluorohexanoate
PFHxS	perfluorohexane sulfonic acid
PFNA	perfluorononanoic acid
PFOA	perfluorooctanoic acid
PFOS	perfluorooctane sulfonic acid
PFOSA or FOSA	perfluorooctane sulfonamide
PFPeA	perfluoropentanoate
PFTeA	perfluorotetradecanoate
PFTeDA	perfluorotetradecanoic acid
PFTrA	perfluorotridecanoate
PFTrDA	perfluorotridecanoic acid
PFUA	perfluoroundecanoate
PFUnA	perfluoroundecanoic acid
PFUnDA	perfluoroundecanoic acid
POE	point of entry
POTW	publicly owned treatment work
POU	point of use
RO	reverse osmosis
Sb-PFOA	branched perfluorooctanoic acid
SD	standard deviation
Sm-PFOS	perfluoromethylheptane sulfonic acid
UCMR	Unregulated Contaminant Monitoring Rule
ww	wet weight

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Abstract

This white paper provides an overview of the published literature on whether personal behavior modifications can demonstrably reduce exposure to per- and polyfluoroalkyl substances (PFAS) (e.g., by showing decreases in serum levels). The reviewed studies are presented by exposure source. The preponderance of the identified literature relates to diet and drinking water. Literature on interventions for other exposure sources, such as dust and consumer products, is more limited. Breastfeeding is an important potential source of exposure for infants; the effect of lactation on mothers' PFAS levels is unclear. For communities with high levels of PFAS in drinking water, interventions related to tap water filtration showed some efficacy in reducing PFAS levels in the water. It is possible that an intervention may reduce PFAS levels in a particular medium, but if this medium is not a major source of overall exposure, then that intervention may not contribute significantly to reduction in human exposures. Overall, the intervention literature is sparse and has many limitations. Thus, the committee may have to rely on assumptions and other bodies of evidence to make recommendations to individuals and communities about exposure reduction.

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E-1

Introduction

Per- and polyfluoroalkyl substances (PFAS) are anthropogenic chemicals that have been produced and utilized globally since the 1940s.[1] PFAS have garnered attention for several reasons, including their ubiquitous presence in the environment (Ahrens and Bundschuh, 2014; von der Trenck et al., 2018) and in humans (Calafat et al., 2019; Göckener et al., 2020; Health Canada, 2019; Kannan et al., 2004), and because—as their epithet "forever chemicals" suggests—many of these chemicals are persistent both in the environment and in humans, with half-lives estimated to be several years (Li et al., 2018; Myers et al., 2012). Exposure to PFAS has been linked with such health endpoints as reduced immune response, lipid metabolism, and kidney function; thyroid disease; liver disease; glycemic parameters and diabetes; cancer; and impaired fetal and child development (ATSDR, 2020).

Activities to limit the production and use of exposure to PFAS compounds include regulatory limits, voluntary reductions in manufacture (Butenhoff et al., 2006) and use in products, cleanup of contaminated sites, and modifications to publicly owned treatment works (POTWs) to reduce PFAS in drinking water. However, as evidenced by studies of measurements of PFAS in serum from nationally representative populations in the United States (Calafat et al., 2019), as well as serum measurements in communities near sites with known contamination (Herrick et al., 2017), exposure to PFAS is ongoing. It has been well documented that PFAS are present in numerous media and products, including drinking water; breast milk; other foods and food packaging material; cosmetics; and household products, including carpets, stain- and water-repellent fabrics, nonstick products, polishes, waxes, paints, and cleaning products (D'Hollander et al., 2010; EFSA, 2020; Eichler and Little, 2020; Fromme et al., 2009; Sajid and Ilyas, 2017; Sunderland et al., 2019).[2]

Communities impacted by PFAS exposure would like advice on how they can prevent its potential health effects. To help clinicians respond to patient concerns about PFAS exposure, the Agency for Toxic Substances and Disease Registry (ATSDR) has published *PFAS: An Overview of the Science and Guidance for Clinicians on Per- and Polyfluoroalkyl Substances* (referred to hereafter as the ATSDR PFAS Clinical Guidance) (ATSDR, 2019). This guidance summarizes general information about PFAS and PFAS health studies and suggests answers to example patient questions. Some people living in PFAS-impacted communities have voiced frustration that the clinical guidance lacks clear recommendations to their physicians about what people can do to protect their health, which prompted the ATSDR and the National Institute of Environmental Health Sciences (NIEHS) to request that the National Academies of Sciences, Engineering, and Medicine convene a committee to provide advice for clinicians about PFAS testing, such as when to test, whom to test, how to test, what to test for, and the risks of testing. The committee is also charged with developing principles clinicians can use to advise patients on exposure reduction.[3] The committee commissioned this white paper to determine whether evidence exists that supports the effectiveness of these types of behavior changes. The literature review in this white paper is intended to help the National Academies committee evaluate possible evidence-based recommendations for improving the ATSDR's PFAS Clinical Guidance. This white paper is not intended to be a comprehensive review of human exposure to PFAS; the National Academies have other sources for that information.

Various organizations have provided suggestions for personal actions to lower individual PFAS exposure. These include avoiding contaminated water or fish and selecting personal care products that do

[1] See https://www.epa.gov/pfas/basic-information-pfas (accessed May 12, 2021).

[2] See also https://www.epa.gov/pfas/basic-information-pfas (accessed May 12, 2021).

[3] See the committee's full Statement of Task at https://www.nationalacademies.org/our-work/guidance-on-pfas-testing-and-health-outcomes (accessed May 19, 2021).

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not contain PFAS and related compounds (ATSDR, 2020; EWG, 2016; Loria, 2019; ODH, 2020). Reducing intake of PFAS should reduce exposure, but people may not necessarily know whether their foods, beverages, or products contain PFAS. This review addresses the following question: Based on current research, are there interventions or *personal* changes that individuals can make to *effectively* reduce their PFAS exposure? This question includes two key concepts. First, "personal" indicates that the focus is on research related to media and products that people may be able to control partly or wholly (see Figure E-1) as opposed to activities that occur on a larger scale, such as contaminated site cleanup, changes in occupational exposures, or modifications to publicly owned water treatment plants. Second, "effectively" refers to changes in personal behavior that can result in measurable or substantial reductions in exposures.

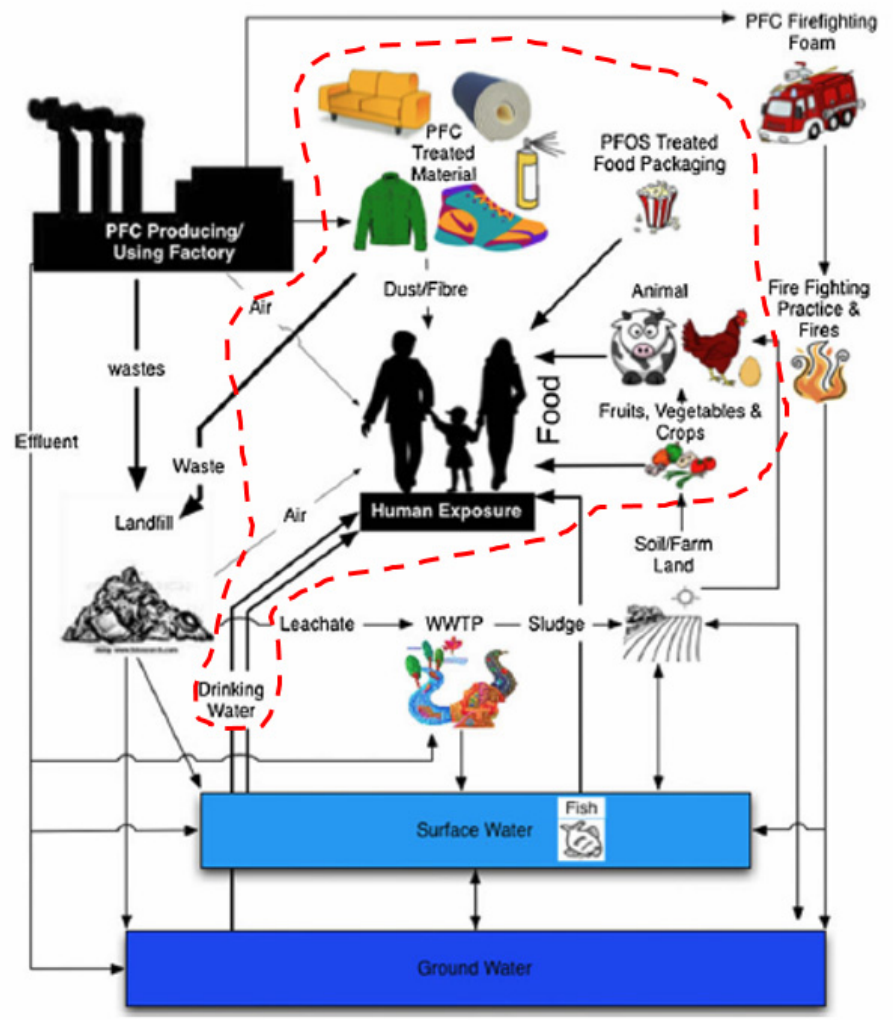


FIGURE E-1 Human PFAS exposure pathways. The area inside the dashed red line denotes pathways for which individual actions may lower PFAS exposures.
NOTE: PFC = per-/polyfluorinated chemical; WWTP = wastewater treatment plant.
SOURCE: Adapted from Oliaei et al., 2013. CC BY 4.0.

To answer the above question, it is important to consider several factors related to reducing human PFAS exposure. First, PFAS must be present in the media or products of interest. While this seems like a straightforward notion, information on PFAS concentrations in local media (e.g., local

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drinking water) and specific products (e.g., cookware) may in fact be sparse or unavailable. Second, there must be a complete exposure pathway between the medium or product and humans. In other words, if PFAS are present in a medium or product but there is no human contact, then exposure will not occur. Third, an exposure pathway may be complete but not contribute substantially to overall human exposure. In this case, a reduction in the PFAS source may not result in a meaningful reduction in human PFAS exposure. Fourth, actions to reduce PFAS exposure from one pathway may result in exposure to PFAS or other chemicals from a new pathway (e.g., an action to remove exposure to one food item with known PFAS levels may result in exposure to another food item that has not yet been analyzed for PFAS or other chemicals). Finally, PFAS tend to have long physiological half-lives; thus, interventions or changes in behavior may not produce near-term changes in internal PFAS levels (i.e., serum levels).

Taking these issues into consideration, this white paper explores the following three questions:

1. *Is there research that links specific interventions or changes to reductions in human exposures?* This paper focuses on studies seeking to establish that an intervention or behavioral change produces a quantifiable reduction in human exposure as evidenced by measurements of PFAS in the media of interest or directly in humans. As noted above, studies relying on biomonitoring to assess the efficacy of an intervention must consider the long half-lives of many PFAS and must be of appropriate duration to enable observation of postintervention decreases in serum levels.
2. *Can information from exposure assessments that estimate human intakes from multiple pathways of exposure and exposure routes be used as the basis for individual or community recommendations?* In this type of study, measurements of PFAS in various media are used to model human PFAS intake (i.e., nanograms [ng] per day or ng/kilogram [kg] per day). If sufficient measurement data are available, it may be possible to estimate the relative importance of various pathways of exposure.
3. *Is the available research sufficiently robust such that recommendations for modifications to behavior can be made?* Studies differ in terms of their quality and generalizability (i.e., how well the results translate from the studied population and conditions to other populations and conditions). They therefore also differ in terms of the confidence one can place in the results. Factors impacting confidence in studies can include sample size, quality assurance, inter- and intrastudy consistency in results, and completeness of reporting. In addition, for recommendations applicable to the United States, it is important to consider whether the studies reflect conditions that apply to behaviors and exposures in this country.

The remainder of this paper first describes our approach to identifying and reviewing the literature. We then provide results by medium. Next, we discuss efforts to model relative contributions of media and products to overall PFAS intakes in the United States.

We note that there are more than 9,000 PFAS compounds (NASEM, 2021), and any given study generally examines only a very small subset of these. We focus here on the 16 PFAS[4] chemicals included in the Centers for Disease Control and Prevention's (CDC's) *National Report on Human Exposure to Environmental Chemicals* (CDC, 2009).

[4] PFBS: perfluorobutane sulfonic acid; PFDA: perfluorodecanoic acid; PFDoA: perfluorododecanoic acid; PFHpA: perfluoroheptanoic acid; PFHxS: perfluorohexane sulfonic acid; PFNA: perfluorononanoic acid; PFOA: perfluorooctanoic acid; n-PFOA: n-perfluorooctanoic acid; Sb-PFOA: branched perfluorooctanoic acid; PFOS: perfluorooctane sulfonic acid; n-PFOS: n-perfluorooctane sulfonic acid; Sm-PFOS: perfluoromethylheptane sulfonic acid; PFOSA or FOSA: perfluorooctane sulfonamide; EtFOSAA: 2-(n-ethyl-perfluorooctane sulfonamido) acetic acid; MeFOSAA: 2-(n-methyl-perfluorooctane sulfonamido) acetic acid; PFUnDA: perfluoroundecanoic acid. The various PFAS abbreviations used in this paper are defined in the listing at the beginning of the paper. For brevity, these abbreviations are not spelled out further in the text of this paper.

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E-2

Methods

This review is a scoping review that aims to (1) "identify the types of available evidence in a given field," (2) "report on the types of evidence that address and inform practice in the field and the way the research has been conducted," (3) "examine how research is conducted on a certain topic," and (4) "identify and analyze gaps in the knowledge base" (Munn et al., 2018).

LITERATURE IDENTIFICATION

Online data sources, including PubMed, EMBASE, and Google Scholar, were used to conduct the initial literature searches. We used such keywords as "(PFBS OR PFDA OR PFDoA OR PFHpA OR PFHxS OR PFNA OR PFOA OR n-PFOA OR sb-PFOA OR PFOS OR n-PFOS OR Sm-PFOS OR PFOSA OR FOSA OR EtFOSAA OR MeFOSAA OR PFUnDA OR PFAS)," "PFAS," "perfluoroalkyl," "human," "exposure," "cooking," "dust," "fish," "shellfish," "water," "nail polish," "cleaning," "consumer products," "filter," "water filter," "filtration," "intervention," "determinant," "reduction," "diet," "vacuum," "popcorn," "biomonitor," "breast milk," "breastfeeding," "infant formula," "milk powder," "carpeting," "packaging," "indoor," "bottled water," "air conditioning," "fabrics," "well water," "water treatment," "apparel," "inhalation," "ventilation," "cosmetics," "dental floss," and "personal care products," as well as various combinations of these and related keywords. We selected articles describing interventions designed to reduce human exposure to PFAS, specifically interventions that could be carried out by individuals (e.g., excluding site cleanups, modifications of publicly owned treatments works [POTWs]). Secondary references of retrieved articles were reviewed to identify publications not identified by the electronic search. Additional literature searches were conducted to identify reviews that contained estimates of human PFAS intakes using the following keywords in different combinations: "(PFBS OR PFDA OR PFDoA OR PFHpA OR PFHxS OR PFNA OR PFOA OR n-PFOA OR sb-PFOA OR PFOS OR n-PFOS OR Sm-PFOS OR PFOSA OR FOSA OR EtFOSAA OR MeFOSAA OR PFUnDA)," "PFAS," "exposure," "review," "biomonitor," "PFOA," and "human." The final search date was March 5, 2021.

The criteria for inclusion in the review were as follows: studies of interventions related to personal modifiable behavior and English-language publications. Exclusion criteria included the following: occupational studies and those interventions requiring professional activities, such as modifications to POTWs. For publications on modeled intake estimates, we were interested primarily in PFAS intakes in the United States. Research focused on clinical interventions is outside the scope of this review (e.g., Ducatman et al., 2021; Genuis et al., 2014).

LITERATURE REVIEW AND DATA EXTRACTION

Each study that met the above inclusion criteria was examined by both authors. The data from each intervention study were tabulated. Information extracted from each study included (where available[5]) the following:

- description of the study population: size, composition, source, and location;
- study design: laboratory and population;
- type of specimen and number of samples;

[5] Because of the disparate nature of the identified studies, it was often possible to include only a portion of the elements.

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- PFAS and concentrations or changes in concentrations; and
- results: percent decrease/increase, concentration decrease/increase, and a measure of precision (e.g., 95% confidence interval [CI], standard deviation [SD]). (For publications with results reported qualitatively, the text was extracted and reproduced verbatim.)

ASSESSMENT OF EVIDENCE

We evaluated the overall strength of evidence for each medium, considering such elements as overall study design, participant selection, sample size, and exposure assessment (LaKind et al., 2014; Vandenbroucke et al., 2007). For studies using biomonitoring data to assess the efficacy of an intervention, we considered whether sampling intervals were designed to capture potential effects of the intervention. To assess the utility of the available evidence for providing recommendations for behavior modifications to reduce PFAS exposures, we examined such factors as (1) the number of available studies for each medium/PFAS chemical/intervention type combination, (2) the quality of the individual studies, (3) the intra- and interstudy consistency in results, and (4) the generalizability of the information to U.S. populations.

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E-3

Results

Studies with the potential to provide information on methods that could be used by individuals for reducing exposure to PFAS were identified. The intervention areas include preparation of fish and other foods, reduction of exposure to PFAS in drinking water either via water filtration at point of entry (POE) or point of use (POU) or via consumption of bottled water, selection of cookware, minimization of indoor dust exposure through modification of indoor products, and use of personal care products or dietary fiber. Literature on breast milk/infant formula and both mother and infant PFAS exposure reduction is discussed. Finally, studies on source contributions to overall PFAS intake in the United States were identified. Each of these bodies of literature is described in the following sections.

FOOD PREPARATION: FISH, SHELLFISH, AND MOLLUSKS

Fish, shellfish, and mollusks have been studied for their potential as a source of PFAS exposure in humans. Nine studies examining the effect of various fish and seafood preparation methods on PFAS levels were identified. The studies included different species, cooking practices, and PFAS. We briefly summarize these studies here.

Alves and colleagues (2017) measured PFOS and PFUnA levels in mackerel and flounder purchased in markets in Spain, Italy, and the Netherlands (25 samples per species/location). PFAS were measured in raw samples and from samples steamed at 105°C for 15 minutes. No significant differences in concentrations were found between the steamed and raw samples. Mean concentrations of PFOS in flounder for raw and steamed samples were 24±1.5 nanograms per gram (ng/g) wet weight (ww) and 22±1.5 ng/g ww, respectively. Mean concentrations of PFUnA in mackerel for raw and steamed samples were 3.1±0.2 ng/g ww and 2.9±0.1 ng/g ww, respectively.

The 16 PFAS compounds identified previously (PFBA, PFPeA, PFHxA, PFHpA, PFOA, PFNA, PFDcA, PFUnA, PFDoA, PFTrA, PFTeA, PFBS, PFHxS, PFHpS, PFOS, and PFDS) were measured in raw and steamed tuna, hake, plaice (n = 25 each; fillets), and mussels (n = 50) purchased in European markets (Barbosa et al., 2018). Steaming was performed at 105°C for 15 minutes (fish) or 5 minutes (mussels). The effects of steaming varied considerably by both species and compound. Selected results shown in Figure E-2 illustrate these differences.

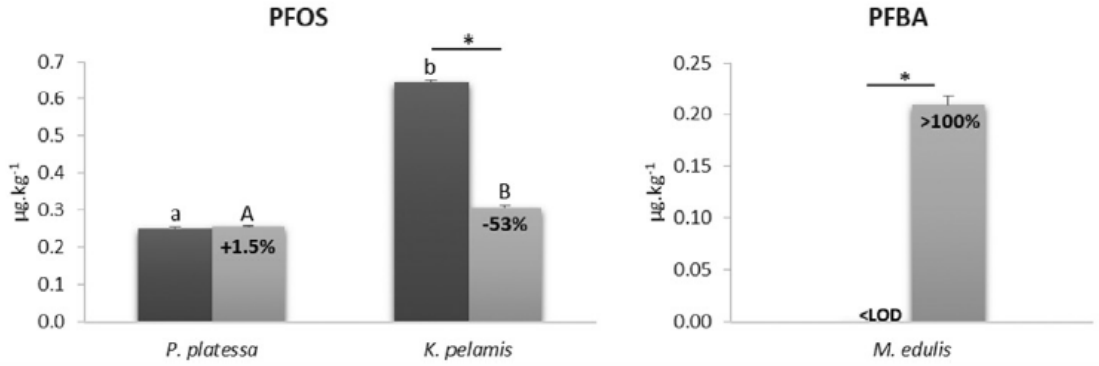


FIGURE E-2 Examples of PFAS content (micrograms per kilogram [μg/kg] wet weight [ww]) in raw and steamed seafood samples and percentages of PFAS content increase (+) and decrease (−) after steaming (mean ± standard deviation [SD]).
NOTE: * = significant differences ($p < 0.05$) between raw and steamed samples.
SOURCE: Partially reprinted from Barbosa et al., 2018.

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Bhavsar and colleagues (2014) measured various PFAS (including perfluoroalkyl carboxylic acids, perfluoroalkane sulfonic acids, perfluoroalkyl phosphonic acids, perfluoroalkyl phosphinic acids, and polyfluoroalkyl phosphoric acid diesters) in fresh-caught Chinook salmon, lake trout, common carp, and walleye obtained in Ontario, Canada (fillets from four or five fish per species). PFAS levels in the cooking oil were below the limit of detection (LOD). Fillet samples were placed on a layer of canola oil and fried, baked, or broiled for 10–15 minutes. PFOS was by far the dominant PFAS in each species; thus, the results were focused on the effects of cooking methods on PFOS fish concentrations. All cooking methods resulted in mostly statistically significant increases in concentrations of PFOS in salmon, trout, and walleye (the increase in walleye after frying did not appear to be statistically significant). For example, PFOS concentrations in raw, baked, broiled, and fried chinook salmon (mean ± standard deviation [SD]) were, respectively, 12.70±12.61 ng/g ww, 16.56±18.00 ng/g ww, 16.45±15.63 ng/g ww, and 16.03±15.19 ng/g ww. For carp, broiling and frying resulted in no significant changes. The authors also assessed the change in amount of PFOS (in contrast with the concentration change) to account for loss in mass due to cooking. With this approach, results were mixed in directionality and mostly nonsignificant.

Del Gobbo and colleagues (2008) analyzed fish and shellfish species purchased in Canadian markets for PFOA, PFNA, PFDA, PFUA, PFDoDA, PFTeDA, and PFOS. Species included catfish, cuttlefish, grey mullet, grouper, monkfish, octopus, red snapper, sea squirt, skate, whiting, and yellow croaker. Composites from each species were comprised of at least three individuals from three different sources or markets (total of nine). The fillets (sometimes with skin) were baked, boiled, or fried in water, sesame oil, rice wine, or vegetable oil. PFAS levels in the cooking oil were below the LOD. Baking and frying times were 15 minutes at 163°C or 325°C. All cooking interventions were found to decrease PFAS concentrations, with boiling and frying reducing total PFAS concentrations by an average of 79 percent and 54 percent, respectively. Baking reduced all measured PFAS to below the LOD (0.03–10 ng/g ww). Boiling appeared to increase concentrations of PFOS in octopus (from nondetect to 0.23 ng/g ww) but not in red snapper, skate, or yellow croaker. The authors hypothesize that large loss of mass in the boiled octopus (87 percent) may have resulted in an increase in levels above the LOD.

Hu and colleagues (2020) measured 13 PFAS compounds (PFBA, PFPeA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnDA, PFDoDA, PFBS, PFHxS, PFOS, and FOSA) in grass carp taken from Tangxun Lake, China (n = 5). The fillets were either steamed, boiled, fried, or grilled, with cooking temperatures for the various processes ranging from 100°C to 210°C. The authors note that in the "cooking blank juice samples all PFAS were below the MLQs [Method Quantitation Limit] except PFBS and PFOS. The concentrations of PFBS and PFOS in cooking blank samples were 1.31–2.43 ng/g and 0.131–0.169 ng/g, respectively" (Hu et al., 2020, p. 4). The effects of the cooking methods on concentrations of various PFAS compounds were found to be inconsistent. For example, median PFOS concentrations increased from 71.3 ng/g ww in uncooked fish to 146 ng/g ww in fried fillets. In contrast, median PFBS concentrations decreased from 20.3 ng/g ww in raw fish to 8.08 ng/g ww after grilling. These results exemplify the difficulty in characterizing the directionality and magnitude of the effectiveness of cooking fish as a proposed type of intervention.

Kim and colleagues (2020) measured 19 PFAS (PFOS, PFDS, PFHxS, PFTeDA, PFTrDA, PFDoA, PFUnDA, PFBS, PFDA, PFNA, PFOA, PFHpA, PFHxA, PFPeA, PFOSA, N-EtPFOSAA, N-MePFOSAA, L-PFHpS, and PFBA) in mackerel bought from a market in Korea (n = 10). Composited fillets from three mackerels each were prepared with various washing, soaking, and cooking (grill, braise, steam, or fry) methods (two composites each). The fish were cooked between 6 and 25 minutes with various ingredients including oil, water, potato, soy sauce, pepper paste, sugar, garlic, and ginger. Preparation methods included such traditional Korean practices as soaking the fillets in sake or rice-washed solutions. These soaking practices reduced PFAS levels by 51 to 80 percent. Washing the mackerel with water resulted in a reduction in PFAS of 74 percent. Similarly, all cooking methods reduced total PFAS content compared with the raw samples: grilling—91 percent, steaming—75 percent, frying—58 percent, and braising—47 percent. While cooking with potatoes further reduced PFAS levels in the mackerel, it also increased the levels in the potatoes.

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Luo and colleagues (2019) assessed the effect of pretreatments and cooking on levels of 19 PFAS (PFOA, PFOS, PFBA, PFPeA, PFHxA, PFHpA, PFNA, PFDA, PFUnDA, PFDoDA, PFTrDA, PFTeDA, PFBS, PFHxS, L-PFHpS, PFDS, PFOSA, N-MePFOSAA, and N-EtPFOSAA) in fish cakes (n = 4 brands) and swimming crabs (n = 100) purchased in markets in Korea. The effects of a wide range of cooking methods were evaluated. Additional foods used in the cooking processes included soybean oil, two types of soy sauce, and Korean radish. Blanching, commonly used as a pretreatment before cooking fish cakes, did not yield significant changes in PFAS levels. Significant reductions in total PFAS were observed after boiling, frying, and stir-frying fish cakes (total PFAS in control, boiled, fried, and stir-fried fish cakes, respectively, were as follows: 2.96 ± 0.6 ng/g, 1.60 ± 0.16 ng/g, 1.93 ± 0.19 ng/g, and 1.94 ± 0.07 ng/g). For the crabs, presoaking reduced PFAS levels. PFAS in the crabs were significantly decreased after steaming and stewing.

Taylor and colleagues (2019) collected school prawn, blue swimmer crab, and dusky flathead from contaminated or reference estuaries in New South Wales and analyzed them for 20 PFAS compounds (PFAS above the limit of quantitation [LOQ] were PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnDA, PFDoDA, PFBS, PFHxS, PFOS, PFDS, FOSA, and NEtFOSE) before and after cooking. Dusky flathead fillets were baked or pan-fried in olive oil, while the crab and prawn were boiled in salted water. PFAS were below LODs in the cooking water and oil. Five or six replicates were used for each species and each cooking treatment (for prawns, each replicate was comprised of a composite of 10 individuals). The effect on PFAS concentrations varied with species, cooking method, and chemical. For instance, PFOS concentrations showed no change in crab following boiling, whereas PFHxS and PFOA concentrations were reduced. However, PFOS, PFHxS, and PFOA concentrations increased in prawns after cooking. For the dusky flathead, PFOS levels did not change significantly after frying, but baking resulted in a small but significant increase. The authors conclude that "cooking does not consistently reduce PFAS concentrations, and cannot mitigate dietary exposure" (Taylor et al., 2019, p. 280).

Vassiliadou and colleagues (2015) obtained several species of fish (anchovy, bogue, hake, picarel, sardine, sand smelt, and striped mullet) and shellfish (Mediterranean mussel, shrimp, and squid) from local markets in Greece and mussels from a mariculture farm. Twelve PFAS compounds (PFBA, PFPeA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnDA, PFDoA, PFBS, PFHxS, and PFOS) were measured in raw or washed samples that were then fried (in virgin olive oil at 170°C) or grilled (at 180°C). Total PFAS concentrations were found to be mostly higher after grilling and after frying, but changes in individual PFAS were inconsistent.

In general, there appear to be numerous factors related to preparation of fish, shellfish, and mollusks that can impact changes (increases or decreases) in concentrations of PFAS. Taylor and colleagues (2019) observe that whether cooking reduces PFAS concentrations in fish depends on the physicochemical properties of the chemical, the cooking method used, and the species. They note several processes that could impact changes in PFAS levels in fish from food preparation: losses to the cooking medium (e.g., cooking oil), moisture loss during cooking, PFAS precursors in fish tissues transforming during cooking to PFAAs, protein loss, or protein increase. Additional factors possibly affecting changes in concentration are the size, shape, and thickness of fish fillets (Hu et al., 2020).

Overall, robust recommendations for fish preparation interventions would ideally be based on consistent intra- and interstudy results; use of fish species and preparation methods common to the United States; and well-powered, replicated studies. The results from the studies reviewed here indicate that the effects of preparation of fish and shellfish on PFAS levels are inconsistent (examples are shown in Figure E-3). It is therefore difficult to use this information to inform recommendations regarding the efficacy of fish and shellfish preparation for reducing PFAS intake.

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FIGURE E-3 Examples of inconsistent changes in selected PFAS concentrations after fish and shellfish preparation (arrows indicate direction of concentration change).
SOURCES: Blue swimmer crab information is from Taylor et al., 2019. Carp information is from Hu et al., 2020. Crab photo: Judy S. LaKind, CC BY SA 3.0, https://commons.wikimedia.org/w/index.php?curid=4963391. Carp photo: Dezidor—Self-photographed, CC BY 3.0, https://commons.wikimedia.org/w/index.php?curid=12661115.

Furthermore, some of the fish species and preparation methods included in this body of research are not common to the United States, limiting the generalizability of results for U.S. communities. Additionally, because very few of these studies used the same cooking methods and species, it is difficult to corroborate even the instances in which a particular intervention appears to have been effective (within this small group of studies, various cooking preparation approaches included soaking followed by stewing, steaming, boiling, frying, or stir-frying; grilling, steaming, baking, boiling, or frying without presoaking; and baking in rice wine or vegetable oil). Furthermore, several of the studies included very small sample sizes. Finally, while some studies considered the effect of moisture or mass variations on PFAS concentration changes, others reported only concentration data. A more relevant metric in terms of human exposure would be the mass of PFAS remaining in the samples after preparation.

FOOD PREPARATION: OTHER

Two studies were identified that examined the effect of preparation on PFAS levels in foods other than fish (Binnington et al., 2017; Jogsten et al., 2009). Jogsten and colleagues (2009) measured several PFAS in various uncooked and cooked foods (see Table E-1).[6] Specifically, composite samples (n = 2) of beef, pork, or chicken were cooked in an oil mixture using nonstick cookware. Only PFHxS, PFOS, PFHxA, and PFOA were detected in at least one of two composite samples. PFOS levels increased in grilled pork, grilled chicken, and fried chicken compared with the raw samples. In contrast, levels did not increase in cooked veal or fried pork. The results from this study are not directly relevant for intervention recommendations. First, foods were purchased outside of the United States, and it is not known whether PFAS in these foods are similar to those in foods found in the United States. Second, only two samples per food type were included; there was no information on brands and limited information on cooking procedures; and results were inconsistent regarding the efficacy of cooking in reducing PFAS concentrations. Finally, it is not clear whether the effects of cooking can be disentangled from those of the Teflon-coated cookware used in this study.

[6] Jogsten and colleagues (2009) also measured PFAS in foods wrapped in different types of packaging. The foods were purchased in Spain, and the brands were not identified, so the relevance to the U.S. population is unclear. Egeghy and Lorber (2011) note that while fluorochemical-treated food packaging can be a source of PFAS in food, it appears that PFAS levels in packaging such as fast-food wrappers have decreased over time.

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Binnington and colleagues (2017) studied the effects of preparation of beluga whale blubber on nutrients and environmental chemicals, including PFAS. They collected samples from two male whales (aged 24 and 37 years) from the Northwest Territories and prepared them using traditional approaches. Measured PFAS (detected in ≥50 percent of the samples) were PFNA, PFDA, PFUnDA, PFDoDA, and PFOS. PFAS were measured in raw and prepared (boiled, roasted, and aged) samples. Roasting increased concentrations of some of the PFAS compared with certain other treatments (e.g., air-drying, hang-drying, and boil pot), but were reduced in oil (Table E-1 includes concentration results for PFOS). According to the authors, issues with sample preparation may prevent these results from being considered representative of the overall mixture.

TABLE E-1 Summary of Results of Studies Examining the Effect of Food Preparation on PFAS Levels

Source	PFAS	Study Location	Food Type	Number	Concentration
Jogsten et al., 2009	PFBuS PFHxS PFOS PFHxA PFHpA PFOA PFNA PFDA PFUnDA PFDoDA	Spain	Beef, pork, chicken	Two composite samples from at least six subsamples for each food type from each of two sampling locations	PFOS, ng/g fresh weight (standard deviation) *Veal* raw: <0.015 grilled: <0.008 fried: <0.018 *Pork* raw: <0.008 grilled: 0.011 (0.009) fried: <0.008 *Chicken* raw: <0.008 grilled: 0.012 (0.01) fried: 0.010 (0.007)
Binnington et al., 2017	PFNA PFDA PFUnDA PFDoDA PFOS	Canadian Arctic	Male beluga whale blubber	Blubber sample divided into portions for different preparation processes	nanograms per gram wet weight (ng/g ww)[a] PFOS: nondetect (ND) to <1 PFNA: ND to <4 PFDA: ND to <4 PFUnDA: ND to <4 PFDoDA: ND to <4

[a] Estimated from publication figures.

In summary, only two studies of changes in PFAS levels associated with cooking foods were identified. These studies included small sample sizes and yielded inconsistent results.

LOCAL FOOD CONSUMPTION ADVISORIES

State advisories for guidance on consumption of locally grown foods could be a source of information to inform exposure reduction decisions. Eleven states have advisory guidelines for consumption of fish, wildlife, and other foods (California [seafood], Connecticut, Hawaii [in process], Maine [fish, beef, and milk], Michigan [fish and deer], Minnesota, New Hampshire, New Jersey, New York, Washington [in process], Wisconsin [fish and deer]) to protect human health from exposure to PFAS.[7] These advisories offer guidance on limiting the quantity of these foods consumed. Depending on the state-specific PFAS and concentrations, different consumption levels are indicated, ranging from do not eat (e.g., fish or deer in Michigan with PFOS concentrations of more than 300 parts per billion [ppb])

[7] See https://www.ecos.org/wp-content/uploads/2021/04/Updated-Standards-White-Paper-April-2021.pdf (accessed June 30, 2022).

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to unlimited consumption (e.g., fish in New Jersey with 0.56 ng/g PFOS). While fish consumption has a role in a healthy diet (Mozaffarian and Rimm, 2006), weighing the risks of PFAS exposure from fish consumption against the benefits of fish consumption is a complex process, and no intervention studies were identified that evaluated the impact of reduced consumption of fish on PFAS levels in blood and urine.

DRINKING WATER

Ingestion of drinking water is thought to be a major pathway for PFAS exposure (Domingo and Nadal, 2019). Research on two types of drinking water interventions is described in this section. The first addresses whether—and the extent to which—the use of water filters at POE into the home, under the sink (POU), or in water pitchers reduces PFAS exposure. The second addresses whether the use of purchased bottled water results in lower PFAS exposure compared with the use of tap water. Because PFAS levels in water can vary widely, we focus on studies that measured PFAS in tap and bottled water obtained from the same geographic area.

Six publications and one agency report evaluating possible drinking water interventions were identified. Four (Ao et al., 2019; Iwabuchi and Sato, 2021; MDH, 2008; Patterson et al., 2019) evaluated use of POE, POU, and water pitcher filtration devices; and three (Ao et al., 2019; Gellrich et al., 2013; Heo et al., 2014) evaluated differences in PFAS concentrations between tap water and bottled water. These studies are summarized here. Also discussed is one association paper assessing the relationship between drinking water source and serum levels in a highly contaminated area (Emmett et al., 2006).

POE, POU, and Water Pitcher Filtration

Ao and colleagues (2019) (also discussed in the next section) measured six PFAS compounds (PFOA, PFOS, PFNA, PFBS, PFHpA, and PFHxS) in tap (n = 9), filtered (n = 9), and bottled (n = 9) water in Shanghai, China. Paired tap and filtered water samples were collected from each of nine homes served by three different water sources. The filtered water samples were collected from the effluent of the home's water purification device. No further information on sampling or quality control in the field was given, nor was information on the type or brand of filter provided. ΣPFAS median concentrations in tap water and filtered water were 4.44 nanograms per Liter (ng/L) and 3.13 ng/L, respectively, but the differences were not statistically significant.

Herkert and colleagues (2020) tested municipal, well, and filtered (n = 89) and unfiltered (n = 87) tap water in residences (n = 73) in North Carolina for 11 PFAS compounds (GenX, PFBS, PFBA, PFHxS, PFOS, PFPA, PFHxA, PFHpA, PFOA, PFNA, and PFDA). The 89 POE and POU filters tested varied in both type (e.g., pitcher, under sink, faucet, whole house) and filtration method (reverse osmosis [RO], granular activated carbon [GAC], single-stage, two-stage). Notably, RO filters and dual-stage filters were found to consistently remove most measured compounds (except PFNA and GenX) at an average of ≥90 percent efficiency. On the other hand, GAC filters had more variable performance and were far less effective in removing short-chain PFAS compounds, with an average removal efficiency of just 41 percent for those chemicals. Whole-house activated carbon POE systems resulted in increased levels of PFAS in half of the tests (n = 4). The authors did not observe any correlations between removal efficiency and brand, source water, loading, or filter age.

Iwabuchi and Sato (2021) tested pitcher-type water filters for their ability to reduce concentrations of six PFAS compounds (PFOA, PFOS, PFHxA, PFDA, PFDoA, and PFHxS). Four different models from four manufacturers were evaluated—two with a carbon, ceramic, and hollow fiber membrane design; and two with an activated carbon (AC) and ion exchange design (brand names not given). One liter of the test water was applied to the water filters 200 times, with filtrate analyzed after 10 L, 100 L, and 200 L had been passed through the filters. For each model, filtration effectiveness decreased with prolonged use, but three of the four models were effective in removing the majority of all PFAS compounds. Removal efficiency did not appear to be related to filter material type. Removal

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efficiency varied by carbon-chain length (i.e., more efficient removal was observed for longer-carbon-chain PFAS) and the PFAS functional group (PFOS >PFOA, and PFHxS >PFHxA). All tests were performed with initial PFAS concentrations of 50 ng/L. It is possible that filter effectiveness may vary with initial concentration levels. Iwabuchi and Sato (2021) conclude that household water purifiers are effective at reducing PFAS levels in drinking water.

Patterson and colleagues (2019) tested five commercially available POU/POE water treatment systems for six PFAS (PFOS, PFOA, PFHpA, PFHxS, PFBS, and PFNA). These included three RO systems (iSpring RCS5T, HydroLogic Evolution RO1000, and Flexeon LP-700) and two GAC systems (Calgon Filtrasorb 600 AR+ and Evoqua 1230CX). The filters were tested at various flow rates. The authors found that both RO and GAC systems had the potential to remove PFAS to below the LOD under their experimental water quality and operational conditions. They note, though, that performance was variable and that the long-term performance of the systems was not tested.

In addition to the publications described above, the Minnesota Department of Health (MDH, 2008) conducted a survey of POU water filtration devices for PFAS. Fourteen filters were lab-tested, and 11 of these that passed initial testing were field-tested using water from municipal wells. The brands were AC (n = 8)—Aquion Rainsoft Hydrefiner P-12 9878, Kinetico MACguard 7500, and Sears Kenmore Elite 625.385010; and RO (n = 6)—GE Smartwater GXRM10GBL and Watts Premier WP-4V. The four AC devices removed PFAS compounds to below the analytical reporting level (0.2 micrograms [µg]/L). The RO devices also removed PFAS to below the reporting limit. In terms of use by consumers, MDH notes, AC filters, RO membranes, and other filter elements have a limited service life and must be periodically replaced. Manufacturer recommendations vary, but many suggest replacing filters after 500 gallons of treatment or every 6 months (MDH, 2008).

MDH also tested a small, inexpensive, faucet-mounted carbon filter (PUR models FM-2000B, FM-3333B) using chlorinated and unchlorinated water with levels of PFAS that exceeded the U.S. Environmental Protection Agency (EPA) Lifetime Health Advisory Levels. The filter removed all PFAS from the unchlorinated water to below the LODs (which ranged from 5 to 10 ng/L). Some breakthrough of PFBA occurred for the chlorinated water test, but even at the filter capacity recommended by the manufacturer (100 gal), the filter was still removing 73 percent of the PFBA.[8]

In the course of their work sampling private well water in Washington County, Minnesota, MDH staff collected incidental samples of water treated by homeowner-installed carbon and RO systems. While many of these systems performed quite well, others achieved only partial removal of PFAS or none at all. MDH did not have the capacity to investigate further regarding the reasons for poor performance, but suspected that inadequate system maintenance may have been the main cause. When conveying the results of such tests, whether the system was performing well or not, MDH cautions homeowners that no guarantee can be made regarding long-term effectiveness of the system and recommends that they work with a qualified water treatment company to ensure proper maintenance of their system (Virginia Yingling, personal communication, July 14, 2021).

For those homes that exceed the state's drinking water guidance values, the Minnesota Pollution Control Agency installs and maintains whole-house GAC filter systems consisting of two 90 lb carbon canisters in series. As there are currently more than 1,000 of these systems in place, the state does not manage them individually, but changes the carbon out annually (which is more frequent than needed for the levels of PFAS present in the groundwater). Testing shows that the state's GAC systems remove all PFAS below reporting limits (4.4 ng/L). However, some trace levels, especially of PFBA, have been detected above the method detection limits (which range from 0.5 to 1.0 ng/L). In a few instances, the filter systems were found not to be working, but these cases were related to the homeowners having accidentally switched the bypass valve or altered the plumbing in a way that bypassed the filter. As a result, the state's contractor now inspects every system and plumbing at the annual filter changeout (Virginia Yingling, personal communication, July 14, 2021).

[8] The information in this and the subsequent two paragraphs was provided by Virginia Yingling, Environmental Health Division, Minnesota Department of Health, Saint Paul, Minnesota.

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A study of residents living in an area served by the Little Hocking water system in Ohio—with water PFOA levels in the low ppb range at the time of the study—examined whether a community relying on highly contaminated public water could significantly reduce exposure through the use of a carbon water filter (Emmett et al., 2006). Serum PFOA was measured in a random sample of study participants (n = 324), who also provided information on their drinking water habits. Those who used only water from the Little Hocking water system in their homes were categorized as using a home carbon water filtration system (n = 64) versus no home water filtration system, a system not known to remove PFOA, or a system of unknown type (n = 209). Participants with home carbon water filters were shown to have statistically significantly (p = 0.008) lower median serum PFOA levels compared with those who did not (318 nanograms per milliliter [ng/ml] versus 421 ng/ml, respectively). The difference in serum PFOA levels in these two groups of participants was not as large as that seen for individuals using bottled, spring, or cistern water (see the next section for more information). The authors ascribe this finding to the limited effectiveness of water filters, as well as reliability issues associated with filter maintenance. They do not recommend use of home filtration systems that were available at the time.

All but one of the studies reviewed here found that various filtration methods showed evidence of their potential effectiveness. These studies suggest that pitcher-type, POE, and POU filtration systems can reduce PFAS levels in drinking water under the conditions tested. It is worth noting that optimal filtration depends on the user's actively maintaining these devices, and no study has yet looked at the effectiveness of these interventions in real-world circumstances.[9]

Bottled Water (Versus Tap Water)

Here we summarize three studies that included measurements of PFAS in bottled, tap, or filtered water from the same area in order to compare levels across drinking water sources. This research was conducted in China, Germany, and Korea.

Ao and colleagues (2019) measured six PFAS compounds (PFOA, PFOS, PFNA, PFBS, PFHpA, and PFHxS) in tap (n = 9), filtered (n = 9), and bottled (n = 9) water in Shanghai, China. Tap and filtered water samples were collected from each of nine families served by three different water sources. Paired tap and filtered water samples were taken from each home. The filtered water samples were collected from the effluent of the home's water purification device. Bottled water was purchased from local markets and represented nine best-selling brands. No further information on sampling or quality control in the field was given. ΣPFAS median concentrations in tap water, filtered water, and bottled water were 4.44 ng/L, 3.13 ng/L, and 2.36 ng/L, respectively; the differences were not statistically significant.

Mineral water (n = 119), tap water (n = 26), and spring water (n = 18) samples were measured for 10 or 19 (tap water only) PFAS compounds (PFBA, PFBS, PFPeA, PFHxA, PFHxS, HPFHpA, PFHpA, PFOA, H4PFOS, PFOS, FOSA, PFNA, H2PFDA, PFDA, PFDS, H4PFUnDA, PFUnDA, PFDoDA, and PFTeDA) (Gellrich et al., 2013). The mineral water samples were from Germany; spring water samples from Switzerland, the Czech Republic, and Germany; and tap water samples from homes in unidentified locations. No further information on water sampling was given. The highest ΣPFAS concentration was in tap water (42.7 ng/L). The proportions of individual PFAS differed across water type. For example, PFOS was below the LOD in all of the spring water samples but was detected in 9 percent of all of the mineral water samples. The authors note that the PFAS concentrations in the three water types were similar and described the concentrations as "low." For example, the median PFOA levels in mineral, spring, and tap water samples were 1.6 ng/L, 1.4 ng/L, and 2.6 ng/L, respectively.

Heo and colleagues (2014) measured 16 PFAS compounds (PFBA, PFPeA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnDA, PFDoDA, PFTrDA, PFTeDA, PFBS, PFHxS, PFHpS, PFOS, and

[9] Information on certified water filters can be found at the following website: https://www.nsf.org/knowledge-library/perfluorooctanoic-acid-and-perfluorooctanesulfonic-acid-in-drinking-water (accessed May 12, 2021). Note that the certification applies only to PFOA and PFOS, and the water filter must be able to reduce these chemicals to under 70 parts per trillion (ppt).

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PFDS) in tap (n = 34) and bottled (n = 8) water from Busan, Korea. The bottled water samples were purchased in markets, and the tap water samples were collected from 16 districts in Busan (no further information on types of bottled water or sampling methods for tap water was provided). PFAS concentrations and detection frequencies were higher in the tap water than in the bottled water samples. For example, mean ΣPFAS levels in bottled and tap water were 0.48 ng/L and 41.3 ng/L, respectively. The authors do not provide information on whether the observed differences were statistically significant.

A study of residents living in an area served by the Little Hocking water system in Ohio suggests that a community relying on highly contaminated public water could significantly reduce exposure through the use of bottled water (Emmett et al., 2006). Serum PFOA was measured in a random sample of study participants (n = 324), who also provided information on their drinking water habits. Residents who reported drinking primarily bottled/spring/cistern water had a median serum PFOA level of 71 ng/mL, compared with a statistically significantly higher level of 374 ng/mL for those who drank only Little Hocking system water. Overall, the authors observed a strong relationship between serum PFOA levels and PFOA concentrations in the drinking water source.

For communities with highly contaminated water supplies, the use of alternative drinking water sources has been shown to be associated with significantly reduced exposures. However, none of the intervention studies reviewed here provide robust evidence for the effectiveness of replacing tap water with bottled water for U.S. locations with background levels of PFAS. The studies reviewed here were conducted in Europe and Asia, and the water PFAS concentrations there may not be generalizable to the United States. Lack of brand information and small sample sizes present additional challenges for evaluating this intervention.

Use of bottled water as a replacement for tap water can be expensive and inconvenient. To be confident that the use of bottled water will result in a reduction in PFAS exposure, an understanding of local water conditions in comparison with PFAS levels in specific types of bottled water is needed. As described above, while PFAS levels in bottled water tend to be approximately between <LOD to <100 ng/L, it cannot be assumed that levels in bottled water are always lower than those in tap water. Outside of highly contaminated areas, the degree of spatial granularity for concentration data required to ensure that replacing tap water with bottled water will reduce PFAS exposure is not well understood. However, based on public data POTWs (EPA, 2017), PFAS levels in treated water can vary widely within regions or states (e.g., PFOS water concentrations in Delaware ranged from <MRL [Minimum Reporting Level] of 0.04 to 1.8 µg/L, while PFOA water concentrations in Pennsylvania ranged from <MRL of 0.02 to 0.349 µg/L).

The EPA's Third Unregulated Contaminant Monitoring Rule (UCMR) (EPA, 2017) includes monitoring data for PFOS, PFOA, PFNA, PFHxS, PFHpA, and PFBS (data from 2013 to 2015 from a representative sample of public water systems serving ≤10,000 people). Figure E-4 shows varying concentrations of these six PFAS across the United States for PFAS levels above the method reporting limits. Levels in drinking water can vary across the United States by at least an order of magnitude. The concentrations are in the low ng/L range, similar to reported levels in bottled water. Also note that for much of the United States, PFAS levels in drinking water are below the method reporting limit (see Figure E-5, green symbols) and so would possibly be similar to those in bottled water.

BREAST MILK AND INFANT FORMULA

In considering the idea of "intervention" as it pertains to breast milk, there are two underlying concepts. The first is whether there are interventions that could reduce exposure to the breastfeeding infant, and the second is whether lactating can be an effective method for reducing the mother's levels of PFAS compounds. We discuss each of these concepts in this section.

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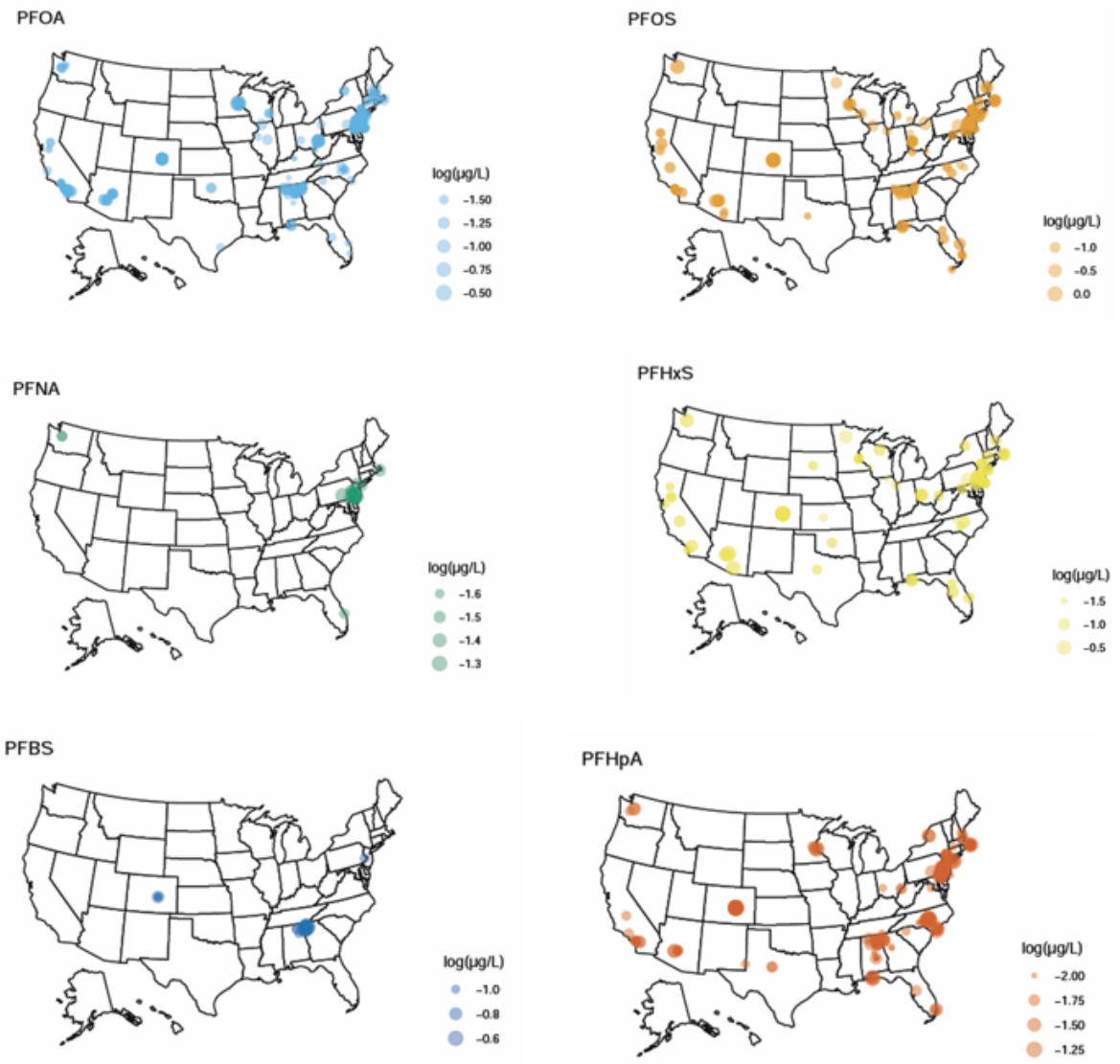


FIGURE E-4 Variation in PFAS levels above the method reporting limit in drinking water in the United States.
NOTES: The relative sizes of the symbols correspond to the actual measured water concentrations. The legends show exemplar concentrations. Plotted: $\log_{10}$(concentration), excluding data below minimum reporting levels. µg/L = microgram per Liter.
SOURCE: Data were extracted from the U.S. Environmental Protection Agency's (EPA's) Third Unregulated Contaminant Monitoring Rule (UCMR 3) (2013–2015) database (EPA, 2017). Individual concentration data for each point on the graphics can be found at http://lakindassociates.com/interactive-map (accessed June 30, 2022).

The Infant's Exposure: Does Formula Feeding in Place of Breastfeeding Reduce Exposure?

Many mothers choose to breastfeed rather than use infant formula. Breastfeeding confers various health advantages to the infant and mother (AAP, 2012; WHO, 2020). At the same time, breast milk includes environmental chemicals (LaKind et al., 2001, 2018; Lehmann et al., 2018) that derive from the mother's body and are transferred to the infant via breastfeeding. Thus, concern has been expressed about the possible health effects on the infant from those environmental chemical exposures. This concern raises the question of whether formula feeding would serve as a method for "intervening," or reducing infant exposure to PFAS.

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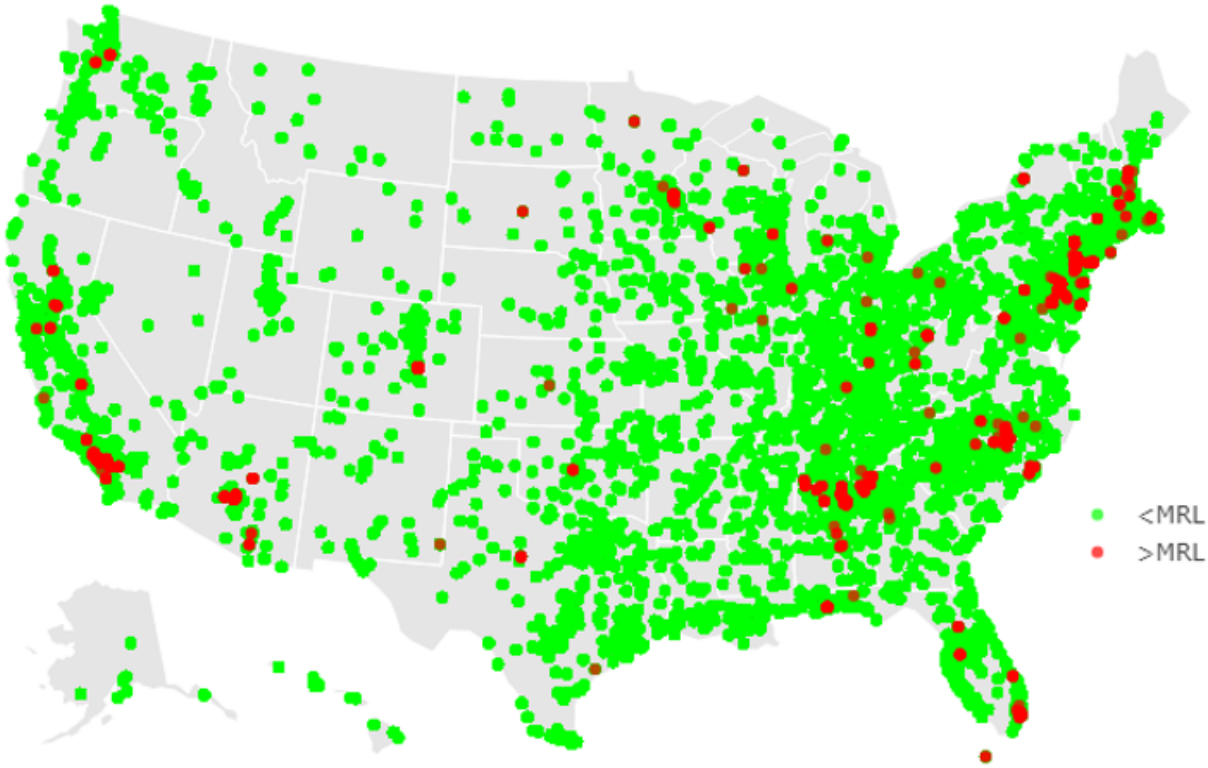


FIGURE E-5 PFAS levels above (red) and below (green) the method reporting limit in drinking water in the United States.
NOTES: The symbols represent a total of 215,963 measurements from the U.S. Environmental Protection Agency's (EPA's) Third Unregulated Contaminant Monitoring Rule (UCMR 3) for PFOA, PFOS, PFHxS, PFBS, PFHpA, and PFNA. MRL = Minimum Reporting Level.
SOURCE: Data were extracted from the EPA's UCMR 3 (2013–2015) database (EPA, 2017).

At least two factors need to be taken into consideration. The first is that infants' exposures begin in utero. Some studies suggest that despite environmental chemical exposures associated with breastfeeding, infants who are breastfed may do better in a number of health-related aspects compared with formula-fed infants (LaKind et al., 2008). The World Health Organization has stated, "in studies of infants, breastfeeding was associated with beneficial effects, in spite of the contaminants present. The subtle effects noted in the studies were found to be associated with transplacental, rather than lactational, exposure" (WHO, 2000, p. 237). These conclusions are drawn from research on persistent chemicals, such as dioxins; to our knowledge, there is no PFAS-specific literature on health effects of breastfed versus formula-fed infants. At present, there does not appear to be sufficient PFAS-related research that would allow for a definitive conclusion regarding infant health and the choice between breastfeeding and use of infant formula. Thus, it is not clear that formula feeding is a scientifically supported "intervention" that would prevent adverse health outcomes.

The second factor is that in choosing formula feeding to reduce infant exposure to PFAS, the assumption is being made that infant formulas do not themselves contain PFAS compounds. While studies have reported on detectable levels of PFAS in infant formula in other countries (e.g., Llorca et al., 2010; Macheka et al., 2021), it is instructive to review available data on levels of PFAS in breast milk versus infant formula in the United States to assess whether levels in formula are lower than those found in breast milk. As an additional complication, it is not uncommon to purchase powdered formula and reconstitute it with drinking water. Therefore, we provide here a synopsis of U.S. levels of PFAS in breast milk, formula, and drinking water.

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PFAS in Breast Milk in the United States

Reviews of PFAS in breast milk (Lehmann et al., 2018; Liu et al., 2020) identify three studies reporting measurements of PFAS levels in breast milk in the United States. One of these studies (Kuklenyik et al., 2004) is an analytical methods study. No information on either the milk donors or the sampling procedures is reported; the information from this study is not relevant to this discussion. In a second study, von Ehrenstein and colleagues (2009) collected milk samples from 34 breastfeeding women in North Carolina at 2–7 weeks and 3–4 months postpartum. Nine PFAS were measured (PFOS, PFOA, PFNA, PFHxS, PFOSA, MeFOSAA, EtFOSAA, PFBS, and PFDA). Measurements below the LOD were assigned a value of LOD/sqrt2. PFAS levels were below the LOQ in most of the 34 milk samples collected at both sample times (note that the LOQs for PFAS in milk ranged from 0.15 to 0.60 ng/ml, or ppb). Specifically, PFAS were detected in samples from only 4 of the 34 women, and of these only three PFAS were above the LOQ: Et-PFOSA-AcOH (1.0 ng/ml) and Me-PFOSA-AcOH (0.7 ng/ml) in one woman, and PFOSA in three women (0.3 ng/ml, 0.5 ng/ml, and 0.6 ng/ml). The remainder of the milk samples from both collections were found to have concentrations <LOQ.

In contrast, Tao and colleagues (2008a) measured PFAS (PFOS, PFOA, PFHxS, PFNA, PFHpA, PFDA, PFUnDA, and PFDoDA) in milk samples collected from 45 primiparous and multiparous women in Massachusetts. Levels that were below the detection limit were assigned a value of zero, while those detected but below the LOQ were assigned a value of one-half the LOQ. Mean levels of PFOS and PFOA were 131±103 parts per trillion (ppt) (mean±SD) and 43.8±33.1 ppt, respectively. Mean PFHxS and PFNA levels were 14.5±13.7 ppt and 7.26±4.70 ppt, respectively. The remainder of the PFAS were detected in only ≤4 samples (<24 ppt).

PFAS in Infant Formula in the United States

In a 2018 review of environmental chemicals in breast milk and infant formulas (Lehmann et al., 2018), only one publication is identified with measurements of PFAS in infant formula in the United States (Tao et al., 2008b). Tao and colleagues (2008b) measured PFAS in 21 formula samples purchased in Washington, DC, and Boston, Massachusetts. The brands represented >99 percent of the U.S. market. Most of the samples were organic or nonorganic milk- or soy-based powders and ready-to-use or concentrated liquids. PFOS was detected in one sample (11.3 ppt; LOQ = 11.0 ppt). PFHxS was detected in two samples (1.36 ppt and 3.59 ppt; LOQ = 1.35 ppt). No other PFAS (PFOA, PFNA, PFBS, PFHpA, PFDA, PFUnDA, and PFDoDA) were detected in any samples.

PFAS in Drinking Water in the United States

An assessment of infant exposure to environmental chemicals would not be complete without considering exposures via drinking water used to reconstitute infant formula (LaKind et al., 2005). ATSDR (2019) has noted that a source of PFAS exposure to infants and toddlers is "formula mixed with PFAS contaminated water." It is important to be able to provide information on what is meant by "contaminated," as PFAS levels in tap water vary widely. For example, Andrews and Naidenko (2020), using national and state databases, estimated that 18−80 million people in the United States use tap water containing at least 10 ng/L (ppt) PFOA and PFOS combined, and more than 200 million people may have water with a combined PFOA and PFOS level of at least 1 ng/L.

Formula may also be reconstituted with bottled water. There is a paucity of data on PFAS levels in bottled water in the United States. Akhbarizadeh and colleagues (2020) reviewed the international literature on PFAS levels in bottled water (with none from the United States) and report levels of various PFAS in the low ng/L range, with some levels as high as the low 100s ng/L depending on the type and number of PFAS included in the reporting. They note that researchers have attributed the PFAS in bottled water to several possible sources, including PFAS from the plastic bottles themselves; introduction of PFAS to the water before bottling or during bottle closure; or contamination of contact materials during

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bottling, handling, and storage of the bottles. In a study of tap, spring, and mineral water from Germany, Gellrich and colleagues (2013) found PFAS levels to be generally in the low ng/L range.

Looking beyond the peer-reviewed literature, *Consumer Reports* (Felton, 2020) conducted a study on PFAS levels in bottled water purchased in stores in the United States and from online retailers. The noncarbonated water levels in 31 brands were less than 1 ppt, and two other brands had levels of 1.21 ppt and 4.64 ppt. These results derive from the averages of two to four samples of each product, but it is not clear whether these were replicates from the same bottle or different bottles. The report does not include information on detection limits or specific PFAS compounds detected, but merely describes "total PFAS as the sum of average concentrations of all PFAS detected in the samples tested of a product."

The U.S. Food and Drug Administration (FDA) measured PFOA and PFOS in carbonated and noncarbonated bottled water (n = 30; brands not identified) and found that levels were below the lower LOQ in all samples (0.004 µg/L).[10]

Studies have also detected PFAS in bottled water from other countries (see, e.g., Le Coadou et al., 2017). As brand names are not provided by these authors, it is not clear how this information could be used for intervention recommendations for the United States.

Based on these very limited data, it is possible that ready-to-use formulas may have lower PFAS levels than formulas reconstituted with tap or bottled water (see Figure E-6). However, the following caveats must be noted: data comparisons across studies are complicated by differences in total PFAS included; measurements may include more legacy PFAS compounds and not include PFAS compounds used to replace the older chemistries; studies use varying detection limits and approaches for assigning values to measurements below the LOD; and sampling in these studies is not representative of regions within the United States.

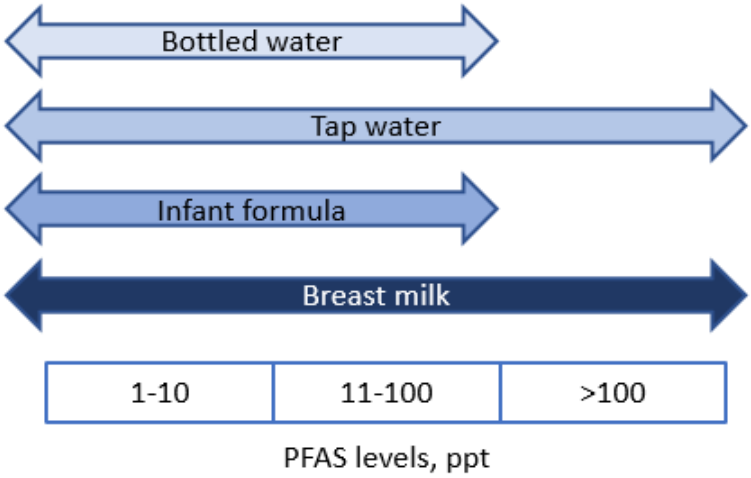


FIGURE E-6 Limited data on PFAS levels in breast milk and infant formula in the United States show general overlapping concentrations, which also overlap with PFAS concentrations in drinking water that could be used to reconstitute formula.
NOTES: Double-headed arrows indicate that these bars could extend in either direction as new data are obtained. ppt = parts per trillion.
SOURCE: Author generated.

[10] See https://www.fda.gov/media/130564/download (accessed May 12, 2021).

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The Mother's Exposure: Does Lactation Reduce Internal Exposure?

There are two likely sources of persistent chemicals in breast milk: legacy stores in the mother from her lifetime exposure, and current sources of exposure, such as diet (LaKind, 2007). So the question arises: If a mother lactates, can she lower the stores of chemicals in her body, thus reducing her internal exposures? (If she disposes of her milk rather than breastfeeding, a process referred to as pumping-and-dumping, can she similarly reduce her exposure?) The reduction in chemical levels in the body via lactation, referred to as depuration, has been studied for such persistent chemicals as dioxins, polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), and chlorinated organic compounds (LaKind, 2007). Those studies have yielded mixed results (reviewed by LaKind et al., 2001), with some showing substantial declines in breast milk levels over time (Klein et al., 1986; Yakushiji et al., 1978) and others showing either minimal declines, no changes, or increasing levels over the course of lactation (Hooper et al., 2007; LaKind et al., 2009).

If lactation is an effective process for reducing the legacy stores of PFAS in the mother, one would expect to see levels of PFAS in serum and breast milk decline as lactation progressed and the mother's stores were depleted. We review here the literature on this topic.

In a review of the literature on breastfeeding and serum levels in mothers, VanNoy and colleagues (2018) conclude that the published studies support an association between breastfeeding and serum PFAS concentrations among women. However, they also observe that key aspects of breastfeeding, including duration, exclusivity, and timing of sample collection, should influence the breastfeeding–serum relationship, yet only one study in their review included all three exposure variables. They further describe the importance of parity for predicting maternal serum PFAS levels and note that most of the studies reviewed were unable to disentangle the effects of breastfeeding versus those of parity on PFAS exposure. The ability to fully assess the impact of breastfeeding on maternal PFAS levels is therefore limited.

Another approach to assessing the impact of breastfeeding on maternal PFAS exposure is to examine changes in PFAS levels in breast milk over the course of lactation. If stores of PFAS predominate over current exposures via diet and other sources, and if lactation resulted in mobilization and excretion of those stores, then lactation (with either breastfeeding or "pump-and-dump") could result in lower internal exposures for the mother. Three studies were identified that followed individual women over the course of lactation and collected multiple breast milk samples, which were analyzed for PFAS.

Lee and colleagues (2018) collected breast milk samples (n = 293) from 127 mothers at four different times postpartum (<7, 15, 30, and 90 days) as part of the Children's Health and Environmental Chemicals in Korea Cohort study. Samples were measured for 16 PFAS. Of the 127 women, only 15 provided samples across the lactation period, and samples from these women were pooled by sampling time. The concentrations of PFOS, PFOA, PFNA, and ΣPFAS 30 days postpartum were statistically significantly higher than in milk from sampling at <7 days postpartum.

In a study in Norway with nine women (Thomsen et al., 2010), milk samples were collected monthly from approximately 2 weeks postpartum up to 1 year postpartum (n = 3–10 per woman). The authors found a consistent decrease in concentrations of PFOS and PFOA, except for PFOS in one woman (these were the only PFAS >LOQ). The modeled depuration rates were reported as 7.7 percent and 3.1 percent reduction per month for PFOA and PFOS, respectively. Regarding generalizing these results, the authors note that because they did not have information on changes in the mothers' body weight or diet during the sampling timeframe, they could not evaluate the influence of these factors on depuration rates.

Fromme and colleagues (2010) also measured PFAS in breast milk samples collected monthly over 5 months from seven women. No significant differences in PFOS levels over the 5 months were observed.

In summary, our understanding of PFAS depuration based on breast milk measurements rests on three studies with between 7 and 15 women that yielded conflicting results. This is not necessarily surprising as depuration rates likely depend on numerous factors, such as current exposures, volume of

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breast milk excreted, and initial levels of PFAS in the body. Thus, the value of lactation as an intervention is unknown and requires additional study.

Would "Real-Time" Testing of Milk Help Guide Decision Making Regarding Infant Feeding?

It is unclear whether "real-time" testing of breast milk is an advantageous approach to guiding new mothers in decision making regarding infant feeding. First, testing requires sampling, shipping to laboratories, and conducting the analyses, which comes with a monetary cost. But even with unlimited resources, the time component is a critical consideration, as results may not be available until well into the infant's first few weeks or months. Second, a single measurement may not capture the infant's actual exposure as PFAS levels in milk may change over the duration of lactation, and the direction and rate of change are not well understood.

INDOOR DUST

Dust is a potential exposure pathway for PFAS compounds (Trudel et al., 2008). One dust-related PFAS intervention study was identified (Young et al., 2021). Dust samples were collected from floors by vacuum in "PFAS-free" refurbished rooms (7 with a full intervention and 28 with a partial intervention) and 12 control rooms at a university in the northeastern United States. Fifteen PFAS compounds were measured (PFOS, PFOA, PFHxA, PFHxS, FOSA, PFHpA, PFPeA, PFNA, PFBS, PFDS, PFBA, PFDA, PFUnDA, PFDoDA, and N-MeFOSAA), with detection limits ranging from 0.06 to 1.5 ng/g. PFAS levels in field blanks were either below the LOD or substantially lower than levels in the samples.

The geometric mean ΣPFAS levels were 481 ng/g (225–1,140 ng/g) in rooms with no intervention, 252 ng/g (18.1–8,310 ng/g) in rooms with partial interventions, and 108 ng/g (43.6–243 ng/g) in rooms with full interventions. Use of PFAS-free furnishing resulted in a statistically significant (78%, 95% CI: 38–92) ΣPFAS reduction in dust compared with control rooms. The results from this study suggest a possible intervention for reducing exposure to PFAS in dust.

While not an intervention study, research conducted by Scher and colleagues (2019) examined associations between PFAS-contaminated soil outside of homes and dust concentrations inside of the homes. The authors collected dust samples from the interior of the house and entryways to determine whether entryway dust levels indicate that "track-in" is an important contributor to house dust PFAS levels. They observed higher PFAS levels in the interior of the homes compared with soil levels, and suggest that soil track-in was not an important source of PFAS in interior dust.

Interpretation of PFAS dust studies is complicated by the lack of standardized sampling techniques for dust collection; the impact of use of different sampling methods on PFAS concentrations is unknown (Scher et al., 2019). Furthermore, for interventions that reduce PFAS in dust, the extent to which overall human intakes of PFAS would be reduced is not known. Estimates of the PFAS intake via dust have varied considerably (Nadal and Domingo, 2014).

OTHER POTENTIAL INTERVENTIONS

Other possible actions that could be considered interventions include limiting the use of PFAS-containing household goods and personal care products and introducing substances to the diet specifically intended to remove PFAS from the body. No intervention studies for these behavioral changes were identified, but here we describe one study on nonstick cookware, one study related to use of dental floss, and one dietary modification approach in the context of future exploration.

Nonstick Cookware

Nonstick cookware has been studied as a source of PFAS exposure. In terms of interventions, at issue is whether replacing nonstick cookware with items that do not contain PFAS would result in a

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measurable decrease in human PFAS exposures. While no intervention studies were identified, we briefly describe one study conducted in the United States that compared the release of PFAS into air and water from nonstick versus stainless steel frying pans. Sinclair and colleagues (2007) purchased four brands of domestic and imported nonstick frying pans and one brand of stainless steel frying pan (three to five of each brand) in New York. The pan brand names are not identified. All pans were precleaned with hot, soapy water; rinsed with Milli-Q water; and dried with a towel. The stainless steel pans were used as controls. The authors report that under normal cooking conditions (179°C to 233°C surface temperature), PFOA in the gas phase was measured at 11–503 picograms per square centimeter (pg/cm^2) from the nonstick frying pans. (Fluorotelemer alcohols were also detected, but these are not chemicals of focus in this paper and are not discussed further.) Gas-phase PFOA decreased after repeated use of one brand of pan but not the others (n = 1 for each brand). The authors also measured PFOA in Milli-Q water boiled for 10 minutes in selected pans and found inconsistent results (certain pans resulted in measurable levels of PFOA in the water, while others did not).

Since brand names are not included in the publication, this information cannot be used as the basis for specific intervention recommendations. Even if brand names were included, given the small sample size and the lack of study replication, it would be difficult to use this information as the basis for general recommendations. Finally, as neither air nor water concentrations are provided, the extent to which this exposure source contributes to overall intake is unclear.

Dental Floss

One study examined the association between use of dental floss and serum PFAS (PFOA, PFNA, PFDeA, PFHxS, PFOS, and Me-PFOSA-AcOH) levels (Boronow et al., 2019). Serum PFAS levels were measured in 178 middle-aged women. An administered questionnaire included one question on use of dental floss: "In the last month, how often did you use Oral-B Glide dental floss?" Response choices were as follows: "Never or almost never, Several times a month, 2 or more times a week, Every day." Only "ever" and "never" were used in the regression analysis. While five of the PFAS compounds did not show significant associations, a 24.9 percent (95% CI: 0.2–55.7) higher level of PFHxS was found in subjects who used Oral-B Glide floss. It is worth noting that the questionnaires were administered several years after blood sample collection.

The authors also analyzed 18 dental floss products (only one or two samples per brand were analyzed, except the Oral-B Glide brand [five samples]) for total fluorine (as an indicator for polytetrafluoroethylene or PTFE), 6 of which yielded detectable levels. Given the lag in time between blood collection and questionnaire administration, the small number of floss samples analyzed, and the mostly nonsignificant results, it is not clear whether dental floss is an important route of human exposure to PFAS.

Fiber Intake

Studies have observed relationships between higher fiber intake and lower serum PFAS levels (e.g., PFOA, PFOS, and PFNA [Dzierlenga et al., 2021]; PFOS and PFOA [Halldorsson et al., 2008]; PFOS, PFOA, PFHxS, EtFOSAA, MeFOSAA, and PFNA [Lin et al., 2020]). This finding is based on studies examining associations between dietary recall information and serum levels in cross-sectional study designs. Thus, it is unknown whether other factors (e.g., whether diets with high fiber generally have lower levels of PFAS) influence this relationship.

MODELED INTAKES AS THE BASIS FOR RECOMMENDATIONS FOR REDUCING EXPOSURE TO PFAS

Well-conducted and generalizable intervention studies can be considered the gold standard for recommending approaches to reductions in personal exposure to PFAS. In the absence of such studies, it

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may be informative to use results from studies designed to model the relative contributions of exposure pathways to overall PFAS exposure. This approach would focus attention and intervention strategies on the most important exposure pathways. In this section, we discuss results from studies using concentration data from several media and products (e.g., soil, water, and food concentration data) in combination with generic intake factors (e.g., g intake/kg body weight per day) to model human intakes of PFAS. We explore whether any common, generalizable themes emerge from such modeling studies regarding dominant intake pathways.

Because of geographic differences in both environmental media and product concentrations, the emphasis here is on studies conducted for the U.S. population. First, however, we provide a brief synopsis of reviews on studies conducted for populations outside of the United States. Although some of the modeling efforts included less well-studied PFAS (e.g., PFHxS), the most commonly assessed PFAS were PFOA and PFOS. Sunderland and colleagues (2019) recently compiled data from the literature on percent source contribution in adults. The results are summarized in Figures E-7 and E-8. The modeling approaches represented by the underlying papers included different concentration data and intake assumptions. Regardless of the approach used, diet appears to be the major pathway of exposure to PFOS, with the percent contribution ranging from 65 to 96 percent. For PFOA, the range of percentages for dietary contributions is wider (6–86 percent). While these results point to the importance of diet, the location of a study and the presence or absence of point sources will influence the relative contributions of various pathways to overall PFAS intake. Behavioral differences across countries can also impact the results and their generalizability to communities in the United States. We therefore focus on two studies that modeled source contributions for PFOA (Lorber and Egeghy, 2011) and PFOS (Egeghy and Lorber, 2011) in the United States.

For PFOA intake modeling, Lorber and Egeghy (2011) used the following data, assumptions, and approach. Their intakes were developed for adults and 2-year-old children. PFOA concentrations in various media from the published literature were used in combination with EPA exposure contact rates. The authors generated intake distributions by inputting different exposure media concentrations into the model. They assumed that PFOA concentrations in indoor air were 20 times higher than those in outdoor air, with outdoor air data being derived from a study in Albany, New York. Whether these air concentrations represent more general U.S. exposures is not discussed. The authors also used dietary data from the Canadian Total Dietary Survey (Tittlemier et al., 2007); thus, it is not known whether this aspect of the model is representative of exposures to PFOA in the United States. PFOA levels in drinking water were estimated from surface water concentrations in various parts of the United States (New York, North Carolina, New Jersey [drinking water], Great Lakes, Tennessee, and Florida). Dust concentration data were obtained from dust samples from homes and day care centers in Ohio and North Carolina.

Lorber and Egeghy (2011)[11] discuss numerous limitations around their estimates of intakes, including the following: no estimates of intake via direct contact with consumer products (e.g., treated carpets or cosmetics), no inclusion of PFOA precursors, and a lack of PFOA data for food in the United States. However, Lorber and Egeghy (2011) also used a simple one-compartment, first-order pharmacokinetic model in combination with National Health and Nutrition Examination Survey (NHANES) 2003–2004 data and back-calculated intakes. Their central tendency intake estimates for adults and children (70 ng/day and 26 ng/day, respectively) are not dissimilar to the intakes back-calculated from the NHANES data (56 ng/day and 37 ng/day for males and females, respectively). These models would benefit from the use of improved measurement data in food and other media specific to—and representative of—the United States (or at least areas or regions of interest within the United States) and the use of more recent NHANES data.

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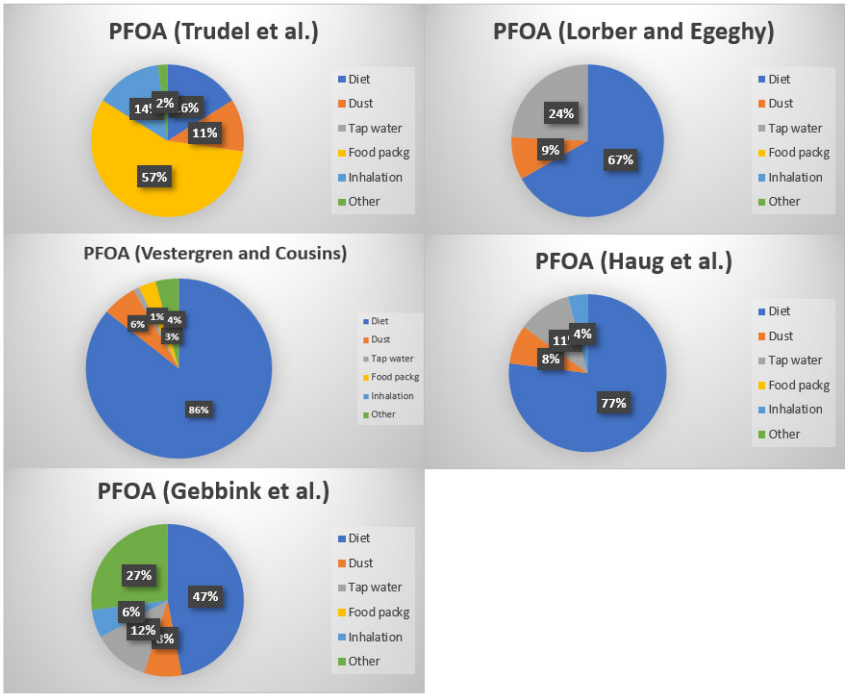


FIGURE E-7 Relative contribution percentiles for various pathways of exposure to PFOA.
NOTE: Values less than 1 percent were assigned a value of zero.
SOURCE: Data from Sunderland et al., 2019.

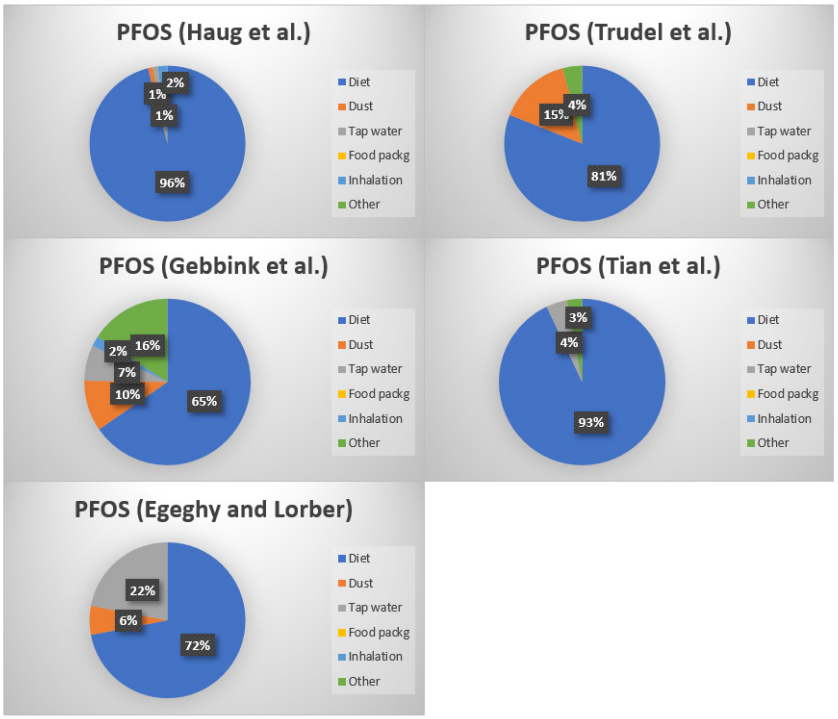


FIGURE E-8 Relative contribution percentiles for various pathways of exposure to PFOS.
NOTE: Values less than 1 percent were assigned a value of zero.
SOURCE: Data from Sunderland et al., 2019.

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Egeghy and Lorber (2011) used a similar approach to model intakes of PFOS and its precursors. In addition to modeling of precursors, this effort differs from the PFOA models described above in that the models were developed for both "typical" and "contaminated" scenarios. (The contaminated scenario was similar to the background scenario except that much higher water concentrations were used.) In terms of available data on environmental concentrations of PFOS and precursors, the authors note that "measurement of PFCs in exposure media in North America was relatively sparse for all media compared with European data with the possible exception of dust. Food data are sparse for both continents. No measurements of PFOS in uncontaminated soil could be found" (Egeghy and Lorber, 2011, p. 158). The authors found that diet was the main route of exposure in the general population, while for children, dust ingestion was almost as important a contributor to intake as diet. Perhaps most important in the context of using modeled uptakes as the basis for recommendations for modifying behaviors to reduce exposure, Egeghy and Lorber (2011) found that the pathway-specific contributions spanned several orders of magnitude and overlapped substantially.[12]

Use of model estimates of dominant pathways of PFAS exposure for making recommendations to individuals regarding exposure reduction faces a number of challenges. First, while diet appears to be a major pathway of exposure, there is little information on PFAS in commercial foods commonly consumed in the United States. The FDA has released PFAS data for certain foods, which could be used in future studies involving modeling of source contributions to PFAS intake.[13] However, the FDA observed that its data for produce, meat, dairy, and grain products are based on a small sample size, and the results "cannot be used to draw definitive conclusions about the levels of PFAS in the general food supply" (para. 3). It is not currently well understood whether the data on commercial foods from other countries used in the intake models are representative of levels in the United States. Wu and colleagues (2015) state, "Information on dietary predictors in U.S. is still limited" and "more data are needed to determine the relative contributions of food and dust to serum PFCs for both adult and child populations" (p. 265). A review of PFAS in foods by Domingo and Nadal (2017) identifies only two recent studies for the United States: one focused on PFAS uptake by lettuce and strawberries irrigated with reclaimed water (Blaine et al., 2014) and the other on concentrations of PFAS in freshwater fish samples from urban rivers and the Great Lakes (Stahl et al., 2014).

Furthermore, the relative importance of different sources varies by study (see Figures E-7 and E-8) and by demographic group and population (Sunderland et al., 2019). In describing the findings of their recent review on nonoccupational intakes via background exposures, De Silva and colleagues (2021) observe that the inconsistency in the relative importance of different exposure sources from one study to the next may be due to differing concentrations of PFAS in media, as well as the assignment of different values for exposure intake factors (e.g., exposure frequency and duration). They conclude, "Without rigorously conducted exposure studies it is challenging to rank order the most important human exposure pathways and without these data, our ability to design evidence-based exposure intervention strategies will be limited" (De Silva, 2021, p. 644).

Within even a small geographic area, one could envision varying exposure characteristics that could, in turn, affect sources of PFAS intakes. Vestergren and Cousins (2009) explored this possibility by estimating relative intakes for those with exposure to background PFAS levels, exposure to higher levels in drinking water or drinking water impacted by a PFAS point source, or occupational exposures; they found substantial differences in the relative contributions to overall intakes (see Figure E-9).

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[13] See https://www.fda.gov/food/cfsan-constituent-updates/update-fdas-continuing-efforts-understand-and-reduce-exposure-pfas-foods (accessed May 12, 2021).

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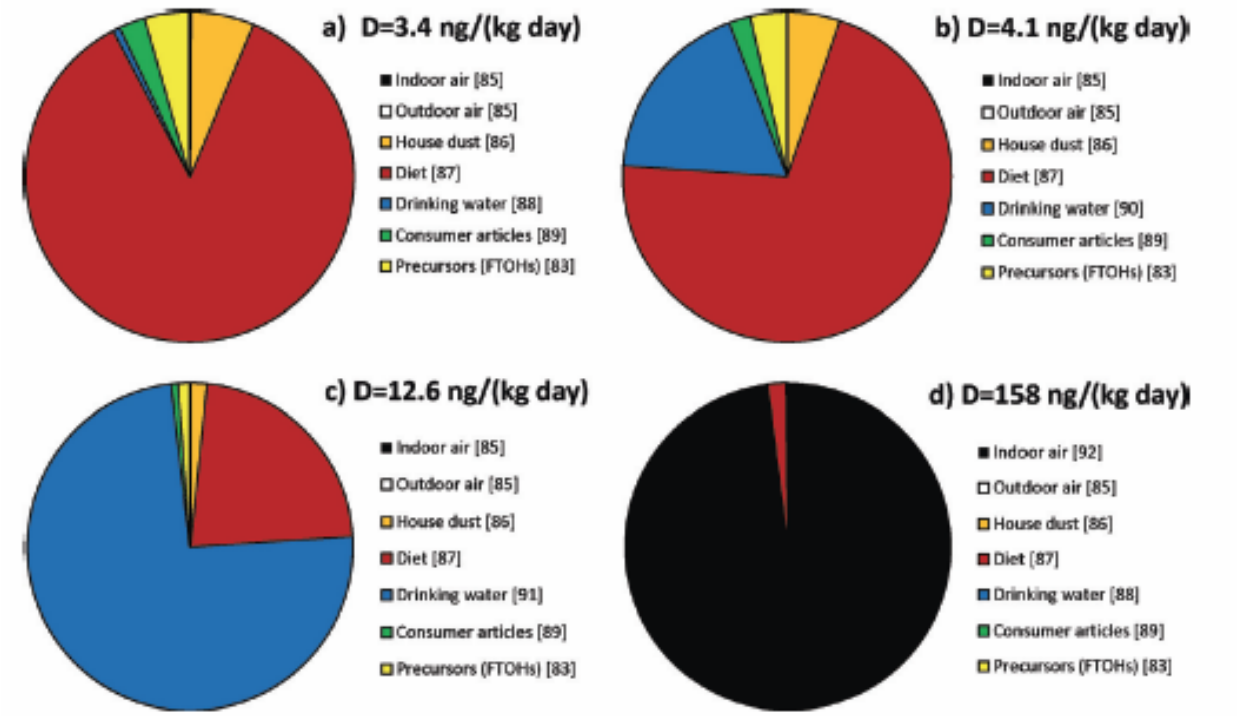


FIGURE E-9 Estimated daily intakes for male adults (D) and relative source contributions.
NOTES: Data for sources are derived from different studies and different countries. Bracketed numbers refer to citations in Vestergren and Cousins, 2009. (a) = background concentrations in drinking water (1.3 [nanograms per Liter [ng/L]); (b) = elevated concentrations in drinking water (40 ng/L); (c) = point sources of drinking water contamination (519 ng/L); and (d) = occupationally exposed individuals (indoor air concentrations 1 micrograms per cubic meter [µg/m^3]). FTOH = fluorotelomer alcohols; ng/(kg day) = nanograms per kilogram per day .
SOURCE: Reprinted with permission from Vestergren and Cousins, 2009. Copyright 2009 American Chemical Society.

Another factor related to modeling principal sources of exposure is the changes in production and use of individual PFAS over time. Sunderland and colleagues (2019) describe the changes in serum PFAS levels following the phase-out of production of PFOS and its precursors, with PFOS declining, but other PFAS, such as PFHxS, increasing. They observe that while exposures to PFOS and PFOA "have been successfully reduced by product phase-outs for many populations, exposures to C-9-C-11 PFCAs have not followed the same trends" (Sunderland et al., 2019, p. 138). Thus, it is important to use recent environmental and consumer product and dietary data to develop robust estimates of current dominant pathways of exposure.

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Discussion

In this review, we have sought to address the following question: Based on current research, are there interventions or *personal* modifiable behavioral changes that individuals can make to *effectively* reduce their PFAS exposure? We have described research on potential ways in which an individual could reduce exposure to PFAS. It is important to acknowledge that communities across the United States have received guidance from state and federal agencies regarding PFAS exposure reduction, including advisories around consumption of drinking water and fish. While it may seem obvious that avoiding exposure to sources of PFAS would result in reduced intake of PFAS and, in turn, lower internal PFAS levels, some caution in assuming that exposure and risk reduction would ensue is warranted. For example, if one is advised to avoid locally caught fish because of known PFAS contamination in that fish, such avoidance could result in reduced exposure. However, if dietary fish is replaced by another food that is also high in PFAS, avoiding the fish may not result in lower PFAS exposure. Another issue to consider is that avoiding one group of chemicals by changing diet or other behaviors can result in increased exposures to other chemicals. Finally, if a certain PFAS source is related to a small portion of overall exposure, then avoidance of that source may not result in appreciable reductions in internal levels of PFAS. Thus, while avoiding known sources of PFAS exposure may be a useful approach to lowering overall exposure, these other considerations should be taken into account; research on the efficacy of various interventions could help shed light on these complexities.

The number of intervention studies available to address the question of whether there are interventions or *personal* modifiable behavioral changes that individuals can make to *effectively* reduce their PFAS exposure ranged from 1 to 11, depending on the source of exposure; for some pathways, no studies were found (see Figure E-10). Overall, only the water filtration studies provided relatively consistent evidence of an effective reduction in PFAS levels. No studies were identified that confirmed reduction in human exposure via biomonitoring. To fully demonstrate the efficacy of an intervention, a study would need to be conducted over a timeframe sufficient to account for the long half-lives of PFAS.

Demonstration that PFAS exposures are reduced in a meaningful way through biomonitoring confirmation is an important step. It is possible that an intervention may reduce PFAS levels in a particular medium, but if this medium is not a major source of overall exposure, then that intervention may not contribute significantly to reduction in human exposures. Overall, the intervention literature is sparse and has many limitations. Thus, the committee may have to rely on assumptions and other bodies of evidence to make recommendations to individuals and communities about exposure reduction.

We also considered whether intake models could assess with some degree of confidence the relative source contributions to overall intake and whether this approach could be used to inform decisions regarding community recommendations. While diet appears to be a major contributor to overall intake, dietary data from the United States were not used in these models. It is not known whether the Canadian data used are representative of levels in the United States. As observed by Macheka-Tendenguwo and colleagues (2018), “numerous investigations have attempted to establish the main exposure pathway of PFAS in humans, but differing viewpoints make the results inconclusive” (p. 36066).

In conclusion, other than using certified POE or POU water filters, the available intervention studies generally do not appear to be sufficiently robust to support recommendations for personal behavioral modifications for communities in the United States. The studies reviewed here were limited by small sample sizes, generally inconsistent study designs and results, and a possible lack of generalizability to the United States. It is also important to consider additional factors when developing recommendations for changes in personal behavior, such as the ease and cost of an intervention, associated trade-offs (i.e., If an intervention lowers concentrations of PFAS, does it increase exposures to other chemicals, including

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other PFAS?), and whether enough is known about varying PFAS levels in the environment and consumer products to understand the necessary scale of an intervention recommendation (e.g., individual, local, or regional). Information will be needed on local levels of PFAS in drinking water, as well as levels in breast milk, for any recommendations regarding infant nutrition to be well supported.

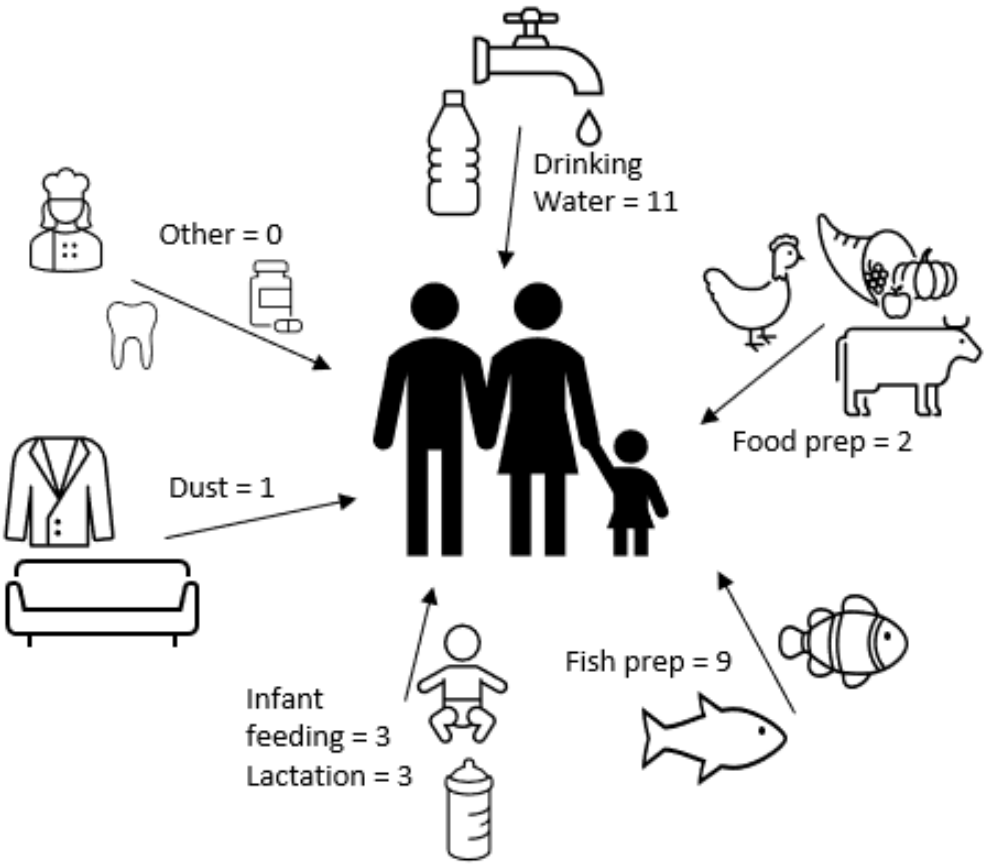


FIGURE E-10 Summary of numbers of studies identified for each PFAS exposure source.
NOTE: "Other" includes nonstick cookware, dental floss, and fiber intake, which these sources are described in the text but for which no intervention studies were identified.
SOURCE: Author generated.

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Review article

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Air emissions during destruction of PFAS-containing materials

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Abstract

Per- and polyfluoroalkyl substances (PFASs) are synthetic chemicals used across numerous industrial and consumer applications. Their persistence and toxicological impacts necessitate their removal from the environment and their complete destruction; however, many PFAS destruction technologies release gas-phase and aerosol-phase fluorinated products of incomplete destruction (PIDs). In this Review, we discuss the PIDs released by PFAS destruction methods and approaches to categorize and measure them. Existing and emerging technologies use thermal, chemical, electrical or biological approaches to degrade PFASs, with varying degrees of success. Although many technologies achieve destruction and removal efficiencies of more than 99.99%, this metric does not account for the formation of airborne PIDs. Methods are being developed to measure PIDs in air emissions to protect exposed communities and facilitate the closure of fluorine mass balances. The choice of sampling and analytical methods depends on the polarity and volatility of the PIDs. Closing the mass balance is important because PIDs such as CF_4 can have unintended global impacts, and potential localized risks from exposure to PIDs remain uncertain. Therefore, diverse analytical methods are needed to comprehensively characterize PIDs; such characterization is important to identify PFAS destruction technologies that minimize atmospheric emissions and their associated environmental and human health risks.

Sections

Introduction

Non-destructive treatment

Destructive treatment and PID formation

Characterization of PIDs

Environmental and toxicological impacts of PIDs

Summary and future perspective

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Introduction

Per- and polyfluoroalkyl substances (PFASs) are a large class of synthetic organic chemicals that have been widely used in industrial processes and consumer products such as textile protectants and aqueous film-forming foams (AFFFs) since the 1940s[1–3]. The high chemical and thermal stability that makes PFASs suitable for these applications also enables them to persist in the environment[4,5] (Box 1). PFASs are released into the environment during PFAS manufacturing, their incorporation into products, and during and at the end of product life cycles[6–8]. The environmental and human health impacts of many PFASs are the subject of much study; for example, exposure to some PFASs such as perfluorooctanoate (PFOA) can lead to an increased risk of certain cancers[9,10]. Therefore, treatment approaches are required to reduce ongoing releases of PFASs and to clean up contaminated sites.

Non-destructive technologies are frequently used to remove PFASs from contaminated water or soil. However, these approaches produce PFAS-laden residuals that pose a substantial risk of secondary pollution if not managed properly. Current management approaches are problematic: the residuals are either sent to landfills where PFASs persist or transform into other PFASs, or are thermally treated, in which case incomplete degradation can lead to the release of products of incomplete destruction (PIDs)[11–15] (Fig. 1). PIDs are formed when conditions are insufficient to completely mineralize PFASs: that is, convert them to inorganic fluorine such as hydrogen fluoride (HF) and fluoride salts, and other inorganic species such as carbon dioxide (CO_2). PIDs can be present in solid and liquid residuals as well as in airborne emissions; they can include carbon tetrafluoride (CF_4), perfluoroethane (C_2F_6), other fluorinated and mixed halogenated organics, and PFASs with well understood toxicity, such as PFOA[16–33].

Numerous treatment technologies are being developed to destroy PFAS-containing wastes such as AFFFs, water, soil, biosolids, pavements, and residuals produced by non-destructive technologies. It is important to assess the performance of destruction technologies not solely based on the disappearance of the parent PFASs, but also with respect to the minimization of PID formation in atmospheric emissions and other residuals. Destruction and removal efficiency (DRE) calculations, which simply account for the loss of individual PFASs by comparing the mass feed rate to the mass emissions rate through the stack exhaust[34], fail to account for the potential release of fluorinated PIDs to the air and for the potential presence of the parent compound or PIDs in residual solids and liquids[35,36]. This definition does not apply to destructive technologies without stacks, for which the DRE concept can be extended to refer to the loss of individual PFASs in the aqueous phase. Therefore, aiming to close the fluorine mass balance, instead of solely relying on DRE measurements, is a critical goal when evaluating the performance of PFAS destruction technologies[12]. The fluorine mass balance and the degree of PFAS mineralization can be calculated from measurements of organic and inorganic fluorine. However, accounting for the fate of every fluorine atom to achieve a complete fluorine mass balance is difficult at all scales and improbable at large scales. This difficulty arises because fluorine reacts with reactor surfaces and matrix constituents, but also because quantitative analytical methods are still being developed for many PIDs.

Despite being neglected by the DRE metric, it is important to characterize air emissions for potential PIDs from PFAS destruction technologies. Gas-phase and aerosol-phase PIDs can represent a substantial portion of fluorine in the fluorine mass balance of destruction processes[37,38]. Once these PIDs are discharged to the atmosphere, they can be transported over large distances and returned to terrestrial and aquatic ecosystems via dry and wet deposition[39–41] (Fig. 1). This atmospheric transport broadens the environmental impacts and the risk of human exposure through inhalation or ingestion of contaminated air, water, and food. Comprehensive characterization of PFAS-derived PIDs is important even when they are produced in trace amounts because some compounds might exhibit high risk even at low concentrations.

In this Review, we evaluate methods to characterize PIDs produced by PFAS destruction technologies, with a particular focus on characterization of gas-phase and aerosol-phase PIDs. First, we introduce existing non-destructive technologies for PFASs. We then discuss potential PIDs generated during PFAS destruction processes and outline existing destructive technologies as well as some emerging destructive technologies. Next, we review the analytical methods available for identifying and quantifying PIDs. Finally, we outline priority research needs to address the knowledge gaps that currently limit understanding of PID formation during PFAS destruction.

Non-destructive treatment

In many remediation strategies, PFAS-containing media are first treated by non-destructive separation processes that concentrate PFASs. Although these methods do not destroy PFASs, they have a critical role in interim containment and exposure reduction. However, they inevitably generate PFAS-laden residuals that require PFAS destruction to achieve long-term environmental protection. In this section, we briefly outline common separation processes used to remove PFASs from soil, water, and air. More detailed information on these non-destructive technologies is available elsewhere[29,42–46].

Separation technologies such as granular activated carbon (GAC) adsorption[47–49], ion exchange[49,50], reverse osmosis[45,51], and foam fractionation[52] are commonly used to treat PFAS-contaminated water. These approaches can economically remove PFASs from the large volumes of water that require treatment (typically 1,000s to 10,000s of cubic metres per day) and concentrate PFASs in residual streams of lower volume[53,54]. Activated carbon is used to remove a wide range of PFASs and many other organic contaminants from water, whereas ion-exchange resins are generally more selective[43,55]. Membrane technologies, such as reverse osmosis, can remove a broad spectrum of PFASs and other constituents via size exclusion and electrostatic interactions, generating a concentrated brine waste[45,51]. After treatment, the spent adsorbents and brines are either landfilled or sent to a destructive process. Foam fractionation exploits the surfactant properties of PFASs, particularly long-chain species, by concentrating them in a collectible foam layer[52,54]. Emerging non-destructive PFAS removal methods, such as electrosorption and adsorbents including metal–organic frameworks and supramolecular macrocycles, show promise but remain at early development stages[56–58].

For soils and sediments, non-destructive approaches aim to immobilize or extract PFASs. Stabilization, solidification, and in situ sorptive amendments (that is, using sorbents such as activated carbon, biochar and organoclays) can substantially reduce PFAS leaching[59,60]. However, the long-term performance of these approaches under field conditions, where pH and/or redox conditions might change, remains uncertain[44]. Ex situ soil washing can remove PFASs from coarse-grained soils[42,61,62] but is often less effective for fine-grained or organic-rich matrices, and it generates PFAS-laden wash water[61], which must be treated.

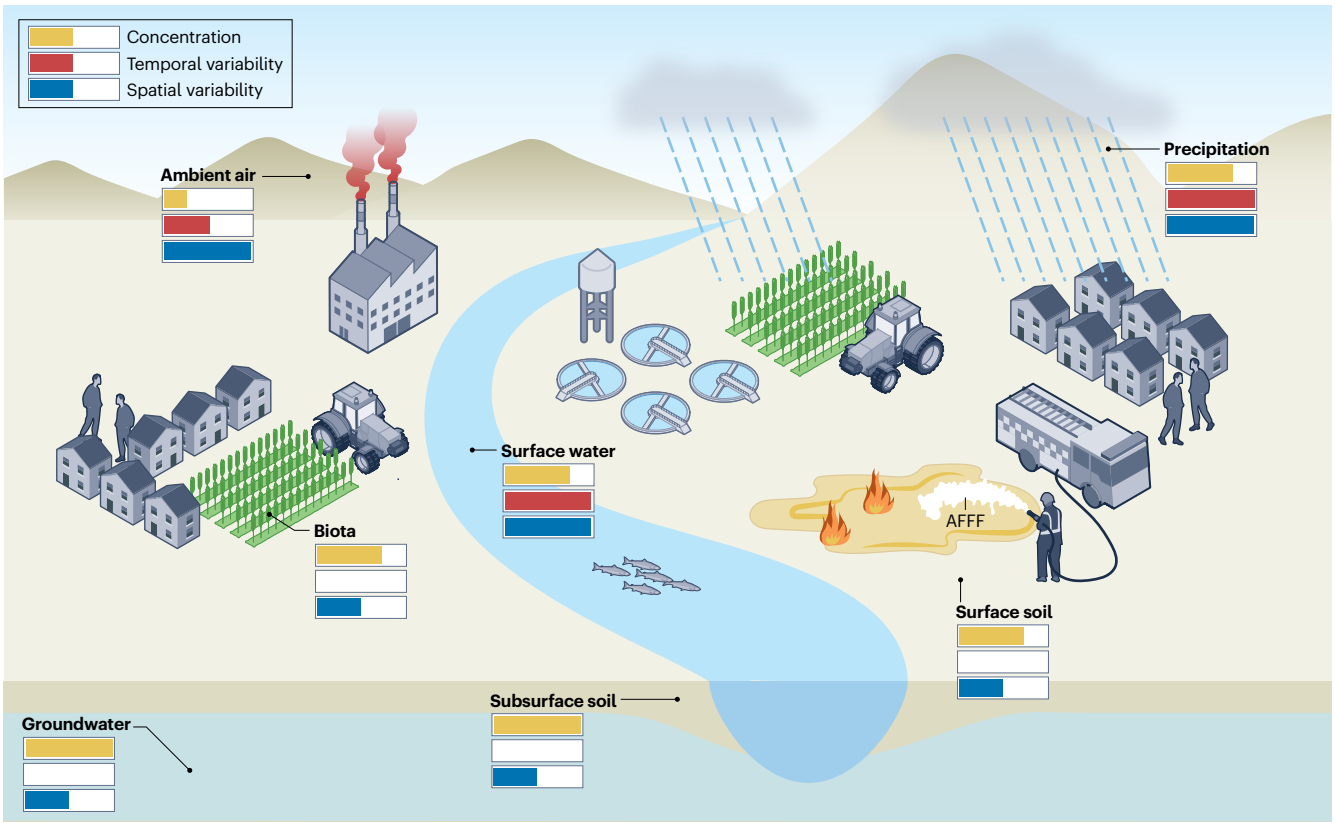
Box 1 | Understanding PFASs

Definition

Per- and polyfluoroalkyl substances (PFASs) are a family of synthetic fluorinated substances that contain at least one perfluorinated methyl ($-CF_3$) or methylene ($-CF_2-$) group, without any H, Cl, Br or I atom attached to the carbon atom, as defined by the Organisation for Economic Co-operation and Development (OECD)[182]. PFASs include polymers (such as polytetrafluoroethylene, PTFE) and non-polymers (such as perfluorooctane sulfonate, PFOS), with non-polymeric PFASs currently drawing the most regulatory and research attention. Subclasses of PFASs are typically categorized by head groups, such as perfluorocarboxylates (PFCAs) and perfluorosulfonates (PFSAs), as well as fluorine saturation levels, with perfluorinated compounds containing no C–H bonds and polyfluorinated compounds containing at least one bond between C and H, Cl, Br or I. PFASs can also be classified as long-chain ($C \geq 8$ for PFCAs and $C \geq 6$ for PFSAs), short-chain and ultrashort-chain ($C \leq 3$) structures depending on their number of C atoms[183].

Persistence, environmental relevance and toxicity

PFASs are highly resistant to degradation or transformation in natural and engineered systems. This persistence arises from the strength of the carbon–fluorine (C–F) bond, which has a bond dissociation energy of ~500 kJ mol^{-1} (refs. 184,185). Consequently, released PFASs can persist in environmental matrices for long periods. Media-specific environmental concentrations of PFASs are highly interrelated, show substantial temporal and spatial variability, and can exceed reference concentrations by several orders of magnitude near release sites, such as fluorochemical manufacturing facilities and sites contaminated with aqueous film-forming foam (AFFF) (see the figure: with partially to fully filled bars indicating an exceedance of 1 to 4 orders of magnitude for the media-specific concentrations relative to reference values (empty bar) of 4–10 ng l^{-1} for water, 0.07 µg m^{-3} for air, and <1 µg kg^{-1} for soil, representing typical background concentrations[186], and the full, half-full and empty bars indicating high, medium, and low spatial and temporal variability, respectively). High concentrations of PFASs can lead to ubiquitous human exposure through contaminated water, food, and air[187–193]. Although risk-based toxicity factors for non-cancer effects and cancer risk are available for some PFASs[118], further examination of the effects of exposure to PFAS mixtures[194] and intermediates[195] and an analysis of toxicological interactions[196] are needed.



Destructive treatment and PID formation

PFAS-impacted waste generated from non-destructive treatment technologies should be subjected to effective destructive treatments to prevent secondary pollution (Fig. 2a). Currently, these materials are often managed by landfilling and, in the case of biosolids and water treatment residuals, land application, but

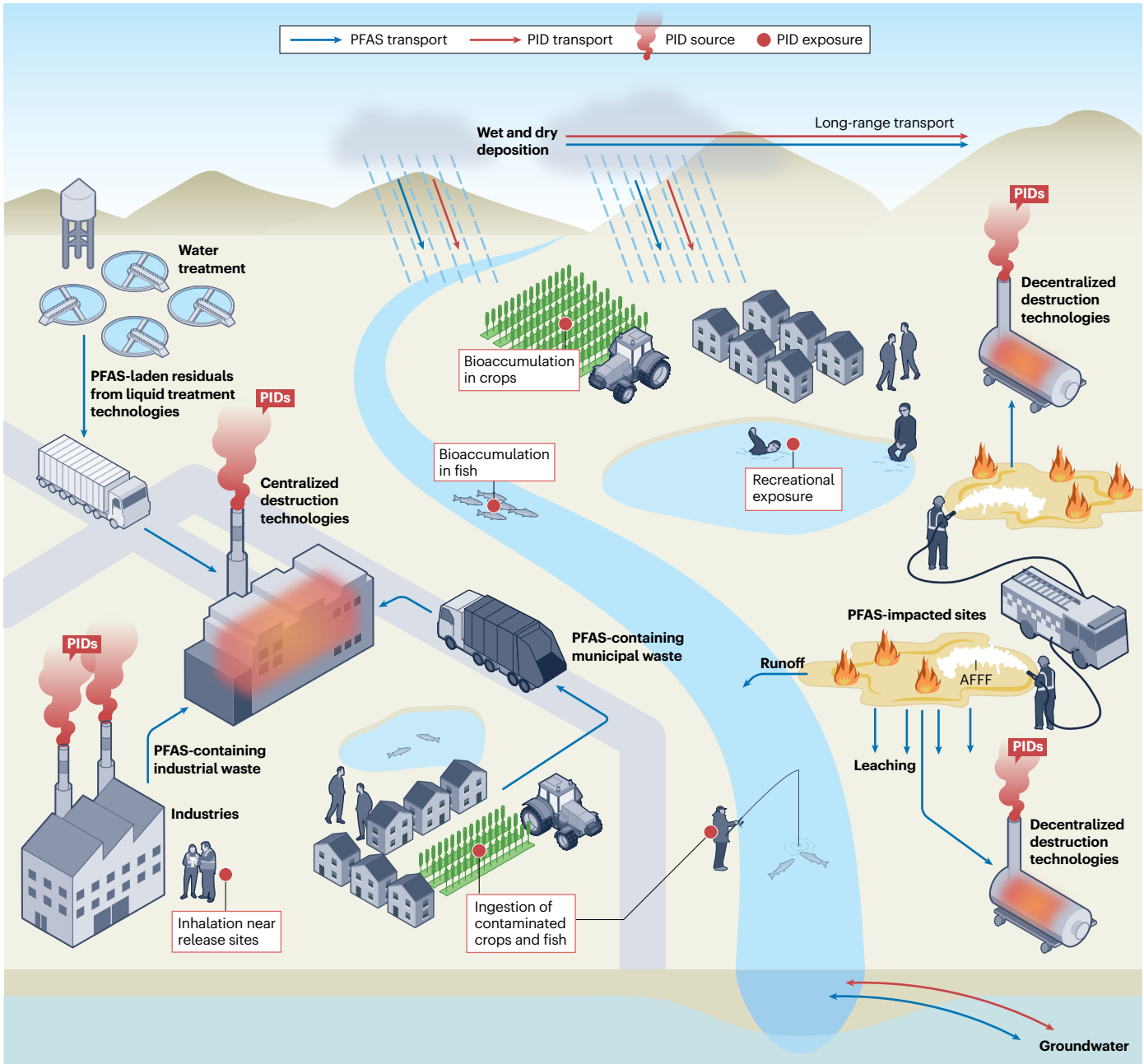


Fig. 1 | Sources, transport and exposure points of PFAS-derived PIDs. Products of incomplete destruction (PIDs) are generated from the destruction of wastes that contain per- and polyfluoroalkyl substances (PFASs). Such wastes include residuals from water treatment technologies, municipal solid waste, industrial waste, and soil contaminated with aqueous film-forming foam (AFFF). These PFAS-containing materials can be transported to centralized or decentralized destruction technologies for processing. The gas-phase and aerosol-phase PIDs from these technologies can be transported across large areas in the atmosphere and through dry or wet deposition. Consequently, people might be exposed through multiple pathways: inhalation near facilities that manufacture, use or treat PFASs; ingestion of contaminated food and water; and through recreation activities such as swimming. The definition for PFAS-derived PIDs is inclusive of PFASs.

such management approaches lead to PFAS cycling in the environment. Various destruction technologies have been investigated and deployed at the laboratory, pilot and commercial (field) scale, yet many of these approaches can lead to the formation of PIDs. This section first summarizes how PIDs are generally produced during PFAS destruction and then outlines destructive technologies that show promise with respect to field-scale application, with an emphasis on their efficiency as characterized by PFAS destruction parameters (such as DREs and PID formation), scalability, and suitability for real-world applications.

PID formation during PFAS destruction

Destruction technologies generate a range of PIDs, although the specific species and their quantities strongly depend on the technology, its operating conditions, matrix constituents of the treated material (for example AFFF, soil, solid waste, ion-exchange resins, GAC), and the presence of other chemicals in the treated material such as co-adsorbed halogenated species on GAC. Although liquid and solid-phase PIDs are produced by some PFAS destruction technologies, airborne PIDs have received substantially less attention and thus are the focus of this Review.

Computational efforts provide insight into PID formation and help to identify the optimal conditions needed to minimize the formation of PIDs during PFAS destruction. Simulations suggest that perfluoroalkenes, perfluoroalkanes, and hydrofluoroalkanes can be the major PIDs formed upon cleavage of the terminal head group during the destruction of PFASs[63–70]. Many of these intermediates and smaller gaseous PIDs (such as CF_4 and C_2F_6) have also been observed experimentally[71–75]. The presence of PIDs indicates that there are operational conditions and kinetic bottlenecks preventing complete PFAS mineralization. Other oxidation and/or pyrolysis intermediates have been identified in theorized reaction pathways[63–67], including perfluorinated α-lactones, acyl fluorides, aldehydes, n-alkyl radicals, and head-group radicals[67]. Some of these classes, along with other intermediates such as non-polar semivolatiles (for example fluorotelomer alcohols[76]), have also been observed experimentally (including some short-lived radicals[77]).

Hydrogen sources are essential for reducing PID formation during PFAS destruction. If fluorinated radical species are formed, for example through the cleavage of carboxylic acid or sulfonic acid head groups, and then recombine, PIDs can potentially be generated that have a higher

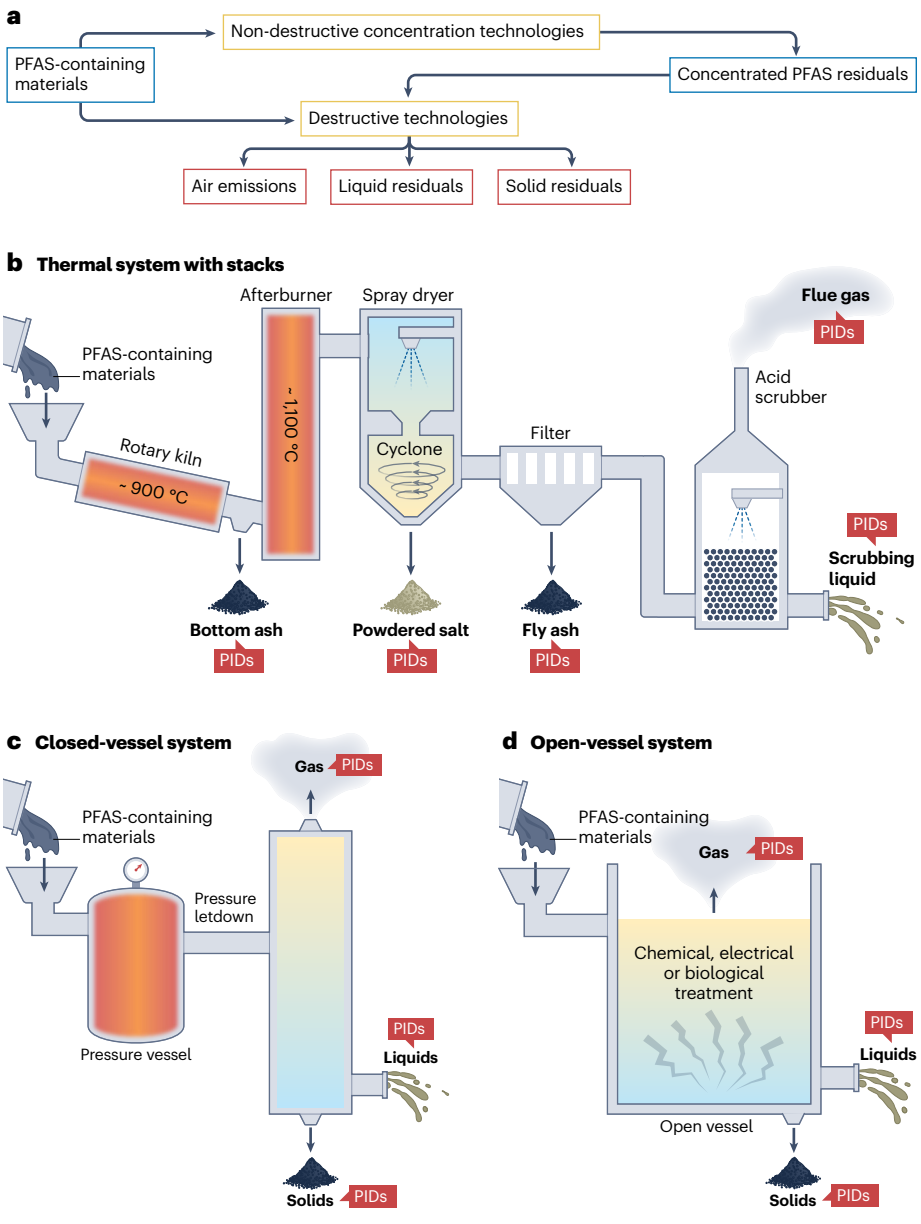


Fig. 2 | Strategies for managing PFASs. **a**, Non-destructive technologies generate concentrated per- and polyfluoroalkyl substance (PFAS) residuals that require subsequent management. Alternatively, destructive technologies aim to break down PFASs but can produce various outputs including air emissions, liquid residuals and solid residuals that might contain products of incomplete destruction (PIDs). **b**, Schematic of a typical hazardous waste incineration system used to manage PFAS-impacted residuals by combusting the waste in an incinerator. The flue gases are typically treated through emission control systems, such as afterburners, spray dryers, filters and scrubbers, before being released. **c**, Schematic of a closed-vessel system where PFAS-impacted liquids and slurries are exposed to high pressure and temperature. The treated effluent is separated in a gas–liquid separator. **d**, Schematic of a general open-vessel system, which uses chemical, electrical or biological means to destroy PFAS-impacted wastes and residuals. PIDs formed by destructive technologies are released into the environment in various phases.

Table 1 | PFAS destruction technologies

Technology	Temperature (°C)	Current technology scale[a]	Major PID types detected	PID detection approach	Air pollution control systems required
Thermal destruction technologies with stacks					
Incinerators[16–20,32]	~650–1,200	Commercial	COF_2, CF_4, C_2F_6, TFA and longer-chain per- and polyfluoroalkanes and -alkenes	FTIR, LC-MS, GC-MS, CIMS	Yes, secondary combustion chamber, quench system, spray dry absorber, baghouses, electrostatic precipitator or scrubber
Pyrolyser and gasifier[33,71,73,74,88]	~500–1,100	Commercial	Perfluoroalkenes	IR spectroscopy, GC-MS	Yes, wet scrubber
Cement kilns[22]	~1,200–1,800	Commercial	Unknown	Unknown	Yes, baghouses, electrostatic precipitator, or scrubbers
GAC reactivation furnaces[23,96,97]	~700–950	Commercial	Perfluoroalkenes	LC-MS, GC-MS	Yes, thermal oxidizer
Closed-vessel systems					
Hydrothermal alkaline treatment[24,98]	~300–350	Pilot scale	Short-chain PFCAs; unknown gas emissions	LC-MS, GC-MS	No
Supercritical water oxidation[25,100]	>374	Commercial	Perfluorohexane, perfluoroheptane	LC-MS, GC-MS, CIC	Yes, activated carbon adsorber
Open-vessel systems					
Advanced oxidation processes[29,30]	Ambient	Bench scale	Short-chain PFASs, perfluorinated alkanes	LC-MS, GC-MS	No
Advanced reduction processes[28]	Ambient	Bench scale	Polyfluorinated alkenes and alkanes, short-chain PFASs	LC-MS, GC-MS	No
Plasma-based methods[27]	Ambient	Pilot scale	Short-chain PFASs; unknown gas emission	LC-MS	No
Sonolysis[26]	Ambient, localized temperatures of up to 4,730 °C	Pilot scale	Short-chain PFASs; unknown gas emissions	LC-MS	No
Biological methods[31]	Ambient	Bench scale	Short-chain PFASs	LC-MS	No

[a]Technology scale reflects the current state and operational scale of these technologies. Commercial-scale systems can process much more material than technologies operating at smaller scales (pilot scale and bench scale). CIMS, chemical ionization mass spectrometry; FTIR, Fourier-transform infrared spectroscopy; GAC, granular activated carbon; GC-MS, gas chromatography–mass spectrometry; IR, infrared; LC-MS, liquid chromatography–mass spectrometry; PFASs, per- and polyfluoroalkyl substances; PFCA, perfluorocarboxylates; PID, products of incomplete destruction; TFA, trifluoroacetic acid.

degree of fluorination and/or a higher number of carbon atoms than the parent PFASs. To avoid the recombination of radical species (particularly in incineration), hydrogen sources such as water or natural gas can be added to generate volatile hydrofluoroalkanes, which can further transform through defluorination to simpler organic fluorine species, potentially resulting in complete mineralization to inorganic fluorine species[70]. Calcium hydroxide ($Ca(OH)_2$) is added in some destruction technologies to decrease PID formation[78–80], by providing hydrogen and a means of forming CaF_2 in place of HF (refs. 78–81). The addition of Ca^{2+} can also enhance gasification reactions during GAC reactivation[82].

Various PFAS destruction technologies are available and differ in their PID formation, energy requirements, and availability at scale (Table 1). Although many technologies achieve high DREs[83,84], only a few have demonstrated complete mineralization of PFASs to inorganic fluorine species such as HF, silicon tetrafluoride (SiF_4, which readily reacts with water), fluoride salts (such as CaF_2) and non-fluorinated gases such as CO_2. The nature and extent of PID formation differs across technologies, but very limited data are available as of yet, and additional research is required to directly characterize and quantify PIDs.

Thermal destruction technologies with stacks

Thermal destruction technologies rely on elevated temperatures, often combined with oxidizing or catalytic environments, to break the strong C–F bonds in PFASs. These technologies generally have high DREs (>99.99%), but PIDs can still form, particularly at low temperatures or under suboptimal operating conditions. Because many thermal technologies produce large volumes of heated air and air emissions, their designs typically include tall stacks to release these emissions to higher elevations, promoting broader atmospheric dispersion.

Incineration. Conventional incinerators, including hazardous waste incinerators (HWIs), municipal waste incinerators (MWIs), and sewage sludge incinerators (SSIs), operate with an oxidizing environment at temperatures ranging from about 650 °C to 1,100 °C or more[16,17,32]. Among these, HWIs are the most effective at mineralizing organic contaminants because they operate at high temperatures and consist of multiple combustion stages (see, for example, Fig. 2b). These stages often include a rotary kiln that serves as the primary combustion chamber with a residence time of 10–90 min at ≥650 °C (ref. 85). The exhaust from the primary chamber is sent to an afterburner, a type of thermal oxidizer, that serves as the secondary combustion chamber with a residence time of 2–4 s at ≥950 °C to enhance mineralization of remaining organic compounds and reduce harmful emissions[16,20,70]. In these incinerators, the waste itself can be used as an energy source rather than external fuels. Under typical operating

conditions of a HWI treating waste feeds at a commercial scale, high DREs (>99.9999%) were achieved for selected PFASs, and emissions showed low to non-detectable levels of PIDs[86]. Additionally, in the afterburner, DREs for spiked C_2F_6, a common PID that is difficult to destroy, were >99.99%. A high DRE for C_2F_6 can serve as a potential indicator for effective PFAS destruction[86]. Apart from afterburners, conventional incinerators also include air pollution control systems, such as scrubbers and baghouses, to manage emissions by further destroying or capturing PIDs[20].

Incinerator operating conditions can have a substantial impact on the formation of PIDs. The presence of a hydrogen source such as the waste, hydrocarbon fuel, or steam is needed to promote the mineralization of PFASs to HF; therefore, low hydrogen-donor content can potentially lead to PID formation[21]. A sufficiently high temperature, temperature uniformity (which can be achieved through mixing), and a sufficiently long residence time are also critical for achieving effective PFAS destruction. For example, although the cleavage of PFAS head groups occurs at temperatures ≤500 °C, temperatures of >950 °C are required for complete mineralization of hydrofluoroalkanes, including 1*H*-perfluoroalkanes, to occur in the gas phase and in the absence of catalysts[70]. There is a substantial knowledge gap regarding air emissions from incinerators that operate at lower temperatures, especially SSIs, as PID formation can reach 15% in SSIs and MWIs[17,32].

Thermal desorption (TD) technologies can be applied to contaminated soils when specific geotechnical characteristics of the soil might be required for final disposal or beneficial reuse. TD systems operate at low temperatures (350–600 °C)[87] in the primary combustion chamber to thermally release PFASs and PIDs that are formed at these temperatures. The flue gas is typically sent to an activated carbon bed to remove the released PFASs and PIDs, generating a waste product, or sent to a thermal oxidizer to destroy the released PFASs and PIDs.

Thermal oxidizers are occasionally used as stand-alone systems for treating gaseous waste streams, such as emissions from fluorochemical manufacturing. They operate at high temperatures (>1,000 °C) and are often equipped for heat recovery[88]. Thermal oxidizers were originally designed to destroy volatile organic compounds (VOCs), but they have been adapted for treating PFAS emissions[89]. Although DREs exceeding 99.99% have been reported for thermal oxidizers, comprehensive data on PID formation and fluorine mass balances remain limited for full-scale applications[33]. For example, a study that reported such high DREs tested for only five PFASs[33]; however, a separate test for volatile organic fluorine compounds in the emissions of the same facility detected only CF_4 (ref. 90).

Laboratory-scale investigations reveal that incineration processes produce various airborne PIDs. In controlled laboratory-scale experiments, the thermal decomposition of perfluorooctane sulfonate (PFOS) formed PIDs at temperatures ranging from 500 °C to 1,000 °C, including trace amounts of perfluoroalkenes and carbonyl difluoride (COF_2), which can rapidly form HF and CO_2 in the presence of water[18]. Additionally, the thermal decomposition of fluoropolymers such as polytetrafluoroethene (PTFE) produced PIDs, including perfluoroalkanes, perfluoroalkenes, and hydrofluoroalkanes[91]. Difluoromethyl radical ($\bullet CHF_2$) fragments identified in the PIDs produced from PFOS combustion in complex environmental matrices that contain hydrogen, such as sewage sludges, point to the formation of 1*H*-perfluoroalkanes[78]. In a pilot-scale test, the combustion of AFFF concentrates at low temperatures (760 °C) produced more short-chain PFASs such as trifluoroacetic acid (TFA) than at higher temperatures (820 °C)[37]. The demonstrated formation of PIDs from incineration processes highlights the importance of thermal oxidizers or afterburners in reducing PID emissions from incineration processes and their role in enhancing PFAS mineralization.

Pyrolysers and gasifiers. Pyrolysis and gasification units limit the amount of oxygen available for reactions during thermal treatment. During pyrolysis at temperatures between 500 °C and 800 °C, PFASs volatilize or partially decompose to form PIDs[92,93]. Although PFAS concentrations in the waste might be reduced by one or more orders of magnitude after treatment[92,93], PFASs might accumulate in the pyrolysis oil[94]. Early bench-scale research investigating the thermal decomposition of salts of long-chain perfluorocarboxylates (PFCAs) in a pyrolytic environment found that volatile perfluoroalkenes formed with yields >97% after cleavage of the carboxylate head group from the parent compound[73]. Similarly, perfluoroalkenes have been identified during the decomposition of PFOS at 450 °C to 1,000 °C (refs. 21,71,74). The pyrolysis of substances that contain complex mixtures of PFASs, such as AFFF and fluorosurfactant concentrates, yielded a broad range of PIDs, including perfluoroalkyl aldehydes, - per- and polyfluoroalkenes, per- and polyfluoroalkanes, and fluorotelomer alcohols (FTOHs)[76]. In general, small amounts of 1*H*-perfluoroalkanes have been observed when hydrogen from NaOH or steam ($H_2O_{(g)}$) was present[21,72].

Gasification units operate at higher temperatures than pyrolysis units, typically up to 1,000 °C, with the goal of producing syngas. Given these elevated temperatures, high DREs (>99.99%) have been reported for gasification systems[33]. However, data on PID formation and fluorine mass balances are limited and PID characterization has largely been confined to controlled laboratory studies. Collectively, these findings indicate that PID formation can be expected in pyrolysers and gasifiers with fluorinated alkenes and alkanes being the dominant species.

Cement kilns. Cement kilns operate at very high temperatures (1,200–1,800 °C) in high-pH environments. When PFAS-containing materials are placed directly in cement kilns, high DREs (>99.99%) for PFASs have been observed[22]. Cement kilns offer the advantage of integrating waste destruction within an existing material production process. PID formation is expected to be low because the high operating temperatures exceed those typically achieved by afterburners or thermal oxidizers. However, further research is needed as only limited field-scale data are available, and no measurements of potential PFAS-derived PID formation have been attempted[22].

GAC reactivation furnaces. Spent GAC is commonly reactivated in furnaces operating at 700 °C to 950 °C, typically with afterburners or thermal oxidizers being used to destroy PFASs present in the flue gas[23]. A full-scale GAC reactivation facility reported DREs >99.99% for PFASs after the thermal oxidizer; however, sampling and quantification of emissions and residuals only accounted for 61% of the fluorine mass balance[95]. Additionally, without a thermal oxidizer, up to 10–20% PID formation has been observed[23,96,97]. A bench-scale study found that PFAS mineralization was enhanced when PFASs were adsorbed to GAC, relative to treating non-adsorbed PFASs, and that this mineralization was further enhanced by the presence of NaOH (refs. 23,96). However, the fluorine mass balance was not closed[23,96]. Tentatively identified PIDs from these systems include perfluoropropene from perfluorobutanoate (PFBA) and perfluoro-1-butene from perfluoropentanoate (PFPeA)[36].

Closed-vessel systems

In addition to conventional thermal treatment methods, several emerging thermal technologies using closed vessels have been developed (Fig. 2c). These vessels are typically closed to maintain higher temperatures and pressures with PFAS-impacted water, so any PIDs formed are released when the effluent from these systems is vented into a receiving storage vessel at ambient pressure.

Hydrothermal alkaline treatment. Hydrothermal alkaline treatment is a closed-system process that can mineralize PFASs using alkaline conditions at moderate temperatures (300–350 °C). The process is performed under elevated pressures (2–22 MPa). Hydroxide additives enhance the reactivity of PFASs, generally leading to stoichiometric fluoride ion generation and complete fluorine mass balances[98]. There is no evidence that PFAS-derived PIDs are formed under optimal conditions; however, more extensive testing is needed for confirmation and to define limitations that would lead to suboptimal treatment such as matrix effects, co-contaminants and reaction times[98]. PID formation can occur if there is insufficient reaction time or if the base additive concentrations are too low[99]. There are limited field data on hydrothermal alkaline treatment, and its scalability remains under evaluation[24], largely because it is difficult to produce reactor materials that can withstand the high temperatures, high pressures and corrosive alkaline conditions involved.

Supercritical water oxidation. Supercritical water oxidation (SCWO) systems operate above the supercritical point of water (>374 °C, >22 MPa), enabling the homogeneous oxidation of PFASs to CO_2, water and fluoride. Temperatures of more than 650 °C are required to achieve complete defluorination of recalcitrant PFASs[25]. SCWO has high DRE potential (>99.9%) and can be designed to produce minimal air emissions by ensuring condensation of water vapour upon release from the pressurized vessel[100]. However, high capital and operational costs, along with the need for corrosion-resistant materials, present challenges for implementation. Additional work is needed to characterize PID formation, although initial results are encouraging. For example, in a pilot-scale study, destruction of AFFFs by SCWO generated only trace levels of aqueous PIDs. Gas-phase PIDs including perfluorohexane, perfluoroheptane and some chlorinated–fluorinated alkanes, were also detected at concentrations <300 μg m^{-3} (ref. 100).

Open-vessel systems

Thermal and non-thermal destruction technologies are being developed to target PFAS destruction through chemical, electrical, or biological means in open-vessel systems (Fig. 2d). These systems, which typically operate at ambient pressures and are thus open to the atmosphere, aim to mineralize PFASs with lower energy inputs and to realize easier integration with existing treatment systems. However, many open-vessel approaches remain in early development stages, with limited full-scale deployment[101].

Advanced oxidation processes. Advanced oxidation processes (AOPs) use highly reactive radicals (for example, sulfate radicals) to oxidize PFASs. These processes typically degrade PFASs into short-chain PFCAs and gaseous PIDs such as fluorinated alkanes. Radicals can be generated in various ways, including through the photolytic or thermal activation of radical precursors such as persulfate, through photocatalysts, and through electrochemical reactions. Conventional oxidants such as ozone and hydrogen peroxide are generally insufficient to decompose PFASs[102]. Some AOPs can partially oxidize PFCAs, but oxidation of perfluoroalkyl sulfonates (PFSAs) is more difficult[29]. Precursors of perfluoroalkyl acids (PFAAs) are generally more susceptible to oxidation but often result in the formation of more recalcitrant PFAAs[103]. AOPs often require high energy input and specific conditions such as high or low pH, the presence of certain chemical reagents, the absence of radical scavengers, and carefully optimized operational parameters[29].

The formation of PIDs in AOPs is common but is not yet well understood. Although most studies focus on production of aqueous PIDs, gaseous PIDs such as perfluoroalkanes have also been identified[30]. For example, oxidation of PFOA with sulfate radicals at room temperature produced a complex mixture of short-chain PFCAs and 18 distinct perfluoroalkane species[30]. The detected perfluoroalkanes constituted up to 25% of the converted PFOA and were predominantly linear with various chain lengths, although branched isomers and traces of 1*H*-perfluoroalkanes (<0.1 mol%) were also detected[30]. Higher temperatures favoured mineralization: oxidation at 70 °C generated only short-chain PFCAs and fluoride, suggesting more complete degradation and reduced formation of gaseous PIDs, although the fluorine mass balance remained incomplete[104]. Incomplete fluorine recovery has also been reported for other advanced oxidation methods, such as photocatalysis and electrochemical oxidation[105,106].

Advanced reduction processes. Advanced reduction processes (ARPs) use strong reducing species such as solvated electrons. These processes, with DREs of over 99.9%, typically defluorinate PFASs by reductively cleaving the C–F bonds to form fluoride, shorter-chain PFASs and polyfluorinated compounds[28]. Although ARPs can make PFASs more biodegradable, achieving complete mineralization is challenging. Additionally, the process can generate PIDs, including fluorinated alkanes and alkenes[107]. For example, volatile iodinated PIDs such as $CFHI_2$, C_2F_4HI and $C_6F_{13}I$ were produced in the reduction of various PFASs using ultraviolet irradiation with iodide, which are presumably from the incorporation of iodine radicals into intermediate products[28]. Under the same conditions, PFOS produced more diverse volatile iodinated intermediates than PFOA[28,108,109], indicating that headgroup chemistry can have important implications with respect to the types of PIDs formed when PFASs are subjected to ARPs.

Non-thermal plasma-based technologies. Non-thermal plasma-based technologies use hydrated, high-energy electrons and radicals produced by plasma at the gas–liquid interface to cleave the C–F bond and cause PFAS fragmentation. These technologies have demonstrated high (>90%) DREs for PFOA and PFOS, but DREs can be reduced by co-contaminants such as salts[110]. Although pilot demonstrations exist, commercial systems are rare[27]. Additionally, understanding of byproduct formation is still evolving, but it seems that the formation of volatile fluorinated cyclic alkanes and short-chain acids is common. Gaseous byproduct formation during plasma treatment of PFOA and PFOS was shown to depend on the exposure time. Concentrations of cyclic perfluoroalkanes (C_4F_8 to C_8F_{16}), which probably formed via intramolecular free-radical cyclization, peaked at 30 minutes (reaching up to 110 μg l^{-1} from an initial 8.3 mg l^{-1} PFOA), then decreased with continued treatment[111]. The concentrations of the airborne byproducts accounted for less than 0.5% of the initially added organic fluorine and were 1–2 orders of magnitude lower than those of the aqueous PIDs[111].

Sonolysis. Sonolysis, which generates localized high temperatures through cavitation, can destroy PFASs with DREs of 50 to >99%[26].

Acoustic fields with frequencies between 20 and 1,000 kHz are applied to produce microbubbles that create extreme localized conditions (~4,730 °C and 2×10^8 Pa) when they collapse, leading to the pyrolysis of water vapour and the formation of reactive species that facilitate PFAS destruction[112]. PFAS destruction primarily occurs via pyrolysis at gas–water interfaces[26]. Although shorter-chain PFCAs can be formed in the aqueous phase[113], potential gas-phase and aerosol-phase emission products of PFASs from sonolysis have not been characterized. This technique consumes very large quantities of electricity, so its potential applications are currently limited to treating highly concentrated, small-volume waste streams[110].

Biological processes. Specialized microorganisms can slowly transform some PFASs under specific conditions, leading to partial defluorination and transformation rather than complete mineralization to CO_2 and fluoride. PIDs can form as PFASs transform into aqueous short-chain or partially fluorinated compounds. Minimal gaseous products are usually formed and typically consist of only CO_2 and methane (CH_4) from substrate consumption[31].

Summary

To summarize, existing PFAS destruction technologies have different strengths and weaknesses (Fig. 3). Thermal methods are the most commonly used PFAS destruction techniques, owing to their effectiveness and implementability within the existing waste infrastructure. However, these approaches typically require high temperatures, substantial energy input, and often dedicated secondary air pollution controls to minimize environmental impacts. Non-thermal approaches might offer lower energy demands and, in some cases, more selective reaction pathways, but they frequently rely on the addition of chemical reagents, and further validation is required to ensure consistent PFAS destruction and control of secondary emissions.

Characterization of PIDs

Methods for characterizing gas-phase and aerosol-phase PIDs are still in the relatively early stages of development, making it difficult to fully understand the PID formation potential of PFAS destruction technologies. High-temperature combustion can achieve PFAS destruction, but it is unclear whether this process can form new fluorinated or mixed halogenated byproducts. Other technologies can exhibit high DREs for certain PFASs, but in many cases, little is known about the extent of mineralization and PID formation. Moreover, DREs alone are insufficient to assess PFAS destruction, because this metric does not ensure the absence of gas-phase or aerosol-phase emissions or residuals containing potentially high risk PIDs that could have an impact at low concentrations. This section discusses the categorization of airborne PIDs and approaches for PID identification and monitoring during PFAS destruction.

Categorizing PFAS-derived PIDs

The wide range of potential PID species combined with concerns about the operation of PFAS destruction processes at suboptimal conditions has spurred efforts to measure specific PIDs. To achieve such measurements, it is important to categorize PIDs, because different analytical approaches must be developed to target different chemical species.

In general, PFAS-derived PIDs can be categorized, at least for the purposes of their chemical determination, according to their polarity and volatility into the following groups: polar volatile, polar semivolatile, non-polar volatile, and non-polar semivolatile (Fig. 4). Examples of PFAS-derived PIDs that represent polar semivolatile fluorinated compounds are PFAAs with four or more carbon atoms. In contrast, examples for non-polar semivolatile PIDs include highly fluorinated alkanes and alkenes with boiling points above about 100 °C. Polar volatile fluorinated compounds are characteristically smaller and include ultrashort-chain PFASs such as TFA. Gaseous non-polar volatile fluorinated PIDs include fluorinated alkanes (C1–C8) and alkenes (C2–C7). Additionally, some PIDs represent intermediates that can further transform; for example, perfluorinated acyl fluorides hydrolyse to form PFCAs in the presence of liquid water[70]. Different sampling and analysis techniques are needed to identify PIDs in each of these categories, owing to the physicochemical properties of each of the PIDs.

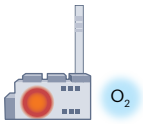
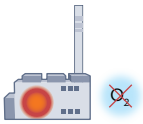
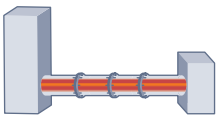
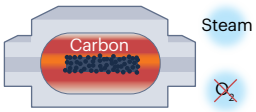
Characterization methods

Efforts to develop an understanding of air emissions from PFAS destruction technologies primarily focus on off-line analytical methods, in which a sample is collected and taken to a laboratory for analysis. These analytical methods for gas-phase and aerosol-phase PIDs can be used for regulatory compliance. Such off-line methods are essential for characterizing potential PIDs[67,114], and quantifying organic and inorganic fluorinated target compounds to determine the fluorine mass balance during PFAS destruction processes[79,96,115,116]. The US Environmental Protection Agency (EPA) has developed additional methods under Other Test Methods (OTMs) for fluorinated organic compounds that are intended to serve as comprehensive tools for characterizing gas-phase and aerosol-phase emissions. Each of these methods has strengths and weaknesses, and some modifications to these methods might be warranted to increase confidence in their ability to comprehensively assess PIDs associated with PFAS destruction (Box 2). To date, use of these OTMs to explore the fate of PFASs in PFAS destruction technologies has been limited.

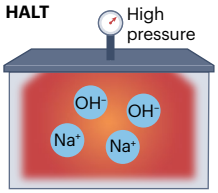
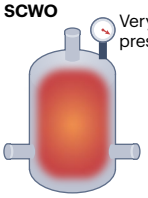
OTM-45. OTM-45 is a standardized method for sampling and analysing polar fluorinated compounds (ionic PFASs). This method was developed to quantify 49 PFASs from stationary sources[117]. It consists of a multicomponent sampling train (involving a filter to capture aerosol-bound PIDs and XAD-2 resins and impingers containing basic water to capture gaseous PIDs) and is modelled after US EPA Method 23 (ref. 118). OTM-45 relies on targeted liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) to determine both gas-phase and aerosol-bound ionic PFASs. The generated extracts can also be used for non-targeted analysis to identify additional PIDs. The extraction and analysis protocols in OTM-45 are influenced by other standardized methods for PFAS analyses, including US EPA Methods 533, 537.1, 1633 and 8327, and ASTM Method D7968[118].

OTM-45 has been used to evaluate PID formation and determine DREs of PFASs in various PFAS-impacted waste streams[17,38,116,119]. For example, OTM-45 stack sampling was used in both a full-scale thermal reactivation study to investigate DREs for PFASs adsorbed to GAC[116] and by the US EPA in pilot-scale thermal destruction experiments with PFAS-based AFFF. Both studies reported DREs of >99.99%[38,116]. A modified OTM-45 sampling train was used to collect emissions during the use of SCWO to mineralize concentrated liquid PFAS waste streams and found equally high DREs[119]. However, only 63% of the fluorine was accounted for, which was likely a result of data being limited only to the PIDs targeted by OTM-45. In a separate effort, emissions from a full-scale SSI were collected using a method based on OTM-45 and analysed for total organic fluorine using combustion ion chromatography and for ionic PFASs[17]. No specific PFASs from

Thermal systems with stacks

	Incinerators	Pyrolyser and gasifiers	Cement kilns	GAC reactivation furnaces
				
PID formation	Low	Low	Low	Low
Temperature	>650 °C	>500 °C	>1,200 °C	>700 °C
Energy consumption	High	High	High	High
Additional chemicals	No	No	No	Yes
Degradation	Non-selective	Non-selective	Non-selective	Non-selective
Air pollution control	Yes	Yes	Yes	Yes

Closed-vessel systems

	HALT	SCWO
		
PID formation	Medium	Low
Temperature	>300 °C	>650 °C
Energy consumption	Medium	Medium
Additional chemicals	Yes	Yes
Degradation	Selective	Non-selective
Air pollution control	No	Yes

Open-vessel systems

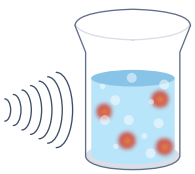
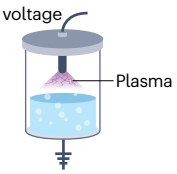
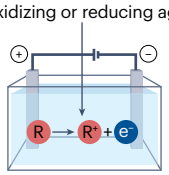
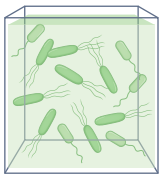
	Sonolysis	Plasma-based methods	AOPs and ARPs	Biological methods
				
PID formation	Medium	Unknown	Medium	High
Temperature	Localized high temperature	Ambient	Ambient	Ambient
Energy consumption	High	Low	Low	Very low
Additional chemicals	No	No	Yes	Yes
Degradation	Non-selective	Non-selective	Selective	Selective
Air pollution control	No	No	No	No

Fig. 3 | Classification of PFAS destruction technologies. An overview of conventional thermal technologies with stacks and emerging thermal and non-thermal technologies with closed or open vessels used for the destruction of per- and polyfluoroalkyl substances (PFASs), highlighting the formation of gas-phase or aqueous-phase products of incomplete destruction (PIDs), temperature, energy consumption, the need for additional chemicals (such as NaOH, oxidants, solvents), selectivity for PFASs and other halogenated organic substances, and whether additional methods of air pollution control are required to treat waste streams to meet regulatory permits[4,20,26,27,29,31,33,98,100,101,105,106,108,110,181]. The energy consumption indicated does not account for energy contributions from potential energy-recovery systems such as those used in incinerators. Common PFAS destruction methods are classified by their thermal and operational characteristics, with each technology evaluated on key criteria that can affect PID formation. AOP, advanced oxidation process; ARP, advanced reduction process; GAC, granular activated carbon; HALT, hydrothermal alkaline treatment; SCWO, supercritical water oxidation.

the targeted methods could be identified as contributing to the total organic fluorine measurements, suggesting that other fluorinated PIDs were being formed.

OTM-50. OTM-50 can characterize PFAS-derived PIDs that are non-polar and volatile. It is similar to US EPA Methods TO-15A and 327 in that evacuated canisters are used to capture a gaseous sample that

is analysed via gas chromatography–mass spectrometry (GC-MS)[118]. In OTM-50, gas samples pass through a heated filter (which removes particulates) and an optional series of impingers containing deionized water (which remove acidic gases such as HCl and HF) before being sampled into the canister. The method targets 30 PIDs with one to eight carbon atoms, including persistent gases, such as CF_4 and C_2F_6, and longer-chain perfluoroalkanes, such as perfluorooctane. Although the method is based on targeted GC-MS analysis, non-targeted GC-MS analyses can also be performed on OTM-50 canister samples to tentatively identify PIDs not quantified in this method[38,120]. If analytical standards are commercially available for tentatively identified PIDs, the identity of such PIDs can be validated, and they can be subsequently incorporated into the target analyte list.

OTM-50 was introduced in 2024; therefore, most prior research characterizing non-polar volatile PFAS-derived PIDs used earlier methods such as TO-15A and was largely exploratory. For example, the collection of gas-phase emissions with TO-15A during the pyrolysis of PFAS-impacted biosolids found that the concentrations of all 17 targeted volatile fluorinated PIDs were below the method detection limits[121]. Despite this finding, it was concluded that the potential for the formation of semivolatile PFAS-derived PIDs raises concerns and warrants further investigation[122]. During a field demonstration of a mobile SCWO system treating AFFF, gaseous effluents were collected using a modified TO-15A sampling train, revealing trace amounts of perfluoroalkanes[100]. Additionally, fluorinated gas-phase emissions collected with TO-15A during the thermal application of fluoropolymer dispersion coatings on fabrics at temperatures up to 400 °C suggested that the dispersions underwent transformation to C1–C8 fluorocarbons including tetrafluoroethylene (TFE) and heptafluoropropyl-1,2,2,2-tetrafluoroethyl ether (fluoroether E1)[120].

Since its release, OTM-50 has been applied in several studies to characterize emissions from PFAS destruction technologies. For example, OTM-50 demonstrated that CF_4 was consistently found in the emissions of a thermal oxidizer unit at a fluorochemical manufacturer[90]. Furthermore, application of OTM-50 showed that all C1–C8 1*H*-perfluoroalkanes were formed at concentrations of 0.2–9 mg m^{-3} during the incineration of PFOS-based AFFF at 810 °C, a non-ideal condition at which DREs ranged from 45.7% for PFBA to >99.97% for most of the targeted PFSAs[38]. At a temperature of 1,090 °C, PID formation decreased substantially, with only pentafluoroethane and trichlorofluoromethane detected at concentrations <1 μg m^{-3}, and DREs were >99.99% for all targeted PFAAs[38]. Such findings demonstrate that it is important to characterize PIDs, particularly non-polar volatile PFAS-derived PIDs, when assessing the performance of destruction technologies. Importantly, to enable the characterization of all gas-phase PFAS-derived PIDs potentially emitted during PFAS destruction, the target analyte lists of OTM-45 and OTM-50 could be expanded to include additional PIDs that have been identified in other investigations (such as perfluoroheptene isomers)[121].

Additional methods. Although OTM-45 and OTM-50 cover a broad range of potential PIDs, a method is still needed to collect and analyse non-polar, semivolatile PFASs such as FTOHs[123,124] and other longer-chain PFAS-derived PIDs[73]. OTM-55 is under development and is expected to combine elements of US EPA Methods 8270E and 0010[125]. Early versions of this method are being incorporated into current evaluations of PFAS destruction technologies[126]. For example, a GC-MS method, modelled after US EPA Method 8270E (SW-846), was used to target five FTOHs in emissions from the incineration of PFAS-laden sewage sludge; none of the samples yielded reportable levels of FTOHs[17]. The lack of a standardized sample collection and analysis method is likely, at present, to be a major impediment to broader efforts to characterize this subclass of PFAS-derived PIDs.

Furthermore, a standard method is still needed to characterize polar volatile PIDs such as TFA. LC-MS/MS methods for the analysis of TFA and other ultrashort-chain PFASs are available[127], but sampling

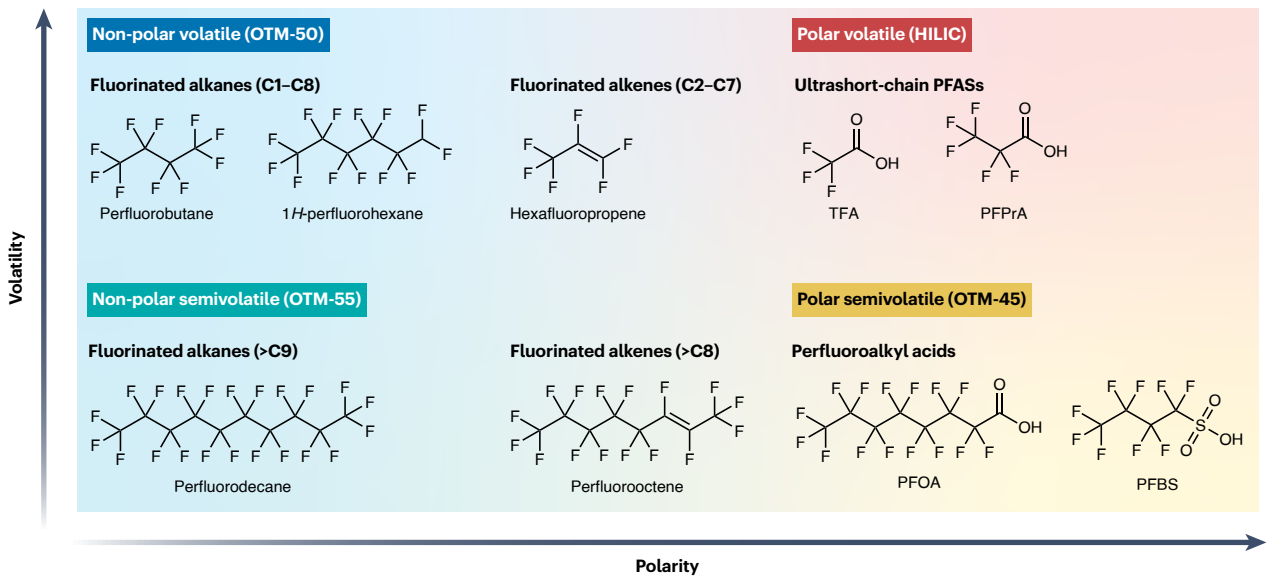


Fig. 4 | Categorizing PIDs. Categories of products of incomplete destruction (PIDs) from per- and polyfluoroalkyl substances (PFASs) based on their polarity and volatility. The analytical methods used to measure PIDs from each category are stated in parenthesis; note that OTM-55 is still under development. Different analytical approaches are needed to target different chemical species. HILIC, hydrophilic interaction liquid chromatography; OTM, Other Test Method; PFBS, perfluorobutanesulfonic acid; PFOA, perfluorooctanoic acid; PFPrA, perfluoropropionic acid; TFA, trifluoroacetic acid.

Box 2 | Analytical methods for PID characterization

OTM-45

Strengths

- Currently includes 49 targeted per- and polyfluoroalkyl substances (PFASs) (C4 and larger)[117]
- Quantifies the targets through isotope dilution, which provides highly accurate quantitative data
- Analysis largely derived from US Environmental Protection Agency (EPA) Methods 533, 537.1 and 1633; this aids in wider adoption by commercial labs and simplifies interpretation[197–199]
- Performance based, and thus can be modified without substantial effort as analytical technologies improve

Weaknesses

- Complex sampling train and only snapshots of emissions
- Potential contamination of targeted analytes due to the complex sampling train, potentially leading to false positives[118]
- Acyl fluorides are converted to perfluorocarboxylates in aqueous impingers, which limits any ability to distinguish between these two classes of compounds[70]
- Analytical costs can be high, leading to less-frequent sampling

Potential modifications

- Non-targeted analysis of extracts can be used to determine additional products of incomplete destruction (PIDs)
- Integration with OTM-50 sampling train lines to enable simpler sample collection
- Sample collection protocol for closed and open reactor systems

Method 320/ASTM D6348

Strengths

- Obtains real-time Fourier-transform infrared spectroscopy data[129,131]
- Can be used to monitor CF_4 and C_2F_6 emissions to provide an indicator of effective PFAS destruction and PID emission
- Can measure inorganic and organic compounds, such as HF, CO_2 and volatile organic fluorine

Weaknesses

- Poor sensitivity unless a very long path cell is used
- Moisture can interfere and impact sensitivity
- Fluorinated compounds can have overlapping peaks
- Reference spectra needed for all potential compounds in the sample

Potential modifications

- Increase path cell length (>100 m) to improve sensitivity
- Use deconvolution and machine learning techniques to circumvent the issue of overlapping peaks

OTM 50

Strengths

- Currently includes 30 known and suspected PIDs[118,200]
- Considers non-targeted analysis to identify new compounds and incorporate them into the target list[38,120]

Weaknesses

- Expansion of current target list limited by the availability of commercially available standards
- Requires specialized instrumentation for analysis
- Analytical costs can be high, leading to less-frequent sampling
- Only snapshots of emissions

Potential modifications

- Include standardized guidance for the identification and incorporation of tentatively identified compounds
- Include guidance on the use of surrogate compounds, such as CF_4 and C_2F_6, as an indicator of effective PFAS destruction and PID emission
- Integration with OTM-45 sampling train lines to enable simpler sample collection

Chemical ionization mass spectrometry

Strengths

- Real-time measurement[143,144]
- High sensitivity[141,147]
- Can do targeted and non-targeted (time-of-flight mass spectrometry only) analysis[146,147]

Weaknesses

- Compound detection is dependent on the reagent gas used
- The generation of quantitative gas-phase standards for semivolatile compounds for calibration is challenging
- No commercial vendor available for field testing
- Requirement of specialized system and high-expertise users will add to costs

Potential modifications

- Design a system with multiple reagent gases to avoid needing to switch

trains need to be evaluated for their efficiency in quantitatively sampling polar volatile PIDs from emissions generated by PFAS destruction technologies.

Real-time analytical methods

Although off-line methods are often used for regulatory compliance sampling, more frequent and (near) real-time analysis of gaseous streams is sometimes helpful for pilot-scale and full-scale evaluations to enable real-time adjustments to operational parameters. Such measurements can be achieved with in-line and on-line methods, which continuously measure the actual gas or side (slip) stream of interest, respectively. There are two promising commercial analytical techniques that have been developed and partially validated for PFASs and PFAS-derived PIDs: Fourier-transform infrared (FTIR) spectroscopy (Method 320/ASTM D6348) and chemical ionization mass spectrometry (CIMS).

FTIR spectroscopy can be used for semiquantitative analysis of incinerator stack emissions[128–130]. The C–F bond absorbs infrared radiation strongly from ~900 cm^{-1} to 1,500 cm^{-1}, enabling the use of FTIR to identify and quantify some PFAS-derived PIDs[131,132]. This approach is a viable candidate for the analysis of gas-phase PIDs and initial laboratory-scale efforts to examine PFAS-derived PIDs relied on infrared spectral analysis[73]. FTIR can also be used for regulatory compliance sampling, particularly for emissions of fluorocarbons containing one to two carbons[129]. Advances in data processing algorithms could enable real-time analysis[133–137]. Additionally, the analytical sensitivity of the technique could be increased by increasing the cell path length, but this development often reduces the temporal resolution. Efforts are being made to establish FTIR reference spectra for PFAS-derived PIDs to increase the accuracy and sensitivity of the measurements[118,131].

CIMS is a commonly used and potentially field-deployable tool for the analysis of volatile and semivolatile compounds in air[138–142]. In CIMS, gas-phase compounds react with a reagent gas to form an ion that can be detected by mass spectrometry[143,144], with different reagent gases being used to selectively ionize different compounds. A specific type of commercially available CIMS system, chemical ionization time-of-flight mass spectrometry (CI-TOF-MS), can acquire data for a broad range of compounds (FTOHs, PFCAs, hexafluoropropylene oxide dimer acid (HFPO-DA) and HF) very quickly (within 100 ms) and can detect target and non-target chemicals. CIMS[145,146] and specifically CI-TOF-MS[147–149] systems have been used for PFAS analysis with various reagent gases and ionization approaches[147–154]. CI-TOF-MS is attractive as a tool for in-line or on-line analysis of gaseous PFASs and PFAS-derived PIDs owing to its low detection limit (1.4 pptv)[147] and the broad range of compounds that can be detected[37,147,151,152]. The success of CIMS depends on the choice of reagent gas and the development of more reliable and robust approaches and algorithms for the quantitative detection of semivolatile compounds.

Other emerging mass spectrometry methods, some of which are field portable[155,156], can theoretically perform real-time measurements. However, these techniques have limited quantitative capabilities or have been limited in their application to PFAS-derived PIDs. Ambient ionization, which can be performed under ambient atmospheric conditions, has emerged as a potential means to achieve real-time analyses[157–160] through approaches such as desorption electrospray ionization[161] and direct analysis in real time (DART)[162]. For example, the DART approach has been used to characterize volatile PFASs in situ[163]. Other approaches using ambient ionization, such as dielectric barrier discharge ionization[164,165], low-temperature plasma ionization[166,167] and microwave-induced plasma ionization[168], have been used to detect gas-phase and volatile organic compounds for alternative applications. Although these systems have not yet been applied to gas-phase PFASs, they can provide suitable ionization sources for in-line, on-line and off-line analysis. Secondary electrospray ionization has also been used for ambient vapour analysis[169–174]. When combined with mass spectrometry, this approach can achieve extremely low detection limits[175] and can be applied to environmental analyses[176,177], suggesting that it could be a strong contender for analysing gaseous PFAS-derived PIDs.

Environmental and toxicological impacts of PIDs

Owing in part to the strength of their C–F bonds, PFAS-derived PIDs can be persistent in the atmosphere, capable of long-distance transport, and subject to complex transformation and degradation pathways. The environmental behaviour, receptor exposure, and toxicological impacts and therefore the risk of PFAS-derived PIDs are largely unknown. However, insights can be gained from extensively studied compounds, specifically perfluoroalkanes such as CF_4 and C_2F_6, and the perfluoroalkene tetrafluoroethene (C_2F_4). Perfluoroalkanes predominantly originate from industrial sources and are persistent gases with extended atmospheric half-lives of the order of 10^5 years[178]. In contrast, perfluoroalkenes have much shorter atmospheric half-lives, typically hours or days (US National Toxicology Program).

Exposure to PFAS-derived PIDs via inhalation is a risk for communities living near PFAS destruction technologies and PID-producing industries (Fig. 1). Inhalation toxicity assessments for PFAS-derived PIDs are very limited[118]. Although exposure to perfluoroalkanes can cause asphyxiation when they displace enough oxygen, with the risk increasing for heavier molecules, their general chemical toxicity is considered low[179]. There is also some evidence that perfluoroalkenes could be human carcinogens (US National Toxicology Program).

The known environmental and toxicological impacts highlight the importance of the development and optimization of effective PFAS destruction technologies. The goal of these technologies should be to avoid the emission of PFAS-derived PIDs with long-term environmental persistence that could pose a risk to human and ecological receptors. To achieve this goal, further efforts are needed to optimize operating conditions, reactor design, and the management of emissions to guide the decomposition process toward complete mineralization.

Summary and future perspective

There is an urgent need to destroy PFASs in materials such as AFFF, contaminated environmental matrices, and residuals from non-destructive concentration technologies that remove PFASs from air, water, and soil. Numerous incumbent and emergent technologies can achieve high DREs for PFASs known to be present in affected materials. However, DREs alone are insufficient to ensure the absence of harmful air emissions and residuals from destructive technologies. There is computational and experimental evidence that PIDs with a wide range of physicochemical characteristics can form during PFAS destruction. Therefore, multiple off-line, in-line, and on-line methods have been developed or are under development to comprehensively characterize gas-phase and aerosol-phase emissions from PFAS destruction technologies. Despite these advances, understanding of what PIDs are produced, and of their risks to humans and the environment, remains limited.

There are very limited data available so far on air emissions and residuals from PFAS destruction technologies. It is important, therefore, to use existing offline and in-line analytical methods to provide confidence that destructive technologies effectively destroy PFASs while limiting PID formation. Additionally, robust data collected under real-world conditions that evaluate DREs and the presence of PIDs will help to identify viable options for PFAS destruction. To this end, the US EPA has released interim guidance on the destruction and disposal of PFASs and materials containing PFASs[85]. It focuses on the current state of the science for large-scale technologies that can destroy PFASs and includes a framework for evaluating emerging technologies for PFAS destruction and disposal. The EPA is required to update the guidance as appropriate, but no less frequently than every three years.

Data for PFAS destruction technologies should be collected at optimal and suboptimal operating conditions to understand the potential for harmful emissions under various conditions, including system failures. Furthermore, existing data and data being collected in ongoing and future research efforts should be deposited in a database. Such a database would

enable meta-analyses to identify the most promising PFAS destruction technologies and potential risks associated with system failures.

It is important to understand the identity of PFAS-derived PIDs to ensure that air pollution control devices are designed and operated to manage undesirable PIDs. Although several methods exist to characterize gas-phase and aerosol-phase emissions from PFAS destruction technologies, they do not cover the entire spectrum of potential PIDs that might form. Therefore, standard methods are needed for non-polar semivolatile PFASs (such as anticipated with OTM-55) and polar volatile PFASs such as ultrashort-chain PFASs, including TFA. Analyte lists for targeted methods, such as OTM-45 and OTM-50, could also be expanded based on findings from non-targeted analysis workflows. Such methods should be complemented by approaches to measure the total gaseous fluorine to assess whether targeted methods are missing important PFAS-derived PIDs[180]. In addition, real-time (or near real-time) methods should be developed and implemented to assess process performance, particularly for identifying compounds that are indicators of system failure or suggest that a system is operating at suboptimal conditions.

The human health and environmental risks associated with gas-phase emissions of PFAS-derived PIDs remain poorly understood. The toxicity of individual PIDs, mixtures of known PIDs, and whole gas-phase emissions should be assessed through various approaches, including effect-directed analysis to identify PIDs of toxicological relevance; screening with high-throughput methods to identify important toxicological endpoints; and toxicity testing to establish reference doses for individual PIDs. Additionally, the potential for environmental harm associated with the operation of and emissions from PFAS destruction technologies needs to be determined. Gases such as CF_4 and C_2F_6 are common byproducts of multiple technologies and are hard to avoid. Therefore, life cycle analyses should be conducted to assess the environmental cost or benefit of PID destruction: for example, comparing the environmental impacts of CF_4 emissions with the emissions from additional energy required to fully destroy CF_4.

Finally, there is a clear need to communicate what is known and what remains unknown with potentially affected communities. Clear and transparent communication should focus on existing methods for PFAS-derived PID sampling and analysis, and the performance of current waste management technologies to provide a comparison baseline for emerging technologies. Such focused communications can also aid in explaining what is currently known about airborne emissions, and how this knowledge can be used in identifying sources of exposure to PFASs and their relative risk. Standard methods should be used to determine PFASs and PFAS-derived PID concentrations in the ambient air of communities near facilities that are manufacturing, using or destroying PFASs. Coupled with source apportionment and risk assessment, this information would be important for community outreach. Although PFAS mineralization can require high energy input to destroy potential PIDs, minimizing the overall human and environmental impacts is paramount to maximizing risk reduction from PFAS destruction.

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S. Silsby provided overall coordination for the Review. S. Silsby, S. Sühnholz, D.R.U.K. and C.P.H. developed the concept for the Review. Initial draft writing was conducted by S. Silsby, S. Sühnholz, M.Q., K.D., C.Y., D.R.U.K. and C.P.H. Figures were drafted by S. Silsby, S. Sühnholz, M.Q. and N.S. All authors contributed views and opinions, and reviewed the final manuscript.

Competing interests

R.S.G. is an employee of Alcoa Corporation, which is involved in bauxite mining, alumina refining and aluminium smelting operations worldwide. R.S.G. is an inventor of patents US9795920B2, US9327237B2, US8894748B2, US8137649B2, US7906089B2, US7645430B2 and EU2265357 in the area of gas exhaust treatment as well as patents US8673152B2, US8206586B2, US8157995B2, US7897049B2 and US5837145 in the area of water treatment. C.P.H., T.J.S. and S.H. are inventors on patent US11577111 related to the use of hydrothermal alkaline conditions for PFAS destruction. T.J.S. is also a paid technical advisor for Aquagga, Inc., which has licensed patent US11577111. The remaining authors declare no competing interests.

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Per- and polyfluoroalkyl substances (PFAS) distribution in landfill gas collection systems: leachate and gas condensate partitioning

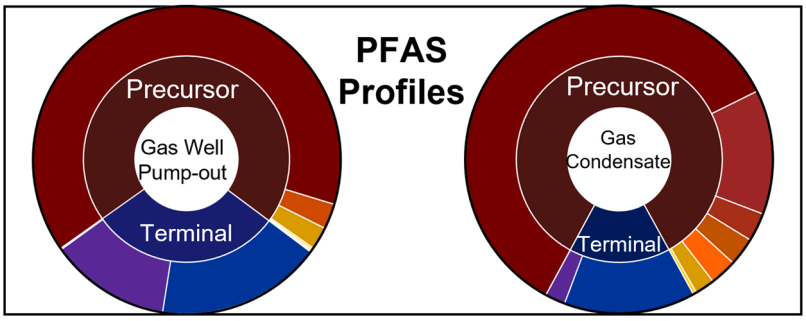
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HIGHLIGHTS

- Two leachates were collected from landfill gas systems: condensate and well pumpout.
- Gas condensate is not colored, contains ammonia, alkalinity, and some metalloids.
- Gas well pump-out is dark in color, resembles leachate, high chloride concentrations.
- Condensate has lower total PFAS abundance, but greater diversity of species.
- PFAS which are more abundant in condensate include semi-volatile FTOHs and FASEs.

GRAPHICAL ABSTRACT



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ABSTRACT

While per- and polyfluoroalkyl substances (PFAS) have been reported extensively in municipal solid waste (MSW) landfill leachate,they have rarely been quantified in landfill gas or in discrete landfill liquids such as landfill gas condensate (LGC), and the potential for PFAS to partition to the condensate has not been reported. LGC and leachate collected from within gas wells known as gas well pump-out (GWP) from three MSW landfills underwent physical-chemical characterization and PFAS analysis to improve understanding of the conditions under which these liquids form and to illuminate PFAS behavior within landfills. LGC was observed to be clear liquid containing ammonia and alkalinity while GWP strongly resembled leachate – dark in color, high in chloride and ammonia. Notably, arsenic and antimony were found in concentrations exceeding regulatory thresholds by over two orders of magnitude in many LGC samples. LGC contained a lower average concentration of ΣPFAS (19,000 ng L) compared to GWP (56,000 ng L); however, LGC contained more diversity of PFAS, with 53 quantified compared to 44 in GWP. LGC contained proportionally more precursor PFAS than GWP, including more semi-volatile PFAS which are rarely

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measured in water matrices, such as fluorotelomer alcohols and perfluoroalkane sulfonamido ethanols. This study provides the first detailed comparison of these matrices to inform timely leachate management decisions.

Environmental Implications Statement

The US EPA is proposing to designate two PFAS (PFOA and PFOS) as hazardous substances. While both were phased out of production decades ago, they continue to enter landfills through consumer waste and are the degradation product of precursor PFAS. PFAS have been measured consistently at high concentrations in landfill leachate; however, little is known about PFAS behavior under full-scale landfill conditions. Here, we characterize PFAS and other constituents in two distinct liquids collected from landfill gas systems to explore each liquid's origin, inform the most appropriate management strategy, and to identify possible sources of PFAS leaving landfills.

Data availability

Data will be made available on request.

1. Introduction

Waste decomposition within a landfill produces gas (landfill gas; LFG) consisting primarily of methane and carbon dioxide, which are often collected and either flared to oxidize methane or processed into a renewable fuel source. During the vacuum collection of LFG, gas from the warm, moist interior of the landfill comes into contact with ambient air temperatures, and that water vapor condenses to form a liquid which can accumulate in the landfill gas collection and control system (GCCS). GCCS liquids are often broadly described as gas condensate [6], but true leachate, derived from within the waste mass itself, which collects in the gas wells (referred to here as gas well pump-out, or GWP), constitutes another major source of liquids in the GCCS. Liquids from a landfill GCCS are normally mixed with leachate intercepted at the bottom of the lined landfill but could be isolated if desired. Commonly referred to as landfill gas condensate (LGC), GCCS liquids are removed from pipes and wells installed and operated to extract and convey the gases produced as a result of waste decomposition. A diagram of a GCCS is included in Fig. 1 to illustrate the discrete sources of these liquids. LGC typically represents a small fraction of a landfill's total leachate generation and is commonly mixed with other sources of landfill leachate for disposal. The US Environmental Protection Agency (US EPA) estimated landfill LGC generation for an individual landfill site in the range of 2700 to 7500 L per day [6]. For comparison, the landfills used in this study generate approximately 100,000 L of leachate per day. An example calculation to estimate LGC generation based on LFG production is included in Section S1 of the supplemental information (SI).

Per- and polyfluoroalkyl substances (PFAS) are an emerging constituent of concern which present an operational challenge with landfill leachate due to their toxicity, persistence, and lack of viable treatment technologies [14,16,20,28,31,39,5,56,59,60]. Studies have reported diverse and significant concentrations of PFAS in landfill leachate worldwide [28,5,8]. The most well-studied PFAS, perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS), have been detected in leachate at concentrations ranging from ng L^{-1} to μg L^{-1} [16]. In the US, PFOA and PFOS have been measured at concentrations as high as 50 μg L^{-1} [47] in landfill leachate, and PFAS regulations are being promulgated across the US and globally [19,42,49,54,53]. Although landfill leachates are not usually subject to regulation, the recent proposal to designate PFOA and PFOS as CERCLA Hazardous Substances [55] may determine how leachate is managed in the future, as any wastewater treatment effluent requirements will have upstream impacts on leachate management decisions.

While leachate has been described extensively in scientific literature, recent studies have placed focus on studying the behavior of PFAS (e.g., partitioning, transformation, and potential volatilization) within municipal solid waste (MSW) landfills [29]. Gas and air measurement of PFAS is comparatively limited in the literature; however, PFAS have been measured in ambient air above landfills and wastewater treatment plants [22], suggesting that they are either volatilizing or aerosolizing and entering the air [23]. To this point, PFAS have been shown to travel great distances through the air and have been measured in soil, water, and biota in remote locations such as the arctic [62]. The term "semi-volatile" is defined in different ways across the literature in reference to PFAS as well as other chemicals and might refer to compounds which possess a specified range of vapor pressures, or by their observed partitioning behaviors. Several PFAS, including fluorotelomer alcohols,

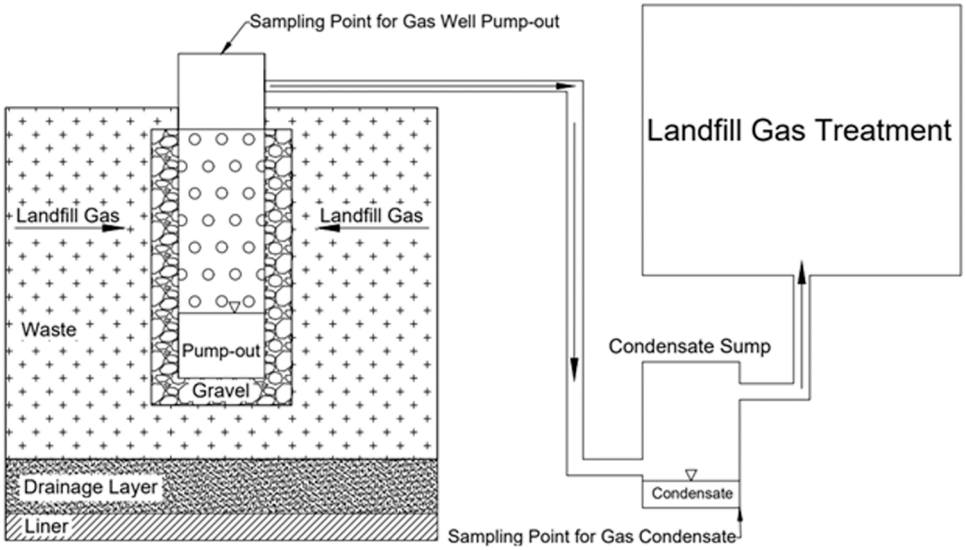


Fig. 1. Schematic of typical gas collection system layout and example gas condensate and gas well pump-out sampling points.

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perfluoroalkane sulfonamides and sulfonamido substances, and terminal perfluoroalkyl acids, have been quantified using both gas and liquid chromatography, indicating their presence in both phases [36,37] Because of their different origins, LGC and GWP are expected to differ significantly from each other in chemical composition. Differences between LGC and GWP are anecdotally recognized within the solid waste industry, although some misconceptions persist. To explore these differences, with a focus on PFAS, LGC and GWP were collected from three MSW landfills in central Florida and underwent PFAS analysis and other characterizations. This is the first study of its kind to evaluate these landfill liquids, and the results of this investigation provide critical insight for the management of discrete sources of leachate within the landfill, and also shed light on the partitioning and transformation behavior of PFAS within MSW landfills.

2. Materials and methods

2.1. Sample collection

Twelve LGC and five GWP samples were collected from three active MSW landfills (referred to as Landfill A, B, and C) in Florida between July and October 2021. Table S1 provides detailed information regarding each site. GWP samples were collected using in-place submerged pumps already installed in the gas wells. The flexible hose connecting these pumps to the leachate collection system was removed from the pipes and used for collection. LGC samples were collected at the condensate knockouts at low points in the GCCS. Pumps installed in the knockouts were used for collection. Samples were collected into one methanol-rinsed 2-L high-density polyethylene (HDPE) bottle for PFAS analysis and two methanol-rinsed 500-mL HDPE bottles, with preservatives, for the physical-chemical parameters. During sampling, an HDPE bottle with PFAS-free water was opened and poured into another HDPE bottle to serve as a field blank. Samples were transported between the field and laboratory on ice and stored at −20 °C. All samples were stored and preserved according to each analysis method specifications in the laboratory (Table S2 in the SI).

2.2. Field and laboratory physical-chemical characterization

LGC and GWP samples were analyzed for pH, conductivity, chloride, biochemical oxygen demand (BOD), chemical oxygen demand (COD), ammonia-nitrogen, alkalinity, and trace metals. Conductivity and pH were measured in the field upon sample collection using a field probe (YSI 556 Multi-probe system, Xylem, Inc., Washington, DC). Ammonia-nitrogen (NH_3-N) in the leachate was measured using the salicylate method (Method 10031; Hach Company, 2012) and spectrophotometric analysis (DR 3900, Hach Company, Loveland, CO). COD was measured in the LGC and GWP using the reactor digestion (Hach Method 10212) and spectrophotometric analysis method (DR 3900, Hach Company, Loveland, CO). Chloride and alkalinity were analyzed using the colorimetric method (HACH Method CD-51 and 10239, respectively). LGC and GWP samples also underwent digestion according to EPA Method 3010 A [50], followed by analysis by inductively coupled plasma – atomic emission spectroscopy (ICP-AES; Thermo Scientific iCAP series 6000) according to EPA Method 200.7 [51] to quantify metals in solution. Select subsamples were sent to Katahdin Analytical Services (Scarborough, ME) on ice for BOD analysis according to Standard Method 5210 B [1].

2.3. PFAS extraction, analysis, and quantitation

The sample extraction method for PFAS was adapted from Robey et al. [44]. Leachate was centrifuged to remove the particulate matter and a 50-mL subsample was taken from the centrifuged sample and adjusted to pH 4–5 using glacial acetic acid (source, location), then spiked with 25 μL of mass-labeled internal standards (Tables S3 and S5 in the SI). Strata-X-AW, 100-μm Phenomenex polymeric weak anion exchange cartridges (500 mg/6 mL), were conditioned with 4 mL of 0.3 % ammonium hydroxide in methanol, followed by 3 mL of Optima-purity grade methanol (Fisher Scientific, Hampton,NH) and 4 mL of an ammonium acetate/acetic acid buffer solution (pH = 4). After loading the sample at a rate of one drop per second, cartridges were washed with the buffer solution (4 mL) then eluted with methanol (2 mL) and 0.3 % ammonium hydroxide in methanol (6 mL). Extracts were concentrated to 1 mL at 32 °C under a constant flow of ultra-high purity nitrogen gas using a Biotage TurboVap II.

Extracts were analyzed for 92 PFAS (full list in the accompanying SI Table S3; 68 were purchased from Wellington Laboratories Inc. [Guelph, ON, Canada] and 24 were donated by Synquest Laboratories Inc. [Alachua, FL, USA, abbreviated as Syn #] and Oakwood Products Inc. [Estill, SC, USA, abbreviated as Oak #]) using a Thermo Scientific Vanquish ultra-high pressure liquid chromatograph (LC) coupled to a TSQ Quantis triple quadrupole mass spectrometer (UHPLC-MS/MS). The LC and MS parameters and accompanying compound abbreviations can be found in Section S3 of the SI (Tables S3 and S4). PFAS were quantified using a 14-level calibration (from 10 to 100,000 ng L^{-1}) developed through dilutions of multiple primary stock solutions. Isotopically-labeled internal standards for 27 compounds (also purchased from Wellington Laboratories, Inc.) were added to each calibration level at 800 ng L^{-1}. If an internal standard was not available for a compound or had poor response on the instrument, another internal standard similar in retention time or structure was used for quantitation. The corresponding mass-labeled standard for each targeted compound are indicated in Table S3 in the SI. Scheduled selected reaction monitoring transitions were used to detect and quantify PFAS in a total of 18 samples and 7 controls (including blanks). The most intense transition was used to quantify PFAS, while the second transition was used to confirm identification (if possible). Acquisition and peak integration were performed using Xcalibur v.4.1 (Thermo Fisher Scientific, Waltham, MA). PFAS were quantified following integration of the peak area for the most intense transition of each analyte. A linear regression model was used to build the calibration curves (individual concentrations listed in Table S5 of the SI), with intercept, slope, and correlation coefficient values calculated over the linear dynamic range for each PFAS species. Instrument detection and quantification limits (LOD and LOQ, respectively) were calculated for each compound using the calibration curve and visual signal-to-noise (S/N) determination (S/N of 3 and 10 for LOD and LOQ, respectively). Detection limits were also calculated using the method described by US EPA; however, for most compounds, the visual method produced a higher (i.e., more conservative) LOD, and seven replicates of each limit showed higher reproducibility (relative standard deviation < 15 %) than the EPA approach, so the visual method was employed in this study.

2.4. PFAS quality control (QC) and extraction efficiency

A detailed discussion of the QA/QC procedures is included in section S4 of the SI. QC samples included field blanks, extraction blanks, solvent blanks, and laboratory control samples. Field blanks consisted of Optima-purity grade water (Fisher Scientific, Hampton, NH) water exposed to ambient air and conditions at the landfills sites in order to capture potential contamination in the entire workflow. Extraction blanks were prepared with Optima water and extracted along with the LGC and GWP samples to identify contamination derived from the extraction process. A solvent blank was run within the instrument method queue every five samples to monitor potential carryover and instrument-derived contamination. Pooled samples of each matrix (LGC and GWP) were used as laboratory control samples to measure extraction efficiencies (see Table S6).

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2.5. Data analysis

To calculate summary statistics across samples, physical-chemical constituents (e.g., chloride, BOD, COD, ammonia-nitrogen, and trace metals), which were not detected, were given a value of one half the detection limit for each type of analysis. Because the data were non-normally distributed, a Mann-Whitney-test (a = 0.05) was used to compare the results between matrix types to determine if they were in fact significantly different from one another. Relative concentrations of individual PFAS in each matrix type (LGC and GWP) were compared as a function of chain length and functional group in order to explore the role of PFAS structure on partitioning behavior within the landfill. A two-tailed spearman correlation was used to find correlations between constituents. A correlation p value less than 0.05 was considered significant.

3. Results and discussion

3.1. Physical-chemical parameters

Although little has been published comparing LGC and GWP matrices, an understanding of the sources of each effluent type informs the expectation of significant differences between the two. For example, GWP, like landfill leachate, is expected to be dark in color and higher in organic matter and water-soluble constituents, such as chloride. Ammonia, on the other hand, might be present under certain conditions as either a gas or water-soluble ion and is likely to be found in both sample types. LGC is expected to be (and was observed to be) clear in appearance, as shown in the picture in Fig. 2. This figure also demonstrates the stark difference between these liquids, which are often conflated or misidentified in the landfill industry. Box-and-whisker plots comparing the range of values for bulk physical-chemical parameters (chloride, alkalinity, COD, pH, ammonia-nitrogen, and conductivity) are included in Fig. 3.

Chloride and conductivity, which are proxies for total ions present in solution, were both significantly higher ($p = 0.002$ for both) in the GWP (mean chloride: 4840 mg L^{-1}, mean conductivity: 24.4 mS cm^{-1}) than in the LGC (mean chloride: 110.8 mg L^{-1}, mean conductivity: 7.33 mS cm^{-1}), with chloride below the detection limit of 20 mg L^{-1} in 58 % of the LGC samples. Average alkalinity and pH were higher in GWP (mean alkalinity: 9200 mg L^{-1}, mean pH: 8.1) than in the LGC (mean alkalinity: 4000 mg L^{-1}, mean pH: 7.2) ($p < 0.02$ for both). While microbially-driven processes within the landfill can contribute to acidic pH in landfill leachate (and, presumably, GWP), especially in the early years of landfill operation [34,43], carbonate-driven alkalinity results from high levels of carbon dioxide generated during organic waste decomposition [35] – this carbonate alkalinity also contributes to highly buffered LGC. The wider range of pH measured in the LGC samples (5.1–8.2) was representative of the difference in landfill conditions; LFG constituents such as hydrogen sulfide can have a significant impact on condensate pH [38].



Fig. 2. Sample of gas well pump-out (GWP) (left) and gas condensate (LGC) (right). The gas well pump-out is a dark brown in color, while the gas condensate is clear.

The decomposition of organic matter, especially food waste, produces ammonia. The distribution of ammonia gas (NH_3) and ammonium ion (NH_4^+) is driven by pH – at pH less than 7, very little ammonia gas is present, and at pH of 9, the distribution is approximately 50 % of each. Within the range of pH measured in the GWP in this study, ammonia gas was likely present in the landfill gas, which could have then partitioned to the LGC. Ammonia-nitrogen, a measurement of the total ammonia/ammonium content of a sample, was lower, on average, in the LGC (mean: 912 mg L^{-1}) than in the GWP (mean: 1870 mg L^{-1}) samples ($p = 0.01$). COD, analyzed in all samples, averaged 5180 mg L^{-1} in the LGC and 13,200 mg L^{-1} in the GWP ($p = 0.04$). BOD, which makes up a fraction of COD and is a measurement of biologically available organic matter, averaged 139 mg L^{-1} in the three LGC samples for which BOD was measured, and 962 mg L^{-1} in the GWP samples ($n = 5$).

While metals concentrations in the GWP were typical of MSW leachate [24,26,35,58], nearly all metals were significantly lower or below detection limits in the LGC samples – with the exception of arsenic and antimony, two metalloids which have been previously reported in MSW landfill gas [40,13,21]. Arsenic concentrations ranged between 0.034 mg L^{-1} and 0.85 mg L^{-1} (mean: 0.47 mg L^{-1}) in the GWP and between 0.055 mg L^{-1} and 2.85 mg L^{-1} (mean: 1.06 mg L^{-1}) in the LGC. Antimony concentrations ranged between 0.015 mg L^{-1} and 0.29 mg L^{-1} (mean: 0.19 mg L^{-1}) in the GWP and between 0.060 mg L^{-1} and 2.20 mg L^{-1} (mean: 0.60 mg L^{-1}) in the LGC. A box and whisker plot of arsenic and antimony concentrations in both matrices is included in Fig. 4. While mean concentrations were not significantly different between the matrices, at the high end of the range, LGC samples were over 100-times the risk-based thresholds for both metalloids. In the top 25 % of samples, arsenic exceeded the Florida groundwater cleanup target level (GCTL) – based on the EPA primary drinking water standard (DWS) of 10 μg L^{-1} – by a factor of more than 150, and antimony exceeded the GCTL of 6 μg L^{-1} by a factor of 200 in the top quartile of samples [12,52]. Although other metals/metalloids are known to volatilize in the environment or under landfill conditions (e.g., Cd, Pb, Se, and Sn) [40,33,41], none of these except for arsenic and antimony were detected consistently in the LGC. Concentrations of all metals in both sample types are included in Table S6 in the SI.

A balance of the total ions measured in the aqueous form for each matrix illustrates their fundamental differences. Among the LGC samples, ammonia and alkalinity (as bicarbonate) constituted 86 % of the 3380 mg L^{-1} total measured solids, while metals and anions made up the remaining 14 %. GWP was more evenly distributed, with metals/metalloids accounting for an average of 18 %, ammonium an average of 11 %, and anions an average of 30 % of the total solids (16,149 mg L^{-1}). The percent difference between anions and cations was 8.1 % and 4.3 % for LGC and GWP, respectively. These distributions are visualized in Fig. 5, with the unaccounted-for portion of the anions/cations represented as the “Balance”.

3.2. PFAS profiles in LGC and GWP

The total PFAS concentration ($\sum_{92}$PFAS) in LGC and GWP samples ranged from 3000 to 50,000 ng L^{-1} and 36,000–74,000 ng L^{-1}, respectively; the average $\sum_{92}$PFAS was three times higher in the GWP (56,000 ng L^{-1}) than in the LGC (19,000 ng L^{-1}). These concentrations are comparable to the range of PFAS concentrations measured in leachates collected from MSW landfill leachate collection systems [10,

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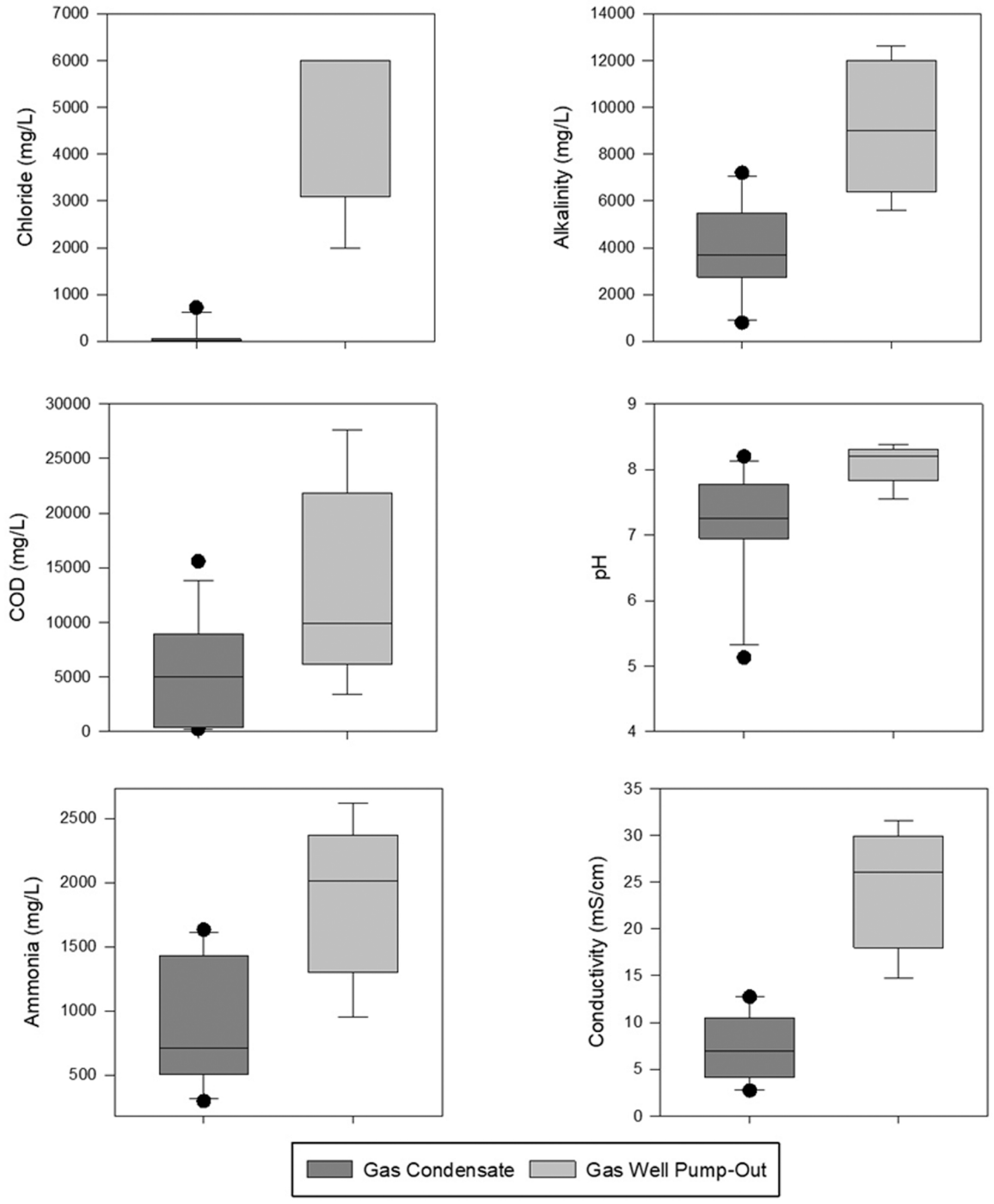


Fig. 3. Chloride, alkalinity, COD, pH, ammonia, and conductivity measurements in gas condensate (LGC) and gas well pump-out (GWP). Box-and-whisker plots represent 10-, 25-, 50-, 75-, and 90th percentile values.

44,46]. Like the physical-chemical constituent profiles of these matrices, the distribution of PFAS in each liquid matrix sheds light on the behavior (i.e., partitioning, transformation) of PFAS within the waste mass of an MSW landfill. Although concentrations were higher in the GWP, the diversity of PFAS (total number of quantified PFAS) was higher in LGC; 53 PFAS were quantified in LGC samples and 44 PFAS were quantified in GWP samples. This difference is illustrated in Figs. 6 and 7, which present the distribution of PFAS among class, subclass, and species for LGC and GWP, respectively. Seventeen PFAS (six PFAAs, six fluorotelomers, and five sulfonamides) were quantified in 100 % of LGC samples, and five PFAS (two PFAAs, two fluorotelomers, and one other precursor) were quantified in 100 % of GWP samples. Additionally, the ratio between terminal species (i.e., the breakdown product of other PFAS and which are known to not break down further under environmental conditions) and precursor species (i.e., PFAS known to degrade into terminal species) varied between the two matrices. Precursor PFAS represented the majority of $\sum_{92}$PFAS in both sample types, but a proportionally larger fraction in the LGC (70 % in the GWP vs 85 % in the LGC).

The detection frequency, mean, and median concentrations for terminal PFAS (which are further broken down into subclasses: perfluoroalkyl carboxylic acids [PFCAs], perfluoroalkane sulfonic acids

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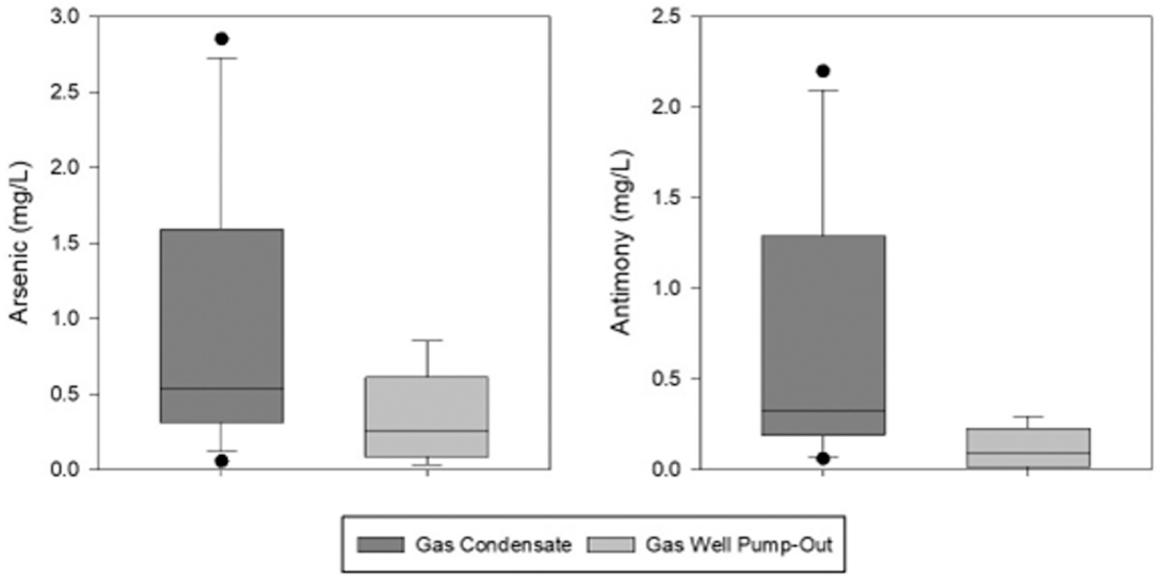


Fig. 4. Arsenic and antimony concentrations in gas condensate (LGC) and gas well pump-out (GWP). Box-and-whisker plots represent 10-, 25-, 50-, 75-, and 90th percentile values.

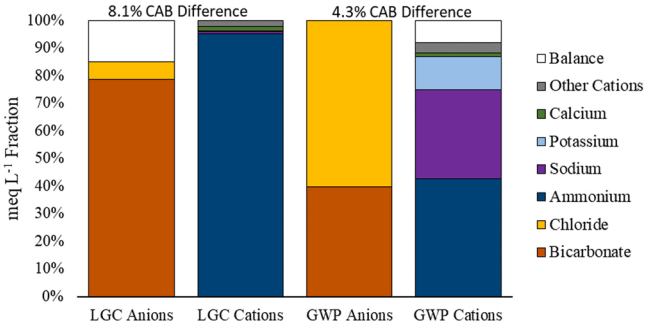


Fig. 5. Cation-anion balance (CAB) of the gas condensate (LGC) and gas well pump-out (GWP) matrices by the dissolved ions measured in solution.

[PFSAs], perfluoroalkane sulfinic acids [PFSiAs], and perfluoroalkyl phosphinic acid (PFPiAs) are detailed in Table 1 and in Fig. S1 in the SI for select species. Among the 28 terminal PFAS targeted in this analytical method, 19 were quantified in LGC or GWP samples. Again, while the total concentration of terminal PFAS ($\sum_{28}$Terminal) was higher in the GWP, the diversity of terminal PFAS in the LGC was higher – with 19 quantified in at least one LGC sample and 16 quantified in at least one GWP. Fifteen terminal PFAS were quantified in over half of the LGC samples and only nine in the GWP. (Tables 1, 2, and 3). Fig. 8.

While GWP was approximately evenly split between the two largest subclasses of terminal PFAS (58 % PFCAs and 42 % PFSAs), LGC was dominated by PFCAs, which represented 86 % of the $\sum_{28}$Terminal. PFSAs require higher temperatures for volatilization than PFCAs [11, 61], suggesting that PFCAs are also more abundant than PFSAs in LFG. This is notable because, while previous studies have reported thermal desorption of PFAS at temperatures between 100 °C and 500 °C [30,48], typical MSW landfill temperatures are only between 35 and 55 °C [27]. Perfluorobutanesulfinic acid (Syn34), a recently identified terminal PFAS [2], which has not previously been identified in landfill leachate, was quantified in over half of the samples of both matrix types. Long-chain PFCAs (>C10) were measured in LGC samples at concentrations similar or higher than those reported previously in landfill leachate [28,29,44] and at higher concentrations in the LGC than the GWP.

The detection frequency,mean and median concentrations for

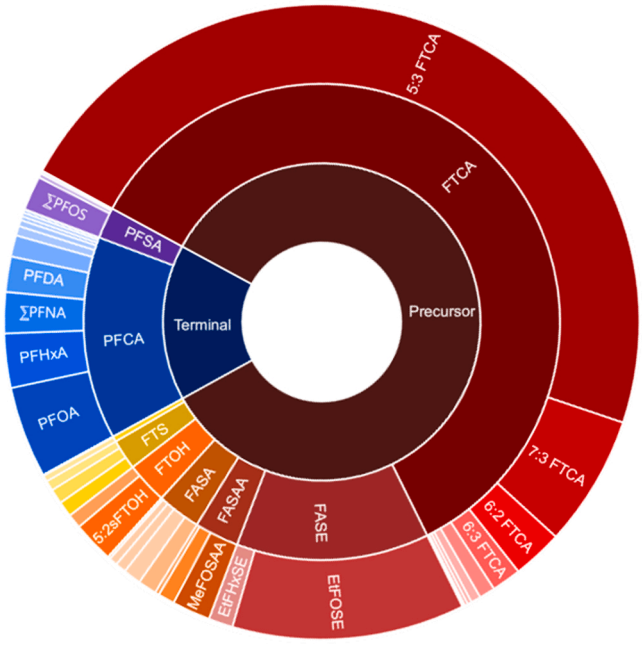


Fig. 6. PFAS distribution in landfill gas condensate (LGC). In the interest of legibility, only compounds which represented > ~1 % of $\sum_{92}$PFAS are labelled, mean concentrations for all PFAS are included in Tables 1, 2, and 3. The center ring of the plot shows the percentage of Terminal and Precursor species. The middle ring shows the classes. The outer ring shows the individual PFAS.

precursor PFAS are detailed in Table 2 (fluorotelomers) and Table 3 (sulfonamides and other precursors). Box and whisker plots of all sample measurements are also included in Figs. S2 (fluorotelomers) and S3 (other precursors) in the SI. Of the 38 PFAA-precursors measured, 35 and 29 were detected in LGC and GWP samples, respectively. Precursors in both matrices were dominated by one PFAS: 5:3 FTCA, a fluorotelomer carboxylic acid which is well documented in landfill leachates and leachate-contaminated media [18,17,25,4,32]. 5:3 FTCA was detected in 100 % of both sample types and accounted for 55 % of the $\sum_{38}$Precursor in LGC and 87 % of the $\sum_{38}$Precursor in GWP. The remaining balance of $\sum_{38}$Precursor in GWP consisted primarily of other

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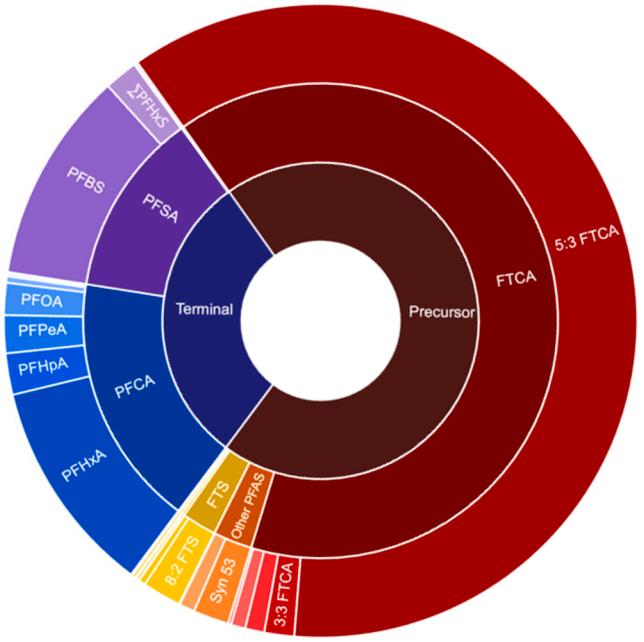


Fig. 7. PFAS distribution in landfill gas well pump-out (GWP). In the interest of legibility, only compounds which represented > ~1 % of $\sum_{92}$PFAS are labelled, mean concentrations for all PFAS are included in Tables 1, 2, and 3. The center ring of the plot shows the percentage of Terminal and Precursor species. The middle ring shows the classes. The outer ring shows the individual PFAS.

fluorotelomer compounds (predominantly FTCAs and fluorotelomer sulfonates [FTSAs]). LGC contained a greater variety of precursor PFAS, especially sulfonamides, which represented not only a more significant fraction of the LGC (18 % of the $\sum_{92}$PFAS) compared to GWP (1.7 % of the $\sum_{92}$PFAS) but were found in higher concentrations in the LGC as well. An emerging PFAS of note, 6:2 fluorotelomer phosphate diester (6:2 diPAP), was measured in both the LGC (77 % detection frequency, mean: 500 ng L^{-1}) and in the GWP (80 % detection frequency, mean: 720 ng L^{-1}).

Select PFAS which have been measured in air samples and described as volatile or semi- volatile, were detected in aqueous matrices in this study. Fluorotelomer alcohols (FTOHs) are a class of PFAS known to volatilize which have been measured in air samples near MSW landfills and wastewater treatment facilities [3,57]. FTOHs are not commonly measured in liquid matrices; however, they are known to degrade into FTCAs and further into PFCAs, both of which are prevalent in MSW landfill leachate [62,7]. The two FTOHs targeted by this analysis (5:2 and 7:2 FTOH) were quantified in 77 % and 69 % of LGC samples, respectively, and in 0 % and 20 % of GWP samples, respectively. The other class of semi-volatile species included in this analytical method which are not frequently measured in liquid matrices, perfluoroalkane sulfonamido ethanols (FASE), were also identified in the LGC at a greater frequency and at higher concentrations than in the GWP. N-ethyl perfluorooctane sulfonamido ethanol (N-EtFOSE), which is a PFOS precursor, was quantified in 100 % of LGC samples at an average concentration of 2200 ng L^{-1} (compare with GWP, where it was measured in 80 % of samples at a mean concentration of 180 ng L^{-1}). N-ethyl perfluorohexane sulfonamido ethanol (N-EtFHxSE), a PFHxS precursor, was measured in 54 % of LGC at an average concentration of 230 ng L^{-1} and was not measured above LOD in any GWP samples.

A spearman correlation analysis was performed on both LGC and GWP to examine the relationships between physical chemical parameters and PFAS within each type of leachate. The results of the spearman correlation on the LGC data showed that there was no significant correlation ($p < 0.05$) between $\sum_{92}$PFAS and any of the physical-chemical parameters. Individual PFAS, however, did show positive correlation with select physical-chemical parameters, suggesting that these PFAS might share common release pathways into the LGC. 5:3 FTCA, which makes up almost half of the $\sum_{92}$PFAS in LGC, was most strongly correlated to chloride ($r = 0.628$, $p = 0.038$). The second most abundant fluorotelomer, 7:3 FTCA, was most strongly correlated with ammonia ($r = 0.800$, $p = 0.010$) and chloride ($r = 0.861$, $p = 0.001$). The small number of GWP samples limited the number of correlations that could be performed. The only significant correlation was a negative correlation between iron and $\sum_{92}$PFAS ($r = -0.900$, $p = 0.037$). Correlation coefficients between the most abundant PFAS across samples and physical-chemical parameters are included in the SI Tables S8 (LGC) and S9 (GWP).

3.3. Gas condensate partitioning as a function of carbon chain length

While $\sum_{92}$PFAS was greater, on average, in GWP than in LGC, the structure of individual PFAS correlated with partitioning behavior and the relative abundance of homologous PFAS in each matrix. The effect of carbon chain length on partitioning behavior has been studied previously in the interfacial layer [15,45,61,9], indicating a positive

Table 1
Percent of samples in which each terminal PFAS (carboxylic acids, sulfonic acids, and sulfinic acids) was detected and mean (standard deviation) and median concentrations of PFAS quantified in gas condensate and gas well pump-out samples. Mean, standard deviation, and median are in units of ng L^{-1}.

Analyte	Gas Condensate n = 12				Gas Well Pump-out n = 5			
	Detection Frequency	Mean (SD)		Median	%	Mean (SD)		Median
PFBA	62 %	39	(73)	5	20 %	180	(370)	BDL
PFPeA	69 %	90	(170)	5	40 %	1100	(1300)	BDL
PFHxA	100 %	510	(1,00)	35	100 %	6000	(1600)	5148
PFHpA	92 %	200	(400)	11	100 %	1100	(300)	1056
PFOA	100 %	860	(1400)	282	40 %	890	(1100)	BDL
$PFNA_{linear}$	100 %	170	(220)	45	60 %	50	(63)	6
$PFNA_{branched}$	100 %	220	(230)	148	40 %	19	(26)	BDL
PFDA	100 %	330	(410)	190	60 %	32	(41)	4
PFUdA	92 %	49	(81)	6	60 %	4	(5)	1
PFDoA	92 %	57	(94)	7	60 %	6	(7)	3
PFTrDA	77 %	29	(43)	4	40 %	7	(10)	BDL
PFHxDA	31 %	2.0	(4)	BDL	0 %	BDL		BDL
PFBS	38 %	20	(41)	BDL	80 %	5900	(4400)	9153
PFHxS	69 %	41	(60)	29	80 %	940	(890)	534
PFHpS	31 %	7	(13)	BDL	0 %	BDL		BDL
$PFOS_{branched}$	92 %	63	(140)	4	20 %	4	(8)	BDL
$PFOS_{linear}$	100 %	260	(450)	78	20 %	16	(32)	BDL
PFDS	38 %	2	(3)	BDL	0 %	BDL		BDL
Syn34	77 %	1	(1)	1	60 %	75	(107)	37

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Table 2
Percent of samples in which each fluorotelomer PFAS (fluorotelomer sulfonates, saturated and unsaturated carboxylic acids) was detected and mean, standard deviation, and median concentrations of PFAS quantified in gas condensate and gas well pump-out samples. Mean, standard deviation and median are in units of ng L^{-1}.

Analyte	Gas Condensate n = 12				Gas Well Pump-out n = 5			
	Detection Frequency	Mean (SD)		Median	%	Mean (SD)		Median
4:2 FTS	15 %	BDL		BDL	80 %	19	(30)	7
6:2 FTS	77 %	95	(150)	27	80 %	180	(150)	230
8:2 FTS	100 %	140	(170)	5	100 %	1100	(1400)	23
10:2 FTS	62 %	150	(340)	4	20 %	4	(8)	BDL
3:3 FTCA (FPrPA)	31 %	18	(42)	BDL	80 %	840	(930)	530
5:3 FTCA (FPePA)	100 %	8700	(12,000)	3000	100 %	34,000	(15,000)	44,000
6:3 FTCA	100 %	280	(470)	73	40 %	38	(62)	BDL
7:3 FTCA (FHpPA)	100 %	1200	(1200)	720	60 %	540	(780)	12
8:3 FTCA	92 %	93	(77)	56	40 %	22	(32)	BDL
6:2 FTCA	85 %	470	(1100)	58	40 %	400	(610)	BDL
8:2 FTCA	100 %	150	(180)	48	20 %	18	(37)	BDL
10:2 FTCA	62 %	32	(47)	15	20 %	3	(6)	BDL
8:2 FTUCA	100 %	36	(64)	8	40 %	8	(11)	BDL
10:2 FTUCA	85 %	7	(11)	2	20 %	1	(1)	BDL

Table 3
Percent of samples in which each precursor PFAS (including diPAPs, sulfonamides, sulfonamido and acetic acids) was detected and mean, standard deviation, and median concentrations of PFAS quantified in gas condensate and gas well pump-out samples. Mean, standard deviation and median are in units of ng L^{-1}.

Analyte	Gas Condensate n = 12				Gas Well Pump-out n = 5		
	Detection Frequency	Mean (SD)		Median	%	Mean (SD)	Median
6:2 diPAP	77 %	500	(1300)	11	80 %	720 (730)	320
FBSA	100 %	1	(1)	1	60 %	2 (2)	3
FHxSA	46 %	1	(1)	BDL	0 %	BDL	BDL
N-AP-FHxSA	31 %	24	(44)	BDL	0 %	BDL	BDL
FOSA	100 %	77	(89)	45	40 %	6 (7)	BDL
FOSAA	77 %	40	(110)	2	40 %	4 (6)	BDL
CMAmP+6:2FOSA	31 %	100	(180)	BDL	20 %	11 (22)	BDL
MeFOSA	77 %	170	(460)	11	0 %	BDL	BDL
EtFOSA + MeFOSA	85 %	170	(470)	6	0 %	BDL	BDL
MeFOSAA	100 %	350	(710)	66	60 %	77 (120)	23
EtFOSAA	100 %	160	(260)	20	60 %	33 (31)	36
EtFOSE	100 %	2200	(4700)	534	80 %	100 (93)	60
EtFHxSE (Syn2)	54 %	230	(540)	10	0 %	BDL	BDL
PFECHS	23 %	1	(2)	BDL	20 %	1 (2)	BDL
Oak10	15 %	1	(2)	BDL	60 %	10 (9)	11
Syn1	23 %	7	(15)	BDL	0 %	BDL	BDL
Syn 41	0 %	BDL		BDL	100 %	440 (120)	430
Syn53	23 %	8	(18)	BDL	60 %	1000 (1400)	320

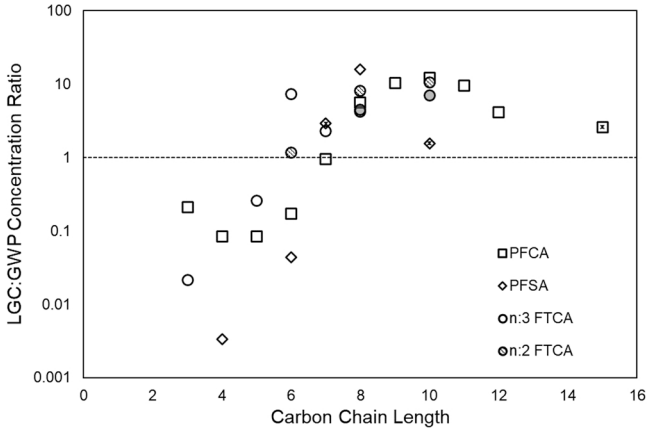


Fig. 8. LGC:GWP concentration ratio as a function of carbon chain length for PFAS in the PFCA and PFSA terminal classes and for FTCAs, the most abundant class of PFAS identified in this study. PFHpS, PFDS, and PFHxDA, indicated with asterisks (*) in the figure, were not measured in any GWP samples and one-half of the approximate detection limit was substituted to allow the calculation of a LGC:GWP ratio.

correlation between chain length and air-water interfacial partitioning. In a study of landfill leachate foam separation, Robey et al. [44] found a similar relationship among short chain PFAAs, which then reversed as air-water interface partitioning appeared to decrease among the longest chain PFAAs. While PFAS volatilization studies are less common, Zhang et al. [63] derived vapor pressures for nine PFAS and found a logarithmic relationship between the number of carbons in the compounds' carbon-fluorine chains and both vapor pressure and sublimation enthalpy. For every two-carbon increase in chain length, vapor pressure was found to decrease by approximately ten times, suggesting longer-chain PFAS have a lower propensity to volatilize. As illustrated in Fig. 7, the results of this study indicate that longer chain-length PFCAs, PFSAs, and FTCAs (the most abundant class identified in these samples) have a greater tendency to partition to the LGC, although this relationship weakens among the longest chain PFAS. These findings, placed in the context of the aforementioned studies, suggest that PFAS in LGC are a result of interfacial partitioning and potentially aerosolization, not volatilization. Compounds like PFHxDA, PFHpS, and PFDS were not measured in any GWP samples, so to estimate a relative concentration, one-half of the detection limit for these compounds was substituted for the mean concentration. This trend can also be seen in the box and whisker plots of the series included in the SI Figs. S1, S2, and S3.

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4. Conclusion

The results of this study shed light on the conditions under which gas condensate and leachate are produced, and these findings provide valuable information about the fate and behavior of PFAS within MSW landfills. As PFAS regulations are promulgated at the state and federal level, as well as internationally, the results of this study contribute to a better understanding of how PFAS in waste contribute to PFAS in landfill effluent, which includes leachate and LFG. While the two leachate matrices studied here are typically combined and managed in the same manner as any other leachate type, separate management might reduce, for instance, precursor PFAS concentrations in leachate, which is treated using standard wastewater treatment techniques that has the potential to transform precursor PFAS to terminal PFAS, which are more likely to be regulated and are of concern. While active landfills may not be able to separate these leachate streams due to the infrastructure already in place, this could be considered for those that are able to as well as new facilities and landfill cells. This study represents the most in-depth characterization of these discrete liquid leachate matrices to date.

Previous research comparing PFAS in landfill leachate collection from waste collection vehicles prior to landfill disposal to leachate collected form a landfill liner system has found that under landfill conditions, PFAS are likely to transform from precursor species to terminal species [29]. Much less is known, however, about transformation, partitioning and potential volatilization of PFAS within the landfilled waste mass itself. In this study, two discrete leachate streams which have not been studied previously – LGC, a clear liquid formed when moisture in landfill gas condenses at ambient air temperatures, and GWP, a dark leachate formed in the upper layers of waste which collects in gas wells and is removed prior to reaching the bottom liner system – from three MSW landfills, were characterized using physical-chemical and PFAS analyses. Unsurprisingly, pH, conductivity, chloride, COD, ammonia-nitrogen, and alkalinity concentrations between the matrices were significantly different from one another based on a Mann-Whitney test for non-parametric data. Concentrations of two metalloids, which have been documented to volatilize under landfill conditions – arsenic and antimony – exceeded risk-based thresholds for groundwater by factors of 150 and 200, respectively, in some LGC samples; however, the mean concentrations in each matrix were not found to be significantly different from one another.

While total concentrations of PFAS ($\sum_{92}$PFAS) were higher in the GWP, the distribution of PFAS among classes was notably different – LGC contained a greater variety of PFAS (53 detected) than GWP (44 detected), including proportionally more precursor PFAS and more semi-volatile PFAS (FTOH and FASE classes). These semi-volatile PFAS have also been documented in air samples close to landfills and are presumed to leave landfills via LFG, although this is the first time this has been demonstrated with the analysis of landfill-collected samples. Among terminal PFAS, LGC contained proportionally more PFCAs than PFSAs, while GWP contained an approximately equivalent fraction of each type of PFAAs – corroborating previous studies which found that PFCAs more readily volatilize than PFSAs. This is the first report of PFAA volatilization at MSW landfill temperatures (35–55 °C). Inherent variability associated with the collection of discrete leachate samples from full scale landfills may limit the representativeness of these samples, however, this study provides a first look at the physical-chemical and PFAS profiles of these unique leachate types. Future work should corroborate and expand upon this data, potentially to include PFAS and physical-chemical parameter partitioning in landfill gas. Members of the solid waste community can use these results to make informed decisions on managing liquids within the landfill, including managing these leachate streams separately.

CRediT authorship contribution statement

Thomas J. Smallwood: Conceptualization, Methodology, Validation, Data curation, Formal analysis, Investigation, Visualization, Writing - original draft, Writing - review & editing. **Nicole M. Robey:** Conceptualization, Methodology, Validation, Investigation, Visualization, Writing - original draft, Writing - review & editing. **Yalan Liu:** Conceptualization, Methodology, Validation, Data curation, Formal analysis, Investigation, Writing - original draft, Writing - review & editing. **John A. Bowden:** Methodology, Validation, Formal analysis, Funding acquisition, Writing - review & editing. **Thabet M. Tolaymat:** Validation, Supervision, Writing - review & editing. **Helena M. Solo-Gabriele:** Funding acquisition, Validation, Writing - review & editing. **Timothy G. Townsend:** Conceptualization, Validation, Funding acquisition, Project administration, Supervision, Writing - review & editing.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Timothy Townsend reports financial support was provided by United States Environmental Protection Agency.

Data availability

Data will be made available on request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jhazmat.2023.130926.

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Special Series

Biological and behavioral responses of European honey bee (*Apis mellifera*) colonies to perfluorooctane sulfonate exposure

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EDITOR'S NOTE:

This article is part of the special series "Ecological Risk Assessment for Per- and Polyfluorinated Alkyl Substances." The series documents and advances the current state of the practice, with respect to ecotoxicological research, environmental exposure monitoring and modeling, ecologically based screening benchmarks, and risk assessment frameworks.

Abstract

Bees provide pollination services to managed and wild ecosystems but are threatened globally due to multiple stressors, including exposure to contaminants. Perfluorooctane sulfonate (PFOS) is a widely detected and persistent contaminant that accumulates and biomagnifies in food chains. In this exposure effect study, small whole colonies of *Apis mellifera* (1000 bees) were exposed to PFOS using a purpose-built cage system over a 4-week period. The PFOS exposure concentrations were provided to bees in sugar syrup at concentrations detected in the environment, ranging from 0 to 1.6 mg L^{-1}. A range of biological and behavioral responses were monitored. Bee tissue, honey, and fecal matter were analyzed using isotope dilution combined with liquid chromatography–tandem mass spectrometry adapted for bee and honey matrix analysis. Bee mortality increased significantly with PFOS exposure at 0.8 mg L^{-1} or greater, and brood development ceased entirely at 0.02 mg L^{-1} or greater. Colony activity, temperament, hive maintenance, and defense were adversely affected in all PFOS exposure treatments compared with the control, even at the lowest PFOS exposure of 0.02 mg L^{-1}. Perfluorooctane sulfonate was detected in bee tissue with a mean bioaccumulation factor of 0.3, and it was also identified in honey and in feces collected from the hive cages. These findings provide the first evidence that PFOS exposure adversely affects honey bee colonies and may transfer to honey. With PFOS contaminating thousands of sites worldwide, our study has implications for exposed bee populations under natural conditions, pollination services, the honey industry, and human health. *Integr Environ Assess Manag* 2021;17:673–683. © 2021 SETAC

KEYWORDS: BAF, Bioaccumulation, Ecotoxicity, Effect trial, Honey, PFAS, PFOS

INTRODUCTION

Perfluorooctane sulfonate (PFOS) is an environmental contaminant that is highly persistent in the environment and causes detrimental effects upon a range of receptors (Jian et al., 2018; Mommaert et al., 2011; Mudumbi et al., 2017; Stahl et al., 2011). PFOS has been used globally in thousands of consumer and industrial applications since the 1950s, including protective coatings on cooking pans and fire-retardant products such as aqueous film-forming foam extensively used from the 1970s to 2000s (Gluge et al., 2020). Widespread use and inappropriate disposal have resulted in PFOS-contaminated soil, sediments, and water worldwide, and hundreds of contaminated sites show PFOS in various environmental compartments at concentrations exceeding current guidelines (AECOM, 2016; Chen et al., 2018; DAWE, 2016; Gilljam et al., 2016; Lin et al., 2014). PFOS accumulates and biomagnifies in food chains and affects the reproduction, development, and general fitness of wildlife (Houde et al., 2006; Squadrone et al., 2015). PFOS is now listed on the International Stockholm Convention for Persistent Organic Pollutants under Annex B, for restriction of production and use (Stockholm Convention, 2019), and over recent years, many jurisdictions have been developing regulations and protective guidelines in response to the emerging concerns.

Bees are ecologically and economically important to wild and managed ecosystems due to the pollination services

This article contains online-only Supporting Information.

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they provide (Ballantyne et al., 2017; Ollerton et al., 2011; Winfree et al., 2011). Honey bees also support a $6 billion (US) global honey market and a range of other bee product industries (Australian Government, Department of Agriculture, 2016). Yet bee populations are under threat due to multiple stressors including land-use change and intensification, pests, diseases, and exposure to a multitude of environmental contaminants (Bekic et al., 2014; Simon-Delso et al., 2014; vanEngelsdorp et al., 2009). Evidence supporting the adverse impact of pesticides on bee health is increasing, particularly for neonicotinoid pesticides (Alburaki et al., 2017; Goulson et al., 2015; Long & Krupke, 2016). Bees, however, may be exposed to many other contaminants in the environment, including PFOS, for which the consequences are little understood.

A number of potential exposure routes for bees to PFOS exist. Plants are reported to uptake PFOS from contaminated soils and water via the roots through a mechanism thought to be similar to systemic pesticide uptake (Alburaki et al., 2015; Long & Krupke, 2016), and relatively high PFOS concentrations are tolerated (up to 800 mg L^{-1}) with no detrimental effect on growth or flowering potential (Felizeter et al., 2014; Qu et al., 2010). For example, Felizeter et al. (2014) demonstrated that zucchini (*Curcubita pepo* var. Black Beauty) and tomatoes (*Solanum lycopersicum* var. Moneymaker) grown hydroponically and exposed to PFOS concentrations ranging from 10 to 10 000 ng L^{-1} accumulated 303 ± 130 ng g^{-1} PFOS in the leaves, but only low concentrations were detected in the fruit (0.22 ± 0.12 ng g^{-1}) (Felizeter et al., 2014). Therefore, bee exposure via plant pollen and nectar while foraging on contaminated plants may occur, although translocation to plant pollen and nectar has not yet been confirmed (Blaine et al., 2014; Felizeter et al., 2014). Furthermore, plant nectar and pollen may become contaminated by deposition of PFOS-contaminated soil or aerosol drift of contaminated water (Alburaki et al., 2017; Long & Krupke, 2016). Water consumption may also provide exposure because bees obtain dietary salt from pools of brackish water and access water for hive cooling (Stabentheiner et al., 2021), and this may provide a direct exposure pathway to PFOS in contaminated water (Lau & Nieh, 2016).

Studies of PFOS effects on bees appear to be limited to two controlled laboratory studies by Mommaert et al. (2011) and Wilkins (2001a, 2001b). Mommaert et al. (2011) studied lethal and sublethal effects on the survival, nest growth, reproduction, and foraging behavior of the bumblebee (*Bombus terrestris* L.) at PFOS concentrations ranging from 0 to 10 mg L^{-1} in spiked sugar syrup. Mortality increased significantly and reproduction ceased at PFOS concentrations of 1 mg L^{-1} or greater with PFOS accumulation reported at a mean of 655 ± 109 ng per worker bee whole body (Mommaert et al., 2011). Wilkins (2001a, 2001b) examined PFOS toxicity on European honey bees (*Apis mellifera*) but used individuals rather than colonies. The authors reported a 72-h LD50 value of 0.40 µg/bee for an oral dose (Wilkins, 2001a) and a 96-h LD50 value of 4.78 µg/bee for a contact dose (Wilkins, 2001b). Little is known about PFOS accumulation and elimination in bees, nor transfer to honey; however, one study analyzing samples from seven European countries reported PFOS detected in honey for a limited number of samples, but at concentrations below the method limit of quantification, which was 0.134 ng g^{-1} (Surma et al., 2016).

Studies on bee colonies, rather than individuals, are critical to fully understand the impacts of exposure to contaminants such as PFOS on bee populations, and the pollination services provided to ecosystems (D'Hollander et al., 2014; Stahl et al., 2009). Exposure effect studies on whole colonies provide multigenerational information on the long-term impact of stressors, including contaminants (Khoury et al., 2011; Torres et al., 2015; Uzunov et al., 2015). To the best of our knowledge, it appears that the effects of sublethal exposure of honey bees or whole colonies to PFOS have not yet been examined. The aim of this study was to evaluate biological and behavioral responses of small colonies of the European honey bee (*A. mellifera*) to PFOS exposures. Whole bee colonies were exposed to environmental concentrations in spiked sugar syrup over a 4-week period in a purpose-built cage environment and a range of colony metrics were assessed together with concentrations in bee tissue, honey, and feces.

MATERIALS AND METHODS

Three replicate PFOS exposure-response assessments were conducted, each for a period of four weeks. These were run consecutively between 9 February to 30 May and 26 October to 29 November 2017. Each replicate comprised two honey bee colonies for each exposure concentration treatment, which included six different PFOS concentrations and a control (i.e., seven treatments for each replicate, with each treatment using two bee colonies). For each replicate, the bee colonies were housed in seven randomly assigned cages (one per exposure treatment) in a glasshouse. Bees were exposed using PFOS-spiked 55% sugar syrup, and biological and behavioral responses were recorded. At the completion of each replicate, the colonies were euthanized and stored in a freezer, and PFOS concentrations in bee tissue, feces, and honey determined.

The specialized cage system (Sonter et al., 2019) used for this exposure trial was installed in a dedicated bay of the glasshouse complex at the University of New England, Armidale, NSW (Australia) (Supporting Information Figure S1). Modifications to the cages and hive boxes to prevent PFOS cross-contamination included removable covers on hive box lids, water feeders, floors, cage walls, and temperature monitors. These covers were completely removed and replaced between replicates, and entire cages were washed with dilute phosphate-free detergent and rinsed with distilled water. Each cage was provided with 2 × 100 mL 55% w/w sugar syrup solution (spiked or not spiked) (Huang, 2010), as a substitute for plant nectar. Water and also pollen and protein supplement as Jarrah pollen (10 g) and Bee Build Protein Sausage™ (20 g) (Penders

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Beekeeping Supplies) were supplied weekly for each bee colony. All hive boxes, including the frames, wax foundation, and wax comb, were frozen for a period of 48 h before the start of each replicate to kill any pest eggs. Ambient temperature in the glasshouse bay was maintained between 24 °C and 28 °C with 40%–60% humidity for the duration of the experiment. Hive box and ambient temperature were monitored (Jumbo Display In/Out Thermometer, model QM7310; Jaycar Electronics Pty Ltd.) with a temperature probe attached to the center of each hive box.

Each replicate was run with 14 individual young bee colonies (two in each of the seven treatments), each comprising approximately 1000 bees and a young fertile queen bee (Root, 1978; Winston, 1987). Each colony was initially housed in a hive box with a brood frame to encourage colony establishment and also two empty frames to allow colony expansion (Eckert et al., 1994). The colonies were acclimatized for one week before exposure during which each cage was provided with nonspiked sugar syrup, water, and food supplements, as previously described. Immediately before PFOS exposure, the brood frames were removed from each hive box and replaced with a frame with a wax comb to enable the queen to lay eggs.

Bee colonies were exposed to PFOS in 55% sugar syrup as a nectar substitute to ensure PFOS intake. Water consumption by bees occurs largely to cool the hive, and in a caged environment, this was considered insufficient to guarantee exposure. Furthermore, Mommaert et al. (2011) have demonstrated that exposure using spiked sugar syrup was successful for individual bees. The PFOS standard solution used to make up exposure concentrations was supplied as 40% in H_2O (the standard available in Australia at the time) (Sigma-Aldrich). Validation at National Measurement Institute (NMI) confirmed the concentration as 63%. The PFOS solution was diluted with ultrapure water for a working stock (TKA Micropure UV/UF-ST 50132372), followed by 55% w/w sugar syrup for PFOS exposure solution concentrations of 0.02, 0.08, 0.2, 0.3, 0.8, and 1.6 mg L^{-1} in 55% w/w sugar syrup, with a control of nonspiked 55% w/w sugar syrup. The exposure concentrations were developed from the range of concentrations reported for surface and groundwater at PFOS-contaminated sites in the absence of ecological guidelines for PFOS at the time of experimental set-up (AECOM, 2016; Ahrens & Bundschuh, 2014; Lin et al., 2014; Oliaei et al., 2014). The exposures also encompassed concentrations considered sublethal to bees, based on available research (Mommaert et al., 2011; Wilkins, 2001a). The working stock solution was prepared fresh at the start of each replicate. The two colonies allocated to each cage shared the same PFOS sugar syrup solution, provided as 2 × 100 ml in two feeding containers, with fresh solution provided each week (Supporting Information Figure S1).

Biological responses after PFOS exposure were recorded weekly and included brood development (i.e., presence of eggs, larvae, pupae, and young adults), hive box temperature, mortality (i.e., worker bee and queen death (Supporting Information Equations 1–3), gastric health (i.e., presence or absence of dysentery in the form of dark, streaky deposits on a scale of 1–4, with 1 being no dark or streaky fecal deposits and 4 being greater than 50 dark or streaky fecal deposits (Supporting Information Table S1 and Figure S2), and bee weight. Population change was measured at the end of the treatment period (Supporting Information Equation 4).

Behavioral responses recorded included colony establishment, colony response to any incidental queen mortality (i.e., remaining in the hive box and raising a replacement queen, or leaving), activity (i.e., the number of bees sitting and/or crawling, and flying, outside the hive boxes), temperament (ranked on a scale of 1–5, with 1 being calm and 5 being aggressive) (Supporting Information Table S2), hive maintenance (i.e., cleanliness of hive box ranked on a scale of 1–5, with 1 being free of dead bees and debris and 5 being greater than 20 dead bees, evidence of debris and/or fecal deposits; Supporting Information Table S3), and pest management (i.e., presence of small hive beetles, wax moths, or cockroaches inside the hive boxes indicating low defense against pests). Bee activity, the response to queen mortality (if applicable), and the colony temperament were monitored twice each week. Hive maintenance (Supporting Information Table S3) and pest management (Supporting Information Table S4) were monitored weekly. Colony establishment was determined by colonies remaining in the hive boxes for the duration of the replicate.

Analyses of bee tissue, honey, and feces were performed by NMI, North Ryde, Australia, using an isotope dilution method combined with liquid chromatography–tandem mass spectrometry (LCMS) adapted for bee tissue (O'Connor et al., 2018). Bee tissue samples (0.5 g) were analyzed from the 0, 0.02, and 1.6 mg L^{-1} PFOS treatment groups for each of the three replicates. Honey was only produced in the control and the 0.02 mg L^{-1} treatment in all replicates, and samples (1 g) were analyzed from each of these treatments for one replicate. Fecal deposits (0.5 g) were analyzed from one replicate of the 1.6-mg L^{-1} treatment. Detailed analytical procedures including quality assurance and quality control are described in the Supporting Information, with the suite of 18 polyfluoroalkyl substance (PFAS) compounds analyzed including PFOS. The limit of quantitation (LOQ) for PFOS in bee tissue, honey, and feces was 0.0003 mg L^{-1}. Mean PFOS recovery for control spike samples was 92% and that of the other PFAS compounds analyzed was 89% to 121% (PFOA = 89%), with precision less than 11% for PFOS based on the recovery of extraction internal standard from bee tissue and honey samples. The bioaccumulation factor (BAF) was calculated for each exposure concentration analyzed, by dividing the mean PFOS concentration in bee tissue samples ($n = 3$) by the PFOS exposure concentration in that treatment group, and the elimination factor was calculated by dividing the fecal PFOS concentration (mg kg^{-1}) by the bee tissue PFOS concentration (mg kg^{-1}) (refer to Supporting Information for Equations).

Generalized linear mixed-effects models (GLMMs) were used to assess the effect of PFOS on measured honey bee

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responses. These were constructed using the 'glmmTMB' function in the *glmmTMB* package in RStudio (Harrison et al., 2018). The effect of PFOS on honey bee biology was determined by the fixed effect of PFOS exposure concentration with the response variables of egg and larvae production, hive box temperature, bee weight, population growth, and gastric health. To determine the effect of PFOS on honey bee behavior, the fixed effect was PFOS concentration and the response variables were temperament, hive maintenance, and pest management. Variability in the responses among weeks and replicates was accounted for by nesting the trial and replicate number within week, as random effects. The most suitable response distribution was applied for each GLMM and was determined by comparing Akaike information criterion values between models for each response variable fitted with different response distributions.

Associations between the bioaccumulation of PFOS in bee tissue, transfer to honey, and the elimination in feces could not be tested due to the small sample size. In-text numerical data are presented as mean (*M*) and standard error (±). Bee and queen mortality were compared between each PFOS concentration treatment using pairwise comparisons in the *lsmeans* package (Lenth, 2016; R Core Team 2016), and significance was determined using false discovery rate (FDR)-corrected *p* values (at $\alpha = 0.05$) (Verhoeven et al., 2005). All statistical analyses were conducted in R version 3.4.3 (R Core Team 2016) and bar graphs were constructed with Excel 2019.

RESULTS

Biological response metrics

Hive box temperature decreased when colonies were exposed to each of the PFOS treatment concentrations over the 4-week exposure period (Supporting Information Figure S3). The highest mean (*M*) temperature ($M = 29.3\,°C \pm 1.1\,°C$) was recorded in the control and the lowest in the 1.6 mg L^{-1} treatment group ($M = 26.4\,°C \pm 1.2\,°C$), with a significant difference ($df = 715$, $p < 0.001$) between the control and all other treatment groups, including the lowest PFOS exposure concentration tested (0.02 mg L^{-1}), but no dose–response effect was evident.

Eggs were laid in all treatment groups, but the maximum number was in the control ($M = 63 \pm 14$), which showed significantly more eggs ($df = 167$, $p < 3.49^{e-05}$) than all the PFOS-treated groups (Figure 1A), although a dose–response relationship was not evident. Due to the reduced number of eggs laid in the PFOS treatment groups, a reduced number of larvae developed, only observed in the 0.02 mg L^{-1} ($M = 2 \pm 2$) and 0.08 mg L^{-1} ($M = 1 \pm 1$) PFOS exposure groups, compared with the controls which had significantly greater larval numbers ($M = 10 \pm 5$) ($df = 167$, $p = 0.0161$). Larvae progressed to pupae in all controls ($M = 15 \pm 6$) and in only one replicate of the 0.08-mg L^{-1} PFOS treatment ($M = 3 \pm 1$), but the pupae in this PFOS treatment were removed by the worker bees, indicating that the pupae were dead. Pupae in the controls for all replicates appeared healthy, and in one control, replicate young adults, of normal size and appearance, emerged by Week 4 ($M = 15 \pm 7$) (Supporting Information Figure S4). The data suggest that when *A. mellifera* colonies were exposed to PFOS (≥0.02 mg L^{-1}) over four weeks, egg viability was compromised. Tests of significance were not undertaken for pupae and young adults because these life stages were only observed in the control treatments.

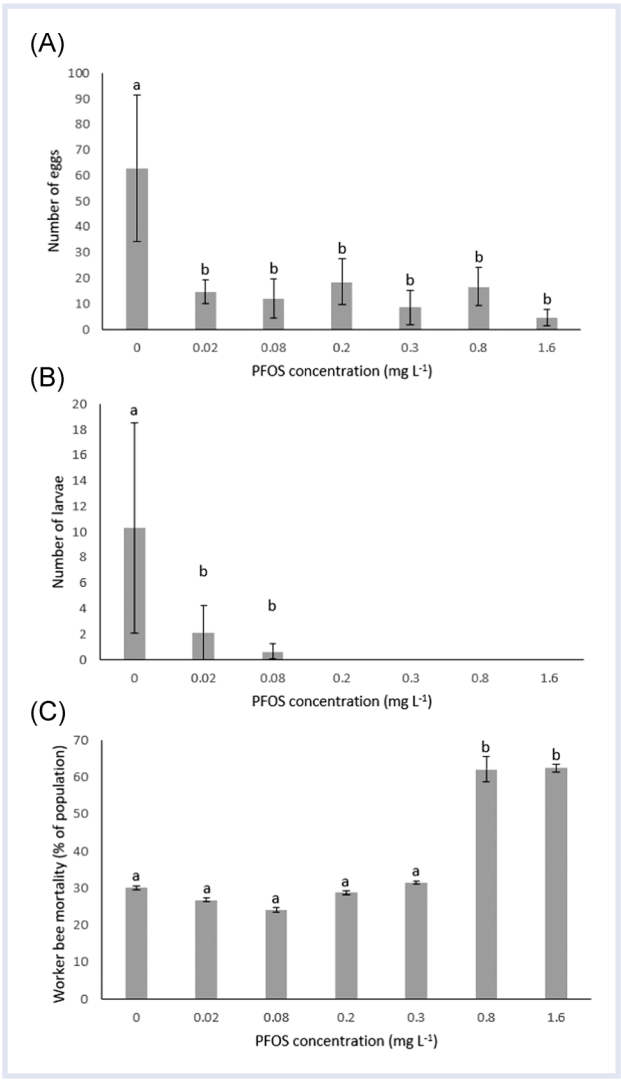


FIGURE 1 Biological responses observed as (A) eggs and (B) larvae in brood development, and (C) worker bee mortality at four weeks of PFOS exposure. Bar graph indicates the mean of three replicates and error bars indicate standard error. Letters indicate significant differences ($p < 0.05$)

Worker bee mortality increased with PFOS exposure and this became significant at exposure concentrations of 0.8 mg L^{-1} or greater ($df = 71$, $p < 4.84^{e-13}$) (mean mortality was 75.6 ± 3.82% at 1.6-mg L^{-1} PFOS exposure) (Figure 1C). Queen bee mortality was least in the control and in the 0.2 mg L^{-1} PFOS exposure treatment, with one death in each of these treatments over the three replicates. Two queens died in the 0.02, 0.08, and 0.8 mg L^{-1} treatment groups and three queens died at 0.3 and 1.6 mg L^{-1} PFOS exposure.

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Queen mortality was significantly lower in the control compared with the 0.3 mg L^{-1} ($df = 17$, $p = 0.0379$) and 1.6 mg L^{-1} ($df = 17$, $p = 0.0105$) treatment groups, but not significantly different from any other PFOS treatment group.

The colony population decreased in the control and all PFOS treatment groups (Supporting Information Figure S5), but the % decrease was larger at higher PFOS exposures and became significant at PFOS exposure of 0.2 mg L^{-1} or greater ($df = 17$, $p < 3^{e-04}$). The mean population decrease in the control was 33.26 ± 12.4%, and 75.6 ± 13.3% in the 1.6 mg L^{-1} treatment group. Mean bee weight was similar in the control ($M = 0.0863 \pm 0.0164$ g) and the 0.02, 0.08, and 0.2 mg L^{-1} PFOS exposure groups, but it was significantly smaller ($df = 1008$, $p < 2^{e-16}$) at PFOS exposures of 0.3 to 1.6 mg L^{-1}, with maximum colony weight loss at 1.6 mg L^{-1} PFOS exposure ($M = 0.0659 \pm 0.0169$ g) (Supporting Information Figure S6). Dysentery was observed only in the PFOS treatments, which started at Week 2, and the incidence increased at Weeks 3 and 4. Dysentery was not observed in any control treatment; however, gastric health measurements did not differ significantly among treatment groups ($df = 89$, $p = 0.0624$) (Supporting Information Table S5).

Behavioral response metrics

During the acclimatization week, the bee colonies successfully established in the hive boxes with no colonies absconding. The consumption of resources and bee activity in the cages increased or did not change over the week. Throughout the subsequent four weeks of PFOS exposure, consumption of resources varied in treatments. All of the sugar syrup, pollen, and protein sausage were consumed in the control treatment each week, but resource consumption decreased with PFOS exposure and ceased almost entirely by Week 4 in the 0.3 mg L^{-1} or greater PFOS treatment groups. Honey production was observed only in the control and lowest, 0.02 mg L^{-1}, PFOS treatment group, with approximately 4 × 4 cm of uncapped honey produced on frames in each of these treatments in all three replicates. When a queen death was observed in a control colony, the colony continued to operate as an individual colony (as opposed to joining the other colony in the cage) and remained in the hive box for the remainder of the trial. The PFOS treatment group colonies responded differently to queen death, and these colonies were observed to combine and, on one occasion, abscond from the hive.

Bee activity, as percent of the remaining population of bees in the cage sitting and/or crawling, and flying in the control was significantly ($df = 143$, $p < 2^{e-16}$) greater at both observation times (10:00 a.m. and 3:00 p.m.), compared with all other treatment groups, except the 0.02-mg L^{-1} for bees sitting and/or crawling (Figure 2). The data for the 0.02-mg L^{-1} treatment were influenced by colony absconding after queen death in one replicate, resulting in a large percentage of bees in the cage at all times. Bee activity was lowest in PFOS exposure 1.6 mg L^{-1} (Figure 2). Temperament in the control colonies was calm for the duration of the trial (*Mode* = 2 and 3), the 0.02 to 0.3 mg L^{-1}

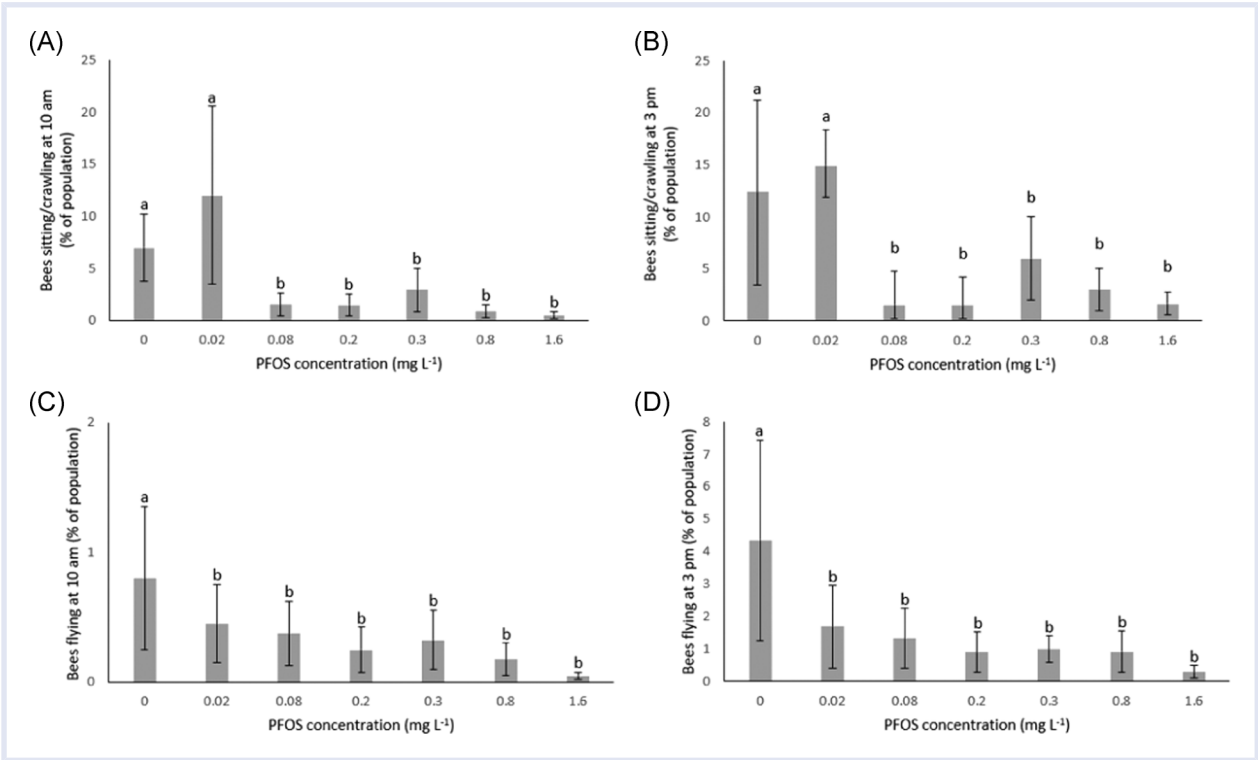


FIGURE 2 Bee activity observed during the PFOS exposure experiment: (A) bees (% of remaining population) sitting and/or crawling in the cage at 10 a.m. and (B) 3 p.m. and (C) bees (% of remaining population) flying at 10 a.m. and (D) 3 p.m.. Bar graphs indicate the mean of three replicates over four weeks of observation, and error bars indicate standard error. Letters indicate significant differences ($p < 0.05$)

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treatment groups displayed agitation (*Mode* = 4), but the 0.8 and 1.6 mg L^{-1} treatment groups were very calm (*Mode* = 1) (Supporting Information Table S6). There was a significant relationship between PFOS exposure and temperament in all treatment groups ($df = 143$, $p < 0.001$) when compared with the control.

Hive maintenance behaviour deteriorated in all PFOS treatment groups as compared with the control, and by Week 4, all PFOS exposure treatments scored a poor maintenance value of 4 in at least one replicate, significantly lower than the control which maintained the optimum value of 1 ($df = 181$, $p < 0.001$) (Supporting Information Table S7). Pests were observed in the 0.08, 0.8, and 1.6 mg L^{-1} treatment groups by Week 2 and in all treatment groups, except the control by Week 4, with a significant increase in pests in 0.08 mg L^{-1} or greater PFOS exposure treatments when compared with the control ($df = 194$, $p < 0.001$), indicative of decreased colony defense.

PFOS accumulation, transfer, and elimination

Perfluorooctane sulfonate was detected in all bee tissue, honey, and feces samples analyzed from the PFOS exposure treatments (0.02 and 1.6 mg L^{-1} for bee tissue, 0.02 mg L^{-1} for honey, and 1.6 mg L^{-1} for feces), but it was not detected in any of the samples collected from the controls (Table 1). The nondetects in controls confirm no cross-contamination between treatments, nor contamination of water, sugar syrup solution, or food supplement. Seven additional PFAS compounds (including PFOA) were detected in samples from PFOS exposure treatments but at significantly smaller concentrations than PFOS. The PFOS accumulated in the bee tissue at a mean BAF of 0.3 for the two exposure concentrations assessed. Assuming the honey was contaminated directly from bees, the transfer factor from bees in tissue to honey was approximately 0.4 at PFOS exposure of 0.02 mg L^{-1}. Fecal deposit analysis from the 1.6 mg L^{-1} treatment group showed a PFOS concentration

TABLE 1 PFAS concentrations (mg kg^{-1}) in bee tissue, honey, and feces (mean ± standard error)

	Bee tissue			Honey		Feces
	Treatment group (mg L^{-1})			Treatment group (mg L^{-1})		
PFAS[a]	Control	0.02	1.6	Control	0.02	1.6
PFPeA	-	-	-	-	-	-
PFHxA	-	-	-	-	-	-
PFHpA	-	-	0.0070 ± 0.0030	-	-	0.0044
PFOA	-	-	0.0003 ± 0	-	-	0.0017
PFNA	-	-	-	-	-	0.0044
PFDA	-	-	-	-	-	0.0014
PFUdA	-	-	-	-	-	-
PFDoA	-	-	-	-	-	-
PFBS	-	-	-	-	-	-
PFPeS	-	-	0.0018 ± 0.0007	-	-	-
PFHxS	-	0.0122 ± 0.0122	0.0425 ± 0.0174	-	0.0072	0.0069
PFHpS	-	0.0049 ± 0.0023	0.0146 ± 0.0054	-	0.0013	0.0014
PFOS	-	0.0887 ± 0.0887	0.3223 ± 0.1130	-	0.033	0.013
6:2 FTS	-	-	-	-	-	-
8:2 FTS	-	-	-	-	-	-
PFOSA	-	-	-	-	-	-
N-MeFOSAA	-	-	-	-	-	-
N-EtFOSAA	-	-	-	-	-	-

Note: "-" refers to lesser than the limit of quantitation. PFAS compounds are perfluoropentanoic acid (PFPeA), perfluorohexanoic acid (PFHxA), perfluoroheptanoic acid (PFHpA), perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA), perfluorodecanoic acid PFDA), perfluoroundecanoic acid (PFUdA), perfluorododecanoic acid (PFDoA), perfluorobutane sulfonate (PFBS), perfluoropentane sulfonate (PFPeS), perfluorohexane sulfonate (PFHxS), perfluoroheptane sulfonate (PFHpS), perfluorooctane sulfonate (PFOS), 6:2 fluorotelomersulfonate (6:2 FTS), 8:2 fluorotelomersulfonate (8:2 FTS), perfluorooctanesulfonamide (PFOSA), N-methylperfluorooctane sulfonamidoacetic acid (N-MeFOSAA), and N-ethyl perfluorooctane sulfonamidoacetic acid (N-EtFOSAA).

[a]PFAS in bold indicates those detected in bee tissue, honey, or feces.

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of 0.013 mg kg^{-1}, indicating that PFOS was eliminated from the bees in feces with an elimination factor of 0.04 (Supporting Information Equation 5). Statistical analysis was not possible for bee tissue, honey, or feces concentrations due to the small sample size.

DISCUSSION

Bees are adversely impacted by organic contaminants, such as neonicotinoid and pyrethroid pesticides (Alburaki et al., 2015; Aliquane et al., 2009; Chaimanee et al., 2016; Oliver et al., 2015; Williamson et al., 2014), but the effect of PFOS on honey bees is little studied and has not been examined previously using whole bee colonies. Understanding the effects on whole colonies is essential because the survival of bees and the services they provide are inherently dependent on colony health. The PFAS compounds, including PFOS, are widespread in the environment and are reported at high concentrations on thousands of contaminated sites (Groffen et al., 2018; Hepburn et al., 2019). Foraging bees are potentially exposed in contaminated areas through contaminated surface water, plant pollen, and nectar; however, the relative importance of these exposure routes has not yet been quantified. To date, LC50 values for bees have been determined only on individuals in two rather limited studies, with almost nothing known about sublethal and chronic exposure, EC50 values, or colony-level effects. This significantly hinders development of robust protective and management guidelines.

Our study is the first to evaluate PFOS effects on whole colonies of the European honey bee (*A. mellifera*). In the absence of ecological guidelines for PFOS at the time of experimental establishment, we used a PFOS exposure concentration range that included concentrations known to cause mortality in individuals (1.01 mg L^{-1} specified as the LD50 value for bumble bees) (Mommaert et al., 2011) and the range of values reported in contaminated surface and groundwater, including data from a contaminated site in Australia, which is within foraging range (<1–8 km) of numerous managed beehives (AECOM, 2016).

A healthy bee colony can generally maintain colony temperature, produce brood, maintain a low incidence of pests, and sustain a low rate of mortality for population growth (Winston, 1987). In our experiment, all of these responses, essential to colony survival, were adversely affected, and at PFOS concentrations not previously known to affect colonies (Wilkins, 2001a). In our experiment, all the colony biological responses monitored were adversely affected to some degree in treatment groups exposed to PFOS. This included brood development, maintenance of colony temperature, worker and queen bee mortality, gastric health, and bee weight. Although the cage environment provided for successful brood development (Winston, 1987), as evidenced by successful brood development in the controls (Sonter et al., 2019), even at the lowest PFOS exposure concentration of 0.02 mg L^{-1}, the development from eggs to young adults ceased. Larval death was possibly due to nurse bees feeding the larvae PFOS-contaminated food, rather than PFOS residue in the brood comb due to the short time period of exposure and use of noncontaminated frames (Wu-Smart & Spivak, 2016; Zhu et al., 2014). Although the mean brood temperature recorded in controls was lower than considered optimum (32–36 °C; Winston, 1987), successful development of brood in controls occurred, in contrast to the PFOS-exposed colonies where temperature decreased with PFOS exposure. This suggests that the PFOS-exposed colonies may not have been able to maintain social homeostasis. This finding may be a consequence of a range of factors including increased mortality resulting in a decreased number of worker bees available to maintain cluster warmth, worker bees not being able to effectively activate wing muscles, a breakdown of communication within the colony, and disorientation of worker bees (Colin et al., 2019; Meikle et al., 2019).

Worker bee mortality significantly increased with PFOS exposure at concentrations of 0.8 mg L^{-1} or greater. Bees in the 1.6 mg L^{-1} treatment group were exposed to approximately 0.02 µg PFOS per bee per day (assuming 11 µL^{-1} syrup ingested; Huang, 2010), with 20 days of exposure required (i.e., 0.02 µg^{-1} per day × 20 days) to attain the 72-h LD50 value of 0.40 µg/bee for an oral dose reported by Wilkins (2001a). In our experiment, mortality increased by more than 50% over the four weeks of exposure at 1.6 mg L^{-1}, which is relatively consistent when extrapolating from data using individuals (Wilkins, 2001a). The slightly higher mortality evident in the control, compared with the 0.02 mg L^{-1} treatment group (although not significant), could be a consequence of brood care requirements in a healthy colony requiring greater resource consumption and energy expenditure (Munch et al., 2013; Wolf & Schmid-Hempel, 1989).

The mechanism of bee toxicity with PFOS exposure is unclear, but Mommaert et al. (2011) reported that PFOS triggers the production of reactive oxygen species at the cellular level, which causes cell death. In line with an increase in bee mortality, populations decreased with PFOS exposure and this became significant at 0.2 mg L^{-1} or above. Although populations decreased also in the control, the decrease was less than that observed in PFOS treatments. Over a greater time period, as young emergent adults develop (observed in replicate 1), a population increase would be expected in a healthy colony (Nye, 1962; van Praagh, 1972). It is not clear whether the increase in mortality observed in the higher PFOS treatment groups was due to chemical toxicity response or a result of reduced feeding. Nevertheless, the increase in mortality observed can be attributed to PFOS exposure, even if indirectly.

Honey bee gastric health deteriorated even at the lowest PFOS exposure concentration of 0.02 mg L^{-1}, and dysentery was observed in all treatments except the control. PFOS-spiked sugar syrup solutions showed no significant difference in pH as compared with the control, confirming that pH was not the cause of dysentery (Demares et al., 2016). Recent research that supports these findings indicates that contaminants can adversely affect the bee gut microbiome,

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compromising the immune system and resulting in susceptibility to viral, bacterial, and/or fungal infections (Blot et al., 2019; Dai et al., 2018; Motta et al., 2018). Blot et al. (2019), for example, reported that glyphosate reduced the growth of beneficial microbiota, thereby allowing pathogen development, such as *Nosema ceranae* which reduces the general health and longevity of honey bees. Bee weight was also reduced with PFOS exposure concentration, and significantly in the 0.3 mg L^{-1} or greater PFOS treatment groups. Factors influencing the reduced bee weight may include poor gastric health (H. Zheng et al., 2017) and reduced consumption of the PFOS-spiked sugar syrup, pollen, and protein supplement due to avoidance or poor health (B. Zheng et al., 2014). In our study, the slightly smaller mean bee weight (not significant) recorded for the control as compared with 0.02 and 0.2 mg L^{-1} treatment groups was possibly influenced by the emergence of the young adult bees in the control which are lighter than mature adult bees (Winston, 1987).

Behavioral response to queen death altered with PFOS exposure. Instead of remaining in the nest (as seen in the controls), the bees from the colony that had lost the queen joined the queened colony in the same cage. It is also unusual for a queened colony to accept a queenless colony, when a queen dies. A possible explanation may be reduced olfactory memory specificity, as observed in honey bees exposed to pesticides at sublethal concentrations (Aliquane et al., 2009; Blacquiere et al., 2012; Chakrabarti et al., 2015; Williamson et al., 2013). Honey bee activity was also adversely affected with PFOS exposure, and flying was reduced even in the lowest PFOS concentration treatment group of 0.02 mg L^{-1}. Temperament was altered with PFOS exposure and bees were more agitated at PFOS exposure of 0.02-mg L^{-1} or above, but calmer in the 0.8-mg L^{-1} or greater PFOS treatment groups. The lower exposure elicited a response that stimulated defensive behavior (possibly due to stress) (Wray et al., 2011), but at higher exposures, metabolic changes as a result of toxicity, or lower energy due to reduced food consumption probably suppressed temperament (Oliver et al., 2015; Williamson et al., 2014). We also observed a deterioration in hive maintenance in all PFOS exposure concentration treatment groups and an increased number of pests, compared with the controls. This may be due to lower bee numbers in PFOS treatments and/or a reduced colony capacity to redirect to normal functional tasks, suggesting a reduced capacity to maintain the hive and defend against pests (Chakrabarti et al., 2015; Couvillon et al., 2010; Williamson et al., 2013).

Our results demonstrated that PFOS exposure adversely affected a wide range of biological and behavioral responses in honey bee colonies. Effects varied depending on response and PFOS exposure concentration. The mechanism of toxicity for honey bees exposed to PFOS is not understood, but honey bees exposed to pyrethroid and neonicotinoid insecticides show symptoms consistent with an impaired nervous system. At sublethal concentrations, overstimulation of the nervous system occurs and death results (Soderlund & Bloomquist, 1989; Williamson et al., 2014). PFOS effects on the honey bee nervous system can be considered as one possible hypothesis for the effects observed in our study, but toxicity response and mechanisms require further exploration.

Our study confirms that PFOS (and other PFAS) accumulated in honey bee tissue in the colony environment exposed to PFOS. The only other study available examined the transfer of PFOS to bee tissue of bumblebee (*Bombus terrestris*) workers directly fed 100 µl^{-1} PFOS-spiked sugar syrup (Mommaert et al., 2011). The PFOS accumulated to 2184 ± 365 ng wet body weight in bees, equivalent to a much higher BAF value of 27.9 (Mommaert et al., 2011) than observed in our study, as would be expected in a direct feeding trial. Moreover, our study also demonstrates that PFOS transfers to honey, which supports the results of the European survey that detected PFOS in honey samples, though the PFOS source was unknown (Surma et al., 2016). In addition, we provide evidence that PFOS can be eliminated from the bee body in the feces, with potential additional contamination of hive parts, bees, and surrounds.

CONCLUSION

We investigated the exposure of European honey bee colonies to the water-soluble and persistent contaminant, PFOS. Our results showed that PFOS exposure resulted in a range of adverse biological and behavioral colony responses. Mortality increased at PFOS exposures of 0.8 mg L^{-1} or above and brood development ceased entirely even at the lowest PFOS exposure concentration of 0.02 mg L^{-1}. Adverse behavioral changes were observed at PFOS exposure of 0.02 mg L^{-1} or above. The PFOS accumulated in bee tissue of the colony; it appeared to be partially eliminated in feces, but it also transferred to honey.

The PFOS exposure pathways for bees in the field are little understood, but foraging bees are potentially exposed in contaminated areas through contaminated surface water, plant pollen and nectar. The relative importance of these exposure routes is not yet quantified, nor have relevant field exposure concentrations been identified. Thus, the next steps to our work are to establish colony-based EC50 and LC50 values, understand toxicity mechanisms and further investigate PFOS transfers and transformations. Field-based investigation is needed to understand the relative importance of exposure routes and field exposure concentrations for the development of comprehensive ecological risk assessment and colony-based protective guidelines for honey bees. Honey bees pollinators in wild and managed ecosystems, and they produce products for human consumption. Hence, the development of this work has important implications for food chain contaminant movement, food security, and the establishment of robust guidelines for the protection of the environment and humans.

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CONFLICT OF INTEREST

The authors declare there are no conflicts of interest.

DISCLAIMER

The peer-review process for this article was managed by the Editorial Board without the involvement of SC Wilson.

DATA AVAILABILITY STATEMENT

Data are available by emailing corresponding author Carolyn Sonter (csonter@myune.edu.au).

SUPPORTING INFORMATION

TABLE S1. Fecal deposits and associated dysentery scale.

TABLE S2. Temperament scale.

TABLE S3. Hive maintenance scale.

TABLE S4. Pest management scale.

TABLE S5. Mode gastric health score for each exposure concentration treatment group (all samples $N = 3$).

TABLE S6. Mode temperament score for 10 a.m. and 3 p.m. for each exposure concentration treatment group (all samples $N = 3$).

TABLE S7. Mode maintenance score for each exposure concentration treatment group (all samples $N = 6$).

FIGURE S1. Cage system showing three of the cages with timber hive boxes and syrup and water feeders.

FIGURE S2. Fecal deposits—Light circular deposits indicating good gastric health and the dark and/or streaky deposits indicating dysentery and poor gastric health.

FIGURE S3. Hive box temperature during the PFOS exposure experiment. Bar graph indicates the mean of three replicates and error bars indicate standard error. Letters show statistical differences between treatments ($p = .05$).

FIGURE S4. Young adults that emerged for each treatment group during the PFOS exposure experiment. Bar graph indicates the mean of three replicates and the error bar indicates standard error.

FIGURE S5. Mean decrease in population (% of initial population) in the PFOS exposure experiment. Bar graph indicates the mean of three replicates and the error bar indicates standard error. Letters indicate significant differences ($p = .05$).

FIGURE S6. Worker bee weight (g) in the PFOS exposure experiment. Bar graph indicates the mean of three replicates and error bar indicates standard error. Letters indicate significant differences ($p = .05$).

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Persistent Organic Pollutants Review Committee
Twelfth meeting
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Report of the Persistent Organic Pollutants Review Committee on the work of its twelfth meeting

Addendum

Risk profile on pentadecafluorooctanoic acid (CAS No: 335-67-1, PFOA, perfluorooctanoic acid), its salts and PFOA-related compounds

At its twelfth meeting, by its decision POPRC-12/2, the Persistent Organic Pollutants Review Committee adopted a risk profile for pentadecafluorooctanoic acid (CAS No: 335-67-1, PFOA, perfluorooctanoic acid), its salts and PFOA-related compounds on the basis of the draft contained in the note by the Secretariat (UNEP/POPS/POPRC.12/3), as revised during the meeting. The text of the risk profile as adopted is set out in the annex to the present addendum. It has not been formally edited.

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Annex

Pentadecafluorooctanoic acid (PFOA, Perfluorooctanoic acid), its salts and PFOA-related compounds

RISK PROFILE

Persistent Organic Pollutants Review Committee

September 2016

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Executive summary

1. The POPs Review Committee concluded that PFOA fulfilled the screening criteria in Annex D and that issues related to the inclusion of PFOA-related compounds that potentially degrade to PFOA and the inclusion of PFOA salts should be addressed in the draft risk profile (see Decision POPRC-11/4). The substances covered by this risk profile include pentadecafluorooctanoic acid (CAS No: 335-67-1, EC No: 206-397-9, PFOA, perfluorooctanoic acid) including its isomers, its salts and PFOA-related compounds.

2. PFOA and its salts are most widely used as processing aids in the production of fluoroelastomers and fluoropolymers, with polytetrafluoroethylene (PTFE) being an important fluoropolymer. PFOA-related compounds are used as surfactants and surface treatment agents (e.g. in textiles, paper and paints, fire-fighting foams) and for the manufacture of side-chain fluorinated polymers. Due to their physico-chemical properties, PFOA, its salts and PFOA-related compounds are used in a wide variety of applications and consumer products across many sectors.

3. PFOA, its salts and PFOA-related compounds are subject to a number of national regulations. Norway has banned its use in consumer products (currently the substance is being phased out); while in the U.S. there is a voluntary initiative to phase out its use. Elsewhere in Canada and the EU, steps are underway towards the setting of legally binding conditions to restrict or ban the substance.

4. From 1951 to 2004, the estimated total global production of PFOA and APFO (ammonium perfluorooctanoic acid) was 3600 – 5700 t. Current production of PFOA is predominantly carried out in China where the production of PFOA and its salts has tripled from around 30 t in 2004 to around 90 t in 2012. The most recent data that is publically available concerning global production of APFO indicates that an average 200-300 t of PFOA were produced annually (1995-2002). It is expected that current production is significantly lower due to the voluntary phasing out of the substance by U.S., EU and Japanese companies. However, its current use at global level in the production of fluoroelastomers and fluoropolymers demonstrates that there is an ongoing production and use.

5. Direct releases to the environment occur from the production of the raw substance (including PFOA as impurity in the manufacturing of PFOA-related compounds and some alternatives), during the processing, use and disposal of the chemical, from treated articles and from products contaminated with PFOA. Main emission vectors of PFOA and its salts are water, wastewater and dust particles. Historic releases to the environment from PFOA manufacturing are available from a plant in the U.S. into air and water between 1951 and 2003. Some estimates of releases during the disposal of the chemical are available, particularly from sewage treatment plants, wastewater treatment plants and landfill sites. Indirect releases occur from the degradation or transformation of precursors. PFOA-related compounds are released to air and (waste) water and will degrade to PFOA in the environment and in organisms. An assessment of sources of PFOA to the Baltic Sea estimated that 30% of the releases were due to transformation of fluorotelomers. Thus releases of PFOA from degradation contribute a major share to the releases of PFOA to the environment.

6. Degradation results show that PFOA is persistent and does not undergo any abiotic or biotic degradation under relevant environmental conditions. The monitoring data show that PFOA in soil leaches over time and can be a long term source to underlying groundwater. On this basis it is concluded that PFOA is highly persistent in all environmental compartments, with a strong resistance to all conventional mechanisms of degradation under relevant environmental conditions.

7. PFOA has a low to moderate potential to accumulate in aquatic (i.e. water breathing) species, but there is evidence that PFOA and its salts accumulate and biomagnify in air breathing terrestrial and marine mammals (BMFs, TMFs > 1).

8. Worldwide monitoring of water, air, sediment and biota at remote locations all detect the presence of PFOA and related compounds. Equally environmental modeling data suggest that the capacity for long range transport does exist, while others have identified key mechanisms which would make long range transport plausible. On this basis it can be concluded that PFOA directly or through precursors is subject to long range transport.

9. PFOA exposure of the general public typically takes place “human via environment” by consumption of drinking water and food including breast feeding, via uptake of contaminated indoor air and dust or from consumer products containing PFOA, its salts and related compounds. PFOA has been detected in humans in blood and breast milk from various countries. Humans are very slow eliminators of PFOA compared with other species with an estimated half-life of PFOA elimination ranging from 2 to 4 years. PFOA accumulates in humans with increasing levels with age.

10. PFOA exhibits low acute toxicity in aquatic organisms. In fish, PFOA inhibited expression of genes involved in thyroid hormone biosynthesis, induced vitellogenin gene expression, developed oocytes in the testes of males and caused ovary degeneration in females. PFOA has the potential to affect endocrine function where visible effects may not be apparent until the organisms reach adulthood.

11. Effects of repeated oral PFOA exposure in animals such as alterations to the liver, reproductive/developmental toxicity and endocrine disruption have been identified. Developmental effects that are of relevance for the oral route are perinatal mortalities, reduced pup body weight gain and development, cyanosis and necrosis, delayed ossification, changes in mammary gland development and delayed maturation.

12. In the European Union, PFOA has a legally-binding harmonized classification as Carc. 2, Repr. 1B and STOT RE 1 (liver). PFOA is quickly absorbed, not metabolised and distributed in the body, transferred to foetus through placenta and infants via breast milk. A considerable number of adverse health effects associated with PFOA exposure in humans have been reported. The C8 Science Panel (a large-scale epidemiological study conducted in the US on PFOA exposure and health effects) concluded that there was a probable link to PFOA exposure for diagnosed high cholesterol, ulcerative colitis, thyroid disease, testicular cancer, kidney cancer and pregnancy-induced hypertension. Scientific data have demonstrated PFOA-mediated immunotoxicity, primarily suppression of antibody response, in humans. There have also been other reported adverse health effects (e.g. altered reproductive or developmental effects, endocrine disruption, impaired neurodevelopment, immunotoxicity, etc.) associated with PFOA exposure in humans.

13. PFOA is persistent, bioaccumulative and toxic to animals including humans. There is widespread occurrence of PFOA and a number of PFOA-related compounds in environmental compartments and in biota and humans. Therefore, it is concluded that PFOA, its salts and related compounds that degrade to PFOA are likely, as a result of their long-range environmental transport, to lead to significant adverse human health and/or environmental effects such that global action is warranted.

1. Introduction

14. In June 2015 the European Union and its Member States submitted a proposal to list pentadecafluorooctanoic acid (CAS No: 335-67-1, PFOA, perfluorooctanoic acid), its salts and PFOA-related compounds in Annex A, B, and/or C of the Stockholm Convention (UNEP/POPS/POPRC.11/5). This proposal was considered by the Persistent Organic Pollutants Review Committee (POPRC) at its eleventh meeting held in October 2015.

15. PFOA, its salts and PFOA-related compounds fall within a family of perfluoroalkyl and polyfluoroalkyl substances (PFASs). PFASs consist of carbon chains of different chain length, where the hydrogen atoms are completely (perfluorinated) or partly (polyfluorinated) substituted by fluorine atoms (Buck et al., 2011; OECD, 2013; ECHA, 2015a). The very stable bond between carbon and fluorine is only breakable with high energy input. Therefore, perfluorinated acids, like PFOA, are not degradable in the environment. Certain polyfluorinated substances can be degraded to persistent perfluorinated substances like PFOA under environmental conditions and are therefore precursors. Those PFASs, which can be degraded to PFOA in the environment, are referred to as PFOA-related compounds. PFOA and its salts are most widely used as processing aids in the production of fluoroelastomers and fluoropolymers, with PTFE being an important fluoropolymer. PFOA-related compounds are used as surfactant and for the manufacture of side-chain fluorinated polymers (ECHA, 2015a). Due to the surfactant properties of both PFOA and its related non-polymeric surfactants, applications exist for the use of these substances, e.g. fire-fighting foams, wetting agents and cleaners. Side-chain fluorinated polymers provide durable water, oil and stain repellency and find use as surface finishes for textiles and apparel, leather, paper and cardboard, paints, lacquers and other uses (non-woven medical garments, floor waxes, and stone/wood sealants, thread sealant tapes and pastes, adhesives, products for apparel) (UNEP/POPS/POPRC.11.5; FluoroCouncil, 2016).

16. PFOA has been manufactured since 1947 when 3M developed the production process via electrochemical fluorination (ACS, 2015). PFOA-related compounds (such as fluorotelomer alcohols) have also been in use, notably with the development of fluorotelomer technologies in the 1960s and their subsequent commercialization in the 1970s and thereafter. The perfluorooctyl iodide fraction of fluorotelomers was oxidized to PFOA and subsequently used as a fluoropolymerization aide by several fluoropolymer producers globally (DuPont, 2010; FluoroCouncil, 2016). However growing concerns regarding health and environmental effects of PFOA have meant stricter controls and phase-out plans under legislation such as the regulation on registration, evaluation, authorization and restriction of

chemicals (REACH EC 1907/2006) in the EU and the *Canadian Environmental Protection Act 1999* (CEPA) as well as voluntary efforts such as the US EPA PFOA Stewardship Program (US EPA, 2015) and work by industry (OECD, 2015). With a voluntary move by the Telomer Research Program (TRP) and Fluoropolymer Manufacturing Group (FMG) members in 2006, eight of the main manufacturers of C8-substances (fluorochemicals related to eight fully fluorinated C8-atoms) in the US, Europe and Japan agreed on a 2-stage stepwise global phase out of PFOA and related long-chain substances by the end of 2015 (ACC, 2015; FluoroCouncil, 2015/2016). A similar program existed in Canada[1]. All Stewardship Program participants were successful at virtually eliminating those chemicals globally from facility emissions and product content. Beyond possible impurities, Stewardship Program participants no longer manufacture, use, or sell PFOA and related long-chain substances. The voluntary phase out does not include manufacturers using PFOA in countries like China, India or Russia (ECHA, 2015a). After the Stewardship participants announced they were phasing out PFOA and related long-chain substances, companies who were not part of the Stewardship Program increased capacities (FluoroCouncil, 2016).

17. In 2011, PFOA-relevant technology and products were added to the Catalogue for the Guidance of Industrial Structure Adjustment in China (NDRC, 2013), including that new installation of PFOA production facilities shall be restricted and that PFOA-containing paints and fluoropolymers that use PFOA in the polymerization shall be eliminated. In 2013, fluoropolymers that use PFOA in the polymerisation were recognized as products with high pollution and high environmental risk ("dual-high" products) in the Comprehensive Catalog for Environmental Protection (China MEP, 2015).

18. The proposal to list PFOA, its salts and PFOA-related compounds in Annex A, B and/or C of the Stockholm Convention (UNEP/POPS/POPRC.11.5) highlighted concerns that the presence of PFOA in the environment is also influenced by the degradation of PFOA-related compounds, including side-chain fluorinated polymers. Hence the addition of PFOA alone to the Stockholm Convention would not be sufficient to protect human health and the environment. For some substances, e.g. for the fluorotelomer alcohol 8:2 FTOH[2] degradation to PFOA has been proven by experiment. Furthermore, potential PFOA-related compounds can be substances containing a perfluorinated alkyl chain with the formula $F(CF_2)_n$- (n=7 or 8) and that is directly bonded to any chemical moiety other than a fluorine, chlorine or bromine atom or a phosphonic, phosphinic or sulfonic group. Such substances can undergo abiotic degradation resulting in release of PFOA (Nielsen, 2013, 2014; Wang et al., 2014a; Ellis et al., 2004a). C8-substances (e.g. PFOA) may occur as impurities in the C6 alternatives. Thus, the C6 alternatives also contain C8 (and longer chain) residual substances, which can be released into the environment. (ECHA, 2015a).

1.1 Chemical identity

19. The proposed substances defined within the screening dossier (UNEP/POPS/POPRC.11/5) include pentadecafluorooctanoic acid (CAS No: 335-67-1, EC No: 206-397-9, PFOA, perfluorooctanoic acid) including its salts and PFOA-related compounds.

20. Taking into account the background document to the restriction proposals for PFOA, PFOA salts and PFOA-related compounds (ECHA 2015a; ECHA 2015c), the risk profile covers:

(a) PFOA (including its isomers);

(b) Its salts; and

(c) PFOA-related compounds which, for the purposes of this risk profile, are any substances that degrade to PFOA, including any substances (including salts and polymers) having a linear or branched perfluoroheptyl group with the moiety (C7F15)C as one of the structural elements, for example:

(i) Polymers with C8 to C16 based fluorinated side chains;[3]

(ii) 8:2 fluorotelomer compounds;

[1] Environmental Performance Agreements and Results: Perfluorinated Carboxylic Acids (PFCAs) and their Precursors (2010-2015), from http://www.ec.gc.ca/epe-epa/default.asp?lang=En&n=0D8C879E-1#X-2013092511492112

[2] FTOHs are polyfluorinated compounds typically characterized by even numbered perfluorinated carbons and two nonfluorinated carbons adjacent to a hydroxyl group (Dinglasan et al., 2004). For FTOHs, the prefix 'C8:2' denotes that for "X:Y", X = the number of perfluorinated carbons within the alkyl chain, and Y = the number of non-fluorinated carbons within the alkyl chain.

[3] DuPont, 1998. Technical information: Zonyl fluorochemical intermediates.

(iii) 10:2 fluorotelomer compounds.

PFOA-related compounds do not include:

(i) C8F17-X, where X= F, Cl, Br;

(ii) Fluoropolymers[4] that are covered by CF3[CF2]n-R', where R'=any group, n>16; or

(iii) PFOS, its salts and PFOSF as listed in Annex B to the Stockholm Convention.

21. Data on PFOA are presented in Table 1 and Table 2. The screening dossier (UNEP/POPS/POPRC.11/5) also further includes information on salts of PFOA and PFOA-related compounds based on the study carried out by the OECD (2007, 2011) as well as information from an assessment conducted by Environment Canada and Health Canada (2012). To maintain a concise document tables of data for PFOA salts and related PFOA compounds are provided in a background document to this risk profile (see section 1.1 of document UNEP/POPS/POPRC.12/INF/5).

Table 1: Identity of PFOA

CAS number:	335-67-1
CAS name:	Octanoic acid, 2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-pentadecafluoro-
IUPAC name:	Pentadecafluorooctanoic acid
EC number:	206-397-9
EC name:	Pentadecafluorooctanoic acid
Molecular formula:	$C_8HF_{15}O_2$
Molecular weight:	414.07 g/mol
Synonyms:	Perfluorooctanoic acid; PFOA; Pentadecafluoro-1-octanoic acid; Perfluorocaprylic acid; Perfluoro-n-octanoic acid; Pentadecafluoro-n-octanoic acid; Pentadecafluorooctanoic acid; n-Perfluorooctanoic acid; 1-Octanoic acid, 2,2,3,3,4,4,5,5,6,6, 7,7,8,8,8-pentadecafluoro

Table 2: Overview of relevant physicochemical properties of PFOA

Property	Value	Reference/Remark
Physical state at 20°C and 101.3 kPa	Solid	Kirk, 1995
Melting/freezing point	54.3 °C 44 — 56.5 °C	Lide, 2003 Beilstein, 2005 cited in ECHA, 2013a
Boiling point	188 °C (1013.25 hPa) 189 °C (981 hPa)	Lide, 2003 Kauck and Diesslin, 1951
Vapour pressure	4.2 Pa (25° C) for PFO; extrapolated from measured data 2.3 Pa (20° C) for PFO; extrapolated from measured data 128 Pa (59.3° C) for PFO; measured	Kaiser et al., 2005; Washburn et al., 2005 Washburn et al., 2005 Washburn et al., 2005
Water solubility	9.5 g/L (25° C) 4.14 g/L (22°C)	Kauck and Diesslin, 1951 Prokop et al., 1989
Dissociation constant	<1.6, e.g. 0.5 1.5 — 2.8	Vierke et al., 2013 Kissa, 2001
pH-value	2.6 (1 g/L at 20 °C)	ECHA 2015a (reliability not assignable)

[4] Fluoropolymers are carbon-only polymer backbone with F directly attached to backbone C atoms.

22. There are two manufacturing processes to produce PFOA, its salts and PFOA-related compounds: electrochemical fluorination (ECF) and telomerization. From 1947 until 2002, the ECF process was mainly used to manufacture ammonium perfluorooctanoate (APFO) worldwide (80-90% in 2000) which results in a mixture of branched and linear isomers (78% linear and 22% branched isomers). The current extent of global ECF manufacturing is unknown; however, most of the manufacturers are using the telomerisation process nowadays, which mainly results in linear compounds (Wang et al., 2014a). Results from Jiang et al. (2015) suggest that ECF is still used by some manufacturers in China. ECHA (2013b) details the physico-chemical properties of APFO in the environment. The free PFOA stays in equilibrium with PFO, the conjugate base, in aqueous media in the environment as well as in the laboratory. The physico-chemical properties of PFOA and PFO are different. Therefore, the expected environmental fate will depend on the environmental conditions, which influence the equilibrium between base and acid (pH and pKa). APFO, which is often used in animal experiments, is very soluble in water. In aqueous solution it is present as anion PFO and the ammonium cation. Goss (2008) suggested that PFOA is expected to have a low pKa, such that >99% of the compound will occur in its anionic form (i.e., PFO) under most environmental conditions suggesting that the environmental partitioning of PFOA will be dominated by the anionic form. The dissolved anion PFO will stay in equilibrium with the corresponding acid in aqueous media. With currently available analytical methods it is not possible to distinguish between PFO and PFOA in samples. In the literature reporting human and environmental monitoring studies the concentrations are referred to as PFOA or APFO, but always both species (PFO and PFOA) are included in the given concentration (ECHA, 2013b).

23. A standardized method for the analysis of presence of PFOA in different matrices is currently not available. The unique chemical and physical properties of PFOA prevent it from being measured using conventional analysis. More complex methodology of liquid chromatography and tandem mass spectrometry (LC/MS-MS) has been proven most reliable for analyzing PFOA in biological and environmental samples and therefore the analytical method of choice (Xu et al., 2013; EFSA, 2008; Loos et al., 2007). This type of analysis has allowed for more sensitive determination of many perfluorinated chemicals (PFCs), including PFOA, in air, water, and soil (ATSDR, 2015).

1.2 Conclusion of the Review Committee regarding Annex D information

24. The POPs Review Committee evaluated the proposal regarding pentadecafluorooctanoic acid (CAS No: 335-67-1, PFOA, perfluorooctanoic acid), its salts and PFOA-related compounds (UNEP/POPS/POPRC.11/5) according to the requirements in Annex D of the Stockholm Convention at its eleventh meeting in Rome. The Committee concluded that PFOA fulfilled the screening criteria in Annex D. The Committee also decided to establish an ad-hoc working group to review the proposal further and prepare a draft risk profile in accordance with Annex E of the Convention and further that issues related to the inclusion of PFOA-related compounds that potentially degrade to PFOA and the inclusion of PFOA salts should be dealt with in developing the draft risk profile (Decision POPRC-11/4).

1.3 Data sources

25. The draft risk profile is based on the following data sources:

(a) Proposal submitted by the European Community and its member states that are Parties to the Convention (UNEP/POPS/POPRC.11/5), 2015;

(b) Information submitted by Parties and observers according to Annex E of the Convention: Albania, Austria, Canada, China, Germany, Hungary, Japan, Monaco, Norway, Romania Estonian Environmental Research Centre, FluoroCouncil, IPEN, Semiconductor Industry Associations;

(c) The Screening Assessment Report on PFOA, its salts and precursors prepared by Environment Canada and Health Canada, 2012;

(d) Screening Information Datasets (SIDS) on PFOA and the ammonium salt of PFOA prepared by the OECD, 2006, 2007, 2011, 2013;

(e) Support documents for the identification of PFOA and the ammonium salt of PFOA as a Substance of Very High Concern (SVHC) included under the EU Regulation on Registration, Evaluation, Authorization and restriction of Chemicals (REACH), (ECHA, 2013a, 2013b);

(f) Committee for Risk Assessment (RAC) and Committee for Socio-economic Analysis (SEAC) background document to the opinion on the Annex XV dossier proposing restrictions on PFOA, PFOA salts and PFOA-related compounds (ECHA, 2015a);

(g) Data from the United States Environmental Protection Agency (US EPA) PFOA Stewardship Programme;

(h) The Australian National Industrial Chemicals Notification and Assessment Scheme (NICNAS) Environment tier II assessment for both direct and indirect precursors of PFOA (NICNAS, 2015a and 2015b).

1.4 Status of the chemical under international conventions

26. PFOA is subject to one international convention. Under the Oslo/Paris Commission for the Protection of Marine Environment of the North-East Atlantic (OSPAR), a review of both PFOS and PFOA was conducted to assess the potential impact upon the environment. This led to the inclusion of PFOS on the list of chemicals for priority action in 2003. PFOA was not added to the list at that time pending additional research and review with the option for inclusion at a later date (OSPAR, 2006).

27. OECD provided a recent overview on risk reduction approaches for PFASs across countries (OECD, 2015). PFOA is subject to a number of national and/or regional regulations:

(a) In 2013, both PFOA and APFO were identified as Substances of Very High Concern (SVHC) upon their persistent, bioaccumulative and toxic properties and were included into the REACH-Candidate List (ECHA, 2013a, 2013b). Inclusion upon this list means the substances can be subject to further review and ultimately phased out under the Authorization process. Moreover, on request industry is obliged to inform consumers on the occurrence to the listed substances in consumer articles;

(b) In 2014 Germany and Norway lodged a joint proposal for the inclusion of PFOA within Annex XVII (restriction) of the REACH regulation within the EU (ECHA 2014a). The aim of the proposal was a total ban on manufacture, placing on the market and use (including import) of PFOA and its salts also including substances that may degrade to PFOA (PFOA-related compounds) in concentrations equal to or greater than 2 ppb. The proposed restriction also covers articles containing these substances. After submission of the proposal to the European Chemicals Agency (ECHA) stakeholders commented this proposal during the public consultation period and delivered new information. Subsequently, the proposal was updated by the Dossier Submitters together with ECHA's scientific committees RAC and SEAC and it has been transmitted to the European Commission for developing the final legislative proposal.

(c) PFOA was included under the Classification, Labelling and Packaging (CLP) Regulation (Regulation (EC) No 1272/2008), by the Commission Regulation (EU) No 944/2013 of 2 October 2013 (index number: 607-704-00-2). PFOA has been classified as Carc. 2 H351, Repr 1B H360D, Lact H362, STOT RE 1 (liver) H372, Acute tox 4 H332, Acute tox 4 H302 and Eye dam 1 H318.

(d) The Norwegian Environment Agency published an amendment to the consumer products regulation in 2014 banning the use of PFOA in consumer products and textiles. This has a transitional period allowing the import and sale of products before phase out;

(e) Within Canada following the screening assessment conducted in 2012, PFOA, its salts and precursors were found to meet the requirements of Section 64a of CEPA and were added to Schedule 1, the List of Toxic Substances. In June 2006, the Government of Canada published a Notice of Action Plan for the assessment and management of perfluorocarboxylic acids and their precursors. The Action Plan included measures to prevent the introduction of new substances into Canada that would contribute to the level of PFCAs (perfluorocarboxylic acids) in the environment, and to seek action from industry to address sources of PFCAs already in Canadian commerce. To this end, a voluntary Environmental Performance Agreement was signed on March 30, 2010. Signatories to the Performance Agreement agreed to reduce the amount of PFOA and long-chain perfluorocarboxylic acids in perfluorinated chemicals in Canadian commerce by 95% by December 31, 2010, and to virtually eliminate them by December 31, 2015. Furthermore in April 2015, the proposed *Regulations Amending the Prohibition of Certain Toxic Substances Regulations, 2012,* were published in Canada. These amendments propose to prohibit PFOA, its salts and precursors and products containing them, unless present in manufactured items. Furthermore, the amendments propose time-limited exemptions and ongoing permitted uses for certain applications where the development of alternatives is underway or where there are currently no known alternatives;

(f) In the United States of America, the US EPA established the PFOA Stewardship Programme in 2006. This is a programme that includes eight major manufacturers of PFOA, its salts and PFOA-related compounds (Arkema, Asahi, BASF, Clariant, Daikin, 3M/Dyneon, DuPont, Solvay

Solexis). The programme is a voluntary initiative to the phase-out the manufacture and use of PFOA, PFOA precursors and related higher homologue substances (US EPA, 2015).

(g) Russia regulates APFO in occupational air. A number of short- and middle chain PFASs are regulated in occupational air and water (OECD, 2013).

2. Summary information relevant to the risk profile

2.1 Sources

2.1.1 Production, trade, stockpiles

28. The following table summarizes information related to the production of PFOA, its salts, APFO and FTOH according to several literature sources. Further information is contained in section 2.1.1 of document UNEP/POPS/POPRC.12/INF/5.

Table 3: Production of PFOA, its salts, APFO and FTOH

Year/Period	Production	Value/Range [in t or t/y]	Reference
1992-2002	PFOA production 3M [in US]	113 t/y	3M communication to US EPA, 2003
2009	Estimated worldwide production of FTOH	11,000-14,000 t/y	Umweltbundesamt, 2009
2014	Production of PFOA related substances in the EU (actual volume is likely to be greater)	100 – 1,000 t/y	ECHA, 2015a
2003	Production of PFOA and its salts in China	30 t/y	Li et al., 2015
2012	Production of PFOA and its salts in China	90 t/y	Li et al., 2015

29. In 2005, producers were located in Italy, U.S., South America, Japan and China. Up until 2010, production was also carried out in Italy. All production of PFOA and its salts in the EU has now ceased, and manufacturing in Japan and the U.S. should now have stopped with the intention to phase out production by the end of 2015 (ECHA, 2015a). However, in China, the production of PFOA and its salts has tripled from around 30 t in 2004 to around 90 t in 2012 (Li et al., 2015).

30. In 2014, production of PFOA-related compounds in the EU was reported to be between 100-1,000 tonnes per annum (ECHA, 2015a). However, this volume is derived from a search in the ECHA-database of four PFOA-related compounds registered under REACH, and in its report ECHA concludes that the actual volume is likely to be greater. No other information concerning the production of PFOA-related compounds was identified in the submissions by parties, or in the literature reviewed.

31. Data concerning imports and exports of PFOA is limited and no information concerning global volume of trade flows has been identified owing to industry confidentiality claims (ECHA, 2015a).

32. PFOA ammonium salt was imported into Canada in quantities ranging between 0.1 to 100 t (2004 survey) (Environment Canada and Health Canada, 2012).

33. ECHA 2015a provides estimates on import figures for the EU. The main information related to imports is summarized in the following (detailed information see section 2.1.1 of document UNEP/POPS/POPRC.12/INF/5).

Table 4: Import of PFOA, its salts and PFOA related substances to the EU

Year	Imports to the EU	Value [in t]
2014	PFOA and its salts (decreasing trend since 2008)	~20
2014	PFOA and its salts (mixtures i.e. in fluoropolymer dispersions)	~10
2014	PFOA and its salts in articles (high uncertainty)	<10
2015	PFOA and its salts forecast to fall after 2015	<0.15
2014	PTFE	3 – 16
2014	PFOA related substances	100 – 1,000
2014	PFOA related substances in textiles (the total volume of PFOA related substances in imported articles is unknown)	1,000 – 10,000

Reference: ECHA (2015a)

34. According to the information gathered in a survey conducted by the OECD in 2009 (OECD, 2011), the reported concentration of PFOA in products containing the substance varies between <1-160 ppm. Concentrations of other PFOA-related compounds in products vary between 1-4,200 ppm, and concentrations of C8:2 fluorotelomers in products range between 5-35,000 ppm. Also ECHA (2015a) provides in its appendix data on concentrations of PFOA and related compounds in products such as outdoor clothing, workers protection clothing, membranes for apparel, treated home textile and upholstery, treated non-woven medical garments, leather finishing, carpets, impregnating sprays/ waterproofing agents, firefighting foams, treated paper, paints and inks, cleaning agents, floor waxes/wood sealants, lubricants and sealant tapes (refer to ECHA, 2015a, Appendix B, Tables A.B.2-4 and A.B.2-9 for more information).

35. Information on imports of products containing PFOA and PFOA-related compounds was only identified for Australia, Albania and the EU, as follows:

(a) Australia reported the import of an antifoam product containing less than 10% of a PFOA-related chemical (constituting ~10 kg of PFOA) in 2005 for use in a dyeing process with sulfur dyes (NICNAS, 2015b). Australia assesses individual chemical entities in consumer products and chemicals released from articles. Articles are not within the scope of the *Industrial Chemicals Notification and Assessment Act* (Australia 2016);

(b) In 2014, the EU reported the import of between 1,000-10,000 tonnes of PFOA-related compounds contained in textile articles (specifically within outdoor jackets). Of note, this volume is expected to decline after 2015 due to the availability of alternatives (estimated imports thereafter are between 300-3,000 tonnes per annum) (ECHA, 2015a);

(c) Albania provided information within the Annex E survey (Albania, 2015) for the quantity of goods imported that may contain PFOA for the years 2012–2015. This ranged between 3–20 tonnes for total net weight of all goods.

36. Stockpiles of firefighting foams containing PFAS including PFOA are likely to be present at military bases, airports, oil production facilities and rigs and other facilities (Baduel et al., 2015; Anderson et al., 2016; FluoroCouncil 2016). For example, the US military did possess approximately 11 million liters of aqueous fire-fighting foam containing PFCs including PFOA (Place and Field, 2012). Two studies report on estimated quantities of AFFF in the U.S. (Darwin, 2004, 2011).

2.1.2 Uses

37. Due to their physico-chemical properties, PFOA, its salts and its related compounds are used in a wide variety of applications and consumer products across many sectors (primarily automotive, electronics, construction and aerospace industries) (ECHA, 2015a; OECD, 2013). ECHA (2015a) provides estimation on the amounts of PFOA and its salts as well as on PFOA related substances used in the EU in different sectors/industries. The main information is summarized in the following.

Table 5: Use of PFOA, its salts and PFOA-related compounds in different sectors in the EU

Use in the EU	Sector/Industry	Value/Range [in t]
PFOA-related compounds	Textile and leather treatment	~1,000
	Paper treatment	>150 – 200
	Firefighting agents	>50 – 100
	Paints and inks	>50 – 100
	Others uses	>0.1 – 0.5
PFOA and its salts	Manufacture of fluoropolymers	<20
	Photo industry	1.0
	Semiconductor industry	<0.05
	Other uses 0.5-1.5 t	0.5-1.5

Reference: ECHA (2015a)

38. PFOA is predominantly used in the form of APFO in aqueous solution as an emulsifier and processing aid in the manufacture of many fluoropolymers, such as PTFE, FEP (fluorinated ethylene propylene), PFA (perfluoroalkoxy alkane) or PVDF (polyvinylidene fluoride) (Emmett et al., 2006; OECD, 2006; ECHA, 2015a). PFOA is a contaminant in fluorochemicals and telomer products (Emmett et al., 2006). Fluoropolymers are used across many sectors for different purposes, including: in the manufacture of hoses, cable and gaskets; non-stick coatings on cookware; and personal care products (Begley et al., 2005; Environment Canada and Health Canada, 2012; U.S.EPA, 2009; van der Putte et al., 2010; ECHA 2015a). APFO is also used in the processing of paint, photographic film additives and in the textile coating industry (OECD, 2006), and can be found in certain aqueous

fire-fighting foams (OECD, 2006; Prevedouros et al. 2006; Environment Canada and Health Canada, 2012). PFOA is also used, as a surfactant and processing aid in the manufacture of semi-conductors used in the photolithographic process (van der Putte et al., 2010; ESIA, 2015) and as a replacement for perfluorooctane sulfonate (PFOS) (ECHA, 2015a).

39. PFOA-related compounds are used as surfactants and/or fluorinated polymers for a variety of products including ski waxes (Freberg et al., 2010; Nilsson et al., 2010a, 2010b) and treatments for leather, other textiles (such as outdoor textiles and carpets) (Washburn et al., 2005; Begley et al., 2005), and paper packaging of microwave popcorn bags (Sinclair et al., 2007). High PFOA levels were identified in ski waxes (up to about 2000 µg/kg PFOA), outdoor textiles (up to 19 µg/m^2 PFOA) and some baking papers (up to 15 µg/m^2 PFOA) (Kotthoff et al., 2015). PFOA-related compounds are used as a surface treatment agent in both the stone and paper industries (China, 2015), in medical devices (Austria, 2015), as an antifoam products for use in the dyeing process using sulfur dyes (NICNAS, 2015b), and as a leveling agent in paints and inks. PFOA-related compounds are used as (1) non-polymeric substances for firefighting foams, wetting agents or cleaners (OECD, 2013; van der Putte et al., 2010) or (2) as part of side-chain fluorinated polymers, such as fluoroacrylate polymers (US EPA, 2009; van der Putte et al., 2010). Side-chain fluorinated polymers are used to provide a water, grease and soil protection, for example in applications such as textiles, products for apparel, leather, paper and cardboard (e.g. in food packaging), paints and lacquers (e.g. exterior and interior architectural paints), non-woven medical garments, floor waxes and stone/wood sealants, thread sealant tapes and pastes, adhesives or products for apparel. Fluorotelomers is a term often used in the literature, referring to substances produced with the telomerization process. Fluorotelomers might be PFOA-related compounds if they contain the respective chain length. For fluorotelomers it was reported that 80% are used in polymers and 20% in non-polymeric applications. Identified major uses of PFOA-related compounds in Europe are surface treated textiles, fire-fighting foam, surface treated paper and paints and inks (ECHA, 2015a).

2.1.3 Releases to the environment

40. Numerous direct and indirect sources of PFOA, its salts and PFOA-related compounds contribute to the overall release of PFOA to the environment. Direct releases to the environment occur from the production of the raw substance, during the processing, use and disposal of the chemical and of products containing the chemical. Main emission vectors are water, wastewater and dust particles. Indirect releases to the environment occur due to the formation of PFOA from PFOA-related compounds. They are released to air and waste water during manufacture of the substances themselves, from side-chain fluorinated polymers and during use and disposal of consumer articles treated with PFOA-related compounds. When emitted to the atmosphere, they can be degraded to PFOA, and deposited on soil or surface waters (see also para 2.1.3 of document UNEP/POPS/POPRC.12/INF/5). They are also washed out from the atmosphere via precipitation (ECHA, 2015a). The estimated global cumulative emission of PFOA ranged from 2078 to 18366 t over the period of 1951 to 2030, thus presenting the compound with the highest cumulative emission of the C_4-C_{14} PFCAs (Wang et al., 2014a). The study also indicates a decrease in estimated emissions in North America, Western Europe and Japan, concurrently with increases in India, Russia and China.

41. The manufacturing of PFOA has been identified as a major direct source of PFOA in the environment (Armitage et al., 2009; Prevedouros et al., 2006). During the manufacturing of PFOA the substance can be emitted into the environment either via waste water or into the air. Prevedouros and co-workers (2006) estimated global PFOA manufacturing emissions: 45 t in 1999, 15 t in 2004, 7 t in 2006. PFOA can also be directly released from manufacturing and use of PFOA-related compounds. (Pistocchi and Loos, 2009; Loos et al., 2008; Dauchy et al., 2012). As detailed by Wang et al. (2014a), releases related to the manufacturing and use of PFOA have likely changed along with changes in practices at manufacturing sites (i.e. with vs. without control measures of waste streams). A recent publication documents the first source-specific inventory for environmental releases of PFOA/PFO in China from 2004 to 2012 and estimates cumulative environmental releases reaching 250 tonnes (t) over a period of nine years (Li et al., 2015) and several studies confirm the relevance of PFOA in China. Chinese samples from river/estuary systems were highly polluted by an industrial point source discharging mainly PFOA (Heydebreck et al., 2015) and an individual plant producing PTFE and other fluoropolymers releases extraordinary high quantities of PFOA into a river (Wang et al., 2016). There is a positive correlation between the proximity to a fluoropolymer production facility and PFOA concentrations in rivers (Shi et al., 2015) and substantial levels of per- and polyfluoroalkyl substances were detected in outdoor dust in China with PFOA being a predominant substance (Yao et al., 2016). Historic releases to the environment from production are available for a U.S. plants in West Virginia (Emmett et al., 2006; Paustenbach et al., 2007; Lerner, 2005) and from other large scale production until 2002 (Minnesota State Dep, 2016; Oliaei et al., 2013; Minnesota Pollution Control Agency, 2016).

42. The manufacture of fluoropolymers is considered the main direct emission source of PFOA, where APFO is used as processing aid (Armitage et al., 2009; Prevedouros et al., 2006). From fluoropolymer production sites, PFOA is emitted to air (mainly particle bound) and water. Fluoropolymer dispersions are often used to coat metal and fabric surfaces. During the dispersion processing and subsequent use and disposal of consumer articles are released. Environmental releases from the direct use of PFOA are also possible from the photo industry and the semiconductor industry (van der Putte et al., 2010; ESIA, 2015; SIA, 2015).

43. Releases to the indoor domestic environment arise from the use of products containing PFOA and its related compounds for several products purchased from retail in the US, with the largest releases from use reported for professional carpet-care liquids, pre-treated carpeting, floor waxes and stone/tile/wood sealers and household textiles and upholstery (US EPA, 2009a). Emissions from outdoor jackets occur mainly during washing and impregnation procedures (Umweltbundesamt, 2014).

44. The use of PFOA-related compounds results in direct and indirect emissions of PFOA These result from impurities in fluorotelomer based products and from degradation of fluorotelomer based products as well as from the manufacture, use and disposal of side-chain fluorinated polymers (ECHA, 2015a; Environment Canada and Health Canada, 2012). The manufacture of side-chain fluorinated polymers represents one major industrial use of PFOA-related compounds (Russell et al., 2008; ECHA 2015a; Danish Ministry of Environment, 2013).

45. Releases to the environment occur from management of waste water and of solid waste consisting of or contaminated with PFOA, its salts or PFOA-related compounds. Industrial wastewater from fluoropolymer manufacturing is considered the most important point source of PFOA to surface water. Waste water treatment plants do not remove PFOA efficiently. Thus, a large share remains in the water phase and enters surface water bodies and degradation of PFOA-related compounds during the treatment can even lead to higher PFOA emissions (ECHA 2015a; Schultz et al., 2006; Bayerisches Landesamt für Umwelt, 2010; Houtz et al., 2016; UNEP/POPS/POPRC.12/INF/5). During solid waste management PFOA releases may result from incineration, landfilling and recycling (Yamada et al., 2005; Poulsen et al., 2005) and it is assumed that recycling of contaminated wastes contributes to environmental releases (ECHA, 2015a). There are a number of studies available related to waste water, sewage sludge or landfill leachate and waste treatment that include measurements of direct releases to the environment from the disposal of PFOA and its related compounds (Muir and Scott, 2003; Boulanger et al., 2005; Yan et al., 2015; Ikonomou, 2006; Busch et al., 2010; Guo, 2008; US EPA, 2009b; OECD 2011).

46. PFOA-related compounds will degrade to PFOA in sludge, soil, water and air (Wang et al., 2005a, 2005b; Moody and Field, 1999; IPEN, 2015; Dasu and Lee, 2016; Bizkarguenaga et al., 2016), and such degradation has been noted across many products containing PFOA-related compounds (Dasu et al., 2013; Ellis et al., 2003; Ellis et al., 2004a; Frömel and Knepper, 2010; Gauthier and Mabury, 2005; Hilal et al., 2004; Jackson and Mabury, 2013; Jackson et al. 2013; Rayne and Forest, 2010; Renner, 2008; Wang et al., 2005a; Wang et al., 2005b; Washington et al., 2009; Young and Mabury, 2010; Young et al., 2008; Zhang et al., 2013; Butt et al., 2014; Rankin et al., 2014; Washington et al., 2015). Release of PFOA to the environment results from its use in fire fighting foams. Aqueous film forming foams (AFFF) were developed in the early 1960s with PFASs as key components and constitute a remaining source of PFOA e.g. in firefighting training pads or around military aviation bases (Baduel et al., 2015; Australia, 2016).

47. There are no known natural sources of PFOA or their related compounds (Kissa, 1994; Environment Canada and Health Canada, 2012; IPEN 2015).

48. These above paragraphs provide overview information on releases to the environment. For more detailed information, please see section 2.1.3 of document UNEP/POPS/POPRC.12/INF/5.

Contribution from salts of PFOA and PFOA-related compounds to the environmental occurrence of PFOA

49. The nomination proposal for PFOA, its salts and PFOA-related compounds (UNEP/POPS/POPRC.11/5) highlights concerns that PFOA-related compounds could be contributing to environmental concentrations of PFOA through degradation. This point was raised by the Danish Ministry of Environment (2013) who suggests that: *"An assessment of PFOA to the Baltic Sea estimated with high uncertainty that 30% of the releases were due to the transformation of fluorotelomers"*.

50. Studies by Prevedouros et al. (2006) and Kim et al. (2012 and 2013) looked at the fate and behaviour of FTOHs (6:2 and 8:2 FTOHs) within terrestrial environments, suggesting that degradation products from 8:2 FTOHs will include PFOA. Prevedouros et al. (2006) estimate that around 1% of

PFCA emissions originate from fluorotelomer raw material degradation which would include PFOA; while Kim et al. (2012) highlights a biotransformation process within soils, whereby soil bacteria degraded FTOHs (both long and short chain) into a range of degradation products which include PFOA. Washington et al (2009) incubated an acrylate-linked fluorotelomer polymer in soil microcosms and monitored the microcosms for possible fluorotelomer and PFC degradation products. These and other results suggest that fluorotelomer-polymer degradation is a source of PFOA to the environment (Washington et al., 2009; Washington et al., 2014; refer to Koch et al., 2006, 2009; Russell et al., 2008, 2010 for a broader view of degradation and half-lives).

51. Thermolysis of PTFE also yields hexafluoropropylene and other compounds including PFOA (Ellis et al., 2001; Schlummer et al., 2015) while monomers used to make long-chain fluorotelomers and degraded from them can be biotransformed to PFOA (Butt et al., 2010a). Taylor (2009) and Taylor et al. (2014) in collaboration with DuPont could not detect PFOA during laboratory-scale combustion testing. Krusik and Roe (2004) and Krusik et al. (2005) studied the thermal decomposition of the ammonium salt of perfluorooctanoic acid (APFO) and PFOA. APFO and PFOA are thermally unstable at waste incineration temperatures (~1000°C). For example, the half-life for APFO is estimated to be less than 0.2 s at 350 °C. In light of the historically large production of fluorotelomer-based polymers (FTPs) and the poor efficacy of conventional treatments for recovery of PFCAs from waste streams, degradation of FTP based materials have the potential to increase environmental levels of PFCAs.

52. FTPs manufactured to date potentially could increase PFCAs 4- to 8-fold over current oceanic loads, largely depending on the integrity of disposal units to contain PFCAs upon hydrolytic generation from FTPs (Washington and Jenkins, 2015; refer to Koch et al., 2006, 2009; Russell et al., 2008, 2010 for alternative assessments indicating lower global loading potential).

53. PFOA is also found in packaging materials. For example, PFOA and related substances in fluoropolymer resin products vary up to 150 mg/kg and 5–3000 mg/kg in fluropolymer dispersion products in non-US based production locations. PFOA can also be extracted at low concentrations from textiles and packaging treated with side-chain-fluorinated polymers. The releases of substances from the different parts of the life cycle and the significance of the exposure of humans and the environment is still not fully understood and more information is needed (Danish Ministry of Environment, 2013).

54. Fluorotelomer carboxylic acids (FTCAs) and the corresponding unsaturated acids (FTUCAs) are intermediates in the biodegradation of industrially produced fluorotelomer alcohols (FTOHs) to environmentally persistent perfluorinated carboxylic acids (PFCAs). The fate of these compounds was investigated in a simple sediment–water microcosm system. Microcosms were spiked with 8:2 FTCA, 10:2 FTCA and 8:2 FTUCA, or 10:2 FTUCA. The FTCAs and FTUCAs investigated degrade rapidly. Identifiable degradation products of FTCAs and FTUCAs were observed and include PFOA for both, 8:2 and 10:2 compounds. (Myers and Mabury 2010; Butt et al., 2014**).**

55. Environment Canada and Health Canada (2012) and ECHA (2015a) compile scientific evidence on the degradation/transformation of PFOA related substances based on different sources (the information from both sources is compiled in section 2.1.3 of document UNEP/POPS/POPRC.12/INF/5).

56. ECHA (2015a) provides further details on the role of PFOA-related compounds to degrade and contribute to environmental concentrations of PFOA: *"The yields of PFOA are in most studies in the range 1.7 – 20%. The duration of the studies varies from 28 days to 90 days. One study was performed with a longer time scale of 7 months. The yield of PFOA in this study was in the range of 10 — 40% (Wang et al., 2009).This indicates that some of the degradation steps may take some time although the estimated half-life of the PFOA-related compounds is in the range of days. Thus, it can be hypothesized that in the environment PFOA yields from PFOA-related compounds are much higher than measured in the short time degradation experiments. We further hypothesize that over a long time frame of 5 — 10 years PFOA yields from PFOA-related compounds degradation are around 80%".*

57. In conclusion, PFOA-related compounds have the capacity to degrade to PFOA in the natural environment contributing to the environmental concentrations of PFOA. The quantities of PFOA generated via degradation from PFOA-related compounds are more debatable with values of between 1.7% - 40% quoted. However, as noted by ECHA (2015a), these results are based on shorter term tests and over longer periods of time the quantity of PFOA may be much higher (around 80%).

2.2 Environmental fate

2.2.1 Persistence

58. PFOA is extremely stable within the natural environment due to its chemical properties and does not degrade under environmentally relevant conditions. This fact is corroborated by the US EPA (2014), Australian NICNAS (2015a and 2015b), Environment Canada and Health Canada (2012) and OECD (2006). In particular, both assessments conducted by Environment Canada and Health Canada and OECD noted that there was no clear degradation of PFOA under abiotic or biotic conditions.

59. Within the aquatic compartment under natural environmental conditions, PFOA has a half-life of greater than 92 years with the most likely value of 235 years and shows no obvious decay from photodegradation (Todd, 1979; Hatfield, 2001; 3M, 2001). In aquatic environments where PFOA undergoes indirect photolysis, the half-life was estimated to be longer than 349 days (Hatfield, 2001; ECHA, 2013a, 2013b). A review of half-life data and mechanisms of decay is presented in Table 6.

Table 6: Reported findings on the persistence of PFOA (adapted from Environment Canada and Health Canada, 2012)

Medium	Mechanism	Degradation Half-life	References
Water	Photolysis	No photodegradation	Todd 1979; Hatfield, 2001
Water	Indirect photolysis	> 349 days	Hatfield, 2001
Water	Hydrolysis	~ 235 years	3M, 2001
Air	Hydroxyl reaction	~ 130 days	Hurley et al., 2004
Sludge	Biodegradation	> 2.5 months	Pace Analytical , 2001
Soil/sludge	Biodegradation	> 259 days	Liou et al., 2010

60. On the basis of the available data, abiotic degradation of PFOA in the atmosphere is expected to be slow. The atmospheric lifetime of PFOA has been predicted to be 130 days (conclusion by analogy from short-chain perfluorinated acids; see OECD, 2006; Hurley et al., 2004).

61. Persistence within the terrestrial environment mirrors the position seen in the aquatic environment, with high persistence, slow degradation and long half-lives. Both the REACH Annex XV restriction dossier (ECHA, 2015a) and REACH proposal for identification of PFOA as an SVHC (ECHA 2013a) state that PFOA was not biodegradable and that, based on high persistence, it was not possible to calculate half-lives in soil or sediment. This was the same conclusion presented within the screening dossier (UNEP/POPS/POPRC.11.5).

62. More detailed information on persistence is provided in section 2.2.1 of document UNEP/POPS/POPRC.12/INF/5.

Summary on persistence

63. Based on the available experimental evidence it is concluded that PFOA is highly persistent in all environmental compartments, with a strong resistance to all conventional mechanisms of degradation under relevant environmental conditions.

2.2.2 Bioaccumulation

Screening assessment based on physical-chemical properties

64. Due to the high surfactant capacity of PFOA and its ability to form multiple layers in octanol/water, it is not possible to analyze log K_{ow} directly (US EPA, 2002). As quoted in Annex D of the Stockholm Convention and reported in Table 2, the derived estimates vary from above to below the critical log K_{ow} value of 5. Studies for BCF and BAF also highlight values below the critical value of 5,000 quoted by Annex D. However, based on the physical properties, PFOA is known to undergo a protein bioaccumulation mechanism rather than lipid partitioning (UNEP/POPS/POPRC.3/INF/8/2003), which makes standard BCF/BAF analysis less meaningful. Thus the use of log K_{ow}, BCF and BAF have been demonstrated to be inappropriate measures of bioaccumulation of PFOA (ECHA, 2013a).

65. To assess whether PFOA meets the bioaccumulation criterion, the focus is placed on the Annex D paragraph c) ii and iii; the evidence for concern within species as a part of monitoring. This includes an approach utilising biomagnification factors (BMF) and trophic magnification factors (TMF), which has been adopted alongside monitoring of higher trophic tier species to substantiate that bioaccumulation occurs in the natural environment. BMF and TMF approaches assess predator-prey relations and the whole food chain, respectively, to evaluate the flow of material and substantiate

whether biomagnification might take place across trophic levels. A number of studies have assessed both BMF and TMF within ecosystems spanning the Arctic, Canada, USA, Netherlands and Brazil with reported values of >1 (critical value), suggesting that bioaccumulation of PFOA is taking place across trophic levels (Houde et al., 2006; Kelly et al., 2009; Martin et al., 2004; Tomy et al., 2004, 2009; Butt et al., 2008).

66. A literature review of studies reporting the BMF and TMF of PFOA indicated wide ranges of BMF (min=0.04; max=125) and TMF (min=0.58; max=13), raising the concern of the reliability of these values as indicators of bioaccumulation potential. This might be a result of several factors such as nonachievement of steady-state conditions, uncertainties in the feeding ecology and the impact of metabolism of precursor compounds. It is suggested that BMFs and TMFs to be determined in dietary studies performed under strictly controlled conditions with consideration of measured elimination half-lives (Franklin, 2016).

Bioconcentration studies in aquatic organisms

67. Because of the high surfactant capacity and solubility of PFOA, fish might excrete PFOA through the gills leading to reduce uptake and bioaccumulation. This explains the often low values seen in BMF/BAF tests using fish. Equally for BMF/TMF analysis where the higher predators within the food chain are fish species it can be seen that the critical values fall below 1. BCF values within the aquatic environment tend to be low. Studies based on water breathing animals provide evidence that the bioaccumulation criterion in the aquatic environment is not met (OECD, 2006; Kelly et al., 2009). There are several studies investigating the isomer profile of PFOA in the aquatic organisms. In a marine food web, the linear isomer of PFOA is the dominant form found in biota. In another study, branched PFOA isomers accumulated in rainbow trout tissue (Fang et al., 2014; De Silva et al., 2009).

68. Studies for air breathing aquatic species are scarce. However, the potential for bioaccumulation with marine mammals is confirmed. There is evidence that PFOA biomagnifies in air-breathing mammals because BMFs range from 1.3 – 125 for selected predator prey relationships and TMFs range from 1.1 to 13 for selected food chains. For non-fish species, particularly air-breathing terrestrial and avian species, bioaccumulation is shown to occur (Houde et al., 2006; Butt et al., 2008; van den Heuvel-Greve et al., 2009; Müller et al., 2011).

69. More detailed information on bioconcentration in aquatic organisms is provided in section 2.2.2 of document UNEP/POPS/POPRC.12/INF/5.

Bioaccumulation studies in terrestrial organisms

70. A number of studies have been completed highlighting the presence of PFOA in higher-tier terrestrial species. Studies include an overview of the presence of perfluorinated chemicals in marine, limnetic and terrestrial biota in Germany (Rüdel et al., 2011). PFOA was detected in liver samples from Canadian bears, and ecosystem studies in Canada highlighted the presence of PFOA above detectable limits in lichen, caribou and wolves. Trophic magnification factors for wolf/caribou/lichen (or vegetation) ranged from 1.1 to 2.4. (Martin et al., 2004; Müller et al., 2011). In these three studies, the potential for PFOA to bioaccumulate within terrestrial species was confirmed. A feeding study with fattening pigs showed that PFAAs accumulated in their tissues. The authors modeled related toxicokinetics and concluded that pigs exhibit longer elimination half-lives (236 days for PFOA) than reported for most organisms (Numata et al., 2014).

71. More detailed information on bioconcentration in terrestrial organisms is provided in section 2.2.2 of document UNEP/POPS/POPRC.12/INF/5.

Summary on bioaccumulation

72. The assessment of bioaccumulation for PFOA is complicated by its physical properties, which make assessment of log K_{ow}, BCF and BAF approaches challenging. PFOA does not accumulate in water-breathing animals according to the criteria of the Stockholm Convention. This can be explained by the way that fish process and excrete PFOA through their gills.

73. PFOA biomagnifies in air breathing mammals. PFOA has been detected within the body tissues of air-breathing aquatic species. For terrestrial species, the presence of PFOA is readily detected, with a number of studies indicating BMF and TMF scores of greater than 1. There is evidence that PFOA bioaccumulates in air-breathing mammals and other terrestrial species, including humans (see specific section 2.3.2 on human exposure).

2.2.3 Potential for long-range environmental transport

74. The screening dossier (UNEP/POPS/POPRC.11/5) highlights the high persistence and stability of PFOA, its salts and PFOA-related compounds, particularly within the air compartment. Hurley et al. (2004) quote that the atmospheric lifetime of PFOA with respect to hydroxyl radicals has been predicted to be 130 days (see Table 6). This would be sufficient amount of time to allow PFOA to travel considerable distances from the point of emission.

75. Franklin (2002) calculated an atmospheric lifetime of PFOA to be in the order of days if PFOA is emitted from a ground source, and therefore, PFOA is likely not subject to long-range transport. However, if PFOA is produced from an atmospheric source (i.e. via precursors) and if the major loss mechanism is wet or dry deposition, then it may have a lifetime of 20-30 days before deposition (Ellis et al, 2004b). This would be sufficient amount of time to allow PFOA transport over many thousands of kilometres.

76. Modeling studies have reported oceanic transport as the dominant pathway for distributing PFOA (primarily from direct sources) to the Arctic. It is believed that ocean circulation and its variations are factors that determine the long-range distribution and fate of PFOA (Stemmler and Lammel, 2010; Armitage et al., 2009).

Presence of PFOA, its salts and PFOA-related compounds in remote areas

77. Alongside the persistent characteristics of PFOA and environmental modeling data to suggest the capacity for long-range transport, environmental monitoring data from a number of studies also exist to help corroborate the model estimates. Table 7 provides details of monitoring for PFOA within surface water, ice, sediment and biota from remote locations far from the point of use and emission.

Table 7: Examples of presence of PFOA in remote areas

Sample	Value	Remarks	Reference
Surface Water			
Canadian Arctic lakes (Meretta, Resolute, Char, Small, North, and 9 Mile)	0.6 – 17 ng/L (range)	2010-2011	(Lescord et al., 2015)
Sea Water / Ice			
Baydaratskaya Bay (Russian Federation)	0.1307 (±0.077.2) ng/L (average ± std dev.)	2007	(Saez et al., 2008)
Greenland Sea	0.020 – 0.111 ng/L (range)		(Theobald et al., 2007; ECHA, 2015a)
Sediment			
Canadian Arctic lakes (Char Lake and Resolute Lake)	1.7 and 7.5 ng/g dw <1.1 and 2.3 ng/g dw 1.2 and <1.8 ng/g dw (range)	0-1 cm (depth) 1-2 cm 2-3 cm	(Stock et al., 2007)
Air			
High volume air samples collected from vessels on Atlantic Ocean, Southern Ocean and Baltic Sea High volume air samples from a land-based site close to Hamburg, Germany	0.7 pg/m^3 (average) 0.3 pg/m^3 (average)	2007-2008	(Dreyer et al., 2009)
Birkenes Zeppelin Andøya (Norway)	0.32 pg/m^3 0.22 pg/m^3 0.19 pg/m^3 (average)	2014	(NILU, 2015)
Cornwallis Island (Canada)	1.4 pg/m^3 (average)	2004	(Stock et al., 2007)
Biota			
Polar bear (liver) (East Greenland)	0.6 – 14 ng/g ww 6.8 – 15.8 ng/g ww 11.8 – 17.6 ng/g ww (range) 14 ng/g ww 12.3 ng/g ww 4.8 ng/g ww (annual average)	1990 (year) 1995 2006 2006 2010 2011	(Dietz et al., 2007) (Rigét et al., 2013)

Sample	Value	Remarks	Reference
Polar bear (liver) (North American Arctic, European Arctic)	2.4 – 36 ng/g ww (range of geometric means)		(Smithwick et al., 2005)
Ringed seal (liver) (Arviat – Canadian Arctic)	<0.85 – 3.6 ng/g ww (range)		(Butt et al., 2007)
Ringed seal (liver) (East Greenland)	1.6 ng/g ww 2.2 ng/g ww <Limit of detection (annual average)	2006 2008 2010	(Rigét et al., 2013)

78. There is evidence of PFOA present in the remotes regions of the Arctic ice cap (US EPA, 2014), surface and sea water in Arctic regions (Butt et al., 2010b; AMAP, 2014; Benskin et al., 2012a, 2012b; Veillette et al., 2012; Kwok et al., 2013; Cai et al., 2012; Zhao et al., 2012a) snow and ice cores from China (e.g. northern China and Tibetan Plateau) (Wang et al., 2014b; Shan et al., 2015), air, snow/ice cores, water, sediment and biota samples from Norway (Xie et al., 2015; NILU, 2015; Kwok et al., 2013), snow samples from Slovakia, Switzerland and Italy (Cobbing, 2015) as well as air, water and fauna samples from Antarctica and the Southern Hemisphere (Zhao et al., 2012a; Tao et al., 2006; Danish Ministry of Environment, 2013; Wang et al., 2015).

79. Lescord et al. (2015) report evidence of PFOA in lake food webs from the Canadian high Arctic. Evidence is also reported to show the contamination in eggs of the high-artic ivory gull (*Pagophila eburnea*) (Lucia et al., 2015) and other Arctic and North Atlantic mammals (Bytingsvik et al., 2012; Rotander et al., 2012; Rigét et al., 2013). Different PFOA isomers were detected in liver samples of polar bears from the Canadian Arctic and eastern Greenland such that Greenland polar bear samples showed a variety of branched isomers while only the linear PFOA isomer was determined in Canadian samples (de Silva and Mabury, 2004). Regarding the Arctic, PFOA was detected in liver samples of killer whales, seals, and polar bears from East Greenland (Gebbink et al., 2016).

80. Fluorotelomer alcohols such as n-$C_8F_{17}CH_2CH_2OH$ appear to be a significant global source of persistent bioaccumulative perfluorocarboxylic acid pollution, and present modeling results show that with current estimates of chemistry and fluxes the atmospheric oxidation of 8:2 FTOH can provide a quantitative explanation for the presence of PFCAs including PFOA in remote regions (Wallington et al., 2006; Young et al., 2007). Besides, biotransformation of 8:2 FTOH to PFOA has been shown in laboratory experiments (Butt et al., 2014).

Potential mechanisms for long range transport

81. Bengtson Nash et al. (2010) and Prevedouros et al. (2006) discuss two key mechanisms to aid the long range transport of PFOA to remote regions. The first mechanism involves a release to environment either as air-borne dust particles contaminated directly by PFOA or salts of PFOA, or a direct release to surface waters. These releases then undergo long range transport through a cycle of deposition and volatization from marine waters, with the transport in water via the marine environment likely to be the dominant delivery method to the Arctic regions. It has been suggested that hydrospheric transport will form a slow but primary input pathway of PFCs to the Antarctic region (Bengtson Nash et al., 2010). The second mechanism involves a delivery through air-borne precursors (such as FTOHs) again through contaminated dust particles and then the degradation of these materials to form PFOA *in situ* on arrival.

82. ECHA discusses the mechanism of atmospheric transport. Due to the relative vapor pressures of APFO, PFOA, and perfluorooctanoate (PFO), the chemical form potentially most subject to gas-phase atmospheric transport is PFOA (ECHA, 2015a). Franklin (2002) suggested that in the presence of water in air, gaseous PFOA condenses to aerosol particles and dissociates to corresponding PFO. This would suggest that cycling between atmosphere and ground through wet/dry deposition and resuspension is possible and would aid the long range transport of PFOA. Climate change leading to melting snow and ice has also been proposed as a potential mechanism (Zhao et al., 2012a).

83. More detailed information on long-range transport is provided in section 2.2.3 of document UNEP/POPS/POPRC.12/INF/5.

Summary on long-range environmental transport

84. PFOA is highly stable and resistant to degradation. Monitoring of water, air, sediment and biota at remote locations all detect the presence of PFOA. Equally, environmental modeling data suggest that the capacity for long range transport (e.g. oceanic transport) does exist while others have identified key mechanisms involving precursors that would make long range transport plausible. On this basis, it can be concluded that PFOA meets the Annex D criterion for long range transport.

2.3 Exposure

2.3.1 Environmental monitoring data

85. Various studies demonstrate that PFOA is ubiquitously present in the environment. ECHA (2015a) contains a selection of studies, which report detections of PFOA and related compounds in several compartments (surface water, deep-sea water, drinking water, wastewater treatment plant, sediment, groundwater, soil, atmosphere, dust, biota, and human) at worldwide sampling locations (see ECHA, 2015a, Table A.B.4-8 in Appendix B; see also section 2.3.1 of document UNEP/POPS/POPRC.12/INF/5). No large-scale monitoring program has been conducted for PFOA and only limited time trend studies are available. There is not sufficient information available to conclude on the trend of environmental concentrations. The available time trends studies indicate both decreasing and increasing trends in biota in Europe as well as in arctic regions (ECHA 2015a, Roos and Benson, 2016, Faxneld et al., 2014; Rigét et al., 2013).As PFOA is not degradable this decreasing trend is not proven by water and sediment samples suggesting that oceans and sediments are sinks of PFOA (ECHA 2015a).

86. Environmental monitoring data has been gathered for a number of sites still producing PFOA and its related compounds in China. PFOA has been found in sediment, river water, surface and groundwater as well as drinking water, primarily near PTFE and perfluorobutane sulfonate production facility or fluorine industry parks (Bao et al., 2011; Chen et al., 2015; Shi et al., 2015, Yao et al., 2014). Although it is widely accepted that telomerization is currently the predominant manufacturing method for PFOA, yielding an isomerically pure and linear product, electrochemical fluorination is still used by some manufacturers in China (Jiang et al., 2015). This is confirmed with the analysis of isomer profiles of PFOA in fresh snow samples collected in northern China, which were in agreement with the signature of the historical 3M electrochemical fluorination products, suggesting that these products were still produced and used in China (Shan et al., 2015).

87. PFOA was found in the majority of ground water samples in the EU and of public drinking water systems in the U.S. (Loos et al., 2010; Post et al., 2009). Reasons for groundwater contamination are e.g. release to surface soils, general atmospheric deposition, landfill leachate, historical use of AFFF e.g. in the vicinity of airports and fire-fighting training sites (Xiao et al., 2015; Eschauzier et al., 2013; Yan et al., 2015; Filipovic et al., 2015; KemI, 2016). Currently, there is a large-scale drinking water monitoring program funded by the U.S. EPA under the Safe Drinking Water Act called the Unregulated Contaminant Monitoring Rule (UCMR), where over 4800 public water systems are being monitored for 30 contaminants including PFOA (US EPA, 2016).

88. An assessment of the relative contribution of historic (i.e. electronically fluorinated) and contemporary (i.e. telomer) sources of PFOA in various seawater samples indicated that the dominant PFOA source(s) to the Pacific and Canadian Arctic Archipelago are either from (1) direct emissions of contemporary PFOA via manufacturing or use in Asia or (2) from atmospheric transport and oxidation of contemporary PFOA-precursors (Benskin et al., 2012c).

89. Environmental monitoring data are available for surface water and sediment in Japan (Japan, 2015), for the river Po in Italy (ECHA, 2014b), landfill leachate, water and gull eggs and sediment in Australia (Gallen et al., 2016; Thompson et al., 2011a) and for river sediments from South Africa PFOA with higher concentrations than those found in other countries (Mudumbi et al. 2014).

90. There is abundant evidence to show detected levels of PFOA in marine, limnetic and terrestrial biota worldwide (de Silva and Mabury, 2004; Martin et al., 2004; Smithwick et al., 2005; Houde et al., 2006; Butt et al., 2007; Dietz et al., 2007; Ishibashi et al., 2008; Sonne et al., 2008; de Silva et al., 2009; Müller et al., 2011; Rüdel et al., 2011; Bytingsvik et al., 2012; Rotander et al., 2012; NILU, 2013; Fang et al., 2014; Lescord et al., 2015; Lucia et al., 2015). As previously discussed in Sections 2.2.2 and 2.2.3, bioaccumulation of PFOA occurs across trophic levels, and the detection of PFOA in biota at remote locations suggest the capacity of PFOA for long range transport, respectively.

91. A previous study reported higher levels of PFOA in pine needles (Pinus mugo) near ski areas (Chropeňová et al. 2016a). PFOA application as stain repellents in modern outdoor clothes and in ski waxes is assumed to be a potential important source in ski resorts for releases to the high mountains environment and potential for human exposure (Chropeňová et al. 2016b).

92. More detailed information on environmental monitoring is provided in section 2.3.1 of document UNEP/POPS/POPRC.12/INF/5.

2.3.2 Human Exposure

93. A large number of studies have been conducted to determine human exposure to PFOA. Among other things, findings identify common routes to exposure and vulnerable groups to exposure as well as reporting concentration levels found in samples.

94. Human exposure typically takes place "human via environment"[5] by consumption of drinking water and food, via uptake of contaminated indoor dust or from consumer products containing PFOA and its related substances and, to a lesser degree, through dermal contact. (ECHA, 2013a ; Post et al., 2009 ; Shoeib et al., 2015 ; Wilhelm et al., 2010 ; Schwanz et al., 2015 ; D'Hollander et al., 2015 ; Eriksson et al., 2013 ; Carlsson et al., 2014 ; Yamada et al., 2014). Indoor air and dust are also important sources of human PFOA exposure (Haug et al., 2011; Gebbink et al., 2015). PFOA then concentrates within the blood, liver, kidney and lungs (ECHA, 2013a).

95. Several studies have suggested that PFOA levels increase with age (Haug et al., 2010, 2011; Christensen et al., 2016) and that breast feeding, diet and indoor environment are important factors for PFOA exposure that need to be addressed in the evaluation of human exposure and accumulation of PFOA (Haug et al., 2010, 2011; Brantsater et al. 2013; ECHA, 2013a).

96. Dietary intake including water is considered as the most important route of human exposure to PFOA based on studies from various countries. There is evidence that for children, the intake is higher (Vestergren et al., 2008; Haug et al., 2010, 2011; Thompson et al., 2011b; Cornelis et al., 2012, Shan et al., 2016). Taking this into consideration, among others, the U.S. EPA has issued a lifetime drinking water Health Advisory for combined PFOA/PFOS of 0.07 micrograms per liter (µg/L) based on a reference dose (RfD) derived from a developmental toxicity study in mice (US EPA, 2016b).

97. The issue of human exposure via consumer products was examined by Vestergren et al. (2015), who modeled emissions from consumer products imported from China and finds that 1.5% of PFOA discharged to wastewater and 0.3% of 8:2 FTOH emissions to air in Norway can be attributed to these products imported to Norway.

98. Analysis of serum samples demonstrate that PFOA is detectable across the general population in various countries (Emmett et al. 2006; Vestergren and Cousins, 2009; Fromme et al., 2009; Health Canada, 2013; CDC, 2015; Cho et al., 2015; Góralczyk et al., 2015; Wuttke et al., 2013; Vorkamp et al., 2014).

99. Based on the existing studies of serum/plasma PFOA concentrations in the general European adult population and in children worldwide, the average median (and maximum) PFOA serum levels were calculated to be 3.5 (21) ng/mL and 6.4 (108) ng/mL for European adults and children worldwide, respectively (ECHA, 2015a; also see UNEP/POPS/POPRC.12/INF/5 for list of studies).[6]

100. It has been estimated that serum and plasma PFOA concentrations in the European populations ranged from <0.5 – 40 µg/mL (Fromme et al., 2009; Vestergren and Cousins, 2009). PFOA was also detected in the general American population and Canadian population including children in Nunavik as well as Canadians living on reserve with geometric mean concentrations ranging from 1.39 – 2.5 µg/L (Health Canada, 2013; Wuttke et al., 2013 AMAP, 2015; CDC, 2015). Levels of PFOA were also found in people throughout the circumpolar Arctic, and in some countries such as Iceland and Norway, PFOA levels within the sampled populations were among the highest compared to other POPs (AMAP, 2015). A US study of older male anglers found PFOA serum levels similar to those of the US general population, and increasing age as well as alcohol consumption were observed to be predictors of PFOA levels (Christensen et al., 2016). Several studies indicated that human PFOA concentrations in Australian adults and Danish pregnant women decreased over time (Toms et al., 2014; Bjerregaard-Olesen et al., 2016) and that concentrations of PFOA in Australian women of child-bearing age are higher compared to Germany and the USA (Toms et al., 2014).

101. There are a few reports of detected PFOA serum levels in the occupationally exposed workers, (Guruge et al., 2005; Fromme et al., 2009; Freberg et al., 2010; Nilsson et al., 2010b). Indoor dust and total suspended particles seem to be important occupational exposure routes in fluorochemical manufacturing and is also considered relevant in domestic settings (Gao et al., 2015; Eriksson and Kärrman, 2015; Tian et al., 2016). In some cases, elevated serum PFOA levels can be largely

[5] Indirect exposure of humans via the environment may occur by consumption of food (fish, crops, meat and milk) and drinking water as well as inhalation of air (ECHA, 2012).

[6] It should be mentioned that for the calculation of average median (and maximum) PFOA serum levels reported in ECHA, 2015a, one outlying value from the study by Mondal et al., (2012) was included in the calculation. By removing this outlying value, the average median (and maximum) PFOA serum levels in children is 2.52 ng/mL (15.9 ng/mL)

attributed to exposure to PFOA-related compounds such as 8:2 FTOH (Nilsson et al., 2010a, 2010b). Persons living in the vicinity of fluorochemical manufacturing plants have higher PFOA levels than the general population (Emmett et al., 2006; Oliaei et al., 2013; Hoffman et al., 2011).

102. Mothers excrete PFOA via breast milk, which is considered an important source of exposure to breast-fed infants, whose PFOA exposure level is considerably higher than adults (Haug et al., 2011). PFOA is also transferred to the fetus through the placenta, and it has been reported that total branched PFOA isomers cross the placenta more efficiently than linear isomers (Beeson et al. 2011). Time interval between pregnancies has been shown to be strongly correlated with increased concentrations of PFOA in pregnant women, possibly reflecting the re-accumulation of PFOA in maternal blood with increasing time between pregnancies (Brantsæter et al., 2013). Due to *in utero* exposure and exposure from breast milk and drinking water, children up to about 12 years of age have higher serum PFOA concentrations than their mothers (Mondal et al., 2012). Breastfeeding is an important exposure pathway to PFOA as well as trans-placental passage (Umweltbundesamt AT, 2012, 2013; Carious et al., 2015; Forns et al., 2015; Papadopoulou et al., 2015; Hanssen et al., 2013; Mogensen et al., 2015, Brantsæter et al., 2013).

103. Studies related to breastfeeding from Jordan, Spain and Italy indicate that PFOA levels are higher in older women and that there is a greater transfer of PFC during breastfeeding by primiparous and thus a higher exposure to these contaminants for the first child (Al-sheyab et al., 2015; Motas Guzmàn et al., 2016; Barbarossa et al., 2013).

104. A recent study reported the detection of PFOA in 27 out of 30 human hair samples with a PFOA concentration range of 25-74 pg/g hair and an average of 46 pg/g hair (Alves et al., 2015).

105. Humans are very slow eliminators of PFOA compared with other species such as rodents, pigs and monkeys (Olsen et al., 2007; Numata et al., 2014) with a half-life ranging between 2 and 4 years (ECHA, 2014a; Olsen et al., 2007; Russell et al., 2015; Bartell et al., 2010; Han et al., 2012).

106. More detailed information on human exposure is provided in section 2.3.2 of document UNEP/POPS/POPRC.12/INF/5.

Summary of human exposure

107. Human exposure typically takes place “human via environment” by consumption of drinking water and food, via uptake of contaminated indoor dust or from consumer products containing PFOA and its related substances. PFOA has been detected in humans in blood and breast milk from various countries. Babies are susceptible to PFOA exposure via breastfeeding or trans-placental passage, and people who live near fluoropolymer manufacturing facilities have been shown to have higher levels of serum PFOA than those from the general population. There is evidence that PFOA levels in humans accumulate and increase with age. Humans are very slow eliminators of PFOA compared with other species with an average half-life of 2-4 years.

2.4 Hazard assessment for endpoints of concern

Adverse effects on aquatic organisms

108. According to several information sources, data currently available for PFOA indicate some adverse effects on a number of aquatic organisms. Generally, acute aquatic toxicity is low in standard ecotoxicity tests (NICNAS, 2015a); moderate to low acute toxicities are seen in pelagic organisms including fish, and low chronic toxicities in benthic organisms (Environment Canada and Health Canada, 2012). Adverse effects include intergenerational toxicity in the first offspring generation (Ji et al., 2008) and some PFOA-mediated toxicity in freshwater algae (Elnabarawy et al., 1981; Ward et al., 1995a, 1995b, 1996a, 1996b, 1996c; Boudreau, 2002; Thompson et al., 2004 as cited in Environment Canada and Health Canada, 2012; Latała et al., 2009) and other aquatic organisms (3M Company 1987a, 1990a, 1996a, b, c; Beach 1995a cited in Environment and Health Canada 2012). Further, PFOA-mediated effects on fish development, particularly in reproduction, have been observed (Ulhaq et al. 2015; Wei et al., 2007; Wei et al., 2008). Studies with other aquatic organisms such as freshwater male tilapia, marine mussels and Baikal seals showed estrogenic effects, hepatotoxicity, inflammation, and chemosensitivity (Liu et al., 2007; Stevenson et al., 2006; Ishibashi et al., 2008). Field studies related to effects of PFOA on immune function and clinical blood parameters in dolphins and sea turtles revealed increases in indicators of inflammation and immunity (Peden-Adams et al., 2004a, 2004b). Increased pro-inflammatory responses in male Japanese medaka were also observed (Yang, 2010). Activation of peroxisome proliferator–activated receptor α were shown in Baikal seals (Ishibashi et al., 2008b). Evidence indicates that PFOA could exacerbate the adverse effects triggered by certain types of pesticides (Rodea-Palomares et al., 2015).

Adverse effects on terrestrial organisms

109. The effects of repeated oral exposure to PFOA have been evaluated in mice (Loveless et al., 2006; Christopher and Marisa, 1977; Griffith and Long, 1980; Lau et al., 2006; Macon et al., 2011; Abbott et al., 2007; Wolf et al., 2007), rats (Metrick and Marisa, 1977; Griffith and Long, 1980; Goldenthal, 1978; Palazzolo, 1993; Biegel et al., 2001; Perkins et al., 2004) and monkeys (Goldenthal, 1978b; Griffith and Long, 1980; Thomford, 2001; Butenhoff et al., 2002). Hepatocellular hypertrophy was observed in all species. At lower doses, reduced body weight as well as increased kidney and liver weight were noted. Also, degeneration and/or focal to multifocal necrosis were reported with increased severity at doses between 1.5 to 15 mg/kg bw/day in rats and mice. Mortality was observed at high doses (ECHA, 2015a).

110. No-observed- and lowest-observed-adverse-effect-levels (NOAELs and LOAELs respectively) were identified from critical and relevant studies for the observed effects. The NOAEL and LOAEL for increased liver weight and hepatocellular hypertrophy based on subchronic toxicity studies in rats are 0.056 mg/kg bw/day and 0.64 mg/kg bw/day, respectively (ECHA, 2015a; Perkins et al., 2004). The LOAEL of maternal toxicity was 1 mg/kg bw/day based on increased liver weight based on a developmental toxicity study in CD-1 mice (Macon et al. 2011). White et al. (2009) identified a LOAEL of 1.0 mg/kg bw/day for delayed mammary gland development in F1 (the first offspring generation). Abbott et al. (2007) calculated a NOAEL of 0.3 mg/kg bw/day for neonatal survival based on developmental exposure of mice.

111. Toxicological studies in rats have shown that PFOA reduces serum lipids while it increases hepatic triglycerides, probably through the activation of the peroxisome proliferator-activated receptor α (PPARα) (Haugom and Spydevold, 1992; Bjork et al., 2011). A study by Butenhoff et al. reported a dose-dependent increase in serum triglycerides in monkeys and only a moderate and non-significant reduction in cholesterol with increasing PFOA (Butenhoff et al., 2002).

112. The induction of tumours has been demonstrated in rats exposed to PFOA. Rats chronically exposed to PFOA resulted in increased incidences of liver adenomas, Leydig cell hyperplasia/adenomas and pancreatic acinar cell tumours (PACT) in male Sprague-Dawley rats (Biegel et al., 2001). Mammary fibroadenoma in the female rats was observed in another chronic PFOA exposure study although this observation has since been disputed after an independent group of pathologists (Pathology Working Group) re-examined the tissue and reached a consensus that incidence of mammary gland neoplasms were not affected by chronic PFOA exposure (Hardisty et al., 2010; Butenhoff et al., 2012).

113. Animal studies show that PFOA increases the incidence of complete litter loss, postnatal mortality, decreases foetal body weight, delays ossification, changes mammary gland development and delays maturation in several developmental studies in mice (and some in rat) depending on strain, dose, time and length of exposure (Lau et al., 2006; Abbott et al., 2007; Macon et al., 2011; White et al., 2007, 2009, 2011; Wolf et al., 2007; Yang et al., 2009; Zhao et al., 2012b; Dixon et al., 2012, Suh et al., 2011; Albrecht et al., 2013). The LOAEL of maternal toxicity was determined as 1 mg/kg bw/day, and the NOAEL for neonatal survival was 0.3 mg/kg bw/day (Lau et al., 2006; Abbott et al., 2007). The National Institute of Environmental Health Sciences (US) reviewed the evidence for the effects of PFOA on foetal growth in animals, and the authors concluded that there is sufficient evidence of decreased foetal growth in non-human mammalian species (Koustas et al., 2014).

114. Effects of gestational exposure to PFOA on the development of the mammary gland from lactating dams and female pups have been demonstrated (White et al., 2007, 2009). PFOA, when exposed in a critical window of susceptibility (GD 10-17), was shown to induce changes in offspring mammary gland development in CD-1 mice (Macon et al., 2011). In addition, chronic low dose exposure of PFOA reduced mammary gland development in F_1 as well as F_2 (Yang et al. 2009). Zhao et al. (2010) observed that PFOA stimulates mammary gland development in C57BL/6 mice. Some of observed PFOA-induced developmental/reproductive effects might be mediated by PPARα (Zhao et al., 2012b; Albrecht et al., 2013). However, it has been mentioned that PFOA-induced alterations in mammary gland development might be to be dependent on steroid production in ovaries and independent of PPARα (Zhao et al., 2010).

115. There are studies suggesting that PFOA may alter steroid hormone production or act indirectly via ovarian effects as a mean of endocrine disruption (Zhao et al., 2010, 2012b; York, 2002; Butenhoff et al., 2004; Suh et al., 2011; Hines et al., 2009). PFOA has been reported to alter sexual maturation and pubertal timing in female and male offspring of rats and in multiple strains of mice (York, 2002; Butenhoff et al., 2004, Yang et al., 2009; Dixon et al. 2012), indicating a disruption of the normal steroid hormone regulation. Also, inhibited expression of the placental prolactin-family hormone genes was observed in the placenta of mice exposed to PFOA during gestation (Suh et al., 2011).

116. There are indications of immune effects mediated by PFOA. Short-term dietary exposure to PFOA resulted in a reduction in thymus weight, decreased number of thymocytes and splenocytes and suppressed IgM antibody synthesis in C57BL/6 mice (DeWitt et al., 2008; DeWitt et al., 2009; Qazi et al., 2009). Adult offspring exposed to PFOA given to dams from gestation through lactation exhibited altered immune responses such as reduced splenic T cells and IL-10 production from these cells (Hu et al., 2012). PFOA was also shown to increase histamine release from mast cells as well as exacerbate the IgE-dependent local allergic reaction in mice (Singh et al., 2012). Lastly, suppressed T-cell-mediated immunity in Japanese quails was observed after exposure to PFOA via drinking water for 8 weeks (Smits and Nain, 2013).

117. The authors of the background document for the restriction of PFOA (ECHA, 2015a) emphasize the importance of assessing mice studies instead of rat studies as basis for DNEL-setting when this is based on animal studies due to the longer half-life of PFOA in mice compared to rats.

118. Studies with chicken showed no significant outcomes such as alterations in embryonic pipping success or in biochemical parameters at concentrations up to 10 µg/g ww of embryo and doses up to 1.0 mg/kg body weight for 3 weeks (O'Brien et al., 2009; Yeung et al., 2009). However, a recent study observed developmental toxicity (namely reduced embryo survival) of PFOA in cormorant (*Phalacrocorax carbo sinensis*), herring gull (*Larus argentatus*) and the domestic White Leghorn chicken (*Gallus gallus domesticus*), with chicken being the most sensitive species (Nordén et al., 2016).

119. The soil-dwelling nematode *Caenorhabditis elegans* showed lethal effects with EC_{50} concentrations of 3.85 mM after 1 hour of exposure and 2.35 mM after 48 hours of exposure (Tominaga et al., 2004).

120. Studies with terrestrial plants such as lettuce (*Lactuca sativa*), cucumber (*Cucumis sativus*) and pakchoi (*Brassica rapa chinensis*), spring wheat, oats, potatoes, maize, and perennial ryegrass showed species-dependent adverse effects (e.g. root growth and necrosis) mediated by PFOA (Li, 2008; Stahl et al., 2009).

121. PFOA is primarily detected in the livers of biota such as polar bears and seals (Martin et al., 2004; Smithwick et al., 2005; Dietz et al., 2007; Sonne et al., 2008; Butt et al., 2007; Ishibashi et al., 2008), but the adverse effects of PFOA in such biota have not yet been elucidated. Ishibashi et al., (2008) showed activation of PPARα in the livers of Baikal seals, but no PFOA-mediated adverse effect in the livers was reported. Also, Sonne et al., (2008) concluded that it is not clear whether chronic exposure to PFOA is associated with liver lesions in polar bears, but it is possible that at sufficient concentrations in polar bears, PFOA might induce hepatic alterations.

122. Environment Canada and Health Canada (2012) concluded that the risk quotients (PEC/PNEC) indicate low likelihood of risk to pelagic organisms, mammalian wildlife from exposures at current concentrations in the environment. However, according to the ECHA (2014), the properties of persistent, bioaccumulative, and toxic (PBT) chemicals create uncertainty in the risk estimation to the environment and human health when performing quantitative risk assessment. ECHA stated that using the currently available methods, a "safe" concentration in the environment cannot be established quantitatively with sufficient reliability for an acceptable risk. Furthermore, due to the persistence of the substance, its tendency to accumulate and biomagnify in a variety of terrestrial and marine mammals, its hepatotoxicity and the upward temporal trend of PFOA concentrations in polar bears and some other species, PFOA concentrations in polar bears may approach exposures resulting in harm. Indeed, a temporal trend analysis indicated an annual increase of 2.3% in PFOA levels in East Greenland polar bears from 1984 to 2006 (Dietz et al., 2007).

123. Detailed information on adverse effects on aquatic and terrestrial organisms is presented in the background document (see section 2.4 of document UNEP/POPS/POPRC.12/INF/5).

Summary of ecotoxicological effects

124. Acute aquatic toxicity is low in standard ecotoxicity tests; moderate to low acute toxicities are seen in pelagic organisms including fish and low chronic toxicities in benthic organisms. Adverse effects include intergenerational toxicity in the first offspring generation and some PFOA-mediated toxicity in freshwater algae and other aquatic organisms.

125. There is experimental evidence in terrestrial organisms showing the potential for PFOA to induce alterations to the liver, endocrine dysfunction, developmental toxicity and tumour formation. Adverse effects include alterations in sexual maturation and pubertal timing, changes in mammary gland development as well as induction of a variety of tumours. There are some indications of PFOA-mediated immunomodulation. In plants, PFOA can cause visible abnormalities and alter root

growth. Because of the tendency of PFOA to bioaccumulate, PFOA concentrations in polar bears might increase over time and approach exposures resulting in harm.

Adverse effects on human health

126. The toxicity of PFOA has been evaluated by ECHA, US EPA, the Canadian ministries and EFSA (ECHA, 2011, 2015a; Environment Canada and Health Canada, 2012; US EPA, 2006, 2016b; EFSA, 2008). In the European Union, PFOA has a legally-binding harmonized classification as Carc. 2, Repr. 1B and STOT RE 1 (liver) according to Regulation (EU) No 944/2013.

127. PFOA is readily absorbed after exposure (ingestion) and accumulates in serum and highly perfused organs, mainly in the liver and kidney, due to PFOA primarily binding to albumin proteins in the blood. PFOA does not undergo metabolism or biotransformation in the body (Environment Canada and Health Canada, 2012; Post et al., 2012). As mentioned earlier, the half-life of PFOA elimination in humans is long, ranging between 2 and 4 years (Olsen et al., 2007; Russell et al., 2015).

128. Animal studies have demonstrated the induction of tumours mediated by PFOA or APFO, and hepatic activation of PPARα has been proposed as a mechanism of induction of hepatic tumours (Klaunig et al., 2003). However, the PPAR-agonist mode of action proposed for rat liver, testes and pancreatic tumours may not be relevant for humans. However, human relevance had not been definitively determined according to established frameworks a decade ago (Meek et al., 2003; Boobis et al., 2006), and PFOA compounds have also not been tested for carcinogenic potential in any laboratory animal species other than rats (Environment Canada and Health Canada, 2012). Therefore, the ECHA's Risk Assessment Committee (RAC) came to the conclusion that data on the mode of action are insufficient to conclude that APFO-induced tumours in animals are not relevant for humans, and therefore, PFOA is classified as Carc 2. Based on limited evidence in humans that PFOA causes testicular and renal cancer as well as limited evidence in experimental animals, IARC has classified PFOA as a Group 2B substance (possibly carcinogenic to humans) (Benbrahim-Tallaa et al., 2014; IARC, 2016).

129. PFOA is known to be transmitted to the fetus in cord blood and to the newborn in breast milk. Developing fetuses and newborns are particularly sensitive to PFOA-induced toxicity (US EPA 2016b). Positive correlation in PFOA level between maternal and cord blood samples has been reported in several birth cohort studies in Spain and Norway (Manzano-Salgado et al., 2015; Gutzkow et al., 2012). As APFO can be transferred to infants through breast-feeding, the RAC agreed on an additional classification of PFOA on lactation effects (CLP: Lact. H362: May cause harm to breast-fed children) (ECHA, 2011).

Epidemiological studies

130. Several epidemiological studies investigated exposure and related health effects in workers and different population groups. One of the most extensive epidemiological studies regarding PFOA in humans is the C8 Health Project, a population-wide health study to assess whether or not there is a probable link between C8 exposure and disease in the community. The background behind the C8 Health Project can be found online (C8 Science Panel, 2013).

131. Exposure of PFOA and elevated cholesterol levels (e.g. hypercholesterolemia) as well as other lipid parameters (e.g. uric acid, serum lipid) have been positively correlated in studies involving occupationally-exposed workers (Sakr et al., 2007), highly exposed community residents (Steenland et al., 2009; Steenland et al., 2010a; Frisbee et al., 2010; Fitz-Simon et al., 2013), the general population (Eriksen et al., 2013; Nelson et al., 2010) and children especially boys (Qin et al., 2016).

132. There is also evidence from the C8 Health Project to suggest potential association between PFOA exposure and increased risk of testicular and/or kidney cancers (Barry et al., 2013; Vieira et al., 2013; Steenland, 2012). There are a few reports where no significant association between PFOA exposure and human cancer risk was observed (Chang et al., 2014; Leonard et al., 2008). The Eriksen study acknowledges the possibility of information bias regarding the exposure related to effect (Eriksen et al., 2009). According to IARC (2016) there is limited evidence in humans for the carcinogenicity of PFOA. A positive association was observed for cancers of the testis and kidney.

133. Ulcerative colitis and rheumatoid arthritis have been positively associated with PFOA exposure among workers (Steenland et al., 2015).

134. Adverse reproductive effects have been reported in some epidemiological studies. A positive association between serum PFOA levels and the rate of menopause has been reported in several studies such that women in the highest quintile of PFOA exposure have higher chances of experiencing earlier menopause (Taylor et al., 2013; Knox et al., 2011a), but reverse causation,

i.e. earlier menopause leading to increased PFOA, has been suggested (Dhingra et al. 2016a, 2016b). This would suggest that women who experience earlier menopause might be at risk of higher body burden of PFOA. Another study in Canada involving more than 1,700 women showed possible reduction in fecundity in women with higher plasma levels of PFOA (Velez et al., 2015). Serum PFOA is positively associated with pregnancy-induced hypertension (Darrow et al., 2013), and preeclampsia is weakly associated with PFOA level (Savitz et al., 2012; Stein et al., 2009). Higher serum PFOA levels have been found in patients with polycystic ovary syndrome (Vagi et al., 2014).

135. Developmental effects in humans may be associated with PFOA exposure. On one hand, an inverse correlation between PFOA and birth weight, ponderal index and head circumference has been reported in several mother-child cohort studies (Fei et al., 2007; Apelberg et al., 2007; Maisonet et al., 2012; Chen et al., 2012, Wu et al., 2012; Whitworth et al., 2012a). A novel meta-analysis methodology was applied to review the evidence of 9 human studies, and the outcomes of this meta-analysis indicated that there was sufficient evidence to suggest that developmental exposure to PFOA reduces foetal growth (Johnson et al., 2014; Lam et al., 2014). On the other hand, mothers of low birth weight babies might have altered renal function such as less plasma volume expansion, therefore leading to reduced clearance of PFOA through glomerular filtration (Whitworth et al., 2012b; Verner et al., 2015; Sagiv et al., 2015). Furthermore, there are other studies that reported no significant association between maternal serum PFOA levels and birth weight (Washino et al., 2009; Stein et al., 2009; Monroy et al., 2008; Hamm et al., 2010).

136. Other reported adverse effects observed in children and adolescents associated with higher levels of serum PFOA include impaired neurodevelopment (see below), adiposity (Halldorsson et al., 2012 ; Braun et al., 2016), dyslipidemia (Geiger et al., 2014), altered renal function (Kataria et al., 2015), reduced humoral immune response (Grandjean et al., 2012; Grandjean and Budtz-Jørgensen, 2013), lower levels of insulin-like growth factor-1 and sex hormones (Lopez-Espinosa et al., 2016), higher odds of self-reported food allergies (Buser and Scinicariello, 2016) and later age of puberty in girls (Lopez-Espinosa et al., 2011; Holtcamp, 2012).

Neurotoxicity in humans

137. Impaired neurodevelopment has been associated with PFOA. An inverse relationship between prenatal PFOA concentrations in mothers and neurodevelopment as determined with the mental development index (MDI) in female (not male) offspring at 6 months of age was observed in a Japanese birth cohort (Hokkaido) study. However, this relationship was not observed with offspring at 18 months of age (Goudarzi et al., 2016). Also, no correlation between PFOA levels and birth weight was observed in the same cohort study (Washino et al., 2009). Statistically significant inverse associations between PFOA and memory impairment has been reported (Gallo et al., 2013). On the other hand, there are studies that reported no association between PFOA exposure and impaired neurodevelopment or behavior (Chen et al., 2013; Stein et al., 2013).

Immunotoxicity

138. The U.S. National Institute of Environmental Health Sciences' National Toxicology Program (NTP) systematically reviewed and evaluated evidence on exposure to PFOS or PFOA and immune-related health effects to determine whether exposure to either chemical is associated with immunotoxicity for humans. Based on the health effects data from 33 human studies, 93 animal studies, and 27 in vitro/mechanistic studies relevant for addressing the objective, NTP published its draft findings in June of 2016 concluding that PFOA is presumed to be an immune hazard to humans. The NTP based this conclusion primarily on evidence of suppression of the antibody response and increased hypersensitivity, as well as additional evidence that is primarily from epidemiological studies that PFOA reduced infectious disease resistance and increased autoimmune disease (US NIEHS, 2016).

139. Reduced humoral immune response has been observed in a few studies (Grandjean et al., 2012; Looker et al., 2014; Kielsen et al., 2015). In particular, elevated PFOA serum levels are associated with reduced antibody titer rise, especially to A/H3N2 influenza virus and an increased risk of not attaining a protection threshold antibody concentration (Looker et al., 2014). In the Danish National Birth Cohort study, it was reported that prenatal exposure to PFOA is not associated with increased risk of infectious diseases leading to hospitalisation in early childhood; however, when the analysis was stratified by gender, girls showed a slightly higher risk of hospitalisation for infections associated with higher maternal PFOA levels (incidence rate ratio of 1.74 at the highest quartile compared with the lowest) (Fei et al., 2010).

140. Using data from a study of immunotoxicity in children (Grandjean et al., 2012), BMDLs were calculated to be approximately 0.3 ng/mL for PFOA, in terms of the serum concentration decrease of specific antibodies. Using an uncertainty factor of ten to take into account individual susceptibility, the BMDLs would result in a reference dose serum concentration of about or below 0.1 ng/mL.

Endocrine Disruption

141. Findings from studies seem to indicate a PFOA-mediated effect on the endocrine system. Prenatal exposure of PFOA may alter testosterone concentrations in females (Maisonet et al., 2015), and an inverse correlation between parathyroid hormone 2 receptor (PTH2R) and PFOA exposure was also reported in a study of 189 women (Galloway et al., 2015). As for men, a study by La Rocca et al. (2015) reported an inverse relationship between PFOA serum level in men and expression of nuclear receptors such as estrogen and androgen receptors. Also, early menopause in women with high PFOA levels have been observed in the C8 Health Project (Knox et al., 2011a, 2011b).

142. PFOA has been implicated to act as a so-called obesogene similar to other endocrine disruptive compounds that can act directly on ligands for nuclear hormone receptors or affect components in metabolic signaling pathways. A human prospective cohort study showed a correlation between low dose PFOA exposure of 655 Danish pregnant women and obesogenic effects in their offspring at 20 years of age. Maternal PFOA concentrations were positively associated with serum insulin and leptin levels and inversely associated with adiponectin levels in female offspring (Halldorsson et al., 2012). On the other hand, the C8 Health Project concluded that PFOA exposure in early life was not associated with overweight and obesity risk in adulthood (Barry et al., 2014).

143. Evidence from several epidemiological studies seems to suggest an association between exposure to PFOA and changes in different thyroid hormones leading to altered thyroid function inducing thyroid disease such as hypothyroidism or hyperthyroidism (Shrestha et al., 2015; Lopez-Espinosa et al., 2012; Knox et al., 2011b; Kim et al., 2011; Melzer et al., 2010; C8 Science Panel, 2013; Yang et al., 2016). However, there have also been studies that reported inconsistent findings between PFOA exposure and thyroid diseases (i.e. inverse relation between subclinical hyperthyroidism and PFOA or no association between hypothyroidism and PFOA) (as summarised in Steenland et al., 2010b and C8 Science Panel, 2013).

144. The potential of PFOA to affect estrogen receptor (ER) and androgen receptor (AR) transactivity as well as aromatase enzyme activity was analysed in an *in vitro* study, and it was shown that PFOA significantly induced ER transactivity yet antagonized AR activity in a concentration-dependent manner. In addition, when PFOA was mixed with 6 other PFCs, a mixture effect more than additive was observed on AR function, emphasizing the importance of considering the combined action of PFCs in assessing related health risks (Kjeldsen and Bonefeld-Jørgensen, 2013).

145. According to the study of Su et al., (2016), PFOA showed a potential protective effect against glucose intolerance and the risk of diabetes.

146. Detailed information on adverse effects on human health is presented in the background document (see section 2.4 of document UNEP/POPS/POPRC.12/INF/5).

Summary of adverse effects on human health

147. PFOA is classified as Carc. 2, Repr. 1B and STOT RE 1 (liver) according to Regulation (EU) No 944/2013. IARC also categorised PFOA as a Group 2B substance (possibly carcinogenic to humans). There have been reported adverse health effects such as elevated cholesterol levels, altered reproductive/developmental effects, endocrine disruption, impaired neurodevelopment, as well as increased risk of cancer associated with PFOA exposure in humans. Scientific data have demonstrated PFOA-mediated immunotoxicity, primarily suppression of antibody response, in humans. Although the findings are limited, the reported adverse health effects suggest additional public health concerns.

3. Synthesis of information

148. PFOA has been manufactured since 1947 and is currently manufactured mainly as the salt APFO. The global volume of production has gradually increased over time with ongoing production, for example, in China.

149. PFOA-related compounds have the capacity to degrade to PFOA and hence to contribute to environmental concentrations of PFOA. It is unlikely that actions against PFOA and APFO alone can fully minimize and eliminate environmental concentrations. In particular, fluorotelomer alcohols (FTOHs) have been identified as a potential source of PFOA. Numerous direct and indirect sources of

PFOA, its salts and PFOA-related compounds contribute to the overall release of PFOA to the environment. Main emission vectors are water, wastewater, dust particles and air.

150. Fluoropolymers and fluorotelomer-based polymers (FTPs) are used across many sectors for different purposes, including the manufacture of stain- and water-resistant coatings on textiles and carpets; hoses, cable and gaskets; non-stick coatings on cookware; and personal care products. Traces of PFOA can also be found in certain aqueous fire-fighting foams.

151. Based on the available experimental evidence, it is concluded that PFOA is highly stable and persistent within the natural environment. PFOA is unlikely to degrade under conditions present in the natural environment and has been shown to have long half-lives within the environment. Monitoring data show that PFOA in soil leaches over time and can be a long term contamination source to underlying groundwater.

152. PFOA has been found in marine, limnetic and terrestrial biota worldwide, and bioaccumulation of PFOA occurs across trophic levels. Assessment of bioaccumulation for PFOA is complicated by its physical properties as a surfactant, which make analysis for development of log K_{ow} values not directly possible. PFOA accumulates and biomagnifies in air breathing animals and other terrestrial species including humans but not in water breathing animals as fish excrete PFOA through their gills.

153. Monitoring of water, snow, air, sediment and biota at remote locations all detect the presence of PFOA. Equally, environmental modeling data and other information enable to conclude that PFOA meets the criterion for long range transport.

154. Human PFOA exposure occurs via dietary intake of food and drinking water, exposure to contaminated indoor dust or consumer products containing PFOA and its related substances. Studies have demonstrated the presence of PFOA within humans, mainly in blood and breast milk samples. Fetuses and newborns are susceptible to PFOA exposure via breastfeeding or trans-placental passage. Occupational exposure or exposure near sites of production resulted in higher serum PFOA levels than exposure of general population. Humans are very slow eliminators of PFOA compared with other species. The half-life of PFOA elimination in human blood is long, ranging from 2-4 years.

155. PFOA exhibits adverse effects for both terrestrial and aquatic species. Ecotoxicity data indicate a low acute toxicity for aquatic organisms. There is also experimental evidence in terrestrial organisms showing the potential for PFOA to induce changes in liver function, endocrine function, development as well as immune responses, and induction of tumours has been shown in rats exposed to PFOA. The adverse effects of PFOA in biota have not yet been elucidated, but because of the tendency of PFOA to bioaccumulate, PFOA concentrations in biota, especially polar bears, might increase over time and approach exposures resulting in harm.

156. PFOA is classified in line with the EU GHS criteria as Carc. 2, Repr. 1B and STOT RE 1 (liver) (Regulation (EU) No 944/2013). IARC has also categorized PFOA a Class 2B carcinogen with particular regard to testicular and kidney cancers. High cholesterol, inflammatory diseases, ulcerative colitis, thyroid disease, immune effects, pregnancy-induced hypertension, endocrine disruption and impaired neuro- as well as reproductive development have been found to be associated with PFOA exposure in humans in epidemiological studies, but more evidence is needed to determine the relationship between PFOA and the observed health effects.

4. Concluding statement

157. PFOA, its isomers, salts (since the late 1940s) and PFOA-related compounds (since the 1960s) have been widely used on a global basis. However, growing evidence of health and environmental effects has meant that concerns regarding the use of PFOA have existed for a number of decades. Stricter regulatory control and voluntary action by industry to phase-out the production and use of PFOA have been in place since 2006.

158. PFOA has been demonstrated to be highly persistent with the capacity to undergo long range transport far from the point of use. This is evidenced by monitoring data of PFOA in air, water, soil/sediment and biota in both local and remote locations like the Arctic. Additionally, PFOA-related compounds exist and have the capacity within the natural environment to degrade and form PFOA, further aiding the long range transport of these substances. PFOA can bioaccumulate in air-breathing mammals and other terrestrial species including humans. Evidence also exists to highlight the potential adverse effects of PFOA upon both environmental species and humans.

159. Based on the persistence, bioaccumulation, toxicity in mammals including humans and widespread occurrence in environmental compartments, it is concluded that PFOA, its isomers, salts and related compounds that degrade to PFOA, as a result of their long-range environmental transport,

are likely to lead to significant adverse human health and environmental effects such that global action is warranted.

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Report of the Persistent Organic Pollutants Review Committee on the work of its second meeting

Addendum

Risk profile on perfluorooctane sulfonate

At its second meeting, the Persistent Organic Pollutants Review Committee adopted the risk profile on perfluorooctane sulfonate, on the basis of the draft contained in document UNEP/POPS/POPRC.2/11. The text of the risk profile, as amended, is provided below. It has not been formally edited.

K0653903 151206

KD2680

PERFLUOROOCTANE SULFONATE

RISK PROFILE

Adopted by the Persistent Organic Pollutants Review Committee
at its second meeting

November 2006

KD2681

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EXECUTIVE SUMMARY

1 INTRODUCTION

1.1 Chemical Identity of the proposed substance

On July 14, 2005, the government of Sweden made a proposal for listing perfluorooctane sulfonate (PFOS) and 96 PFOS-related substances in Annex A of the Stockholm Convention on Persistent Organic Pollutants (POPs).

Chemical name: Perfluorooctane Sulfonate (PFOS)

Molecular formula: $C_8F_{17}SO_3^-$

PFOS, as an anion, does not have a specific CAS number. The parent sulfonic acid has a recognised CAS number (CAS No. 1763-23-1). Some examples of its commercially important salts are listed below:

Potassium salt (CAS No. 2795-39-3)

Diethanolamine salt (CAS No. 70225-14-8)

Ammonium salt (CAS No. 29081-56-9)

Lithium salt (CAS No. 29457-72-5)

Structural formula:

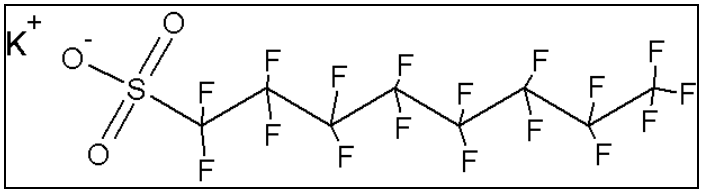


Figure 1. Structural formula of PFOS shown as its potassium salt

PFOS is a fully fluorinated anion, which is commonly used as a salt or incorporated into larger polymers. PFOS and its closely related compounds, which contain PFOS impurities or substances which can give rise to PFOS, are members of the large family of perfluoroalkyl sulfonate substances. In its regulatory measures on PFOS, the EU has addressed all molecules having the following molecular formula: $C_8F_{17}SO_2Y$, where Y = OH, metal or other salt, halide, amide and other derivatives including polymers (European Union 2006).

The physical and chemical properties of the potassium salt of PFOS are listed in Table 2.

Table 2. Physical and chemical properties of PFOS potassium salt.

(Data from OECD, 2002, unless otherwise noted).

Property	Value
Appearance at normal temperature and pressure	White powder
Molecular weight	538 g/mol
Vapour Pressure	3,31 x 10^{-4} Pa
Water solubility in pure water	519 mg/L (20 ± 0,5ºC) 680 mg/L (24 - 25ºC)
Melting point	> 400 ºC
Boiling point	Not measurable
Log K_{OW}	Not measurable
Air-water partition coefficient	< 2 x 10^{-6} (3M, 2003a)
Henry's Law Constant	3,09 x 10^{-9} atm m^3/mol pure water

PFOS can be formed (by environmental microbial degradation or by metabolism in larger organisms) from PFOS-related substances, i.e., molecules containing the PFOS-moiety depicted in Figure 1. Although the ultimate net contribution of individual PFOS-related substances to the environmental loadings of PFOS cannot be predicted readily, there is a potential that any molecule containing the PFOS moiety could be a precursor to PFOS.

The majority of PFOS-related substances are polymers of high molecular weights in which PFOS is only a fraction of the polymer and final product (OECD, 2002). PFOS-related substances have been defined somewhat differently in different contexts and there are currently a number of lists of PFOS-related substances (Table 3). The lists contain varying numbers of PFOS-related substances that are thought to have the potential to break down to PFOS. The lists overlap to varying extents depending on the substances under consideration and the overlap between national lists of existing chemicals.

Table 3. Number of PFOS-related substances as proposed by UK – DEFRA, US – EPA, OECD, OSPAR, and Canada

Source	Number of PFOS-related substances
RPA and BRE (2004)	96
US - EPA (2002, 2006)	88[1] + 183[1]
OECD (2002)	172[1] (22 classes of perfluoroalkyl sulfonate substances)
OSPAR (2002)	48
Environment Canada (2006)	57

[1] Perfluorinated substances with different carbon chain lengths are included in the list.

A large number of substances may give rise to PFOS and thus contribute to the contamination problem. DEFRA in the United Kingdom (RPA and BRE, 2004) has recently proposed a list of 96 PFOS-related substances. However, the properties of the 96 substances have not generally been determined. According to 3M (submission to the secretariat of Stockholm Convention (SC), 2006), they may have very different environmental characteristics such as solubility, stability and ability to be absorbed or metabolised. Nevertheless, the document by the United Kingdom infers that all of these substances would give rise to the final degradation product of PFOS (RPA and BRE, 2004).

Environment Canada's ecological risk assessment defines PFOS precursors as substances containing the perfluorooctylsulfonyl ($C_8F_{17}SO_2$, $C_8F_{17}SO_3$,, or $C_8F_{17}SO_2N$) moiety that have the potential to transform or degrade to PFOS (Environment Canada, 2006). The term "precursor" applies to, but is not limited to, some 51 substances identified in the ecological assessment. However, this list is not considered exhaustive, as there may be other perfluorinated alkyl compounds that are also PFOS precursors. This information was compiled based on a survey to industry, expert judgement and CATABOL modelling, in which 256 perfluorinated alkyl compounds were examined to determine whether non-fluorinated components of each substance were expected to degrade chemically and/or biochemically and whether the final perfluorinated degradation product was predicted to be PFOS. While the assessment did not consider the additive effects of PFOS and its precursors, it is recognized that the precursors to PFOS contribute to the ultimate environmental loading of PFOS. Precursors may also play a key role in the long-range transport and subsequent degradation to PFOS in remote areas, such as the Canadian Arctic.

1.2 Conclusion of the POP Review Committee on Annex D information

The Persistent Organic Pollutants Review Committee (POPRC) evaluated Annex D information at the First meeting of the POPRC, Geneva, 7-11 November 2005, and concluded that PFOS information meets the screening criteria specified in Annex D (decision POPRC-1/7: Perfluorooctane sulfonate).

1.3 Data sources

This document on PFOS mainly builds on information that has been gathered in the hazard assessment report prepared by the UK and the USA for the OECD, and in the UK risk reduction strategy:

OECD (2002) Co-operation on Existing Chemicals - Hazard Assessment of Perfluorooctane Sulfonate and its Salts, Environment Directorate Joint Meeting of the Chemicals Committee and the Working Party on Chemicals, Pesticides and Biotechnology, Organisation for Economic Co-operation and Development, Paris, 21 November 2002.

RPA AND BRE (2004) Perfluorooctane Sulfonate – Risk reduction strategy and analysis of advantages and drawbacks, Final Report prepared for Department for Environment, Food and Rural Affairs and the Environment Agency for England and Wales.

Recent relevant information from the open scientific literature (up to May 2006) is also included. Data submitted by Parties and observers, which have been considered, are also included in this report when they add new information.

1.4 Summary of assessment and management under other programs

The hazard assessment of PFOS, prepared by the OECD in 2002, concluded that the presence and the persistence of PFOS in the environment, as well as its toxicity and bioaccumulation potential, indicate a cause of concern for the environment and human health.

An environmental risk assessment, prepared by the UK-Environment Agency, and discussed by the EU member states under the umbrella of the existing substances regulation (ESR DIR 793/93) shows that PFOS is of concern.

The final Environment Canada/Health Canada assessments of PFOS, its salts and its precursors were released in July 2006. The ecological risk assessment has concluded that PFOS and its salts are persistent and bioaccumulative, and that PFOS, its salts and its precursors have immediate or long-term harmful effects on the environment (Environment Canada, 2006).

The EU has recently decided on restrictions on the marketing and use of PFOS (European Union, 2006). The measures cover PFOS acid, its salts and PFOS derivatives, including PFOS polymers. The decision prohibits the placing on the market and use of these compounds as a substance or constituent of preparations in a concentration equal to or higher than 0,005% by mass. Furthermore, semi-finished products and articles, containing PFOS more than 0,1% by mass are prohibited. Some derogations are, however, granted in the decision. These include certain uses in photolithography processes, in photographic coatings and in metal plating, hydraulic fluids for aviation and fire fighting foams that have already been placed on the market.

The UK and Sweden have proposed the following classification for PFOS in EU (2005):

T Toxic

R40 Carcinogen category 3; limited evidence of carcinogenic effect

R48/25 Toxic; danger of serious damage to health by prolonged exposure if swallowed

R61 May cause harm to the unborn child

R51/53 Toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment has.

Norway is now considering a proposal to prohibit the use of fire fighting foams containing PFOS and PFOS-related compounds, which is the major use of these compounds today in Norway.

The Environmental Protection Agency (EPA) in the USA finalized two Significant New Use Rules (SNURs) in 2002, requiring companies to inform the EPA before manufacturing or importing 88 listed PFOS-related substances. The EPA proposed an additional SNUR under section 5(a)(2) of the Toxic Substances Control Act (TSCA) in March 2006 to include within the scope of this regulation another 183 perfluoroalkyl sulfonates with carbon chain lengths of five carbons and higher. The EPA further proposed an amendment to the Polymer Exemption rule in March 2006 which would remove from exemption polymers containing certain perfluoroalkyl moieties consisting of CF3- or longer chains, and would require that new chemical notifications be submitted on such polymers.

1.5 Status of the chemical under international conventions

OSPAR: PFOS was added to the list of Chemicals for Priority Action in June 2003.

Persistent Organic Pollutants Protocol to the Long-Range Transboundary Air Pollution Convention ("LRTAP"): The Executive Body of the UNECE LRTAP Convention agreed that PFOS be considered a POP as defined under the Protocol on POPs and requested that the UNECE Task Force on POPs continue with the review of the substance and exploring management strategies.

2 SUMMARY INFORMATION RELEVANT FOR THE RISK PROFILE

2.1 Sources

2.1.1 Production and trade

The main production process of PFOS and PFOS-related substances is electro-chemical fluorination (ECF), utilized by 3M, the major global producer of PFOS and PFOS-related substances prior to 2000.

Direct fluorination, electro-chemical fluorination (ECF):

$$C_8H_{17}SO_2Cl + 18\ HF \rightarrow C_8F_{17}SO_2F + HCl + \text{by products}$$

The reaction product, perfluorooctanesulfonyl fluoride (PFOSF)[1] is the primary intermediate for synthesis of PFOS and PFOS-related substances. The ECF method results in a mixture of isomers and homologues with about 35-40% 8-carbon straight chain PFOSF. However, the commercial PFOSF products were a mixture of approximately 70% linear and 30% branched PFOSF derivate impurities. The global production of PFOSF by 3M until the production ceased is estimated to have been 13,670 metric tonnes (1985 to 2002), with the largest yearly production volume, 3700 metric tonnes of PFOS and PFOS related substances, in 2000 (3M, Submission to SC, 2006). PFOSF may be further reacted with methyl- or ethylamine to form *N*-ethyl- and *N*-methyl perfluorooctane sulfamide and subsequently with ethylene carbonate resulting in *N*-ethyl- and -methyl-perfluorooctane sulfamidoethanol (*N*-EtFOSE and *N*-MeFOSE). *N*-EtFOSE and *N*-MeFOSE were the principal building blocks of 3M's product lines. PFOS is formed after the chemical or enzymatic hydrolysis of PFOSF (3M, 1999).

Other production methods for perfluoroalkylated substances are telomerisation and oligomerisation. However, to which extent these methods are applied for production of PFOS and PFOS-related substances is not evident.

[1] In the OECD report, 2002, perfluorooctanesulfonyl fluoride is abbreviated POSF.

On 16 May 2000, 3M announced that the company would phase-out the manufacture of PFOS and PFOS-related substances voluntarily from 2001 onwards. By the end of 2000, about 90 % of 3M's production of these substances had stopped and in the beginning of 2003 the production ceased completely.

3M´s voluntary phase-out of PFOS production has led to a reduction in the use of PFOS-related substances. This is due not only to the limited availability of these substances (3M had at the time the greatest production capacity of PFOS-related substances in the world), but also to action within the relevant industry sectors to decrease companies´ dependence on these substances.

The US Environmental Protection Agency (US EPA) compiled a list of non-US companies which are believed to supply PFOS-related substances to the global market. Of these (and excluding the plant of 3M in Belgium), six plants are located in Europe, six are located in Asia (of which four are in Japan) and one in Latin America (OECD, 2002). However, this list may not be exhaustive or current.

According to the recent submission from Japan to the secretariat of the Stockholm Convention, 2006, there is one manufacturer in Japan still producing PFOS and with a production amount of 1-10 tonnes (2005). The submission from Brazil states that lithium salt of PFOS is produced but that no quantitative data is available.

2.1.2 Uses

Perfluorinated substances with long carbon chains, including PFOS, are both lipid-repellent and water-repellent. Therefore, the PFOS-related substances are used as surface-active agents in different applications. The extreme persistence of these substances makes them suitable for high temperature applications and for applications in contact with strong acids or bases. It is the very strong carbon-fluorine binding property that causes the persistence of perfluorinated substances.

The historical use of PFOS-related substances in the following applications has been confirmed in the US and the EU.

- Fire fighting foams
- Carpets
- Leather/apparel
- Textiles/upholstery
- Paper and packaging
- Coatings and coating additives
- Industrial and household cleaning products
- Pesticides and insecticides

In the UK study (RPA and BRE, 2004), detailed information has been received from the following sectors that currently use PFOS-related substances:

- Use of existing fire fighting foam stock
- Photographic industry
- Photolithography and semiconductor
- Hydraulic fluids
- Metal plating

The sectors presented above account for the UK but are considered to be representative for EU. However, deviation in the current use pattern between EU countries cannot be excluded.

PFOS and its precursors are not manufactured in Canada but rather are imported as chemicals or products for Canadian uses. They may also be components in imported manufactured articles. It is estimated that the majority of PFOS has been used as water, oil, soil and grease repellents (e.g. on fabric, leather, paper, packaging, rugs and carpets) and as surfactants (e.g. in fire fighting foams and coating additives) (Environment Canada, 2006).

PFOS and its precursors are not manufactured in the US, but can be imported either as chemicals or in products for the specific limited uses that were excluded from regulation. These comprise use as an anti-erosion additive in aviation hydraulic fluids; use as a component of a photoresist substance, including a photo acid generator or surfactant, or as a component of an anti-reflective coating, used in a photomicrolithography process to produce semiconductors or similar components of electronic or other miniaturized devices; use in coatings for surface tension, static discharge, and adhesion control for analog and digital imaging films, papers, and printing plates, or as a surfactant in mixtures used to process imaging films; and use as an intermediate only to produce other chemical substances to be used solely for these uses. Historically, PFOS and its precursors were also used as surfactants in fire fighting foams and in industrial and household cleaning products; in carpet, textile, leather, and paper coatings; and in termite and ant bait insecticide products. Stocks of PFOS and PFOS-containing products that were in existence at the time the US regulations were promulgated in 2002 could continue to be used in any application until they were consumed without violating the regulation, except that the PFOS-related insecticide products are subject to a phase-out agreement prohibiting their use after 2015.

The table below outlines the estimated current demand for PFOS-related substances in these applications in the EU (RPA and BRE, 2004).

Estimated Current (2004) Demand for PFOS Related Substances in the EU	
Industry Sector	Quantity (kg/year)
Photographic industry	1,000
Photolithographic and semi-conductors	470
Hydraulic fluids	730
Metal plating	10,000

In the survey on production and use of PFOS and related substances performed by OECD in 2004 (published 2005), data concerning PFOS were difficult to separate from data on other perfluoroalkyl sulfonates.

Fire Fighting Foams

The fire fighting foams can be grouped in two main categories:

- Fluorine-containing foam types (some of them consist of PFOS-related substances)
- Fluorine-free foam types

Since the announcement of the voluntary cessation of production of PFOS-related substances by 3M, the presence of PFOS in fire fighting foams has gradually decreased (RPA and BRE, 2004).

Historically, in Canada, the most significant imports of PFOS, itself, were in the form of the potassium salt, used for fire-fighting foams (Environment Canada, 2006). Canada has also identified that existing stocks of PFOS-containing fire fighting foams could be a continued significant source of releases.

An industry survey conducted in the US by the Fire Fighting Foam Coalition in 2004 reported that the total inventory of aqueous film-forming foam in the US was approximately 9.9 million gallons, of which about 45% was PFOS-based stocks produced before 2003, with the other 55% comprised of telomer-based foams.

Textile, Carpet and Leather Protection

PFOS-related substances have been used to provide soil, oil and water resistance to textiles, apparels, home furnishings and upholstery, carpets, and leather products. Since 3M´s withdrawal from the market, PFOS-related substances are used to a much smaller extent for these applications (RPA AND BRE, 2004).

Paper and Packaging Protection

PFOS-related substances have been used in the packaging and paper industries in both food packaging and commercial applications to impart grease, oil and water resistance to paper, paperboard and packaging substrates. According to 3M, fluorochemicals were used for both food contact applications (plates, food containers, bags and wraps) and non-food applications (folding cartons, containers and carbonless forms and masking papers). Since 3M´s withdrawal from the market, PFOS related substances are used to a much smaller extent for these applications (RPA and BRE, 2004).

Coatings and Coating Additives

3M indicates that prior to its voluntary phase-out of PFOS production, the company would sell fluorochemical polymer coatings and coating additives which were used undiluted or diluted with water or butyl acetate to impart soil or water repellence to surfaces (including printing circuit boards and photographic film) (RPA and BRE, 2004).These polymers contained fluorocarbon residuals at a concentration of 4% or less. Other applications for aqueous coatings are to protect tile, marble and concrete. It is unclear which of these products were actually based on PFOS-related substances.

A survey in the UK among members of the British Coatings Federation (BCF) showed that the use of PFOS-related substances for these purposes is very limited (RPA and BRE, 2004).

Industrial and Household Cleaning Products (Surfactants)

3M PFOS-based products were sold in the past to a variety of formulators to improve the wetting of water-based products marketed as alkaline cleaners, floor polishes (to improve wetting and levelling), denture cleansers and shampoos. Several of these products (alkaline cleaners, floor polishes, shampoos) were marketed to consumers; some products were also sold to janitorial and commercial services. A number of the alkaline cleaners were spray-applied.

With regard to the UK cleaning products industry, the responses received do not indicate the use of PFOS-related substances in industrial and household cleaning products. Based on information provided in product registers, the Swedish National Chemicals Inspectorate (KemI) has indicated that PFOS-related substances are still being used in Sweden for both industrial and household use (RPA and BRE, 2004).

Photographic Industry

PFOS-based chemicals are used for the following purposes in mixtures, in coatings applied to photographic films, papers, and printing plates (RPA and BRE, 2004):

- Surfactants
- Electrostatic charge control agents;

- Friction control agents;
- Dirt repellent agents; and
- Adhesion control agents

Photolithography and Semiconductors

Photoresist

Semiconductor manufacturing comprises up to 500 steps, of which there are four fundamental physical processes:

- Implant
- Deposition
- Etch
- Photolithography

Photolithography is the most important step towards the successful implementation of each of the other steps and, indeed, the overall process. It shapes and isolates the junctions and transistors; it defines the metallic interconnects; it delineates the electrical paths that form the transistors; and joins them together. Photolithography reportedly represents 150 of the total of 500 steps mentioned above. Photolithography is also integral to the miniaturization of semiconductors (RPA and BRE, 2004).

PFOS is used as a photoacid generator (PAG) in a mechanism called chemical amplification that increases the sensitivity of photoresist to allow etching images smaller than wavelength of light.

Antireflective Coatings

A number of resist suppliers sell antireflective coatings (ARC), subdivided into Top (TARC) and Bottom (BARC) coatings and used in combination with deep ultra violet (DUV) photoresist. The process involves placing a thin, top coating on the resist to reduce reflective light, in much the same way and for the same purposes that eyeglasses and camera lenses are coated.

Hydraulic Fluids for the Aviation Industry

Hydraulic fluids were initially used in aircraft to apply brake pressure. As larger and faster aircraft were designed, greater use of hydraulic fluids became necessary. An increase in the number of hydraulic fluid fires in the 1940s necessitated work towards developing fire resistant fluids. The first of these fluids was developed around 1948, when fire resistant hydraulic fluids based on phosphate ester chemistry were developed.

Perfluorinated anions act by altering the electrical potential at the metal surface, thereby preventing the electrochemical oxidation of the metal surface under high fluid flow conditions (RPA and BRE, 2004). As a result, hydraulic fluids based on phosphate ester technology and incorporating additives based on perfluorinated anions are used in all commercial aircraft, and in many military and general aviation aircraft throughout the world, as well as by every airframe manufacturer (RPA and BRE, 2004).

Metal Plating

The main uses of PFOS-related substances in metal plating are for chromium plating, and anodising and acid pickling. PFOS related substances lower the surface tension of the plating solution so that mist containing chromic acid from the plating activity is trapped in solution and is not released to air (RPA and BRE, 2004).

Other

There is information on other historical or current PFOS applications such as in pesticides, medical applications, mining and oil surfactants, flame retardants and in adhesives. Based on current understanding, these applications represent a minor part of known PFOS applications and are therefore not further elaborated in this profile.

2.1.3 Releases to the environment

There is to date very limited information regarding the emissions and pathways of PFOS to the environment. The occurrence of PFOS in the environment is a result of anthropogenic manufacturing and use, since PFOS is not a naturally occurring substance.

Releases of PFOS and its related substances are likely to occur during their whole life cycle. They can be released at their production, at their assembly into a commercial product, during the distribution and industrial or consumer use as well as from landfills and sewage treatment plants after the use of the products (3M, 2000).

Manufacturing processes constitute a major source of PFOS to the local environment. During these processes, volatile PFOS-related substances may be released to the atmosphere. PFOS and PFOS-related substances could also be released via sewage effluents (3M, 2000). High local emissions are indicated by one study that showed extremely high concentrations of PFOS in wood mice collected in the immediate vicinity to 3M's fluorochemical plant in Antwerpen, Belgium (Hoff et al., 2004). High concentrations of PFOS were also found in liver and blood from fish collected in the Mississippi River at the immediate vicinity of another 3M fluorochemical plant at Cottage Grove in Minnesota (MPCA, 2006).

Fire training areas have also been revealed to constitute a source of PFOS emissions due to the presence of PFOS in fire-fighting foams. High levels of PFOS have been detected in neighbouring wetlands of such an area in Sweden (Swedish EPA, 2004) as well as in groundwater in the US close to a fire-training area (Moody et al., 2003).

An investigation on the uses of PFOS and PFOS-related compounds in Norway in 2005 shows that approximately 90% of the total use is in fire extinguishers (Submission to SC, 2006). Estimated releases of PFOS related to fire extinguishers are at least 57 tonnes since 1980 to 2003 (2002; 13-15 tonnes). Remaining quantities of fire extinguisher foam in Norway are estimated to be a minimum of 1.4 million litres, which corresponds to an amount of approximately 22 tonnes PFOS. Releases from the municipal sector in Norway, 2002, were estimated to be 5-7 tonnes (Submission to SC, 2006).

The use of PFOS in semiconductors is estimated to result in a release of 43 kg per year in the EU, according to the Semiconductur Industry Association (SIA) (SIA, Submission to SC, 2006). This corresponds to 12 % of the total PFOS use in this application. PFOS released in the USA from semiconductors is estimated to be in the same range (SIA, 2006).

The releases of sulfonated perfluorochemicals, including PFOS or PFOS-related substances, from different product usages have been estimated (3M Speciality Materials, 2002). For example, garments treated with home-applied products, are expected to lose 73 % of the treatment during cleaning over a 2-year life span. A loss of 34 % to air is expected from spray can products during use, while up to 12.5 % of the original content may be remaining in the cans at the time of disposal.

One route for PFOS and PFOS-related substances to the environment may be through sewage treatment plants (STPs) and landfills, where elevated concentrations have been observed compared to background concentrations. Once released from STPs, PFOS will partially adsorb to sediment and organic matter. A substantial amount of PFOS may also end up in agricultural soil, due to the

application of sewage sludge. The primary compartments for PFOS are therefore believed to be water, sediment and soil (RIKZ, 2002).

Dispersion of PFOS in the environment is thought to occur through transport in surface water, or oceanic currents (Yamashita *et al*., 2005, Caliebe *et al*., 2004), transport in air (volatile PFOS-related substances), adsorption to particles (in water, sediment or air) and through living organisms (3M, 2003a).

One major obstacle when trying to estimate the releases of PFOS to the environment is that PFOS can be formed through degradation of PFOS-related substances. The rate and the extent of that formation are presently unknown. In a study on Swedish STPs, higher concentrations of PFOS were found in the effluents compared to incoming sewage water, which could indicate that PFOS was formed from PFOS-related substances (Posner and Järnberg, 2004).

2.2 Environmental fate

2.2.1 Persistence

PFOS is extremely persistent. It does not hydrolyse, photolyse or biodegrade in any environmental condition tested (OECD, 2002).

A study on the hydrolysis of PFOS in water has been performed following US-EPA OPPTS protocol 835.2210. The study was conducted at pH varying from 1.5 – 11.0 and at a temperature of 50°C, to facilitate hydrolysis, but did not indicate any degradation of PFOS. The half-life of PFOS was set to be greater than 41 years.

A study on the photolysis of PFOS in water following US-EPA OPPTS protocol 835.5270 has been conducted. No evidence of direct or indirect photolysis was observed under any of the conditions tested. The indirect photolytic half-life of PFOS at 25°C was calculated to be more than 3.7 years.

Biodegradation of PFOS has been evaluated in a variety of tests. Aerobic biodegradation of PFOS has been tested in activated sewage sludge, sediment cultures and soil cultures in several studies. Anaerobic biodegradation has been tested in sewage sludge. None of the studies demonstrated any signs of biodegradation.

Modelling with a simulator program of microbial degradation, the CATABOL system, and expert judgment predicted that of 171 studied perfluorinated substances over 99% would biodegrade to extremely persistent perfluorinated acids. Of them, 109 substances were predicted to end up as perfluorinated sulfonic acids, including PFOS, and 61 as perfluorinated carboxylic acids (Dimitrov et al., 2004).

The only known condition whereby PFOS is degraded is through high temperature incineration under correct operating conditions (3M, 2003a). Potential degradation at low temperature incineration is unknown.

2.2.2 Bioaccumulation

It should be noted that PFOS does not follow the “classical” pattern of partitioning into fatty tissues followed by accumulation, which is typical of many persistent organic pollutants. This is because PFOS is both hydrophobic and lipophobic. Instead, PFOS binds preferentially to proteins in the plasma, such as albumin and β-lipoproteins (Kerstner-Wood et al., 2003), and in the liver, such as liver fatty acid binding protein (L-FABP; Luebker et al., 2002). Because of the unusual physical-chemical characteristics of PFOS, the mechanism of bioaccumulation probably differs from other POPs.

In a study following OECD protocol 305, the bioaccumulation of PFOS in bluegill sunfish (*Lepomis macrochirus*) has been tested. The whole-fish kinetic bioconcentration factor (BCFK) was determined to be 2796 (3M, 2002).

In another study on rainbow trout (*Oncorhynchus mykiss*), a bioconcentration factor (BCF) in liver and plasma was estimated to be 2900 and 3100, respectively (Martin, *et al*., 2003).

When strictly looking at the BCF values, it is clear that these values are below the numeric BCF criteria in Stockholm Convention Annex D (the reported BCF values are below 5000) but, in this particular case, as noted above, the BCF numeric criteria may not adequately represent the bioaccumulation potential of the substance. Monitoring data from top predators at various locations show highly elevated levels of PFOS and demonstrate substantial bioaccumulation and biomagnification (BMF) properties of PFOS. It is notable that the concentrations of PFOS found in livers of Arctic polar bears exceed the concentrations of all other known individual organohalogens (Martin et al., 2004a). Based on the concentration of PFOS in predators (e.g., the polar bear) in relation to the concentration in their principal food (e.g., seals), hypothetical BMF values can be calculated. Such data are reported in Table 4. It should be noted that there are uncertainties in these comparisons. Even if either liver or blood concentrations are compared in two species, species differences in specific protein binding in that particular compartment may affect the concentration in the organ without having affected the whole-body concentration of the substance.

Table 4. Measured concentrations of PFOS in biota from various locations. Calculated BMF is shown where applicable.

Species and Location	Concentrations of PFOS	Reference
• Polar Bear, Canadian Arctic	- Concentrations of PFOS in liver (1700 – > 4000 ng/g) exceeding all other individual organohalogens. - BMF > 160 based on concentrations in Arctic seals.	Martin et al., 2004a.
• Arctic fox, Canadian Arctic	- Very high concentrations of PFOS in liver (6.1 - 1400 ng/g)	Martin et al., 2004a.
• Mink, US	- Very high concentrations of PFOS in liver (40 - 4870 ng/g). - BMF = 22 based on data from fish in the same area. - nother mink study also show very high concentrations of PFOS in liver (1280 - 59 500 ng/g, mean 18 000 ng/g,) - BMF ~145 to ~4000 based on data from their prey such as crayfish (whole body), carp (muscle) and turtles (liver	Giesy and Kannan, 2001 Kannan et al., 2005

• Bald Eagle, US	- Very high concentrations of PFOS in plasma (1 – 2570 ng/g).	Giesy and Kannan 2001.
• Dolphin, US	- Very high concentrations of PFOS in liver (10 – 1520 ng/g).	3M, 2003a.
• Seal in the Bothnian Sea, Finland	- Very high concentrations of PFOS in liver (130 – 1100 ng/g). - BMF > 60 based on data from salmon in the same area.	Kannan et al., 2002

In a study by Kannan et al. (2005), the whole body BCF for round gobies (*Neogobius melanostomus)* was calculated to be approximately 2400, which is comparable with laboratory data. PFOS concentrations in fish (whole body of round gobies) compared to concentrations in liver of salmon results in BMFs of approximately 10-20. In bald eagles, the mean PFOS concentration in the livers, 400 ng/g ww, gives a BMF of four to five when compared to fish at higher trophic levels in the study. For mink, BMFs from 145 to 4000 can be calculated when based on the mean liver concentration, 18 000 ng/g ww, compared to their prey items such as crayfish (whole body), carp (muscles) and turtles (liver).

In general, data show that animals at higher trophic levels have higher concentrations of PFOS than animals at lower trophic levels, indicating that biomagnification is taking place. For instance, a trophic magnification factor (TMF) of 5,9 was calculated for PFOS based on a pelagic food web including: one invertebrate species, Mysis; two forage fish species, rainbow smelt and alewife; and a top predator fish species, lake trout. A diet-weighted bioaccumulation factor of approximately 3 was determined for the trout (Martin et al., (2004b).

Morikawa *et al.* (2005) showed a high bioaccumulation in turtles. Results from a study performed by Tomy *et al*. (2004a) indicated that PFOS biomagnified in an eastern Arctic marine food web (liver concentrations of PFOS were used for seabirds and marine mammals). Houde *et al.* (2006) showed PFOS biomagnification in the Atlantic Ocean bottlenose dolphin food web.

A study by Bossi *et al*. (2005a) further supports that biomagnification is taking place. In this study, a preliminary screening of PFOS and related compounds has been performed in liver samples of fish, birds and marine mammals from Greenland and the Faroe Islands. PFOS was the predominant fluorochemical in the biota analyzed, followed by perfluorooctane sulfonamide (PFOSA). The results from Greenland showed a biomagnification of PFOS along the marine food chain (shorthorn sculpin < ringed seal < polar bear).

It is assumed that the main and most relevant route of exposure to PFOS for birds is through the diet as biomagnification in bird tissues can occur this way. BMFs above one are reported for several bird species collected in the Gulf of Gdansk (Gulkowska *et. al.* 2005). Kannan *et al*. (2005) reported a BMF of 10 to 20 in bald eagles (relative to prey items). Tomy *et al*. (2004a) calculated a trophic level BMF for black-legged kittiwake:cod of 5.1 and a BMF for glaucous gull:cod of 9.0. Newsted *et al.* (2005) indicated that PFOS has relatively shorter half-lives in blood and liver tissue in birds compared to mammals. For example, the estimated elimination half-life for PFOS from serum is 13.6 days in male mallards whereas in male rats, it is greater than 90 days. A recent study has suggested that PFOS is excreted relatively rapidly from birds (Kannan *et al*., 2005). However, if birds are chronically exposed to PFOS in their diet, biomagnification can still occur. Environmental monitoring of birds in northern parts of their range in fact indicates accumulation of PFOS.

The fact that PFOS binds to proteins leads to the relevant question -- *at what concentrations of PFOS will the binding sites on these proteins be saturated?* Serum albumin is most likely the binding pool of PFOS (Jones *et al*., 2003) and several studies have been carried out with regard to bioconcentration in plasma. In Ankley *et al*. (2005), the bioconcentration in fish was studied at concentrations of PFOS in water up to 1 mg /L; the concentration of PFOS in water and plasma followed an almost linear relationship in the doses tested up to 0.3 mg/l without any signs of saturation (1 mg/l was not tested due to mortality at that dose). This is far above environmentally relevant concentrations.

In a study by 3M (2003a), the bioconcentration factor (BCF) in whole fish was determined to be approximately 2800 at a PFOS concentration of 86 µg/l, based on calculations of uptake and depuration of PFOS. Steady-state levels were attained after 49 days of exposure. Depuration occurred slowly and 50% clearance for whole fish tissues was estimated to be 152 days. Due to mortality, a BCF could not be calculated for the other concentration used, 870 µg/l. Thus, it is not likely that saturation of serum protein binding sites will limit the bioconcentration of PFOS in fish. In Cynomolgus monkeys, cumulative doses of PFOS (0,03, 0,15, or 0,75 mg/kg/day, orally, for 182 days) showed a linear increase in plasma at the low- and mid-dose groups while a nonlinear response was showed in the high-dose group (Covance Laboratories, Inc. 2002a). We are not aware of similar data in other mammals, but considering the high level of bioaccumulation observed in mammals, and that mammalian serum contains high concentrations of protein, binding sites are not likely to limit the bioaccumulation of PFOS in environmentally exposed mammals.

2.2.3 Long-range environmental transport

The potassium salt of PFOS has a measured vapour pressure of 3.31×10^{-4} Pa (OECD, 2002). Due to this vapour pressure and a low air-water partition coefficient ($< 2 \times 10^{-6}$), PFOS itself is not expected to volatilise significantly. It is therefore assumed to be transported in the atmosphere predominantly bound to particles, because of its surface-active properties, rather than in a gaseous state.

Some of the PFOS-related substances have a considerably higher vapour pressure than PFOS itself, and are as a result more likely to be volatile. The vapour pressures of precursors, such as N-EtFOSEA and N-MeFOSEA, may exceed 0.5 Pa (1000 times greater than that of PFOS) (Giesy and Kannan 2002). Other PFOS precursors considered volatile include N-EtFOSE alcohol, N-MeFOSE alcohol, N-MeFOSA and N-EtFOSA (3M, 2000). These precursors to PFOS could evaporate into the atmosphere and be more widely transported through air than is possible for PFOS itself. Once in the atmosphere, they can remain in gas phase, condense on particles present in the atmosphere and be carried or settle out with them, or be washed out with rain (3M, 2000). Martin *et al*. (2002) measured the air in Toronto and Long Point, Ontario, for some precursors of PFOS. They found an average N-MeFOSE alcohol concentration of 101 pg/m^3 in Toronto and 35 pg/m^3 at Long Point. The average concentrations of N-EtFOSE alcohol were 205 pg/m^3 in Toronto and 76 pg/m^3 in Long Point.

For precursors released to water, the vapour pressure may be significant enough to allow the substance to enter into the atmosphere. For N-EtFOSE alcohol, the tendency to leave the water phase is indicated by its relatively high Henry's law constant (1.9×10^3 Pa·m^3·mol^{-1}) (Hekster *et al*. 2002). It has been reported that when these PFOS precursors are present as residuals in products, they could evaporate into the atmosphere when the products containing them are sprayed and dried (3M, 2000).

PFOS has been detected in rainwater from an urban center in Canada with a concentration of 0.59 ng/L. Whether or not PFOS originates from precursors either being transported and subsequently wet deposited and degraded to PFOS, or atmospherically degraded and then wet deposited, is unclear. Measurements of potential precursors for PFOS were not performed in this study (Loewen *et al*, 2005)

The atmospheric half-life of PFOS is expected to be greater than two days. This statement, while not specifically tested, is based on the fact that PFOS has exhibited extreme resistance to degradation in all tests performed. However, an atmospheric half-life of 114 days has been calculated for PFOS using an AOP computer modeling program v1.91 (Environment Agency,, 2004). The indirect photolytic half-life of PFOS at 25°C has been estimated to be more than 3.7 years (OECD, 2002).

How perfluoralkyl acid substances have come to be globally disseminated in the environment has been the key question, since, for example, the vapour pressure and Henry's law constant of PFOS indicates it is too involatile and therefore unlikely to enter directly into the atmosphere (Stock *et al.* 2004). Therefore it has been hypothesized that PFOS must be globally distributed via more volatile, neutral airborne contaminants that undergo long-range transport and then degrade to yield the free acids.

In support, Stock *et al.* (2004) have recently reported that polyfluorinated sulfonamides are widely distributed throughout the North American troposphere. Mean concentrations ranged from 22-403 pg/m^3 with the dominant polyfluorinated contaminant dependent on the sampling location.

High mean concentrations of N-methyl perfluorooctane sulfonamidoethanol (NMeFOSE) of 359 pg/m^3, were identified in the air of Griffin, Georgia. The authors speculate that, as Griffin is located in the midst of the main carpet manufacturing and treatment zone of the US, it probably is entering the environment from carpet treatment products, many of which consist of fluorinated molecules linked to polymeric materials. For example, it is possible that free chemical may be left in the carpet fibres, with publicly available information on 3M produced products indicating the concentration of free polyfluorinated sulfonamides is typically 1-2% or less. Alternatively, it is postulated that chemically bound NMeFOSE may also be released from carpets due to chemical, physical, and/or biological degradation processes.

Support for this hypothesis comes from Shoeib *et al.* (2004), who measured both NMeFOSE and the related N-ethyl perfluorooctane sulfonamidoethanol (NEtFOSE) in both indoor and outdoor air. Mean indoor air concentrations for these were 2590 and 770 pg/m^3, respectively, and the ratios between indoor and outdoor air were 110 and 85, respectively. Again carpets were identified as a possible source of NMeFOSE, and high usage of paper in the building as a possible source of NEtFOSE. Paper products were also suggested by Stock *et al.* (2004) as a possible source for the high levels of NEtFOSE in the air of Reno, Nevada.

Recently Dinglasan-Panlilio and Maybury (2006) have demonstrated that residual fluorinated substances detected in materials, including 0.39% of a perfluoroalkyl sulfonamido alcohol present in a commercially available carpet protector product, are the likely sources for these volatile precursors. Further N-methyl perfluorobutane sulfonamidoethanol (NMeFBSE) has been demonstrated in the laboratory to degrade to perfluorobutane sulfonate (PFBS), albeit in low yield (D'eon et al, 2006).

PFOS has been measured in a wide range of biota in the Northern Hemisphere such as the Canadian Arctic, Sweden, the US and the Netherlands. In a study by Martin *et al.* (2004a), the levels of PFOS were measured in liver samples from biota in the Canadian Arctic and were found in the vast majority of the species examined. The presence of PFOS in Arctic biota, far from anthropogenic sources, demonstrates the potential of PFOS for long-range transport. The mechanisms of this transport are not known, but it could be due to the transport of volatile PFOS-related substances that eventually degrade to PFOS.

While precursors will undergo degradation once released to the environment, transformation rates may vary widely. Precursors that reach a remote region through the atmosphere or other media may be subject to both abiotic and biotic degradation routes to PFOS (Giesy and Kannan 2002a; Hekster *et al*. 2002). The mechanisms of this degradation are not well understood. When rats metabolize N-MeFOSE-based compounds, several metabolites have been confirmed in tissue samples, including PFOS and N-MeFOSE alcohol (3M Environmental Laboratory 2001a, 2001b). PFOS appears to be the final product of rat and probably other vertebrate metabolism of POSF-based substances.

A recent study performed with rainbow trout (*Onchorhynchus mykiss)* liver microsomes has demonstrated that *N*-ethyl perfluorooctanesulfonamide (N-EtPFOSA) is a precursor of PFOS in fish (Tomy et al., 2004b). These findings combined with the recent measurements of concentrations up to 92.8 ± 41.9 ng/g wet weight of *N*-EtPFOSA in aquatic organisms from Arctic regions (Tomy et al., 2004a) strengthen the hypothesis that perfluorinated sulfonamides are one of the volatile precursors of PFOS transported over long distances to the Arctic. However, the hypothesis that these volatile precursors reach the Arctic latitudes by atmospheric transport has not yet been confirmed by atmospheric measurements (Bossi et al., 2005b)

2.3 Exposure

2.3.1 Measured environmental levels

A screening study was assigned by the Swedish Environmental Protection Agency (Swedish EPA) and performed by ITM, Institute of Applied Environmental Research, on the levels of PFOS in the Swedish environment (Swedish EPA, 2004). The results showed highly elevated levels of PFOS in a wetland in the vicinity of a fire drill area with a declining gradient out in the adjacent bay (2.2 – 0.2µg/L). Elevated levels were also detected outside sewage treatment plants (STPs) and landfills. Effluents from STPs contained levels of PFOS up to 0.020 µg/L and leachate levels from landfills were between 0.038 – 0.152 µg/L.

The occurrence of PFOS and other perfluoroalkyl sulfonate substances in open ocean waters such as the Atlantic and the Pacific Ocean have been investigated. The detection of PFOS in oceanic waters suggests another potential long-range transport mechanism to remote locations such as the Arctic. The results showed that PFOS is present in central to western Pacific Ocean regions in concentrations ranging from 15 – 56 pg/L, comparable to the concentrations in the mid-Atlantic ocean. These values appear to be the background values for remote marine waters far from local sources (Taniyasu *et al*., 2004). PFOS was also detected in oceanic waters in several coastal seawaters from Asian countries (Japan, China, and Korea) at concentrations ranging from 1.1 - 57 700 pg.L^{-1} (Jin *et al.*, 2004; Yamashita *et al*., 2005). PFOS was also observed in the North Sea (estuary of the river Elbe, German Bight, southern and eastern North Sea) (Caliebe *et al*., 2004).

In a study in cities across China, PFOS was detected in all water samples (surface and sea water, groundwater, municipal and industrial effluents and tap water), showing that PFOS pollution existed generally in water compartments in China. Concentrations were generally at levels of approximately 1 ngéL (Jin *et al.*, 2004).

Studies in the US have identified the presence of PFOS in surface water and sediment downstream of a production facility, as well as in wastewater treatment plant effluent, sewage sludge and landfill leachate at a number of urban centres in the US (3M Multi City study, reviewed in OECD (2002) and 3M (2003a). Four of the cities (Decatur (AL), Mobile, Columbus (GA), Pensacola) were cities that have manufacturing or industrial use of fluorochemicals; two of the cities (Cleveland (TN), Port St. Lucie) were control cities that do not have significant fluorochemical activities. The ranges of PFOS levels in these cities are provided in Table 5.

Table 5. Environmental Levels of PFOS in Six US Urban Centres in the US (from OECD, 2002)

Medium	Range of PFOS levels (µg/L or µg/kg)
Municipal wastewater treatment plant effluent	0.041 - 5.29
Municipal wastewater treatment plant sludge	0.2 - 3.120 (dry weight)
Drinking water	ND - 0.063
Sediment	ND - 53.1 (dry weight)
Surface water	ND - 0.138
'Quiet' water	ND - 2.93

Note: ND: not detected

The control cities' samples generally inhabited the lower end of the above ranges, except for the municipal wastewater treatment plant effluent and sludge findings for one of the control cities (Cleveland), which were intermediate in their ranges, and the 'quiet' water samples at control city (Port St. Lucie), which were the highest. In Canada, suspended sediment samples were collected annually at Niagara-on-the-Lake in the Niagara River over a 22 year period (1980-2002). PFOS concentrations ranged from 5 to 1100 $pg.g^{-1}$ (Furdui *et al.,* 2005). Preliminary findings suggest that PFOS concentrations increased during the study period from < 400 $pg.g^{-1}$ in the early 1980s to > 1000 $pg.g^{-1}$ in 2002.

Samples of effluent from fifteen representative industry sectors have been analysed for PFOS (Hohenblum *et al*, 2003). The industry sectors were printing (1 site), electronics (3), leather, metals, paper (6), photographic and textiles (2). The PFOS levels ranged from 0-2.5 µg/L (2.5 µg/L for leather, 0.120 µg/l for metal, 0.140-1.2 µg/l at four paper sites, 1.2 µg/l for photographic, not found in textiles or electronics).

Groundwater from below an air force base in Michigan, US, has been sampled (Moody et al, 2003). Fire fighting foams containing PFOS had been used there in training exercises from the 1950s to 1993 when the base was decommissioned. The groundwater was found to contain PFOS, at levels from 4 - 110 µg/l.

Sixteen Great Lakes water samples (eight locations) were analysed for perfluorooctane surfactants. PFOS was present in all samples with a concentration range of 21-70 ng/L. Three PFOS precursors were also found in the water samples. N-EtFOSAA (4.2-11 ng/L) and PFOSA (0.6 -1.3 ng/L) were present in nearly all samples while PFOSulfinate was identified at six out of eight locations (2.2-17 ng/L) (Boulanger et al, 2004). PFOS was detected in surface water as a result of a spill of fire-fighting foam from the Toronto International Airport into nearby Etobicoke Creek. Concentrations

of PFOS ranging from <0.017 to 2210 $\mu g.L^{-1}$ were detected in creek water samples over a 153-day sampling period. PFOS was not detected at the upstream sample site (Moody *et al*. 2003).

PFOS and related fluorochemicals have been detected in animals in a number of studies in a variety of locations around the globe. Generally, the highest concentrations are found in top predators in food chains containing fish. The highest North American or circumpolar concentration of PFOS in mammal tissue reported in the published literature is 59 500 $\mu g.kg^{-1}$ ww in mink liver from USA (Kannan *et al*., 2005a).

Martin *et al.* (2004a) measured the levels of PFOS in liver samples from biota in the Canadian Arctic. PFOS was found in the vast majority of the samples and higher levels were found in animals at the top of the food chain. The highest levels were found in polar bear, with a mean level of 3100 ng/g from seven animals (maximum value > 4000 ng/g). The concentrations of PFOS in polar bear are 5-10 times higher than the concentration of all other perfluoroalkyl substances and were higher than any other previously reported concentrations of persistent organochlorine chemicals (e.g., PCBs, chlordane or hexachlorocyclohexane) in polar bear fat (Martin *et al.,* 2004a). PFOSA, a precursor to PFOS, was also found in most of the samples. The concentration of PFOSA was higher than that of PFOS in fish, but not in mammals. This could indicate that PFOSA has been metabolised to PFOS in mammals and the high concentrations may be the result of both direct exposure to PFOS and metabolism from PFOSA.

PFOS is found in birds worldwide. In North America, PFOS has been found in eagles in the Great Lakes, mallards in the Niagara River, loons in northern Quebec, gulls in the Arctic and in Canadian migratory species in the United States (e.g., common loon in North Carolina). In Canadian or Canada-US migratory species, concentrations have been measured in liver ranging from not detectable to 1780 ng/g for loon in northern Quebec and bald eagle in Michigan, in blood plasma ranging from <1- 2220 ng/g blood plasma in bald eagles, and in eggs and egg yolk ranging from 21-220 ng/g in double-crested cormorant in Manitoba. In several monitoring studies, piscivorous water birds were found to have some of the highest liver and serum PFOS concentrations compared to other species (Newsted *et al.,* 2005). In a study of birds in the Niagara River Region, piscivorous birds (common merganser, bufflehead) contained significantly greater PFOS concentrations than non-piscivorous birds (Sinclair *et al*., 2006). Preliminary data on temporal trends show an increase in bird PFOS concentrations, in two Canadian Arctic species (thick-billed murres and northern fulmars) from 1993 to 2004 (Butt *et al*., 2005). It is noted that concentrations of PFOS in plasma have been reported in eagle, gulls and cormorants around the Great Lakes and in the Norwegian Arctic ranging from <1 ng/g to 2220 ng/g .

Kannan and Giesy (2002b) have summarised results of analyses on archived tissue samples. The tissues analysed came from marine mammals, birds, fish, reptiles and amphibians from around the world, including the Arctic and Antarctic Oceans. Samples collected in the 1990s were used. Around 1700 samples were analysed, with concentrations in liver, egg yolk, muscle or blood plasma determined. The detection limit varied from 1 ng/g to 35 ng/g wet weight. A summary of the results is shown in Table 6.

Table 6. Maximum concentrations of PFOS in various species as well as frequency of detection. Based on Kannan and Giesy (2002a)

Species	Maximum concentration ng/g wwt	Frequency of detection
Marine mammals	1520	77%
Mink and otter	4900	100%
Birds	2570	60%
Fish	1000	38%

PFOS was detectable in most of the samples, including those from remote marine locations, at concentrations >1 ng/g. The authors compared the results from remote areas with those from more industrial locations and noted that PFOS is widely distributed in remote regions, including the Polar Regions, but that the levels found in more urban and industrial areas (e.g. the Baltic, Great Lakes) are several times higher. The tissues of fish-eating birds in Canada, Italy, Japan and Korea all contained detectable levels of PFOS, suggesting that they are exposed through the fish they consume. A summary of several studies is given in Table 7.

Table 7. Monitored levels of PFOS in animals (data from selected studies, based on OECD, 2002)

Description	Reference	Reported Highest Concentrations (Max, Mean)	Location
Global monitoring survey of marine mammals (Florida, California, Alaska, northern Baltic Sea, Mediterranean Sea, Arctic, Sable Island (Canada)	A	Bottlenose dolphin (liver, n = 26): Max: 1520 ng/g wet wt. Mean: 420 ng/g wet wt.	Florida
		Ringed seal (liver, n = 81): Max: 1100 ng/g wet wt. Mean: 240 ng/g wet wt.	Northern Baltic Sea
Survey of mammals, birds and fish in the Canadian Arctic	B	Polar bear (liver, n = 7): Max: > 4000 ng/g wet wt. Mean: 3100 ng/g wet wt.	Canadian Arctic

Description	Reference	Reported Highest Concentrations (Max, Mean)	Location
		Arctic fox (liver, n = 10): Max: 1400 ng/g wet wt. Mean: 250 ng/g wet wt.	
Survey of fish (US, Europe, North Pacific Ocean, Antarctic)	C	Fish (muscle, n = 172): Max: 923 ng/g wet wt. Mean. 40 ng/g wet wt.	Belgian estuary
		Carp (muscle, n = 10): Max: 296 ng/g wet wt. Mean: 120 ng/g wet wt.	US Great Lakes
Survey of fish-eating birds (US, Baltic Sea, Mediterranean Sea, Japanese coast, Korean coast)	D	Bald eagle (plasma, n = 42): Max: 2570 ng/mL Mean: 520 ng/mL	Midwest US
Survey of mink and river otter in the US	E	Mink (liver, n = 77): Max: 4870 ng/g wet wt. Mean: 1220 ng/g wet wt.	US
		River otter (liver, n = 5): Max: 994 ng/g wet wt. Mean: 330 ng/g wet wt.	US
Survey of oysters in the US (Chesapeake Bay & Gulf of Mexico)	F	Oyster (Whole body, n =77) Max: 100 ng/g wet wt. Mean: 60 ng/g wet wt.	US
Fish samples upstream and downstream of 3M facility in Decatur, Alabama, US	G	Fish (whole body): Mean (upstream): 59.1 µg/kg wet wt. Mean (downstream): 1,332 µg/kg wet wt.	Decatur, US
Swedish urban and background	H	Perch: 3 - 8 ng/g (urban sites in the vicinity of	Sweden (Lake Mälaren)

Description	Reference	Reported Highest Concentrations (Max, Mean)	Location
fish samples		municipal STPs); 20-44 ng/g in Lake Mälaren and near Stockholm	

Sources: A: 3M (2003a), B: Martin *et al.* (2004a); C: Giesy and Kannan (2001c) in 3M (2003a); D: Giesy and Kannan (2001b) in 3M (2003); E: Giesy and Kannan (2001d) in 3M (2003a); F: Giesy and Kannan (2001e) in 3M (2003); G: Giesy and Newsted (2001) in OECD (2002); H: Holmström *et al.* (2003).

Concentrations of PFOS in guillemot (*Uria aalge*) eggs from Stora Karlsö in the Baltic Sea have been measured retrospectively from 1968 to 2003 (Holmström et al, 2005). The results shown in Figure 2 display a trend of increasing concentrations since 1968 (17 – 623 ng/g).

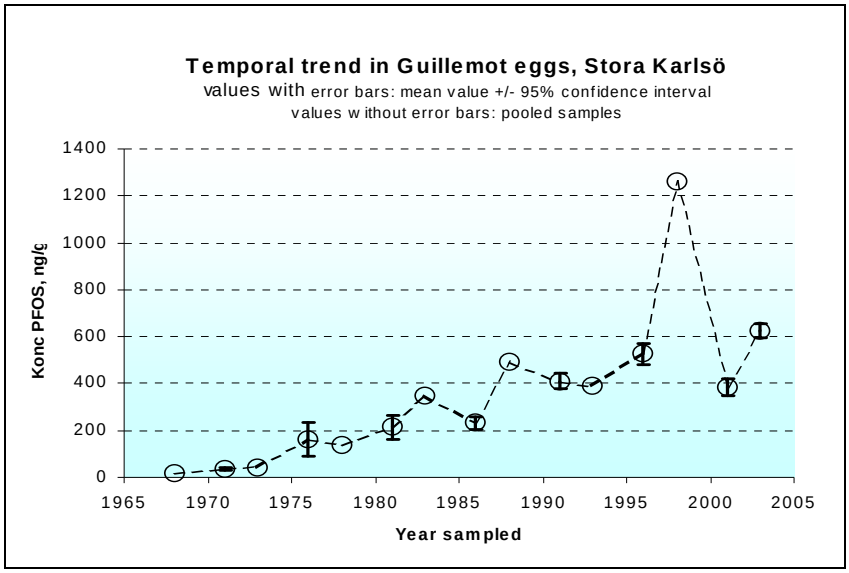


Figure 2. Measured concentrations of PFOS in Guillemot (*Uria aalge*) eggs sampled at Stora Karlsö in the Baltic Sea between the years 1968 – 2003. The graph is taken from the report "Screening av perfluorerade ämnen" by the Swedish EPA, Environmental Assessment Department (2004).

2.3.2 Bioavailability

Studies on fish have shown that PFOS has bioconcentration properties. In studies on bluegill sunfish (*Lepomis macrochirus*) and rainbow trout (*Oncorhynchus mykiss*), bioconcentration factors (BCFs) have been estimated to be 2796 (whole fish) as well as 2900 (liver) and 3100 (plasma), respectively. The major route of uptake is believed to be through the gills (Martin *et al.*, 2003).

Since PFOS is released from sewage treatment plants to the environment i.e. through water, one major route for PFOS into local food chains could be through fish. PFOS has shown a high oral uptake (95%) within 24 hours in the gastro-intestinal (GI) tract in studies on rats (OECD, 2002). Taken together, this could constitute the basis of the highly elevated levels that have been observed in top predators in food chains containing fish.

This could also be corroborated by two separate human monitoring studies on the Swedish population where the levels of PFOS in whole blood was higher (27.2 ng/g, 3.0 – 67, n = 10) in females with a high consumption of fish (Berglund, 2004) compared to samples from females in the general population (17.8 (ng/g, 4.6 – 33, n = 26) (Kärrman *et al.,* 2004).

In humans, the highest concentrations of PFOS have been detected in workers at 3M's manufacturing plant for perfluorochemicals in Decatur, US, where the levels in serum in the last year of measurement (2000) ranged between 0.06 – 10.06 ug/g (n = 263, OECD, 2002).

In a study of the general population, blood samples from families including three generations living in 12 European countries were tested for a large number of chemicals including PFOS and PFOSA. PFOS was present in 37 of 38 samples with concentrations from 0.36 to 35.3 ng/g blood, while PFOSA was present in 36 of 38 samples with concentrations from 0.15 to 2.04 ng/g blood (WWF, 2005).

Pooled serum samples from 3802 Australian residents, collected 2002-2003 and divided in relation to age, gender and region, were analysed for perfluoroalkylsulfonates, perfluoroalkylcarboxylates and PFOSA (Kärrman et al., 2006). PFOS and PFOSA were quantified in all pooled serum samples with a total range of 12.7-29.5 ng/ml (mean 17.2 ng/ml) and 0.36-2.4 ng/ml (mean 0.81 ng/ml), respectively. For PFOS, a significant correlation between age and concentration was shown. No substantial difference was found in levels of perfluorinated compounds between the urban and rural regions. According to gender some differences were shown for some of the age groups.

2.4 Hazard assessment for endpoints of concern

2.4.1 Mammalian Toxicity

Evidence of the mammalian toxicity of PFOS is available from acute, sub-chronic and chronic exposures to rats, sub-chronic exposures to monkeys, and a two-generation study on rats. Results are available from reproductive and teratogenicity studies on rats and rabbits. Details of these studies are not included here, they can be found in the assessment made by OECD (2002). The most relevant data for this risk profile are:

- A 90-day study on rhesus monkeys exposed to PFOS potassium salt via gavage at the doses 0, 0.5, 1.5 and 4.5 mg/kg bw/day. At 4.5 mg/kg bw/day all monkeys (4) died or were sacrificed in moribound condition. No deaths were observed at 0.5 or 1.5 mg/kg bw/day, but there were signs of gastrointestinal toxicity. A NOAEL could not be established since the lowest dose was a LOAEL (Goldenthal et al., 1978a).
- A 90-day oral repeated dose toxicity study in rats that were fed diets containing 0, 30, 100, 300, 1000 and 3000 mg PFOS potassium salt per kg diet. All rats died when fed diets containing 300 mg/kg PFOS and above (equivalent to 18 mg/kg bw/day and above). At 100 mg/kg (6 mg/kg bw/day), 50% (5/10) of the animals died. All rats receiving diets containing 30 mg/kg PFOS (2.0 mg/kg/day) survived until the end of the study, but small changes in body and organ weights were reported. Since the lowest dose tested was a LOAEL, a NOAEL could not be established (Goldenthal et al., 1978b).
- A two-generation reproductive toxicity study on rats that were fed PFOS potassium salt via gavage at the doses 0.1, 0.4, 1.6, and 3.2 mg/kg bw/day. At the doses 1.6 and 3.2 mg/kg bw/day a significant reduction in the viability of the F1 generation was observed. In the 1.6 mg/kg bw/day group, 34% (86/254) of the F1 pups died within four days after birth. In the 3.2 mg/kg bw/day group, 45% (71/156), of the F1 pups died within one day after delivery. None of these pups survived beyond day 4. Maternal toxicity at 1.6 and 3.2 mg/kg bw/day was manifested as reduced

food consumption, body weight gain, and terminal bodyweight. Localised alopecia was also observed at 3.2 mg/kg bw/day. The LOAEL in this study was 0.4 mg/kg bw/day based on significant reductions in pup weight gain in the F1 generation animals. The NOAEL was 0.1 mg/kg bw/day (Christian et al., 1999). A new study by Luebker *et al.* (2005) supports these results.

- Cynomolgus monkeys administered PFOS for 26 weeks were observed to have thymic atrophy (females), and reduced high density lipoprotein, cholesterol, triiodothyronine, total bilirubin levels (males) (Covance Laboratories, Inc. 2002a). The LOEL dose was 0.03 $mg.kg^{-1}$ bw/day at which average mean female and male concentrations in sera and liver were 19.8 $\mu g.mL^{-1}$ and 14.5 $\mu g.g^{-1}$, respectively.
- A 2-year dietary rat study in which histopathological effects in the liver were seen in males and females at intakes as low as 0.06–0.23 mg PFOS/kg bw per day and 0.07–0.21 mg PFOS/kg bw per day, respectively (Covance Laboratories, Inc. 2002b). Average values were determined for males and females to establish LOELs of 40.8 ug/g in liver and 13.9 mg/L in serum.

A study by Grasty *et al.* (2003) concluded that exposure of pregnant rats to PFOS late in gestation, at 25 mg/kg b.w. PFOS by oral gavage on gestation day (GD) 17-20 or 50 mg/kg PFOS on GD 19-20, is sufficient to induce 100% pup mortality and that the causative factor may be inhibition of lung maturation. However, in a subsequent study by Grasty *et al.* (2005), the mechanism behind pup mortality could not be established.

2.4.2 Ecotoxicity

Environmental toxicity data for PFOS is predominantly found for aquatic organisms such as fish, invertebrates and algae, and for birds.

PFOS has shown moderate acute toxicity to fish. The lowest observed LC_{50} (96h) was estimated to be 4.7 mg/l in a study where fathead minnow (*Pimephales promelas*) were exposed to the lithium salt of PFOS. The lowest NOEC, 0.3 mg/l, has been observed in *Pimephales promelas* at prolonged exposure (42d) and was based on mortality (OECD, 2002). The lowest LC_{50} (96h) for aquatic invertebrates has been observed in the mysid shrimp (*Mysidopsis bahia)* and was estimated to be 3.6 mg/l. The lowest NOEC value has been observed in *Mysidopsis bahia* at 0.25 mg/l (OECD, 2002).

A study by Macdonald *et al*. (2004) reported a 10-day NOEC of 0.0491 mg/L for the growth and survival of the aquatic midge (Chironomous *tentans*). The authors concluded that PFOS is 2-3 orders of magnitude more toxic to chironomids than to other aquatic organisms possibly through some kind of interaction with haemoglobin, which is present at all levels of dissolved oxygen (DO) in chironomids as opposed to daphnids, where haemoglobin is produced only in response to declining DO levels.

The most sensitive algae appear to be the green algae *Pseudokirchnerilla subcapitata* with a IC_{50} (96h, cell density) of 48.2 mg/L. The lowest NOEC value for algae was determined in the same study for *Pseudokirchnerilla subcapitata*, 5.3 mg/L (Boudreau *et al.,* 2003).

Mallard and bobwhite quail were exposed to PFOS in feed for up to 21 weeks and a variety of endpoints examined including changes in adult body and organ weights, feed consumption rate, fertility, hatchability, and offspring survival. At a dose of 10 mg/kg diet PFOS, effects in male mallards (*Anas platyrhyncos)* included reduced testes size and decreased spermatogenesis (3M, 2003b). At this dose, the concentrations of PFOS in serum and liver were 87.3 ug/mL and 60.9 ug/g, respectively (3M, 2004). For quail (*Colinus virginianus*), at 10 mg/kg in diet, minor effects were observed in adults, including an increase in liver weight (females), an increase in the incidence of small testes size (males), and reduction in survivability in quail chicks as a percentage

of eggs set. Concentrations in serum and liver of adult quail females was 84 $\mu g.mL^{-1}$ serum (week 5, pre-reproductive phase), and 8.7 $\mu g.mL^{-1}$ serum (week 21) and 4.9 $\mu g.kg^{-1}$ wet weight liver; in adult quail males, concentrations were 141 $\mu g.mL^{-1}$ serum and 88.5 $\mu g.g^{-1}$ wet weight liver (3M, 2003c).

3 SYNTHESIS OF THE INFORMATION

Perfluorooctane sulfonate (PFOS) is a fully fluorinated anion, which is commonly used as a salt in some applications or incorporated into larger polymers. Due to its surface-active properties, it has historically been used in a wide variety of applications, typically including fire fighting foams and surface resistance/repellency to oil, water, grease or soil. PFOS can be formed by degradation from a large group of related substances, referred to as PFOS-related substances (see definition on page 4).

Due to their intrinsic properties, PFOS and its related substances have been used in a wide variety of applications. While historically, PFOS and PFOS-related substances have been used in eight different sectors as shown in Section 2.1.2. above, the present use in industrialized countries seems to be limited to five sectors, see 2.1.2. It is not known whether this also reflects the global use.

PFOS and PFOS-related substances can be released to the environment at their manufacture, during their use in industrial and consumer applications and from disposal of the chemicals or of products or articles containing them after their use.

The rate and the extent of the formation of PFOS from its related chemicals are largely unknown. Lack of data makes it very difficult to estimate the net contribution of the transformation of each of the PFOS-related substances to the environmental loadings of PFOS. However, based on its extreme stability, it is expected that PFOS is likely to be the final degradation product of all PFOS-related substances.

PFOS is extremely persistent. It has not shown any degradation in tests of hydrolysis, photolysis or biodegradation in any environmental condition tested. The only known condition whereby PFOS is degraded is through high temperature incineration.

With regard to bioaccumulation potential, PFOS meets the Annex D criteria given the highly elevated concentrations that have been found in top predators such as the polar bear, seal, bald eagle and mink. Based on the concentrations found in their prey, high BMFs have been estimated for these predators. BCF values in fish, although (rather) high do not in themselves meet the specific numeric criteria. However, due to the properties of PFOS, which binds preferentially to proteins in non-lipid tissues, application of numeric criteria for BCF or BAF, which are derived based on consideration of lipid-partitioning substances, may be inappropriate for PFOS. Most notable and alarming are the high concentrations of PFOS that have been found in Arctic animals, far from anthropogenic sources. PFOS has been detected in higher trophic level biota and predators such as fish, piscivorous birds, mink, and Arctic biota. Also, predator species, such as eagles, have been shown to accumulate higher PFOS concentrations than birds from lower trophic levels. Even with reductions in manufacturing of PFOS by some manufacturers, wildlife, such as birds, can continue to be exposed to persistent and bioaccumulative substances such as PFOS simply by virtue of its persistence and long-term accumulation.

According to available data, PFOS meets the criteria for the potential for long-range transport. This is evident through monitoring data showing highly elevated levels of PFOS in various parts of the northern hemisphere. It is especially evident in the Arctic biota, far from anthropogenic sources. PFOS also fulfils the specific criteria for atmospheric half-life.

PFOS fulfils the criteria for adverse effects. It has demonstrated toxicity towards mammals in sub-chronic repeated dose studies at low concentrations, as well as rat reproductive toxicity with mortality of pups occurring shortly after birth. PFOS is toxic to aquatic organisms with mysid shrimp and *Chironomus tentans* being the most sensitive organisms.

Table 8. POP characteristics of PFOS (studies performed with the potassium salt of PFOS, unless otherwise noted).

Criterion	Meets the criterion (Yes/No)	Remark
Persistence	Yes	Extremely persistent. No degradation recorded in chemical or biological tests
Bioaccumulation	Yes	Found in highly elevated concentrations in top predators. Calculated hypothetical BMFs = 22 - 160. BCF in fish = 2796 - 3100.
Potential for Long-Range Environmental Transport	Yes	Atmospheric half life > 2 days (estimated value based on photolytic half life > 3.7 years)
Toxicity	Yes	Sub-chronic exposure: Mortality in monkeys at 4.5 mg/kg bw/day. Reproductive toxicity: mortality in rat pups at 1.6 mg/kg bw/day. Acute toxicity to Mysid shrimp (*Mysidopsis bahia)*: LC_{50} (96h) = 3.6 mg/L Acute toxicity to fish, Fathead minnow (*Pimephales promelas*): LC_{50} = 4.7 mg/L[1]

[1]The study compound was the lithium salt of PFOS

A risk quotient analysis, where known or potential exposures are integrated with known or potential adverse environmental effects, have been performed on PFOS for the wildlife in Canada (Environment Canada, 2006). The results indicate that the higher trophic level mammals may be at risk at current environmental concentrations of PFOS.

In the risk quotient analyses for polar bear, the highest concentration was found in South Hudson Bay with a maximum concentration of 3.77 $\mu g.g^{-1}$ ww liver (range 2.00-3.77 $\mu g.g^{-1}$, mean 2.73 $\mu g.g^{-1}$ ww liver, Smithwick *et al*. 2005). In comparing this value of 3.77 $\mu g.g^{-1}$ ww liver of PFOS in polar bear with a critical toxicity value of 40.8 $\mu g.g^{-1}$ ww liver for histopathological effects in liver

of rats (a 2-year study, Covance Laboratories, Inc. 2002), the difference is only about a factor 10. Using an application factor of 100[2], as was used in the Canadian Ecological Screening Assessment Report, a risk quotient of 9.2 was calculated, where values above one indicate risk. Risk quotients were also calculated on toxicological endpoints from other studies in rats and monkeys but with the same maximum exposure concentration from the south Hudson Bay polar bear, showing risk quotients from 2.1 to 19.

Concentrations in Canadian Arctic polar bear are among the highest in polar bears worldwide but the exposure concentrations are not considered an anomaly given similar concentrations in polar bears in other North America and European Arctic locations and high concentrations in other wildlife globally as shown above.

Risk quotients were also calculated for a number of bird species that are native to Canada, including many piscivorous birds and migratory species. The range of risk quotients is either above or approaching one that indicates potential for harm at concentrations observed in native species, including migratory species (Environment Canada, 2006).

4 CONCLUDING STATEMENT

PFOS is a synthetic substance of anthropogenic origin with no known natural occurrence. It can be concluded therefore that the presence of PFOS and its precursors in the environment are the result of anthropogenic activities and that PFOS found in remote areas far from possible sources has been brought there through long-range environmental transport. While PFOS related substances may be degraded to PFOS, PFOS itself is extremely persistent in all media and can bioaccumulate and biomagnify in mammals and piscivorous birds.

The voluntary phase out of PFOS production by the major producer in the USA has led to a reduction in the current use of PFOS-related substances. However, it can be assumed that it is still produced in some countries and it continues to be used in many countries. Given the inherent properties of PFOS,[3] together with demonstrated or potential environmental concentrations that may exceed the effect levels for certain higher trophic level biota such as piscivorous birds and mammals; and given the widespread occurrence of PFOS in biota, including in remote areas; and given that PFOS precursors may contribute to the overall presence of PFOS in the environment, it is concluded that PFOS is likely, as a result of its long-range environmental transport, to lead to significant adverse human health and environmental effects, such that global action is warranted.

[2] An application factor of 100 applied for extrapolation from laboratory to field conditions and for intraspecies and interspecies variations in sensitivity, and extrapolation from the observed effects level to a no-effect level.

[3] A decision on the inclusion of PFOS precursors has been postponed until the Committee has evaluated the information requested under Annex F.

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PFAS Destruction Testing at Veolia's Port Arthur Incinerator

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Executive Summary

PFAS incineration tests were performed in two campaigns in July and October 2024 at the Veolia Port Arthur, Texas facility to demonstrate that the facility can effectively destroy PFAS-contaminated waste with minimal release of PFAS into the environment.

The campaigns were performed at the Veolia RCRA incinerator in Port Arthur, Texas in collaboration with the stack testing company, AECOM. Eurofins laboratory was used for analysis of all solid and liquid samples and for stack gas samples obtained using EPA methods OTM-45 and OTM-50.

Four different streams were incinerated: A3F, PFAS-contaminated soil, spent activated carbon and CF4. The results of the testing were:

- Stack testing during PFAS incineration showed that PFAS were either non detect or emitted at very low levels (< 2 10^-5 lb/hr) in normal conditions. For PFAS with high feed concentration, thermal treatment can remove or destroy up to 99.9999% of PFAS.
- CF4 incineration indicated a DRE of less than 50%, consistent with predictions that temperatures above 2,550 °F are necessary to destroy this compound.
- Products of incomplete combustion (PIC) listed in OTM-50 were either non-detect at stack, or close to Method Detection Limit (MDL). Tetrafluoromethane (three detections) was the only PIC measured at or above the Level of Quantification (LoQ) in these samples.
- For a majority of solid residues, no PFAS were detected. Only 7 solid samples out of 69 showed PFAS levels close to the MDL
- Liquid residues contained PFAS at ppt level due to background PFAS present in the process water, unrelated to PFAS incineration. This level remains very low and did not increase during PFAS incineration.
- HF measurement at the stack was within regulatory limits.

The results demonstrated high destruction and removal efficiency for PFAS compounds during the incineration process, with values up to 99.9999%. These findings suggest that hazardous waste incineration, when properly operated under high-temperature conditions, can effectively break down PFAS compounds. Notably, very few PICs were detected in any of the output streams, suggesting a complete mineralization of the PFAS compounds rather than partial decomposition. The consistent achievement of high DRE values across multiple test runs, coupled with the absence of PICs, provides strong confidence in the reliability of the thermal treatment process for PFAS destruction.

Analysis of solid residues and liquid residues showed relatively low to no detectable concentrations of PFAS compounds, further supporting the effectiveness of the thermal treatment process. The minimal presence of PFAS compounds in these residual streams indicates successful destruction rather than mere phase transfer of the contaminants.

These results present compelling evidence that high-temperature incineration is an effective solution for PFAS disposal, addressing a critical data gap in PFAS destruction methods as mentioned in the EPA 2024 guidance on the Destruction and Disposal of PFAS and Materials Containing PFAS. The comprehensive study demonstrated efficient PFAS destruction under normal operating conditions. These results conclusively demonstrate that high-temperature incineration at Port Arthur is a viable and environmentally responsible method for PFAS disposal.

The study demonstrates Veolia's commitment to innovation and leadership in addressing emerging environmental challenges. With minimal environmental releases, effective handling of various waste streams, and compliance with regulatory standards, this achievement reinforces Veolia's position as an industry leader committed to providing cutting-edge environmental solutions while ensuring the highest standards of environmental protection and customer service.

Note : There are more than 10,000 different PFAS in nature. Due to analytical limitations, the PFAS campaign conducted at Port Arthur focused on a limited list of 79 targeted PFAS listed in OTM-45, OTM-50 and EPA 1633.

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3. List of Acronyms and Abbreviations

AFFF - Aqueous Film Forming Foam
APCS - Air Pollution Control System
DE - Destruction Efficiency
DRE - Destruction and Removal Efficiency
DSCFM - Dry Standard Cubic Feet per Minute
GC/MS - Gas Chromatography/Mass Spectrometry
GPM - Gallons Per Minute
HAP - Hazardous Air Pollutant
LC/MS - Liquid Chromatography/Mass Spectrometry
LoQ - Limit of Quantification
MDL - Method Detection Limit
NTS - Non-Target Screening
PFCA - Perfluoroalkyl Carboxylic Acid
PFAS - Per- and Polyfluoroalkyl Substances
PFSA - Perfluoroalkyl Sulfonic Acid
PIC - Products of Incomplete Combustion
POHC - Principal Organic Hazardous Constituent
POP - Persistent Organic Pollutant defined by the Stockholm Convention
PPB - Parts Per Billion
PPT - Parts Per Trillion
QA/QC - Quality Assurance/Quality Control
SCC - Secondary Combustion Chamber
TCEQ - Texas Commission on Environmental Quality
TSCA - Toxic Substances Control Act
VFC - Volatile Fluorinated Compounds

4. Introduction

4.1 Background

In July and October 2024, a groundbreaking full-scale test of PFAS thermal treatment was conducted at Veolia RCRA-permitted hazardous waste combustor facility in Port Arthur, Texas. As one of North America's leading hazardous waste management service providers, Veolia initiated this testing to confront the growing issue of per- and poly-fluorinated alkyl substances (PFAS) in environmental media. The volume of PFAS-containing waste streams continues to surge across industries, presenting an urgent need for effective disposal solutions. As regulatory scrutiny intensifies and remediation efforts expand, stakeholders are increasingly seeking viable treatment options for these persistent compounds.

High-temperature incineration has emerged as a potentially promising disposal pathway, particularly given its demonstrated success in destroying other challenging chemical compounds. While the exceptional stability of PFAS molecules poses unique challenges due to the strength of the multiple carbon-fluorine (C-F) chemical bonds in these compounds, properly engineered thermal treatment systems could offer a practical approach to permanently eliminating these substances from the waste stream. This is especially relevant for concentrated PFAS wastes, such as aqueous film-forming foam (AFFF) stockpiles, where other treatment technologies may prove less effective or economically feasible.

In early 2024, the EPA released its *Guidance on the Destruction and Disposal of PFAS and Materials Containing PFAS,* and in that report acknowledged a lack of data on PFAS fate via incineration. The agency highlighted the need to evaluate destruction efficiency of targeted PFAS, along with the potential creation of products of incomplete combustion (PIC). In January 2024, the EPA released OTM-50, a new method to sample PFAS in air emissions and detect volatile short-chain PFAS that are thermally stable and could potentially form as PICs.

To bridge the knowledge gap regarding PFAS incineration, Veolia developed and implemented a comprehensive full-scale testing program to evaluate the efficiency of PFAS destruction via high temperature incineration. The test program was conducted in collaboration with AECOM to conduct PFAS stack testing, and Eurofins to perform laboratory analysis of the collected samples. Veolia also involved the EPA in the planning process and shared the protocol of its testing program as well as the results of the study.

In a significant demonstration of its technological leadership and innovative capabilities, Veolia implemented the cutting-edge OTM-50 sampling methodology for PFAS stack emissions testing at full-scale. This achievement marks a notable advancement in PFAS analysis, as OTM-50 represents the most recent and sophisticated approach to quantifying short-chain PFAS, or volatile fluorinated compounds (VFC) in air emissions. By mastering this newly developed sampling technique, Veolia has positioned itself at the forefront of environmental testing technology, showcasing its ability to adapt and implement emerging methodologies.

The test was performed at Veolia RCRA incinerator Port Arthur, TX, one of the three RCRA incineration facilities that Veolia owns and operates in the U.S.A. The two others are Gum Spring, AR, and Sauget, IL. Worldwide, Veolia owns or operates 51 hazardous waste incineration plants in 18 different countries. Prior to the US campaign, Veolia has already performed PFAS testing campaigns in other parts of the world: the company's European operations, in particular, has showcased state-of-the-art technology for PFAS treatment since 2022.

Note : There are more than 10,000 different PFAS in nature. Due to analytical limitations, the PFAS campaign conducted at Port Arthur focused on a limited list of 79 targeted PFAS listed in OTM-45,

OTM-50 and EPA 1633.

Therefore, all the findings and conclusions below are limited to this list of PFAS. When it is said that no PFAS was detected in a waste or at stack, or that a sample is PFAS-free, this means that none of the targeted PFAS were detected. It is possible that other PFAS were present in the waste/sample but could not be detected with today's analytical technique.

4.2 Test objectives

The data quality objective of the Veolia test program was to demonstrate the ability of the Port Arthur RCRA incinerator to destroy PFAS in the waste feed without releasing PICs in air emissions, or PFAS in the solid and liquid residues during normal process operating conditions. To demonstrate this, Veolia incinerated four different waste streams contaminated with PFAS in various concentrations.

The primary objective was to determine if PICs in the form of Volatile Fluorinated Compounds (VFC) are formed during PFAS incineration via OTM-50. OTM-50 is a new sampling method for PFAS released by the EPA in 2024 to detect PICs, that is to say short-chain PFAS or VFCs. PICs may form from PFAS incineration when their destruction is incomplete. OTM-50 sampling was performed at the stack during PFAS incineration. The absence of PICs measured at the stack indicates, within the limit of current analytical detection limits, that the degradation of PFAS is complete. This data supports complete mineralization of PFAS to HF.

The second objective was to establish the destruction and removal efficiency (DRE) for a list of targeted PFAS. DRE is defined by 40 Code of Federal Regulations [CFR] §264.343 as follows:

$$DRE = (1 - Waste\ out/Waste\ in) * 100$$

Where:

- Waste in = mass feed rate of PFAS in the waste stream feeding the incinerator
- Waste out = mass emission rate of the same PFAS present in exhaust emissions prior to release to the atmosphere

DRE is only calculated for medium to long-chain PFAS (C>4) listed in OTM-45 and EPA 1633, i.e. PFAS that can be measured both in the PFAS waste and at the stack.

A related objective was to study the total DRE from OTM-45 sampling. Total DRE is defined as follows:

$$Total\ DRE_{OTM\ 45} = (1 - Sum\ of\ PFAS\ out_{OTM\ 45}/Sum\ of\ PFAS\ in_{1633}) * 100$$

Where:

- $Sum\ of\ PFAS\ out_{OTM\ 45}$ is the sum of PFAS detected at the stack via OTM-45
- $Sum\ of\ PFAS\ in$ is the sum of PFAS detected in the waste via EPA Method 1633 or EPA Method 537

In case of PFAS undetected at the stack or in the waste, the MDL is used in the calculations. In case of PFAS measured below the LoQ, the LoQ is used in the calculations.

The last objective was to calculate the Total Destruction Efficiency based on OTM-50, OTM-45 and PFAS detected in liquid and solid residues.

$$DE = (1 - (PFAS\ in\ liq + PFAS\ in\ solids + PFAS\ in\ air)/PFAS\ in) * 100$$

Where:

- PFAS in = total mass feed rate of PFAS fed into the incinerator
- PFAS in air = total mass emission rate of PFAS present in exhaust emissions and detected via in OTM 45 or OTM 50.
- PFAS in liquids = total mass emission rate PFAS present in liquid residues
- PFAS in solids = mass emission rate PFAS present in solid residues

4.3. Incinerator and Process Description

Veolia Environmental Services Technical Solutions, L.L.C., (Veolia) operates a hazardous waste incinerator at their Port Arthur, Texas location. This incinerator processes hazardous materials in accordance with the Resource Conservation and Recovery Act (RCRA) Permit HW-50212, Air Permit 42450 from the Texas Commission on Environmental Quality (TCEQ), and a Toxic Substances Control Act (TSCA) permit from the United States Environmental Protection Agency (US EPA).

The following sections provide a description of the Veolia Port Arthur incineration system. Two process flow diagrams (PFD) are included in Figures 1 and 2. The PFD are a representation of the combustion and air pollution control systems and include all major process flow streams.

Description of Combustion System

The incinerator at the Port Arthur facility consists of waste receiving and waste feeding equipment, rotary kiln, an ash/slag removal system, a secondary combustion chamber (SCC), a quench tower, two acid gas absorbers, four cooling towers, a four-stage ionizing wet scrubber, a wet electrostatic precipitator, an induced draft fan, an exhaust stack, and a scrubber water treatment/recycle system.

Description of Rotary Kiln

The rotary kiln measures 60 feet in length and the slope of the kiln from inlet to ash discharge is approximately one degree. The nominal rotation speed is one rpm with an average solid residence time of approximately one hour. The shell of the kiln is 16 feet in outside diameter and is lined with refractory material which protects the carbon steel shell from the high temperatures of incineration. The inside diameter of the kiln with the insulation in place is approximately 14 feet. The kiln is normally operated under negative pressure maintained between zero- and one-inch water column vacuum to prevent fugitive emissions from the kiln ends. The kiln temperature is measured with three thermocouples inserted through the roof of the ash dropout chamber, approximately 4 feet past the end of the kiln.

Secondary Combustion Chamber Design

Hot flue gases from the transition section enter the cylindrical secondary combustion chamber (SCC) approximately 14 feet above the base of the tower. The SCC is 78 feet tall and equipped with eight burners evenly arranged around the perimeter of the SCC tower. The combustion gases are raised to the current, minimum temperature of 2,012 °F. The burners, which fire tangentially, are rated at 15 million Btu/hr each. The burners are dual fuel burners and have the capability to fire waste liquid and/or fossil fuel. The burners are equipped with external steam and air atomizers and natural gas fired pilot lights. A compressed gas cylinder feed system is designed to feed waste gases to the secondary combustion chamber. The SCC temperature is measured with three thermocouples inserted through the top of the hot cross-over duct between the SCC and quench, approximately 7.5 feet downstream of the SCC.

Quench Tower

Cooling of the combustion gases occurs in the quench tower. Three rows of water spray nozzles are arranged around the circumference of the tower and spray water directly into the hot combustion gas.

As the gas flows downward, it is cooled to an adiabatic saturation temperature of about 185°F. Water is supplied from the scrubber water recycle loops and from clean process water. A caustic solution is added to the quench water for initial neutralization of acid gases. The ash particulate collected by the water sprays falls to the bottom of the quench tower.

Air Pollution Control System

Removal of particulate matter and acid gases occur in the Air Pollution Control System (APCS). The APCS consists of the following equipment:

- Two parallel Acid Gas Absorbers;
- An Ionizing Wet Scrubber; and
- A Wet Electrostatic Precipitator.

Process Parameter Continuous Monitoring Systems

The incinerator uses process instruments, which include thermocouples, flowmeters, pH meters, and pressure transmitters to document compliance with applicable operating parameters. The process instruments continuously monitor and record operating parameters of the incinerator.

Veolia operates two identical EcoChem MC3 multi-component gas analyzers (one for compliance and one for redundancy). Each individual multi-component instrument analyzes the stack gas for O2, CO, CO2, SO2 and HCl.

Incineration Residues

The liquid residues from incineration are injected into an on-site Class IV underground injection well after treatment. The three solid residues (ashes from the kiln, slag from the quench and SCC, and filter cake from the water treatment) are sent to an off-site Subtitle C landfill.

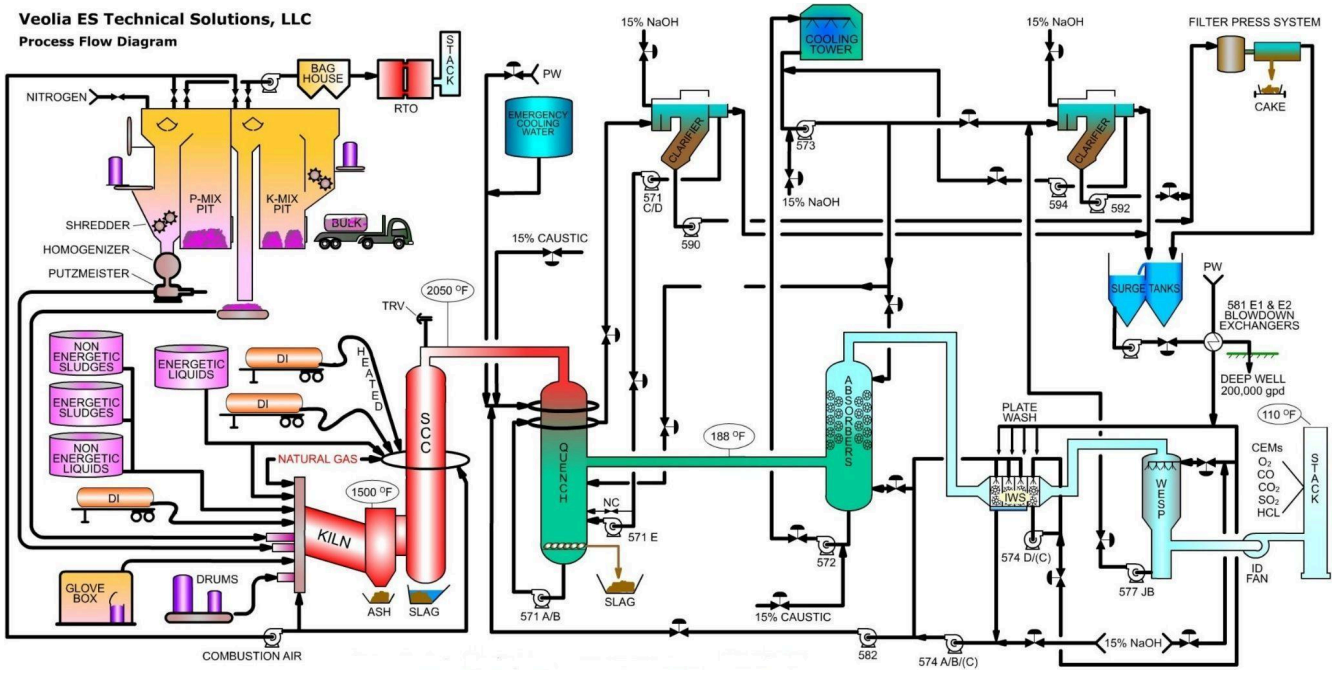


Figure 1 - Process Diagram at Port Arthur

5. Protocol and Sampling

The PFAS testing program at Port Arthur was performed in 2 campaigns :

Campaign	Date	Analysis	PFAS waste
Campaign 1	July 29 to August 5, 2024	Analysis of air emissions, liquid (blowdown) and solids (ashes, slag and filter cake)	AFFF (3 runs) AFFF-contaminated soil (3 runs)
Campaign 2	October 6th to October 24th, 2024	Analysis of air emissions, liquid (blowdown) and solids (ashes, slag and filter cake)	AFFF (2 runs) AFFF with lime injection (1 run) Contaminated soil (3 runs) Spent activated carbon (2 runs) Carbon tetrafluoride CF4 (1 run)

Campaign 1 & 2 were performed in collaboration with AECOM, a reputable stack testing company, who was tasked with performing OTM-45 & OTM-50[1] and HF sampling at stack. Eurofins USA performed the laboratory analysis on all matrix samples at their laboratory facilities in Knoxville, TN and Lancaster, PA.

Campaign 2 was performed in collaboration with the EPA. Veolia shared with EPA the protocol in advance and the results of the test. The testing program followed *EPA Guidance to Conduct PFAS Emissions Field Testing at Commercial Thermal Destruction Sources (Appendix A of the 2024 EPA guidance on PFAS waste disposal).*

Before each new matrix, a baseline run was performed : liquid and solid residues are sampled, OTM-45, 50 and HF sampling is performed at the stack while no PFAS is incinerated in the kiln.

5.1. Sampling Procedure

All residues (liquid, solid and air emissions) were sampled for PFAS during PFAS incineration either during the run or at the end. The average duration of a run was between 3-4 hours. The list of sample points is provided below :

- 1 liquid sample at the end of the water treatment line at the end of each PFAS run and 24 hours afterwards when no PFAS has been incinerated in between.
- 3 solid samples: ashes in the kiln, slag from the SCC and quench, and the filtercake in the water treatment line. Solids are sampled once they have cooled (<24 hours after the run).
- 3 air emission samples at the stack: OTM-45, OTM-50 and HF samples were collected..

Parameter	Stream	Analytical Method	Sampling method
HF	Stack Gas	IC - EPA Method 26A	EPA Method 26A

[1] OTM-45 and OTM-50 are 2 sampling methods for PFAS developed by the EPA. OTM-45 targets 49 semi-volatile PFAS and OTM-50 targets 30 volatile non-polar PFAS.

PFAS	Stack Gas	LC – MS EPA Method 537 (modified)	OTM-45
VFC	Stack Gas	GC/MS - ASTM D5466-21	OTM-50
O_2, CO_2	Stack Gas	Instrument Analyzers	EPA Method 3A
Moisture	Stack Gas	Gravimetric	EPA Method 4
Process water	Tap	LC – MS EPA Method 537 (modified)	S004
Liquid residues	Blowdowns	LC – MS EPA Method 537 (modified)	S004
Solid residues	Ashes, Slag, Filtercake	LC – MS EPA Method 537 (modified)	S007
PFAS	Waste Feeds	LC – MS EPA Method 537 (modified) or EPA Method 1633	

Table 1 - Summary of analytical and sampling methods

5.1.1. Waste Feed Sampling Procedures

Samples of the liquid and solid waste feed streams were collected in glass bottles or plastic containers by Veolia staff and handed to AECOM at the end of the program. Pre-cleaned bottles were purchased and used to collect the samples.

Liquid waste feed samples were collected using the tap sampling procedure specified in U.S. EPA Method S004, *"Sampling and Analysis Methods for Hazardous Waste Combustion."* The sample tap was flushed each time (allowed to flow briefly) before the sample was collected to ensure that any stagnant accumulation of solids, or other contaminants that may be present in the tap, did not affect the sample integrity or its representation of the stream being sampled.

Solid waste feed samples were collected using the scoop sampling procedure specified in U.S. EPA Method S007, *"Sampling and Analysis Methods for Hazardous Waste Combustion."* Samples of the bulk solid wastes (i.e., "regular wastes") fed to the kiln were collected by Veolia personnel for each of the three test runs. In the case where multiple samples of "regular wastes" were collected, the average analysis result for each test run was utilized.

5.1.2. Stack Gas Sampling Procedures

The stack gas emissions were sampled for determination of the parameters indicated in **Table 2**. The sampling methods used are described below.

For all of the stack gas emission parameters measured using sampling trains (i.e., Method OTM-45, OTM-50 and Method 26A) a field blank sample was collected by assembling the sampling train, taking it to the stack sampling location, and then performing a leak check. The field blank was then recovered and analyzed in the same manner as actual samples. The primary purpose of the field blank samples is to evaluate any bias to sample results due to contamination of the sampling system and/or sampling media.

5.1.3. EPA Methods 2 and 4 (Flowrate and Moisture)

Concurrent with the performance of isokinetic sampling trains, measurements were made to determine gas velocity by EPA Method 2, and moisture by EPA Method 4.

5.1.4. EPA Method OTM-45 for PFAS

Samples of exhaust emissions of PFAS compounds were collected according to OTM-45 for determination of PFAS.

A schematic diagram of the sampling train is shown in **Figure 2-1**. According to this method, sample gas was removed isokinetically from the stack and passed through the following components:

- Glass (quartz) nozzle;
- Heated, glass (quartz)-lined probe;
- Heated glass-fiber filter with a glass filter support;
- Coil condenser;
- Sorbent trap containing XAD-2® resin;
- One short-stem Knockout impingers empty;
- Modified Greenburg-Smith impinger containing 100 mL of water;
- Greenburg-Smith impinger containing 100 mL of water;
- Modified Greenburg-Smith impinger containing 100 mL of water;
- Second sorbent trap containing XAD-2® resin; and
- Modified Greenburg-Smith impinger containing silica gel.

After sample collection, the sampling train was recovered to provide the following fractions:

- Solvent (5% ammonium hydroxide) rinse of the nozzle, probe and front half of the filter holder;
- Filter;
- 5% ammonium hydroxide rinse of back half of the filter holder, transfer line, and condenser;
- XAD-2® sorbent trap; and
- Condensate catch from Impingers 1 through 4;
- 5% ammonium hydroxide rinse of Impingers 1 through 4 and connecting glassware; and
- 2nd XAD-2® sorbent trap.

All samples, except the particulate filter were maintained cold and on ice prior to transportation. The particulate filter is stored at 68±5 °F and shipped unrefrigerated. These samples were shipped to the Eurofins Test America laboratory in Knoxville, TN, for analysis as follows:

- PFAS analysis using liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) multiple reaction monitoring (MRM).

Sampling data sheets are presented in Appendix I (campaign 1) and II (campaign 2).

OTM-45 fractions were sent to Eurofins for analysis. Eurofins analysed separately the 4 fractions shown in Figure 2-1:

- Fraction 1 : #1 Filter and #2 Front Half Rinse
- Fraction 2 : #3 XAD1, #4 Back Half Rinse, #6 Impinger Rinse
- Fraction 3 : #5 Condensate and Impingers Water

- Fraction 4 : #7 Breakthrough XAD2

5.1.5. EPA Method OTM-50 for PICs in the form of Volatile Fluorinated Compounds (VFC)

Samples of exhaust emissions of PICs in the form of VFCs were collected according to OTM-50 for determination of volatile fluorinated compounds

A schematic diagram of the sampling train is shown in **Figure 2-2.** In this method, a gas sample is extracted from the gas stream and collected in an evacuated canister. Sample conditioning was necessary for sources with moisture content exceeding 3% volume/volume (v/v) or for sample gas temperatures above 250 °F. For Veolia sampling, sample conditioning is included due to the high moisture content in the gas stream. The stack sample passes through the following components:

- Heated, glass (quartz)-lined probe;
- Heated glass-fiber filter with a glass filter support;
- Perfluoroalkoxy (PFA) heated transfer line;
- One short-stem Knockout impingers empty;
- Mini impinger with open stem containing 15 mL of water;
- Mini impinger with tapered stem containing 15 mL of water;
- Mini impinger with tapered stem containing 15 mL of water;
- Mini impinger with open stem, empty; and
- Stainless steel canister.

After sample collection, the sampling train was recovered to provide the following fractions:

- Stainless steel canister.

These samples were transferred to the Eurofins Test America laboratory in Knoxville, TN, for analysis as follows:

- VFC analysis using gas chromatography coupled with mass spectrometry (GC/MS).

Sampling data sheets are presented in Appendix I (campaign 1) and II (campaign 2).

5.1.6. EPA Method 26A (HF)

Samples for the determination of HF in stack emissions were collected using a single sampling train meeting the requirements of EPA Method 26A. A schematic diagram of the sampling train is shown in **Figure 2-3**. This sample train consists of the following components:

- Glass (quartz) nozzle;
- Heated, glass (quartz)-lined probe;
- Heated Teflon mat filter with a Teflon filter support;
- Greenburg-Smith impinger containing 100 mL of 0.1 N H2SO4;
- Greenburg-Smith impinger containing 100 mL of 0.1 N H2SO4;
- Modified Greenburg-Smith impinger containing 100 mL of 0.1 N NaOH;
- Modified Greenburg-Smith impinger containing 100 mL of 0.1 N NaOH;
- Optional empty knockout impinger, modified Greenburg-Smith; and
- Modified Greenburg-Smith impinger containing silica gel.

These procedures require the isokinetic extraction of particulate matter on a filter maintained at a controlled temperature. In accordance with the requirements of EPA Method 26A, the filter and probe were kept at a temperature between 248°F and 273°F, and a Teflon-backed filter was used. A Teflon union was used to connect the quartz nozzle to the quartz probe liner.

Fluoride analysis was performed on the impinger contents using ion chromatography according to Method 26A.

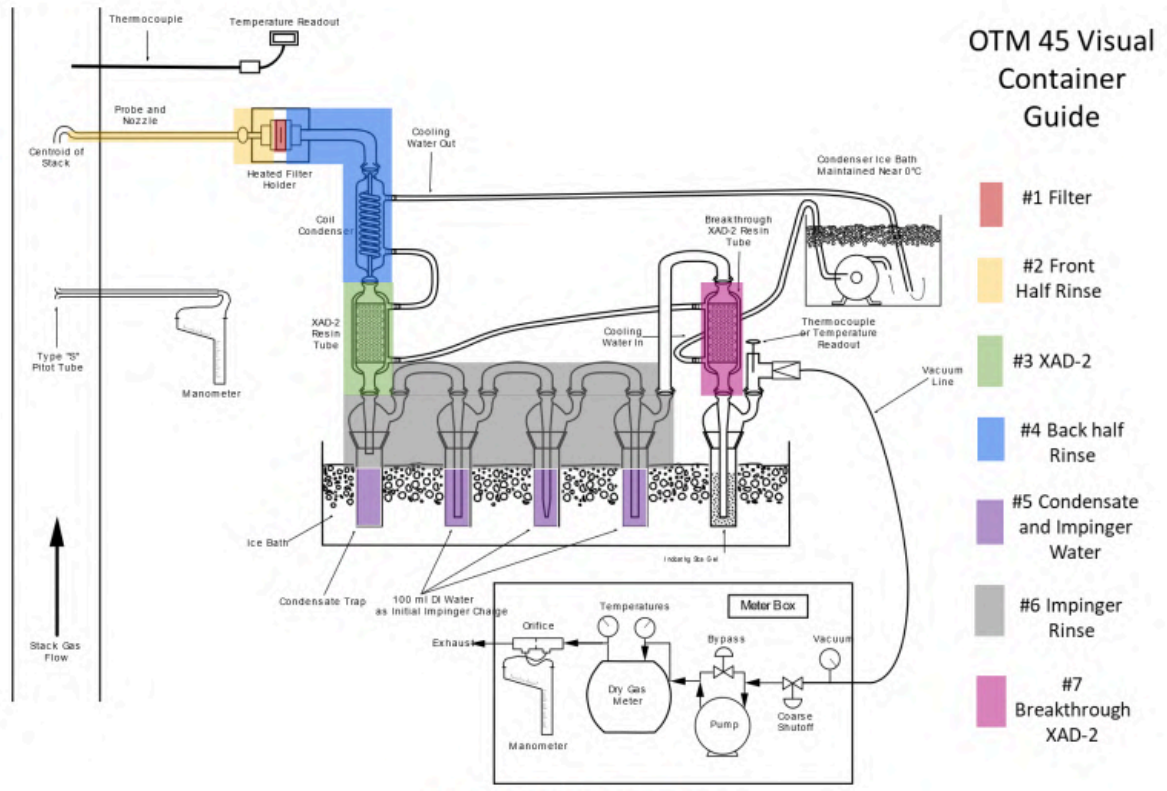


Figure 2-1 - OTM-45 sampling train

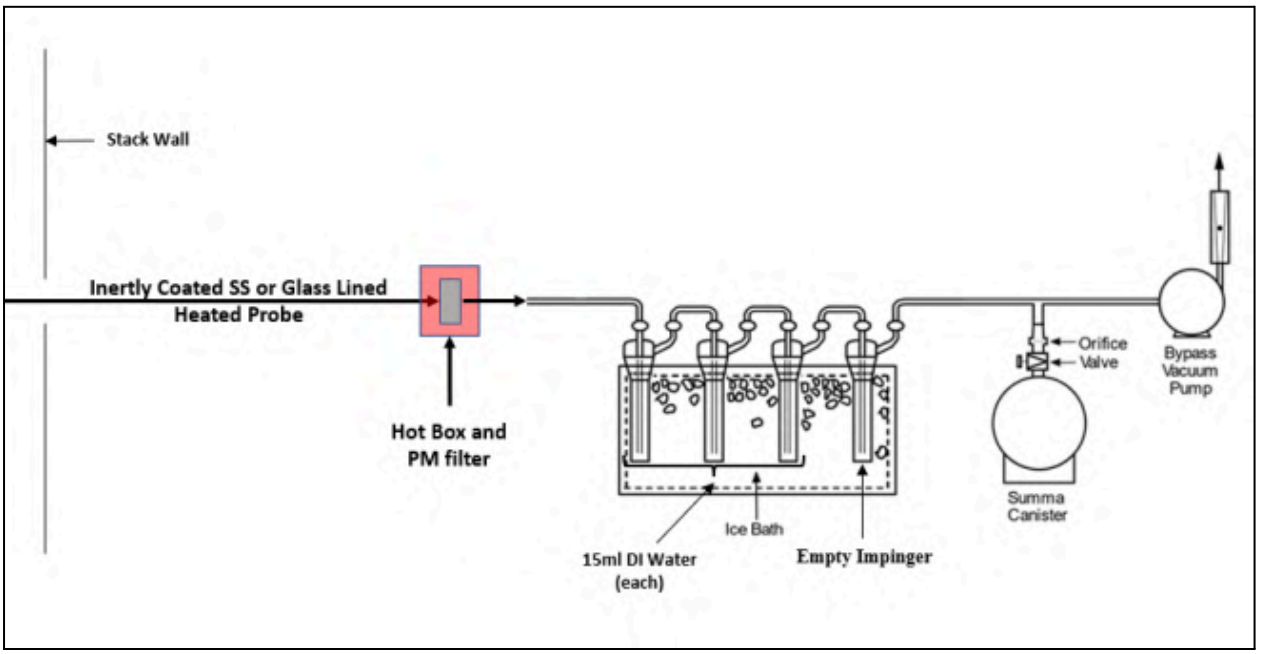


Figure 2-2 - OTM-50 sampling train

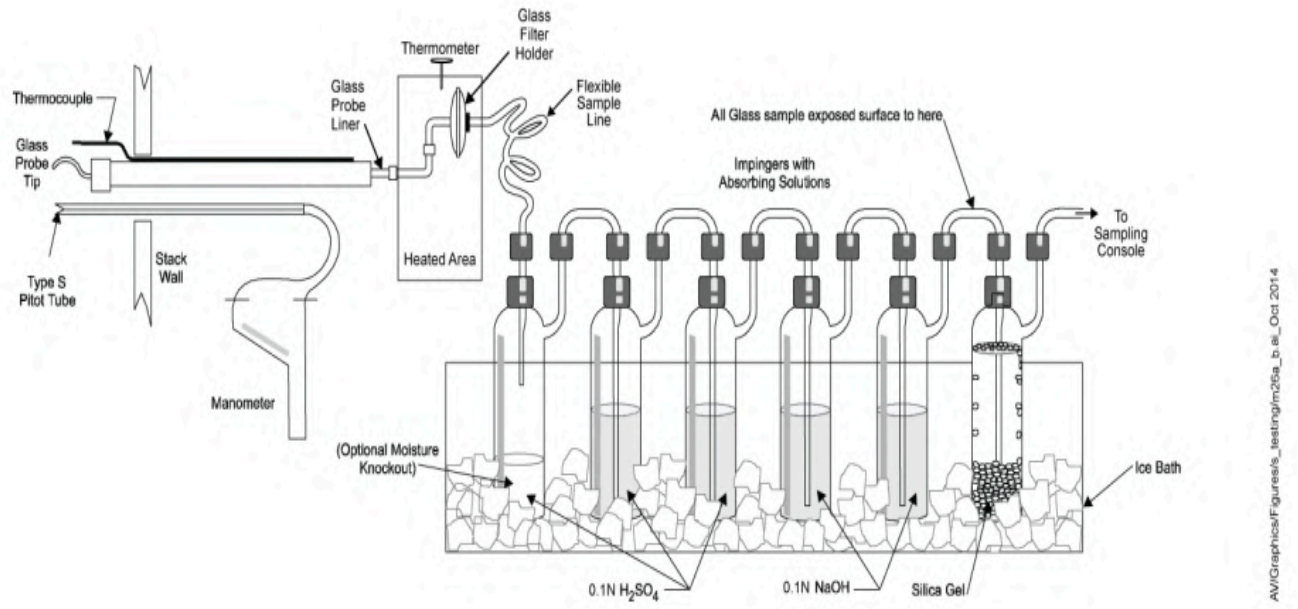


Figure 2-3 – Sampling Train Schematic – EPA Method 2

5.2. Operating conditions

The incinerator was operated under normal conditions in terms of temperature and residence time. In addition to the PFAS waste, regular waste was injected into the kiln, with the exception of waste with F content > 2%. Regular waste includes a wide variety of liquid and solid hazardous waste including waste with high H content like aqueous waste. A high ratio H/F is known to be useful to enhance HF formation and avoid incomplete PFAS destruction.

Tables 2-1 and 2-2 present additional information regarding the conditions evaluated in the final campaign phase. **Table 2-3** summarizes the combustion conditions in the SCC during the test.

Date		Run	Avg Feedrate (lb/hr)	Stack Gas Flow (DSCFM)	Blowdown Flow (GPM)	SCC (Deg F)	Solid residual Generation (lb/hr)
7/31	Morning	**Baseline**	NA			2049 *	
7/31	Afternoon	**AFFF #1**	2420.8	28987.8	124.17		5403.3
8/01	Morning	**AFFF #2**	2175	30049	120.91		5403.3
8/01	Afternoon	**AFFF #3**	2235.2	29605.5	128.32		5403.3
8/03	Morning	**Baseline**	NA				
8/03	Afternoon	**Soil #1**	4824.7	28587	124.35		5584.2
8/04	Morning	**Soil #2**	3909.8	29143.7	115.51		5584.2
8/04	Morning	**Soil #3**	2936.9	28793.4	119.65		5584.2

Table 2-1 - Process data for the July campaign. * Temperature in the SCC is the average temperature during the test, temperature for each run is not available.

Date	Time	Run	Feed Rate (lb/h)	Stack Gas Flow (DSCFM)	Blowdown (GPM)	Kiln Face* (Deg F)	Kiln Exit* (Deg F)	SCC* (Deg F)	Solid residual generation (lb/hr)
10/9	Morning	**Baseline**	NA	29878	104.9	1550	1554	2034	6,618
10/9	Afternoon	**A3F #1**	2184	29521	96.5	1535	1572	2035	8,104
10/10	Afternoon	**A3F #2**	2294	29942	105.4	1706	1628	2042	8,410
10/12	Morning	**Baseline**	NA	29019	124.4	1559	1636	2058	9,225
10/12	Afternoon	**A3F #3 lime**	2378	28866	100	1532	1778	2055	8,165
10/14	Morning	**Baseline**	NA	29206	105.1	1492	1667	2048	10,935
10/14	Afternoon	**Soil #1**	4061	28688	108.6	1490	1673	2052	12,195
10/17	Morning	**Baseline**	NA	29931	115.6	1496	1568	2053	7,203
10/17	Afternoon	**CF4**	30.9	29730	99	1427	1717	2054	8,565
10/19	Morning	**Baseline**	NA	29723	103.3	1455	1643	2040	7,010
10/19	Afternoon	**Carbon #1**	2187	29387	105.2	1392	1689	2046	6,230
10/20	Morning	**Carbon #2**	2422	29668	105.9	1519	1644	2066	5,140
10/22	Morning	**Baseline**	NA	31208	96.8	1595	1643	2056	2,838
10/22	Afternoon	**Soil #2**	2565	29925	95.9	1414	1799	2049	6,745
10/23	Morning	**Soil #3**	3875	30253	104	1484	1697	2046	13,320

Table 2-2 - Process data for the October campaign. * indicates average temperature during test

	Campaign 1			Campaign 2		
Parameter	**Min**	**Max**	**Average**	**Min**	**Max**	**Average**
Residence time (calculated)	2.3 sec					
O2 at the stack - % dry (measured)	7.0	8.1	7.5	7.2	8.4	8.1
CO2 at the stack - % dry at the stack (measured)	9.1	10.2	9.7	9.1	9.9	9.3
Moisture at the stack - %v/v (measured)	7.7	8.7	8.0	5.5	7.2	6.2

Table 2-3 - Combustion Conditions in the SCC and at the stack

6. Quality Assurance/Quality Control

The primary objectives of the QA/QC effort were to control, assess, and document data quality. To accomplish these objectives, the QA/QC approach consisted of the following key elements:

- Definition of data quality objective that reflects the overall technical objectives of the measurement program (see section 4.2);
- Design of a sampling, analytical, QA/QC and data analysis system to meet those objectives;
- Evaluation of the performance of the measurement system; and
- Initiation of corrective action when measurement system performance does not meet the specifications

These QA procedures include sampling and analytical procedures, along with specified calibration requirements, QC checks, data reduction and validation procedures, and sample tracking. A review of analysis results for QA/QC samples and assessment of overall data quality is presented in this section.

The sections below present discussions of the QA/QC activities associated with sampling and analysis, as well as with data quality assessment.

6.1. Analysis of Waste Feeds for PFAS

Samples of waste feed materials were analyzed for PFAS using SW-846 Method 537 (Modified). These same samples were analyzed for PFAS using Liquid Chromatography/Tass Spectrometry. These samples were prepared for analysis using appropriate extraction or dilution techniques. Quality assurance and quality control activities associated with these analyses included:

- Sample handling and preservation;
- Preparation and analysis of samples within specified holding times;
- Preparation and analysis of laboratory blanks;

- Preparation and analysis of laboratory control samples;
- Preparation and analysis of samples collected in duplicate; and
- Preparation and analysis of matrix spike and matrix spike duplicate samples.

Review of these field-related QA/QC activities indicates that these data are supportable and usable. See the detailed data quality assessment in Appendix III (campaign 1) and IV (campaign 2). Any issues with the sample analysis were identified in the laboratory reports. Media checks were sufficient to qualify the data, and a sufficient number of reagents blanks were collected and analysed to further qualify the data.

6.2. Stack Gas

6.2.1. Isokinetic Sample Collection

The following isokinetic sampling trains were used for the collection of air emission compliance samples:

- Hydrogen fluoride using a sampling train meeting the requirements of EPA Method 26A; and
- PFAS in accordance with OTM-45.

Quality assurance and quality control activities associated with the collection of these samples included:

- Collection of the specified volumes of stack gas over the specified duration;
- Collection of stack gas within 90-110 percent of isokinetic;
- Maintaining the probe, filter, and heated transfer line at the specified temperatures, as applicable;
- Maintaining the impinger exit and the condenser exit at the specified temperature, as applicable;
- Performing sampling train leak checks before sample collection, at port changes, and after sample collection;
- Performing pitot tube leak checks before and after sample collection; and
- Recording all data on pre-printed data sheets.

6.2.2. Non-Isokinetic Sample Collection

The following non-isokinetic sampling trains were used for the collection of air emission compliance samples:

- VFC using EPA Method OTM-50.

Quality assurance and quality control activities associated with the collection of these samples included:

- Collection of the specified volumes of stack gas over the specified duration;
- Maintaining the probe, filter, and heated transfer line at the specified temperatures, as applicable;
- Maintaining the impinger exit and the condenser exit at the specified temperature, as applicable;
- Performing sampling train leak checks before sample collection, at port changes, and after sample collection;
- Performing pitot tube leak checks before and after sample collection; and
- Recording all data on pre-printed data sheets.

6.2.3. Monitoring of Stack Gas for Oxygen and Carbon Dioxide

The stack gas was monitored for oxygen (O_2) and carbon dioxide (CO_2) according to EPA Methods 3A (O_2 and CO_2).

Quality assurance and quality control activities associated with continuous emission monitoring include:

- Use of calibration gas standards of known and acceptable quality;
- Use of calibration gas standards in the specified ranges;
- Performance of calibration error tests;
- Performance of system bias checks;
- Performance of zero and span drift checks; and
- Performance of a NOx analyzer converter efficiency check.

Review of these QA/QC activities indicates that these data are supportable and usable. See the data quality assessment in Appendix III (campaign 1) and IV (campaign 2). Copies of certifications of calibration gas standards; the raw data for continuous emission monitors is presented in Appendix V (campaign1) and VI (campaign 2).

7. Results and discussion

7.1. Campaign 1 : Incineration of A3F and PFAS-contaminated soil

Campaign 1 Summary :

Campaign 1 demonstrated that Port Arthur incinerator can efficiently treat PFAS waste and reach DRE at stack above 99.9999% for the most concentrated PFAS, including PFOS, PFHxS, PFHpS and PFPeS.

OTM-50 results demonstrated that no PICs were detected in any runs of the campaign, showing that PFAS mineralization was complete, within the limit of current analytical detection limits.

Solid residues were PFAS-free, or contained PFAS at levels close to the MDL.

Liquid residues contained PFAS at low levels (ppt), reflecting the background presence of PFAS in the pond water supplying the process water to the incinerator.

7.1.1. Waste analysis

Three different A3F were incinerated in three different runs, with PFAS feed rate in the kiln ranging from 8 g/hr to 188 g/hr. A3F profiles are in **Appendix VI.**

3 runs of contaminated soil were incinerated with a PFAS injection rate of 29 g/hr in the kiln. The solid mix was spiked with A3F to create the contaminated soil. The soil profile is in **Appendix VIII.**

7.1.2. Solid and liquid residues

No PFAS were detected in significant quantities in the solid residues. 2 PFAS (PFOSAm and 6:2 FTS) were detected at the MDL in the ashes and filtercake in 2 samples out of 18. Detailed results are provided in **Appendix IX-A and X-A.**

All blowdowns had the same PFAS concentration and signature. A dozen PFAS were detected at ppt level (Figure 3), that is to say at the order of magnitude of the EPA drinking water standard[2]. Results were similar for samples collected during baseline (at the beginning of the campaign, before PFAS incineration) and in the samples collected after PFAS incineration.

Detecting PFAS at ppt level in the blowdown was unexpected. It was later explained by the background presence of PFAS in the pond water supplying the process water. This pond is supplied by rain water and is likely contaminated by PFAS from various industrial sources as Port Arthur is an industrial area. PFAS contamination in water bodies is not uncommon across the USA.

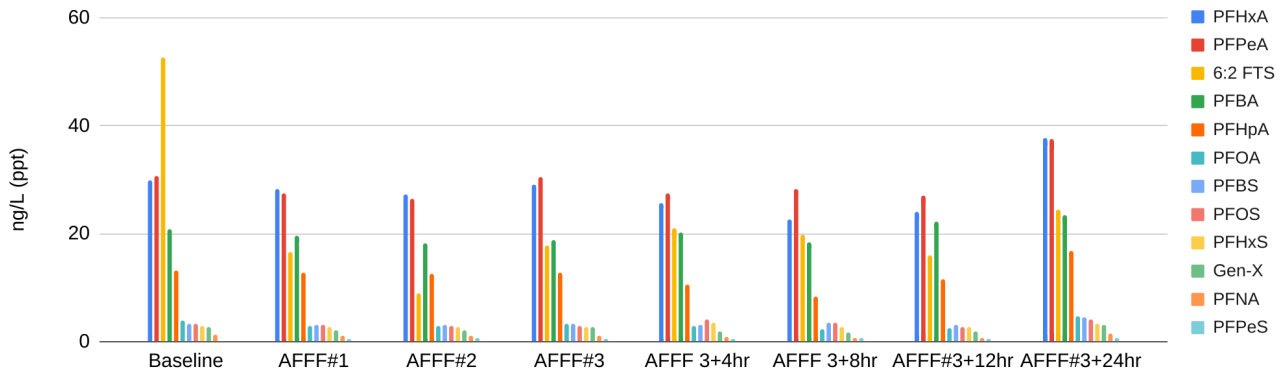


Figure 3 - PFAS detected in blowdowns during A3F incineration

This PFAS presence in liquid residues is due to PFAS background presence in the pond water used in the incinerator : the same signature of PFAS was observed in the process water and in the blowdown sampled during the baseline (without PFAS incineration), with PFAS being twice as concentrated in the blowdowns than in the pond water because of evaporation (Table 3-1). The only difference between the PFAS signature in the process water and blowdown is the presence of 6:2 FTS in the blowdown, detected in every run even during the baseline. During campaign 1, 6:2 FTS was not detected at the stack, so it seems unlikely that 6:2 FTS is a PIC generated during the incineration process. However, 6:2 FTS was detected at the stack in campaign 2. Port Arthur is currently studying the possibility that some equipment inside the incinerator releases PFAS during normal operations. Otherwise 6:2 FTS could be a residual PIC from previous PFAS incineration.

The process water comes from a nearby pond, supplied by rainfall. As many water sources in the USA, this pond is contaminated with PFAS at low level (< 100 ppt). Veolia was not aware that the pond water was contaminated with PFAS before performing the PFAS campaign and sampling process water. Veolia is currently studying PFAS removal technology to treat the pond water prior to injecting process water in the incinerator.

The process water sample was not collected during campaign 1 but 6 weeks after, when the first results of blowdowns were analysed. Therefore, PFAS contamination in process water during campaign 1 may have been slightly different. However, the second sample of process collected in

[2] EPA Maximum Contaminant Level : PFOA & PFOS : 4 ng/L each PFHxS, PFNA, HFPO-DA : ng/L

campaign 2 shows a similar PFAS signature and concentration compared to the one obtained for campaign 1, suggesting that PFAS contamination in the process water does not vary, at least over short time periods.

PFAS	Process water (ng/L)	Blowdown baseline (ng/L)
6:2 FTS	ND	52.6
PFBA	11.20	20.8
PFPeA	16.10	30.8
PFHxA	14.90	29.9
PFHpA	8.41	13.1
PFOA	2.88	3.88
PFNA	1.26	1.33
HFPO-DA	2.02	2.77
PFBS	1.81	3.33
PFHxS	1.49	2.84
PFOS	2.48	3.4

Table 3-1 : PFAS concentration in process water and blowdown baseline (ng/L). *The column "blowdown baseline" is an average of the 2 baselines collected during campaign 1. All PFAS listed in EPA 1633 were tested but only the ones detected are shown in this table.*

Table 3-2 shows the difference in PFAS concentration entering the incinerator via the process water, vs PFAS exiting the incinerator via the liquid residue (blowdowns). In all runs except Soil run#3, there are more PFAS entering via the process water than exiting in the liquid residues. That confirms that PFAS in the process water pass through the stack gases and are emitted at the stack.

	AFFF run 1	AFFF run 2	AFFF run 3	Soil run 1	Soil run 3
PFAS in process water entering the incinerator - PFAS in (lb/hr)	1.10E-05	1.07E-05	1.09E-05	9.67E-05	1.32E-04
PFAS in liquid residue exiting the incinerator - PFAS out (lb/hr)	8.22E-06	7.29E-06	8.44E-06	8.91E-05	2.02E-04
% PFAS out / PFAS in	74.73%	68.13%	77.43%	92.14%	153.03%

Table 3-2 : Comparison of PFAS flowrate in liquid entering and exiting the incinerator. *Soil run 2 is not shown because process water flowrate was not measured.*

Port Arthur is equipped with a wet flue gas treatment. The stack gases are in contact with process water after the incineration process, and it is possible that some PFAS transfer from water to stack gases after thermal treatment.

Detailed results for PFAS concentrations in liquid residues are provided in **Appendix IX-B (AFFF) and X-B (contaminated soil).**

7.1.3. Air Emissions

7.1.3.1. PFAS Mass Emission Rate at Stack (OTM-45)

Table 4-1 and 4-2 present PFAS mass emission rate at the stack during campaign 1 for baselines, AFFF incineration and contaminated-soil incineration.

> Analysis of OTM-45 during baseline :

Analysis of PFAS flowrate at the stack during baseline tests shows that most of the OTM-45 listed PFAS are not detected at the stack.

In the baseline performed before AFFF was fed, 10 PFAS are detected at low level, close to the MDL during the baseline. These PFAS were PCFAs only, and 3 of them are present in the process water (PFPeA, PFHxA, PFOA) which could explain their detection at the stack. Among the 7 other PFAS (PFDA, PFDoA, perfluorohexadecanoic acid, perfluorooctadecanoic acid, PFTeA, PFTriA and PFUnA), perfluorohexadecanoic acid and perfluorooctadecanoic acid could not be measured in the process water due to analytical limitations and may be in the water.

There is no apparent reason to explain the presence of PFDA, PFDoA, PFTriA, PFUnA, PFTeA at the stack while no PFAS were incinerated during the baseline.

Results for the baseline before contaminated soil was fed were similar to the AFFF baseline except for the additional presence of PFHxS at the stack. PFHxS was not detected in the process water. Its measurement at the stack during the soil baseline was very close to the MDL.

> Analysis of OTM-45 mass emission rate during AFFF and contaminated soil incineration

During AFFF incineration, only 13 PFAS out of the 49 listed in OTM 45 were detected above MDL in at least one run. They are in bold in Table 4-1. They are exclusively PFCAs:

- PFBA, PFHpA, PFHxA, PFOA, PFPeA, PFNA, which are present in the process water. Their presence at the stack during AFFF incineration most likely comes from the process water.
- PFDA, PFDoA, PFTeA, PFTriA, PFUnA, perfluoroctadecanoic acid, perfluorohexadecanoic acid, which were detected during the AFFF baseline at the stack.

Comparison of OTM-45 between the baseline and AFFF or soil incineration shows that the same PFAS are detected at the stack, reflecting mainly the presence of PFAS in the process water.

Comparing total PFAS emission at the stack between baselines and PFAS incineration, OTM-45 results indicated higher total PFAS emissions for the PFAS waste incineration tests compared to baseline tests. However, given that PFAS were present in the water fed to the quench and wet scrubber downstream of the incineration process, these data are not considered conclusive.

	Baseline	Run 1		Run 2		Run 3	
PFAS	Stack emission (lb/hr)	Waste feed rate (lb/hr)	Stack emission (lb/hr)	Waste feed rate (lb/hr)	Stack emission (lb/hr)	Waste feed rate (lb/hr)	Stack emission (lb/hr)
PFBS	<0.000000083 DLL	0.024500	<0.000000075 DLL	0.002170	ND (0.000000076)	ND (0.000032)	<0.000000079 DLL
PFBA	<0.00000084 DLL	0.004730	<0.00000087 DLL	0.001080	**0.000000589**	0.000858	<0.00000080 DLL
PFDS	ND (0.000000025)	0.000173	ND (0.000000025)	0.000894	ND (0.000000025)	ND (0.000032)	ND (0.000000025)

PFDA	**0.0000000504**	0.000180	**0.000000183**	0.000005	**0.000000164**	ND (0.000032)	<0.000000036 DLL
PFDoS	ND (0.000000023)	0.000109	ND (0.000000022)	0.000025	ND (0.000000023)	ND (0.000032)	ND (0.000000023)
PFDoA	**0.0000000649**	0.000717	**0.0000000805**	ND (0.000043)	**0.0000000718**	0.000131	**0.0000000116**
PFHpS	ND (0.000000028)	0.033400	ND (0.000000027)	0.000594	ND (0.000000027)	ND (0.000032)	ND (0.000000028)
PFHpA	<0.00000024 DLL	0.006120	**0.000000912**	0.000561	**0.000000398**	0.000295	**0.00000130**
Perfluorohexadecanoic acid	**0.000000116**	--	**0.0000000727**	--	**0.0000000577**	--	**0.0000000442**
PFHxS	ND (0.000000028)	0.179000	ND (0.000000027)	0.018000	ND (0.000000028)	0.000396	<0.000000029 DLL
PFHxA	**0.000000104**	0.022300	**0.000000605**	0.004090	**0.000000338**	0.006460	**0.000000339**
PFNS	ND (0.000000019)	0.000392	ND (0.000000019)	0.000763	ND (0.000000019)	ND (0.000032)	ND (0.000000019)
PFNA	<0.000000099 DLL	0.000228	**0.000000412**	0.000644	**0.000000289**	ND (0.000032)	<0.000000075 DLL
Perfluorooctadecanoic acid	**0.0000000880**	--	**0.0000000468**	--	**0.0000000501**	--	**0.0000000450**
FOSA	ND (0.000000025)	0.000228	ND (0.000000024)	0.000644	ND (0.000000025)	ND (0.000032)	ND (0.000000025)
PFOS	<0.000000042 DLL	0.007190	<0.000000038 DLL	0.005830	<0.000000035 DLL	0.004380	<0.000000038 DLL
PFOA	**0.000000101**	0.000806	**0.000000782**	0.000653	**0.000000378**	0.000490	**0.000000147**
PFPeS	ND (0.000000025)	0.000178	ND (0.000000024)	0.000144	ND (0.000000025)	0.000108	ND (0.000000025)
PFPeA	**0.0000000696**	0.000301	**0.000000329**	0.000744	**0.000000271**	0.000183	**0.0000000821**
PFTeA	**0.0000000843**	0.000070	**0.0000000920**	0.000006	**0.0000000575**	0.000004	**0.0000000352**
PFTriA	**0.0000000919**	0.000671	**0.0000000825**	0.000543	**0.0000000658**	0.000408	**0.0000000223**
PFUnA	**0.0000000604**	0.000622	**0.000000116**	0.000504	**0.000000101**	0.000379	<0.000000036 DLL
9-Chloro.	ND (0.000000022)	ND (0.000059)	ND (0.000000021)	ND (0.000053)	ND (0.000000022)	ND (0.0000054)	ND (0.000000022)
NEtFOSA	ND (0.000000066)	0.000865	ND (0.000000064)	ND (0.000043)	ND (0.000000066)	ND (0.000043)	ND (0.000000066)
NEtFOSAA	ND (0.000000025)	--	ND (0.000000024)	--	ND (0.000000025)	--	ND (0.000000025)
NMeFOSAA	ND (0.000000021)	ND (0.000047)	ND (0.000000020)	ND (0.000043)	ND (0.000000021)	ND (0.000043)	ND (0.000000021)
11-Chloro.	<0.000000027 DLL	ND (0.0000059)	ND (0.000000025)	ND (0.0000053)	ND (0.000000026)	ND (0.0000054)	ND (0.000000026)
NEtFOSE	ND (0.000000038)	0.000627	ND (0.000000037)	0.000396	ND (0.000000038)	ND (0.000043)	ND (0.000000038)
NMeFOSE	<0.0000017 DLL	0.000207	ND (0.0000016)	ND (0.000053)	<0.0000017 DLL	ND (0.000054)	<0.0000017 DLL
PES	ND (0.000000022)	--	ND (0.000000021)	--	ND (0.000000022)	--	ND (0.000000022)
PFECA	ND (0.000000032)	--	ND (0.000000031)	--	ND (0.000000031)	--	ND (0.000000032)
PFECA	ND (0.000000028)	--	ND (0.000000027)	--	ND (0.000000028)	--	ND (0.000000028)
PFECA	ND	--	ND	--	<0.000000027	--	ND

	(0.000000025)		(0.000000025)		DLL		(0.000000025)
PFECHS	ND (0.000000031)	--	ND (0.000000030)	--	ND (0.000000030)	--	ND (0.000000031)
DONA	ND (0.000000099)	ND (0.000059)	ND (0.000000096)	ND (0.000053)	ND (0.000000099)	ND (0.0000054)	ND (0.000000099)
HFPO-DA	ND (0.0000010)	0.000006	ND (0.00000099)	ND (0.000043)	ND (0.0000010)	ND (0.000043)	ND (0.0000010)
NMeFOSA	ND (0.000000071)	0.000264	ND (0.000000068)	ND (0.000043)	ND (0.000000070)	ND (0.000043)	ND (0.000000070)
10:2 FTCA	ND (0.000000077)	--	ND (0.000000075)	--	ND (0.000000076)	--	ND (0.000000077)
10:2 FTS	ND (0.000000033)	--	ND (0.000000032)	--	ND (0.000000032)	--	ND (0.000000033)
3:3 FTCA	ND (0.000000051)	ND (0.000012)	ND (0.000000049)	ND (0.000053)	ND (0.000000050)	ND (0.0000054)	ND (0.000000051)
4:2 FTS	ND (0.000000012)	0.000035	ND (0.000000012)	0.000857	ND (0.000000012)	0.000380	ND (0.000000013)
5:3 FTCA	ND (0.000000093)	0.000022	ND (0.000000090)	0.000091	ND (0.000000092)	0.000198	ND (0.000000093)
6:2 FTCA	ND (0.00000011)	--	ND (0.00000011)	--	ND (0.00000011)	--	ND (0.00000011)
6:2 FTS	ND (0.00000084)	0.004410	ND (0.00000080)	0.008680	ND (0.00000083)	0.010700	ND (0.00000083)
6:2 FTUCA	ND (0.000000027)	--	ND (0.000000026)	--	ND (0.000000027)	--	ND (0.000000027)
7:3 FTCA	ND (0.000000070)	ND (0.000013)	ND (0.000000068)	ND (0.000012)	ND (0.000000069)	0.000286	ND (0.000000070)
8:2 FTCA	ND (0.000000066)	--	ND (0.000000064)	--	ND (0.000000065)	--	ND (0.000000066)
8:2 FTS	ND (0.000000027)	0.002090	ND (0.000000026)	0.000097	ND (0.000000026)	0.001113	ND (0.000000027)
8:2 FTUCA	ND (0.000000038)	--	ND (0.000000037)	--	ND (0.000000037)	--	ND (0.000000038)
Total PFAS (lb/hr)	**1.36E-06**	**2.91E-01**	**9.33E-06**	**4.85E-02**	**7.74E-06**	**2.73E-02**	**7.90E-06**

Table 4-1 - OTM-45 results for AFFF incineration in campaign 1. *PFAS highlighted in blue were detected in the process water. Values in bold were PFAS detected above MDL in at least one of the OTM-45 fractions. ND means PFAS was not detected at the stack. DLL means that some portions of the sample were above the MDL, while the others were ND.*

	Baseline	Run 1		Run 2		Run 3	
PFAS	**Stack emission (lb/hr)**	**Feed rate (lb/hr)**	**Stack emission (lb/hr)**	**Waste feed rate (lb/hr)**	**Stack emission (lb/hr)**	**Waste feed rate (lb/hr)**	**Stack emission (lb/hr)**
PFBS	ND (0.000000074)	0.00013800	<0.000000074 DLL	0.00011200	ND (0.000000074)	0.00008400	ND (0.000000073)
PFBA	<0.00000078 DLL	0.00036000	<0.00000077 DLL	0.00029200	<0.00000082 DLL	0.00021900	<0.00000085 DLL

PFDS	ND (0.000000025)	0.00000414	ND (0.000000025)	0.00000336	ND (0.000000025)	0.00000252	ND (0.000000024)
PFDA	**0.00000007**	0.00010500	**0.00000012**	0.00008480	**0.00000024**	0.00006370	<0.000000033 DLL
PFDoS	ND (0.000000022)	0.00000122	ND (0.000000022)	0.00000985	ND (0.000000022)	0.00000740	ND (0.000000022)
PFDoA	**0.00000005**	0.00000152	**0.00000006**	0.00000124	**0.00000009**	0.00000928	**0.00000001**
PFHpS	ND (0.000000027)	0.00035200	ND (0.000000027)	0.00028500	ND (0.000000027)	0.00021400	ND (0.000000027)
PFHpA	<0.00000022 DLL	0.00045400	0.00000024	0.00036800	0.00000082	0.00027700	ND (0.00000021)
Perfluorohexadecanoic acid	**0.00000004**	--	**0.00000005**	--	**0.00000003**	--	**0.00000003**
PFHxS	**0.00000003**	0.00101000	**0.00000002**	0.00081700	<0.000000028 DLL	0.00061400	**0.00000001**
PFHxA	**0.00000011**	0.00122000	**0.00000023**	0.00139000	**0.00000071**	0.00105000	**0.00000013**
PFNS	ND (0.000000019)	0.00000207	ND (0.000000019)	0.00000168	ND (0.000000019)	0.00000126	ND (0.000000019)
PFNA	<0.00000011 DLL	0.00000385	**0.00000018**	0.00000312	**0.00000047**	0.00000235	ND (0.000000071)
Perfluorooctadecanoic acid	<0.000000072 DLL	--	**0.00000004**	--	ND (0.000000064)	--	**0.00000003**
FOSA	ND (0.000000024)	0.00038700	ND (0.000000024)	0.00031400	ND (0.000000024)	0.00023600	ND (0.000000024)
PFOS	<0.000000037 DLL	0.00038700	<0.000000033 DLL	0.00058300	<0.000000033 DLL	0.00438000	<0.000000035 DLL
PFOA	**0.00000011**	0.00080600	**0.00000023**	0.00065300	**0.00000079**	0.00049000	**0.00000006**
PFPeS	ND (0.000000024)	0.00017800	ND (0.000000024)	0.00014400	0.00000001	0.00010800	0.00000001
PFPeA	**0.00000005**	0.00030100	**0.00000015**	0.00024400	**0.00000045**	0.00018300	**0.00000003**
PFTeA	**0.00000004**	0.00000700	**0.00000004**	0.00000567	**0.00000007**	0.00000426	**0.00000002**
PFTriA	**0.00000006**	0.00067100	**0.00000005**	0.00054300	**0.00000009**	0.00040800	**0.00000001**
PFUnA	**0.00000007**	0.00062200	**0.00000007**	0.00050400	**0.00000014**	0.00037900	ND (0.000000031)
9-Chloro.	ND (0.000000022)	ND(0.00000093)	ND (0.000000021)	ND(0.00000075)	ND (0.000000022)	ND(0.00000056)	ND (0.000000021)

NEtFOSA	ND (0.000000064)	0.00000550	ND (0.000000064)	0.00000446	ND (0.000000064)	0.00000335	ND (0.000000063)
NEtFOSAA	ND (0.000000025)	ND(0.00000093)	ND (0.000000024)	ND(0.00000075)	ND (0.000000024)	ND(0.00000056)	ND (0.000000024)
NMeFOSAA	ND (0.000000021)	ND(0.00000093)	ND (0.000000021)	ND(0.00000075)	ND (0.000000021)	ND(0.00000056)	ND (0.000000020)
11-Chloro.	ND (0.000000026)	ND(0.00000093)	ND (0.000000025)	ND(0.00000075)	ND (0.000000026)	ND(0.00000056)	ND (0.000000025)
NEtFOSE	ND (0.000000037)	0.00013800	ND (0.000000037)	0.00011200	ND (0.000000037)	0.00084300	ND (0.000000036)
NMeFOSE	ND (0.0000016)	0.00004380	ND (0.0000016)	0.00003550	ND (0.0000017)	0.00002670	ND (0.0000016)
PES	ND (0.000000022)	--	ND (0.000000022)	--	ND (0.000000022)	--	ND (0.000000021)
PFECA	ND (0.000000031)	--	ND (0.000000031)	--	<0.000000037 DLL	--	ND (0.000000031)
PFECA	ND (0.000000028)	--	ND (0.000000028)	--	ND (0.000000028)	--	ND (0.000000027)
PFECA	ND (0.000000025)	--	<0.000000025 DLL	--	<0.000000033 DLL	--	ND (0.000000024)
PFECHS	ND (0.000000030)	--	ND (0.000000030)	--	ND (0.000000030)	--	ND (0.000000029)
DONA	ND (0.000000096)	ND(0.00000093)	ND (0.000000095)	ND(0.00000075)	ND (0.000000096)	ND(0.00000056)	ND (0.000000094)
HFPO-DA	ND (0.00000100)	0.00045100	ND (0.00000099)	0.00036500	ND (0.0000010)	0.00027400	ND (0.00000098)
NMeFOSA	ND (0.000000068)	ND (0.0000023)	ND (0.000000068)	ND (0.0000019)	ND (0.000000068)	ND (0.0000014)	ND (0.000000067)
10:2 FTCA	ND (0.000000075)	--	ND (0.000000075)	--	ND (0.000000075)	--	ND (0.000000074)
10:2 FTS	ND (0.000000032)	--	ND (0.000000032)	--	ND (0.000000032)	--	<0.000000034 DLL
3:3 FTCA	ND (0.000000049)	0.00000156	ND (0.000000049)	0.00000127	ND (0.000000049)	0.00000952	ND (0.000000048)
4:2 FTS	ND (0.000000012)	0.00002310	ND (0.000000012)	0.00001870	ND (0.000000012)	0.00001400	ND (0.000000012)
5:3 FTCA	ND (0.000000091)	0.00029000	ND (0.000000090)	0.00002350	ND (0.000000091)	0.00001770	ND (0.000000089)
6:2 FTCA	ND (0.00000011)	--	ND (0.00000011)	--	ND (0.00000011)	--	ND (0.00000011)

6:2 FTS	ND (0.00000081)	0.01170000	ND (0.00000080)	0.00946000	ND (0.00000081)	0.00711000	ND (0.00000079)
6:2 FTUCA	ND (0.000000026)	--	ND (0.000000026)	--	ND (0.000000026)	--	ND (0.000000026)
7:3 FTCA	ND (0.000000068)	0.00000326	ND (0.000000068)	0.00000264	ND (0.000000068)	0.00000199	ND (0.000000067)
8:2 FTCA	ND (0.000000064)	--	ND (0.000000064)	--	ND (0.000000064)	--	ND (0.000000063)
8:2 FTS	ND (0.000000026)	0.00010500	ND (0.000000026)	0.00008480	ND (0.000000026)	0.00006370	<0.000000030 DLL
8:2 FTUCA	ND (0.000000037)	--	ND (0.000000037)	--	ND (0.000000037)	--	ND (0.000000037)
Total PFAS (lb/hr)	**6.58E-06**	**1.98E-02**	**6.98E-06**	**1.65E-02**	**9.65E-06**	**1.71E-02**	**6.20E-06**

Table 4-2 - OTM-45 results for contaminated soil incineration in campaign 1. *PFAS highlighted in blue were detected in the process water. Values in bold were PFAS detected above MDL in at least one of the OTM-45 fractions. ND means PFAS was not detected at the stack. DLL means that some portions of the sample were above the MDL, while the others were ND.*

> Comparison between total PFAS mass emission rate during baseline vs PFAS incineration.

Table 4-3 compares total PFAS mass emission rate at the stack between baseline and PFAS incineration. The OTM-45 results indicated higher total PFAS emissions for the PFAS waste incineration tests compared to baseline tests in Campaign 1. However, given that PFAS were present in the water fed to the quench and wet scrubber downstream of the incineration process, these data are not considered conclusive.

Campaign 1	**Average Baseline**	**Average AFFF**	**Average Soil**	**Average AFFF & Soil**
Total PFAS mass emission rate at the stack : OTM-45 (lb/hr)	**3.97E-06**	**8.32E-06**	**7.61E-06**	**7.97E-06**
Ratio PFAS incineration/Baseline		**2.1**	**1.9**	**2.0**

Table 4-3 : Total PFAS mass emission rate at the stack during baselines and PFAS incineration. *Average baselines refers to the average total stack emission rate of PFAS observed during the 2 baselines in Campaign 1. Average AFFF (resp. Soil) refers to the average total stack emission rate of PFAS observed during the 3 runs of AFFF (resp. soil) in Campaign 1.*

Figure 4-1 and 4-2 shows the PFAS signature at the stack (4-1) and in the 3 different AFFF feed streams (4-2). While the 3 incinerated AFFF samples have different PFAS composition (especially AFFF3 vs AFFF1 and AFFF2), the PFAS signature at the stack does not significantly vary to reflect AFFF signature. The same 13 PFCAs were detected in the process water and in the baseline. By comparison, the AFFFs are rich in PFHxS, 6:2 FTS, PFOS. The same result was observed for the contaminated soil. This suggests that AFFF and contaminated soil incineration produce similar PFAS emissions at the stack.

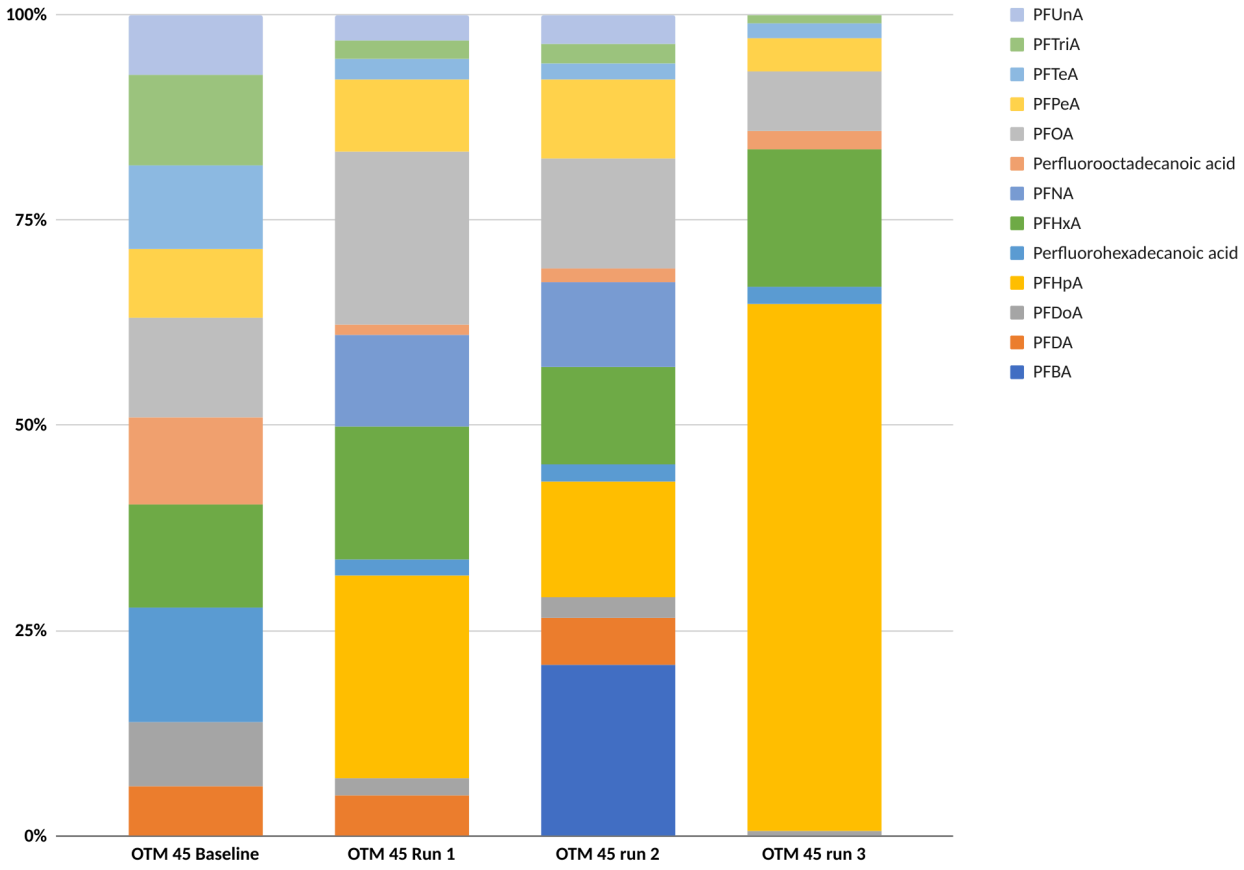


Figure 4-1 : PFAS signature at the stack (OTM-45) during AFFF incineration and baseline

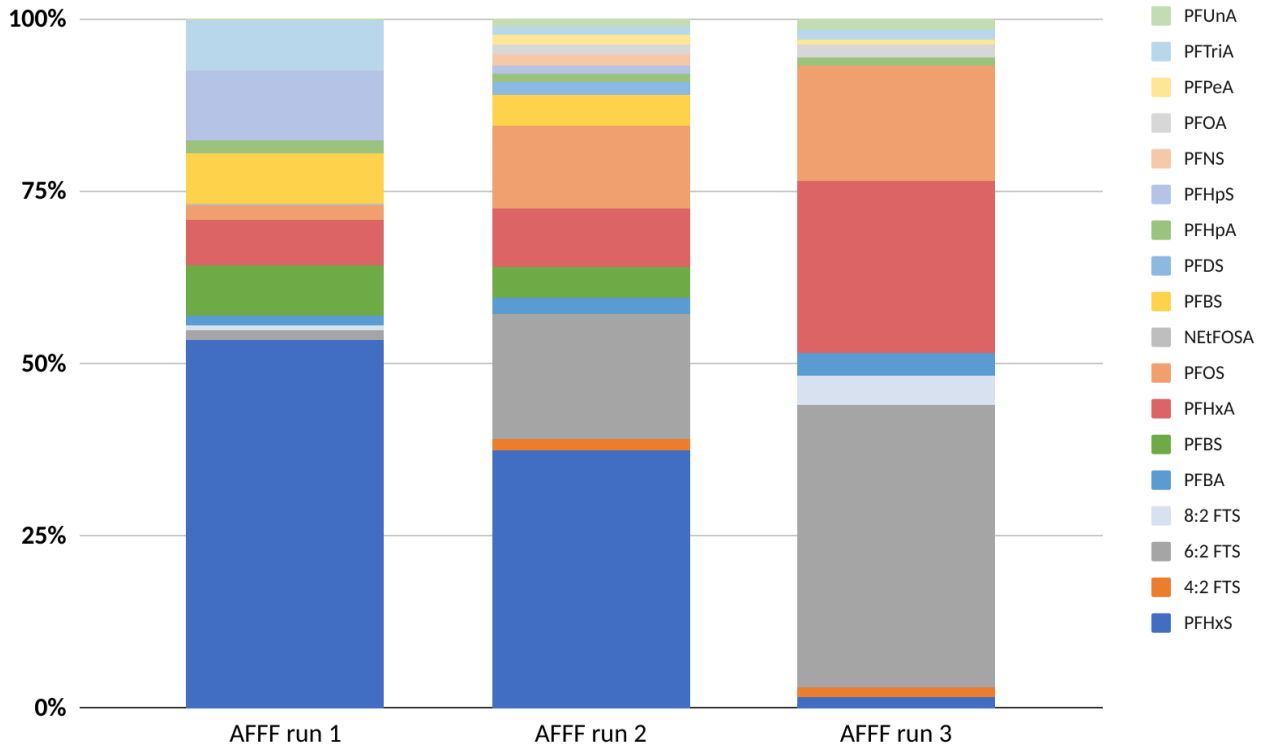


Figure 4-2 : PFAS signature in the AFFF

7.1.3.2.Destruction and Removal Efficiency

The DRE was computed for each run, as it is a parameter of interest to assess the performance of thermal treatment of PFAS according to the 2024 EPA interim guidance on destruction and disposal of PFAS material. When a PFAS is not detected at the stack, the MDL was used. When a PFAS is detected between the MDL and LoQ, the LoQ is used. This approach underestimates DRE.

DRE for the 6 runs of campaign 1 are provided in **Appendix XI (AFFF) and XII (contaminated soil).** Table 5 gives an overview of the DRE obtained in AFFF run#1 for the 20 most concentrated PFAS. PFAS in blue were not detected at the stack, while PFAS in yellow were detected below the LoQ.

Of the 20 most concentrated PFAS in the waste, 14 were either undetected at the stack or below the LoQ. The 6 PFAS detected above the MDL at the stack include 4 coming from process water (PFHxA, PFOA, PFPeA, PFHpA). In conclusion, only 2 PFAS were detected at the stack above LoQ while present in the waste and not coming from process water (PFDA, PFDoA).

	AFFF TF Feed Stream	R1 Stack Emissions	DRE
	Avg lb/hr	lb/hr	
Feed Rate	2,421		
Target Compounds	**lb/hr**	**lb/hr**	**%**
Perfluorohexanesulfonic acid (PFHxS)	0.179	0.000000027	> 99.9999
Perfluorooctanesulfonic acid (PFOS)	0.0436	0.000000038	> 99.9999
Perfluorobutanesulfonic acid (PFBS)	0.0245	0.000000075	> 99.9997
Perfluoroheptanesulfonic acid (PFHpS)	0.0232	0.000000027	> 99.9999
Perfluoropentanesulfonic acid (PFPeS)	0.0226	0.000000024	> 99.9999

Perfluorohexanoic acid (PFHxA)	0.0223	0.000000605	99.9973
Perfluorooctanoic acid (PFOA)	0.01790	0.00000078	99.9956
Perfluoropentanoic acid (PFPeA)	0.00593	0.00000033	99.9945
Perfluoroheptanoic acid (PFHpA)	0.00583	0.000000912	99.9844
Perfluorobutanoic acid (PFBA)	0.00443	0.00000087	> 99.9804
6:2 FTS	0.00426	0.00000080	> 99.9812
8:2 FTS	0.00197	0.000000026	> 99.9987
2-(N-ethylperfluoro-1-octanesulfonamido) ethanol	0.000455	0.000000037	> 99.9919
Perfluorononanesulfonic acid (PFNS)	0.000327	0.000000019	> 99.9942
2-(N-methylperfluoro-1-octanesulfonamido) ethanol	0.000259	0.0000016	> 99.3822
Perfluorooctanesulfonamide (FOSA)	0.000223	0.000000024	> 99.9892
Perfluorodecanoic acid (PFDA)	0.000178	0.000000183	99.8972
Perfluorodecanesulfonic acid (PFDS)	0.000164	0.000000025	> 99.9848
Perfluorododecanesulfonic acid (PFDoS)	0.000087	0.000000022	> 99.9746
Perfluorododecanoic acid (PFDoA)	0.0000675	0.000000081	99.8807

Table 5 - DRE obtained for the 20 most concentrated PFAS in AFFF run #1. *PFAS in blue were not detected at the stack, while PFAS in yellow were detected below the LoQ.*

Figure 5 shows how the DRE increases with the PFAS feed rate in the kiln. This is, in part, an effect of the conservative approach to quantify PFAS not detected at the stack (considered as present at MDL). But the same correlation is observed for PFAS detected at the stack. There seems to be a residual level of PFAS detected at the stack, independent of the PFAS concentration in the waste. As an effect, DRE increases with the PFAS concentration in the waste. For the same feed rate, PFSAs seem to have a higher DRE than PFCAs. This may be explained by the background presence of PFCAs in the process water.

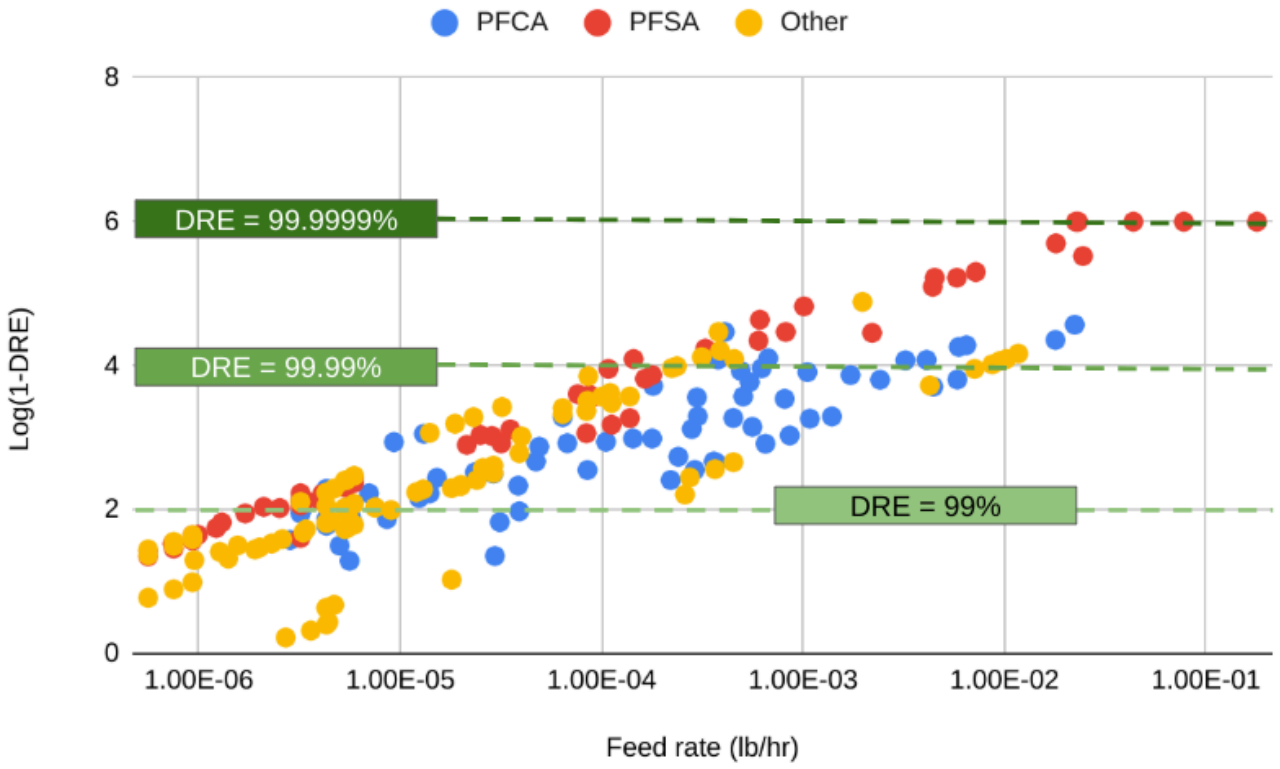


Figure 5 - Relation between DRE and feed rate. Log scale used for feed rate in the x-axis.

DRE > 99.9999% was obtained for the 4 of the 5 most concentrated PFAS : PFOS, PFHxS, PFPeS and PFHpS. For the 3 PFAS classified as Persistent Organic Pollutant (POP) according to Stockholm convention (PFOS,PFOA,PFHxS), the DRE was above 99.99% and even above 99.9999% for 2 of them (PFOS, PFHxS). PFOS and PFOA are also listed as hazardous substances under CERCLA.

Because of the stack gas contamination from process water, the DRE are artificially lower than they should be for the following PFAS: PFHxA, PFPeA, PFHpA, PFOA, HFPO-DA, PFHxS, PFOS, PFBA, and PFBS. **Table 6** shows the total DRE for OTM 45[3] obtained during campaign 1 for each run.

	A3F #1	A3F #2	A3F #3	Soil#1	Soil#2	Soil #3
PFAS feedrate (lb/hr)	0.3575	0.1236	0.0187	0.0265	0.0215	0.0161
Total DRE from OTM 45 train	99.9974%	99.9937%	99.9584%	99.9741%	99.9540%	99.9613%

Table 6 - Total DRE for the 6 runs of campaign 1

7.1.3.3. Products of Incomplete Destruction (OTM-50)

OTM-50 is a sampling method to trap and detect 30 PICs, that is to say, volatile short-chain PFAS potentially formed during AFFF or contaminated soil incineration. OTM-50 results were negative for all runs, showing that either no PICs were formed during the incineration process, or they were present at levels below the MDL. OTM-50 measurements for campaign 1 are presented in **Appendix XII.**

7.1.4. Total Destruction Efficiency

The Total Destruction Efficiency was computed for each run, as described in section 4.2. When a PFAS was not detected in the liquid, solid residue or at the stack, the MDL is used. When a PFAS was detected below the LoQ, the LoQ was used.

The total DE for the A3F and contaminated soil runs were very low or even negative (ranging from negative to 74%). This is due to the high MDL for OTM-50 (0.01 lb/hr to 0.1 lb/hr). The PFAS concentration in the waste was sometimes lower than the MDL for OTM-50. This demonstrates the need to improve detection levels for OTM-50 to align them with EPA Methods 537 and 1633.

The comparison between PFAS mass emission rate in the process water (PFAS entering the incinerator) vs PFAS mass emission rate in the blowdown (PFAS exiting the incinerator) shows that there is a transmission of PFAS from the process water to the stack gas. PFAS mass emission rate in the process water is higher than in the blowdown.

7.1.5 HF Measurements

HF measurements are available in **Appendix XIV** for campaign 1. HWC MACT standards for existing hazardous waste incinerators from §63.1219 applicable to the incinerator at Veolia's Port Arthur facility require HF at the stack to be below 32 ppmv, dry, at 7% O2. HF remained below the regulatory limit during campaign 1 testing.

7.1.6. Non Targeted Screening

Veolia has developed its own methodology to perform Non Targeted Screening (NTS) analysis on some of the pre-test samples. The objective of this innovative methodology is to detect PFAS that are not targeted in OTM-45 and OTM-50. It was developed by a Veolia R&D team based in France.

3

Veolia has looked for non-targeted PFAS in the AFFF used in run#1, and in the OTM-45 fractions of run #1 and the related baseline run.

Prior to non-targeted screening, sample preparation must be as non-selective as possible. The analysis was performed on AFFF after dilution and on OTM-45 fractions, which allows the extraction of a wide range of PFAS.

The non-targeted screening approach uses Liquid Chromatography coupled to High-Resolution Mass Spectrometry (LC/HRMS) as the analytical technique. It was performed on an Exploris 120 system (Thermo Scientific).

For the detection part, two acquisition methods are used to maximize the amount of recorded information and enable efficient data processing:

- Fullscan ESI+/ESI- R 60,000 for peak detection
- Fullscan ESI+/ESI- + DDA-MS2 (Data-dependent analysis) for identification.

All samples are analyzed in triplicates with both methods. Data processing is the most complex part of non-targeted screening analyses. A complete workflow in three main campaigns was developed using Compound Discoverer software (Thermo Scientific):

- Pre-processing for peak detection
- Signal prioritization to isolate data corresponding to potential PFAS compounds
    - Number of Fluorine in proposed molecular formulas
    - KMD (Kendrick Mass Defect) to identify homologue series
    - PFAS-specific fragments
- Annotation or identification of prioritized signals
    - Use of spectral libraries
    - In-silico fragmentation
    - Comparison with literature data

NTS results on the AFFF used in run #1

To test the reliability of the NTS method developed by Veolia, results from NTS (performed by Veolia) were compared with targeted analysis (performed by Eurofins) for the AFFF. If the NTS method is valid, it should detect all the targeted compounds detected by Eurofins.

It appears that most of the compounds detected in targeted analysis are also detected in NTS. 6 out of 32 detected in the targeted analysis were not detected in the NTS. One PFAS listed in the targeted analysis was not detected by Eurofins but by the NTS.

Analyte	Targeted Analyis	NTS Analysis	Suspect Analysis
Perfluorohexanesulfonic acid (PFHxS)	■	■	
Perfluorooctanesulfonic acid (PFOS)	■	■	
Perfluoropentanesulfonic acid (PFPeS)	■	■	
Perfluoroheptanesulfonic acid (PFHpS)	■	■	
Perfluorohexanoic acid (PFHxA)	■	■	
Perfluorooctanoic acid (PFOA)	■	■	
Perfluoropentanoic acid (PFPeA)	■	■	
Perfluoroheptanoic acid (PFHpA)	■	■	
Perfluorobutanoic acid (PFBA)	■	■	
1H,1H,2H,2H-Perfluorooctane sulfonic acid (6:2 FTS)	■	■	
Perfluorobutanesulfonic acid (PFBS)	■	■	
1H,1H,2H,2H-Perfluorodecane sulfonic acid (8:2 FTS)	■	■	
N-ethylperfluorooctane sulfonamidoethanol (NEtFOSE)	■		■
Perfluorononanesulfonic acid (PFNS)	■	■	
N-methylperfluorooctane sulfonamidoethanol (NMeFOSE)	■		
Perfluorooctanesulfonamide (PFOSAm)	■		■
Perfluorodecanoic acid (PFDA)	■	■	
Perfluorodecanesulfonic acid (PFDS)	■	■	
Perfluorododecanesulfonic acid (PFDoS)	■	■	
Perfluorododecanoic acid (PFDoA)	■	■	

Analyte	Targeted Analyis	NTS Analysis	Suspect Analysis
Perfluorononanoic acid (PFNA)	■	■	
N-ethylperfluorooctane sulfonamide (NEtFOSA)	■	■	
1H,1H,2H,2H-Perfluorohexane sulfonic acid (4:2 FTS)	■		■
Perfluorotetradecanoic acid (PFTeDA)	■		■
N-methylperfluorooctane sulfonamide (NMeFOSA)	■		■
Perfluoro-4-methoxybutanoic acid (PFMBA)	■		
3-Perfluoropentylpropanoic acid (5:3 FTCA)	■		
Perfluoro-3-methoxypropanoic acid (PFMePA)	■		
Perfluorotridecanoic acid (PFTrDA)	■		
Perfluoroundecanoic acid (PFUnA)	■		■
Perfluoro (2-ethoxyethane) sulfonic acid (PFEESA)	■	■	
N-ethylperfluorooctanesulfonamidoacetic acid (NEtFOSAA)	■		
N-methylperfluorooctanesulfonamidoacetic acid (NMeFOSAA)			
Hexafluoropropylene Oxide Dimer Acid (HFPO-DA)			
4,8-Dioxa-3H-perfluorononanoic acid (DONA)			■
Nonafluoro-3,6-dioxaheptanoic acid (NFDHA)			
9-Chlorohexadecafluoro-3-oxanonan-1-sulfonic acid (9Cl-PF3ONS)			
11-Chloroeicosafluoro-3-oxaundecan-1-sulfonic acid (11Cl-PF3OUdS)			
3-Perfluoropropylpropanoic acid (3:3 FTCA)			

Table 7 - comparing PFAS detected in the targeted analysis (Eurofins) vs NTS (Veolia)

In total, 321 compounds suspected to be PFAS were detected in the AFFF, many more than the 32 detected in the targeted analysis. Among the 20 compounds with the highest intensities in the NTS, only 5 are in the targeted analysis. However, for the LC/HRMS used for NTS, the intensity can be different for compounds at the same concentration, as it is not fully proportional to concentration.

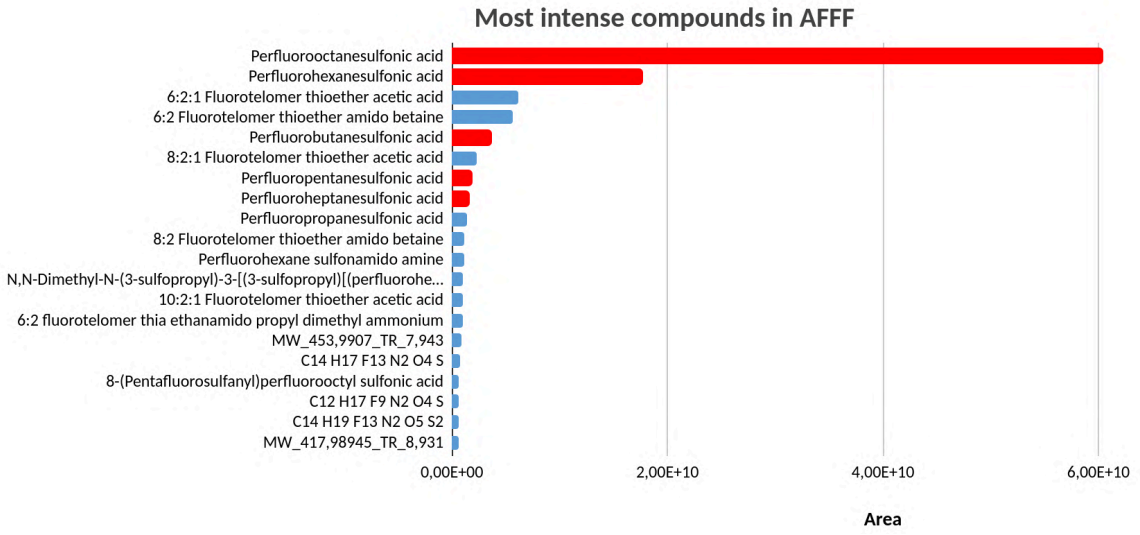


Figure 6 - Results of NTS for AFFF. The compounds with the highest intensity according to NTS. In red, compounds were also detected in the targeted analysis.

NTS results on OTM-45 fractions (air emissions)

PFAS detected in OTM-45 fractions from AFFF incineration were compared to PFAS detected in OTM-45 fractions from the baseline run. Only 8 PFAS compounds were detected in the OTM-45 fractions from AFFF incineration exclusive of being detected in the baseline. All these compounds had very low responses, indicating low concentration. None of the most intense compounds detected in the AFFF were detected in the OTM-45 fractions.

	Fraction 1		Fraction 2		Fraction 3		Fraction 4		Presence in AFFF solution
	Specific to Run with AFFF	Common to Run with and without AFFF	Specific to Run with AFFF	Common to Run with and without AFFF	Specific to Run with AFFF	Common to Run with and without AFFF	Specific to Run with AFFF	Common to Run with and without AFFF	
(Perfluoroheptyl)(sulfonylamino)acetic acid									No
Bisphenol AF									No
MW_1379.81621@RT_11.861									No
MW_165.95489@RT_0.661									Yes
MW_197.99153@RT_4.872									Yes
MW_394.98705@RT_9.191									No
MW_443.93372@RT_7.576	3*								No
MW_453.99070@RT_7.943									Yes
MW_507.96432@RT_8.292									No
MW_517.98348@RT_9.606									Yes
MW_670.96845@RT_10.031									No
MW_724.43756@RT_10.777									No
Perfluorobutanesulfonic acid									Yes
Perfluorobutanoic acid									Yes
Perfluorodecanoic acid			4*						Yes
Perfluorododecanoic acid									Yes
Perfluoroheptanoic acid									Yes
Perfluorohexanoic acid									Yes
Perfluorononanoic acid									Yes
Perfluorooctanoic acid									Yes
Perfluoropentanoic acid									Yes
Perfluoroundecanoic acid			3*						Yes

Table 8 - Results of NTS for OTM 45 fractions from AFFF incineration and from the baseline. *MW means "molecular weight" : in that case, a PFAS is identified but its formula is unknown.*

Conclusions

NTS shows that more PFAS were present in the AFFF than the 32 PFAS detected in the targeted analysis. Results show that these non-targeted PFAS were not detected in OTM-45 fractions, which suggests they were destroyed during the incineration process, as observed for targeted PFAS. It must be noted, however, as the OTM-45 sampling and sample preparation used for the NTS are selective to semi-volatile PFAS (boiling point > 212°F), it is possible that some PFAS are emitted in the air but not trapped in OTM-45.

7.2. Campaign 2 : Incineration of A3F, contaminated soil, CF4 and spent carbon

Campaign 2 summary :

Campaign 2 confirms campaign 1 results.

In a majority of runs (11 out of 15), no PICs were detected via OTM-50. CF4 was detected in three runs : during the CF4 run, a baseline run and an AFFF incineration run. In these 2 latter runs, the CF4 level is close to the MDL. The source of the CF4 in the latter two tests is unclear. It is unlikely to form as a PIC due to the high H/F ratio in the SCC during these tests. The incineration test where CF4 was fed into the SCC indicated a DRE of less than 50%, consistent with predictions that temperatures above 2,550 F are necessary to destroy this compound. Another OTM-50 PFAS was detected during a baseline at a level close to MDL. It is unlikely to be a PIC due to its low thermal stability (polyfluoroether) and is more likely a contaminant from the analytical laboratory.

DRE > 99.999% is reached for PFOS in 3 runs. The waste streams incinerated in this campaign are less concentrated in PFAS than during campaign 1 which explains why the 99.9999% could not be reached.

Solid residues are PFAS-free, or contain PFAS at levels close to MDL.

Liquid residues contain PFAS at low level (ppt), reflecting the background presence of PFAS on the pond water supplying the process water of the incinerator.

Contrary to campaign 1, a contamination issue is suspected in the OTM-45 results: 4 runs contained background >C10 PFAS detected in OTM-45 fractions, whose source is still under investigation.

7.2.1. Waste Analysis

Four waste streams were injected separately: A3F, spent carbon, AFFF-contaminated soil and CF4. Waste profiles are available in **Appendix XV for AFFF, spent carbon and AFFF-contaminated soil.**

Run 2 and 3 of A3F were performed with the same A3F but under different conditions. In run 3, lime was injected at 2 gpm, simultaneously with A3F injection, to study its ability to enhance PFAS destruction.

7.2.2. Solid and Liquid Residues

In the solid residues, no PFAS was detected in significant quantities. All slag and filtercake samples were PFAS-free. PFAS were detected in four samples of ashes, with detection level close to MDL. Detailed results of these 4 samples are available in **Appendix XVII. Table 10** gives a summary of the results for PFAS in solid residues.

	Baseline	Run 1	Baseline 2	Run 2	Run 3[4]
A3F	**ashes : PFAS detected close to MDL** filtercake : ND slag : ND	ashes : ND slag : ND Filter cake : ND	**ashes : PFAS detected close to MDL** filtercake : ND slag : ND	**ashes : PFAS detected close to MDL** filtercake : ND slag : ND	ashes : ND slag : ND Filter cake : ND
Carbon	ashes : ND slag : ND Filter cake : ND	ashes : ND slag : ND Filter cake : ND	N/A	ashes : ND slag : ND Filter cake : ND	N/A
CF4	Ashes : ND Filter cake : ND Slag : ND	Ashes : ND Filter cake : ND Slag : ND	N/A	N/A	N/A
Contaminated soil	**ashes : PFAS detected close to MDL** filtercake : ND slag : ND	Ashes : ND Filter cake : ND Slag : ND	Ashes : ND Filter cake : ND Slag : ND	Ashes : ND Filter cake : ND Slag : ND	Ashes : ND Filter cake : ND Slag : ND

Table 9 - PFAS analysis in solid residues of the campaign 2

For the liquid residues, 20 blowdown samples were collected. 16 had the same signature and PFAS concentration as observed in the campaign 1, while 4 of them had PFAS levels above 200 ppt (A3F

[4] In run#3 A3F, lime is injected simultaneously to A3F injection

baseline #1, A3F run#2, A3F run3+24hr, carbon run#2+24 hr). Three of these samples were collected at a time when no PFAS were being incinerated. It is unclear whether these results come from laboratory background or from PFAS in the blowdown water that is transferred from the stack gas. The profile of these 4 atypical samples is available in **Figure 7.**

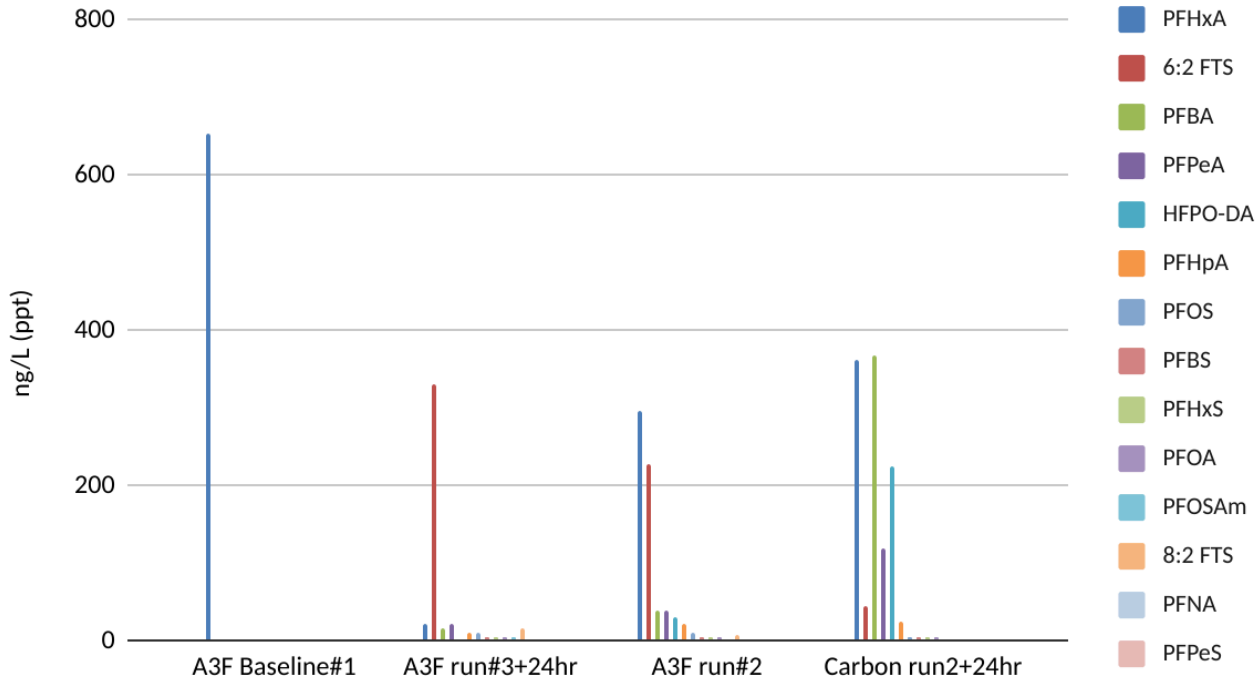


Figure 7 - Profile of the atypical samples of blowdowns obtained in campaign 2

In the 16 other blowdown samples, the same results as in campaign 1 were obtained. Because of background PFAS presence in the pond water, a dozen PFAS were detected at ppt level, both in the baseline sampled at the beginning of the campaign (before PFAS incineration), and in the samples collected during PFAS incineration. PFAS are twice to four times more concentrated in the blowdowns than in the pond water because of evaporation. Results for the typical blowdown samples are shown in **Figure 8.**

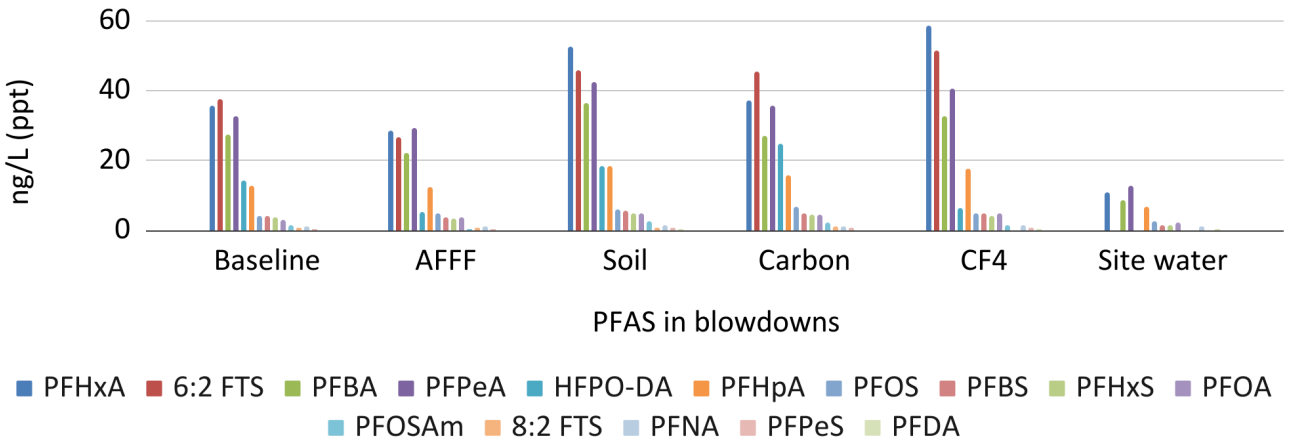


Figure 8 - PFAS signature in the blowdown (left) vs process water (on the right side of the graph)

Table 10 shows the results for process water collected in campaign 1 and 2. PFAS concentration in process water is similar in campaign 1 and 2, which means that the PFAS concentrations in the pond water are stable. The only differences are :

- the detection of PFDA in campaign 2 at very low level (<1 ppt), undetected in campaign 1
- the detection of HFPO-DA in campaign 1 at very low level (<3 ppt) undetected in campaign 2

PFAS	Concentration process water campaign 1 (ng/L)	Concentration process water campaign 2 (ng/L)	Concentration in blowdown baseline campaign 2 (ng/L)
PFHxA	14.90	11.1	35.73
PFBA	11.20	8.57	27.50
PFPeA	16.10	12.9	32.61
PFHpA	8.41	6.81	12.80
PFOS	2.48	2.57	4.10
PFBS	1.81	1.36	4.14
PFHxS	1.49	1.53	3.78
PFOA	2.88	2.11	2.89
PFNA	1.26	1.21	1.02
PFDA	ND (0.293)	0.344	ND (0.292)
HFPO-DA	2.02	ND (1.56)	14.14
6:2 FTS	ND (1.07)	ND (1.07)	37.59
PFOSAm	ND (0.293)	ND (0.293)	1.56
8:2 FTS	ND (0.586)	ND (0.586)	0.86
PFPeS	ND (0.293)	ND (0.293)	0.57

Table 10 - PFAS concentration in process water in campaign 1 and 2 and in the blowdown baseline in campaign 2. *All PFAS listed in EPA 1633 were tested but only the ones detected are shown. Concentration in the blowdown baseline in campaign 2 is an average of all the baselines collected, excluding the 1st baseline collected during AFFF incineration which is considered not representative.*

In campaign 2, the only differences between the PFAS signature in the process water and blowdown is the presence of:

- **6:2 FTS** in the blowdown - could be a PIC from previous PFAS incineration or from the PFAS campaign.
- **HFPO-DA -** was detected in every blowdown sample, and was also detected in the process water during campaign 1 but not campaign 2. It is likely that this was a false negative and HFPO-DA was also in the process water during the campaign 2 at very low level, maybe non detectable. Evaporation during the process makes it detectable in the blowdown.
- **8:2 FTS, PFOSAm and PFPeS** were found in low quantities (1 ppt or less, very close to MDL) in some blowdowns but not the process water. It is possible that they were also present in the process water below MDL, or that there was a false positive in the blowdowns samples.

Detailed results for PFAS concentration in liquid residues for Campaign 2 are available in **Appendix XVI.**

7.2.3. Air Emissions

7.2.3.1. PFAS Mass Emission Rate at Stack (OTM-45)

The following OTM-45 samples were collected during the campaign 2, with 2 patterns emerging :

- **Pattern 1 :** PFAS concentration & signature at stack is similar to campaign 1. PFAS < C10 were found at stack at < 2 10^-5 lb/hr in total. PFAS signature at the stack reflects the PFAS signature in process water.
- **Pattern 2** : High concentration at stack for C>10 PFAS: PFDA, PFUnA, PFDoA, PFTriA, PFTeA. Total PFAS emissions < 9.5 10^-5 lb/hr)

Runs	Comment on OTM-45 results
A3F Baseline#1	Pattern 1
A3F run#1	**Pattern 2**
A3F run #2	Pattern 1
A3F Baseline #2	Pattern 1
A3F run#3 (lime)	Pattern 1
Soil Baseline #1	Pattern 1
Soil run #1	**Pattern 2**
Carbon baseline #1	Pattern 1
Carbon run #1	**Pattern 2**
Carbon run #2	Pattern 1
CF4 baseline	Pattern 1
CF4 run#1	Pattern 1
Soil baseline #2	Pattern 1
Soil run#2	Pattern 1
Soil run #3	**Pattern 2**

Table 11 -Repartition of each run to pattern 1 and 2

Table 12 shows the PFAS stack emissions (OTM-45) for the different baselines performed prior to each incineration test. Two baselines were performed for AFFF because there were 2 conditions (with and without lime). Two baselines were performed for contaminated soil because run#2 and run#3 were performed 5 days after run#1 because of equipment damage.

The 6 baseline runs were similar in terms of PFAS signature and PFAS stack emissions. Compared to the baseline tests in campaign 1, there are the same dominant PFAS, with the additional presence of FOSA, NEtFOSE, NMeFOSA, 6:2 FTS and 6:2 FTUCA at very low levels close to MDL.

PFAS	Baseline before AFFF #1	Baseline before AFFF #2	Baseline before Soil #1	Baseline before Soil #2	Baseline before Carbon	Baseline before CF4
PFBS	0.0000000601	0.0000000482	0.0000000463	0.0000000497	0.000000049	0.0000000585
PFBA	0.0000007150	0.0000007620	0.000000573	0.000000821	0.000000521	0.000000400
PFDS	ND (0.000000018)	0.0000003860	ND (0.000000018)	ND (0.000000018)	ND (0.000000018)	ND (0.000000018)
PFDA	0.0000001480	0.0000002500	0.000000324	0.000000995	0.000000515	0.000000118
PFDoS	ND (0.000000026)	ND (0.000000026)	ND (0.000000026)	ND (0.000000026)	ND (0.000000026)	ND (0.000000026)
PFDoA	0.0000000787	0.0000001280	0.000000171	0.000000446	0.000000252	0.0000000628
PFHpS	ND (0.000000023)	ND (0.000000023)	ND (0.000000023)	ND (0.000000023)	ND (0.000000023)	ND (0.000000023)
PFHpA	0.0000005390	0.0000006590	0.000000692	0.00000164	0.000000890	0.000000382
Perfluorohexadecanoic acid	0.0000000283	0.0000000542	0.0000000710	0.000000206	0.000000133	0.0000000267
PFHxS	0.0000000168	0.0000000123	0.0000000162	0.0000000115	0.000000011	<0.000000019 DLL
PFHxA	0.0000005770	0.0000006340	0.000000556	0.00000108	0.000000585	0.000000379
PFNS	ND (0.000000017)	ND (0.000000017)	ND (0.000000017)	ND (0.000000017)	ND (0.000000017)	ND (0.000000018)
PFNA	0.0000002720	0.0000004280	0.000000526	0.00000160	0.000000800	0.000000220
Perfluorooctadecanoic acid	<0.000000034 DLL	0.0000000154	0.0000000263	0.0000000656	0.000000031	ND (0.000000029)
FOSA	<0.000000077 DLL	<0.000000079 DLL	<0.000000077 DLL	<0.000000075 DLL	<0.000000079 DLL	<0.000000078 DLL
PFOS	0.0000000971	0.0000000480	0.0000000646	<0.000000049 DLL	<0.000000046 DLL	0.0000000213
PFOA	0.0000006080	0.0000006770	0.000000704	0.00000170	0.000000899	0.000000319
PFPeS	ND (0.000000013)	ND (0.000000013)	ND (0.000000013)	ND (0.000000013)	ND (0.000000013)	ND (0.000000013)
PFPeA	0.0000004290	0.0000004630	0.000000385	0.000000717	0.000000422	0.000000193
PFTeA	0.0000000783	0.0000001260	0.000000135	0.000000348	0.000000216	0.0000000590
PFTriA	0.0000000652	0.0000001180	0.000000152	0.000000445	0.000000238	0.0000000506
PFUnA	0.0000000922	0.0000001530	0.000000189	0.000000667	0.000000352	0.0000000706
9-chloro.	ND (0.000000017)	ND (0.000000017)	ND (0.000000017)	ND (0.000000017)	ND (0.000000017)	ND (0.000000017)
NEtFOSA	ND (0.000000039)	ND (0.000000039)	ND (0.000000040)	ND (0.000000040)	ND (0.000000039)	<0.000000041 DLL
NEtFOSAA	ND (0.000000025)	ND (0.000000025)	ND (0.000000025)	ND (0.000000025)	ND (0.000000025)	ND (0.000000025)
NMeFOSAA	ND (0.000000027)	ND (0.000000027)	ND (0.000000028)	ND (0.000000028)	ND (0.000000027)	ND (0.000000028)
11-chloro.	ND (0.000000023)	ND (0.000000023)	ND (0.000000023)	ND (0.000000023)	ND (0.000000023)	ND (0.000000023)
NEtFOSE	ND (0.000000039)	ND (0.000000038)	ND (0.000000039)	ND (0.000000039)	ND (0.000000039)	0.0000000193
NMeFOSE	ND (0.00000021)	<0.00000022 DLL	<0.00000022 DLL	<0.00000022 DLL	ND (0.00000022)	<0.00000024 DLL
PES	ND (0.000000020)	ND (0.000000021)	ND (0.000000021)	ND (0.000000021)	ND (0.000000020)	ND (0.000000020)
PFECA	<0.000000030 DLL	<0.000000031 DLL	<0.000000033 DLL	0.0000000206	<0.000000030 DLL	ND (0.000000027)
PFECA	ND (0.000000030)	ND (0.000000030)	ND (0.000000030)	ND (0.000000030)	ND (0.000000030)	ND (0.000000030)
PFECA	ND (0.000000058)	ND (0.000000058)	ND (0.000000058)	<0.000000065 DLL	ND (0.000000058)	ND (0.000000059)
PFECHS	ND (0.000000026)	ND (0.000000026)	ND (0.000000027)	ND (0.000000026)	ND (0.000000026)	ND (0.000000026)
DONA	ND (0.000000031)	ND (0.000000031)	ND (0.000000032)	ND (0.000000031)	ND (0.000000031)	ND (0.000000031)
HFPO-DA	ND (0.00000037)	ND (0.00000037)	ND (0.00000037)	ND (0.00000037)	ND (0.00000037)	ND (0.00000038)
NMeFOSA	0.0000004070	0.0000001190	0.0000000942	0.0000000427	0.000000048	0.0000000755
10:2 FTCA	ND (0.00000011)	ND (0.00000011)	ND (0.00000011)	ND (0.00000011)	ND (0.00000011)	ND (0.00000011)
10:2 FTS	ND (0.000000036)	ND (0.000000036)	ND (0.000000037)	ND (0.000000037)	ND (0.000000037)	ND (0.000000037)
3:3 FTCA	ND (0.000000046)	ND (0.000000046)	ND (0.000000047)	ND (0.000000047)	ND (0.000000046)	ND (0.000000047)
4:2 FTS	ND (0.000000014)	ND (0.000000014)	ND (0.000000015)	ND (0.000000015)	ND (0.000000014)	ND (0.000000015)
5:3 FTCA	ND (0.000000063)	ND (0.000000064)	ND (0.000000064)	ND (0.000000064)	ND (0.000000063)	ND (0.000000064)
6:2 FTCA	ND (0.000000091)	ND (0.000000091)	ND (0.000000092)	ND (0.000000092)	ND (0.000000091)	ND (0.000000092)
6:2 FTS	<0.00000024 DLL	<0.00000023 DLL	<0.00000030 DLL	<0.00000024 DLL	0.000000148	0.000000973
6:2 FTUCA	<0.000000025 DLL	ND (0.000000019)	<0.000000021 DLL	ND (0.000000019)	ND (0.000000019)	<0.000000024 DLL
7:3 FTCA	ND (0.000000054)	ND (0.000000055)	ND (0.000000055)	ND (0.000000055)	ND (0.000000055)	ND (0.000000055)
8:2 FTCA	ND (0.000000079)	ND (0.000000079)	ND (0.000000080)	ND (0.000000080)	ND (0.000000079)	ND (0.000000080)
8:2 FTS	ND (0.000000024)	ND (0.000000024)	ND (0.000000025)	ND (0.000000025)	ND (0.000000024)	ND (0.000000024)
8:2 FTUCA	ND (0.000000031)	ND (0.000000032)	ND (0.000000032)	ND (0.000000032)	ND (0.000000031)	ND (0.000000032)
Total PFAS (lb/hr)	6.18E-06	7.00E-06	6.74E-06	1.28E-05	7.86E-06	5.18E-06

Table 12 - PFAS emissions at the stack (OTM-45) during baselines. *PFAS highlighted in blue were detected in the process water. Values in bold when PFAS was detected above MDL in at least one of the OTM-45 fractions.*

Tables 13-1 to 13-4 presents PFAS mass emission rate at stack based on OTM-45 for the four different PFAS-containing wastes: AFFF, contaminated soil, carbon, and CF4.

	Run 1		Run 2		Run 3	
PFAS	Waste feed rate (lb/hr)	Stack emission (lb/hr)	Waste feed rate (lb/hr)	Stack emission (lb/hr)	Waste feed rate (lb/hr)	Stack emission (lb/hr)
PFBS	0.0000612000	**0.0000001190**	0.0001220000	**0.0000000745**	0.0001270000	**0.0000000606**
PFBA	0.0000725000	**0.0000004360**	0.0004010000	**0.0000009040**	0.0004160000	**0.0000004470**
PFDS	ND (0.0000052)	ND (0.000000018)	ND (0.0000057)	ND (0.000000018)	ND (0.0000059)	ND (0.000000019)
PFDA	0.0000125000	**0.0000005170**	0.0001120000	**0.0000004880**	0.0001160000	**0.0000001220**
PFDoS	ND (0.0000052)	ND (0.000000025)	ND (0.0000057)	ND (0.000000025)	ND (0.0000059)	ND (0.000000027)
PFDoA	0.0000053700	**0.0000017200**	0.0000447000	**0.0000001830**	0.0000464000	**0.0000002420**
PFHpS	0.0000143000	ND (0.000000023)	0.0000255000	ND (0.000000023)	0.0000264000	**<0.000000026 DLL**
PFHpA	0.0000319000	**0.0000002250**	0.0001100000	**0.0000014300**	0.0001140000	**0.0000002300**
Perfluorohexadecanoic acid	--	**0.0000019900**	--	**0.0000000396**	--	**0.0000001260**
PFHxS	0.0005680000	**0.0000000097**	0.0012800000	**0.0000000158**	0.0013200000	**0.0000000378**
PFHxA	0.0007450000	**0.0000003300**	0.0023200000	**0.0000011500**	0.0024000000	**0.0000002280**
PFNS	ND (0.0000052)	ND (0.000000017)	ND (0.0000057)	ND (0.000000017)	ND (0.0000059)	ND (0.000000018)
PFNA	ND (0.0000052)	**0.0000003180**	0.0000182000	**0.0000009920**	0.0000189000	**0.0000001280**
Perfluorooctadecanoic acid	--	**0.0000005490**	--	**<0.000000038 DLL**	--	**0.0000000595**
FOSA	0.0000240000	**<0.000000078 DLL**	0.0000661000	**<0.000000078 DLL**	0.0000685000	**<0.000000079 DLL**
PFOS	0.0030800000	**0.0000000478**	0.0085300000	**0.0000000837**	0.0088500000	**0.0000005860**
PFOA	0.0001200000	**0.0000002600**	0.0004820000	**0.0000013900**	0.0004990000	**0.0000008870**
PFPeS	0.0000775000	ND (0.000000013)	0.0002520000	ND (0.000000013)	0.0002620000	**0.0000000074**
PFPeA	0.0000505000	**0.0000002190**	0.0002660000	**0.0000007220**	0.0002760000	**0.0000001700**
PFTeA	ND (0.0000052)	**0.0000030500**	0.0000165000	**0.0000001650**	0.0000171000	**0.0000002750**
PFTriA	0.0000059400	**0.0000025300**	0.0000116000	**0.0000001580**	0.0000120000	**0.0000002800**
PFUnA	0.0000099600	**0.0000008970**	0.0000759000	**0.0000002480**	0.0000787000	**0.0000001610**
9-chloro.	ND (0.0000052)	ND (0.000000017)	ND (0.0000057)	ND (0.000000017)	ND (0.0000059)	ND (0.000000017)
NEtFOSA	ND (0.000011)	ND (0.000000039)	ND (0.000011)	ND (0.000000039)	ND (0.000012)	ND (0.000000041)
NEtFOSAA	0.0000073400	ND (0.000000025)	0.0000340000	ND (0.000000025)	0.0000352000	ND (0.000000026)
NMeFOSAA	ND (0.0000063)	ND (0.000000027)	ND (0.0000069)	ND (0.000000027)	ND (0.0000071)	ND (0.000000028)
11-chloro.	ND (0.0000052)	ND (0.000000023)	ND (0.0000057)	ND (0.000000023)	ND (0.0000059)	ND (0.000000024)
NEtFOSE	ND (0.000011)	ND (0.000000038)	0.0000243000	ND (0.000000038)	0.0000252000	ND (0.000000040)
NMeFOSE	ND (0.000011)	ND (0.00000021)	ND (0.000011)	ND (0.00000021)	ND (0.000012)	**<0.00000023 DLL**
PES	--	ND (0.000000020)	--	ND (0.000000020)	--	ND (0.000000021)
PFECA	--	ND (0.000000027)	--	**0.0000000171**	--	ND (0.000000029)
PFECA	--	ND (0.000000030)	--	ND (0.000000030)	--	ND (0.000000031)
PFECA	--	ND (0.000000058)	--	ND (0.000000058)	--	ND (0.000000060)
PFECHS	--	ND (0.000000026)	--	ND (0.000000026)	--	ND (0.000000027)
DONA	ND (0.0000052)	ND (0.000000031)	ND (0.0000057)	ND (0.000000031)	ND (0.0000059)	ND (0.000000032)
HFPO-DA	0.0000227000	ND (0.00000037)	0.0000202000	ND (0.00000037)	0.0000210000	ND (0.00000038)
NMeFOSA	ND (0.000011)	**0.0000000713**	ND (0.000011)	**0.0000001140**	ND (0.000012)	**0.0000001460**
10:2 FTCA	--	ND (0.00000011)	--	ND (0.00000011)	--	ND (0.00000011)
10:2 FTS	--	ND (0.000000036)	--	ND (0.000000036)	--	ND (0.000000038)
3:3 FTCA	ND (0.0000031)	ND (0.000000046)	ND (0.0000034)	ND (0.000000046)	ND (0.0000036)	ND (0.000000048)
4:2 FTS	0.0000151000	ND (0.000000014)	0.0000150000	ND (0.000000014)	0.0000156000	ND (0.000000015)
5:3 FTCA	0.0000024500	ND (0.000000063)	0.0001150000	ND (0.000000063)	0.0001190000	ND (0.000000066)
6:2 FTCA	--	ND (0.000000090)	--	ND (0.000000090)	--	ND (0.000000094)
6:2 FTS	0.0050900000	**<0.00000022 DLL**	0.0061900000	**<0.00000023 DLL**	0.0064200000	**<0.00000021 DLL**
6:2 FTUCA	--	**<0.000000023 DLL**	--	**0.0000000128**	--	ND (0.000000020)
7:3 FTCA	ND (0.0000031)	ND (0.000000054)	0.0000278000	ND (0.000000054)	0.0000288000	ND (0.000000057)
8:2 FTCA	--	ND (0.000000079)	--	ND (0.000000079)	--	ND (0.000000083)
8:2 FTS	0.0003280000	ND (0.000000024)	0.0016700000	ND (0.000000024)	0.0017300000	ND (0.000000025)
8:2 FTUCA	--	ND (0.000000031)	--	ND (0.000000032)	--	ND (0.000000033)
Total PFAS (lb/hr)	**1.04E-02**	**1.52E-05**	**2.23E-02**	**1.01E-05**	**2.31E-02**	**6.15E-06**

Table 13-1: PFAS emissions at the stack (OTM-45) from AFFF incineration. *Run 1 and 2: AFFF incineration. Run 3: AFFF incineration with lime injection. PFAS highlighted in blue were detected in the process water. Values in bold when PFAS was detected above MDL in at least one OTM-45 fractions. DLL means that some portions of the sample were above the MDL, while others were below the detection limit (ND).*

	Run 1		Run 2		Run 3	
PFAS	Waste feed rate (lb/hr)	Stack emissions (lb/hr)	Waste feed rate (lb/hr)	Stack emissions (lb/hr)	Waste feed rate (lb/hr)	Stack emissions (lb/hr)
PFBS	ND (0.00016)	**<0.000000097 DLL**	0.000149	**0.0000000455**	0.000226	**<0.00000010 DLL**
PFBA	ND (0.00032)	**<0.0000010 DLL**	0.000459	**0.000000792**	0.000694	**<0.0000010 DLL**
PFDS	ND (0.000081)	ND (0.000000035)	0.00000295	ND (0.000000018)	0.00000446	ND (0.000000035)
PFDA	ND (0.000081)	**0.00000406**	0.0000457	**0.00000138**	0.0000690	**0.00000427**
PFDoS	ND (0.000081)	ND (0.000000059)	0.00000103	ND (0.000000025)	0.00000156	ND (0.000000059)
PFDoA	ND (0.000081)	**0.0000168**	0.0000214	**0.000000525**	0.0000323	**0.0000162**
PFHpS	ND (0.000081)	ND (0.000000045)	0.0000173	ND (0.000000023)	0.0000261	ND (0.000000046)
PFHpA	0.000127	**0.000000715**	0.000107	**0.00000350**	0.000162	**0.000000725**
Perfluorohexadecanoic acid	--	**0.00000778**	--	**0.000000189**	--	**0.00000474**
PFHxS	ND (0.000081)	ND (0.000000033)	0.000913	**0.0000000128**	0.00138	**<0.000000034 DLL**
PFHxA	0.000439	**0.000000575**	0.000929	**0.00000197**	0.00140	**0.000000435**
PFNS	ND (0.000081)	ND (0.000000038)	0.000000575	ND (0.000000017)	0.000000868	ND (0.000000038)
PFNA	ND (0.000081)	**0.00000203**	0.0000272	**0.00000288**	0.0000411	**0.00000228**
Perfluorooctadecanoic acid	--	**0.00000107**	--	**0.0000000972**	--	**0.000000251**
FOSA	ND (0.000081)	**<0.00000021 DLL**	0.00000862	**<0.000000074 DLL**	0.0000130	**<0.00000021 DLL**
PFOS	0.000364	ND (0.000000074)	0.00634	**0.0000000445**	0.00957	**<0.000000076 DLL**
PFOA	0.000135	**0.00000109**	0.000243	**0.00000374**	0.000367	**0.00000106**
PFPeS	ND (0.000081)	ND (0.000000022)	0.000188	ND (0.000000013)	0.000284	ND (0.000000022)
PFPeA	ND (0.000081)	**0.000000415**	0.000159	**0.00000101**	0.000240	**0.000000272**
PFTeA	ND (0.000081)	**0.0000248**	0.00000705	**0.000000459**	0.0000107	**0.0000233**
PFTriA	ND (0.000081)	**0.0000223**	0.00000772	**0.000000542**	0.0000117	**0.0000212**
PFUnA	ND (0.000081)	**0.00000809**	0.00000421	**0.000000834**	0.00000635	**0.00000893**
9-chloro.	ND (0.000081)	ND (0.000000034)	ND (0.00000048)	ND (0.000000017)	ND (0.00000072)	ND (0.000000034)
NEtFOSA	ND (0.00020)	ND (0.000000087)	ND (0.0000012)	ND (0.000000039)	ND (0.0000018)	ND (0.000000088)
NEtFOSAA	ND (0.000081)	ND (0.000000054)	0.00000196	ND (0.000000025)	0.00000296	ND (0.000000054)
NMeFOSAA	ND (0.000081)	ND (0.000000059)	ND (0.00000048)	ND (0.000000027)	ND (0.00000072)	ND (0.000000059)
11-chloro.	ND (0.000081)	ND (0.000000044)	ND (0.00000048)	ND (0.000000023)	ND (0.00000072)	ND (0.000000044)
NEtFOSE	ND (0.00020)	ND (0.000000096)	0.00000305	ND (0.000000038)	0.00000461	ND (0.000000097)
NMeFOSE	ND (0.00020)	ND (0.00000026)	0.000111	**<0.00000023 DLL**	0.000167	ND (0.00000026)
PES	--	ND (0.000000034)	--	ND (0.000000020)	--	ND (0.000000034)
PFECA	--	ND (0.000000040)	--	**0.0000000298**	--	ND (0.000000040)
PFECA	--	ND (0.000000059)	--	ND (0.000000030)	--	ND (0.000000059)
PFECA	--	ND (0.00000014)	--	**<0.000000062 DLL**	--	ND (0.00000014)
PFECHS	--	ND (0.000000041)	--	ND (0.000000026)	--	ND (0.000000040)
DONA	ND (0.000081)	ND (0.000000064)	ND (0.00000048)	ND (0.000000031)	ND (0.00000072)	ND (0.000000064)
HFPO-DA	ND (0.00041)	ND (0.00000072)	0.0000127	ND (0.00000037)	0.0000192	ND (0.00000073)
NMeFOSA	ND (0.00020)	**<0.000000098 DLL**	ND (0.0000012)	**0.0000000321**	ND (0.0000018)	**<0.00000010 DLL**
10:2 FTCA	--	ND (0.00000024)	--	ND (0.00000011)	--	ND (0.00000025)
10:2 FTS	--	ND (0.000000071)	--	ND (0.000000036)	--	ND (0.000000071)
3:3 FTCA	ND (0.000081)	ND (0.000000088)	0.00000125	ND (0.000000046)	0.00000189	ND (0.000000088)
4:2 FTS	ND (0.00024)	ND (0.000000029)	0.00000492	ND (0.000000014)	0.00000744	ND (0.000000029)
5:3 FTCA	ND (0.000081)	ND (0.00000011)	0.0000184	ND (0.000000063)	0.0000278	ND (0.00000011)
6:2 FTCA	--	ND (0.00000020)	--	ND (0.000000090)	--	ND (0.00000020)
6:2 FTS	0.00108	ND (0.00000022)	0.00212	**<0.00000020 DLL**	0.00320	ND (0.00000023)
6:2 FTUCA	--	ND (0.000000035)	--	ND (0.000000019)	--	ND (0.000000035)
7:3 FTCA	ND (0.000081)	ND (0.00000011)	0.00000492	ND (0.000000054)	0.00000744	ND (0.00000011)
8:2 FTCA	--	ND (0.00000017)	--	ND (0.000000079)	--	ND (0.00000017)
8:2 FTS	0.000322	ND (0.000000045)	0.000318	ND (0.000000024)	0.000480	ND (0.000000045)
8:2 FTUCA	--	ND (0.000000052)	--	ND (0.000000032)	--	ND (0.000000052)
Total PFAS (lb/hr)	**6.18E-03**	**9.45E-05**	**1.22E-02**	**2.00E-05**	**1.85E-02**	**8.85E-05**

Table 13-2 - PFAS emissions at the stack (OTM-45) from Contaminated Soil Incineration. *PFAS highlighted in blue were detected in the process water. Values in bold when PFAS was detected*

above MDL in at least one OTM-45 fractions. DLL means that some portions of the sample were above the MDL, while others were below the detection limit (ND).

	Run 1		Run 2	
PFAS	Waste feed rate (lb/hr)	Stack emission (lb/hr)	Waste feed rate (lb/hr)	Stack emission (lb/hr)
PFBS	0.000053400	**<0.000000094 DLL**	0.000059100	**0.000000050**
PFBA	0.000196000	**<0.00000099 DLL**	0.000217000	**0.000000345**
PFDS	0.000000249	ND (0.000000035)	0.000000276	ND (0.000000018)
PFDA	0.000017100	**0.000003010**	0.000019000	**0.000000109**
PFDoS	ND (0.00000020)	ND (0.000000058)	ND (0.00000022)	ND (0.000000026)
PFDoA	0.000003850	**0.000012000**	0.000004260	**0.000000140**
PFHpS	0.000024900	ND (0.000000045)	0.000027600	ND (0.000000023)
PFHpA	0.000077200	**0.000000560**	0.000085500	**0.000000236**
Perfluorohexadecanoic acid	--	**0.000005220**	--	**0.000000062**
PFHxS	0.000562000	**<0.000000034 DLL**	0.000622000	**0.000000012**
PFHxA	0.000286000	**0.000000340**	0.000317000	**0.000000190**
PFNS	0.000000332	ND (0.000000038)	0.000000368	ND (0.000000017)
PFNA	0.000056000	**0.000001420**	0.000062000	**0.000000145**
Perfluorooctadecanoic acid	--	**0.000000257**	--	**<0.000000030 DLL**
FOSA	0.000003130	**<0.00000021 DLL**	0.000003460	**<0.000000084 DLL**
PFOS	0.001210000	**<0.000000088 DLL**	0.001340000	**0.000000046**
PFOA	0.000700000	**0.000000796**	0.000775000	**0.000000380**
PFPeS	0.000056200	ND (0.000000021)	0.000062200	ND (0.000000013)
PFPeA	0.000078300	**0.000000236**	0.000086700	**0.000000133**
PFTeA	0.000001390	**0.000019100**	0.000001540	**0.000000142**
PFTriA	0.000000879	**0.000018200**	0.000000974	**0.000000154**
PFUnA	0.000003350	**0.000006050**	0.000003710	**0.000000114**
9-chloro.	ND (0.00000020)	ND (0.000000034)	ND (0.00000022)	ND (0.000000017)
NEtFOSA	ND (0.00000051)	ND (0.000000087)	ND (0.00000056)	ND (0.000000039)
NEtFOSAA	ND (0.00000020)	ND (0.000000053)	ND (0.00000022)	ND (0.000000025)
NMeFOSAA	ND (0.00000020)	ND (0.000000059)	ND (0.00000022)	ND (0.000000027)
11-chloro.	ND (0.00000020)	ND (0.000000044)	ND (0.00000022)	ND (0.000000023)
NEtFOSE	0.000000575	ND (0.000000096)	0.000000637	ND (0.000000039)
NMeFOSE	ND (0.00000051)	ND (0.00000025)	ND (0.00000056)	**<0.00000023 DLL**
PES	--	ND (0.000000033)	--	ND (0.000000020)
PFECA	--	**ND (0.000000040)**	--	ND (0.000000027)
PFECA	--	ND (0.000000058)	--	ND (0.000000030)
PFECA	--	ND (0.00000014)	--	ND (0.000000058)
PFECHS	--	ND (0.000000040)	--	ND (0.000000026)
DONA	ND (0.00000020)	ND (0.000000064)	ND (0.00000022)	ND (0.000000031)
HFPO-DA	0.002670000	ND (0.00000072)	0.002950000	ND (0.00000037)
NMeFOSA	ND (0.00000051)	<0.000000096 DLL	ND (0.00000056)	**0.000000089**
10:2 FTCA	--	ND (0.00000024)	--	ND (0.00000011)
10:2 FTS	--	ND (0.000000070)	--	ND (0.000000037)
3:3 FTCA	0.000005380	ND (0.000000087)	0.000005960	ND (0.000000046)
4:2 FTS	0.000003430	ND (0.000000028)	0.000003800	ND (0.000000014)
5:3 FTCA	0.000004480	ND (0.00000011)	0.000004970	ND (0.000000063)
6:2 FTCA	--	ND (0.00000020)	--	ND (0.000000091)
6:2 FTS	0.000359000	ND (0.00000022)	0.000397000	**<0.00000024 DLL**
6:2 FTUCA	--	ND (0.000000034)	--	ND (0.000000019)
7:3 FTCA	0.000000733	ND (0.00000011)	0.000000811	ND (0.000000055)
8:2 FTCA	--	ND (0.00000017)	--	ND (0.000000080)
8:2 FTS	0.000085700	ND (0.000000044)	0.000094900	ND (0.000000024)
8:2 FTUCA	--	ND (0.000000051)	--	ND (0.000000032)
Total PFAS (lb/hr)	**6.46E-03**	**7.20E-05**	**7.15E-03**	**4.33E-06**

Table 13-3 - PFAS emissions at the stack (OTM-45) from Spent Activated Carbon Incineration. *PFAS highlighted in blue were detected in the process water. Values in bold when PFAS was detected above MDL in at least one OTM-45 fractions.DLL means that some portions of the sample were above the MDL, while others were below the detection limit (ND).*

PFAS	Baseline stack emission (lb/hr)	CF4 run Stack emission (lb/hr)
PFBS	**0.0000000585**	**0.0000000475**
PFBA	**0.000000400**	**0.000000250**
PFDS	ND (0.000000018)	ND (0.000000018)
PFDA	**0.000000118**	**0.0000000243**
PFDoS	ND (0.000000026)	ND (0.000000025)
PFDoA	**0.0000000628**	**0.0000000268**
PFHpS	ND (0.000000023)	ND (0.000000023)
PFHpA	**0.000000382**	**<0.00000013 DLL**
Perfluorohexadecanoic acid	**0.0000000267**	**<0.000000038 DLL**
PFHxS	**<0.000000019 DLL**	**0.0000000163**
PFHxA	**0.000000379**	**0.000000118**
PFNS	ND (0.000000018)	ND (0.000000017)
PFNA	**0.000000220**	**0.0000000337**
Perfluorooctadecanoic acid	ND (0.000000029)	ND (0.000000029)
FOSA	**<0.000000078 DLL**	**<0.000000075 DLL**
PFOS	**0.0000000213**	**0.000000189**
PFOA	**0.000000319**	**0.000000158**
PFPeS	ND (0.000000013)	**0.00000000602**
PFPeA	**0.000000193**	**0.0000000481**
PFTeA	**0.0000000590**	**0.0000000305**
PFTriA	**0.0000000506**	**0.0000000265**
PFUnA	**0.0000000706**	**0.0000000235**
9-chloro.	ND (0.000000017)	ND (0.000000017)
NEtFOSA	**<0.000000041 DLL**	ND (0.000000039)
NEtFOSAA	ND (0.000000025)	ND (0.000000025)
NMeFOSAA	ND (0.000000028)	ND (0.000000027)
11-chloro.	ND (0.000000023)	ND (0.000000023)
NEtFOSE	**0.0000000193**	ND (0.000000038)
NMeFOSE	**<0.00000024 DLL**	**<0.00000022 DLL**
PES	ND (0.000000020)	ND (0.000000020)
PFECA	ND (0.000000027)	ND (0.000000027)
PFECA	ND (0.000000030)	ND (0.000000029)
PFECA	ND (0.000000059)	ND (0.000000057)
PFECHS	ND (0.000000026)	ND (0.000000026)
DONA	ND (0.000000031)	ND (0.000000031)
HFPO-DA	ND (0.00000038)	ND (0.00000037)
NMeFOSA	**0.0000000755**	**0.0000000249**
10:2 FTCA	ND (0.00000011)	ND (0.00000011)
10:2 FTS	ND (0.000000037)	ND (0.000000036)
3:3 FTCA	ND (0.000000047)	ND (0.000000046)
4:2 FTS	ND (0.000000015)	ND (0.000000014)
5:3 FTCA	ND (0.000000064)	ND (0.000000063)
6:2 FTCA	ND (0.000000092)	ND (0.000000090)
6:2 FTS	**0.000000973**	**<0.00000025 DLL**
6:2 FTUCA	**<0.000000024 DLL**	**<0.000000019 DLL**
7:3 FTCA	ND (0.000000055)	ND (0.000000054)
8:2 FTCA	ND (0.000000080)	ND (0.000000078)
8:2 FTS	ND (0.000000024)	ND (0.000000024)
8:2 FTUCA	ND (0.000000032)	ND (0.000000031)
Total PFAS (lb/hr)	**5.18E-06**	**3.14E-06**

Table 13-4 : PFAS emissions at the stack (OTM-45) for CF4 incineration. *PFAS highlighted in blue were detected in the process water. Values in bold when PFAS was detected above MDL in at least one OTM-45 fractions. DLL means that some portions of the sample were above the MDL, while others were below the detection limit (ND).*

Pattern 1: AFFF run 2 &3, soil #2, carbon #2 → PFAS emission < 2.10^-5 lb/hr and PFAS signature at the stack during PFAS incineration similar to baseline

As in the campaign 1, even if the PFAS containing wastes fed in the kiln had different signatures, the PFAS signature at stack is the same for all runs. The same 6 PFAS are found at stack substantially above MDL in all runs: PFBA, PFHpA, PFHxA, PFNA, PFOA, PFPeA. These PFAS are also found in the PFAS-contaminated process water entering the incinerator, and in contact with the stack gases after thermal treatment.

As in the campaign 1, the flowrate at the stack of these 6 PFAS is higher during PFAS incineration than during the baseline.

Figure 9-1 shows the PFAS stack emissions for pattern 1.

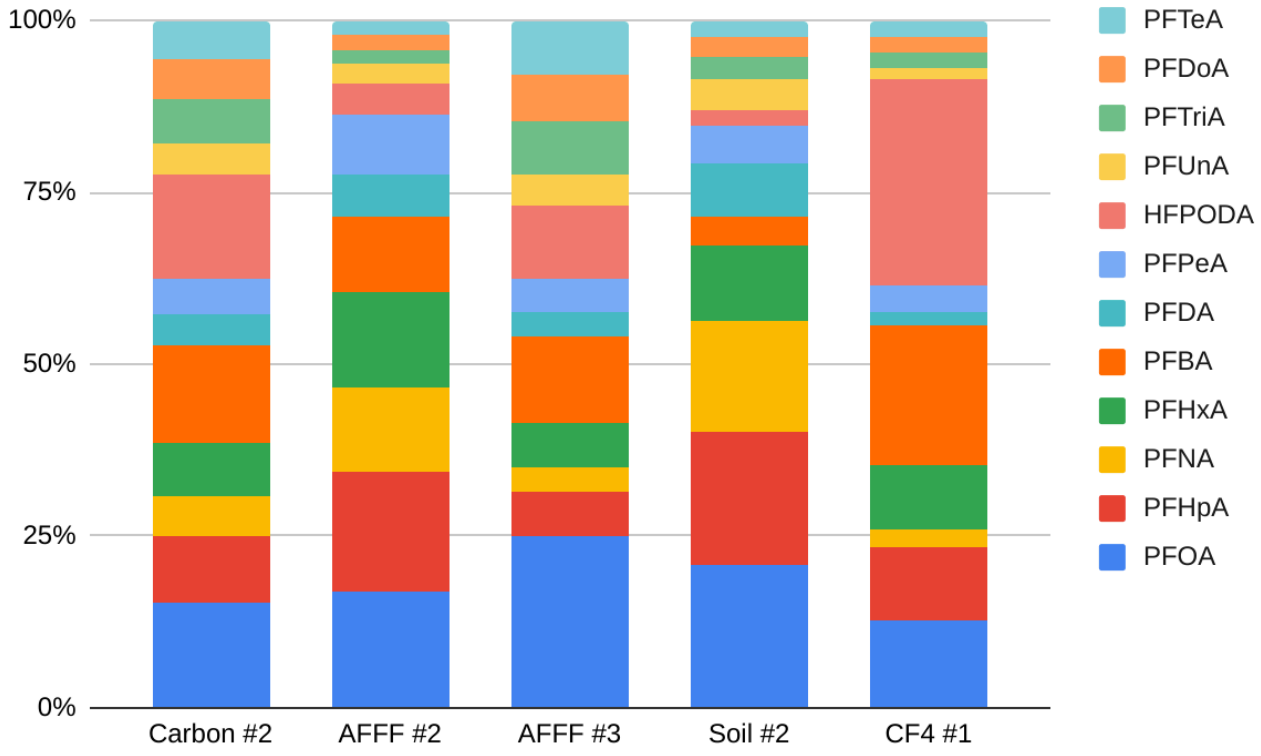


Figure 9-1 - PFAS stack emission observed in pattern 1 (OTM-45). *For readability, only the 12 dominant PFAS are represented.*

Comments on lime injection during the A3F runs:

The same AFFF was incinerated in run 2 and 3, but in run 3, lime was injected during AFFF incineration. Lime injection decreases PFAS stack emission for PFAS with less than 7 carbons like PFBA, PFHxA, PFPeA. Stack emission for these PFAS in AFFF run 3 (lime injection) is lower than in run 2 (without lime) and lower than the baseline. The lime effect on PFAS with C>7 is less certain. PFOA emissions decrease with lime but PFOS, PFTeA, PFTriA, PFDoA emissions increase. Lime was not tested for PFAS content, but according to manufacturer description there is no PFAS in it. Overall, lime injection decreases total PFAS emissions at the stack to a small degree, PFAS emissions at the stack are 1.10^-5 lb/hr without lime injection (AFFF run 2) and 6.15.10^-6 lb/hr with

lime injection (AFFF run 3). The impact of lime was only tested in one run; more tests should be performed to confirm the results.

Comments on the carbon run :

PFAS mass emission rate at stack during the carbon incineration run was lower than the baseline.A possible explanation could be an effect of unique surfaces provided by the activated carbon that may enhance PFAS degradation during incineration.

Pattern 2: AFFF run#1, carbon #1, soil #1 & #3

Figure 9-2 shows the PFAS stack emissions for 4 runs that showed a different PFAS signature (pattern 2) and concentration than the one established during campaign 1. There were: AFFF#1, Soil#1, Carbon#1, and Soil#3. In these runs, high emissions of long chain PFAS like perfluorohexadecanoic acid (C16), PFDoA (C12), PFTeA (C14), PFTriA (C13), PFUnA (C11) and perfluorohexane (C6) were detected at the stack. These 4 runs have the same PFAS signature at stack while the other runs involving AFFF, carbon, soil and CF4 and all baseline runs had PFAS signatures consistent with pattern 1.

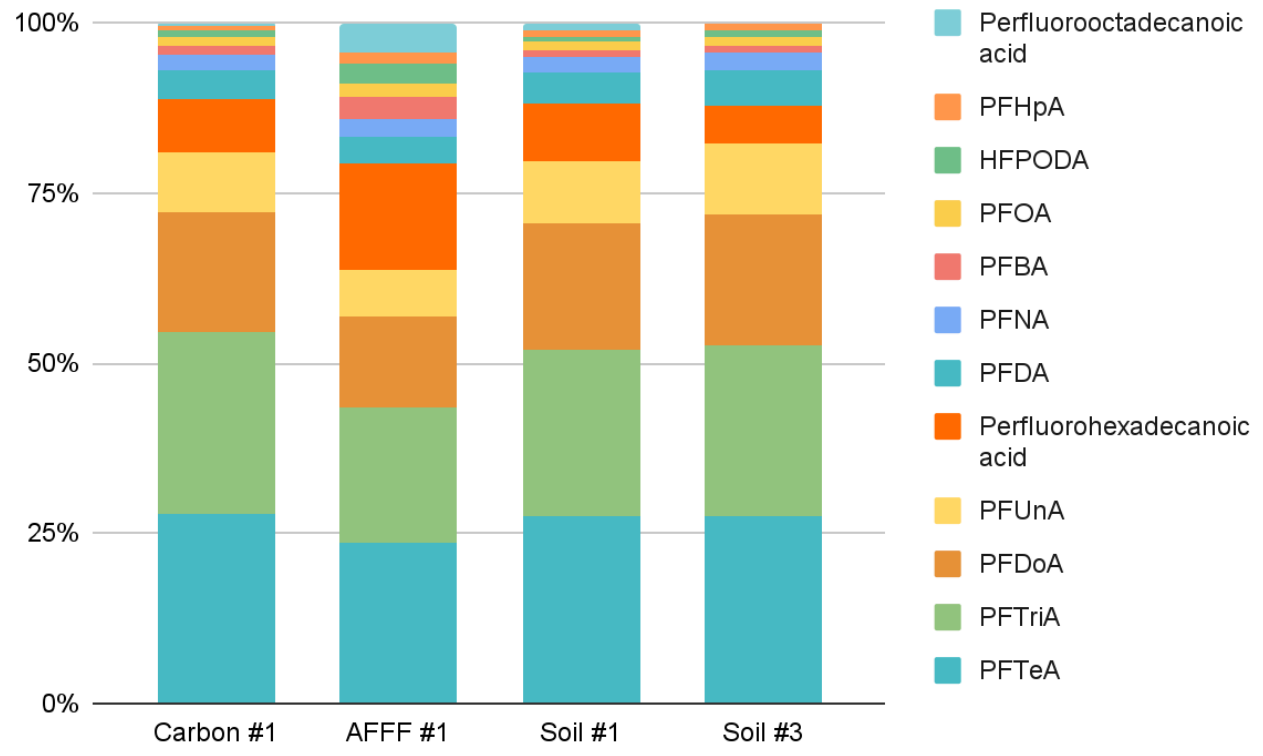


Figure 9-2 - PFAS stack emission observed in pattern 2 (OTM-45). *For readability, only the 12 dominant PFAS are represented.*

For carbon run#1 and run#2, the same carbon mix was incinerated, but very different OTM-45 results were obtained (run#2 gave pattern 1 while run#1 showed pattern 2). This is the same for soil#2 and soil#3. The same soil mix was used but with a different pattern at the stack. These inconsistencies confirm that pattern #2 is likely contaminated by background PFAS. The source of this background PFAS presence is unknown. It is unlikely that emissions from PFAS with C>10 (pattern 2) are PICs from PFAS incineration, because campaign 1 has shown that the incinerator can efficiently destroy PFAS with C<9 like PFOS, PFHxS, PFPeS.

> Comparing PFAS incineration vs baseline in Campaign 2

Table 14 compares the total PFAS mass emission rate at the stack (OTM-45 results) during baseline vs PFAS incineration in Campaign 2.

As in campaign 1, the OTM-45 results indicated higher total PFAS emissions for the PFAS waste incineration tests (excluding the CF4 tests) compared to baseline tests in campaign 2. However, given that PFAS was present in the water fed to the quench and wet scrubber downstream of the incineration process, these data are not considered conclusive.

Campaign 2	Average Baseline	Average AFFF	Average soil	Average carbon	CF4
Total PFAS mass emission rate at the stack : OTM-45 (lb/hr)	7.63E-06	1.05E-05	6.77E-05	3.82E-05	3.14E-06
Ratio PFAS incineration/Baseline		**1.4**	**8.9**	**5.0**	**0.4**

Table 14 : **Total PFAS mass emission rate at the stack during baselines and PFAS incineration.** *Average baselines refers to the average total stack emission rate of PFAS observed during the 6 baselines in Campaign 2. Average AFFF, average soil, and average carbon refers to the average total stack emission rate of PFAS observed during the runs of AFFF, contaminated soil and spent activated carbon in Campaign 2.*

7.2.3.2. Destruction and Removal Efficiency

Tables 15 and 16 present the DRE and total DRE measured in campaign 2. Overall, total DRE >98% were measured in all runs, despite the background presence of long-chain PFAS (pattern 2) from unknown sources. PFAS concentrations in the waste streams in campaign 2 are lower than in campaign 1, leading to lower DRE.

PFAS	AFFF run#1	AFFF # 2	AFFF # 3	Soil # 1	Soil # 2	Soil # 3	Carbon # 1	Carbon # 2
PFAS feed rate (lb/hr)	0.0105	0.0223	0.0231	0.0065	0.0123	0.0186	0.0065	0.0072
Total DRE (%)	99.8552%	99.9547%	99.9734%	98.5462%	99.8374%	99.5242%	98.8923%	99.9399%

Table 15 - Total DRE obtained during campaign 2

	AFFF run #1			AFFF run #2			AFFF run #3			Soil run #1		
PFAS	PFAS feed rate (lb/hr)	DRE %	Non-detected at stack	PFAS feed rate (lb/hr)	DRE %	Non-detected at stack	PFAS feed rate (lb/hr)	DRE %	Non-detected at stack	PFAS feed rate (lb/hr)	DRE %	Non-detected at stack
PFBS	0.0000612	99.8056		0.0001220	99.9389		0.0001270	99.9523		0.00016*	99.9394	DLL
PFBA	0.0000725	99.3986		0.0004010	99.7746		0.0004160	99.8925		0.00032*	99.6875	DLL
PFDS	0.0000052*	99.6538	nd	0.0000057*	99.6842	nd	0.0000059*	99.6780	nd	0.000081*	99.9568	nd
PFDA	0.0000125	95.8640		0.0001120	99.5643		0.0001160	99.8948		0.000081*	94.9877	
PFDoS	0.0000052*	99.5192	nd	0.0000057*	99.5614	nd	0.0000059*	99.5424	nd	0.000081*	99.9272	nd
PFDoA	0.0000054	67.9702		0.0000447	99.5906		0.0000464	99.4784		0.000081*	79.2593	
PFHpS	0.0000143	99.8392	nd	0.0000255	99.9098	nd	0.0000264	99.9015	DLL	0.000081*	99.9444	nd
PFHpA	0.0000319	99.2947		0.0001100	98.7000		0.0001140	99.7982		0.0001270	99.4370	
PFHxS	0.0005680	99.9983		0.0012800	99.9988		0.0013200	99.9971		0.000081*	99.9593	nd
PFHxA	0.0007450	99.9557		0.0023200	99.9504		0.0024000	99.9905		0.0004390	99.8690	
PFNS	0.0000052*	99.6731	nd	0.0000057*	99.7018	nd	0.0000059*	99.6949	nd	0.000081*	99.9531	nd
PFNA	0.000005*	93.8846		0.0000182	94.5495		0.0000189	99.3228		0.000081*	97.4938	
FOSA	0.0000240	99.6750	DLL	0.0000661	99.8820	DLL	0.0000685	99.8847	DLL	0.000081*	99.7407	DLL
PFOS	0.0030800	99.9984		0.0085300	99.9990		0.0088500	99.9934		0.0003640	99.9797	nd
PFOA	0.0001200	99.7833		0.0004820	99.7116		0.0004990	99.8222		0.0001350	99.1926	
PFPeS	0.0000775	99.9832	nd	0.0002520	99.9948	nd	0.0002620	99.9972		0.000081*	99.9728	nd
PFPeA	0.0000505	99.5663		0.0002660	99.7286		0.0002760	99.9384		0.000081*	99.4877	
PFTeA	0.000005*	41.3462		0.0000165	99.0000		0.0000171	98.3918		0.000081*	69.3827	
PFTriA	0.0000059	57.4074		0.0000116	98.6379		0.0000120	97.6667		0.000081*	72.4691	
PFUnA	0.0000100	90.9940		0.0000759	99.6733		0.0000787	99.7954		0.000081*	90.0123	
9-chloro.	0.0000052*	99.6731	nd	0.0000057*	99.7018	nd	0.0000059*	99.7119	nd	0.000081*	99.9580	nd
NEtFOSA	0.000011*	99.6455	nd	0.000011*	99.6455	nd	0.000012*	99.6583	nd	0.00020*	99.9565	nd
NEtFOSAA	0.000011*	99.7727	nd	0.0000243	99.8971	nd	0.0000252	99.8968	nd	0.00020*	99.9730	nd
NMeFOSAA	0.0000073	99.6322	nd	0.0000340	99.9206	nd	0.0000352	99.9205	nd	0.000081*	99.9272	nd
11-chloro.	0.0000052*	99.5577	nd	0.0000057*	99.5965	nd	0.0000059*	99.5932	nd	0.000081*	99.9457	nd
NEtFOSE	0.000011*	99.6545	nd	0.000011*	99.6545	nd	0.000012*	99.6667	nd	0.00020*	99.9520	nd
NMeFOSE	0.0000063*	96.6667	nd	0.0000069*	96.9565	nd	0.0000071*	96.7606	DLL	0.000081*	99.6790	nd
DONA	0.0000052*	99.4038	nd	0.0000057*	99.4561	nd	0.0000059*	99.4576	nd	0.000081*	99.9210	nd
HFPO-DA	0.0000227	98.3700	nd	0.0000202	98.1683	nd	0.0000210	98.1905	nd	0.0004100	99.8244	nd
NMeFOSA	0.000011*	99.3518		0.000011*	98.9636		0.000012*	98.7833		0.00020*	99.9510	DLL
3:3 FTCA	0.0000031*	98.5161	nd	0.0000034*	98.6471	nd	0.0000036*	98.6667	nd	0.000081*	99.8914	nd
4:2 FTS	0.0000151	99.9073	nd	0.0000150	99.9067	nd	0.0000156	99.9038	nd	0.00024*	99.9879	nd
5:3 FTCA	0.0000025	97.4286	nd	0.0001150	99.9452	nd	0.0001190	99.9445	nd	0.000081*	99.8642	nd
6:2 FTS	0.0050900	99.9957	DLL	0.0061900	99.9963	DLL	0.0064200	99.9967	DLL	0.0010800	99.9796	nd
7:3 FTCA	0.0000031*	98.2581	nd	0.0000278	99.8058	nd	0.0000288	99.8021	nd	0.000081*	99.8642	nd
8:2 FTS	0.0003280	99.9927	nd	0.0016700	99.9986	nd	0.0017300	99.9986	nd	0.0003220	99.9860	nd
ADONA	0.0000021*	--		0.0000023*	--		0.0000024*	--		0.000081*	--	
PFEESA	0.0000021*	--		0.0000023*	--		0.0000024*	--		0.000081*	--	
PFMPA	0.0000021*	--		0.0000023*	--		0.0000024*	--		0.000081*	--	
PFMBA	0.0000021*	--		0.0000023*	--		0.0000024*	--		0.000081*	--	
Total (lb/hr)	**1.05E-02**			**2.23E-02**			**2.31E-02**			**6.50E-03**		

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Table 16-1 - DRE for campaign 2. Part 1. PFAS highlighted in blue were detected in the process water. *indicates that values were below the MDL or the LoQ. ND indicates PFAS was not detected at the stack. DLL indicates that the PFAS was detected above MDL in one of the OTM-45 fractions while the others were ND.

	Soil run #2			Soil run#3			Carbon run #1			Carbon run #2		
PFAS	PFAS feed rate (lb/hr)	DRE %	Non-detected at stack	PFAS feed rate (lb/hr)	DRE %	Non-detected at stack	PFAS feed rate (lb/hr)	DRE %	Non-detected at stack	PFAS feed rate (lb/hr)	DRE %	Non-detected at stack
PFBS	0.0001490	99.9695		0.0002260	99.9558	DLL	0.0000534	99.8240	DLL	0.0000591	99.9161	
PFBA	0.0004590	99.8275		0.0006940	99.8559	DLL	0.0001960	99.4949	DLL	0.0002170	99.8410	
PFDS	0.0000030	99.3898	nd	0.0000045	99.2152	nd	0.0000002	85.9438	nd	0.0000003	93.4783	nd
PFDA	0.0000457	96.9803		0.0000690	93.8116		0.0000171	82.3977		0.0000190	99.4263	
PFDoS	0.0000010	97.5728	nd	0.0000016	96.2179	nd	0.00000020*	71.0000	nd	0.00000022*	88.1818	nd
PFDoA	0.0000214	97.5467		0.0000323	49.8452		0.0000039	**		0.0000043	96.7136	
PFHpS	0.0000173	99.8671	nd	0.0000261	99.8238	nd	0.0000249	99.8193	nd	0.0000276	99.9167	nd
PFHpA	0.0001070	96.7290		0.0001620	99.5525		0.0000772	99.2746		0.0000855	99.7240	
PFHxS	0.0009130	99.9986		0.0013800	99.9975	DLL	0.0005620	99.9940	DLL	0.0006220	99.9982	
PFHxA	0.0009290	99.7879		0.0014000	99.9689		0.0002860	99.8811		0.0003170	99.9401	
PFNS	0.0000006	97.0435	nd	0.0000009	95.6221	nd	0.0000003	88.5542	nd	0.0000004	95.3804	nd
PFNA	0.0000272	89.4118		0.0000411	94.4526		0.0000560	97.4643		0.0000620	99.7661	
FOSA	0.0000086	99.1415	DLL	0.0000130	98.3846	DLL	0.0000031	93.2907	DLL	0.0000035	97.5723	DLL
PFOS	0.0063400	99.9993		0.0095700	99.9992	DLL	0.0012100	99.9927	DLL	0.0013400	99.9966	
PFOA	0.0002430	98.4609		0.0003670	99.7112		0.0007000	99.8863		0.0007750	99.9510	
PFPeS	0.0001880	99.9931	nd	0.0002840	99.9923	nd	0.0000562	99.9626	nd	0.0000622	99.9791	nd
PFPeA	0.0001590	99.3648		0.0002400	99.8867		0.0000783	99.6986		0.0000867	99.8466	
PFTeA	0.0000071	93.4894		0.0000107	**		0.0000014	**		0.0000015	90.7792	
PFTriA	0.0000077	92.9793		0.0000117	**		0.0000009	**		0.0000010	84.1889	
PFUnA	0.0000042	80.1900		0.0000064	**		0.0000034	**		0.0000037	96.9272	
9-chloro.	0.00000048*	96.4583	nd	0.00000072*	95.2778	nd	0.00000020*	83.0000	nd	0.00000022*	92.2727	nd
NEtFOSA	0.00000120*	96.7500	nd	0.00000180*	95.1111	nd	0.00000051*	82.9412	nd	0.00000056*	93.0357	nd
NEtFOSAA	0.0000031	99.1803	nd	0.0000046	98.8286	nd	0.0000006	90.7826	nd	0.0000006	96.0754	nd
NMeFOSAA	0.0000020	98.6224	nd	0.0000030	98.0068	nd	0.00000020*	70.5000	nd	0.00000022*	87.7273	nd
11-chloro.	0.00000048*	95.2083	nd	0.00000072*	93.8889	nd	0.00000020*	78.0000	nd	0.00000022*	89.5455	nd
NEtFOSE	0.0001110	99.9658	nd	0.0001670	99.9419	nd	0.00000051*	81.1765	nd	0.00000056*	93.0357	nd
NMeFOSE	0.00000048*	52.0833	DLL	0.00000072*	63.8889	nd	0.00000020*	**	nd	0.00000022*	**	DLL
DONA	0.00000048*	93.5417	nd	0.00000072*	91.1111	nd	0.00000020*	68.0000	nd	0.00000022*	85.9091	nd
HFPO-DA	0.0000127	97.0866	nd	0.0000192	96.1979	nd	0.0026700	99.9730	nd	0.0029500	99.9875	nd
NMeFOSA	0.00000120*	97.3250		0.00000180*	94.4444	DLL	0.00000051*	81.1765	DLL	0.00000056*	84.1071	
3:3 FTCA	0.0000013	96.3200	nd	0.0000019	95.3439	nd	0.0000054	98.3829	nd	0.0000060	99.2282	nd
4:2 FTS	0.0000049	99.7154	nd	0.0000074	99.6102	nd	0.0000034	99.1837	nd	0.0000038	99.6316	nd
5:3 FTCA	0.0000184	99.6576	nd	0.0000278	99.6043	nd	0.0000045	97.5446	nd	0.0000050	98.7324	nd
6:2 FTS	0.0021200	99.9906	DLL	0.0032000	99.9928	nd	0.0003590	99.9387	nd	0.0003970	99.9395	DLL
7:3 FTCA	0.0000049	98.9024	nd	0.0000074	98.5215	nd	0.0000007	84.9932	nd	0.0000008	93.2182	nd
8:2 FTS	0.0003180	99.9925	nd	0.0004800	99.9906	nd	0.0000857	99.9487	nd	0.0000949	99.9747	nd
NFDHA	0.0000400	--		0.0000604	--		0.0000258	--		0.0000286	--	
PFEESA	0.00000048*	--		0.00000072*	--		0.00000020*	--		0.00000022*	--	
PFMPA	0.0000346	--		0.0000523	--		0.0000049	--		0.0000054	--	
PFMBA	0.0000014	--		0.0000021	--		0.0000015	--		0.0000016	--	
Total (lb/hr)	1.23E-02			1.86E-02			6.49E-03			7.18E-03		

Table 16-2 - DRE for campaign 2. Part 2. PFAS highlighted in blue were detected in the process water. *indicates that values were below the MDL or the LoQ. ND indicates PFAS was not detected at the stack. DLL indicates that the PFAS was detected above MDL in one of the OTM-45 fractions while the others were ND.

Tables 16-1 and 16-2 shows that a large number of PFAS were undetected at the stack, despite being detected in the PFAS waste. Eight PFAS including 8:2 FTS, 7:3 FTCA, 5:3 FTCA, 4:2 FTS, HFPO-DA, NEtFOSE, NETFOSAA and PFDS were systematically not detected at the stack. DRE for PFOS was >99.999% in 3 runs. DRE >99.9999% could not be reached because of the limited PFOS concentration in campaign 2.

PFAS with DRE inferior to 99% were :

- PFDA - detected in the process water, stack gas likely contaminated with PFDA from process water
- PFDoA - contamination issue suspected in AFFF run #1, soil run #3 and carbon run #1. Very low concentration in waste for soil run #2 leads to low DRE.
- PFHpA - detected in the process water, stack gas likely contaminated with PFHpA from process water
- PFNA - detected in the process water, stack gas likely contaminated with PFNA from process water
- FOSA - very low concentration in waste for soil run 3 and carbon 1,2 leads to low DRE.
- PFOA - DRE close to 99%, PFOA detected at the stack likely from process water rather than waste incineration
- PFTeA - contamination issue suspected in carbon run 1 and soil run 3. Low concentration in soil run#2 leads to low DRE.
- PFTriA - contamination issue suspected in AFFF run#1, soil run 3 and carbon run #1. Very low concentration in waste for AFFF run 2 and 3 and carbon 2 and soil 2 leads to low DRE.
- PFUnA - contamination issue suspected in AFFF run#1 and carbon run#1. Very low concentration in waste for carbon 2 and soil 2 leads to low DRE.
- NMeFOSA - very low concentration in waste in soil run 2 leads to low DRE

7.2.3.3 Products of Incomplete Destruction (OTM-50)

PICs measured from OTM-50 sampling were detected in 3 runs out of 13. Detailed OTM-50 tables are available in **Appendix XVIII.**

	Baseline	Run 1	Run2	Run 3
A3F	Blank #1: C5 detected at 9.9X10^-5 lb/hr (MDL: 8.4X10^-5 lb/hr)	ND	ND	CF4 detected at 5.1X10^-3 lb/hr (MDL : 4.4X10^-4 ;b/hr)
	Blank #2: CF4 detected at 2.4X10^-3 lb/hr (MDL : 4.4X10^-4 lb/hr)			

Carbon	ND	ND	ND	N/A
CF4	ND	CF4 detected at 17.6 lb/hr	N/A	N/A
Contaminated soil	ND	ND	ND	ND

Table 17 - OTM-50 results in campaign 2. ND means no OTM-50 PFAS was detected at stack during the run. N/A indicates no additional run was performed

The baseline for AFF #1 could be a false positive as no PFAS was supposed to be incinerated at that time.

CF4 was detected in the baseline performed before AFFF run #3 and during AFFF run #3. The high H/F ratio reached in the incinerator prevents CF4 formation during PFAS incineration. The detection of CF4 during these 2 runs could be a false positive, as the level is close to the MDL.

CF4 was also detected at stack during CF4 incineration. The DRE for CF4 is 43%. According to literature, CF4 destruction requires a temperature > 2500 F, while the incinerator temperature is 2040 F in the SCC. The fact that 43% of the CF4 was destroyed suggests that in some parts of the incinerator, temperatures above 2500 F are reached.

OTM-50 results show that in most runs, no PICs were detected at stack during the campaign 2.

The C5 compound detected in AFFF baseline run#1 is a polyfluorinated ether. It is a very unlikely PIC due to its low thermal stability and is more likely a contaminant from the analytical laboratory.

7.2.4 HF measurement.

HF measures are available in **Appendix XIX** for campaign 2. HWC MACT standards for existing hazardous waste incinerators from §63.1219 applicable to the incinerator at Veolia's Port Arthur facility require HF at the stack to be below 32 ppmv, dry, at 7% O2. HF remained well below the regulatory limit during PFAS incineration.

7.2.5 Total Destruction Efficiency

The Total Destruction Efficiency was computed for each run, as described in 4.3. When PFAS was not detected in the liquid, solid residue or at stack, the MDL is used. When a PFAS is detected below the LoQ, the LoQ is used.

The total DE for all runs were very low or even negative. This is due to the high MDL for OTM-50 (0.01 lb/hr to 0.1 lb/hr). The PFAS concentration in the waste was sometimes lower than the MDL for OTM-50. This demonstrates the need to improve detection levels for OTM-50 to align them with EPA Method 537 and EPA Method 1633.

7.3 Conclusions and Recommendations for Further Study

The results demonstrated high destruction and removal efficiency for PFAS compounds during the incineration process, with values up to 99.9999%. These findings suggest that hazardous waste incineration, when properly operated under high-temperature conditions, can effectively break down PFAS compounds. Notably, a single VFC, CF4, was detected in one sample near the LOQ,

suggesting a complete mineralization of the PFAS compounds rather than partial decomposition. The consistent achievement of high DRE values across multiple test runs, coupled with the absence of PICs, provides strong confidence in the reliability of the thermal treatment process for PFAS destruction.

Analysis of solid residues and liquid residues showed relatively low to no concentrations of PFAS compounds, further supporting the effectiveness of the thermal treatment process. The minimal presence of PFAS compounds in these residual streams indicates successful destruction rather than mere phase transfer of the contaminants.

Initial findings from this Veolia PFAS incineration campaign have brought to light several important challenges that warrant further investigation. One significant hurdle remains finding waste streams with sufficiently high PFAS concentrations to demonstrate DRE higher than 99.9999%.

The current detection limits of samples obtained from OTM-50 are significantly higher than samples analyzed from OTM-45, making it difficult to quantify thermally stable, short-chain PFAS in the gaseous emissions. This limitation severely impacts the ability to calculate total PFAS destruction efficiency compared to DRE calculations of the PFAS being fed to the incinerator.

A significant challenge encountered during the study was the detection of background PFAS in some OTM-45 fractions. The source of this PFAS presence remains unclear and this observation highlights the complexity of PFAS sampling near industrial sources and the need for more comprehensive monitoring strategies.

While the targeted analysis of 79 PFAS compounds listed in OTM-45 and OTM-50 provides valuable insights, the potential presence of unknown PFAS necessitates the implementation of non-targeted analytical approaches. Such comprehensive screening would help identify previously uncharacterized PFAS compounds and transformation products that may form during the incineration process. This broader analytical scope would provide a more complete understanding of the treatment effectiveness and potential environmental implications.

An interesting avenue for future research would be investigating the potential catalytic effect of lime on PFAS destruction. As lime is commonly used in air pollution control systems for acid gas removal, understanding its role in PFAS decomposition mechanisms could provide valuable insights into process optimization. Preliminary results suggest that the presence of lime in the system might enhance PFAS destruction through surface-mediated or catalytic effects, potentially allowing for more efficient treatment at lower temperatures or shorter residence times. This knowledge could lead to improved treatment efficiencies and inform future design modifications of incineration systems.

Appendix I -Isokinetic and Non-Isokinetic Data Sheets for Campaign 1

Appendix II - Isokinetic and Non-Isokinetic Data Sheets for Campaign 2

Appendix III - Data quality assessment for Campaign 1

Appendix IV - Data quality assessment for Campaign 2

Appendix V - Calibration data for Campaign 1

Appendix VI - Calibration data for Campaign 1

Appendix VII - PFAS concentration in AFFF in Campaign 1

Non-detected PFAS (ND) are considered to be at MDL.

Parameter	Units	C1-R1	C1-R2	C1-R3
Date Tested	-	7/31/2024	8/1/2024	8/1/2024
Sample Collection Time	minutes	180	180	180
Waste Input Rate - Liquid A3F (lb/hr)	lb/hr	2,421	2,175	2,235
11-Chloroeicosafluoro-3-oxaundecane-1-sulfonic acid (11Cl-PF3OUdS)		ND (0.0000059)	ND (0.0000053)	ND (0.0000054)
1H,1H,2H,2H-Perfluorodecane sulfonic acid (8:2 FTS)		0.00209	0.0000968	0.000113
1H,1H,2H,2H-Perfluorohexane sulfonic acid (4:2 FTS)		0.0000353	0.0000857	0.000380
1H,1H,2H,2H-Perfluorooctane sulfonic acid (6:2 FTS)		0.00441	0.00868	0.0102
3-Perfluoroheptylpropanoic acid (7:3 FTCA)		ND (0.000013)	ND (0.000012)	0.0000286
3-Perfluoropentylpropanoic acid (5:3 FTCA)		0.0000220	0.00000905	0.0000198
3-Perfluoropropylpropanoic acid (3:3 FTCA)		0.00000612	ND (0.0000053)	ND (0.0000054)
4,8-Dioxa-3H-perfluorononanoic acid (DONA)		ND (0.0000059)	ND (0.0000053)	ND (0.0000054)
9-Chlorohexadecafluoro-3-oxanonane-1-sulfonic acid (9Cl-PF3ONS)		ND (0.0000059)	ND (0.0000053)	ND (0.0000054)
Hexafluoropropylene Oxide Dimer Acid (HFPO-DA)		0.00000586	ND (0.0000043)	ND (0.0000043)
N-ethylperfluorooctane sulfonamide (NEtFOSA)		0.0000685	ND (0.0000043)	ND (0.0000043)
N-ethylperfluorooctane sulfonamidoethanol (NEtFOSE)		0.000627	0.0000396	ND (0.0000043)
N-ethylperfluorooctanesulfonamidoacetic acid (NEtFOSAA)		0.0000207	ND (0.0000053)	ND (0.0000054)
N-methylperfluorooctane sulfonamide (NMeFOSA)		0.0000458	ND (0.0000075)	ND (0.0000075)
N-methylperfluorooctane sulfonamidoethanol (NMeFOSE)		0.000264	0.0000181	ND (0.0000043)
N-methylperfluorooctanesulfonamidoacetic acid (NMeFOSAA)		ND (0.0000047)	ND (0.0000043)	ND (0.0000043)
Nonafluoro-3,6-dioxaheptanoic acid (NFDHA)		ND (0.0000035)	ND (0.0000032)	ND (0.0000032)
Perfluoro (2-ethoxyethane) sulfonic acid (PFEESA)		0.00000625	ND (0.0000032)	ND (0.0000032)
Perfluoro-3-methoxypropanoic acid (PFMPA)		0.0000154	ND (0.0000032)	ND (0.0000032)
Perfluoro-4-methoxybutanoic acid (PFMBA)		0.0000231	ND (0.0000032)	ND (0.0000032)
Perfluorobutanesulfonic acid (PFBS)	lb/hr	0.0245	0.00217	ND (0.0000032)
Perfluorobutanoic acid (PFBA)		0.00453	0.00108	0.000858
Perfluorodecanesulfonic acid (PFDS)		0.000173	0.0000894	ND (0.0000032)
Perfluorodecanoic acid (PFDA)		0.000180	0.00000518	ND (0.0000032)
Perfluorododecanesulfonic acid (PFDoS)		0.000109	0.0000250	ND (0.0000032)
Perfluorododecanoic acid (PFDoA)		0.0000717	ND (0.0000043)	0.0000131
Perfluoroheptanesulfonic acid (PFHpS)		0.0334	0.000594	ND (0.0000032)
Perfluoroheptanoic acid (PFHpA)		0.00612	0.000561	0.0000295
Perfluorohexanesulfonic acid (PFHxS)		0.179	0.0180	0.00000396
Perfluorohexanoic acid (PFHxA)		0.0223	0.00409	0.00646
Perfluorononanesulfonic acid (PFNS)		0.000392	0.0000763	ND (0.0000032)
Perfluorononanoic acid (PFNA)		0.0000409	0.00000561	0.00000284
Perfluorooctanesulfonamide (PFOSAm)		0.000228	0.0000644	ND (0.0000032)
Perfluorooctanesulfonic acid (PFOS)		0.0835	0.0776	0.0000317
Perfluorooctanoic acid (PFOA)		0.0179	0.00241	0.000143
Perfluoropentanesulfonic acid (PFPeS)		0.0266	0.00450	ND (0.0000032)
Perfluoropentanoic acid (PFPeA)		0.00603	0.00322	0.000297
Perfluorotetradecanoic acid (PFTeDA)		0.0000295	ND (0.0000043)	0.00000474
Perfluorotridecanoic acid (PFTrDA)		0.0000180	0.0000492	ND (0.0000043)
Perfluoroundecanoic acid (PFUnA)		0.00000932	0.0000472	ND (0.0000032)

Appendix VIII - PFAS concentration in contaminated soil used in Campaign 1

Non-detected PFAS (ND) are considered to be at MDL.

Parameter	Units	C2-R1	C2-R2	C2-R3
Date Tested	-	8/3/2024	8/4/2024	8/4/2024
Sample Collection Time	minutes	240	240	240
Waste Input Rate - Liquid Contaminated Soil (lb/hr)	lb/hr	4,825	3,910	2,937
11-Chloroeicosafluoro-3-oxaundecane-1-sulfonic acid (11Cl-PF3OUdS)		ND(0.00000093)	ND(0.00000075)	ND(0.00000056)
1H,1H,2H,2H-Perfluorodecane sulfonic acid (8:2 FTS)		0.000105	0.0000848	0.0000637
1H,1H,2H,2H-Perfluorohexane sulfonic acid (4:2 FTS)		0.0000231	0.0000187	0.0000140
1H,1H,2H,2H-Perfluorooctane sulfonic acid (6:2 FTS)		0.0117	0.00946	0.00711
3-Perfluoroheptylpropanoic acid (7:3 FTCA)		0.00000326	0.00000264	0.00000199
3-Perfluoropentylpropanoic acid (5:3 FTCA)		0.0000290	0.0000235	0.0000177
3-Perfluoropropylpropanoic acid (3:3 FTCA)		0.00000156	0.00000127	0.000000952
4,8-Dioxa-3H-perfluorononanoic acid (DONA)		ND(0.00000093)	ND(0.00000075)	ND(0.00000056)
9-Chlorohexadecafluoro-3-oxanonane-1-sulfonic acid (9Cl-PF3ONS)		ND(0.00000093)	ND(0.00000075)	ND(0.00000056)
Hexafluoropropylene Oxide Dimer Acid (HFPO-DA)		0.000451	0.000365	0.000274
N-ethylperfluorooctane sulfonamide (NEtFOSA)		0.00000550	0.00000446	0.00000335
N-ethylperfluorooctane sulfonamidoethanol (NEtFOSE)		0.000138	0.000112	0.0000843
N-ethylperfluorooctanesulfonamidoacetic acid (NEtFOSAA)		ND(0.00000093)	ND(0.00000075)	ND(0.00000056)
N-methylperfluorooctane sulfonamide (NMeFOSA)		ND (0.0000023)	ND (0.0000019)	ND (0.0000014)
N-methylperfluorooctane sulfonamidoethanol (NMeFOSE)		0.00000438	0.00000355	0.00000267
N-methylperfluorooctanesulfonamidoacetic acid (NMeFOSAA)		ND(0.00000093)	ND(0.00000075)	ND(0.00000056)
Nonafluoro-3,6-dioxaheptanoic acid (NFDHA)		0.0000120	0.00000974	0.00000731
Perfluoro (2-ethoxyethane) sulfonic acid (PFEESA)		ND(0.00000093)	ND(0.00000075)	ND(0.00000056)
Perfluoro-3-methoxypropanoic acid (PFMPA)		0.00000310	0.00000251	0.00000189
Perfluoro-4-methoxybutanoic acid (PFMBA)		ND(0.00000093)	ND(0.00000075)	ND(0.00000056)
Perfluorobutanesulfonic acid (PFBS)	lb/hr	0.000138	0.000112	0.0000840
Perfluorobutanoic acid (PFBA)		0.000360	0.000292	0.000219
Perfluorodecanesulfonic acid (PFDS)		0.00000414	0.00000336	0.00000252
Perfluorodecanoic acid (PFDA)		0.000105	0.0000848	0.0000637
Perfluorododecanesulfonic acid (PFDoS)		0.00000122	0.000000985	0.000000740
Perfluorododecanoic acid (PFDoA)		0.0000152	0.0000124	0.00000928
Perfluoroheptanesulfonic acid (PFHpS)		0.0000352	0.0000285	0.0000214
Perfluoroheptanoic acid (PFHpA)		0.000454	0.000368	0.000277
Perfluorohexanesulfonic acid (PFHxS)		0.00101	0.000817	0.000614
Perfluorohexanoic acid (PFHxA)		0.00172	0.00139	0.00105
Perfluorononanesulfonic acid (PFNS)		0.00000207	0.00000168	0.00000126
Perfluorononanoic acid (PFNA)		0.0000385	0.0000312	0.0000235
Perfluorooctanesulfonamide (PFOSAm)		0.000387	0.000314	0.000236
Perfluorooctanesulfonic acid (PFOS)		0.00719	0.00583	0.00438
Perfluorooctanoic acid (PFOA)		0.000806	0.000653	0.000490
Perfluoropentanesulfonic acid (PFPeS)		0.000178	0.000144	0.000108
Perfluoropentanoic acid (PFPeA)		0.000301	0.000244	0.000183
Perfluorotetradecanoic acid (PFTeDA)		0.00000700	0.00000567	0.00000426
Perfluorotridecanoic acid (PFTrDA)		0.000671	0.000543	0.000408
Perfluoroundecanoic acid (PFUnA)		0.000622	0.000504	0.000379

Appendix IX-A PFAS concentration in solid residues from AFFF incineration Campaign 1

Non-detected PFAS (ND) are considered to be at MDL.

Parameter	C1-R1	C1-R2	C1-R3
Date Tested	7/31/2024	8/1/2024	8/1/2024
Sample Collection Time	13:45	18:45, 4:00	18:30
Waste Input Rate - Solids (lb/hr)	5,403	5,403	5,403
11-Chloroeicosafluoro-3-oxaundecane-1-sulfonic acid (11Cl-PF3OUdS)	ND (0.0000031)	ND (0.0000031)	ND (0.0000032)
1H,1H,2H,2H-Perfluorodecane sulfonic acid (8:2 FTS)	ND (0.0000092)	ND (0.0000094)	ND (0.0000095)
1H,1H,2H,2H-Perfluorohexane sulfonic acid (4:2 FTS)	ND (0.0000092)	ND (0.0000094)	ND (0.0000095)
1H,1H,2H,2H-Perfluorooctane sulfonic acid (6:2 FTS)	ND (0.0000092)	ND (0.0000094)	ND (0.0000095)
3-Perfluoroheptylpropanoic acid (7:3 FTCA)	ND (0.0000031)	ND (0.0000031)	ND (0.0000032)
3-Perfluoropentylpropanoic acid (5:3 FTCA)	ND (0.0000031)	ND (0.0000031)	ND (0.0000032)
3-Perfluoropropylpropanoic acid (3:3 FTCA)	ND (0.0000031)	ND (0.0000031)	ND (0.0000032)
4,8-Dioxa-3H-perfluorononanoic acid (DONA)	ND (0.0000031)	ND (0.0000031)	ND (0.0000032)
9-Chlorohexadecafluoro-3-oxanonane-1-sulfonic acid (9Cl-PF3ONS)	ND (0.0000031)	ND (0.0000031)	ND (0.0000032)
Hexafluoropropylene Oxide Dimer Acid (HFPO-DA)	ND (0.000015)	ND (0.000016)	ND (0.000016)
N-ethylperfluorooctane sulfonamide (NEtFOSA)	ND (0.0000077)	ND (0.0000079)	ND (0.0000079)
N-ethylperfluorooctane sulfonamidoethanol (NEtFOSE)	ND (0.0000077)	ND (0.0000079)	ND (0.0000079)
N-ethylperfluorooctanesulfonamidoacetic acid (NEtFOSAA)	ND (0.0000031)	ND (0.0000031)	ND (0.0000032)
N-methylperfluorooctane sulfonamide (NMeFOSA)	ND (0.0000077)	ND (0.0000079)	ND (0.0000079)
N-methylperfluorooctane sulfonamidoethanol (NMeFOSE)	ND (0.0000077)	ND (0.0000079)	ND (0.0000079)
N-methylperfluorooctanesulfonamidoacetic acid (NMeFOSAA)	ND (0.0000031)	ND (0.0000031)	ND (0.0000032)
Nonafluoro-3,6-dioxaheptanoic acid (NFDHA)	ND (0.0000031)	ND (0.0000031)	ND (0.0000032)
Perfluoro (2-ethoxyethane) sulfonic acid (PFEESA)	ND (0.0000031)	ND (0.0000031)	ND (0.0000032)
Perfluoro-3-methoxypropanoic acid (PFMPA)	ND (0.0000031)	ND (0.0000031)	ND (0.0000032)
Perfluoro-4-methoxybutanoic acid (PFMBA)	ND (0.0000031)	ND (0.0000031)	ND (0.0000032)
Perfluorobutanesulfonic acid (PFBS)	ND (0.0000062)	ND (0.0000063)	ND (0.0000063)
Perfluorobutanoic acid (PFBA)	ND (0.000012)	ND (0.000013)	ND (0.000013)
Perfluorodecanesulfonic acid (PFDS)	ND (0.0000031)	ND (0.0000031)	ND (0.0000032)
Perfluorodecanoic acid (PFDA)	ND (0.0000031)	ND (0.0000031)	ND (0.0000032)
Perfluorododecanesulfonic acid (PFDoS)	ND (0.0000031)	ND (0.0000031)	ND (0.0000032)
Perfluorododecanoic acid (PFDoA)	ND (0.0000031)	ND (0.0000031)	ND (0.0000032)
Perfluoroheptanesulfonic acid (PFHpS)	ND (0.0000031)	ND (0.0000031)	ND (0.0000032)
Perfluoroheptanoic acid (PFHpA)	ND (0.0000031)	ND (0.0000031)	ND (0.0000032)
Perfluorohexanesulfonic acid (PFHxS)	ND (0.0000031)	ND (0.0000031)	ND (0.0000032)
Perfluorohexanoic acid (PFHxA)	ND (0.0000031)	ND (0.0000031)	ND (0.0000032)
Perfluorononanesulfonic acid (PFNS)	ND (0.0000031)	ND (0.0000031)	ND (0.0000032)
Perfluorononanoic acid (PFNA)	ND (0.0000031)	ND (0.0000031)	ND (0.0000032)
Perfluorooctanesulfonamide (PFOSAm)	<0.0000032 DLL	<0.0000034 DLL	ND (0.0000032)
Perfluorooctanesulfonic acid (PFOS)	ND (0.0000031)	ND (0.0000031)	ND (0.0000032)
Perfluorooctanoic acid (PFOA)	ND (0.0000031)	ND (0.0000031)	ND (0.0000032)
Perfluoropentanesulfonic acid (PFPeS)	ND (0.0000031)	ND (0.0000031)	ND (0.0000032)
Perfluoropentanoic acid (PFPeA)	ND (0.0000031)	ND (0.0000031)	ND (0.0000032)
Perfluorotetradecanoic acid (PFTeDA)	ND (0.0000031)	ND (0.0000031)	ND (0.0000032)
Perfluorotridecanoic acid (PFTrDA)	ND (0.0000031)	ND (0.0000031)	ND (0.0000032)
Perfluoroundecanoic acid (PFUnA)	ND (0.0000031)	ND (0.0000031)	ND (0.0000032)

Appendix IX-B PFAS concentration in liquid residues (blowdowns) from AFFF incineration (campaign 1)

Non-detected PFAS (ND) are considered to be at MDL.

Parameter	C1-R1	C1-R2	C1-R3
Date Tested	7/31/2024	8/1/2024	8/1/2024
Sample Collection Time	13:45	18:45	18:30
Waste Input Rate - Liquid (gal/hr)	7,450	7,255	7,699
11-Chloroeicosafluoro-3-oxaundecane-1-sulfonic acid (11Cl-PF3OUdS)	ND (0.000000031)	ND (0.000000029)	ND (0.000000031)
1H,1H,2H,2H-Perfluorodecane sulfonic acid (8:2 FTS)	ND (0.000000037)	ND (0.000000035)	ND (0.000000038)
1H,1H,2H,2H-Perfluorohexane sulfonic acid (4:2 FTS)	ND (0.000000031)	ND (0.000000029)	ND (0.000000031)
1H,1H,2H,2H-Perfluorooctane sulfonic acid (6:2 FTS)	0.00000104	0.000000542	0.00000125
3-Perfluoroheptylpropanoic acid (7:3 FTCA)	ND (0.000000068)	ND (0.000000064)	ND (0.000000069)
3-Perfluoropentylpropanoic acid (5:3 FTCA)	ND (0.000000031)	ND (0.000000029)	ND (0.000000031)
3-Perfluoropropylpropanoic acid (3:3 FTCA)	ND (0.000000031)	0.0000000306	ND (0.000000031)
4,8-Dioxa-3H-perfluorononanoic acid (DONA)	ND (0.000000031)	ND (0.000000029)	ND (0.000000031)
9-Chlorohexadecafluoro-3-oxanonane-1-sulfonic acid (9Cl-PF3ONS)	ND (0.000000031)	ND (0.000000029)	ND (0.000000031)
Hexafluoropropylene Oxide Dimer Acid (HFPO-DA)	0.000000131	0.000000130	0.000000146
N-ethylperfluorooctane sulfonamide (NEtFOSA)	ND (0.000000025)	ND (0.000000023)	ND (0.000000025)
N-ethylperfluorooctane sulfonamidoethanol (NEtFOSE)	ND (0.000000025)	ND (0.000000023)	ND (0.000000025)
N-ethylperfluorooctanesulfonamidoacetic acid (NEtFOSAA)	ND (0.000000031)	ND (0.000000029)	ND (0.000000031)
N-methylperfluorooctane sulfonamide (NMeFOSA)	ND (0.000000043)	ND (0.000000041)	ND (0.000000044)
N-methylperfluorooctane sulfonamidoethanol (NMeFOSE)	ND (0.000000025)	ND (0.000000023)	ND (0.000000025)
N-methylperfluorooctanesulfonamidoacetic acid (NMeFOSAA)	ND (0.000000025)	ND (0.000000023)	ND (0.000000025)
Nonafluoro-3,6-dioxaheptanoic acid (NFDHA)	ND (0.000000018)	ND (0.000000018)	ND (0.000000019)
Perfluoro (2-ethoxyethane) sulfonic acid (PFEESA)	ND (0.000000018)	ND (0.000000018)	ND (0.000000019)
Perfluoro-3-methoxypropanoic acid (PFMPA)	ND (0.000000018)	ND (0.000000018)	ND (0.000000019)
Perfluoro-4-methoxybutanoic acid (PFMBA)	ND (0.000000018)	ND (0.000000018)	ND (0.000000019)
Perfluorobutanesulfonic acid (PFBS)	0.000000190	0.000000191	0.000000213
PFBA	0.00000122	0.00000110	0.00000126
Perfluorodecanesulfonic acid (PFDS)	ND (0.000000018)	ND (0.000000018)	ND (0.000000019)
Perfluorodecanoic acid (PFDA)	ND (0.000000018)	ND (0.000000018)	ND (0.000000019)
Perfluorododecanesulfonic acid (PFDoS)	ND (0.000000018)	ND (0.000000018)	ND (0.000000019)
Perfluorododecanoic acid (PFDoA)	ND (0.000000025)	ND (0.000000023)	ND (0.000000025)
Perfluoroheptanesulfonic acid (PFHpS)	ND (0.000000018)	ND (0.000000018)	ND (0.000000019)
PFHpA	0.000000796	0.000000757	0.000000695
Perfluorohexanesulfonic acid (PFHxS)	0.000000171	0.000000162	0.000000179
PFHxA	0.00000175	0.00000165	0.00000163
Perfluorononanesulfonic acid (PFNS)	ND (0.000000018)	ND (0.000000018)	ND (0.000000019)
PFNA	0.0000000647	0.0000000702	0.0000000561
Perfluorooctanesulfonamide (PFOSAm)	ND (0.000000018)	ND (0.000000018)	0.0000000188
Perfluorooctanesulfonic acid (PFOS)	0.000000200	0.000000179	0.000000205
PFOA	0.000000183	0.000000174	0.000000177
Perfluoropentanesulfonic acid (PFPeS)	0.0000000338	0.0000000325	0.0000000439
PFPeA	0.00000170	0.0000016	0.00000183
Perfluorotetradecanoic acid (PFTeDA)	ND (0.000000025)	0.000000023	ND (0.000000025)
Perfluorotridecanoic acid (PFTrDA)	ND (0.000000025)	0.000000023	ND (0.000000025)
Perfluoroundecanoic acid (PFUnA)	ND (0.000000018)	0.000000018	ND (0.000000019)

Appendix X-A : PFAS concentration in solid residues from contaminated soil incineration (campaign 1)

Non-detected PFAS (ND) are considered to be at MDL.

Parameter	C2-R1	C2-R2	C2-R3
Date Tested	8/3/2024	8/4/2024	8/4/2024
Sample Collection Time	13:15	19:00	19:15
Waste Input Rate - Solids (lb/hr)	5,584	5,584	5,584
11-Chloroeicosafluoro-3-oxaundecane-1-sulfonic acid (11Cl-PF3OUdS)	ND (0.0000033)	ND (0.0000032)	ND (0.0000032)
1H,1H,2H,2H-Perfluorodecane sulfonic acid (8:2 FTS)	ND (0.0000098)	ND (0.0000096)	ND (0.0000095)
1H,1H,2H,2H-Perfluorohexane sulfonic acid (4:2 FTS)	ND (0.0000098)	ND (0.0000096)	ND (0.0000095)
1H,1H,2H,2H-Perfluorooctane sulfonic acid (6:2 FTS)	ND (0.0000098)	ND (0.0000096)	ND (0.0000095)
3-Perfluoroheptylpropanoic acid (7:3 FTCA)	ND (0.0000033)	ND (0.0000032)	ND (0.0000032)
3-Perfluoropentylpropanoic acid (5:3 FTCA)	ND (0.0000033)	ND (0.0000032)	ND (0.0000032)
3-Perfluoropropylpropanoic acid (3:3 FTCA)	ND (0.0000033)	ND (0.0000032)	ND (0.0000032)
4,8-Dioxa-3H-perfluorononanoic acid (DONA)	ND (0.0000033)	ND (0.0000032)	ND (0.0000032)
9-Chlorohexadecafluoro-3-oxanonane-1-sulfonic acid (9Cl-PF3ONS)	ND (0.0000033)	ND (0.0000032)	ND (0.0000032)
Hexafluoropropylene Oxide Dimer Acid (HFPO-DA)	ND (0.000016)	ND (0.000016)	ND (0.000016)
N-ethylperfluorooctane sulfonamide (NEtFOSA)	ND (0.0000081)	ND (0.0000080)	ND (0.0000079)
N-ethylperfluorooctane sulfonamidoethanol (NEtFOSE)	ND (0.0000081)	ND (0.0000080)	ND (0.0000079)
N-ethylperfluorooctanesulfonamidoacetic acid (NEtFOSAA)	ND (0.0000033)	ND (0.0000032)	ND (0.0000032)
N-methylperfluorooctane sulfonamide (NMeFOSA)	ND (0.0000081)	ND (0.0000080)	ND (0.0000079)
N-methylperfluorooctane sulfonamidoethanol (NMeFOSE)	ND (0.0000081)	ND (0.0000080)	ND (0.0000079)
N-methylperfluorooctanesulfonamidoacetic acid (NMeFOSAA)	ND (0.0000033)	ND (0.0000032)	ND (0.0000032)
Nonafluoro-3,6-dioxaheptanoic acid (NFDHA)	ND (0.0000033)	ND (0.0000032)	ND (0.0000032)
Perfluoro (2-ethoxyethane) sulfonic acid (PFEESA)	ND (0.0000033)	ND (0.0000032)	ND (0.0000032)
Perfluoro-3-methoxypropanoic acid (PFMPA)	ND (0.0000033)	ND (0.0000032)	ND (0.0000032)
Perfluoro-4-methoxybutanoic acid (PFMBA)	ND (0.0000033)	ND (0.0000032)	ND (0.0000032)
Perfluorobutanesulfonic acid (PFBS)	ND (0.0000065)	ND (0.0000064)	ND (0.0000063)
Perfluorobutanoic acid (PFBA)	ND (0.000013)	ND (0.000013)	ND (0.000013)
Perfluorodecanesulfonic acid (PFDS)	ND (0.0000033)	ND (0.0000032)	ND (0.0000032)
Perfluorodecanoic acid (PFDA)	ND (0.0000033)	ND (0.0000032)	ND (0.0000032)
Perfluorododecanesulfonic acid (PFDoS)	ND (0.0000033)	ND (0.0000032)	ND (0.0000032)
Perfluorododecanoic acid (PFDoA)	ND (0.0000033)	ND (0.0000032)	ND (0.0000032)
Perfluoroheptanesulfonic acid (PFHpS)	ND (0.0000033)	ND (0.0000032)	ND (0.0000032)
Perfluoroheptanoic acid (PFHpA)	ND (0.0000033)	ND (0.0000032)	ND (0.0000032)
Perfluorohexanesulfonic acid (PFHxS)	ND (0.0000033)	ND (0.0000032)	ND (0.0000032)
Perfluorohexanoic acid (PFHxA)	ND (0.0000033)	ND (0.0000032)	ND (0.0000032)
Perfluorononanesulfonic acid (PFNS)	ND (0.0000033)	ND (0.0000032)	ND (0.0000032)
Perfluorononanoic acid (PFNA)	ND (0.0000033)	ND (0.0000032)	ND (0.0000032)
Perfluorooctanesulfonamide (PFOSAm)	ND (0.0000033)	ND (0.0000032)	ND (0.0000032)
Perfluorooctanesulfonic acid (PFOS)	ND (0.0000033)	ND (0.0000032)	ND (0.0000032)
Perfluorooctanoic acid (PFOA)	ND (0.0000033)	ND (0.0000032)	ND (0.0000032)
Perfluoropentanesulfonic acid (PFPeS)	ND (0.0000033)	ND (0.0000032)	ND (0.0000032)
Perfluoropentanoic acid (PFPeA)	ND (0.0000033)	ND (0.0000032)	ND (0.0000032)
Perfluorotetradecanoic acid (PFTeDA)	ND (0.0000033)	ND (0.0000032)	ND (0.0000032)
Perfluorotridecanoic acid (PFTrDA)	ND (0.0000033)	ND (0.0000032)	ND (0.0000032)
Perfluoroundecanoic acid (PFUnA)	ND (0.0000033)	ND (0.0000032)	ND (0.0000032)

Appendix X-B : PFAS concentration in liquid residues from contaminated soil incineration (campaign 1)

Non-detected PFAS (ND) are considered to be at MDL.

Parameter	C2-R1	C2-R2	C2-R3
Date Tested	8/3/24 – 8/4/24	8/4/2024	8/4/24 – 8/5/24
Sample Collection Time	13:15, 19:00, 22:30, 02:30	19:15	23:15, 03:15, 07:15, 19:15
Waste Input Rate - Liquid (gal/hr)	7,461	6,931	7,179
11-Chloroeicosafluoro-3-oxaundecane-1-sulfonic acid (11Cl-PF3OUdS)	ND (0.00000012)	ND (0.000000028)	ND (0.00000011)
1H,1H,2H,2H-Perfluorodecane sulfonic acid (8:2 FTS)	0.000000194	ND (0.000000034)	0.000000365
1H,1H,2H,2H-Perfluorohexane sulfonic acid (4:2 FTS)	<0.00000013 DLL	ND (0.000000028)	0.000000119
1H,1H,2H,2H-Perfluorooctane sulfonic acid (6:2 FTS)	0.0000461	0.00000821	0.000151
3-Perfluoroheptylpropanoic acid (7:3 FTCA)	ND (0.00000027)	ND (0.000000061)	ND (0.00000025)
3-Perfluoropentylpropanoic acid (5:3 FTCA)	ND (0.00000012)	ND (0.000000028)	ND (0.00000011)
3-Perfluoropropylpropanoic acid (3:3 FTCA)	ND (0.00000012)	ND (0.000000028)	ND (0.00000011)
4,8-Dioxa-3H-perfluorononanoic acid (DONA)	ND (0.00000012)	ND (0.000000028)	ND (0.00000011)
9-Chlorohexadecafluoro-3-oxanonane-1-sulfonic acid (9Cl-PF3ONS)	ND (0.00000012)	ND (0.000000028)	ND (0.00000011)
Hexafluoropropylene Oxide Dimer Acid (HFPO-DA)	0.00000150	0.000000179	0.00000139
N-ethylperfluorooctane sulfonamide (NEtFOSA)	ND (0.000000097)	ND (0.000000022)	ND (0.000000089)
N-ethylperfluorooctane sulfonamidoethanol (NEtFOSE)	ND (0.000000097)	ND (0.000000022)	ND (0.000000089)
N-ethylperfluorooctanesulfonamidoacetic acid (NEtFOSAA)	ND (0.00000012)	ND (0.000000028)	ND (0.00000011)
N-methylperfluorooctane sulfonamide (NMeFOSA)	ND (0.00000017)	ND (0.000000039)	0.000000113
N-methylperfluorooctane sulfonamidoethanol (NMeFOSE)	ND (0.000000097)	ND (0.000000022)	ND (0.000000089)
N-methylperfluorooctanesulfonamidoacetic acid (NMeFOSAA)	ND (0.000000097)	ND (0.000000022)	ND (0.000000089)
Nonafluoro-3,6-dioxaheptanoic acid (NFDHA)	ND (0.000000072)	ND (0.000000017)	<0.000000068 DLL
Perfluoro (2-ethoxyethane) sulfonic acid (PFEESA)	ND (0.000000072)	ND (0.000000017)	ND (0.000000067)
Perfluoro-3-methoxypropanoic acid (PFMPA)	ND (0.000000072)	ND (0.000000017)	ND (0.000000067)
Perfluoro-4-methoxybutanoic acid (PFMBA)	ND (0.000000072)	ND (0.000000017)	ND (0.000000067)
Perfluorobutanesulfonic acid (PFBS)	0.00000114	0.000000222	0.000000908
Perfluorobutanoic acid (PFBA)	0.00000634	0.00000127	0.00000740
Perfluorodecanesulfonic acid (PFDS)	ND (0.000000072)	ND (0.000000017)	ND (0.000000067)
Perfluorodecanoic acid (PFDA)	0.0000000413	ND (0.000000017)	<0.000000072 DLL
Perfluorododecanesulfonic acid (PFDoS)	ND (0.000000072)	ND (0.000000017)	<0.000000071 DLL
Perfluorododecanoic acid (PFDoA)	ND (0.000000097)	ND (0.000000022)	ND (0.000000089)
Perfluoroheptanesulfonic acid (PFHpS)	ND (0.000000072)	ND (0.000000017)	ND (0.000000067)
Perfluoroheptanoic acid (PFHpA)	0.00000511	0.000000706	0.00000555
Perfluorohexanesulfonic acid (PFHxS)	0.000000857	0.000000199	0.000000719
Perfluorohexanoic acid (PFHxA)	0.0000131	0.00000178	0.0000188
Perfluorononanesulfonic acid (PFNS)	ND (0.000000072)	ND (0.000000017)	ND (0.000000067)
Perfluorononanoic acid (PFNA)	0.000000342	0.0000000508	0.000000162
Perfluorooctanesulfonamide (PFOSAm)	0.000000305	0.000000152	0.0000000527
Perfluorooctanesulfonic acid (PFOS)	0.000000973	0.000000227	0.000000723
Perfluorooctanoic acid (PFOA)	0.00000106	0.000000165	0.00000104
Perfluoropentanesulfonic acid (PFPeS)	0.000000187	0.0000000334	0.000000155
Perfluoropentanoic acid (PFPeA)	0.00000920	0.00000182	0.0000114
Perfluorotetradecanoic acid (PFTeDA)	ND (0.000000097)	ND (0.000000022)	0.0000000611
Perfluorotridecanoic acid (PFTrDA)	ND (0.000000097)	ND (0.000000022)	<0.000000090 DLL
Perfluoroundecanoic acid (PFUnA)	ND (0.000000072)	ND (0.000000017)	ND (0.000000067)

Appendix XI-A OTM-45 and DRE for the AFFF run#1 in campaign 1

	AFFF TF Feed Stream	R1 Stack Emissions	DRE
	Avg lb/hr	lb/hr	
Feed Rate	2,421		
Target Compounds	**lb/hr**	**lb/hr**	**%**
Perfluorobutanesulfonic acid (PFBS)	0.0245	0.000000075*	>99.9997
Perfluorobutanoic acid (PFBA)	0.00443	0.00000087*	>99.9804
Perfluorodecanesulfonic acid (PFDS)	0.000164	0.000000025*	>99.9848
Perfluorodecanoic acid (PFDA)	0.000178	0.000000183	99.8972
Perfluorododecanesulfonic acid (PFDoS)	0.000087	0.000000022*	>99.9746
Perfluorododecanoic acid (PFDoA)	0.0000675	0.000000081	99.8807
Perfluoroheptanesulfonic acid (PFHpS)	0.0334	0.000000027*	>99.9999
Perfluoroheptanoic acid (PFHpA)	0.00583	0.000000912	99.9844
Perfluorohexadecanoic acid	--	0.000000073	--
Perfluorohexanesulfonic acid (PFHxS)	0.179	0.000000027*	>99.9999
Perfluorohexanoic acid (PFHxA)	0.0223	0.000000605	99.9973
Perfluorononanesulfonic acid (PFNS)	0.000327	0.000000019*	>99.9942
Perfluorononanoic acid (PFNA)	0.0000409	0.00000041	98.9927
Perfluorooctadecanoic acid	--	0.000000047*	--
Perfluorooctanesulfonamide (FOSA)	0.000223	0.000000024*	>99.9892
Perfluorooctanesulfonic acid (PFOS)	0.0835	0.000000038*	>99.9999
Perfluorooctanoic acid (PFOA)	0.01790	0.00000078	99.9956
Perfluoropentanesulfonic acid (PFPeS)	0.0266	0.000000024*	>99.9999
Perfluoropentanoic acid (PFPeA)	0.00603	0.00000033	99.9945
Perfluorotetradecanoic acid (PFTeA)	0.0000295	0.0000001	99.6881
Perfluorotridecanoic acid (PFTriA)	0.000018	0.00000008	99.5417
Perfluoroundecanoic acid (PFUnA)	0.00000932	0.00000012	98.7554
9-Chlorohexadecafluoro-3-oxanonane-1-sulfonic acid	0.0000059*	0.000000021*	>99.6441
N-ethylperfluoro-1-octanesulfonamide	0.0000685	0.000000064*	>99.9066
N-methylperfluorooctanesulfonamidoacetic acid (NMeFOSAA)	0.0000047*	0.000000024*	>99.4894
N-ethylperfluorooctanesulfonamidoacetic acid (NEtFOSAA)	0.0000207	0.000000020*	>99.9034
11-Chloroeicosafluoro-3-oxaundecane-1-sulfonic acid	0.0000059*	0.000000025*	>99.5763
2-(N-ethylperfluoro-1-octanesulfonamido) ethanol	0.000455	0.000000037*	>99.9919
2-(N-methylperfluoro-1-octanesulfonamido) ethanol	0.000264	0.0000016*	>99.3939
PES	--	0.000000021*	--
PFECA A	--	0.000000031*	--
PFECA B	--	0.000000027*	--
PFECA F	--	0.000000025*	--
PFECHS	--	0.000000030*	--
DONA	0.0000059*	0.000000096*	>98.3729
HFPO-DA	0.00000586	0.00000099*	>83.1058
NMeFOSA	0.0000458	0.000000068*	>99.8515
10:2 FTCA	--	0.000000075*	--
10:2 FTS	--	0.000000032*	--
3:3 FTCA	0.00000612	0.000000049*	>99.1993
4:2 FTS	0.0000353	0.000000012*	>99.9660
5:3 FTCA	0.000022	0.000000090*	>99.5909
6:2 FTCA	--	0.000000110*	--

6:2 FTS	0.00426	0.00000080*	>99.9812
6:2 FTUCA	--	0.000000026*	--
7:3 FTCA	0.000013*	0.000000068*	>99.4769
8:2 FTCA	--	0.000000064*	--
8:2 FTS	0.00197	0.000000026*	>99.9987
8:2 FTUCA	--	0.000000037*	--
Nonafluoro-3,6-dioxaheptanoic acid (NFDHA)	0.0000035*	--	--
Perfluoro (2-ethoxyethane) sulfonic acid (PFEESA)	0.00000625	--	--
Perfluoro-3-methoxypropanoic acid (PFMPA)	0.0000154	--	--
Perfluoro-4-methoxybutanoic acid (PFMBA)	0.0000231	--	--

* Values are below the MDL or have a qualifier for values above the MDL but below the LoQ.
For DRE calculation, PFAS not detected in the waste are considered to be at MDL level in the waste. PFAS detected below the MDL and LoQ are assumed to be at LoQ in the waste. In those cases, DRE is shown with >.

Appendix XI-B OTM-45 and DRE for the AFFF run#2 in campaign 1

Feed Rate	AFFF TF Feed Stream Avg lb/hr 2,175	R2 Stack Emissions lb/hr	DRE
Target Compounds	**lb/hr**	**lb/hr**	**%**
Perfluorobutanesulfonic acid (PFBS)	0.0022	0.000000076*	>99.9965
Perfluorobutanoic acid (PFBA)	0.00108	0.00000059*	99.9455
Perfluorodecanesulfonic acid (PFDS)	0.000089	0.000000025*	>99.9720
Perfluorodecanoic acid (PFDA)	0.000005	0.000000164	96.8340
Perfluorododecanesulfonic acid (PFDoS)	0.000025	0.000000023*	>99.9080
Perfluorododecanoic acid (PFDoA)	0.0000043*	0.000000072	>98.3302
Perfluoroheptanesulfonic acid (PFHpS)	0.0006	0.000000027*	>99.9955
Perfluoroheptanoic acid (PFHpA)	0.00056	0.000000398	99.9291
Perfluorohexadecanoic acid	--	0.000000058	--
Perfluorohexanesulfonic acid (PFHxS)	0.018	0.000000028*	>99.9998
Perfluorohexanoic acid (PFHxA)	0.0041	0.000000338	99.9917
Perfluorononanesulfonic acid (PFNS)	0.000076	0.000000019*	>99.9751
Perfluorononanoic acid (PFNA)	0.0000056	0.00000029	94.8485
Perfluorooctadecanoic acid	--	0.000000050*	--
Perfluorooctanesulfonamide (FOSA)	0.000064	0.000000025*	>99.9612
Perfluorooctanesulfonic acid (PFOS)	0.0776	0.000000035*	>100.0000
Perfluorooctanoic acid (PFOA)	0.00241	0.00000038	99.9843
Perfluoropentanesulfonic acid (PFPeS)	0.0045	0.000000025*	>99.9994
Perfluoropentanoic acid (PFPeA)	0.00322	0.00000027	99.9916
Perfluorotetradecanoic acid (PFTeA)	0.0000043*	0.0000001	98.6628
Perfluorotridecanoic acid (PFTriA)	0.000049	0.00000007	99.8663
Perfluoroundecanoic acid (PFUnA)	0.00004720	0.00000010	99.7860
9-Chlorohexadecafluoro-3-oxanonane-1-sulfonic acid	0.0000053*	0.000000022*	>99.5849
N-ethylperfluoro-1-octanesulfonamide	0.0000043*	0.000000066*	>98.4651
N-methylperfluorooctanesulfonamidoacetic acid (NMeFOSAA)	0.0000043*	0.000000025*	>99.4186
N-ethylperfluorooctanesulfonamidoacetic acid (NEtFOSAA)	0.0000053*	0.000000021*	>99.6038
11-Chloroeicosafluoro-3-oxaundecane-1-sulfonic acid	0.0000053*	0.000000026*	>99.5094
2-(N-ethylperfluoro-1-octanesulfonamido) ethanol	0.000040	0.000000038*	>99.9040
2-(N-methylperfluoro-1-octanesulfonamido) ethanol	0.000018	0.0000017*	>90.6077
PES	--	0.000000022*	--
PFECA A	--	0.000000031*	--
PFECA B	--	0.000000028*	--
PFECA F	--	0.000000027*	--
PFECHS	--	0.000000030*	--
DONA	0.0000053*	0.000000099*	>98.1321
HFPO-DA	0.0000043*	0.00000100*	>76.7442
NMeFOSA	0.0000075*	0.000000070*	>99.0667
10:2 FTCA	--	0.000000076*	--
10:2 FTS	--	0.000000032*	--
3:3 FTCA	0.0000053*	0.000000050*	>99.0566
4:2 FTS	0.0000857	0.000000012*	>99.9860
5:3 FTCA	0.000009	0.000000092*	>98.9834
6:2 FTCA	--	0.000000110*	--
6:2 FTS	0.00868	0.00000083*	>99.9904
6:2 FTUCA	--	0.000000027*	--
7:3 FTCA	0.000012*	0.000000069*	>99.4250
8:2 FTCA	--	0.000000065*	--

8:2 FTS	0.00010	0.000000026*	>99.9731
8:2 FTUCA	--	0.000000037*	--
Nonafluoro-3,6-dioxaheptanoic acid (NFDHA)	0.0000032*	--	--
Perfluoro (2-ethoxyethane) sulfonic acid (PFEESA)	0.0000032*	--	--
Perfluoro-3-methoxypropanoic acid (PFMPA)	0.0000032*	--	--
Perfluoro-4-methoxybutanoic acid (PFMBA)	0.0000032*	--	--

* Values are below the MDL or have a qualifier for values above the MDL but below the LoQ.
For DRE calculation, PFAS not detected in the waste are assumed to be at MDL level in the waste. PFAS detected below the MDL and LoQ are assumed to be at LoQ in the waste. In those cases, DRE is shown with >.

Appendix XI-C OTM-45 and DRE for the AFFF run#3 in campaign 1

Feed Rate	AFFF TF Feed Stream Avg lb/hr 2,235	R3 Stack Emissions lb/hr	DRE
Target Compounds	**lb/hr**	**lb/hr**	**%**
Perfluorobutanesulfonic acid (PFBS)	0.0000032*	0.000000079*	>97.5313
Perfluorobutanoic acid (PFBA)	0.000858	0.00000080*	>99.9068
Perfluorodecanesulfonic acid (PFDS)	0.0000032*	0.000000025*	>99.2188
Perfluorodecanoic acid (PFDA)	0.0000032*	0.000000036*	>98.8750
Perfluorododecanesulfonic acid (PFDoS)	0.0000032*	0.000000023*	>99.2813
Perfluorododecanoic acid (PFDoA)	0.0000131	0.000000012*	99.9115
Perfluoroheptanesulfonic acid (PFHpS)	0.0000032*	0.000000028*	>99.1250
Perfluoroheptanoic acid (PFHpA)	0.0000295	0.000001300*	95.5932
Perfluorohexadecanoic acid	--	0.000000044*	--
Perfluorohexanesulfonic acid (PFHxS)	0.00000396	0.000000029*	>99.2677
Perfluorohexanoic acid (PFHxA)	0.00646	0.000000339*	99.9948
Perfluorononanesulfonic acid (PFNS)	0.0000032*	0.000000019*	>99.4063
Perfluorononanoic acid (PFNA)	0.00000284	0.000000075*	>97.3592
Perfluorooctadecanoic acid	--	0.000000045*	--
Perfluorooctanesulfonamide (FOSA)	0.0000032*	0.000000025*	>99.2188
Perfluorooctanesulfonic acid (PFOS)	0.0000317	0.000000038*	>99.8801
Perfluorooctanoic acid (PFOA)	0.000143	0.000000147*	99.8972
Perfluoropentanesulfonic acid (PFPeS)	0.0000032*	0.000000025*	>99.2188
Perfluoropentanoic acid (PFPeA)	0.000297	0.000000082*	99.9724
Perfluorotetradecanoic acid (PFTeA)	0.0000032*	0.000000035*	98.9000
Perfluorotridecanoic acid (PFTriA)	0.0000043*	0.000000022*	99.4814
Perfluoroundecanoic acid (PFUnA)	0.0000032*	0.000000036*	98.8750
9-Chlorohexadecafluoro-3-oxanonane-1-sulfonic acid	0.0000054*	0.000000022*	>99.5926
N-ethylperfluoro-1-octanesulfonamide	0.0000043*	0.000000066*	>98.4651
N-methylperfluorooctanesulfonamidoacetic acid (NMeFOSAA)	0.0000043*	0.000000025*	>99.4186
N-ethylperfluorooctanesulfonamidoacetic acid (NEtFOSAA)	0.0000054*	0.000000021*	>99.6111
11-Chloroeicosafluoro-3-oxaundecane-1-sulfonic acid	0.0000054*	0.000000026*	>99.5185
2-(N-ethylperfluoro-1-octanesulfonamido) ethanol	0.0000043*	0.000000038*	>99.1163
2-(N-methylperfluoro-1-octanesulfonamido) ethanol	0.0000043*	0.0000017*	>60.4651
PES	--	0.000000022*	--
PFECA A	--	0.000000032*	--
PFECA B	--	0.000000028*	--
PFECA F	--	0.000000025*	--
PFECHS	--	0.000000031*	--
DONA	0.0000054*	0.000000099*	>98.1667
HFPO-DA	0.0000043*	0.0000010*	>76.7442
NMeFOSA	0.0000075*	0.000000070*	>99.0667
10:2 FTCA	--	0.000000077*	--
10:2 FTS	--	0.000000033*	--
3:3 FTCA	0.0000054*	0.000000051*	>99.0556
4:2 FTS	0.0003800	0.000000013*	>99.9966
5:3 FTCA	0.000020	0.000000093*	>99.5303
6:2 FTCA	--	0.00000011*	--
6:2 FTS	0.0102	0.00000083*	>99.9919
6:2 FTUCA	--	0.000000027*	--
7:3 FTCA	0.000029*	0.000000070*	>99.7552
8:2 FTCA	--	0.000000066*	--

8:2 FTS	0.00011	0.000000027*	>99.9761
8:2 FTUCA	--	0.000000038*	--
Nonafluoro-3,6-dioxaheptanoic acid (NFDHA)	0.0000032*	--	--
Perfluoro (2-ethoxyethane) sulfonic acid (PFEESA)	0.0000032*	--	--
Perfluoro-3-methoxypropanoic acid (PFMPA)	0.0000032*	--	--
Perfluoro-4-methoxybutanoic acid (PFMBA)	0.0000032*	--	--

* Values are below the MDL or have a qualifier for values above the MDL but below the LoQ.
For DRE calculation, PFAS not detected in the waste are assumed to be at MDL level in the waste. PFAS detected below the MDL and LoQ are assumed to be at LoQ in the waste. In those cases, DRE is shown with >.

Appendix XII-A OTM-45 and DRE for the contaminated soil run#1 in campaign 1

Feed Rate	Cont. Soil Feed Stream Avg lb/hr 4,825	R1 Stack Emissions lb/hr	DRE
Target Compounds	**lb/hr**	**lb/hr**	**%**
Perfluorobutanesulfonic acid (PFBS)	0.000138	0.000000074*	>99.9464
Perfluorobutanoic acid (PFBA)	0.00036	0.00000077*	>99.7861
Perfluorodecanesulfonic acid (PFDS)	0.0000041	0.000000025*	>99.3961
Perfluorodecanoic acid (PFDA)	0.000105	0.000000120	99.8857
Perfluorododecanesulfonic acid (PFDoS)	0.00000122	0.000000022*	>98.1967
Perfluorododecanoic acid (PFDoA)	0.0000152	0.0000000551	99.6375
Perfluoroheptanesulfonic acid (PFHpS)	0.0000352	0.000000027*	>99.9233
Perfluoroheptanoic acid (PFHpA)	0.00045	0.000000242	99.9467
Perfluorohexadecanoic acid	--	0.0000000488	--
Perfluorohexanesulfonic acid (PFHxS)	0.00101	0.0000000156	99.9985
Perfluorohexanoic acid (PFHxA)	0.00172	0.000000233	99.9865
Perfluorononanesulfonic acid (PFNS)	0.0000021	0.000000019*	>99.0821
Perfluorononanoic acid (PFNA)	0.0000385	0.000000179	99.5351
Perfluorooctadecanoic acid	--	0.0000000361	--
Perfluorooctanesulfonamide (FOSA)	0.000387	0.000000024*	>99.9938
Perfluorooctanesulfonic acid (PFOS)	0.0072	0.000000033*	>99.9995
Perfluorooctanoic acid (PFOA)	0.00081	0.000000233	99.9711
Perfluoropentanesulfonic acid (PFPeS)	0.000178	0.000000024*	>99.9865
Perfluoropentanoic acid (PFPeA)	0.00030	0.000000152	99.9495
Perfluorotetradecanoic acid (PFTeA)	0.0000070	0.0000000416	99.4057
Perfluorotridecanoic acid (PFTriA)	0.000671	0.0000000528	99.9921
Perfluoroundecanoic acid (PFUnA)	0.000622	0.0000000678	99.9891
9-Chlorohexadecafluoro-3-oxanonane-1-sulfonic acid	0.00000093*	0.000000021*	>97.7419
N-ethylperfluoro-1-octanesulfonamide	0.0000055	0.000000064*	>98.8364
N-methylperfluorooctanesulfonamidoacetic acid (NMeFOSAA)	0.00000093*	0.000000024*	>97.4194
N-ethylperfluorooctanesulfonamidoacetic acid (NEtFOSAA)	0.00000093*	0.000000021*	>97.7419
11-Chloroeicosafluoro-3-oxaundecane-1-sulfonic acid	0.00000093*	0.000000025*	>97.3118
2-(N-ethylperfluoro-1-octanesulfonamido) ethanol	0.000138	0.000000037*	>99.9732
2-(N-methylperfluoro-1-octanesulfonamido) ethanol	0.0000044	0.0000016*	>63.4703
PES	--	0.000000022*	--
PFECA A	--	0.000000031*	--
PFECA B	--	0.000000028*	--
PFECA F	--	0.000000025*	--
PFECHS	--	0.000000030*	--
DONA	0.00000093*	0.000000095*	>89.7849
HFPO-DA	0.000451	0.00000099*	>99.7805
NMeFOSA	0.00000230*	0.000000068*	>97.0435
10:2 FTCA	--	0.000000075*	--
10:2 FTS	--	0.000000032*	--
3:3 FTCA	0.00000156	0.000000049*	>96.8590
4:2 FTS	0.0000231	0.000000012*	>99.9481
5:3 FTCA	0.000029	0.000000090*	>99.6897
6:2 FTCA	--	0.00000011*	--
6:2 FTS	0.0117	0.00000080*	>99.9932
6:2 FTUCA	--	0.000000026*	--
7:3 FTCA	0.0000033*	0.000000068*	>97.9141
8:2 FTCA	--	0.000000064*	--

8:2 FTS	0.00011	0.000000026*	>99.9752
8:2 FTUCA	--	0.000000037*	--
Nonafluoro-3,6-dioxaheptanoic acid (NFDHA)	0.0000120*	--	--
Perfluoro (2-ethoxyethane) sulfonic acid (PFEESA)	0.00000093*	--	--
Perfluoro-3-methoxypropanoic acid (PFMPA)	0.0000031	--	--
Perfluoro-4-methoxybutanoic acid (PFMBA)	0.00000093*	--	--

* Values are below the MDL or have a qualifier for values above the MDL but below the LoQ.
For DRE calculation, PFAS not detected in the waste are assumed to be at MDL level in the waste. PFAS detected below the MDL and LoQ are assumed to be at LoQ in the waste. In those cases, DRE is shown with >.

Appendix XII-B OTM-45 and DRE for the contaminated soil run#2 in campaign 1

Feed Rate	Cont. Soil Feed Stream Avg lb/hr 3,910	R2 Stack Emissions lb/hr	DRE
Target Compounds	**lb/hr**	**lb/hr**	**%**
Perfluorobutanesulfonic acid (PFBS)	0.000112	0.000000074*	>99.9339
Perfluorobutanoic acid (PFBA)	0.00029	0.00000082*	>99.7192
Perfluorodecanesulfonic acid (PFDS)	0.00000336	0.000000025*	>99.2560
Perfluorodecanoic acid (PFDA)	0.000085	0.000000239	99.7182
Perfluorododecanesulfonic acid (PFDoS)	0.00000099	0.000000022*	>97.7665
Perfluorododecanoic acid (PFDoA)	0.0000124	0.0000000855	99.3105
Perfluoroheptanesulfonic acid (PFHpS)	0.0000285	0.000000027*	>99.9053
Perfluoroheptanoic acid (PFHpA)	0.00037	0.000000818	99.7777
Perfluorohexadecanoic acid	--	0.0000000345	--
Perfluorohexanesulfonic acid (PFHxS)	0.00082	0.000000028*	>99.9966
Perfluorohexanoic acid (PFHxA)	0.00139	0.000000706	99.9492
Perfluorononanesulfonic acid (PFNS)	0.0000017	0.000000019*	>98.8690
Perfluorononanoic acid (PFNA)	0.0000312	0.000000465	98.5096
Perfluorooctadecanoic acid	--	0.000000064*	--
Perfluorooctanesulfonamide (FOSA)	0.000314	0.000000024*	>99.9924
Perfluorooctanesulfonic acid (PFOS)	0.0058	0.000000033*	>99.9994
Perfluorooctanoic acid (PFOA)	0.00065	0.000000793	99.8786
Perfluoropentanesulfonic acid (PFPeS)	0.000144	0.0000000116	99.9919
Perfluoropentanoic acid (PFPeA)	0.00024	0.000000450	99.8156
Perfluorotetradecanoic acid (PFTeA)	0.0000057	0.0000000711	98.7460
Perfluorotridecanoic acid (PFTriA)	0.000543	0.0000000919	99.9831
Perfluoroundecanoic acid (PFUnA)	0.000504	0.000000135	99.9732
9-Chlorohexadecafluoro-3-oxanonane-1-sulfonic acid	0.00000075*	0.000000022*	>97.0667
N-ethylperfluoro-1-octanesulfonamide	0.0000045	0.000000064*	>98.5650
N-methylperfluorooctanesulfonamidoacetic acid (NMeFOSAA)	0.00000075*	0.000000024*	>96.8000
N-ethylperfluorooctanesulfonamidoacetic acid (NEtFOSAA)	0.00000075*	0.000000021*	>97.2000
11-Chloroeicosafluoro-3-oxaundecane-1-sulfonic acid	0.00000075*	0.000000026*	>96.5333
2-(N-ethylperfluoro-1-octanesulfonamido) ethanol	0.000112	0.000000037*	>99.9670
2-(N-methylperfluoro-1-octanesulfonamido) ethanol	0.0000036	0.0000017*	>52.1127
PES	--	0.000000022*	--
PFECA A	--	0.000000037*	--
PFECA B	--	0.000000028*	--
PFECA F	--	0.000000033*	--
PFECHS	--	0.000000030*	--
DONA	0.00000075*	0.000000096*	>87.2000
HFPO-DA	0.000365	0.00000100*	>99.7260
NMeFOSA	0.00000190*	0.000000068*	>96.4211
10:2 FTCA	--	0.000000075*	--
10:2 FTS	--	0.000000032*	--
3:3 FTCA	0.00000127	0.000000049*	>96.1417
4:2 FTS	0.0000187	0.000000012*	>99.9358
5:3 FTCA	0.000024	0.000000091*	>99.6128
6:2 FTCA	--	0.00000011*	--
6:2 FTS	0.0095	0.00000081*	>99.9914
6:2 FTUCA	--	0.000000026*	--
7:3 FTCA	0.0000026*	0.000000068*	>97.4242
8:2 FTCA	--	0.000000064*	--

8:2 FTS	0.000085	0.000000026*	>99.9693
8:2 FTUCA	--	0.000000037*	--
Nonafluoro-3,6-dioxaheptanoic acid (NFDHA)	0.0000097*	--	--
Perfluoro (2-ethoxyethane) sulfonic acid (PFEESA)	0.00000075*	--	--
Perfluoro-3-methoxypropanoic acid (PFMPA)	0.0000025	--	--
Perfluoro-4-methoxybutanoic acid (PFMBA)	0.00000075*	--	--

* Values are below the MDL or have a qualifier for values above the MDL but below the LoQ.
For DRE calculation, PFAS not detected in the waste are assumed to be at MDL level in the waste. PFAS detected below the MDL and LoQ are assumed to be at LoQ in the waste. In those cases, DRE is shown with >.

Appendix XII-C OTM-45 and DRE for the contaminated soil run#3 in campaign 1

	Cont. Soil Feed Stream	R3 Stack Emissions	DRE
	Avg lb/hr	lb/hr	
Feed Rate	2,937		
Target Compounds	**lb/hr**	**lb/hr**	**%**
Perfluorobutanesulfonic acid (PFBS)	0.000084	0.000000073*	>99.9131
Perfluorobutanoic acid (PFBA)	0.00022	0.00000085*	>99.6119
Perfluorodecanesulfonic acid (PFDS)	0.00000252	0.000000024*	>99.0476
Perfluorodecanoic acid (PFDA)	0.000064	0.000000033*	>99.9482
Perfluorododecanesulfonic acid (PFDoS)	0.00000074	0.000000022*	>97.0270
Perfluorododecanoic acid (PFDoA)	0.0000093	0.0000000107	99.8847
Perfluoroheptanesulfonic acid (PFHpS)	0.0000214	0.00000003*	>99.8738
Perfluoroheptanoic acid (PFHpA)	0.00028	0.00000021*	>99.9242
Perfluorohexadecanoic acid	--	0.0000000285	--
Perfluorohexanesulfonic acid (PFHxS)	0.00061	0.0000000140	99.9977
Perfluorohexanoic acid (PFHxA)	0.00105	0.000000129	99.9877
Perfluorononanesulfonic acid (PFNS)	0.0000013	0.000000019*	>98.4921
Perfluorononanoic acid (PFNA)	0.0000235	0.000000071*	>99.6979
Perfluorooctadecanoic acid	--	0.0000000287	--
Perfluorooctanesulfonamide (FOSA)	0.000236	0.000000024*	>99.9898
Perfluorooctanesulfonic acid (PFOS)	0.0044	0.000000035*	>99.9992
Perfluorooctanoic acid (PFOA)	0.00049	0.0000000576	99.9882
Perfluoropentanesulfonic acid (PFPeS)	0.000108	0.0000000120	99.9889
Perfluoropentanoic acid (PFPeA)	0.00018	0.0000000349	99.9809
Perfluorotetradecanoic acid (PFTeA)	0.0000043	0.0000000220	99.4836
Perfluorotridecanoic acid (PFTriA)	0.000408	0.0000000139	99.9966
Perfluoroundecanoic acid (PFUnA)	0.000379	0.000000031*	>99.9918
9-Chlorohexadecafluoro-3-oxanonane-1-sulfonic acid	0.00000056*	0.000000021*	>96.2500
N-ethylperfluoro-1-octanesulfonamide	0.0000034	0.000000063*	>98.1194
N-methylperfluorooctanesulfonamidoacetic acid (NMeFOSAA)	0.00000056*	0.000000024*	>95.7143
N-ethylperfluorooctanesulfonamidoacetic acid (NEtFOSAA)	0.00000056*	0.000000020*	>96.4286
11-Chloroeicosafluoro-3-oxaundecane-1-sulfonic acid	0.00000056*	0.000000025*	>95.5357
2-(N-ethylperfluoro-1-octanesulfonamido) ethanol	0.000084	0.000000036*	>99.9573
2-(N-methylperfluoro-1-octanesulfonamido) ethanol	0.0000027	0.0000016*	>40.0749
PES	--	0.000000021*	--
PFECA A	--	0.000000031*	--
PFECA B	--	0.000000027*	--
PFECA F	--	0.000000024*	--
PFECHS	--	0.000000029*	--
DONA	0.00000056*	0.000000094*	>83.2143
HFPO-DA	0.000274	0.00000098*	>99.6423
NMeFOSA	0.00000140*	0.000000067*	>95.2143
10:2 FTCA	--	0.000000074*	--
10:2 FTS	--	0.000000034*	--
3:3 FTCA	0.00000095	0.000000048*	>94.9580
4:2 FTS	0.0000140	0.000000012*	>99.9143
5:3 FTCA	0.000018	0.000000089*	>99.4972
6:2 FTCA	--	0.00000011*	--
6:2 FTS	0.0071	0.00000079*	>99.9889
6:2 FTUCA	--	0.000000026*	--
7:3 FTCA	0.0000020*	0.000000067*	>96.6332
8:2 FTCA	--	0.000000063*	--

8:2 FTS	0.000064	0.000000030*	>99.9529
8:2 FTUCA	--	0.000000037*	--
Nonafluoro-3,6-dioxaheptanoic acid (NFDHA)	0.0000073*	--	--
Perfluoro (2-ethoxyethane) sulfonic acid (PFEESA)	0.00000056*	--	--
Perfluoro-3-methoxypropanoic acid (PFMPA)	0.0000019	--	--
Perfluoro-4-methoxybutanoic acid (PFMBA)	0.00000056*	--	--

* Values are below the MDL or have a qualifier for values above the MDL but below the LoQ.
For DRE calculation, PFAS not detected in the waste are assumed to be at MDL level in the waste. PFAS detected below the MDL and LoQ are assumed to be at LoQ in the waste. In those cases, DRE is shown with >.

Appendix XIII - A OTM-50 tables for AFFF - baseline run in campaign 1

Parameter	Units	BL-R1
Date Tested	-	07/31/2024
Sample Collection Time	minutes	180
Gas Volume Collected	dscf	12.690
Volumetric Flow Rate	dscfm	34,447
1,1,1,2,2-Pentafluoroethane (HFC-125)	lb/hr	ND (0.0027)
1,1,1-Trifluoroethane		ND (0.0019)
1,1,2-Trichloro-1,2,2-trifluoroethane		ND (0.0043)
1H-Heptafluoropropane		ND (0.0046)
1H-Nonafluorobutane		ND (0.0050)
1H-Perfluoroheptane		ND (0.0084)
1H-Perfluorohexane		ND (0.0073)
1H-Perfluorooctane		ND (0.0096)
1H-Perfluoropentane		ND (0.0062)
2H-Perfluoro-5-methyl-3,6-dioxanonane (E2)		ND (0.010)
Chlorodifluoromethane		ND (0.0020)
Chlorotrifluoroethene		ND (0.0026)
Chlorotrifluoromethane		ND (0.0024)
Decafluorobutane		ND (0.0054)
Difluoromethane (HFC-32)		ND (0.0012)
Dodecafluoropentane		ND (0.0066)
Ethane, 1,1,1,2-tetrafluoro-		ND (0.0023)
Fluoromethane		ND (0.00078)
Heptafluoropropyl 1,2,2,2-tetrafluoroethyl ether		ND (0.0065)
Hexadecafluoroheptane		ND (0.0088)
Hexafluoroethane		ND (0.0031)
Hexafluoropropene		ND (0.0034)
Hexafluoropropylene oxide		ND (0.0038)
Octadecafluorooctane		ND (0.0100)
Octafluorocyclobutane (FC-C318)		ND (0.0046)
Octafluorocyclopentene (FC-C1418)		ND (0.0048)
Octafluoropropane		ND (0.0043)
Tetradecafluorohexane		ND (0.0077)
Tetrafluoroethene		ND (0.0023)
Trichlorofluoromethane		ND (0.0031)
Trifluoromethane		ND (0.0016)
Vinyl Fluoride		ND (0.0010)
Tetrafluoromethane		ND (0.010)

Appendix XIII - B OTM-50 tables for AFFF - run #1 and 2 and 3 in campaign 1

Parameter	Units	C1-R1	C1-R2	C1-R3
Date Tested	-	7/31/2024	8/1/2024	8/1/2024
Sample Collection Time	minutes	180	180	180
Gas Volume Collected	dscf	12.200	12.490	12.610
Volumetric Flow Rate	dscfm	32,923	34,582	34,142
1,1,1,2,2-Pentafluoroethane (HFC-125)	lb/hr	ND (0.0017)	ND (0.0015)	ND (0.0019)
1,1,1-Trifluoroethane		ND (0.0012)	ND (0.0010)	ND (0.0013)
1,1,2-Trichloro-1,2,2-trifluoroethane		ND (0.0026)	ND (0.0023)	ND (0.0030)
1H-Heptafluoropropane		ND (0.0028)	ND (0.0025)	ND (0.0032)
1H-Nonafluorobutane		ND (0.0031)	ND (0.0027)	ND (0.0035)
1H-Perfluoroheptane		ND (0.0051)	ND (0.0045)	ND (0.0059)
1H-Perfluorohexane		ND (0.0044)	ND (0.0039)	ND (0.0051)
1H-Perfluorooctane		ND (0.0058)	ND (0.0052)	ND (0.0067)
1H-Perfluoropentane		ND (0.0038)	ND (0.0033)	ND (0.0043)
2H-Perfluoro-5-methyl-3,6-dioxanonane (E2)		ND (0.0063)	ND (0.0056)	ND (0.0072)
Chlorodifluoromethane		ND (0.0012)	ND (0.0011)	ND (0.0014)
Chlorotrifluoroethene		ND (0.0016)	ND (0.0014)	ND (0.0018)
Chlorotrifluoromethane		ND (0.0015)	ND (0.0013)	ND (0.0017)
Decafluorobutane		ND (0.0033)	ND (0.0029)	ND (0.0038)
Difluoromethane (HFC-32)		ND (0.00072)	ND (0.00064)	ND (0.00082)
Dodecafluoropentane		ND (0.0040)	ND (0.0035)	ND (0.0046)
Ethane, 1,1,1,2-tetrafluoro-		ND (0.0014)	ND (0.0013)	ND (0.0016)
Fluoromethane		ND (0.00047)	ND (0.00042)	ND (0.00054)
Heptafluoropropyl 1,2,2,2-tetrafluoroethyl ether		ND (0.0040)	ND (0.0035)	ND (0.0045)
Hexadecafluoroheptane		ND (0.0054)	ND (0.0048)	ND (0.0061)
Hexafluoroethane		ND (0.0019)	ND (0.0017)	ND (0.0022)
Hexafluoropropene		ND (0.0021)	ND (0.0018)	ND (0.0024)
Hexafluoropropylene oxide		ND (0.0023)	ND (0.0020)	ND (0.0026)
Octadecafluorooctane		ND (0.0061)	ND (0.0054)	ND (0.0069)
Octafluorocyclobutane (FC-C318)		ND (0.0028)	ND (0.0025)	ND (0.0032)
Octafluorocyclopentene (FC-C1418)		ND (0.0029)	ND (0.0026)	ND (0.0034)
Octafluoropropane		ND (0.0026)	ND (0.0023)	ND (0.0030)
Tetradecafluorohexane		ND (0.0047)	ND (0.0042)	ND (0.0054)
Tetrafluoroethene		ND (0.0014)	ND (0.0012)	ND (0.0016)
Trichlorofluoromethane		ND (0.0019)	ND (0.0017)	ND (0.0022)
Trifluoromethane		ND (0.00097)	ND (0.00086)	ND (0.0011)
Vinyl Fluoride		ND (0.00064)	ND (0.00057)	ND (0.00073)
Tetrafluoromethane		ND (0.0061)	ND (0.0054)	ND (0.0070)

Appendix XIII - C OTM-50 tables for contaminated soil - baseline run in campaign 1

Parameter	Units	C2-BL-R1
Date Tested	-	08/03/2024
Sample Collection Time	minutes	240
Gas Volume Collected	dscf	18.370
Volumetric Flow Rate	dscfm	32,866
1,1,1,2,2-Pentafluoroethane (HFC-125)	lb/hr	ND (0.0021)
1,1,1-Trifluoroethane		ND (0.0015)
1,1,2-Trichloro-1,2,2-trifluoroethane		ND (0.0033)
1H-Heptafluoropropane		ND (0.0035)
1H-Nonafluorobutane		ND (0.0038)
1H-Perfluoroheptane		ND (0.0064)
1H-Perfluorohexane		ND (0.0056)
1H-Perfluorooctane		ND (0.0073)
1H-Perfluoropentane		ND (0.0047)
2H-Perfluoro-5-methyl-3,6-dioxanonane (E2)		ND (0.0078)
Chlorodifluoromethane		ND (0.0015)
Chlorotrifluoroethene		ND (0.0020)
Chlorotrifluoromethane		ND (0.0018)
Decafluorobutane		ND (0.0041)
Difluoromethane (HFC-32)		ND (0.00090)
Dodecafluoropentane		ND (0.0050)
Ethane, 1,1,1,2-tetrafluoro-		ND (0.0018)
Fluoromethane		ND (0.00059)
Heptafluoropropyl 1,2,2,2-tetrafluoroethyl ether		ND (0.0050)
Hexadecafluoroheptane		ND (0.0067)
Hexafluoroethane		ND (0.0024)
Hexafluoropropene		ND (0.0026)
Hexafluoropropylene oxide		ND (0.0029)
Octadecafluorooctane		ND (0.0076)
Octafluorocyclobutane (FC-C318)		ND (0.0035)
Octafluorocyclopentene (FC-C1418)		ND (0.0037)
Octafluoropropane		ND (0.0033)
Tetradecafluorohexane		ND (0.0059)
Tetrafluoroethene		ND (0.0017)
Trichlorofluoromethane		ND (0.0024)
Trifluoromethane		ND (0.0012)
Vinyl Fluoride		ND (0.00080)
Tetrafluoromethane		ND (0.0077)

Appendix XIII-D : OTM-50 tables for contaminated soil - run 1,2,3 in campaign 1

Parameter	Units	C2-R1	C2-R2	C2-R3
Date Tested	-	8/3/2024	8/4/2024	8/4/2024
Sample Collection Time	minutes	240	240	240
Gas Volume Collected	dscf	18.730	18.860	20.080
Volumetric Flow Rate	dscfm	33,565	33,554	32,572
1,1,1,2,2-Pentafluoroethane (HFC-125)	lb/hr	ND (0.0022)	ND (0.0021)	ND (0.0025)
1,1,1-Trifluoroethane		ND (0.0015)	ND (0.0014)	ND (0.0017)
1,1,2-Trichloro-1,2,2-trifluoroethane		ND (0.0034)	ND (0.0032)	ND (0.0038)
1H-Heptafluoropropane		ND (0.0036)	ND (0.0034)	ND (0.0041)
1H-Nonafluorobutane		ND (0.0040)	ND (0.0038)	ND (0.0045)
1H-Perfluoroheptane		ND (0.0067)	ND (0.0063)	ND (0.0076)
1H-Perfluorohexane		ND (0.0058)	ND (0.0055)	ND (0.0065)
1H-Perfluorooctane		ND (0.0076)	ND (0.0072)	ND (0.0086)
1H-Perfluoropentane		ND (0.0049)	ND (0.0046)	ND (0.0055)
2H-Perfluoro-5-methyl-3,6-dioxanonane (E2)		ND (0.0082)	ND (0.0077)	ND (0.0092)
Chlorodifluoromethane		ND (0.0016)	ND (0.0015)	ND (0.0018)
Chlorotrifluoroethene		ND (0.0021)	ND (0.0020)	ND (0.0023)
Chlorotrifluoromethane		ND (0.0019)	ND (0.0018)	ND (0.0021)
Decafluorobutane		ND (0.0043)	ND (0.0041)	ND (0.0049)
Difluoromethane (HFC-32)		ND (0.00095)	ND (0.00089)	ND (0.0011)
Dodecafluoropentane		ND (0.0052)	ND (0.0049)	ND (0.0059)
Ethane, 1,1,1,2-tetrafluoro-		ND (0.0019)	ND (0.0017)	ND (0.0021)
Fluoromethane		ND (0.00062)	ND (0.00058)	ND (0.00070)
Heptafluoropropyl 1,2,2,2-tetrafluoroethyl ether		ND (0.0052)	ND (0.0049)	ND (0.0058)
Hexadecafluoroheptane		ND (0.0071)	ND (0.0066)	ND (0.0079)
Hexafluoroethane		ND (0.0025)	ND (0.0024)	ND (0.0028)
Hexafluoropropene		ND (0.0027)	ND (0.0026)	ND (0.0031)
Hexafluoropropylene oxide		ND (0.0030)	ND (0.0028)	ND (0.0034)
Octadecafluorooctane		ND (0.0080)	ND (0.0075)	ND (0.0090)
Octafluorocyclobutane (FC-C318)		ND (0.0036)	ND (0.0034)	ND (0.0041)
Octafluorocyclopentene (FC-C1418)		ND (0.0039)	ND (0.0036)	ND (0.0043)
Octafluoropropane		ND (0.0034)	ND (0.0032)	ND (0.0038)
Tetradecafluorohexane		ND (0.0061)	ND (0.0058)	ND (0.0069)
Tetrafluoroethene		ND (0.0018)	ND (0.0017)	ND (0.0020)
Trichlorofluoromethane		ND (0.0025)	ND (0.0023)	ND (0.0028)
Trifluoromethane		ND (0.0013)	ND (0.0012)	ND (0.0014)
Vinyl Fluoride		ND (0.00084)	ND (0.00079)	ND (0.00094)
Tetrafluoromethane		ND (0.0080)	ND (0.0075)	ND (0.0090)

Appendix XIV -A : Method 26A HF Results for baseline and AFFF run 1,2,3 in Campaign 1

Run No.		BL	1	2	3	
Date		31-Jul-24	31-Jul-24	01-Aug-24	01-Aug-24	
Start Time	Units	09:25	15:26	09:00	14:50	
Stop Time		13:20	18:47	12:40	18:15	AVGS
Sampling Parameters --						
Barometric Pressure	in. Hg	30.01	30.01	30.09	30.07	30.05
Volume Metered	dcf	95.878	98.949	100.239	100.290	98.839
Volume of Gas Collected	dscf	91.247	94.426	95.629	95.241	94.136
Moisture	% v/v	8.1	8.0	7.7	7.8	7.9
O_2 at Stack	% dry	7.72	7.02	7.18	7.46	7.35
CO_2 at Stack	% dry	9.98	10.16	10.01	9.14	9.82
Avg. Stack Temp.	°F	108	109	108	109	108
Stack Flowrate	dscfm	32,073	32,054	32,974	33,301	32,600
Isokinetics	%	102	105	104	102	103
HF Emission Results --						
Total HF Detected Acid Imp	µg	< 131.0	< 139.0	< 135.0	< 130.0	< 133.8
Total HF Detected Alkaline Imp	µg	< 187.0	< 168.0	< 179.0	< 184.0	< 179.5
Total HF Concentration	ppm	< 0.148	< 0.138	< 0.139	< 0.140	< 0.141
Conc. @ 7% O2	ppm	< 0.156	< 0.138	< 0.141	< 0.144	< 0.145
HF Emission Rate	lb/hr	< 0.015	< 0.014	< 0.014	< 0.015	< 0.014

Appendix XIV-B : Method 26A HF Results for baseline and contaminated soil run 1,2,3 in Campaign 1

Run No.		BL	1	2	3	
Date		03-Aug-24	03-Aug-24	04-Aug-24	04-Aug-24	
Start Time	Units	08:45	14:25	09:20	14:25	
Stop Time		13:07	18:50	13:35	18:40	**AVGS**
Sampling Parameters --						
Barometric Pressure	in. Hg	29.96	29.91	29.90	29.89	29.92
Volume Metered	dcf	136.380	125.097	123.140	118.601	125.805
Volume of Gas Collected	dscf	130.369	117.583	119.215	112.153	119.830
Moisture	% v/v	8.1	8.7	7.8	8.1	8.2
O_2 at Stack	% dry	7.66	8.14	7.23	7.43	7.62
CO_2 at Stack	% dry	9.98	9.28	9.85	9.53	9.66
Avg. Stack Temp.	°F	109	111	107	109	109
Stack Flowrate	dscfm	34,770	31,307	32,001	29,775	31,963
Isokinetics	%	101	101	100	101	101
HF Emission Results --						
Total HF Detected Acid Imp	µg	< 131.0	< 139.0	< 135.0	< 130.0	< 133.8
Total HF Detected Alkaline Imp	µg	< 187.0	< 168.0	< 179.0	< 184.0	< 179.5
Total HF Concentration	ppm	< 0.103	< 0.111	< 0.112	< 0.119	< 0.111
Conc. @ 7% O2	ppm	< 0.108	< 0.120	< 0.113	< 0.122	< 0.116
HF Emission Rate	lb/hr	< 0.011	< 0.011	< 0.011	< 0.011	< 0.011

Appendix XV - A Waste profile, OTM-45 and DRE for AFFF run #1 in campaign 2

	AFFF TF Feed Stream	R1 Stack Emissions	DRE
	Avg lb/hr	lb/hr	
Feed Rate	2,184		
Target Compounds	lb/hr	lb/hr	%
Perfluorobutanesulfonic acid (PFBS)	0.000061	0.000000119	>99.8056
Perfluorobutanoic acid (PFBA)	0.00007	0.000000436	>99.3986
Perfluorodecanesulfonic acid (PFDS)	0.0000052*	0.000000018*	>99.6538
Perfluorodecanoic acid (PFDA)	0.000013	0.000000517	>95.8640
Perfluorododecanesulfonic acid (PFDoS)	0.0000052*	0.000000025*	>99.5192
Perfluorododecanoic acid (PFDoA)	0.000005	0.000001720	>67.9702
Perfluoroheptanesulfonic acid (PFHpS)	0.000014	0.000000023*	>99.8392
Perfluoroheptanoic acid (PFHpA)	0.000032	0.000000225	>99.2947
Perfluorohexadecanoic acid	--	0.000001990	--
Perfluorohexanesulfonic acid (PFHxS)	0.000568	0.000000010	>99.9983
Perfluorohexanoic acid (PFHxA)	0.00075	0.000000330	>99.9557
Perfluorononanesulfonic acid (PFNS)	0.0000052*	0.000000017*	>99.6731
Perfluorononanoic acid (PFNA)	0.000005*	0.00000032	>93.8846
Perfluorooctadecanoic acid	--	0.000000549*	--
Perfluorooctanesulfonamide (FOSA)	0.000024	0.000000078*	>99.6750
Perfluorooctanesulfonic acid (PFOS)	0.0031	0.000000048	>99.9984
Perfluorooctanoic acid (PFOA)	0.00012	0.00000026	>99.7833
Perfluoropentanesulfonic acid (PFPeS)	0.000078	0.000000013*	>99.9832
Perfluoropentanoic acid (PFPeA)	0.000051	0.00000022	>99.5663
Perfluorotetradecanoic acid (PFTeA)	0.000005*	0.00000305	>41.3462
Perfluorotridecanoic acid (PFTriA)	0.000006	0.00000253	>57.4074
Perfluoroundecanoic acid (PFUnA)	0.000010	0.00000090	>90.9940
9-Chlorohexadecafluoro-3-oxanonane-1-sulfonic acid	0.0000052*	0.000000017*	>99.6731
N-ethylperfluoro-1-octanesulfonamide	0.000011*	0.000000039*	>99.6455
N-ethylperfluorooctanesulfonamidoacetic acid (NEtFOSAA)	0.000011*	0.000000025*	>99.7727
N-methylperfluorooctanesulfonamidoacetic acid (NMeFOSAA)	0.000007	0.000000027*	>99.6322
11-Chloroeicosafluoro-3-oxaundecane-1-sulfonic acid	0.0000052*	0.000000023*	>99.5577
2-(N-ethylperfluoro-1-octanesulfonamido) ethanol	0.000011*	0.000000038*	>99.6545
2-(N-methylperfluoro-1-octanesulfonamido) ethanol	0.0000063*	0.00000021*	>96.6667
PES	--	0.000000020*	--
PFECA A	--	0.000000027*	--
PFECA B	--	0.000000030*	--
PFECA F	--	0.000000058*	--
PFECHS	--	0.000000026*	--
DONA	0.0000052*	0.000000031*	>99.4038
HFPO-DA	0.000023	0.00000037*	>98.3700
NMeFOSA	0.000011*	0.000000071	>99.3518
10:2 FTCA	--	0.00000011*	--
10:2 FTS	--	0.00000004*	--
3:3 FTCA	0.0000031*	0.000000046*	>98.5161
4:2 FTS	0.000015	0.000000014*	>99.9073
5:3 FTCA	0.000002	0.000000063*	>97.4286
6:2 FTCA	--	0.000000090*	--
6:2 FTS	0.0051	0.000000220*	>99.9957
6:2 FTUCA	--	0.000000023*	--
7:3 FTCA	0.0000031*	0.000000054*	>98.2581
8:2 FTCA	--	0.000000079*	--

8:2 FTS	0.0003	0.000000024*	>99.9927
8:2 FTUCA	--	0.000000031*	--
Nonafluoro-3,6-dioxaheptanoic acid (NFDHA)	0.0000021*	--	--
Perfluoro (2-ethoxyethane) sulfonic acid (PFEESA)	0.0000021*	--	--
Perfluoro-3-methoxypropanoic acid (PFMPA)	0.0000021*	--	--
Perfluoro-4-methoxybutanoic acid (PFMBA)	0.0000021*	--	--

* Values are below the MDL or have a qualifier for values above the MDL but below the LoQ.
For DRE calculation, PFAS not detected in the waste are assumed to be at MDL level in the waste. PFAS detected below the MDL and LoQ are assumed to be at LoQ in the waste. In those cases, DRE is shown with >.

Appendix XV - B Waste profile, OTM-45 and DRE for AFFF run #2 in campaign 2

	AFFF TF Feed Stream	R2 Stack Emissions	DRE
	Avg lb/hr	lb/hr	
Feed Rate	2,294		
Target Compounds	**lb/hr**	**lb/hr**	**%**
Perfluorobutanesulfonic acid (PFBS)	0.000122	0.000000075	>99.9389
Perfluorobutanoic acid (PFBA)	0.00040	0.000000904	>99.7746
Perfluorodecanesulfonic acid (PFDS)	0.0000057*	0.000000018*	>99.6842
Perfluorodecanoic acid (PFDA)	0.000112	0.000000488	>99.5643
Perfluorododecanesulfonic acid (PFDoS)	0.0000057*	0.000000025*	>99.5614
Perfluorododecanoic acid (PFDoA)	0.000045	0.000000183	>99.5906
Perfluoroheptanesulfonic acid (PFHpS)	0.000026	0.000000023*	>99.9098
Perfluoroheptanoic acid (PFHpA)	0.000110	0.000001430	>98.7000
Perfluorohexadecanoic acid	--	0.000000040	--
Perfluorohexanesulfonic acid (PFHxS)	0.001280	0.000000016	>99.9988
Perfluorohexanoic acid (PFHxA)	0.00232	0.000001150	>99.9504
Perfluorononanesulfonic acid (PFNS)	0.0000057*	0.000000017*	>99.7018
Perfluorononanoic acid (PFNA)	0.000018*	0.00000099	>94.5495
Perfluorooctadecanoic acid	--	0.000000038*	--
Perfluorooctanesulfonamide (FOSA)	0.000066	0.000000078*	>99.8820
Perfluorooctanesulfonic acid (PFOS)	0.0085	0.000000084	>99.9990
Perfluorooctanoic acid (PFOA)	0.00048	0.00000139	>99.7116
Perfluoropentanesulfonic acid (PFPeS)	0.000252	0.000000013*	>99.9948
Perfluoropentanoic acid (PFPeA)	0.000266	0.00000072	>99.7286
Perfluorotetradecanoic acid (PFTeA)	0.000017*	0.00000017	>99.0000
Perfluorotridecanoic acid (PFTriA)	0.000012	0.00000016	>98.6379
Perfluoroundecanoic acid (PFUnA)	0.000076	0.00000025	>99.6733
9-Chlorohexadecafluoro-3-oxanonane-1-sulfonic acid	0.0000057*	0.000000017*	>99.7018
N-ethylperfluoro-1-octanesulfonamide	0.000011*	0.000000039*	>99.6455
N-ethylperfluorooctanesulfonamidoacetic acid (NEtFOSAA)	0.000024*	0.000000025*	>99.8971
N-methylperfluorooctanesulfonamidoacetic acid (NMeFOSAA)	0.000034	0.000000027*	>99.9206
11-Chloroeicosafluoro-3-oxaundecane-1-sulfonic acid	0.0000057*	0.000000023*	>99.5965
2-(N-ethylperfluoro-1-octanesulfonamido) ethanol	0.000011*	0.000000038*	>99.6545
2-(N-methylperfluoro-1-octanesulfonamido) ethanol	0.0000069*	0.00000021*	>96.9565
PES	--	0.000000020*	--
PFECA A	--	0.000000017*	--
PFECA B	--	0.000000030*	--
PFECA F	--	0.000000058*	--
PFECHS	--	0.000000026*	--
DONA	0.0000057*	0.000000031*	>99.4561
HFPO-DA	0.000020	0.00000037*	>98.1683
NMeFOSA	0.000011*	0.000000114	>98.9636
10:2 FTCA	--	0.00000011*	--
10:2 FTS	--	0.00000004*	--
3:3 FTCA	0.0000034*	0.000000046*	>98.6471
4:2 FTS	0.000015	0.000000014*	>99.9067
5:3 FTCA	0.000115	0.000000063*	>99.9452
6:2 FTCA	--	0.000000090*	--
6:2 FTS	0.0062	0.000000230*	>99.9963
6:2 FTUCA	--	0.000000013*	--
7:3 FTCA	0.000028*	0.000000054*	>99.8058
8:2 FTCA	--	0.000000079*	--

8:2 FTS	0.0017	0.000000024*	>99.9986
8:2 FTUCA	--	0.000000032*	--
Nonafluoro-3,6-dioxaheptanoic acid (NFDHA)	0.0000023*	--	--
Perfluoro (2-ethoxyethane) sulfonic acid (PFEESA)	0.0000023*	--	--
Perfluoro-3-methoxypropanoic acid (PFMPA)	0.0000023*	--	--
Perfluoro-4-methoxybutanoic acid (PFMBA)	0.0000023*	--	--

* Values are below the MDL or have a qualifier for values above the MDL but below the LoQ.
For DRE calculation, PFAS not detected in the waste are assumed to be at MDL level in the waste. PFAS detected below the MDL and LoQ are assumed to be at LoQ in the waste. In those cases, DRE is shown with >.

Appendix XV - C Waste profile, OTM-45 and DRE for AFFF run #3 in campaign 2

	AFFF+ TF Feed Stream	R1 Stack Emissions	DRE
	Avg lb/hr	lb/hr	
Feed Rate	2,378		
Target Compounds	**lb/hr**	**lb/hr**	**%**
Perfluorobutanesulfonic acid (PFBS)	0.000127	0.000000061	>99.9523
Perfluorobutanoic acid (PFBA)	0.00042	0.000000447	>99.8925
Perfluorodecanesulfonic acid (PFDS)	0.0000059*	0.000000019*	>99.6780
Perfluorodecanoic acid (PFDA)	0.000116	0.000000122	>99.8948
Perfluorododecanesulfonic acid (PFDoS)	0.0000059*	0.000000027*	>99.5424
Perfluorododecanoic acid (PFDoA)	0.000046	0.000000242	>99.4784
Perfluoroheptanesulfonic acid (PFHpS)	0.000026	0.000000026*	>99.9015
Perfluoroheptanoic acid (PFHpA)	0.000114	0.000000230	>99.7982
Perfluorohexadecanoic acid	--	0.000000126	--
Perfluorohexanesulfonic acid (PFHxS)	0.001320	0.000000038	>99.9971
Perfluorohexanoic acid (PFHxA)	0.00240	0.000000228	>99.9905
Perfluorononanesulfonic acid (PFNS)	0.0000059*	0.000000018*	>99.6949
Perfluorononanoic acid (PFNA)	0.000019*	0.00000013	>99.3228
Perfluorooctadecanoic acid	--	0.000000060*	--
Perfluorooctanesulfonamide (FOSA)	0.000069	0.000000079*	>99.8847
Perfluorooctanesulfonic acid (PFOS)	0.0089	0.000000586	>99.9934
Perfluorooctanoic acid (PFOA)	0.00050	0.00000089	>99.8222
Perfluoropentanesulfonic acid (PFPeS)	0.000262	0.000000007*	>99.9972
Perfluoropentanoic acid (PFPeA)	0.000276	0.00000017	>99.9384
Perfluorotetradecanoic acid (PFTeA)	0.000017*	0.00000028	>98.3918
Perfluorotridecanoic acid (PFTriA)	0.000012	0.00000028	>97.6667
Perfluoroundecanoic acid (PFUnA)	0.000079	0.00000016	>99.7954
9-Chlorohexadecafluoro-3-oxanonane-1-sulfonic acid	0.0000059*	0.000000017*	>99.7119
N-ethylperfluoro-1-octanesulfonamide	0.000012*	0.000000041*	>99.6583
N-ethylperfluorooctanesulfonamidoacetic acid (NEtFOSAA)	0.000025*	0.000000026*	>99.8968
N-methylperfluorooctanesulfonamidoacetic acid (NMeFOSAA)	0.000035	0.000000028*	>99.9205
11-Chloroeicosafluoro-3-oxaundecane-1-sulfonic acid	0.0000059*	0.000000024*	>99.5932
2-(N-ethylperfluoro-1-octanesulfonamido) ethanol	0.000012*	0.000000040*	>99.6667
2-(N-methylperfluoro-1-octanesulfonamido) ethanol	0.0000071*	0.00000023*	>96.7606
PES	--	0.000000021*	--
PFECA A	--	0.000000029*	--
PFECA B	--	0.000000031*	--
PFECA F	--	0.000000060*	--
PFECHS	--	0.000000027*	--
DONA	0.0000059*	0.000000032*	>99.4576
HFPO-DA	0.000021	0.00000038*	>98.1905
NMeFOSA	0.000012*	0.000000146	>98.7833
10:2 FTCA	--	0.00000011*	--
10:2 FTS	--	0.000000038*	--
3:3 FTCA	0.0000036*	0.000000048*	>98.6667
4:2 FTS	0.000016	0.000000015*	>99.9038
5:3 FTCA	0.000119	0.000000066*	>99.9445
6:2 FTCA	--	0.000000094*	--
6:2 FTS	0.0064	0.000000210*	>99.9967
6:2 FTUCA	--	0.000000020*	--
7:3 FTCA	0.000029*	0.000000057*	>99.8021
8:2 FTCA	--	0.000000083*	--

8:2 FTS	0.0017	0.000000025*	>99.9986
8:2 FTUCA	--	0.000000033*	--
Nonafluoro-3,6-dioxaheptanoic acid (NFDHA)	0.0000024*	--	--
Perfluoro (2-ethoxyethane) sulfonic acid (PFEESA)	0.0000024*	--	--
Perfluoro-3-methoxypropanoic acid (PFMPA)	0.0000024*	--	--
Perfluoro-4-methoxybutanoic acid (PFMBA)	0.0000024*	--	--

* Values are below the MDL or have a qualifier for values above the MDL but below the LoQ.
For DRE calculation, PFAS not detected in the waste are assumed to be at MDL level in the waste. PFAS detected below the MDL and LoQ are assumed to be at LoQ in the waste. In those cases, DRE is shown with >.

Appendix XV - D Waste profile, OTM-45 and DRE for contaminated soil run #1 in campaign 2

	MX Solid Feed Stream	R1 Stack Emissions	DRE
	Avg lb/hr	lb/hr	
Feed Rate	**4,061**		
Target Compounds	**lb/hr**	**lb/hr**	**%**
Perfluorobutanesulfonic acid (PFBS)	0.00016*	0.000000097*	>99.9394
Perfluorobutanoic acid (PFBA)	0.00032*	0.0000010*	>99.6875
Perfluorodecanesulfonic acid (PFDS)	0.000081*	0.000000035*	>99.9568
Perfluorodecanoic acid (PFDA)	0.000081*	0.000004060	>94.9877
Perfluorododecanesulfonic acid (PFDoS)	0.000081*	0.000000059*	>99.9272
Perfluorododecanoic acid (PFDoA)	0.000081*	0.000016800	>79.2593
Perfluoroheptanesulfonic acid (PFHpS)	0.000081*	0.000000045*	>99.9444
Perfluoroheptanoic acid (PFHpA)	0.000127	0.000000715	>99.4370
Perfluorohexadecanoic acid	--	0.000007780	--
Perfluorohexanesulfonic acid (PFHxS)	0.000081*	0.000000033*	>99.9593
Perfluorohexanoic acid (PFHxA)	0.00044	0.000000575	>99.8690
Perfluorononanesulfonic acid (PFNS)	0.000081*	0.000000038*	>99.9531
Perfluorononanoic acid (PFNA)	0.000081*	0.00000203	>97.4938
Perfluorooctadecanoic acid	--	0.000001070*	--
Perfluorooctanesulfonamide (FOSA)	0.000081*	0.00000021*	>99.7407
Perfluorooctanesulfonic acid (PFOS)	0.0004	0.000000074	>99.9797
Perfluorooctanoic acid (PFOA)	0.00014	0.00000109	>99.1926
Perfluoropentanesulfonic acid (PFPeS)	0.000081*	0.000000022*	>99.9728
Perfluoropentanoic acid (PFPeA)	0.000081*	0.00000042	>99.4877
Perfluorotetradecanoic acid (PFTeA)	0.000081*	0.00002480	>69.3827
Perfluorotridecanoic acid (PFTriA)	0.000081*	0.00002230	>72.4691
Perfluoroundecanoic acid (PFUnA)	0.000081*	0.00000809	>90.0123
9-Chlorohexadecafluoro-3-oxanonane-1-sulfonic acid	0.000081*	0.000000034*	>99.9580
N-ethylperfluoro-1-octanesulfonamide	0.00020*	0.000000087*	>99.9565
N-ethylperfluorooctanesulfonamidoacetic acid (NEtFOSAA)	0.00020*	0.000000054*	>99.9730
N-methylperfluorooctanesulfonamidoacetic acid (NMeFOSAA)	0.000081*	0.000000059*	>99.9272
11-Chloroeicosafluoro-3-oxaundecane-1-sulfonic acid	0.000081*	0.000000044*	>99.9457
2-(N-ethylperfluoro-1-octanesulfonamido) ethanol	0.00020*	0.000000096*	>99.9520
2-(N-methylperfluoro-1-octanesulfonamido) ethanol	0.000081*	0.00000026*	>99.6790
PES	--	0.000000034*	--
PFECA A	--	0.000000040*	--
PFECA B	--	0.000000059*	--
PFECA F	--	0.00000014*	--
PFECHS	--	0.000000041*	--
DONA	0.000081*	0.000000064*	>99.9210
HFPO-DA	0.00041	0.00000072*	>99.8244
NMeFOSA	0.00020*	0.000000098*	>99.9510
10:2 FTCA	--	0.00000024*	--
10:2 FTS	--	0.000000071*	--
3:3 FTCA	0.000081*	0.000000088*	>99.8914
4:2 FTS	0.00024*	0.000000029*	>99.9879
5:3 FTCA	0.000081*	0.000000110*	>99.8642
6:2 FTCA	--	0.000000200*	--
6:2 FTS	0.0011	0.000000220*	>99.9796

6:2 FTUCA	--	0.000000035*	--
7:3 FTCA	0.000081*	0.000000110*	>99.8642
8:2 FTCA	--	0.000000170*	--
8:2 FTS	0.0003	0.000000045*	>99.9860
8:2 FTUCA	--	0.000000052*	--
Nonafluoro-3,6-dioxaheptanoic acid (NFDHA)	0.000081*	--	--
Perfluoro (2-ethoxyethane) sulfonic acid (PFEESA)	0.000081*	--	--
Perfluoro-3-methoxypropanoic acid (PFMPA)	0.000081*	--	--
Perfluoro-4-methoxybutanoic acid (PFMBA)	0.000081*	--	--

* Values are below the MDL or have a qualifier for values above the MDL but below the LoQ.
For DRE calculation, PFAS not detected in the waste are assumed to be at MDL level in the waste. PFAS detected below the MDL and LoQ are assumed to be at LoQ in the waste. In those cases, DRE is shown with >.

Appendix XV -E Waste profile, OTM-45 and DRE for contaminated soil run #2 in campaign 2

Feed Rate	MX Feed Stream Avg lb/hr 2,565	R1 Stack Emissions lb/hr	DRE
Target Compounds	**lb/hr**	**lb/hr**	**%**
Perfluorobutanesulfonic acid (PFBS)	0.0001490000	0.000000046	>99.9695
Perfluorobutanoic acid (PFBA)	0.00046	0.000000792	>99.8275
Perfluorodecanesulfonic acid (PFDS)	0.0000030*	0.000000018*	>99.3898
Perfluorodecanoic acid (PFDA)	0.000046	0.000001380	>96.9803
Perfluorododecanesulfonic acid (PFDoS)	0.00000103*	0.000000025*	>97.5728
Perfluorododecanoic acid (PFDoA)	0.000021	0.000000525	>97.5467
Perfluoroheptanesulfonic acid (PFHpS)	0.000017	0.000000023*	>99.8671
Perfluoroheptanoic acid (PFHpA)	0.000107	0.000003500	>96.7290
Perfluorohexadecanoic acid	--	0.000000189	--
Perfluorohexanesulfonic acid (PFHxS)	0.000913	0.000000013	>99.9986
Perfluorohexanoic acid (PFHxA)	0.00093	0.000001970	>99.7879
Perfluorononanesulfonic acid (PFNS)	0.0000006*	0.000000017*	>97.0435
Perfluorononanoic acid (PFNA)	0.000027*	0.00000288	>89.4118
Perfluorooctadecanoic acid	--	0.000000097*	--
Perfluorooctanesulfonamide (FOSA)	0.000009	0.000000074*	>99.1415
Perfluorooctanesulfonic acid (PFOS)	0.0063	0.000000045	>99.9993
Perfluorooctanoic acid (PFOA)	0.0002	0.00000374	>98.4609
Perfluoropentanesulfonic acid (PFPeS)	0.000188	0.000000013*	>99.9931
Perfluoropentanoic acid (PFPeA)	0.000159	0.00000101	>99.3648
Perfluorotetradecanoic acid (PFTeA)	0.000007*	0.00000046	>93.4894
Perfluorotridecanoic acid (PFTriA)	0.000008	0.00000054	>92.9793
Perfluoroundecanoic acid (PFUnA)	0.000004	0.00000083	>80.1900
9-Chlorohexadecafluoro-3-oxanonane-1-sulfonic acid	0.00000048*	0.000000017*	>96.4583
N-ethylperfluoro-1-octanesulfonamide	0.00000120*	0.000000039*	>96.7500
N-ethylperfluorooctanesulfonamidoacetic acid (NEtFOSAA)	0.000003*	0.000000025*	>99.1803
N-methylperfluorooctanesulfonamidoacetic acid (NMeFOSAA)	0.00000196*	0.000000027*	>98.6224
11-Chloroeicosafluoro-3-oxaundecane-1-sulfonic acid	0.00000048*	0.000000023*	>95.2083
2-(N-ethylperfluoro-1-octanesulfonamido) ethanol	0.00011100*	0.000000038*	>99.9658
2-(N-methylperfluoro-1-octanesulfonamido) ethanol	0.00000048*	0.00000023*	>52.0833
PES	--	0.000000020*	--
PFECA A	--	0.000000030*	--
PFECA B	--	0.000000030*	--
PFECA F	--	0.000000062*	--
PFECHS	--	0.000000026*	--
DONA	0.00000048*	0.000000031*	>93.5417
HFPO-DA	0.000013	0.00000037*	>97.0866
NMeFOSA	0.00000120*	0.000000032	>97.3250
10:2 FTCA	--	0.00000011*	--
10:2 FTS	--	0.00000004*	--
3:3 FTCA	0.0000013*	0.000000046*	>96.3200
4:2 FTS	0.000005	0.000000014*	>99.7154
5:3 FTCA	0.000018	0.000000063*	>99.6576
6:2 FTCA	--	0.000000090*	--
6:2 FTS	0.0021	0.000000200*	>99.9906
6:2 FTUCA	--	0.000000019*	--
7:3 FTCA	0.0000049*	0.000000054*	>98.9024
8:2 FTCA	--	0.000000079*	--

8:2 FTS	0.0003	0.000000024*	>99.9925
8:2 FTUCA	--	0.000000032*	--
Nonafluoro-3,6-dioxaheptanoic acid (NFDHA)	0.0000400*	--	--
Perfluoro (2-ethoxyethane) sulfonic acid (PFEESA)	0.00000048*	--	--
Perfluoro-3-methoxypropanoic acid (PFMPA)	0.0000346*	--	--
Perfluoro-4-methoxybutanoic acid (PFMBA)	0.0000014*	--	--

* Values are below the MDL or have a qualifier for values above the MDL but below the LoQ.
For DRE calculation, PFAS not detected in the waste are assumed to be at MDL level in the waste. PFAS detected below the MDL and LoQ are assumed to be at LoQ in the waste. In those cases, DRE is shown with >.

Appendix XV - F : Waste profile, OTM-45 and DRE for contaminated soil run #3 in campaign 2

	MX Feed Stream	R2 Stack Emissions	DRE
	Avg lb/hr	lb/hr	
Feed Rate	3,875		
Target Compounds	lb/hr	lb/hr	%
Perfluorobutanesulfonic acid (PFBS)	0.0002260000	0.00000010*	>99.9558
Perfluorobutanoic acid (PFBA)	0.00069	0.0000010*	>99.8559
Perfluorodecanesulfonic acid (PFDS)	0.0000045*	0.000000035*	>99.2152
Perfluorodecanoic acid (PFDA)	0.000069	0.000004270	>93.8116
Perfluorododecanesulfonic acid (PFDoS)	0.00000156*	0.000000059*	>96.2179
Perfluorododecanoic acid (PFDoA)	0.000032	0.000016200	>49.8452
Perfluoroheptanesulfonic acid (PFHpS)	0.000026	0.000000046*	>99.8238
Perfluoroheptanoic acid (PFHpA)	0.000162	0.000000725	>99.5525
Perfluorohexadecanoic acid	--	0.000004740	--
Perfluorohexanesulfonic acid (PFHxS)	0.001380	0.000000034*	>99.9975
Perfluorohexanoic acid (PFHxA)	0.00140	0.000000435	>99.9689
Perfluorononanesulfonic acid (PFNS)	0.0000009*	0.000000038*	>95.6221
Perfluorononanoic acid (PFNA)	0.000041*	0.00000228	>94.4526
Perfluorooctadecanoic acid	--	0.000000251*	--
Perfluorooctanesulfonamide (FOSA)	0.000013	0.00000021*	>98.3846
Perfluorooctanesulfonic acid (PFOS)	0.0096	0.000000076	>99.9992
Perfluorooctanoic acid (PFOA)	0.0004	0.00000106	>99.7112
Perfluoropentanesulfonic acid (PFPeS)	0.000284	0.000000022*	>99.9923
Perfluoropentanoic acid (PFPeA)	0.000240	0.00000027	>99.8867
Perfluorotetradecanoic acid (PFTeA)	0.000011*	0.00002330	**
Perfluorotridecanoic acid (PFTriA)	0.000012	0.00002120	**
Perfluoroundecanoic acid (PFUnA)	0.000006	0.00000893	**
9-Chlorohexadecafluoro-3-oxanonane-1-sulfonic acid	0.00000072*	0.000000034*	>95.2778
N-ethylperfluoro-1-octanesulfonamide	0.00000180*	0.000000088*	>95.1111
N-ethylperfluorooctanesulfonamidoacetic acid (NEtFOSAA)	0.000005*	0.000000054*	>98.8286
N-methylperfluorooctanesulfonamidoacetic acid (NMeFOSAA)	0.00000296*	0.000000059*	>98.0068
11-Chloroeicosafluoro-3-oxaundecane-1-sulfonic acid	0.00000072*	0.000000044*	>93.8889
2-(N-ethylperfluoro-1-octanesulfonamido) ethanol	0.00016700*	0.000000097*	>99.9419
2-(N-methylperfluoro-1-octanesulfonamido) ethanol	0.00000072*	0.00000026*	>63.8889
PES	--	0.000000034*	--
PFECA A	--	0.000000040*	--
PFECA B	--	0.000000059*	--
PFECA F	--	0.00000014*	--
PFECHS	--	0.000000040*	--
DONA	0.00000072*	0.000000064*	>91.1111
HFPO-DA	0.000019	0.00000073*	>96.1979
NMeFOSA	0.00000180*	0.00000010*	>94.4444
10:2 FTCA	--	0.00000025*	--
10:2 FTS	--	0.000000071*	--
3:3 FTCA	0.0000019*	0.000000088*	>95.3439
4:2 FTS	0.000007	0.000000029*	>99.6102
5:3 FTCA	0.000028	0.00000011*	>99.6043
6:2 FTCA	--	0.00000020*	--
6:2 FTS	0.0032	0.00000023*	>99.9928
6:2 FTUCA	--	0.000000035*	--
7:3 FTCA	0.0000074*	0.00000011*	>98.5215
8:2 FTCA	--	0.00000017*	--

8:2 FTS	0.0005	0.000000045*	>99.9906
8:2 FTUCA	--	0.000000052*	--
Nonafluoro-3,6-dioxaheptanoic acid (NFDHA)	0.0000604*	--	--
Perfluoro (2-ethoxyethane) sulfonic acid (PFEESA)	0.00000072*	--	--
Perfluoro-3-methoxypropanoic acid (PFMPA)	0.0000523*	--	--
Perfluoro-4-methoxybutanoic acid (PFMBA)	0.0000021*	--	--

* Values are below the MDL or have a qualifier for values above the MDL but below the LoQ.
For DRE calculation, PFAS not detected in the waste are assumed to be at MDL level in the waste. PFAS detected below the MDL and LoQ are assumed to be at LoQ in the waste. In those cases, DRE is shown with >.

Appendix XV - G : Waste profile, OTM-45 and DRE for spent carbon run #1 in campaign 2

Feed Rate	MX Feed Stream Avg lb/hr 2,187	R1 Stack Emissions lb/hr	DRE
Target Compounds	**lb/hr**	**lb/hr**	**%**
Perfluorobutanesulfonic acid (PFBS)	0.0000534000	0.000000094	>99.8240
Perfluorobutanoic acid (PFBA)	0.00020	0.000000990	>99.4949
Perfluorodecanesulfonic acid (PFDS)	0.0000002*	0.000000035*	>85.9438
Perfluorodecanoic acid (PFDA)	0.000017	0.000003010	>82.3977
Perfluorododecanesulfonic acid (PFDoS)	0.00000020*	0.000000058*	>71.0000
Perfluorododecanoic acid (PFDoA)	0.000004	0.000012000	**
Perfluoroheptanesulfonic acid (PFHpS)	0.000025	0.000000045*	>99.8193
Perfluoroheptanoic acid (PFHpA)	0.000077	0.000000560	>99.2746
Perfluorohexadecanoic acid	--	0.000005220	--
Perfluorohexanesulfonic acid (PFHxS)	0.000562	0.000000034	>99.9940
Perfluorohexanoic acid (PFHxA)	0.00029	0.000000340	>99.8811
Perfluorononanesulfonic acid (PFNS)	0.0000003*	0.000000038*	>88.5542
Perfluorononanoic acid (PFNA)	0.000056*	0.00000142	>97.4643
Perfluorooctadecanoic acid	--	0.000000257*	--
Perfluorooctanesulfonamide (FOSA)	0.000003	0.000000210*	>93.2907
Perfluorooctanesulfonic acid (PFOS)	0.0012	0.000000088	>99.9927
Perfluorooctanoic acid (PFOA)	0.0007	0.00000080	>99.8863
Perfluoropentanesulfonic acid (PFPeS)	0.000056	0.000000021*	>99.9626
Perfluoropentanoic acid (PFPeA)	0.000078	0.00000024	>99.6986
Perfluorotetradecanoic acid (PFTeA)	0.000001*	0.00001910	**
Perfluorotridecanoic acid (PFTriA)	0.000001	0.00001820	**
Perfluoroundecanoic acid (PFUnA)	0.000003	0.00000605	**
9-Chlorohexadecafluoro-3-oxanonane-1-sulfonic acid	0.00000020*	0.000000034*	>83.0000
N-ethylperfluoro-1-octanesulfonamide	0.00000051*	0.000000087*	>82.9412
N-ethylperfluorooctanesulfonamidoacetic acid (NEtFOSAA)	0.000001*	0.000000053*	>90.7826
N-methylperfluorooctanesulfonamidoacetic acid (NMeFOSAA)	0.00000020*	0.000000059*	>70.5000
11-Chloroeicosafluoro-3-oxaundecane-1-sulfonic acid	0.00000020*	0.000000044*	>78.0000
2-(N-ethylperfluoro-1-octanesulfonamido) ethanol	0.00000051*	0.000000096*	>81.1765
2-(N-methylperfluoro-1-octanesulfonamido) ethanol	0.00000020*	0.00000025*	**
PES	--	0.000000033*	--
PFECA A	--	0.000000040*	--
PFECA B	--	0.000000058*	--
PFECA F	--	0.000000140*	--
PFECHS	--	0.000000040*	--
DONA	0.00000020*	0.000000064*	>68.0000
HFPO-DA	0.002670	0.00000072*	>99.9730
NMeFOSA	0.00000051*	0.000000096	>81.1765
10:2 FTCA	--	0.00000024*	--
10:2 FTS	--	0.00000007*	--
3:3 FTCA	0.0000054*	0.000000087*	>98.3829
4:2 FTS	0.000003	0.000000028*	>99.1837
5:3 FTCA	0.000004	0.000000110*	>97.5446
6:2 FTCA	--	0.000000200*	--
6:2 FTS	0.0004	0.000000220*	>99.9387
6:2 FTUCA	--	0.000000034*	--
7:3 FTCA	0.0000007*	0.000000110*	>84.9932
8:2 FTCA	--	0.000000170*	--

8:2 FTS	0.0001	0.000000044*	>99.9487
8:2 FTUCA	--	0.000000051*	--
Nonafluoro-3,6-dioxaheptanoic acid (NFDHA)	0.0000258*	--	--
Perfluoro (2-ethoxyethane) sulfonic acid (PFEESA)	0.00000020*	--	--
Perfluoro-3-methoxypropanoic acid (PFMPA)	0.0000049*	--	--
Perfluoro-4-methoxybutanoic acid (PFMBA)	0.0000015*	--	--

* Values are below the MDL or have a qualifier for values above the MDL but below the LoQ.
For DRE calculation, PFAS not detected in the waste are assumed to be at MDL level in the waste. PFAS detected below the MDL and LoQ are assumed to be at LoQ in the waste. In those cases, DRE is shown with >.

Appendix XV - H : Waste profile, OTM-45 and DRE for spent carbon run #2 in campaign 2

	MX Feed Stream	R2 Stack Emissions	DRE
	Avg lb/hr	lb/hr	
Feed Rate	2,422		
Target Compounds	lb/hr	lb/hr	%
Perfluorobutanesulfonic acid (PFBS)	0.0000591000	0.000000050	>99.9161
Perfluorobutanoic acid (PFBA)	0.00022	0.000000345	>99.8410
Perfluorodecanesulfonic acid (PFDS)	0.0000003*	0.000000018*	>93.4783
Perfluorodecanoic acid (PFDA)	0.000019	0.000000109	>99.4263
Perfluorododecanesulfonic acid (PFDoS)	0.00000022*	0.000000026*	>88.1818
Perfluorododecanoic acid (PFDoA)	0.000004	0.000000140	>96.7136
Perfluoroheptanesulfonic acid (PFHpS)	0.000028	0.000000023*	>99.9167
Perfluoroheptanoic acid (PFHpA)	0.000086	0.000000236	>99.7240
Perfluorohexadecanoic acid	--	0.000000062	--
Perfluorohexanesulfonic acid (PFHxS)	0.000622	0.000000012	>99.9982
Perfluorohexanoic acid (PFHxA)	0.00032	0.000000190	>99.9401
Perfluorononanesulfonic acid (PFNS)	0.0000004*	0.000000017*	>95.3804
Perfluorononanoic acid (PFNA)	0.000062*	0.00000015	>99.7661
Perfluorooctadecanoic acid	--	0.000000030*	--
Perfluorooctanesulfonamide (FOSA)	0.000003	0.000000084*	>97.5723
Perfluorooctanesulfonic acid (PFOS)	0.0013	0.000000046	>99.9966
Perfluorooctanoic acid (PFOA)	0.0008	0.00000038	>99.9510
Perfluoropentanesulfonic acid (PFPeS)	0.000062	0.000000013*	>99.9791
Perfluoropentanoic acid (PFPeA)	0.000087	0.00000013	>99.8466
Perfluorotetradecanoic acid (PFTeA)	0.000002*	0.00000014	>90.7792
Perfluorotridecanoic acid (PFTriA)	0.000001	0.00000015	>84.1889
Perfluoroundecanoic acid (PFUnA)	0.000004	0.00000011	>96.9272
9-Chlorohexadecafluoro-3-oxanonane-1-sulfonic acid	0.00000022*	0.000000017*	>92.2727
N-ethylperfluoro-1-octanesulfonamide	0.00000056*	0.000000039*	>93.0357
N-ethylperfluorooctanesulfonamidoacetic acid (NEtFOSAA)	0.000001*	0.000000025*	>96.0754
N-methylperfluorooctanesulfonamidoacetic acid (NMeFOSAA)	0.00000022*	0.000000027*	>87.7273
11-Chloroeicosafluoro-3-oxaundecane-1-sulfonic acid	0.00000022*	0.000000023*	>89.5455
2-(N-ethylperfluoro-1-octanesulfonamido) ethanol	0.00000056*	0.000000039*	>93.0357
2-(N-methylperfluoro-1-octanesulfonamido) ethanol	0.00000022*	0.00000023*	**
PES	--	0.000000020*	--
PFECA A	--	0.000000027*	--
PFECA B	--	0.000000030*	--
PFECA F	--	0.000000058*	--
PFECHS	--	0.000000026*	--
DONA	0.00000022*	0.000000031*	>85.9091
HFPO-DA	0.002950	0.00000037*	>99.9875
NMeFOSA	0.00000056*	0.000000089	>84.1071
10:2 FTCA	--	0.00000011*	--
10:2 FTS	--	0.00000004*	--
3:3 FTCA	0.0000060*	0.000000046*	>99.2282
4:2 FTS	0.000004	0.000000014*	>99.6316
5:3 FTCA	0.000005	0.000000063*	>98.7324
6:2 FTCA	--	0.000000091*	--
6:2 FTS	0.0004	0.000000240*	>99.9395
6:2 FTUCA	--	0.000000019*	--
7:3 FTCA	0.0000008*	0.000000055*	>93.2182
8:2 FTCA	--	0.000000080*	--

8:2 FTS	0.0001	0.000000024*	>99.9747
8:2 FTUCA	--	0.000000032*	--
Nonafluoro-3,6-dioxaheptanoic acid (NFDHA)	0.0000286*	--	--
Perfluoro (2-ethoxyethane) sulfonic acid (PFEESA)	0.00000022*	--	--
Perfluoro-3-methoxypropanoic acid (PFMPA)	0.0000054*	--	--
Perfluoro-4-methoxybutanoic acid (PFMBA)	0.0000016*	--	--

* Values are below the MDL or have a qualifier for values above the MDL but below the LoQ.
For DRE calculation, PFAS not detected in the waste are assumed to be at MDL level in the waste. PFAS detected below the MDL and LoQ are assumed to be at LoQ in the waste. In those cases, DRE is shown with >.

Appendix XVI - A : PFAS concentrations in liquid residues from AFFF incineration for baseline (CA-BL), run 1 (CA-R1)and 2 (CA-R2) in campaign 2

PFAS flowrates are in lb/hr. When a PFAS is non-detected, it is considered to be at MDL. 24 hrs means that sample was taken 24 hours after the end of the run

Parameter	CA-BL	CA-R1	CA-R2	CA-R2-24H4
Date Tested	**10/09/2024**	**45574**	**45575**	**10/10/2024**
Sample Collection Time (minutes)	**240**	**240**	**240**	**240**
Waste Input Rate - Liquid A3F (lb/hr)	**52,492.0**	**48288.6**	**52742.16**	**52,742.2**
11-Chloroeicosafluoro-3-oxaundecane-1-sulfonic acid (11Cl-PF3OUdS)	ND (0.000013)	ND (0.000000024)	ND (0.000000026)	ND (0.000000026)
1H,1H,2H,2H-Perfluorodecane sulfonic acid (8:2 FTS)	ND (0.000025)	ND (0.000000028)	0.000000386	0.000000818
1H,1H,2H,2H-Perfluorohexane sulfonic acid (4:2 FTS)	ND (0.000013)	ND (0.000000024)	0.0000000290	0.0000000294
1H,1H,2H,2H-Perfluorooctane sulfonic acid (6:2 FTS)	ND (0.00011)	0.000000739	0.0000121	0.0000174
3-Perfluoroheptylpropanoic acid (7:3 FTCA)	ND (0.0000077)	ND (0.000000052)	ND (0.000000056)	ND (0.000000056)
3-Perfluoropentylpropanoic acid (5:3 FTCA)	ND (0.0000051)	ND (0.000000024)	ND (0.000000026)	ND (0.000000026)
3-Perfluoropropylpropanoic acid (3:3 FTCA)	ND (0.0000077)	ND (0.000000024)	ND (0.000000026)	ND (0.000000026)
4,8-Dioxa-3H-perfluorononanoic acid (DONA)	ND (0.000013)	ND (0.000000024)	ND (0.000000026)	ND (0.000000026)
9-Chlorohexadecafluoro-3-oxanonane-1-sulfonic acid (9Cl-PF3ONS)	ND (0.000013)	ND (0.000000024)	ND (0.000000026)	ND (0.000000026)
Hexafluoropropylene Oxide Dimer Acid (HFPO-DA)	ND (0.000025)	0.000000151	0.00000161	0.000000129
N-ethylperfluorooctane sulfonamide (NEtFOSA)	ND (0.000025)	ND (0.000000019)	ND (0.000000020)	ND (0.000000020)
N-ethylperfluorooctane sulfonamidoethanol (NEtFOSE)	ND (0.000025)	ND (0.000000019)	ND (0.000000020)	ND (0.000000020)
N-ethylperfluorooctanesulfonamidoacetic acid (NEtFOSAA)	ND (0.000013)	ND (0.000000024)	ND (0.000000026)	ND (0.000000026)
N-methylperfluorooctane sulfonamide (NMeFOSA)	ND (0.000025)	ND (0.000000033)	ND (0.000000036)	ND (0.000000036)
N-methylperfluorooctane sulfonamidoethanol (NMeFOSE)	ND (0.000025)	ND (0.000000019)	ND (0.000000020)	ND (0.000000020)
N-methylperfluorooctanesulfonamidoacetic acid (NMeFOSAA)	ND (0.000015)	ND (0.000000019)	ND (0.000000020)	ND (0.000000020)
Nonafluoro-3,6-dioxaheptanoic acid (NFDHA)	ND (0.0000051)	ND (0.000000014)	ND (0.000000015)	ND (0.000000015)
Perfluoro (2-ethoxyethane) sulfonic acid (PFEESA)	ND (0.0000051)	ND (0.000000014)	ND (0.000000015)	ND (0.000000015)
Perfluoro-3-methoxypropanoic acid (PFMPA)	ND (0.0000051)	ND (0.000000014)	ND (0.000000015)	ND (0.000000015)
Perfluoro-4-methoxybutanoic acid (PFMBA)	ND (0.0000051)	ND (0.000000014)	ND (0.000000015)	ND (0.000000015)
Perfluorobutanesulfonic acid (PFBS)	ND (0.000013)	0.000000152	0.000000164	0.000000147
Perfluorobutanoic acid (PFBA)	ND (0.000051)	0.000000966	0.00000198	0.000000886
Perfluorodecanesulfonic acid (PFDS)	ND (0.000013)	ND (0.000000014)	ND (0.000000015)	ND (0.000000015)
Perfluorodecanoic acid (PFDA)	ND (0.000013)	ND	0.0000000360	0.0000000291

		(0.000000014)		
Perfluorododecanesulfonic acid (PFDoS)	ND (0.000013)	ND (0.000000014)	ND (0.000000015)	ND (0.000000015)
Perfluorododecanoic acid (PFDoA)	ND (0.000013)	ND (0.000000019)	ND (0.000000020)	ND (0.000000020)
Perfluoroheptanesulfonic acid (PFHpS)	ND (0.000013)	ND (0.000000014)	ND (0.000000015)	ND (0.000000015)
Perfluoroheptanoic acid (PFHpA)	ND (0.000013)	0.000000473	0.00000117	0.000000564
Perfluorohexanesulfonic acid (PFHxS)	ND (0.000013)	0.000000141	0.000000199	0.000000199
Perfluorohexanoic acid (PFHxA)	0.0000343	0.00000128	0.0000155	0.00000120
Perfluorononanesulfonic acid (PFNS)	ND (0.000013)	ND (0.000000014)	ND (0.000000015)	ND (0.000000015)
Perfluorononanoic acid (PFNA)	ND (0.000013)	0.0000000589	0.0000000828	0.0000000622
Perfluorooctanesulfonamide (PFOSAm)	ND (0.000018)	ND (0.000000014)	0.0000000601	0.000000159
Perfluorooctanesulfonic acid (PFOS)	ND (0.000025)	0.000000235	0.000000472	0.000000549
Perfluorooctanoic acid (PFOA)	ND (0.000013)	0.000000133	0.000000254	0.000000238
Perfluoropentanesulfonic acid (PFPeS)	ND (0.000013)	0.0000000226	0.0000000257	0.0000000270
Perfluoropentanoic acid (PFPeA)	ND (0.000013)	0.00000136	0.00000205	0.00000117
Perfluorotetradecanoic acid (PFTeDA)	ND (0.000013)	ND (0.000000019)	ND (0.000000020)	ND (0.000000020)
Perfluorotridecanoic acid (PFTrDA)	ND (0.000013)	ND (0.000000019)	ND (0.000000020)	ND (0.000000020)
Perfluoroundecanoic acid (PFUnA)	ND (0.000013)	ND (0.000000014)	ND (0.000000015)	ND (0.000000015)

Appendix XVI - B : PFAS concentrations in liquid residues from AFFF incineration for baseline (CA+-BL) and run#1 (CA+-R1) AFFF + lime

PFAS flowrates are in lb/hr. When a PFAS is non-detected, it is assumed to be at MDL. 24 hrs means that sample was taken 24 hours after the end of the run

Parameter	CA+-BL	CA+-R1	CA+-R1 24Hr
Date Tested	10/12/2024	12/10/2024	12/10/2024
Sample Collection Time (Minutes)	240	240	240
Waste Input Rate - Liquid A3F (lb/hr)	62,249.8	50,040	50,040
11-Chloroeicosafluoro-3-oxaundecane-1-sulfonic acid (11Cl-PF3OUdS)	ND (0.000000030)	ND (0.00000025)	ND (0.000000024)
1H,1H,2H,2H-Perfluorodecane sulfonic acid (8:2 FTS)	ND (0.000000036)	ND (0.00000030)	0.0000000366
1H,1H,2H,2H-Perfluorohexane sulfonic acid (4:2 FTS)	ND (0.000000030)	ND (0.00000025)	ND (0.000000024)
1H,1H,2H,2H-Perfluorooctane sulfonic acid (6:2 FTS)	0.00000277	0.00000105	0.00000210
3-Perfluoroheptylpropanoic acid (7:3 FTCA)	ND (0.000000066)	ND (0.00000055)	ND (0.000000053)
3-Perfluoropentylpropanoic acid (5:3 FTCA)	ND (0.000000030)	ND (0.00000025)	ND (0.000000024)
3-Perfluoropropylpropanoic acid (3:3 FTCA)	ND (0.000000030)	ND (0.00000025)	ND (0.000000024)
4,8-Dioxa-3H-perfluorononanoic acid (DONA)	ND (0.000000030)	ND (0.00000025)	ND (0.000000024)
9-Chlorohexadecafluoro-3-oxanonane-1-sulfonic acid (9Cl-PF3ONS)	ND (0.000000030)	ND (0.00000025)	ND (0.000000024)
Hexafluoropropylene Oxide Dimer Acid (HFPO-DA)	0.00000164	ND (0.00000080)	0.000000387
N-ethylperfluorooctane sulfonamide (NEtFOSA)	ND (0.000000024)	ND (0.00000020)	ND (0.000000019)
N-ethylperfluorooctane sulfonamidoethanol (NEtFOSE)	ND (0.000000024)	ND (0.00000020)	ND (0.000000019)
N-ethylperfluorooctanesulfonamidoacetic acid (NEtFOSAA)	ND (0.000000030)	ND (0.00000025)	ND (0.000000024)
N-methylperfluorooctane sulfonamide (NMeFOSA)	ND (0.000000042)	ND (0.00000035)	ND (0.000000034)
N-methylperfluorooctane sulfonamidoethanol (NMeFOSE)	ND (0.000000024)	ND (0.00000020)	ND (0.000000019)
N-methylperfluorooctanesulfonamidoacetic acid (NMeFOSAA)	ND (0.000000024)	ND (0.00000020)	ND (0.000000019)
Nonafluoro-3,6-dioxaheptanoic acid (NFDHA)	ND (0.000000018)	ND (0.00000015)	ND (0.000000015)
Perfluoro (2-ethoxyethane) sulfonic acid (PFEESA)	ND (0.000000018)	ND (0.00000015)	ND (0.000000015)
Perfluoro-3-methoxypropanoic acid (PFMPA)	ND (0.000000018)	ND (0.00000015)	ND (0.000000015)
Perfluoro-4-methoxybutanoic acid (PFMBA)	ND (0.000000018)	ND (0.00000015)	ND (0.000000015)
Perfluorobutanesulfonic acid (PFBS)	0.000000171	0.000000265	0.000000146
Perfluorobutanoic acid (PFBA)	0.00000174	0.00000121	0.00000111
Perfluorodecanesulfonic acid (PFDS)	ND (0.000000018)	ND (0.00000015)	ND (0.000000015)
Perfluorodecanoic acid (PFDA)	ND	ND (0.00000015)	ND

	(0.000000018)		(0.000000015)
Perfluorododecanesulfonic acid (PFDoS)	ND (0.000000018)	ND (0.00000015)	ND (0.000000015)
Perfluorododecanoic acid (PFDoA)	ND (0.000000024)	ND (0.00000020)	ND (0.000000019)
Perfluoroheptanesulfonic acid (PFHpS)	ND (0.000000018)	ND (0.00000015)	ND (0.000000015)
Perfluoroheptanoic acid (PFHpA)	0.000000853	0.000000726	0.000000656
Perfluorohexanesulfonic acid (PFHxS)	0.000000169	0.000000212	0.000000162
Perfluorohexanoic acid (PFHxA)	0.00000252	0.00000149	0.00000150
Perfluorononanesulfonic acid (PFNS)	ND (0.000000018)	ND (0.00000015)	ND (0.000000015)
Perfluorononanoic acid (PFNA)	0.0000000722	ND (0.00000010)	0.0000000560
Perfluorooctanesulfonamide (PFOSAm)	0.0000000214	ND (0.00000015)	0.0000000172
Perfluorooctanesulfonic acid (PFOS)	0.000000219	ND (0.00000025)	0.000000231
Perfluorooctanoic acid (PFOA)	0.000000213	0.000000249	0.000000163
Perfluoropentanesulfonic acid (PFPeS)	0.0000000240	ND (0.00000015)	0.0000000227
Perfluoropentanoic acid (PFPeA)	0.00000214	0.00000163	0.00000137
Perfluorotetradecanoic acid (PFTeDA)	ND (0.000000024)	ND (0.00000020)	ND (0.000000019)
Perfluorotridecanoic acid (PFTrDA)	ND (0.000000024)	ND (0.00000020)	ND (0.000000019)
Perfluoroundecanoic acid (PFUnA)	ND (0.000000018)	ND (0.00000015)	ND (0.000000015)

Appendix XVI - C : PFAS concentrations in liquid residues from contaminated soil incineration for baseline #1 (CS-BL) and run#1 (CS-R1)

PFAS flowrates are in lb/hr. When a PFAS is non-detected, it is assumed to be at MDL. 24 hrs means that sample was taken 24 hours after the end of the run

Parameter	CS-BL	CS-R1
Date Tested	10/14/2024	14/10/2024
Sample Collection Time (Minutes)	240	240
Waste Input Rate - Liquid A3F (lb/hr)	52,592.0	54,343
11-Chloroeicosafluoro-3-oxaundecane-1-sulfonic acid (11Cl-PF3OUdS)	ND (0.000000026)	ND (0.000000027)
1H,1H,2H,2H-Perfluorodecane sulfonic acid (8:2 FTS)	ND (0.000000031)	0.0000000492
1H,1H,2H,2H-Perfluorohexane sulfonic acid (4:2 FTS)	ND (0.000000026)	ND (0.000000027)
1H,1H,2H,2H-Perfluorooctane sulfonic acid (6:2 FTS)	0.00000280	0.00000452
3-Perfluoroheptylpropanoic acid (7:3 FTCA)	ND (0.000000057)	ND (0.000000059)
3-Perfluoropentylpropanoic acid (5:3 FTCA)	ND (0.000000026)	ND (0.000000027)
3-Perfluoropropylpropanoic acid (3:3 FTCA)	ND (0.000000026)	ND (0.000000027)
4,8-Dioxa-3H-perfluorononanoic acid (DONA)	ND (0.000000026)	ND (0.000000027)
9-Chlorohexadecafluoro-3-oxanonane-1-sulfonic acid (9Cl-PF3ONS)	ND (0.000000026)	ND (0.000000027)
Hexafluoropropylene Oxide Dimer Acid (HFPO-DA)	0.000000621	0.000000685
N-ethylperfluorooctane sulfonamide (NEtFOSA)	ND (0.000000021)	ND (0.000000022)
N-ethylperfluorooctane sulfonamidoethanol (NEtFOSE)	ND (0.000000021)	ND (0.000000022)
N-ethylperfluorooctanesulfonamidoacetic acid (NEtFOSAA)	ND (0.000000026)	ND (0.000000027)
N-methylperfluorooctane sulfonamide (NMeFOSA)	ND (0.000000036)	ND (0.000000038)
N-methylperfluorooctane sulfonamidoethanol (NMeFOSE)	ND (0.000000021)	ND (0.000000022)
N-methylperfluorooctanesulfonamidoacetic acid (NMeFOSAA)	ND (0.000000021)	ND (0.000000022)
Nonafluoro-3,6-dioxaheptanoic acid (NFDHA)	ND (0.000000016)	ND (0.000000016)
Perfluoro (2-ethoxyethane) sulfonic acid (PFEESA)	ND (0.000000016)	ND (0.000000016)
Perfluoro-3-methoxypropanoic acid (PFMPA)	ND (0.000000016)	ND (0.000000016)
Perfluoro-4-methoxybutanoic acid (PFMBA)	ND (0.000000016)	ND (0.000000016)
Perfluorobutanesulfonic acid (PFBS)	0.000000165	0.000000262
Perfluorobutanoic acid (PFBA)	0.00000114	0.00000169
Perfluorodecanesulfonic acid (PFDS)	ND (0.000000016)	ND (0.000000016)
Perfluorodecanoic acid (PFDA)	ND (0.000000016)	0.0000000190
Perfluorododecanesulfonic acid (PFDoS)	ND (0.000000016)	ND (0.000000016)
Perfluorododecanoic acid (PFDoA)	ND (0.000000021)	ND (0.000000022)
Perfluoroheptanesulfonic acid (PFHpS)	ND (0.000000016)	ND (0.000000016)
Perfluoroheptanoic acid (PFHpA)	0.000000631	0.00000115

Perfluorohexanesulfonic acid (PFHxS)	0.000000160	0.000000247
Perfluorohexanoic acid (PFHxA)	0.00000177	0.00000304
Perfluorononanesulfonic acid (PFNS)	ND (0.000000016)	ND (0.000000016)
Perfluorononanoic acid (PFNA)	0.0000000320	0.0000000864
Perfluorooctanesulfonamide (PFOSAm)	ND (0.000000016)	0.0000000490
Perfluorooctanesulfonic acid (PFOS)	0.000000129	0.000000347
Perfluorooctanoic acid (PFOA)	0.000000114	0.000000280
Perfluoropentanesulfonic acid (PFPeS)	0.0000000241	0.0000000417
Perfluoropentanoic acid (PFPeA)	0.00000145	0.00000233
Perfluorotetradecanoic acid (PFTeDA)	ND (0.000000021)	ND (0.000000022)
Perfluorotridecanoic acid (PFTrDA)	ND (0.000000021)	ND (0.000000022)
Perfluoroundecanoic acid (PFUnA)	ND (0.000000016)	ND (0.000000016)

Appendix XVI - D : PFAS concentrations in liquid residues from contaminated soil incineration for baseline #2 (CCS2-BL) and run#2 (CCS2-R1) and #3 (CCS2-R2)

PFAS flowrates are in lb/hr. When a PFAS is non-detected, it is assumed to be at MDL. 24 hrs means that sample was taken 24 hours after the end of the run

Parameter	CCS2-BL	CCS2-R1	CCS2-R2	CCS2-R2-24Hr
Date Tested	10/22/2024	22/10/2024	23/10/2024	23/10/2024
Sample Collection Time (Minutes)	240	240	240	240
Waste Input Rate - Liquid A3F (lb/hr)	48,438.7	47,988	52,042	52,042
11-Chloroeicosafluoro-3-oxaundecane-1-sulfonic acid (11Cl-PF3OUdS)	ND (0.000000024)	ND (0.000000024)	ND (0.000000026)	ND (0.000000026)
1H,1H,2H,2H-Perfluorodecane sulfonic acid (8:2 FTS)	ND (0.000000029)	ND (0.000000029)	ND (0.000000031)	ND (0.000000031)
1H,1H,2H,2H-Perfluorohexane sulfonic acid (4:2 FTS)	ND (0.000000024)	ND (0.000000024)	ND (0.000000026)	ND (0.000000026)
1H,1H,2H,2H-Perfluorooctane sulfonic acid (6:2 FTS)	0.000000906	0.00000154	0.00000178	0.00000173
3-Perfluoroheptylpropanoic acid (7:3 FTCA)	ND (0.000000053)	ND (0.000000052)	ND (0.000000057)	ND (0.000000056)
3-Perfluoropentylpropanoic acid (5:3 FTCA)	ND (0.000000024)	ND (0.000000024)	ND (0.000000026)	ND (0.000000026)
3-Perfluoropropylpropanoic acid (3:3 FTCA)	ND (0.000000024)	ND (0.000000024)	ND (0.000000026)	ND (0.000000026)
4,8-Dioxa-3H-perfluorononanoic acid (DONA)	ND (0.000000024)	ND (0.000000024)	ND (0.000000026)	ND (0.000000026)
9-Chlorohexadecafluoro-3-oxanonane-1-sulfonic acid (9Cl-PF3ONS)	ND (0.000000024)	ND (0.000000024)	ND (0.000000026)	ND (0.000000026)
Hexafluoropropylene Oxide Dimer Acid (HFPO-DA)	0.000000620	0.00000123	0.00000105	0.000000817
N-ethylperfluorooctane sulfonamide (NEtFOSA)	ND (0.000000019)	ND (0.000000019)	ND (0.000000021)	ND (0.000000021)
N-ethylperfluorooctane sulfonamidoethanol (NEtFOSE)	ND (0.000000019)	ND (0.000000019)	ND (0.000000021)	ND (0.000000021)
N-ethylperfluorooctanesulfonamidoacetic acid (NEtFOSAA)	ND (0.000000024)	ND (0.000000024)	ND (0.000000026)	ND (0.000000026)
N-methylperfluorooctane sulfonamide (NMeFOSA)	ND (0.000000034)	ND (0.000000033)	ND (0.000000036)	ND (0.000000036)
N-methylperfluorooctane sulfonamidoethanol (NMeFOSE)	ND (0.000000019)	ND (0.000000019)	ND (0.000000021)	ND (0.000000021)
N-methylperfluorooctanesulfonamidoacetic acid (NMeFOSAA)	ND (0.000000019)	ND (0.000000019)	ND (0.000000021)	ND (0.000000021)
Nonafluoro-3,6-dioxaheptanoic acid (NFDHA)	ND (0.000000014)	ND (0.000000014)	ND (0.000000016)	ND (0.000000015)
Perfluoro (2-ethoxyethane) sulfonic acid (PFEESA)	ND (0.000000014)	ND (0.000000014)	ND (0.000000016)	ND (0.000000015)
Perfluoro-3-methoxypropanoic acid (PFMPA)	ND (0.000000014)	ND (0.000000014)	ND (0.000000016)	ND (0.000000015)
Perfluoro-4-methoxybutanoic acid (PFMBA)	ND (0.000000014)	ND (0.000000014)	ND (0.000000016)	ND (0.000000015)
Perfluorobutanesulfonic acid (PFBS)	0.000000275	0.000000272	0.000000285	0.000000440
Perfluorobutanoic acid (PFBA)	0.00000163	0.00000178	0.00000208	0.00000173
Perfluorodecanesulfonic acid (PFDS)	ND (0.000000014)	ND (0.000000014)	ND (0.000000016)	ND (0.000000015)

Perfluorodecanoic acid (PFDA)	ND (0.000000014)	0.0000000198	0.0000000189	0.0000000296
Perfluorododecanesulfonic acid (PFDoS)	ND (0.000000014)	ND (0.000000014)	ND (0.000000016)	ND (0.000000015)
Perfluorododecanoic acid (PFDoA)	ND (0.000000019)	ND (0.000000019)	ND (0.000000021)	ND (0.000000021)
Perfluoroheptanesulfonic acid (PFHpS)	ND (0.000000014)	ND (0.000000014)	ND (0.000000016)	ND (0.000000015)
Perfluoroheptanoic acid (PFHpA)	0.000000562	0.000000619	0.000000843	0.00000117
Perfluorohexanesulfonic acid (PFHxS)	0.000000228	0.000000217	0.000000253	0.000000366
Perfluorohexanoic acid (PFHxA)	0.00000171	0.00000230	0.00000284	0.00000264
Perfluorononanesulfonic acid (PFNS)	ND (0.000000014)	ND (0.000000014)	ND (0.000000016)	ND (0.000000015)
Perfluorononanoic acid (PFNA)	0.0000000543	0.0000000600	0.0000000796	0.000000109
Perfluorooctanesulfonamide (PFOSAm)	0.000000178	0.000000197	0.000000196	0.000000115
Perfluorooctanesulfonic acid (PFOS)	0.000000272	0.000000249	0.000000316	0.000000368
Perfluorooctanoic acid (PFOA)	0.000000150	0.000000163	0.000000220	0.000000316
Perfluoropentanesulfonic acid (PFPeS)	0.0000000314	0.0000000322	0.0000000396	0.0000000755
Perfluoropentanoic acid (PFPeA)	0.00000174	0.00000192	0.00000215	0.00000227
Perfluorotetradecanoic acid (PFTeDA)	ND (0.000000019)	ND (0.000000019)	ND (0.000000021)	ND (0.000000021)
Perfluorotridecanoic acid (PFTrDA)	ND (0.000000019)	ND (0.000000019)	ND (0.000000021)	ND (0.000000021)
Perfluoroundecanoic acid (PFUnA)	ND (0.000000014)	ND (0.000000014)	ND (0.000000016)	ND (0.000000015)

Appendix XVI - E : PFAS concentrations in liquid residues from spent carbon incineration for baseline (CC-BL) and run 1 (CC-R1) and run 2 (CC-R2)

PFAS flowrates are in lb/hr. When a PFAS is non-detected, it is assumed to be at MDL. 24 hrs means that sample was taken 24 hours after the end of the run

Parameter	CC-BL	CC-R1	CC-R2	CC-R2-24Hr
Date Tested	10/19/2024	19/10/2024	20/10/2024	20/10/2024
Sample Collection Time (Minutes)	240	240	240	240
Waste Input Rate - Liquid A3F (lb/hr)	51,691.3	52,642	52,992	52,992
11-Chloroeicosafluoro-3-oxaundecane-1-sulfonic acid (11Cl-PF3OUdS)	ND (0.000000026)	ND (0.000000026)	ND (0.000000026)	ND (0.000000026)
1H,1H,2H,2H-Perfluorodecane sulfonic acid (8:2 FTS)	ND (0.000000031)	0.0000000616	0.0000000657	ND (0.000000031)
1H,1H,2H,2H-Perfluorohexane sulfonic acid (4:2 FTS)	ND (0.000000026)	ND (0.000000026)	ND (0.000000026)	0.0000000444
1H,1H,2H,2H-Perfluorooctane sulfonic acid (6:2 FTS)	0.00000178	0.00000240	0.00000250	0.00000233
3-Perfluoroheptylpropanoic acid (7:3 FTCA)	ND (0.000000057)	ND (0.000000057)	ND (0.000000058)	ND (0.000000058)
3-Perfluoropentylpropanoic acid (5:3 FTCA)	ND (0.000000026)	ND (0.000000026)	ND (0.000000026)	ND (0.000000026)
3-Perfluoropropylpropanoic acid (3:3 FTCA)	ND (0.000000026)	ND (0.000000026)	ND (0.000000026)	ND (0.000000026)
4,8-Dioxa-3H-perfluorononanoic acid (DONA)	ND (0.000000026)	ND (0.000000026)	ND (0.000000026)	ND (0.000000026)
9-Chlorohexadecafluoro-3-oxanonane-1-sulfonic acid (9Cl-PF3ONS)	ND (0.000000026)	ND (0.000000026)	ND (0.000000026)	ND (0.000000026)
Hexafluoropropylene Oxide Dimer Acid (HFPO-DA)	0.000000359	0.00000130	0.000000594	0.0000118
N-ethylperfluorooctane sulfonamide (NEtFOSA)	ND (0.000000021)	ND (0.000000021)	ND (0.000000021)	ND (0.000000021)
N-ethylperfluorooctane sulfonamidoethanol (NEtFOSE)	ND (0.000000021)	ND (0.000000021)	ND (0.000000021)	ND (0.000000021)
N-ethylperfluorooctanesulfonamidoacetic acid (NEtFOSAA)	ND (0.000000026)	ND (0.000000026)	ND (0.000000026)	ND (0.000000026)
N-methylperfluorooctane sulfonamide (NMeFOSA)	ND (0.000000036)	ND (0.000000037)	ND (0.000000037)	ND (0.000000037)
N-methylperfluorooctane sulfonamidoethanol (NMeFOSE)	ND (0.000000021)	ND (0.000000021)	ND (0.000000021)	ND (0.000000021)
N-methylperfluorooctanesulfonamidoacetic acid (NMeFOSAA)	ND (0.000000021)	ND (0.000000021)	ND (0.000000021)	ND (0.000000021)

Nonafluoro-3,6-dioxaheptanoic acid (NFDHA)	ND (0.000000016)	ND (0.000000016)	ND (0.000000016)	ND (0.000000016)
Perfluoro (2-ethoxyethane) sulfonic acid (PFEESA)	ND (0.000000016)	ND (0.000000016)	ND (0.000000016)	ND (0.000000016)
Perfluoro-3-methoxypropanoic acid (PFMPA)	ND (0.000000016)	ND (0.000000016)	ND (0.000000016)	0.0000000404
Perfluoro-4-methoxybutanoic acid (PFMBA)	ND (0.000000016)	ND (0.000000016)	ND (0.000000016)	ND (0.000000016)
Perfluorobutanesulfonic acid (PFBS)	0.000000257	0.000000264	0.000000257	0.000000245
Perfluorobutanoic acid (PFBA)	0.00000139	0.00000143	0.00000140	0.0000194
Perfluorodecanesulfonic acid (PFDS)	ND (0.000000016)	ND (0.000000016)	ND (0.000000016)	ND (0.000000016)
Perfluorodecanoic acid (PFDA)	ND (0.000000016)	ND (0.000000016)	ND (0.000000016)	0.0000000252
Perfluorododecanesulfonic acid (PFDoS)	ND (0.000000016)	ND (0.000000016)	ND (0.000000016)	ND (0.000000016)
Perfluorododecanoic acid (PFDoA)	ND (0.000000021)	ND (0.000000021)	ND (0.000000021)	ND (0.000000021)
Perfluoroheptanesulfonic acid (PFHpS)	ND (0.000000016)	ND (0.000000016)	ND (0.000000016)	ND (0.000000016)
Perfluoroheptanoic acid (PFHpA)	0.000000724	0.000000832	0.000000832	0.00000121
Perfluorohexanesulfonic acid (PFHxS)	0.000000239	0.000000241	0.000000242	0.000000205
Perfluorohexanoic acid (PFHxA)	0.00000176	0.00000197	0.00000208	0.0000192
Perfluorononanesulfonic acid (PFNS)	ND (0.000000016)	ND (0.000000016)	ND (0.000000016)	ND (0.000000016)
Perfluorononanoic acid (PFNA)	0.0000000625	0.0000000658	0.0000000684	0.0000000938
Perfluorooctanesulfonamide (PFOSAm)	0.0000000347	0.000000119	0.0000000546	0.0000000228
Perfluorooctanesulfonic acid (PFOS)	0.000000248	0.000000355	0.000000397	0.000000226
Perfluorooctanoic acid (PFOA)	ND (0.000000016)	0.000000231	0.000000243	0.000000286
Perfluoropentanesulfonic acid (PFPeS)	0.0000000419	0.0000000417	0.0000000390	0.0000000319
Perfluoropentanoic acid (PFPeA)	0.00000171	0.00000188	0.00000182	0.00000631
Perfluorotetradecanoic acid (PFTeDA)	ND (0.000000021)	ND (0.000000021)	ND (0.000000021)	ND (0.000000021)
Perfluorotridecanoic acid (PFTrDA)	ND (0.000000021)	ND (0.000000021)	ND (0.000000021)	ND (0.000000021)
Perfluoroundecanoic acid (PFUnA)	ND (0.000000016)	ND (0.000000016)	ND (0.000000016)	ND (0.000000016)

Appendix XVI - F : PFAS concentrations in liquid residues from CF4 for baseline (CW+-BL) and run 1 (CW-R1)

PFAS flowrates are in lb/hr. When a PFAS is non-detected, it is assumed to be at MDL. 24 hrs means that sample was taken 24 hours after the end of the run

Parameter	CW+-BL	CW+-R1	CW+-R24Hr
Date Tested	10/17/2024	17/10/2024	17/10/2024
Sample Collection Time	240	240	240
Waste Input Rate - Liquid A3F (lb/hr)	57,846.2	49,540	49,540
11-Chloroeicosafluoro-3-oxaundecane-1-sulfonic acid (11Cl-PF3OUdS)	ND (0.000000029)	ND (0.000000025)	ND (0.000000024)
1H,1H,2H,2H-Perfluorodecane sulfonic acid (8:2 FTS)	ND (0.000000034)	ND (0.000000029)	ND (0.000000029)
1H,1H,2H,2H-Perfluorohexane sulfonic acid (4:2 FTS)	ND (0.000000029)	ND (0.000000025)	ND (0.000000024)
1H,1H,2H,2H-Perfluorooctane sulfonic acid (6:2 FTS)	0.00000688	0.00000256	0.000000931
3-Perfluoroheptylpropanoic acid (7:3 FTCA)	ND (0.000000063)	ND (0.000000054)	ND (0.000000054)
3-Perfluoropentylpropanoic acid (5:3 FTCA)	ND (0.000000029)	ND (0.000000025)	ND (0.000000024)
3-Perfluoropropylpropanoic acid (3:3 FTCA)	ND (0.000000029)	ND (0.000000025)	ND (0.000000024)
4,8-Dioxa-3H-perfluorononanoic acid (DONA)	ND (0.000000029)	ND (0.000000025)	ND (0.000000024)
9-Chlorohexadecafluoro-3-oxanonane-1-sulfonic acid (9Cl-PF3ONS)	ND (0.000000029)	ND (0.000000025)	ND (0.000000024)
Hexafluoropropylene Oxide Dimer Acid (HFPO-DA)	0.000000499	0.000000318	0.000000252
N-ethylperfluorooctane sulfonamide (NEtFOSA)	ND (0.000000023)	ND (0.000000020)	ND (0.000000020)
N-ethylperfluorooctane sulfonamidoethanol (NEtFOSE)	ND (0.000000023)	ND (0.000000020)	ND (0.000000020)
N-ethylperfluorooctanesulfonamidoacetic acid (NEtFOSAA)	ND (0.000000029)	ND (0.000000025)	ND (0.000000024)
N-methylperfluorooctane sulfonamide (NMeFOSA)	ND (0.000000040)	ND (0.000000034)	ND (0.000000034)
N-methylperfluorooctane sulfonamidoethanol (NMeFOSE)	ND (0.000000023)	ND (0.000000020)	ND (0.000000020)
N-methylperfluorooctanesulfonamidoacetic acid (NMeFOSAA)	ND (0.000000023)	ND (0.000000020)	ND (0.000000020)
Nonafluoro-3,6-dioxaheptanoic acid (NFDHA)	ND (0.000000017)	ND (0.000000015)	ND (0.000000015)
Perfluoro (2-ethoxyethane) sulfonic acid (PFEESA)	ND (0.000000017)	ND (0.000000015)	ND (0.000000015)
Perfluoro-3-methoxypropanoic acid (PFMPA)	ND (0.000000017)	ND (0.000000015)	ND (0.000000015)
Perfluoro-4-methoxybutanoic acid (PFMBA)	ND (0.000000017)	ND (0.000000015)	ND (0.000000015)
Perfluorobutanesulfonic acid (PFBS)	0.000000296	0.000000238	0.000000236
Perfluorobutanoic acid (PFBA)	0.00000316	0.00000163	0.00000134
Perfluorodecanesulfonic acid (PFDS)	ND (0.000000017)	ND (0.000000015)	ND (0.000000015)
Perfluorodecanoic acid (PFDA)	0.0000000263	0.0000000186	ND

			(0.000000015)
Perfluorododecanesulfonic acid (PFDoS)	ND (0.000000017)	ND (0.000000015)	ND (0.000000015)
Perfluorododecanoic acid (PFDoA)	ND (0.000000023)	ND (0.000000020)	ND (0.000000020)
Perfluoroheptanesulfonic acid (PFHpS)	ND (0.000000017)	ND (0.000000015)	ND (0.000000015)
Perfluoroheptanoic acid (PFHpA)	0.00000119	0.000000882	0.000000624
Perfluorohexanesulfonic acid (PFHxS)	0.000000239	0.000000209	0.000000188
Perfluorohexanoic acid (PFHxA)	0.00000717	0.00000290	0.00000185
Perfluorononanesulfonic acid (PFNS)	ND (0.000000017)	ND (0.000000015)	ND (0.000000015)
Perfluorononanoic acid (PFNA)	0.0000000966	0.0000000733	0.0000000505
Perfluorooctanesulfonamide (PFOSAm)	0.0000000576	0.0000000807	0.000000124
Perfluorooctanesulfonic acid (PFOS)	0.000000285	0.000000238	0.000000188
Perfluorooctanoic acid (PFOA)	0.000000344	0.000000251	0.000000161
Perfluoropentanesulfonic acid (PFPeS)	0.0000000452	0.0000000356	0.0000000338
Perfluoropentanoic acid (PFPeA)	0.00000349	0.00000202	0.00000166
Perfluorotetradecanoic acid (PFTeDA)	ND (0.000000023)	ND (0.000000020)	ND (0.000000020)
Perfluorotridecanoic acid (PFTrDA)	ND (0.000000023)	ND (0.000000020)	ND (0.000000020)
Perfluoroundecanoic acid (PFUnA)	ND (0.000000017)	ND (0.000000015)	ND (0.000000015)

Appendix XVII - A : PFAS in solid residues from AFFF incineration for Baseline (BL-R1), run #1 (CA-R1) and run #2 (CA-R2) for AFFF incineration.

Units for PFAS in ng/g

When a PFAS is non-detected, it is considered to be at MDL.

Parameter	BL-R1	CA-R1	CA-R2
Date Tested	10/09/2024	9/10/2024	10/10/2024
Sample Collection Time (minutes)	240	240	240
11-Chloroeicosafluoro-3-oxaundecane-1-sulfonic acid (11Cl-PF3OUdS)	ND (0.58)	ND (0.58)	ND (0.59)
1H,1H,2H,2H-Perfluorodecane sulfonic acid (8:2 FTS)	ND (1.7)	ND (1.7)	ND (1.8)
1H,1H,2H,2H-Perfluorohexane sulfonic acid (4:2 FTS)	ND (1.7)	ND (1.7)	ND (1.8)
1H,1H,2H,2H-Perfluorooctane sulfonic acid (6:2 FTS)	ND (1.7)	ND (1.7)	ND (1.8)
3-Perfluoroheptylpropanoic acid (7:3 FTCA)	ND (0.59)	ND (0.58)	ND (0.59)
3-Perfluoropentylpropanoic acid (5:3 FTCA)	ND (0.59)	ND (0.58)	ND (0.59)
3-Perfluoropropylpropanoic acid (3:3 FTCA)	ND (0.58)	ND (0.58)	ND (0.59)
4,8-Dioxa-3H-perfluorononanoic acid (DONA)	ND (0.58)	ND (0.58)	ND (0.59)
9-Chlorohexadecafluoro-3-oxanonane-1-sulfonic acid (9Cl-PF3ONS)	ND (0.59)	ND (0.58)	ND (0.59)
Hexafluoropropylene Oxide Dimer Acid (HFPO-DA)	ND (2.9)	ND (2.9)	ND (2.9)
N-ethylperfluorooctane sulfonamide (NEtFOSA)	ND (1.5)	ND (1.5)	ND (1.5)
N-ethylperfluorooctane sulfonamidoethanol (NEtFOSE)	ND (1.5)	ND (1.5)	ND (1.5)
N-ethylperfluorooctanesulfonamidoacetic acid (NEtFOSAA)	ND (0.58)	ND (0.58)	ND (0.59)
N-methylperfluorooctane sulfonamide (NMeFOSA)	ND (1.5)	ND (1.5)	ND (1.5)
N-methylperfluorooctane sulfonamidoethanol (NMeFOSE)	ND (1.5)	ND (1.5)	ND (1.5)
N-methylperfluorooctanesulfonamidoacetic acid (NMeFOSAA)	ND (0.59)	ND (0.58)	ND (0.59)
Nonafluoro-3,6-dioxaheptanoic acid (NFDHA)	ND (0.59)	ND (0.58)	ND (0.59)
Perfluoro (2-ethoxyethane) sulfonic acid (PFEESA)	ND (0.59)	ND (0.58)	ND (0.59)
Perfluoro-3-methoxypropanoic acid (PFMPA)	ND (0.58)	ND (0.58)	ND (0.59)
Perfluoro-4-methoxybutanoic acid (PFMBA)	ND (0.58)	ND (0.58)	ND (0.59)
Perfluorobutanesulfonic acid (PFBS)	ND (1.2)	ND (1.2)	ND (1.2)
Perfluorobutanoic acid (PFBA)	ND (2.3)	ND (2.3)	ND (2.3)
Perfluorodecanesulfonic acid (PFDS)	ND (0.58)	ND (0.58)	ND (0.59)
Perfluorodecanoic acid (PFDA)	ND (0.58)	ND (0.58)	ND (0.59)
Perfluorododecanesulfonic acid (PFDoS)	ND (0.58)	ND (0.58)	ND (0.59)
Perfluorododecanoic acid (PFDoA)	ND (0.59)	ND (0.58)	ND (0.59)
Perfluoroheptanesulfonic acid (PFHpS)	ND (0.59)	ND (0.58)	ND (0.59)
Perfluoroheptanoic acid (PFHpA)	ND (0.58)	ND (0.58)	ND (0.59)
Perfluorohexanesulfonic acid (PFHxS)	ND (0.59)	ND (0.58)	ND (0.59)
Perfluorohexanoic acid (PFHxA)	ND (0.59)	ND (0.58)	0.593
Perfluorononanesulfonic acid (PFNS)	ND (0.59)	ND (0.58)	ND (0.59)
Perfluorononanoic acid (PFNA)	ND (0.59)	ND (0.58)	ND (0.59)
Perfluorooctanesulfonamide (PFOSAm)	ND (0.59)	ND (0.58)	0.668
Perfluorooctanesulfonic acid (PFOS)	ND (0.58)	ND (0.58)	ND (0.59)
Perfluorooctanoic acid (PFOA)	ND (0.59)	ND (0.58)	<0.59 DLL
Perfluoropentanesulfonic acid (PFPeS)	ND (0.58)	ND (0.58)	ND (0.59)
Perfluoropentanoic acid (PFPeA)	ND (0.58)	ND (0.58)	ND (0.59)
Perfluorotetradecanoic acid (PFTeDA)	ND (0.59)	ND (0.58)	ND (0.59)
Perfluorotridecanoic acid (PFTrDA)	ND (0.59)	ND (0.58)	ND (0.59)
Perfluoroundecanoic acid (PFUnA)	ND (0.58)	ND (0.58)	ND (0.59)

Appendix XVII-B : PFAS in solid residues for Baseline (BL), run #1 (CA-R1) for AFFF incineration with lime injection

Units for PFAS in ng/g. When a PFAS is non-detected, it is considered to be at MDL.

Parameter	CA+-BL	CA+-R1
Date Tested	10/12/2024	12/10/2024
Sample Collection Time	240	240
11-Chloroeicosafluoro-3-oxaundecane-1-sulfonic acid (11Cl-PF3OUdS)	ND (0.57)	ND (0.57)
1H,1H,2H,2H-Perfluorodecane sulfonic acid (8:2 FTS)	ND (1.7)	ND (1.7)
1H,1H,2H,2H-Perfluorohexane sulfonic acid (4:2 FTS)	ND (1.7)	ND (1.7)
1H,1H,2H,2H-Perfluorooctane sulfonic acid (6:2 FTS)	ND (1.7)	ND (1.7)
3-Perfluoroheptylpropanoic acid (7:3 FTCA)	ND (0.57)	ND (0.57)
3-Perfluoropentylpropanoic acid (5:3 FTCA)	ND (0.57)	ND (0.57)
3-Perfluoropropylpropanoic acid (3:3 FTCA)	ND (0.57)	ND (0.57)
4,8-Dioxa-3H-perfluorononanoic acid (DONA)	ND (0.57)	ND (0.57)
9-Chlorohexadecafluoro-3-oxanonane-1-sulfonic acid (9Cl-PF3ONS)	ND (0.57)	ND (0.57)
Hexafluoropropylene Oxide Dimer Acid (HFPO-DA)	ND (2.8)	ND (2.9)
N-ethylperfluorooctane sulfonamide (NEtFOSA)	ND (1.4)	ND (1.4)
N-ethylperfluorooctane sulfonamidoethanol (NEtFOSE)	ND (1.4)	ND (1.4)
N-ethylperfluorooctanesulfonamidoacetic acid (NEtFOSAA)	ND (0.57)	ND (0.57)
N-methylperfluorooctane sulfonamide (NMeFOSA)	ND (1.4)	ND (1.4)
N-methylperfluorooctane sulfonamidoethanol (NMeFOSE)	ND (1.4)	ND (1.4)
N-methylperfluorooctanesulfonamidoacetic acid (NMeFOSAA)	ND (0.57)	ND (0.57)
Nonafluoro-3,6-dioxaheptanoic acid (NFDHA)	ND (0.57)	ND (0.57)
Perfluoro (2-ethoxyethane) sulfonic acid (PFEESA)	ND (0.57)	ND (0.57)
Perfluoro-3-methoxypropanoic acid (PFMPA)	ND (0.57)	ND (0.57)
Perfluoro-4-methoxybutanoic acid (PFMBA)	ND (0.57)	ND (0.57)
Perfluorobutanesulfonic acid (PFBS)	ND (1.1)	ND (1.1)
Perfluorobutanoic acid (PFBA)	ND (2.3)	ND (2.3)
Perfluorodecanesulfonic acid (PFDS)	ND (0.57)	ND (0.57)
Perfluorodecanoic acid (PFDA)	ND (0.57)	ND (0.57)
Perfluorododecanesulfonic acid (PFDoS)	ND (0.57)	ND (0.57)
Perfluorododecanoic acid (PFDoA)	ND (0.57)	ND (0.57)
Perfluoroheptanesulfonic acid (PFHpS)	ND (0.57)	ND (0.57)
Perfluoroheptanoic acid (PFHpA)	ND (0.57)	ND (0.57)
Perfluorohexanesulfonic acid (PFHxS)	ND (0.57)	ND (0.57)
Perfluorohexanoic acid (PFHxA)	ND (0.57)	ND (0.57)
Perfluorononanesulfonic acid (PFNS)	ND (0.57)	ND (0.57)
Perfluorononanoic acid (PFNA)	ND (0.57)	ND (0.57)
Perfluorooctanesulfonamide (PFOSAm)	0.354	ND (0.57)
Perfluorooctanesulfonic acid (PFOS)	ND (0.57)	ND (0.57)
Perfluorooctanoic acid (PFOA)	ND (0.57)	ND (0.57)
Perfluoropentanesulfonic acid (PFPeS)	ND (0.57)	ND (0.57)
Perfluoropentanoic acid (PFPeA)	ND (0.57)	ND (0.57)
Perfluorotetradecanoic acid (PFTeDA)	ND (0.57)	ND (0.57)
Perfluorotridecanoic acid (PFTrDA)	ND (0.57)	ND (0.57)
Perfluoroundecanoic acid (PFUnA)	ND (0.57)	ND (0.57)

Appendix XVII-C : PFAS in solid residues from contaminated soil for Baseline (CS-BL), run #1 (CS-R1)

Units for PFAS in ng/g. When a PFAS is non-detected, it is assumed to be at MDL.

Parameter	CS-BL	CS-R1
Date Tested	10/14/2024	14/10/2024
Sample Collection Time (Minutes)	180	180
11-Chloroeicosafluoro-3-oxaundecane-1-sulfonic acid (11Cl-PF3OUdS)	ND (0.57)	ND (0.57)
1H,1H,2H,2H-Perfluorodecane sulfonic acid (8:2 FTS)	ND (0.57)	ND (0.57)
1H,1H,2H,2H-Perfluorohexane sulfonic acid (4:2 FTS)	ND (0.57)	ND (0.57)
1H,1H,2H,2H-Perfluorooctane sulfonic acid (6:2 FTS)	ND (0.57)	ND (0.57)
3-Perfluoroheptylpropanoic acid (7:3 FTCA)	ND (0.57)	ND (0.57)
3-Perfluoropentylpropanoic acid (5:3 FTCA)	ND (0.57)	ND (0.57)
3-Perfluoropropylpropanoic acid (3:3 FTCA)	ND (0.57)	ND (0.57)
4,8-Dioxa-3H-perfluorononanoic acid (DONA)	ND (0.57)	ND (0.57)
9-Chlorohexadecafluoro-3-oxanonane-1-sulfonic acid (9Cl-PF3ONS)	ND (0.57)	ND (0.57)
Hexafluoropropylene Oxide Dimer Acid (HFPO-DA)	ND (0.57)	ND (0.57)
N-ethylperfluorooctane sulfonamide (NEtFOSA)	ND (0.57)	ND (0.57)
N-ethylperfluorooctane sulfonamidoethanol (NEtFOSE)	ND (0.57)	ND (0.57)
N-ethylperfluorooctanesulfonamidoacetic acid (NEtFOSAA)	ND (0.57)	ND (0.57)
N-methylperfluorooctane sulfonamide (NMeFOSA)	ND (0.57)	ND (0.57)
N-methylperfluorooctane sulfonamidoethanol (NMeFOSE)	ND (0.57)	ND (0.57)
N-methylperfluorooctanesulfonamidoacetic acid (NMeFOSAA)	ND (0.57)	ND (0.57)
Nonafluoro-3,6-dioxaheptanoic acid (NFDHA)	ND (0.57)	ND (0.57)
Perfluoro (2-ethoxyethane) sulfonic acid (PFEESA)	ND (0.57)	ND (0.57)
Perfluoro-3-methoxypropanoic acid (PFMPA)	ND (0.57)	ND (0.57)
Perfluoro-4-methoxybutanoic acid (PFMBA)	ND (0.57)	ND (0.57)
Perfluorobutanesulfonic acid (PFBS)	ND (0.57)	ND (0.57)
Perfluorobutanoic acid (PFBA)	ND (0.57)	ND (0.57)
Perfluorodecanesulfonic acid (PFDS)	ND (0.57)	ND (0.57)
Perfluorodecanoic acid (PFDA)	ND (0.57)	ND (0.57)
Perfluorododecanesulfonic acid (PFDoS)	ND (0.57)	ND (0.57)
Perfluorododecanoic acid (PFDoA)	ND (0.57)	ND (0.57)
Perfluoroheptanesulfonic acid (PFHpS)	ND (0.57)	ND (0.57)
Perfluoroheptanoic acid (PFHpA)	ND (0.57)	ND (0.57)
Perfluorohexanesulfonic acid (PFHxS)	ND (0.57)	ND (0.57)
Perfluorohexanoic acid (PFHxA)	ND (0.57)	ND (0.57)
Perfluorononanesulfonic acid (PFNS)	ND (0.57)	ND (0.57)
Perfluorononanoic acid (PFNA)	ND (0.57)	ND (0.57)
Perfluorooctanesulfonamide (PFOSAm)	ND (0.57)	ND (0.57)
Perfluorooctanesulfonic acid (PFOS)	ND (0.57)	ND (0.57)
Perfluorooctanoic acid (PFOA)	ND (0.57)	ND (0.57)
Perfluoropentanesulfonic acid (PFPeS)	ND (0.57)	ND (0.57)
Perfluoropentanoic acid (PFPeA)	ND (0.57)	ND (0.57)
Perfluorotetradecanoic acid (PFTeDA)	ND (0.57)	ND (0.57)
Perfluorotridecanoic acid (PFTrDA)	ND (0.57)	ND (0.57)
Perfluoroundecanoic acid (PFUnA)	ND (0.57)	ND (0.57)

Appendix XVII-D : PFAS in solid residues from contaminated soil for Baseline #2 (CCS2-BL), run #2 (CCS2-R1) and run #3 (CCS2-R3)

Units for PFAS in ng/g When a PFAS is non-detected, it is assumed to be at MDL.

Parameter	CCS2-BL	CCS2-R1	CCS2-R2
Date Tested	10/22/2024	22/10/2024	23/10/2024
Sample Collection Time (Minutes)	240	240	240
11-Chloroeicosafluoro-3-oxaundecane-1-sulfonic acid (11Cl-PF3OUdS)	ND (0.57)	ND (0.58)	ND (0.58)
1H,1H,2H,2H-Perfluorodecane sulfonic acid (8:2 FTS)	ND (1.7)	ND (1.8)	ND (1.7)
1H,1H,2H,2H-Perfluorohexane sulfonic acid (4:2 FTS)	ND (1.7)	ND (1.8)	ND (1.7)
1H,1H,2H,2H-Perfluorooctane sulfonic acid (6:2 FTS)	ND (1.7)	ND (1.8)	ND (1.7)
3-Perfluoroheptylpropanoic acid (7:3 FTCA)	ND (0.57)	ND (0.58)	ND (0.58)
3-Perfluoropentylpropanoic acid (5:3 FTCA)	ND (0.57)	ND (0.58)	ND (0.58)
3-Perfluoropropylpropanoic acid (3:3 FTCA)	ND (0.57)	ND (0.58)	ND (0.58)
4,8-Dioxa-3H-perfluorononanoic acid (DONA)	ND (0.57)	ND (0.58)	ND (0.58)
9-Chlorohexadecafluoro-3-oxanonane-1-sulfonic acid (9Cl-PF3ONS)	ND (0.57)	ND (0.58)	ND (0.58)
Hexafluoropropylene Oxide Dimer Acid (HFPO-DA)	ND (2.8)	ND (2.9)	ND (2.9)
N-ethylperfluorooctane sulfonamide (NEtFOSA)	ND (1.4)	ND (1.5)	ND (1.4)
N-ethylperfluorooctane sulfonamidoethanol (NEtFOSE)	ND (1.4)	ND (1.5)	ND (1.4)
N-ethylperfluorooctanesulfonamidoacetic acid (NEtFOSAA)	ND (0.57)	ND (0.58)	ND (0.58)
N-methylperfluorooctane sulfonamide (NMeFOSA)	ND (1.4)	ND (1.5)	ND (1.4)
N-methylperfluorooctane sulfonamidoethanol (NMeFOSE)	ND (1.4)	ND (1.5)	ND (1.4)
N-methylperfluorooctanesulfonamidoacetic acid (NMeFOSAA)	ND (0.57)	ND (0.58)	ND (0.58)
Nonafluoro-3,6-dioxaheptanoic acid (NFDHA)	ND (0.57)	ND (0.58)	ND (0.58)
Perfluoro (2-ethoxyethane) sulfonic acid (PFEESA)	ND (0.57)	ND (0.58)	ND (0.58)
Perfluoro-3-methoxypropanoic acid (PFMPA)	ND (0.57)	ND (0.58)	ND (0.58)
Perfluoro-4-methoxybutanoic acid (PFMBA)	ND (0.57)	ND (0.58)	ND (0.58)
Perfluorobutanesulfonic acid (PFBS)	ND (1.1)	ND (1.2)	ND (1.2)
Perfluorobutanoic acid (PFBA)	ND (2.3)	ND (2.3)	ND (2.3)
Perfluorodecanesulfonic acid (PFDS)	ND (0.57)	ND (0.58)	ND (0.58)
Perfluorodecanoic acid (PFDA)	ND (0.57)	ND (0.58)	ND (0.58)
Perfluorododecanesulfonic acid (PFDoS)	ND (0.57)	ND (0.58)	ND (0.58)
Perfluorododecanoic acid (PFDoA)	ND (0.57)	ND (0.58)	ND (0.58)
Perfluoroheptanesulfonic acid (PFHpS)	ND (0.57)	ND (0.58)	ND (0.58)
Perfluoroheptanoic acid (PFHpA)	ND (0.57)	ND (0.58)	ND (0.58)
Perfluorohexanesulfonic acid (PFHxS)	ND (0.57)	ND (0.58)	ND (0.58)
Perfluorohexanoic acid (PFHxA)	ND (0.57)	ND (0.58)	ND (0.58)
Perfluorononanesulfonic acid (PFNS)	ND (0.57)	ND (0.58)	ND (0.58)
Perfluorononanoic acid (PFNA)	ND (0.57)	ND (0.58)	ND (0.58)
Perfluorooctanesulfonamide (PFOSAm)	ND (0.57)	ND (0.58)	ND (0.58)
Perfluorooctanesulfonic acid (PFOS)	ND (0.57)	ND (0.58)	ND (0.58)
Perfluorooctanoic acid (PFOA)	ND (0.57)	ND (0.58)	ND (0.58)
Perfluoropentanesulfonic acid (PFPeS)	ND (0.57)	ND (0.58)	ND (0.58)
Perfluoropentanoic acid (PFPeA)	ND (0.57)	ND (0.58)	ND (0.58)
Perfluorotetradecanoic acid (PFTeDA)	ND (0.57)	ND (0.58)	ND (0.58)
Perfluorotridecanoic acid (PFTrDA)	ND (0.57)	ND (0.58)	ND (0.58)
Perfluoroundecanoic acid (PFUnA)	ND (0.57)	ND (0.58)	ND (0.58)

Appendix XVII-E : PFAS in solid residues from spent carbon incineration for Baseline (CC-BL), run #1 (CC-R1) and run #2 (CC-R2)

Units for PFAS in ng/g. When a PFAS is non-detected, it is assumed to be at MDL.

Parameter	CC-BL	CC-R1	CC-R2
Date Tested	10/19/2024	10/19/2024	10/20/2024
Sample Collection Time (Minutes)	240	240	240
11-Chloroeicosafluoro-3-oxaundecane-1-sulfonic acid (11Cl-PF3OUdS)	ND (0.58)	ND (0.58)	ND (0.56)
1H,1H,2H,2H-Perfluorodecane sulfonic acid (8:2 FTS)	ND (1.7)	ND (1.8)	ND (1.7)
1H,1H,2H,2H-Perfluorohexane sulfonic acid (4:2 FTS)	ND (1.7)	ND (1.8)	ND (1.7)
1H,1H,2H,2H-Perfluorooctane sulfonic acid (6:2 FTS)	ND (1.7)	ND (1.8)	ND (1.7)
3-Perfluoroheptylpropanoic acid (7:3 FTCA)	ND (0.58)	ND (0.58)	ND (0.56)
3-Perfluoropentylpropanoic acid (5:3 FTCA)	ND (0.58)	ND (0.58)	ND (0.56)
3-Perfluoropropylpropanoic acid (3:3 FTCA)	ND (0.58)	ND (0.58)	ND (0.56)
4,8-Dioxa-3H-perfluorononanoic acid (DONA)	ND (0.58)	ND (0.58)	ND (0.56)
9-Chlorohexadecafluoro-3-oxanonane-1-sulfonic acid (9Cl-PF3ONS)	ND (0.58)	ND (0.58)	ND (0.56)
Hexafluoropropylene Oxide Dimer Acid (HFPO-DA)	ND (2.9)	ND (2.9)	ND (2.8)
N-ethylperfluorooctane sulfonamide (NEtFOSA)	ND (1.5)	ND (1.5)	ND (1.4)
N-ethylperfluorooctane sulfonamidoethanol (NEtFOSE)	ND (1.5)	ND (1.5)	ND (1.4)
N-ethylperfluorooctanesulfonamidoacetic acid (NEtFOSAA)	ND (0.58)	ND (0.58)	ND (0.56)
N-methylperfluorooctane sulfonamide (NMeFOSA)	ND (1.5)	ND (1.5)	ND (1.4)
N-methylperfluorooctane sulfonamidoethanol (NMeFOSE)	ND (1.5)	ND (1.5)	ND (1.4)
N-methylperfluorooctanesulfonamidoacetic acid (NMeFOSAA)	ND (0.58)	ND (0.58)	ND (0.56)
Nonafluoro-3,6-dioxaheptanoic acid (NFDHA)	ND (0.58)	ND (0.58)	ND (0.56)
Perfluoro (2-ethoxyethane) sulfonic acid (PFEESA)	ND (0.58)	ND (0.58)	ND (0.56)
Perfluoro-3-methoxypropanoic acid (PFMPA)	ND (0.58)	ND (0.58)	ND (0.56)
Perfluoro-4-methoxybutanoic acid (PFMBA)	ND (0.58)	ND (0.58)	ND (0.56)
Perfluorobutanesulfonic acid (PFBS)	ND (1.2)	ND (1.2)	ND (1.1)
Perfluorobutanoic acid (PFBA)	ND (2.3)	ND (2.3)	ND (2.3)
Perfluorodecanesulfonic acid (PFDS)	ND (0.58)	ND (0.58)	ND (0.56)
Perfluorodecanoic acid (PFDA)	ND (0.58)	ND (0.58)	ND (0.56)
Perfluorododecanesulfonic acid (PFDoS)	ND (0.58)	ND (0.58)	ND (0.56)
Perfluorododecanoic acid (PFDoA)	ND (0.58)	ND (0.58)	ND (0.56)
Perfluoroheptanesulfonic acid (PFHpS)	ND (0.58)	ND (0.58)	ND (0.56)
Perfluoroheptanoic acid (PFHpA)	ND (0.58)	ND (0.58)	ND (0.56)
Perfluorohexanesulfonic acid (PFHxS)	ND (0.58)	ND (0.58)	ND (0.56)
Perfluorohexanoic acid (PFHxA)	ND (0.58)	ND (0.58)	ND (0.56)
Perfluorononanesulfonic acid (PFNS)	ND (0.58)	ND (0.58)	ND (0.56)
Perfluorononanoic acid (PFNA)	ND (0.58)	ND (0.58)	ND (0.56)

Perfluorooctanesulfonamide (PFOSAm)	ND (0.58)	ND (0.58)	ND (0.56)
Perfluorooctanesulfonic acid (PFOS)	ND (0.58)	ND (0.58)	ND (0.56)
Perfluorooctanoic acid (PFOA)	ND (0.58)	ND (0.58)	ND (0.56)
Perfluoropentanesulfonic acid (PFPeS)	ND (0.58)	ND (0.58)	ND (0.56)
Perfluoropentanoic acid (PFPeA)	ND (0.58)	ND (0.58)	ND (0.56)
Perfluorotetradecanoic acid (PFTeDA)	ND (0.58)	ND (0.58)	ND (0.56)
Perfluorotridecanoic acid (PFTrDA)	ND (0.58)	ND (0.58)	ND (0.56)
Perfluoroundecanoic acid (PFUnA)	ND (0.58)	ND (0.58)	ND (0.56)

Appendix XVII-F : PFAS in solid residues from CF4 incineration for Baseline (CW+-BL), run #1 (CW+-R1)

Units for PFAS in ng/g. When a PFAS is non-detected, it is assumed to be at MDL.

Parameter	CW+-BL	CW+-R1
Date Tested	10/17/2024	17/10/2024
Sample Collection Time (Minutes)	240	240
11-Chloroeicosafluoro-3-oxaundecane-1-sulfonic acid (11Cl-PF3OUdS)	ND (0.57)	ND (0.57)
1H,1H,2H,2H-Perfluorodecane sulfonic acid (8:2 FTS)	ND (1.7)	ND (1.7)
1H,1H,2H,2H-Perfluorohexane sulfonic acid (4:2 FTS)	ND (1.7)	ND (1.7)
1H,1H,2H,2H-Perfluorooctane sulfonic acid (6:2 FTS)	ND (1.7)	ND (1.7)
3-Perfluoroheptylpropanoic acid (7:3 FTCA)	ND (0.57)	ND (0.57)
3-Perfluoropentylpropanoic acid (5:3 FTCA)	ND (0.57)	ND (0.57)
3-Perfluoropropylpropanoic acid (3:3 FTCA)	ND (0.57)	ND (0.57)
4,8-Dioxa-3H-perfluorononanoic acid (DONA)	ND (0.57)	ND (0.57)
9-Chlorohexadecafluoro-3-oxanonane-1-sulfonic acid (9Cl-PF3ONS)	ND (0.57)	ND (0.57)
Hexafluoropropylene Oxide Dimer Acid (HFPO-DA)	ND (2.8)	ND (2.9)
N-ethylperfluorooctane sulfonamide (NEtFOSA)	ND (1.4)	ND (1.4)
N-ethylperfluorooctane sulfonamidoethanol (NEtFOSE)	ND (1.4)	ND (1.4)
N-ethylperfluorooctanesulfonamidoacetic acid (NEtFOSAA)	ND (0.57)	ND (0.57)
N-methylperfluorooctane sulfonamide (NMeFOSA)	ND (1.4)	ND (1.4)
N-methylperfluorooctane sulfonamidoethanol (NMeFOSE)	ND (1.4)	ND (1.4)
N-methylperfluorooctanesulfonamidoacetic acid (NMeFOSAA)	ND (0.57)	ND (0.57)
Nonafluoro-3,6-dioxaheptanoic acid (NFDHA)	ND (0.57)	ND (0.57)
Perfluoro (2-ethoxyethane) sulfonic acid (PFEESA)	ND (0.57)	ND (0.57)
Perfluoro-3-methoxypropanoic acid (PFMPA)	ND (0.57)	ND (0.57)
Perfluoro-4-methoxybutanoic acid (PFMBA)	ND (0.57)	ND (0.57)
Perfluorobutanesulfonic acid (PFBS)	ND (1.1)	ND (1.1)
Perfluorobutanoic acid (PFBA)	ND (2.3)	ND (2.3)
Perfluorodecanesulfonic acid (PFDS)	ND (0.57)	ND (0.57)
Perfluorodecanoic acid (PFDA)	ND (0.57)	ND (0.57)
Perfluorododecanesulfonic acid (PFDoS)	ND (0.57)	ND (0.57)
Perfluorododecanoic acid (PFDoA)	ND (0.57)	ND (0.57)
Perfluoroheptanesulfonic acid (PFHpS)	ND (0.57)	ND (0.57)
Perfluoroheptanoic acid (PFHpA)	ND (0.57)	ND (0.57)
Perfluorohexanesulfonic acid (PFHxS)	ND (0.57)	ND (0.57)
Perfluorohexanoic acid (PFHxA)	ND (0.57)	ND (0.57)
Perfluorononanesulfonic acid (PFNS)	ND (0.57)	ND (0.57)
Perfluorononanoic acid (PFNA)	ND (0.57)	ND (0.57)
Perfluorooctanesulfonamide (PFOSAm)	ND (0.57)	ND (0.57)
Perfluorooctanesulfonic acid (PFOS)	ND (0.57)	ND (0.57)
Perfluorooctanoic acid (PFOA)	ND (0.57)	ND (0.57)
Perfluoropentanesulfonic acid (PFPeS)	ND (0.57)	ND (0.57)
Perfluoropentanoic acid (PFPeA)	ND (0.57)	ND (0.57)
Perfluorotetradecanoic acid (PFTeDA)	ND (0.57)	ND (0.57)
Perfluorotridecanoic acid (PFTrDA)	ND (0.57)	ND (0.57)
Perfluoroundecanoic acid (PFUnA)	ND (0.57)	ND (0.57)

Appendix XVIII-A - OTM-50 table for Baseline (CA-BL), AFFF run#1 (CA-R1) and AFFF run#2 (CA-R2)

Parameter	Units	CA-BL	CA-R1	CA-R2
Date Tested	-	10/09/2024	9/10/2024	10/10/2024
Sample Collection Time	minutes	240	240	240
Gas Volume Collected	dscf	113.712	117.847	116.075
Volumetric Flow Rate	dscfm	32,608	33,480	32,776
1,1,1,2,2-Pentafluoroethane (HFC-125)	lb/hr	ND (0.000083)	ND (0.000084)	ND (0.000083)
1,1,1-Trifluoroethane		ND (0.00019)	ND (0.00019)	ND (0.00019)
1,1,2-Trichloro-1,2,2-trifluoroethane		ND (0.000048)	ND (0.000049)	ND (0.000048)
1H-Heptafluoropropane		ND (0.000040)	ND (0.000041)	ND (0.000040)
1H-Nonafluorobutane		ND (0.000084)	ND (0.000086)	ND (0.000084)
1H-Perfluoroheptane		ND (0.00012)	ND (0.00013)	ND (0.00012)
1H-Perfluorohexane		ND (0.000069)	ND (0.000071)	ND (0.000069)
1H-Perfluorooctane		ND (0.000091)	ND (0.000093)	ND (0.000091)
1H-Perfluoropentane		ND (0.00012)	ND (0.00013)	ND (0.00012)
2H-Perfluoro-5-methyl-3,6-dioxanonane (E2)		ND (0.00010)	ND (0.00010)	ND (0.00010)
Chlorodifluoromethane		ND (0.00012)	ND (0.00013)	ND (0.00012)
Chlorotrifluoroethene		ND (0.000037)	ND (0.000038)	ND (0.000037)
Chlorotrifluoromethane		ND (0.000089)	ND (0.000092)	ND (0.000090)
Decafluorobutane		ND (0.000062)	ND (0.000063)	ND (0.000062)
Difluoromethane (HFC-32)		ND (0.00011)	ND (0.00011)	ND (0.00011)
Dodecafluoropentane		ND (0.00017)	ND (0.00017)	ND (0.00017)
Ethane, 1,1,1,2-tetrafluoro-		ND (0.000095)	ND (0.000097)	ND (0.000095)
Fluoromethane		ND (0.00070)	ND (0.00072)	ND (0.00070)
Heptafluoropropyl 1,2,2,2-tetrafluoroethyl ether		0.0000996	ND (0.000087)	ND (0.000086)
Hexadecafluoroheptane		ND (0.000080)	ND (0.000082)	ND (0.000080)
Hexafluoroethane		ND (0.000044)	ND (0.000045)	ND (0.000044)
Hexafluoropropene		ND (0.000043)	ND (0.000044)	ND (0.000043)
Hexafluoropropylene oxide		ND (0.0034)	ND (0.0035)	ND (0.0034)
Octadecafluorooctane		ND (0.00010)	ND (0.00011)	ND (0.00010)
Octafluorocyclobutane (FC-C318)		ND (0.000041)	ND (0.000042)	ND (0.000041)
Octafluorocyclopentene (FC-C1418)		ND (0.000096)	ND (0.000098)	ND (0.000096)
Octafluoropropane		ND (0.000093)	ND (0.000095)	ND (0.000093)
Tetradecafluorohexane		ND (0.00016)	ND (0.00016)	ND (0.00015)
Tetrafluoroethene		ND (0.000038)	ND (0.000039)	ND (0.000038)
Trichlorofluoromethane		ND (0.00013)	ND (0.00013)	ND (0.00013)
Trifluoromethane		ND (0.00011)	ND (0.00011)	ND (0.00011)
Vinyl Fluoride		ND (0.000062)	ND (0.000063)	ND (0.000062)
Tetrafluoromethane		ND (0.00044)	ND (0.00045)	ND (0.00044)

Appendix XVIII-B - OTM-50 table for Baseline (CA+-BL), AFFF run#3 (CA+-R1)

Parameter	Units	CA+-BL	CA+-R1
Date Tested	-	10/12/2024	12/10/2024
Sample Collection Time	minutes	240	240
Gas Volume Collected	dscf	112.753	114.395
Volumetric Flow Rate	dscfm	31,960	33,486
1,1,1,2,2-Pentafluoroethane (HFC-125)	lb/hr	ND (0.000083)	ND (0.000084)
1,1,1-Trifluoroethane		ND (0.00019)	ND (0.00019)
1,1,2-Trichloro-1,2,2-trifluoroethane		ND (0.000048)	ND (0.000049)
1H-Heptafluoropropane		ND (0.000040)	ND (0.000041)
1H-Nonafluorobutane		ND (0.000084)	ND (0.000085)
1H-Perfluoroheptane		ND (0.00012)	ND (0.00012)
1H-Perfluorohexane		ND (0.000069)	ND (0.000070)
1H-Perfluorooctane		ND (0.000091)	ND (0.000092)
1H-Perfluoropentane		ND (0.00012)	ND (0.00012)
2H-Perfluoro-5-methyl-3,6-dioxanonane (E2)		ND (0.00010)	ND (0.00010)
Chlorodifluoromethane		ND (0.00012)	ND (0.00013)
Chlorotrifluoroethene		ND (0.000037)	ND (0.000038)
Chlorotrifluoromethane		ND (0.000089)	ND (0.000091)
Decafluorobutane		ND (0.000062)	ND (0.000063)
Difluoromethane (HFC-32)		ND (0.00011)	ND (0.00011)
Dodecafluoropentane		ND (0.00017)	ND (0.00017)
Ethane, 1,1,1,2-tetrafluoro-		ND (0.000094)	ND (0.000096)
Fluoromethane		ND (0.00070)	ND (0.00071)
Heptafluoropropyl 1,2,2,2-tetrafluoroethyl ether		ND (0.000085)	ND (0.000087)
Hexadecafluoroheptane		ND (0.000080)	ND (0.000081)
Hexafluoroethane		ND (0.000044)	ND (0.000045)
Hexafluoropropene		ND (0.000043)	ND (0.000044)
Hexafluoropropylene oxide		ND (0.0034)	ND (0.0035)
Octadecafluorooctane		ND (0.00010)	ND (0.00011)
Octafluorocyclobutane (FC-C318)		ND (0.000041)	ND (0.000042)
Octafluorocyclopentene (FC-C1418)		ND (0.000096)	ND (0.000098)
Octafluoropropane		ND (0.000093)	ND (0.000095)
Tetradecafluorohexane		ND (0.00016)	ND (0.00016)
Tetrafluoroethene		ND (0.000038)	ND (0.000039)
Trichlorofluoromethane		ND (0.00013)	ND (0.00013)
Trifluoromethane		ND (0.00011)	ND (0.00011)
Vinyl Fluoride		ND (0.000062)	ND (0.000063)
Tetrafluoromethane		0.00239	0.00509

Appendix XVIII-C : OTM-50 table for baseline (CC-BL), carbon run#1 (CC-R1) and carbon run#2 (CC-R2)

Parameter	Units	CC-BL	CC-R1	CC-R2
Date Tested	-	10/19/2024	19/10/2024	20/10/2024
Sample Collection Time	minutes	240	240	240
Gas Volume Collected	dscf	114.613	114.081	117.867
Volumetric Flow Rate	dscfm	32,906	32,160	33,931
1,1,1,2,2-Pentafluoroethane (HFC-125)	lb/hr	ND (0.00013)	ND (0.00013)	ND (0.00014)
1,1,1-Trifluoroethane		ND (0.00029)	ND (0.00029)	ND (0.00032)
1,1,2-Trichloro-1,2,2-trifluoroethane		ND (0.000074)	ND (0.000073)	ND (0.000080)
1H-Heptafluoropropane		ND (0.000062)	ND (0.000061)	ND (0.000067)
1H-Nonafluorobutane		ND (0.00013)	ND (0.00013)	ND (0.00014)
1H-Perfluoroheptane		ND (0.00019)	ND (0.00019)	ND (0.00020)
1H-Perfluorohexane		ND (0.00011)	ND (0.00010)	ND (0.00011)
1H-Perfluorooctane		ND (0.00014)	ND (0.00014)	ND (0.00015)
1H-Perfluoropentane		ND (0.00019)	ND (0.00019)	ND (0.00020)
2H-Perfluoro-5-methyl-3,6-dioxanonane (E2)		ND (0.00016)	ND (0.00016)	ND (0.00017)
Chlorodifluoromethane		ND (0.00019)	ND (0.00019)	ND (0.00021)
Chlorotrifluoroethene		ND (0.000057)	ND (0.000056)	ND (0.000062)
Chlorotrifluoromethane		ND (0.00014)	ND (0.00014)	ND (0.00015)
Decafluorobutane		ND (0.000094)	ND (0.000094)	ND (0.00010)
Difluoromethane (HFC-32)		ND (0.00017)	ND (0.00017)	ND (0.00019)
Dodecafluoropentane		ND (0.00025)	ND (0.00025)	ND (0.00028)
Ethane, 1,1,1,2-tetrafluoro-		ND (0.00014)	ND (0.00014)	ND (0.00016)
Fluoromethane		ND (0.0011)	ND (0.0011)	ND (0.0012)
Heptafluoropropyl 1,2,2,2-tetrafluoroethyl ether		ND (0.00013)	ND (0.00013)	ND (0.00014)
Hexadecafluoroheptane		ND (0.00012)	ND (0.00012)	ND (0.00013)
Hexafluoroethane		ND (0.000067)	ND (0.000067)	ND (0.000073)
Hexafluoropropene		ND (0.000066)	ND (0.000066)	ND (0.000072)
Hexafluoropropylene oxide		ND (0.0052)	ND (0.0052)	ND (0.0057)
Octadecafluorooctane		ND (0.00016)	ND (0.00016)	ND (0.00017)
Octafluorocyclobutane (FC-C318)		ND (0.000063)	ND (0.000063)	ND (0.000068)
Octafluorocyclopentene (FC-C1418)		ND (0.00015)	ND (0.00015)	ND (0.00016)
Octafluoropropane		ND (0.00014)	ND (0.00014)	ND (0.00015)
Tetradecafluorohexane		ND (0.00024)	ND (0.00023)	ND (0.00026)
Tetrafluoroethene		ND (0.000058)	ND (0.000058)	ND (0.000063)
Trichlorofluoromethane		ND (0.00020)	ND (0.00020)	ND (0.00022)
Trifluoromethane		ND (0.00017)	ND (0.00016)	ND (0.00018)
Vinyl Fluoride		ND (0.000094)	ND (0.000094)	ND (0.00010)
Tetrafluoromethane		ND (0.00068)	ND (0.00067)	ND (0.00073)

Appendix XVIII-D : OTM-50 table for Baseline (CCS-BL) and contaminated soil run 1 (CCS-R1)

Parameter	Units	CCS-BL	CCS-R1
Date Tested	-	10/14/2024	14/10/2024
Sample Collection Time	minutes	240	240
1,1,1,2,2-Pentafluoroethane (HFC-125)	lb/hr	ND (0.000083)	ND (0.000081)
1,1,1-Trifluoroethane		ND (0.00019)	ND (0.00019)
1,1,2-Trichloro-1,2,2-trifluoroethane		ND (0.000048)	ND (0.000047)
1H-Heptafluoropropane		ND (0.000040)	ND (0.000039)
1H-Nonafluorobutane		ND (0.000084)	ND (0.000082)
1H-Perfluoroheptane		ND (0.00012)	ND (0.00012)
1H-Perfluorohexane		ND (0.000069)	ND (0.000068)
1H-Perfluorooctane		ND (0.000091)	ND (0.000089)
1H-Perfluoropentane		ND (0.00012)	ND (0.00012)
2H-Perfluoro-5-methyl-3,6-dioxanonane (E2)		ND (0.00010)	ND (0.00010)
Chlorodifluoromethane		ND (0.00013)	ND (0.00012)
Chlorotrifluoroethene		ND (0.000037)	ND (0.000036)
Chlorotrifluoromethane		ND (0.000090)	ND (0.000087)
Decafluorobutane		ND (0.000062)	ND (0.000060)
Difluoromethane (HFC-32)		ND (0.00011)	ND (0.00011)
Dodecafluoropentane		ND (0.00017)	ND (0.00016)
Ethane, 1,1,1,2-tetrafluoro-		ND (0.000095)	ND (0.000093)
Fluoromethane		ND (0.00070)	ND (0.00069)
Heptafluoropropyl 1,2,2,2-tetrafluoroethyl ether		ND (0.000086)	ND (0.000084)
Hexadecafluoroheptane		ND (0.000080)	ND (0.000078)
Hexafluoroethane		ND (0.000044)	ND (0.000043)
Hexafluoropropene		ND (0.000043)	ND (0.000042)
Hexafluoropropylene oxide		ND (0.0034)	ND (0.0033)
Octadecafluorooctane		ND (0.00010)	ND (0.00010)
Octafluorocyclobutane (FC-C318)		ND (0.000041)	ND (0.000040)
Octafluorocyclopentene (FC-C1418)		ND (0.000096)	ND (0.000094)
Octafluoropropane		ND (0.000093)	ND (0.000091)
Tetradecafluorohexane		ND (0.00015)	ND (0.00015)
Tetrafluoroethene		ND (0.000038)	ND (0.000037)
Trichlorofluoromethane		ND (0.00013)	ND (0.00013)
Trifluoromethane		ND (0.00011)	ND (0.00011)
Vinyl Fluoride		ND (0.000062)	ND (0.000060)
Tetrafluoromethane		ND (0.00045)	ND (0.00043)

Appendix XVIII- E : OTM-50 table for baseline (CCS2-BL), contaminated oil run#2 (CCS2-R1), contaminated soil run#3 (CCS2-R2)

Parameter	Units	CCS2-BL	CCS2-R1	CCS2-R2
Date Tested	-	10/22/2024	22/10/2024	23/10/2024
Sample Collection Time	minutes	240	240	240
Gas Volume Collected	dscf	118.352	113.312	114.699
Volumetric Flow Rate	dscfm	33,831	31,972	32,829
1,1,1,2,2-Pentafluoroethane (HFC-125)	lb/hr	ND (0.00014)	ND (0.00013)	ND (0.00012)
1,1,1-Trifluoroethane		ND (0.00031)	ND (0.00030)	ND (0.00028)
1,1,2-Trichloro-1,2,2-trifluoroethane		ND (0.000079)	ND (0.000076)	ND (0.000072)
1H-Heptafluoropropane		ND (0.000066)	ND (0.000063)	ND (0.000060)
1H-Nonafluorobutane		ND (0.00014)	ND (0.00013)	ND (0.00012)
1H-Perfluoroheptane		ND (0.00020)	ND (0.00019)	ND (0.00018)
1H-Perfluorohexane		ND (0.00011)	ND (0.00011)	ND (0.00010)
1H-Perfluorooctane		ND (0.00015)	ND (0.00014)	ND (0.00013)
1H-Perfluoropentane		ND (0.00020)	ND (0.00019)	ND (0.00018)
2H-Perfluoro-5-methyl-3,6-dioxanonane (E2)		ND (0.00017)	ND (0.00016)	ND (0.00015)
Chlorodifluoromethane		ND (0.00020)	ND (0.00020)	ND (0.00019)
Chlorotrifluoroethene		ND (0.000061)	ND (0.000059)	ND (0.000055)
Chlorotrifluoromethane		ND (0.00015)	ND (0.00014)	ND (0.00013)
Decafluorobutane		ND (0.00010)	ND (0.000097)	ND (0.000092)
Difluoromethane (HFC-32)		ND (0.00018)	ND (0.00018)	ND (0.00017)
Dodecafluoropentane		ND (0.00027)	ND (0.00026)	ND (0.00025)
Ethane, 1,1,1,2-tetrafluoro-		ND (0.00015)	ND (0.00015)	ND (0.00014)
Fluoromethane		ND (0.0011)	ND (0.0011)	ND (0.0010)
Heptafluoropropyl 1,2,2,2-tetrafluoroethyl ether		ND (0.00014)	ND (0.00013)	ND (0.00013)
Hexadecafluoroheptane		ND (0.00013)	ND (0.00013)	ND (0.00012)
Hexafluoroethane		ND (0.000072)	ND (0.000069)	ND (0.000065)
Hexafluoropropene		ND (0.000071)	ND (0.000068)	ND (0.000064)
Hexafluoropropylene oxide		ND (0.0056)	ND (0.0054)	ND (0.0051)
Octadecafluorooctane		ND (0.00017)	ND (0.00016)	ND (0.00015)
Octafluorocyclobutane (FC-C318)		ND (0.000067)	ND (0.000065)	ND (0.000061)
Octafluorocyclopentene (FC-C1418)		ND (0.00016)	ND (0.00015)	ND (0.00014)
Octafluoropropane		ND (0.00015)	ND (0.00015)	ND (0.00014)
Tetradecafluorohexane		ND (0.00025)	ND (0.00024)	ND (0.00023)
Tetrafluoroethene		ND (0.000062)	ND (0.000060)	ND (0.000057)
Trichlorofluoromethane		ND (0.00021)	ND (0.00020)	ND (0.00019)
Trifluoromethane		ND (0.00018)	ND (0.00017)	ND (0.00016)
Vinyl Fluoride		ND (0.00010)	ND (0.000097)	ND (0.000092)
Tetrafluoromethane		ND (0.00072)	ND (0.00070)	ND (0.00066)

Appendix XVIII-F : OTM-50 table for Baseline (CCF4-BL) and CF4 run (CCF4-R1)

Parameter	Units	CCF4-BL	CCF4-R1
Date Tested	-	10/17/2024	17/10/2024
Sample Collection Time	minutes	240	240
Gas Volume Collected	dscf	115.328	119.429
Volumetric Flow Rate	dscfm	33,597	33,976
1,1,1,2,2-Pentafluoroethane (HFC-125)	lb/hr	ND (0.00013)	ND (0.00013)
1,1,1-Trifluoroethane		ND (0.00029)	ND (0.00031)
1,1,2-Trichloro-1,2,2-trifluoroethane		ND (0.000074)	ND (0.000078)
1H-Heptafluoropropane		ND (0.000061)	ND (0.000065)
1H-Nonafluorobutane		ND (0.00013)	ND (0.00014)
1H-Perfluoroheptane		ND (0.00019)	ND (0.00020)
1H-Perfluorohexane		ND (0.00011)	ND (0.00011)
1H-Perfluorooctane		ND (0.00014)	ND (0.00015)
1H-Perfluoropentane		ND (0.00019)	ND (0.00020)
2H-Perfluoro-5-methyl-3,6-dioxanonane (E2)		ND (0.00016)	ND (0.00016)
Chlorodifluoromethane		ND (0.00019)	ND (0.00020)
Chlorotrifluoroethene		ND (0.000057)	ND (0.000060)
Chlorotrifluoromethane		ND (0.00014)	ND (0.00014)
Decafluorobutane		ND (0.000094)	ND (0.000099)
Difluoromethane (HFC-32)		ND (0.00017)	ND (0.00018)
Dodecafluoropentane		ND (0.00025)	ND (0.00027)
Ethane, 1,1,1,2-tetrafluoro-		ND (0.00014)	ND (0.00015)
Fluoromethane		ND (0.0011)	ND (0.0011)
Heptafluoropropyl 1,2,2,2-tetrafluoroethyl ether		ND (0.00013)	ND (0.00014)
Hexadecafluoroheptane		ND (0.00012)	ND (0.00013)
Hexafluoroethane		ND (0.000067)	ND (0.000071)
Hexafluoropropene		ND (0.000066)	ND (0.000070)
Hexafluoropropylene oxide		ND (0.0052)	ND (0.0055)
Octadecafluorooctane		ND (0.00016)	ND (0.00017)
Octafluorocyclobutane (FC-C318)		ND (0.000063)	ND (0.000066)
Octafluorocyclopentene (FC-C1418)		ND (0.00015)	ND (0.00015)
Octafluoropropane		ND (0.00014)	ND (0.00015)
Tetradecafluorohexane		ND (0.00024)	ND (0.00025)
Tetrafluoroethene		ND (0.000058)	ND (0.000061)
Trichlorofluoromethane		ND (0.00020)	ND (0.00021)
Trifluoromethane		ND (0.00016)	ND (0.00017)
Vinyl Fluoride		ND (0.000094)	ND (0.000099)
Tetrafluoromethane		ND (0.00068)	17.6

Appendix XIX-A : Method 26A HF Results for baseline and AFFF run #2 and #3 in campaign 2

Run No.		BL	1	2	
Date		09-Oct-24	09-Oct-24	10-Oct-24	
Start Time	Units	08:30	13:45	14:30	
Stop Time		12:50	18:05	18:40	**AVGS**
Sampling Parameters --					
Barometric Pressure	in. Hg	29.93	29.92	29.99	29.95
Volume Metered	dcf	117.433	122.651	120.042	120.042
Volume of Gas Collected	dscf	113.774	117.257	114.596	115.209
Moisture	% v/v	6.6	6.9	5.8	6.4
O_2 at Stack	% dry	7.82	7.27	7.48	7.52
CO_2 at Stack	% dry	9.78	10.36	9.93	10.02
Avg. Stack Temp.	°F	101	104	104	103
Stack Flowrate	dscfm	32,670	33,241	32,929	32,947
Isokinetics	%	100	101	99	100
HF Emission Results --					
Total HF Detected Acid Imp	µg	< 76.5	< 95.7	< 77.7	< 83.3
Total HF Detected Alkaline Imp	µg	< 110.0	< 132.0	< 116.0	< 119.3
Total HF Concentration	ppm	< 0.069	< 0.082	< 0.072	< 0.074
Conc. @ 7% O2	ppm	< 0.074	< 0.084	< 0.074	< 0.077
HF Emission Rate	lb/hr	< 0.007	< 0.009	< 0.007	< 0.008

Appendix XIX-B : Method 26A HF Results for baseline (A+-BL) and AFFF run #3 (A+-R1) in campaign 2

Run No.		A+BL	A+R1	
Date		12-Oct-24	12-Oct-24	
Start Time	Units	08:15	13:35	
Stop Time		12:30	17:47	AVGS
Sampling Parameters --				
Barometric Pressure	in. Hg	30.09	30.11	30.10
Volume Metered	dcf	111.785	118.600	115.193
Volume of Gas Collected	dscf	107.824	114.745	111.285
Moisture	% v/v	7.0	7.0	7.0
O_2 at Stack	% dry	7.60	7.06	7.33
CO_2 at Stack	% dry	9.45	9.83	9.64
Avg. Stack Temp.	°F	103	104	104
Stack Flowrate	dscfm	30,493	32,670	31,582
Isokinetics	%	101	100	101
HF Emission Results --				
Total HF Detected Acid Imp	µg	< 83.9	< 79.7	< 81.8
Total HF Detected Alkaline Imp	µg	< 129.0	< 115.0	< 122.0
Total HF Concentration	ppm	< 0.084	< 0.072	< 0.078
Conc. @ 7% O2	ppm	< 0.087	< 0.072	< 0.080
HF Emission Rate	lb/hr	< 0.008	< 0.007	< 0.008

Appendix XV-C : Method 26A HF Results for baseline (CS-BL) and contaminated soil run #1 (Cs-R1) in campaign 2

Run No.		CS-BL	CS-R1	
Date		14-Oct-24	14-Oct-24	
Start Time	Units	08:05	14:45	
Stop Time		12:20	18:55	AVGS
Sampling Parameters --				
Barometric Pressure	in. Hg	30.06	30.04	30.05
Volume Metered	dcf	112.785	120.225	116.505
Volume of Gas Collected	dscf	110.187	115.689	112.938
Moisture	% v/v	7.0	7.3	7.1
O_2 at Stack	% dry	7.19	7.26	7.23
CO_2 at Stack	% dry	9.27	9.54	9.41
Avg. Stack Temp.	°F	104	106	105
Stack Flowrate	dscfm	31,456	32,840	32,148
Isokinetics	%	100	101	100
HF Emission Results --				
Total HF Detected Acid Imp	µg	< 81.3	< 78.5	< 79.9
Total HF Detected Alkaline Imp	µg	< 124.0	< 127.0	< 125.5
Total HF Concentration	ppm	< 0.079	< 0.075	< 0.077
Conc. @ 7% O2	ppm	< 0.080	< 0.077	< 0.078
HF Emission Rate	lb/hr	< 0.008	< 0.008	< 0.008

Appendix XIX-D : Method 26A HF Results for baseline (W+-BL) and CF4 run #1 (W+-R1) in campaign 2

Run No.		W+BL	W+-R1	
Date		17-Oct-24	17-Oct-24	
Start Time	Units	09:05	14:15	
Stop Time		13:20	18:27	AVGS
Sampling Parameters --				
Barometric Pressure	in. Hg	30.22	30.21	30.22
Volume Metered	dcf	115.138	120.270	117.704
Volume of Gas Collected	dscf	115.104	120.264	117.684
Moisture	% v/v	5.5	5.8	5.6
O_2 at Stack	% dry	8.40	8.40	8.40
CO_2 at Stack	% dry	9.10	9.10	9.10
Avg. Stack Temp.	°F	96	98	97
Stack Flowrate	dscfm	33,082	34,380	33,731
Isokinetics	%	99	100	100
HF Emission Results --				
Total HF Detected Acid Imp	µg	< 79.3	< 77.6	< 78.5
Total HF Detected Alkaline Imp	µg	< 123.0	< 119.0	< 121.0
Total HF Concentration	ppm	< 0.074	< 0.069	< 0.072
Conc. @ 7% O2	ppm	< 0.083	< 0.077	< 0.080
HF Emission Rate	lb/hr	< 0.008	< 0.007	< 0.008

Appendix XIX-E : Method 26A HF Results for baseline (C-BL) and carbon run #1 #2 (C-1 and C-2) in campaign 2

Run No.		C-BL	C-1	C-2	
Date		19-Oct-24	19-Oct-24	20-Oct-24	
Start Time	Units	08:15	13:55	09:00	
Stop Time		12:30	18:10	13:16	**AVGS**
Sampling Parameters --					
Barometric Pressure	in. Hg	30.24	30.26	30.26	30.25
Volume Metered	dcf	116.898	118.024	113.235	116.052
Volume of Gas Collected	dscf	115.564	116.443	111.395	114.467
Moisture	% v/v	5.8	6.2	5.8	5.9
O_2 at Stack	% dry	8.40	7.80	8.40	8.20
CO_2 at Stack	% dry	9.10	9.90	9.10	9.37
Avg. Stack Temp.	°F	98	100	98	99
Stack Flowrate	dscfm	33,358	33,134	31,743	32,745
Isokinetics	%	99	100	100	100
HF Emission Results --					
Total HF Detected Acid Imp	µg	< 77.7	< 72.9	< 70.4	< 73.7
Total HF Detected Alkaline Imp	µg	< 127.0	< 218.0	< 108.0	< 151.0
Total HF Concentration	ppm	< 0.075	< 0.106	< 0.068	< 0.083
Conc. @ 7% O2	ppm	< 0.083	< 0.112	< 0.075	< 0.090
HF Emission Rate	lb/hr	< 0.008	< 0.011	< 0.007	< 0.008

Appendix XIX-F : Method 26A HF Results for baseline (CS2-BL) and contaminated soil run #2 #3 (CS-R1 and CS-R2) in campaign 2

Run No.		CS2 - BL	CS2-R1	CS2-R2	
Date		22-Oct-24	22-Oct-24	10-Oct-24	
Start Time	Units	10:00	15:05	08:25	
Stop Time		14:15	19:20	12:40	AVGS
Sampling Parameters --					
Barometric Pressure	in. Hg	30.10	30.08	30.12	30.10
Volume Metered	dcf	118.713	121.132	114.735	118.193
Volume of Gas Collected	dscf	115.259	118.882	110.602	114.914
Moisture	% v/v	7.2	6.9	6.2	6.8
O_2 at Stack	% dry	8.40	7.80	8.40	8.20
CO_2 at Stack	% dry	9.10	9.90	9.10	9.37
Avg. Stack Temp.	°F	104	103	100	102
Stack Flowrate	dscfm	32,882	33,680	31,459	32,674
Isokinetics	%	100	101	100	101
HF Emission Results --					
Total HF Detected Acid Imp	µg	< 82.6	< 82.3	< 72.2	< 79.0
Total HF Detected Alkaline Imp	µg	< 121.0	< 106.0	< 108.0	< 111.7
Total HF Concentration	ppm	< 0.075	< 0.067	< 0.069	< 0.070
Conc. @ 7% O2	ppm	< 0.083	< 0.071	< 0.077	< 0.077
HF Emission Rate	lb/hr	< 0.008	< 0.007	< 0.007	< 0.007

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City Government

City of Edmonds update: Resolution of wastewater treatment plant operational issues ongoing

May 7, 2026

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The Edmonds Wastewater Treatment Plant

As questions continue to swirl over the operational issues affecting the Edmonds Wastewater Treatment Plant, the City of Edmonds issued a statement Thursday regarding recent efforts to address the issues that have blocked the plant's carbon recovery system from performing as originally promised.

Edmonds embarked on the carbon recovery project in 2018, aiming to replace the plant's aging incinerator with a more environmentally sustainable gasification system designed to produce biochar, a potentially marketable byproduct. The technology, developed by EcoRemedy, promised a greener option to disposing of the city's solid wastes.

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Amaresco served as the general contractor for the EcoRemedy installation under a guaranteed-performance contract managed by the Washington State Department of Enterprise Services (DES). Under the contract term, Ameresco guaranteed that the work would be completed, finished on time and that the system would function as intended. Construction began in 2021.

However, the project has been marred by significant delays and technical failures since construction began.

According to City documents, the project cost escalated from $26 million to $31 million between 2020 and 2023. Commissioning efforts in 2023 and 2024 were hindered by numerous issues, including clogged conveyance systems and dryers, equipment breakage, smoke plumes exiting the facility, and consistent failure to achieve steady operations.

While these issues persisted, the city has been forced to haul wastewater solids to a landfill in eastern Oregon. As of late 2025, the total cost for this has been more than $5 million. However, under the Edmonds Regional Wastewater Treatment Plant agreement, the City of Edmonds pays approximately 51% of these costs, while its partners (City of Mountlake Terrace, Olympic View Water and Sewer District and Shoreline/Ronald Sewer District) split the remaining 49%.

In the most recent effort to address the plant's operational issues, on April 14 the Edmonds City Council approved a $415,000 contract with Jacobs Engineering, an engineering consultant, to assist the City with operating the technology. While Ecoremedy and Ameresco remain responsible for the actual commissioning and construction of the system, Jacobs will serve as an independent consultant to ensure the City can operate the facility once substantial completion is reached.

Funding for the contract comes from the City's wastewater utility fund.

Here is the full statement from City of Edmonds on the current status of the efforts to address the wastewater issues:

"The City Council originally approved Ameresco's Energy Services Proposal for Phase 6 of the wastewater treatment plant upgrades based on two key understandings: first, that the aging incinerator needed to be replaced, and second, that the new gasification system would provide long-term economic and environmental benefits for the City and its ratepayers.

Although the project has experienced significant delays, the City has remained committed to the project and to holding Ameresco accountable for delivering the solids handling facility that was promised.

At the same time, the City has been clear that construction progress has not met expectations. Following an early 2025 attempt to operate the gasifier, a number of operational issues became apparent. Earlier this year, the State, acting as the City's contract administrator, involved Ameresco's bonding company to help ensure contractors returned to the site to continue work toward making the system operational.

As progress on the plant was approaching a critical trial run, and with Ameresco's primary subcontractor preparing to move to another job site, the Council made the difficult decision to bring in independent experts with the experience needed to give the project the best possible opportunity for long-term success. The Jacobs Engineering contract gives the City the best chance of achieving the project's original goals.

At the end of April 2026, Ameresco and its subcontractor conducted a multi-day trial run of the gasifier. Jacobs Engineering is still evaluating the results. While some elements of the test appeared encouraging, other issues remain a concern and require further review.

Throughout this process, the City's focus has remained consistent: to ensure Edmonds either receives the facility it was promised or is compensated to the fullest extent allowed under the law.

The City's ability to comment further is limited given the status of the project and possible disputes that will require resolution. The City commits to being transparent to the extent possible without jeopardizing the City's legal rights."

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WASTEDIVE

Aries Clean Technologies looks for fresh start with funding infusion

The company's Linden, New Jersey, plant was idled last year due to mechanical challenges. But with new capital from its lead investor, Aries is plotting a restart and second facility in Maine.

Published May 21, 2026



Jacob Wallace
Senior Reporter



Aries Clean Technologies began construction of its first biosolids gasification plant in Linden, New Jersey, in 2019 and began operations in 2024. The plant suffered from intermittent shutdowns, forcing Aries to pursue a redesign.
Permission granted by Aries Clean Technologies

Aries Clean Technologies is looking for a fresh start thanks to a new infusion of capital.

Last month, lead investor Spring Lane Capital announced it would purchase two bond series issued by Aries. The announcement gave Aries sufficient runway to resume payments on two newer bond series and move ahead with a restart of the company's first facility.

Aries, which built its first biosolids gasification facility in Linden, New Jersey and plans to build a second in Maine, has gone through a transition since last year. It idled its only plant in November after failing to keep it operating consistently since it first began operations in 2024.

And last month, former CEO Jon Cozens transitioned to a role as strategic advisor, with senior vice president of operations Kari Mueller stepping in to take the top position. She now plans to lead the company as it moves toward construction on its new plant in Sanford, Maine.

"These facilities take a while to build, and you have to be very, very attention-to-detail focused," Mueller said. "This whole job is to

execute."

Lessons from New Jersey

Aries began construction on its Linden plant in 2019. The project was designed to process 130,000 tons of biosolids annually from the region. It was designed to run with two biosolids dryers and a gasification system, producing biochar as an output.

The system began operating five years later, but the company repeatedly ran into issues with equipment that needed to come offline, according to executives. The trouble centered mostly on the facility's dryers — if one unit needed to come offline for maintenance, the entire facility had to shut down, said Jason Dana, Aries' vice president of technology.

He said the Linden plant worked well as a demonstration of Aries' process. But moving forward, the company plans to install a third dryer at Linden and all future facilities, plus intermediate storage after the biosolids drying process to ensure redundancy and minimize downtime.

"When it came to commercial operation and profitability, it just wasn't where it needed to be," Dana said. "The dryer is the primary area that we're looking to improve upon."

The company also faced local pushback in 2022 when it attempted to build a second facility in Newark. It eventually abandoned those plans, which was celebrated by local advocates who were fearful of added air emissions from the facility and trucks bringing in feedstock.

Aries executives said the decision to move on from that site was motivated mostly by size constraints. Once the company realized it needed at least three dryers to run its plants efficiently, the site it had been looking at in Newark no longer seemed big enough. Aries explored another site in Taunton, Massachusetts, but later abandoned it for similar reasons.

Second facility in Maine

Aries landed on Sanford as the best location for the second facility for multiple reasons, according to company executives. It was big enough to accommodate the new expanded facility design, but it also served a state looking for solutions for its biosolids.

Maine has some of the most restrictive biosolids disposal rules in the nation. The state banned land application of the material in 2022 after concerns grew about contamination from per- and polyfluoroalkyl substances on farmland where biosolids were used as fertilizer. That led to significant increases in disposal costs as

more biosolids were routed to landfills both in the state and in other states and Canada.

Mark Lyons, Aries' senior director of business development, said Sanford currently sends its biosolids up to 150 miles away. While the company has faced tough questions from nearby residents, community leaders have been broadly supportive of Aries' project, Lyons said. He also noted the proposed site has several advantages for Aries, including underlying water, sewer and power infrastructure and good truck access.

"The city is aware of the biosolids disposal problem. They're very encouraged by our PFAS destruction capabilities, and they're ... looking for a new technology that can safely manage biosolids, reduce volume, create value-added products, and destroy PFAS," Lyons said.

Aries hopes to bring the site online by the end of 2028, though it still has permitting hurdles to get through. Mueller said the company began the process two years ago, before the issues at Linden became apparent. But it's always been the company's goal to expand, and it now sees a clear path to move forward.

"We expect Linden to come online shortly after Sanford. We're still working on that schedule with our engineering to understand it,"

Mueller said. “Did we set out to do it this way? No. Has it worked out to be this way? Yes.”

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Critical Review

Critical Review of Thermal Decomposition of Per- and Polyfluoroalkyl Substances: Mechanisms and Implications for Thermal Treatment Processes

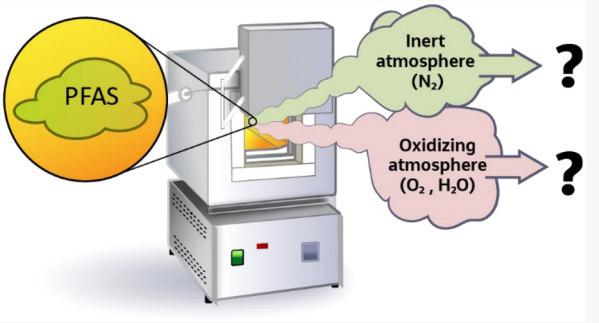
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ABSTRACT: Per- and polyfluoroalkyl substances (PFASs) are fluorinated organic chemicals that are concerning due to their environmental persistence and adverse human and ecological effects. Remediation of environmental PFAS contamination and their presence in consumer products have led to the production of solid and liquid waste streams containing high concentrations of PFASs, which require efficient and cost-effective treatment solutions. PFASs are challenging to defluorinate by conventional and advanced destructive treatment processes, and physical separation processes produce waste streams (e.g., membrane concentrate, spent activated carbon) requiring further post-treatment. Incineration and other thermal treatment processes are widely available, but their use in managing PFAS-containing wastes remains poorly understood. Under specific operating conditions, thermal treatment is expected to mineralize PFASs, but the degradation mechanisms and pathways are unknown. In this review, we critically evaluate the thermal decomposition mechanisms, pathways, and byproducts of PFASs that are crucial to the design and operation of thermal treatment processes. We highlight the analytical capabilities and challenges and identify research gaps which limit the current understanding of safely applying thermal treatment to destroy PFASs as a viable end-of-life treatment process.



KEYWORDS: *Thermal treatment, pyrolysis, combustion, incineration, PFAS*

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1. INTRODUCTION

Per- and polyfluoroalkyl substances (PFASs) are synthetic organic chemicals containing a completely or partially fluorinated alkyl chain. Fluorine is strongly electronegative, causing a short bond length between the C and F. While these structures are desirable for the numerous applications of PFASs, C−F bonds are highly stable and thus poorly susceptible to degradation by hydrolysis, oxidation, photolysis, and biological processes.[1]

The unique chemical structures of PFASs make them naturally hydrophobic, oleophobic, and thermodynamically stable. PFASs have been utilized for over 70 years to produce consumer products with water, stain, and chemical repellence, and/or resistance to thermal degradation. PFASs have been used in food packaging, cookware, textiles and carpets, cosmetics, ski wax, building and automotive materials (e.g., wiring, piping, sealants, adhesives, paints, roofing), and mist-suppression in chrome-plating factories.[2−6] They have also been used in aqueous film-forming foams (AFFFs), which represent a significant source of environmental contamination stemming from military and nonmilitary fire training and hydrocarbon fire suppression.[7−13] Approximately 2000 PFASs have been identified in the global market for intentional use, and at least 6000 CAS-indexed PFASs exist with more than 5000 having defined structures.[14] As a result, PFASs are ubiquitous in the environment,[15,16] and they are detectable in wildlife and humans.[17−19] The likelihood of exposure in combination with the potential for adverse ecological and health effects[20−25] has caused tremendous concern.

In the United States (U.S.), the regulatory landscape for PFASs at the state and federal levels is rapidly changing. In 2016, the U.S. Environmental Protection Agency (US EPA) announced a drinking water health advisory limit of 70 ng/L for the sum of perfluorooctanoic acid (PFOA) and perfluorooctanesulfonate (PFOS),[26,27] and some U.S. states have adopted more stringent guidance.[28] As of 2021, no additional federal regulations have been set, but drinking water primary

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standards under the the Safe Drinking Water Act and waste disposal rules under the Resource Conservation and Recovery Act are expected.[29] While replacements for PFOS and PFOA have been developed with increased renal clearance rates and/or biodegradability, for example, shorter-chain perfluorocarboxylic acids (PFCAs), perfluoroether carboxylic acids, and perfluorosulfonic acids (PFSAs),[30,31] recent toxicological studies have shown they are potentially harmful.[32] For example, in 2021, the US EPA established a chronic oral reference dose for one of the most common PFOA replacements, hexafluoropropylene oxide (i.e., GenX, 3 ng/kg-day), which is ∼10 times lower than for PFOA and PFOS and 100 times lower than for perfluorobutanesulfonate (PFBS).[33]

Various physical and chemical treatment processes have been explored to remove PFASs from environmental media.[34] Physical treatment processes such as adsorption,[35−37] ion exchange,[37−39] and membrane filtration[16,40] are effective for contaminated water or aqueous waste streams (e.g., soil washing[41]), but they produce solid or liquid wastes containing high concentrations of PFASs that still require defluorination to alleviate long-term environmental impacts. While conventional and advanced chemical treatment methods such as oxidation (e.g., ozone, UV/H_2O_2) are largely ineffective for destruction of PFASs, a number of advanced methods are being developed including electrochemical oxidation, sonolysis, bioremediation, advanced reduction, and nonthermal plasma.[42−48] In general, these latter technologies are unproven at scale and/or poorly defluorinate PFASs in time frames that are useful for full-scale application. There is therefore a need for a proven and scalable destruction method for waste streams which contain PFASs.

Thermal treatment processes (e.g., incineration) offer a common, scalable, and widely available approach for managing contaminated solids, liquids, or gases,[49] and many incineration facilities are already knowingly or unknowingly treating PFASs (e.g., consumer products, activated carbon regeneration). Thermal treatment of PFASs has been demonstrated at scale by several commercial interests and is thought to result in mineralization.[50] While thermal treatment processes are generally energy intensive and costly, the technology has already been deployed, and together, these advantages place them as a key solution for managing PFAS-containing wastes.

Many unknowns remain about the thermal behavior of PFASs, despite a growing body of research. In this review, we critically evaluate and summarize the volatility, decomposition mechanisms and pathways, and products of the thermal degradation of PFASs. We further discuss the implications of these mechanisms on thermal degradation of PFASs for industrial-scale thermal treatment systems and highlight analytical methods and needs.

2. SURFACE INTERACTIONS AND VOLATILITY

Thermal decomposition of PFASs sorbed to materials can be initiated via two pathways. First, as the temperature of a solid increases, some PFASs volatilize and undergo gas-phase reactions. Second, some less volatile PFASs undergo thermal degradation in/on the solids. Thus, the extent and type of thermal degradation byproducts are likely to differ based upon the parent PFAS compounds volatility. Aqueous PFASs that are deprotonated or adsorbed to solids via electrostatic or ion exchange interactions have very low propensity to enter the gas phase until the temperature rises above 100 °C, when water evaporates and interactions with the solid weaken. For example, during regeneration of a PFAS containing media, PFOA did not volatilize below 90 °C, then gradually decreased in surface concentration as the temperature increased from 90 to 400 °C.[51]

Vapor pressure describes the equilibrium between a pure liquid and the gas phase in a closed system. This is reflective of the volatility of a pure compound and is useful in understanding thermal treatment processes. In Figure 1a, we show the vapor pressure for different groups of PFASs as a function of chain length. Note that these are computational estimates by the Antoine equation, rather than experimentally derived values. Two other semiempirical models are shown in Figure S1 for comparison, and a comparison of modeled and experimentally measured vapor pressures of fluorotelomer alcohols (FTOHs) is listed in Table S1. Volatility of various structures followed the order of perfluorocarbons (PFCs) > fluorotelomer carboxylic acids (FTCAs) > FTOHs ≈ PFCAs > PFSAs > fluorotelomer sulfonates (FTSAs). Across these same functional groups, vapor pressure increased with decreasing chain length. These modeled trends are further supported by the experimentally derived data shown in Figure 1b and an additional study which reported boiling points of C4−C8 PFCAs and PFSAs increasing from 121 to 192 °C and 210 to 249 °C, respectively.[35] Lower sublimation

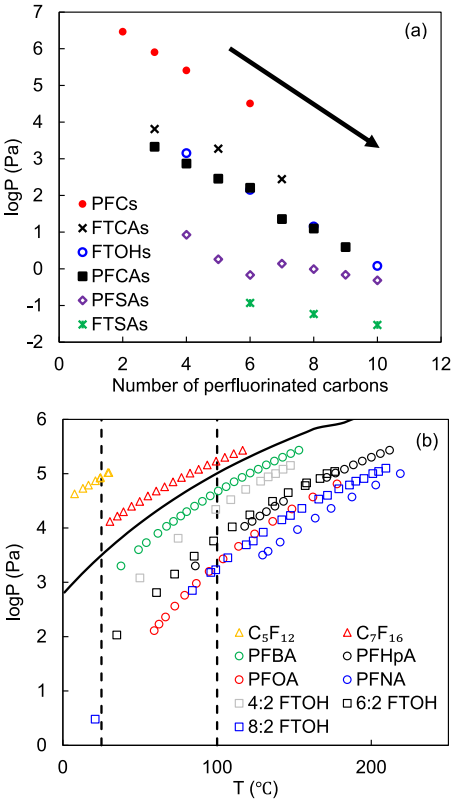


Figure 1. (a) Perfluorocarbon (PFC), fluorotelomer carboxylic acid (FTCA), fluorotelomer alcohol (FTOH), perfluorinated carboxylic acid (PFCA), perfluorinated sulfonic acid (PFSA), and fluorotelomer sulfonate (FTSA) vapor pressures (P) at 25 °C from the semiempirical Antoine method in EPISuite.[57] Arrow indicates a trend of decreasing vapor pressure for longer-chain PFAS. (b) Experimentally determined vapor pressure of specific PFASs over a range of temperatures adapted from Ding and Peijnenburg.[53] Compounds with the same number of fluorinated carbons are shown in the same color, and compounds with similar structures or functional groups have the same marker. The black line shows water vapor pressure. Additional abbreviations are perfluorobutanoic acid (PFBA), perfluoroheptanoic acid (PFHpA), perfluorooctanoic acid (PFOA), and perfluorononanoic acid (PFNA).

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vapor pressures have been reported for an increasing number of fluorinated carbons,[52] again providing evidence that shorter-chain PFASs are more volatile.

Experimental vapor pressures versus temperature for a limited set of PFASs[53] are shown in Figure 1b. PFCs were more volatile than water, indicating that PFCs are likely to volatilize before water. At temperatures lower than the boiling point of water, some liquid phase water may remain, and this is particularly meaningful for perfluoroalkyl acids (PFAAs). However, very few experimentally determined Henry's Law constants are available in published literature which presents a substantial weakness in the understanding of PFASs' volatilities.[53,54] Further, some PFASs decompose at temperatures lower than the temperature required for vaporization.[55]

However, considerations of pure-phase PFASs, PFASs on surfaces, or even single-solute solutions are probably over-simplifications. The presence of cations in solution can form salts with PFASs that influence the ability to predict volatilization. Also, the composition and heterogeneity of surfaces on which PFASs exist will likely influence the propensity of a PFAS to volatilize from a surface. For example, research on soil demonstrated that volatilization was minimal for both PFCAs and PFSAs at 220 °C over 14 days,[56] despite PFCAs boiling points suggesting they would volatilize at temperatures below 200 °C. The mechanisms and roles of surface hydrophobicity on temperatures at which PFASs volatilize from surfaces remain poorly understood. However, as temperatures increase to far above predicted temperatures for volatilization, the dependence of PFAS volatilization on the PFAS structure decreases.

3. DECOMPOSITION INITIATION, PRODUCTS, MECHANISMS, AND KINETICS

Reported primary thermolytic mechanisms of PFASs include cleavage of intramolecular bonds through transition states (TS), direct homolytic cleavage, radical reactions, hydrolysis, and oxidation. Each mechanism and pathway produce some distinct, but also some overlapping, decomposition products. Temperatures at which PFASs volatilize from surfaces may be higher than temperatures at which thermal decomposition initiates, making it difficult to differentiate between these two different mechanisms.[58,59] While acknowledging the potentially synergistic role of surfaces (see Section 4.2 for more discussion), the temperatures required to initiate PFAS decomposition appear to be related to both the number of perfluorinated carbons and functional groups. Pyrolysis (absence of oxygen) and combustion (presence of oxygen) are influenced by functional groups because pyrolysis is unimolecular and initiates with the decomposition of the least stable group, and combustion is initiated by reactions between O_2 and the most reactive group. A summary of experimentally derived thermal decomposition products of PFASs are provided in Table S2. Various perfluorinated gases and solids such as coke and polymeric materials may be formed during pyrolysis (in dry inert conditions) and may persist even at temperatures above 1000 °C.[60−65] Generally, PFASs are pyrolyzed into other forms of organofluorine, including volatile organofluorine (VOF), especially at temperatures less than 500 °C. The lack of closed mass balances (>90% F accounted for) for nearly all experiments strongly implies that some fluorinated products may escape thermal and post-treatment processes, elude detection, and be released to the environment. Additionally, nearly all studies of thermal decomposition of PFASs are conducted under pyrolytic conditions, and therefore, our discussion primarily focuses on pyrolysis, with combustion discussed where data are available.

3.1. Temperatures Required to Initiate Decomposition. Temperatures required to initiate decomposition in terms of structure decrease as follows: perfluorocarbons (PFCs) > perfluoroacyl fluorides > PFSAs > PFCAs > perfluoroether carboxylic acids.[66] The order of the decomposition rates of PFCs (C_xF_y) between 1000 °C and 1250 °C was CF_4 < C_2F_6 < cyclo-C_5F_{10} < C_3F_8 < n-C_4F_{10} < n-C_5F_{12},[61] indicating that the temperature required to decompose PFCs decreases with an increasing number of carbons, although isothermal rates (i.e., rates measured at a fixed temperature) may not perfectly represent overall thermal stability. Perfluorohexane (n-C_6F_{14}) did not decompose at temperatures less than 400 °C[67] nor in 450 °C air even in the presence of a palladium catalyst.[68] Pure 2H-heptafluoropropane (HFP, C_3HF_7) did not decompose at temperatures less than 640 °C,[65] and perfluoropentane (n-C_5F_{12}) was not decomposed below 840 °C.[60]

Xiao et al.[66] studied thermal stability of PFASs sorbed to GAC and found that the temperatures needed to degrade PFCAs increased with an increasing number of perfluorinated carbons. The decomposition of PFSAs generally requires higher temperatures (450 °C) than PFCAs (200 °C), and perfluoroether carboxylic acids are more readily decomposed than PFCAs with the same number of fluorinated carbons, demonstrating that ether bonds weaken the molecule.[66] For acids, the counterion may also impact the thermal stability. For example, Lines and Sutcliffe[69] reported that the thermal stability of PFOA salts increased with the following counterions: ammonium < cesium < potassium < silver < lead < sodium < calcium = barium < lithium.

The temperatures required to decompose fluoropolymers are related to monomeric structure, extent of fluorination, and physiochemical conditions (e.g., reactor material, atmosphere).[70−72] Baker and Kasprzak found that $-CF_3$ branching leads to reduced Teflon thermal stability.[73] Overall, polymers of perfluoroethylene are more stable than perfluorophenylene.[74] Fully fluorinated polymers made up of tetrafluoroethylene and/or hexafluoropropylene are the most thermally stable.[71,74] Also, increasing molecular weight tends to reduce thermal stability,[29,30] and some copolymers containing trifluoronitrosomethane (CF_3NO) or (OCF_2) monomers are poorly stable, decomposing at temperatures as low as 200 °C.[71,72]

Reactor materials and reaction conditions (e.g., presence of O_2) also influence the thermal stability of PFASs. For example, heptafluorobutyric anhydride decomposed at as low as 150 °C in a reactor containing alumina but was stable up to 850 °C in a tantalum or silver reactor.[75] Several fluoropolymers such as fluorosilicone B and polyvinylidene fluoride decomposed more rapidly in the presence of O_2 than in a vacuum/inert atmosphere,[71,72] although others have demonstrated that vacuum/inert vs oxidizing atmosphere causes minimal differences in decomposition rates.[70] Even some copolymers (e.g., CF_3NO/C_2F_3H) have been shown to decompose slower in an oxidizing atmosphere than an inert one.[71,72]

Overall, the thermal decomposition initiating temperatures are determined by the least stable part of a molecule, which is typically a nonfluorinated functional group. PFCs, which contain no such functional groups, are thus the most stable, and their stability decreases with increasing perfluoroalkyl chain length. This indicates that at a specific temperature the loss of a longer-chain PFAS should not be interpreted as mineralization,

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as it is possible or likely that it was decomposed to a shorter-chain, more thermally stable, PFC or PFAS.

3.2. Decomposition Products. *3.2.1. Perfluorocarboxylic Acids.* Products of the pyrolysis of PFCA salts include perfluoroacyl fluorides and anhydrides, corresponding acids, and even dimers of the perfluoroalkyl chains (Figure 2).[76,77]

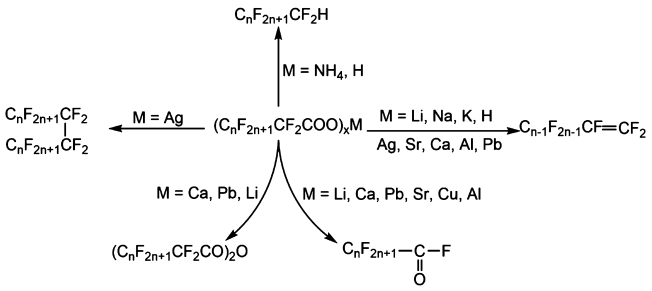


Figure 2. Pathways and products of PFCAs and their salts at temperatures of 200 to 305 °C during pyrolysis. Produced from results presented by LaZerte et al.[76]

Longer-chain PFCA pyrolysis products include shorter-chain PFCAs such as 1*H*-perfluoroalkanes, perfluoro-1-alkenes, and unidentified VOF, CO, CO_2, and HF (Table S2). Perfluoro-1-alkenes are a dominant product of metal salts of PFCAs (except trifluoroacetic acid [TFA] and its salt), and the yield varies with the species of counterion. TFA salts form trifluoroacetyl fluoride and trifluoroacetic anhydride as primary products.[76,78] Sodium or potassium salts of longer-chain PFCAs (C > 3) consistently had the greatest yields of the primary product, perfluoro-1-alkenes, and the yield increased with shortening carbon chain length.[76] Ammonium salts of PFCAs yield neither alkenes nor acetyl-containing compounds but instead almost quantitatively forms 1*H*-perfluoroalkanes.[76,79] Notably, the formation of dimers of the perfluoroalkyl chains was observed for the silver salts of PFCAs. Such detailed observations of varying counterions are not available for PFASs outside of PFCAs during pyrolysis, but given the significant differences between decomposition products and the likelihood of a diversity of counterions present in environmental samples, additional research is warranted, particularly for combustion of soils where PFCAs are likely to be present as salts.[80]

3.2.2. Perfluorosulfonic Acids. PFSAs appear to be thermally defluorinated to a greater extent than PFCAs (i.e., producing stoichiometrically more mineralized fluorine), although the number of investigations available to draw this conclusion is limited.[81−83] Quantification of organic intermediates are rarely presented in these studies, although mass fragments (CF_3^+, $C_2F_3^+$, $C_3F_3^+$, $C_2F_4^+$, $C_2F_5^+$, $C_3F_5^+$, $C_4F_7^+$, $C_5F_9^+$) of some products were observed by GC-MS after thermal decomposition in the presence of lime.[83] The presence of these mass fragments strongly suggests that VOF is a product of thermolysis of PFSAs.[58] Recently, some fluorinated olefins have been identified as thermal decomposition products of PFOS.[84]

3.2.3. Perfluorocarbons. Pyrolysis products of perfluoroalkanes and perfluoroalkenes are similar and include C_2F_6, C_2F_4, C_3F_8, C_3F_6, polymeric compounds, and solid-phase charred residues (Table S2). These products are thought to form as a result of homolysis of C−C bonds (each C retains one electron that formed the bond), forming radicals which in many cases yield stable PFASs such as polymers and cyclo-compounds.[60,64,65] Formation of the products has been shown to be temperature dependent. The thermal decomposition of C_2F_4 was studied from 300 to 800 °C, and the primary product was dimerization to cyclo-C_4F_8 at temperatures below 550 °C. *n*-C_3F_6 and *n*-C_4F_8 were generated between 550 and 700 °C, and C_2F_6, perfluoroisobutene (PFIB, $(CF_3)_2C{=}CF_2$), and other nonvolatiles were formed between 700 and 750 °C.[85,86] In another study, the thermal decomposition of cyclo-C_4F_8 formed *n*-C_3F_6 and C_2F_4 as principal products between 360 and 560 °C.[87] Matula[88] examined *n*-C_3F_6 from 550 to 675 °C and found that perfluoro-2-butene ($CF_3CF{=}CFCF_3$) and PFIB were the primary reaction products.

3.2.4. Fluoropolymers. The thermal decomposition products of fluoropolymers include monomers, dimers, trimers, perfluorocarbons, CO_2, and SiF_4 (when quartz is present).[74,89] C_2F_4 was the primary pyrolysis product of polytetrafluoroethylene (PTFE).[73] The subsequent decomposition of C_2F_4 is discussed in Section 3.2.3.[90] The copolymer-fluorinated ethylene propylene (FEP) more readily forms PFIB than PTFE likely because of the inclusion of a −CF-CF_3− monomer along with −CF_2− in FEP. PFIB is relatively stable and highly toxic.[73,91] Zuev et al.[89] investigated the thermal decomposition of acrylic polymers with fluorinated side chains at 450 to 750 °C and found that the key decomposition pathway of the polymer was scission of the primary polymer carbon bonds. The fluorinated alkyl ester side chains were highly stable and transformed into corresponding fluorinated cycloalkanes.

In the oxidizing atmospheres, carbonyl fluoride (COF_2) is a common gaseous product.[72,73] Defluorination occurs to a greater extent in air (∼60%) than in nitrogen (∼17%).[72,73] TFA and longer-chain perfluoro- and/or polychlorofluoro carboxylic acids (C3−C14) were formed during thermolysis of PTFE and Kel-F at temperatures lower than 500 °C in the presence of O_2 and H_2O.[92] Combustion of fluorinated polymers did not tend to produce PFOA when the temperature was above 1000 °C, similar to municipal and/or medical waste incineration.[50,93] This suggests that the risk of releasing well-known PFASs from incinerators when polymers are combusted may be limited, but there is still the potential to release low molecular weight VOF (Table S2).

Figure 3 is a visual representation of the thermochemical stability and defluorination of PFASs for which there are sufficient data. Although some of the regions are approximate because the experimental data are somewhat coarse, the figure demonstrates overall that mineralization tends to occur above 700 °C in oxidizing atmospheres and that at lower temperatures and in inert atmospheres VOF is formed. This has broad implications for incinerators, which are operated with supplemented air at temperatures greater than 700 °C, potentially limiting the release of VOF. Again, there are few studies of combustion of PFASs, and these studies are limited to select groups of compounds. There is a significant need to study additional PFAS decomposition during combustion to inform treatment approaches of potential gas-phase products. PFAS groups that are not included in this figure have no or too limited published data to make broad conclusions. For example, FTOHs are volatile and thus may escape in the gas phase prior to degradation, but there are limited data available regarding temperatures required to achieve thermal decomposition and/or products.[94] Study of significantly more PFASs (e.g., alcohols, ethers) that are expected to be present in environmental samples and waste streams is warranted to understand the byproducts in relation to the thermolysis temperature.

3.3. Mechanisms and Pathways. *3.3.1. HF Elimination via Transition States. 3.3.1.1. Perfluorocarboxylic Acids.*

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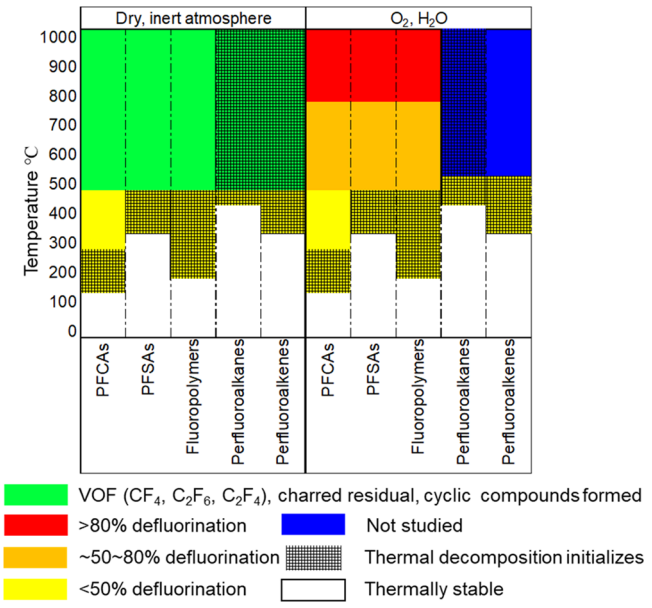


Figure 3. Summary of approximate thermal decomposition in inert and oxidizing atmospheres based on available data provided in Table S2.

Figure 4 illustrates several PFCA decomposition pathways based upon both experimental or computational studies.[95−99] It is worth mentioning that density functional theory calculations are generally carried out at zero Kelvin and that more rigorous ab initio molecular dynamics can simulate thermal effects.[100,101] Pathway 1 depicts the formation of 1*H*-perfluoroalkanes, which begins with HF elimination via a five-membered TS, resulting in the formation of CO_2 and a carbene (C_nF_{2n-1}−:CF). The carbene then reacts with HF to form a 1*H*-perfluoroalkane.[102] 1*H*-Perfluoroalkane formation via pathway 5 has also been proposed.[98] Further, 1*H*-perfluoroalkanes may not be the only products during PFCA pyrolysis. Perfluoro-1-alkenes via a six-membered TS2 may also be possible for long-chain PFCAs (pathway 2), and moderate amounts of perfluoro-1-heptene have been observed during PFOA pyrolysis.[102] Also, through TS2, PFPrA can form perfluoroacetyl fluorides, but this product is specific to PFPrA because it contains only two fluorinated carbons.[66]

Recently, Xiao et al.[66] proposed that thermal decomposition of PFOA on GAC surfaces is initiated by loss of a fluorine atom and −COOH to generate a perfluorocarbon radical (pathway 4), followed by loss of fluorine or CF_2 based on GC-MS identification of the organic fluorine products. There is, however, some debate over the decomposition pathway.[103,104]

Rayne and Forest[77] proposed a terminal perfluoroalkyl carbanion as an intermediate via thermal decarboxylation (pathway 3), yielding 1*H*-perfluoroalkanes by reacting with ambient protons or yielding perfluoroalkenes through loss of a fluorine. However, this pathway is more likely in polar solvents than the gas phase, because the dissociation energy of heterolysis is thought to be greater than homolysis for the same covalent bond in the gas phase.[105]

At least two computational studies of the pyrolysis of PFCAs indicated that direct decarboxylation is not likely to be the main pathway because of the high activation energy.[95,98] The primary decomposition pathway in these studies is HF elimination and the formation of an α-lactone intermediate (M1) via pathway 6, which is not thermally stable and is then degraded to a perfluoroacyl fluoride (M2) and CO at relatively low temperatures (<500 °C).[98] At high temperatures (>1000 °C), M1 tends to be degraded into CO_2 and M3.[98] The formation of M2 is supported by experimental studies of the pyrolysis of the simplest PFCA, TFA (CF_3COOH), which forms COF_2, likely first through the loss of HF and formation of a lactone, followed by loss of CO. This pathway is further supported by the observed

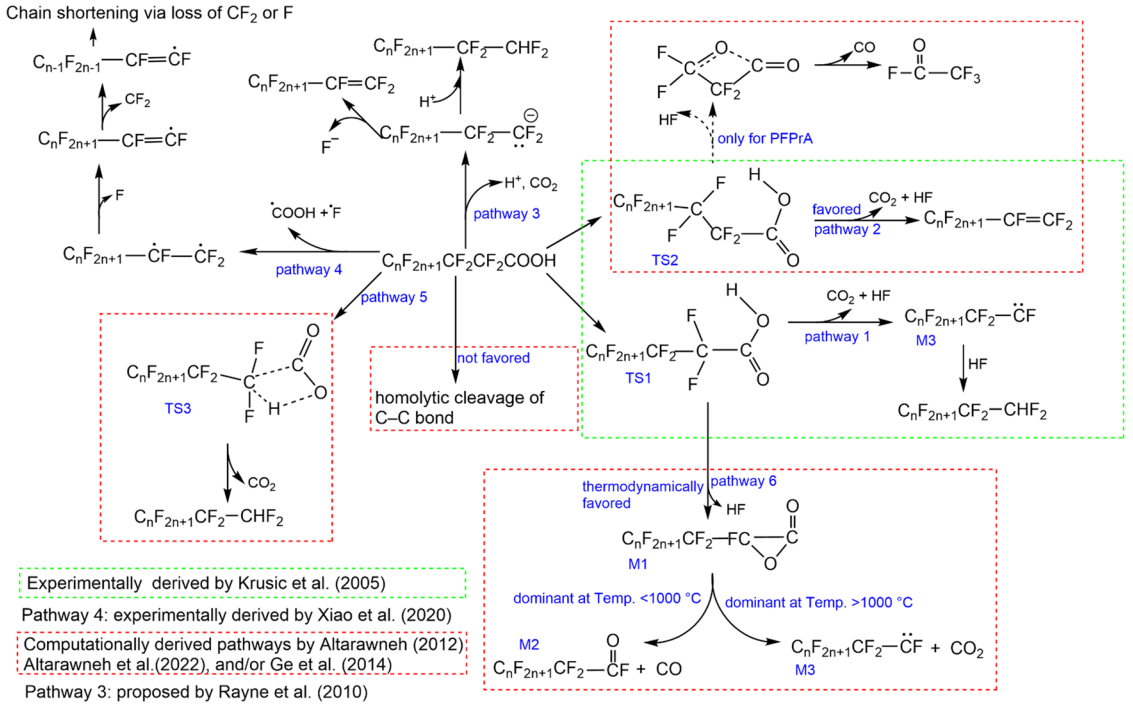


Figure 4. Published experimentally and computationally derived pyrolysis pathways of PFCAs. Dashed boxes indicate the literature source (see bottom left).

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formation of M2 during pyrolysis of PFCA metal salts, both agreeing well with the products in Table S2.

Although M2 has been computationally predicted to be the primary product of PFCAs and has been observed during the thermal decomposition of TFA and PFCA metal salts, M2 was not detected in other experiments of PFCA acids (Table S2). M2 (i.e., CF_3COF) is thermally stable up to ~600 °C (Table S2), and thus, the lack of detection is surprising. The lack of M2 detection may be because either the experimental conditions (e.g., reactor materials) altered the thermal decomposition pathway or because M2 readily reacts with HF and H_2O to form other compounds (Section 3.3.3).

Another poorly understood area of the decomposition of PFCAs is which pathway dominates the formation of the observed intermediates/products (e.g., 1*H*-perfluoroalkanes, perfluoro-1-alkenes). Pathways 1 and 2 support the observed formation of 1*H*-perfluoroalkanes and perfluoro-1-alkenes but fail to explain the traces of short-chained PFBA and perfluoropentanoic acid (PFPeA) present during PFOA pyrolysis.[82] Further, the carbene intermediate in pathway 1 does not explain why water promotes the decomposition of PFOA[77] or how the counterion influences PFCA thermal stability and product formation. Additional products not described well by these pathways have been observed during the pyrolysis of TFA,[106,107] further indicating that the mechanisms may be more complicated than current publications explain.

3.3.1.2. Perfluorosulfonic Acids. For PFSAs, only computationally derived decomposition pathways are available (Figure 5). HF elimination (hydrogen from the sulfonic acid and

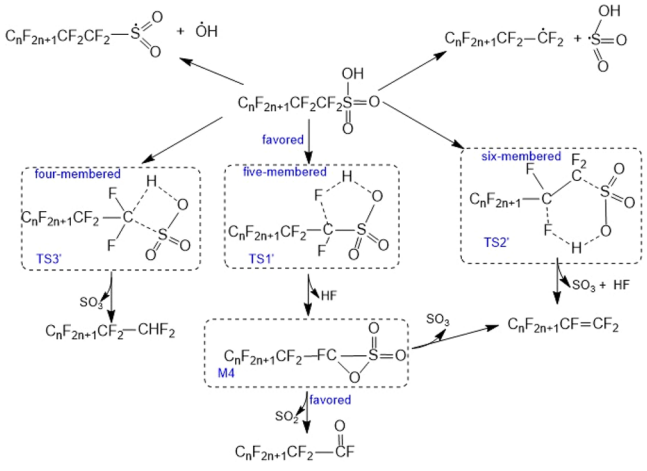


Figure 5. Computationally derived pyrolysis pathways of PFSAs.[96,99]

fluorine from the α carbon) via a five-membered TS1′ is thought to dominate, resulting in formation of an intermediate α-sultone (M4), which is then degraded to a perfluoroacyl fluoride and SO_2 at all studied temperatures.[96,99] Competing pathways include HF elimination leading to the formation of perfluoro-1-ene and SO_3 via six-membered TS2′ and 1*H*-perfluoroalkane formation via TS3′.[96,99]

3.3.1.3. Other Per- and Polyfluoroalkyl Substances. HF elimination is thought to also occur for other PFASs. HF elimination was confirmed experimentally and computationally during the thermal decomposition of 2*H*-heptafluoropropane (R1).[65,108,109] HF elimination was also demonstrated as a pathway for perfluoroalkanol pyrolysis.[110] Thus, HF elimination appears to be a component of multiple pathways of the pyrolysis of PFASs that contain hydrogen in the carbon chain or in a functional group. This is advantageous to incinerators that capture off gases using wet scrubbers, where HF would be captured and retained.

$$CF_3CHFCF_3 \rightarrow CF_3CF = CF_2 + HF \quad \text{(R1)}$$

3.3.2. Direct Cleavage of Intramolecular Bonds. The direct cleavage of intramolecular bonds has been demonstrated to be an initiating mechanism during thermal decomposition of PFASs. During pyrolysis of perfluoropolymers[89,92] and perfluorocarbons,[60,61,64,65,111] intramolecular C−C bond cleavage is dominant in initial reactions. The cleavage proceeds through (1) homolytic cleavage of C−C bonds resulting in the formation of perfluorocarbon radicals[60,61,64] and (2) radical recombination to form relatively thermally stable short-chain VOF or reactions with other matter (e.g., O_2, H_2O, •OH) if available.

One example of the direct cleavage of intermolecular bonds is the decomposition of perfluoroacyl fluorides, which proceeds initially through cleavage of the CF_2−COF bond (R2), leading to a perfluorocarbon radical and •COF.[99] Another example is the cleavage of C−F bonds, which has been observed during thermal decomposition of COF_2 (R3).[112]

$$C_nF_{2n+1}CF_2COF \rightarrow C_nF_{2n+1} - \bullet CF_2 + \bullet COF \quad \text{(R2)}$$

$$COF_2 \rightarrow \bullet COF + \bullet F \quad \text{(R3)}$$

Pyrolysis of perfluorocarbons also proceeds via cleavage of intramolecular bonds and generally yields similar gaseous products independent of the parent structure (Table S2). We summarize the potential pathways for the formation of the most common products (C_2F_6, C_2F_4, C_3F_8, C_3F_6, C_4F_8) with *n*-C_4F_{10} as an example in Figure 6. Additional information related to minor, high activation energy perfluorocarbon radical reactions is available in works by Buravtsev and Kolbanovskii[113] and Yu et al.[64]

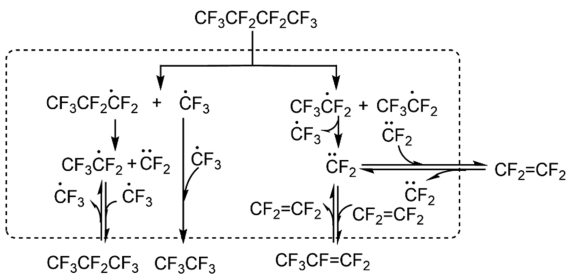


Figure 6. Summarized pyrolysis pathways of perfluorocarbons based on work by Yu et al.[64]

3.3.3. Intermolecular Reactions. 3.3.3.1. Reactions with Radicals and Oxygen. H_2O thermolysis yields •OH and •H at extremely high temperatures (>2000 °C).[114,115] Although the formation of radicals from water thermolysis at typical incinerator temperatures is likely to be low, we discuss their impact on decomposition during thermolysis because they promote decomposition of PFASs by allowing for new decomposition pathways and acting as a source of H for defluorination. In the presence of •OH and •H, abstraction of hydrogen is the dominant reaction pathway for *H*-fluoroalkanes (Figure 7, green box).[116] For sulfonic acids, H abstraction from the sulfonic acid group forms an intermediate M5 which is then decomposed into a corresponding perfluorocarbon radical and

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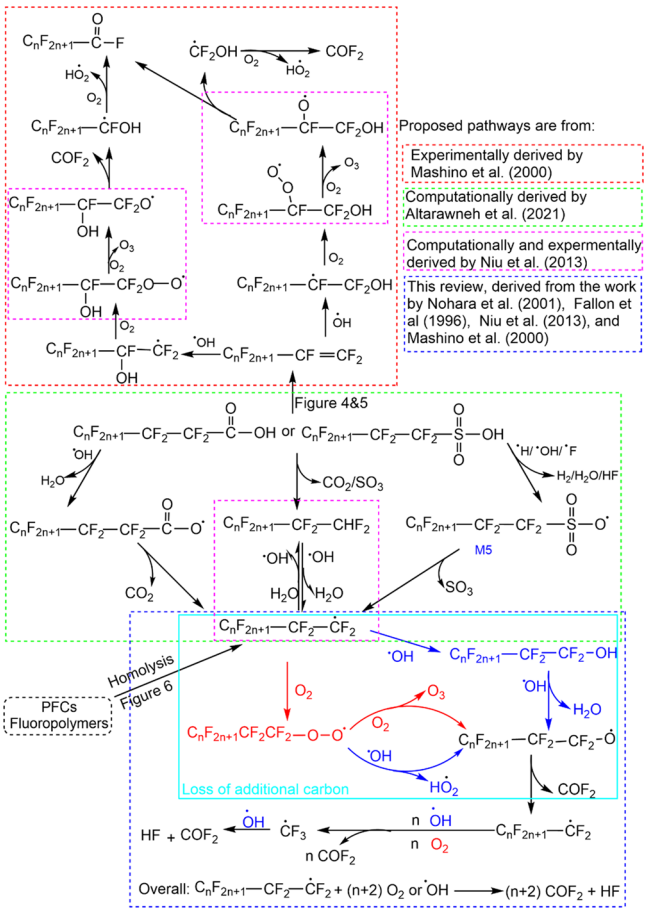


Figure 7. Reactions with •OH and O_2 with PFASs and PFAS decomposition products.[99,117−121] Pathways are generalized regardless of carbon chain length. Dashed boxes indicate literature source (see top right).

SO_3 (Figure 7, green box, right).[99] Similarly, •OH reacts with PFCAs to form a perfluorocarbon radical and CO_2 (Figure 7, green box, left), which has been experimentally observed during drinking water advanced oxidation processes.[117] Although fluorine abstraction is a possible pathway, it is not thermodynamically favored and therefore not illustrated.[116] •OH also attacks carbon−carbon double bonds, initiating the oxidation of perfluoroalkenes in the presence of O_2 to produce corresponding perfluoroacyl fluorides and COF_2 (Figure 7, red box).[118]

On the basis of the mechanisms depicted in the magenta and blue boxes of Figure 7, combined with the appearance of the corresponding expected products in Table S2, we find that the thermal defluorination of PFASs is promoted in the presence of O_2 and H_2O because they participate in reactions with perfluorocarbon radicals. O_2 reacts with perfluorocarbon radicals and the perfluorocarbon chain, and the carbon chain is shortened stepwise through release of COF_2. This has been demonstrated experimentally by the oxidation of PFOA by •OH in an electrochemical system[119] and a photochemical reaction system.[120] The observed formation of COF_2 during thermal decomposition of fluoropolymers provides additional experimental support for this pathway.[72,73]

3.3.3.2. Hydropyrolysis. Water promotes the thermal decomposition of PFASs by hydrolyzing the common intermediate products, perfluoroacyl fluorides (Figure 4, pathway 6; Figure 5, favored pathway; and Figure 7, red box). Perfluoroacyl fluorides are highly thermally stable but react with water to form corresponding PFCAs which are then decomposed to shorter-chain perfluoroacyl fluorides by HF elimination.[122,123] Alternating HF elimination and hydrolysis then occurs, forming shorter and shorter-chain PFCAs (Figure 8).[99,123] An example of such is the formation of PFBA and

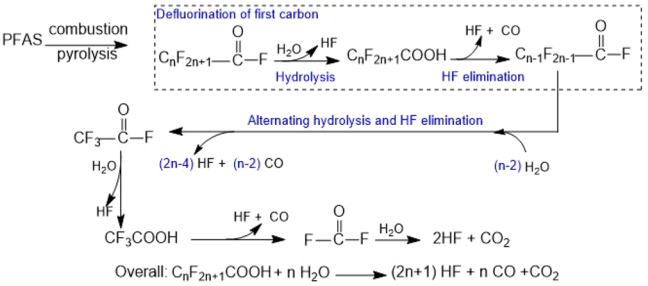


Figure 8. Mineralization of PFASs by alternating hydrolysis and HF elimination during hydropyrolysis.[99,122,123] Combustion and pyrolysis of PFASs to perfluoroacyl fluorides are described in Figures 4, 5, and 7.

PFPeA during PFOA/PFOS hydropyrolysis.[82] The final decomposition product, COF_2, readily reacts with water to produce CO_2 and HF.[124] These reactions have been shown to result in mineralization of F in seconds at temperature as low as 780 °C,[125] but the kinetics at varying temperatures have not been formally measured to our knowledge and are needed to inform hydropyrolytic thermal treatment processes. In the absence of water, the only mechanism which is known to decompose perfluoroacyl fluorides is direct cleavage of intramolecular bonds (Section 3.3.2, R2), resulting in relatively poor defluorination.

At alkaline pH, hydrothermal decomposition of PFOS occurs via OH^- attack of the C alpha to the $-SO_3$, forming a perfluorinated alcohol and SO_3^{2-} (R4).[110,126] The perfluorinated alcohol undergoes HF elimination to form a perfluoroacyl fluoride,[110] which is then hydrolyzed to a corresponding PFCA. The PFCA is ultimately mineralized by decarboxylation and releases F^- (see Figure 8).

$$C_nF_{2n+1} - CF_2 - SO_3^- + OH^- \rightarrow C_nF_{2n+1} - CF_2 - OH + SO_3^{2-} \quad \text{(R4)}$$

3.3.3.3. Reactions with HF. Perfluoroacyl fluorides react with water to form a corresponding PFCA and HF, and this reaction is reversible (R5).[106,127] This mechanism likely competes with those shown in Figure 8, where alternating HF elimination and hydrolysis result in PFAS chain shortening. As an example, perfluoroacyl fluorides have been shown to be formed during pyrolysis of TFA.[106,107,127]

Perfluoroacyl fluorides also react with HF to form perfluoroalkanols by R6.[99] HF may also reversibly react with perfluoroalkenes to form *H*−perfluoroalkanes (R7).[97] Finally, it has been demonstrated that addition of lime to PFAS-containing sludge sequesters F as CaF_2 at temperatures between 300 and 600 °C, but F is released back to the gas phase as HF or SiF_4 at higher temperatures (700−900 °C).[83] These products and decomposition pathways demonstrate that the mechanisms are complex and in many cases lead to the formation of new PFASs which are not part of routine monitoring analyses.

$$C_nF_{2n+1} - COOH + HF \rightleftharpoons C_nF_{2n+1} - COF + H_2O \quad \text{(R5)}$$

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$$C_nF_{2n+1} - COF + HF \rightarrow C_nF_{2n+1} - CF_2 - OH \quad (R6)$$

$$C_nF_{2n+1} - CF = CF_2 + HF \rightleftharpoons C_nF_{2n+1} - CF_2 - CF_2H \quad (R7)$$

3.3.3.4. Surface Catalyzed Reactions. Some oxides (e.g., SiO_2 and Al_2O_3) used as thermolysis reactor materials can participate in the reactions of PFAS thermal decomposition. For example, during pyrolysis of cyclo-C_4F_8 in an inert atmosphere, Butler[87] demonstrated that CO, CO_2, SiF_4, and black solids were produced, and they also noted that the Pyrex reactor was etched. Notably, no oxygen is present in the parent compound, and CO_2 is not a known product of the direct cleavage of fluorocarbon intramolecular bonds (Figure 6). Thus, Butler postulated that R9 and R10 are responsible for the formation of the observed CO, CO_2, and SiF_4. Similarly, CO_2 was formed during the pyrolysis of *n*-C_4F_{10} in an alumina reactor, and once the surface alumina was completely reacted, the formation of CO_2 was significantly reduced.[64]

$$2SiO_2 + \text{cyclo-}C_4F_8 \rightarrow 2SiF_4 + 4CO \quad (R9)$$

$$2SiO_2 + \text{cyclo-}C_4F_8 \rightarrow 2SiF_4 + 2CO_2 + 2C \quad (R10)$$

However, the intermediates during these reactions and reactions of nonfluorocarbon PFASs are not completely resolved. A possible mechanism for fluorocarbons is that some fluoroalkane radicals are formed by direct cleavage of intramolecular bonds (e.g., $\bullet CF_2$, $\bullet CF_3$, and $\bullet C_2F_5$, see Section 3.3.2) which subsequently react with surface oxides.[64,128] This is supported by Ng et al.[129] who found that hydroxyl groups on an Al_2O_3 surface were necessary to degrade a model perfluoroether [$(C_2F_5)_2O$], suggesting that surface hydroxyl groups play a key role in the catalyzed oxidation of PFASs, particularly in inert atmospheres where decomposition of PFASs would typically terminate at the corresponding perfluoroacyl fluoride. This is relevant to incineration of contaminated soils, where surface hydroxyl groups are in abundance but there is a lack of water.

There is also emerging evidence that some metals catalyze thermal PFAS defluorination. For example, nickel catalyzed the decomposition of cyclo-C_4F_8, forming the decomposition product C_3F_8 ~55 times faster than in a Pyrex reactor.[87] Ag and Cu catalyzed the defluorination of perfluoropentacene at ~200 °C.[130] Platinum has been shown in one case to catalyze the pyrolysis of fluorocarbons.[61] Overall, research of catalyzed thermolysis of PFASs is not well studied.

3.4. Thermolysis Kinetics. Published kinetics data are limited, and we are not aware of measured kinetics in the presence O_2 or H_2O. From what has been published, it is evident that thermal unimolecular decomposition of PFASs follows first-order kinetics,[66,87,96] and the relationships between rate and temperature are explained well by the Arrhenius equation.[64,87,95,96,131] Temperature not only has a strong influence on the decomposition rates but also on reaction equilibria.[95] For example, HF elimination (Figure 4, M1) is endothermic, and the equilibrium constant of this reaction increases by approximately 11 orders of magnitude as the temperature is increased from 127 to 727 °C.[95]

Perfluorocarbons are relatively recalcitrant with likely the slowest decomposition rates during pyrolysis. This is evident for perfluoroalkanes (C < 5) which have half-lives greater than 1 h even at temperatures as high as 1000 °C,[61] compared to half-life of PFCAs and PFSAs (<1 s).[96] This is particularly relevant for incinerators because the half-lives of perfluoroalkanes far exceed the typical gas-phase retention time of incinerators (seconds). Perfluoroalkanes may be present in such systems in the feed stream or as decomposition products from other processes.

4. IMPLICATIONS FOR THERMAL TREATMENT PROCESSES

4.1. Treatment Processes. Thermal treatment processes use heat to initiate desorption and degradation of contaminants from a solid or liquid waste. They can be used as a targeted approach to remove PFASs from contaminated matrices such as soil or spent water treatment media (e.g., activated carbon, ion exchange brine), or they can be used as a broad approach to manage mixed municipal solid and hazardous wastes that contain PFASs and are not landfilled.

Thermal decomposition is part of thermal treatment, but thermal treatment encompasses the entire process of thermally treating PFASs, PFAS-containing materials, and the associated products. Thermal treatment processes are often associated generally with the term *incineration*, but incineration is a specific process that is outlined by the US EPA. Title 40 of the Code of Federal Regulations (CFR) Part 260.10 defines an incinerator as any enclosed device that uses controlled flame combustion and is not defined by the CFR as a boiler, sludge dryer, carbon regeneration unit, or industrial furnace, or it is an enclosed device that is operated as an infrared or plasma arc incinerator that has a controlled flame combustion afterburner. Thus, when discussing the thermal treatment of PFASs, one should use the specific terms (i.e., desorption, combustion, pyrolysis) and avoid the use of the term incineration, unless referring to a process described by the CFR.

Thermal desorption uses relatively low temperatures (<600 °C) to separate the contaminant from the solid or liquid waste matrix without the intent to degrade the contaminants, although in some cases they are incidentally decomposed.[132] Thermal desorption includes the energy required to overcome the interaction energy between the PFAS and the solid as well as the energy required to drive the PFAS to the gas phase (volatilization). Thermal desorption can be performed in situ or ex situ. In situ thermal desorption is a widely used method because it eliminates additional costs for transport and/or off-site treatment. It is effective for removing some contaminants at a lower temperature and thus at a lower cost, and those that may form more harmful degradation byproducts at higher temperatures. Although, with thermal desorption, a post-treatment process is always required, which incurs additional costs and complexities to the treatment train. Sörengård et al.[133] demonstrated the use of thermal desorption at temperatures ranging from 150 to 550 °C for a mixture of nine PFASs spiked into two soil types at temperatures ranging from 150 to 550 °C. Greater than 99% desorption of these PFASs from the soil was achieved at 550 °C. The temperature that PFASs desorb can be impacted by the matrix. For example, Crownover et al.[56] showed that desorption was minimal for both PFCAs and PFSAs when the soil was heated to 220 °C for 14 days, despite PFCAs having boiling points below 200 °C.[36] The soil needed to be heated to at least 300 °C to desorb 90% of the PFOA and 60% of the PFOS. Shorter-chain perfluorohexanesulfonic acid desorbed to a lesser extent than PFOS at temperatures below 350 °C. Specific matrix effects that change the required temperature to desorb PFASs are unknown and should be a topic of future studies.

Thermal degradation, which follows or is in conjunction with thermal desorption, is the process of using elevated temperatures (300−1000 °C), often in combination with other

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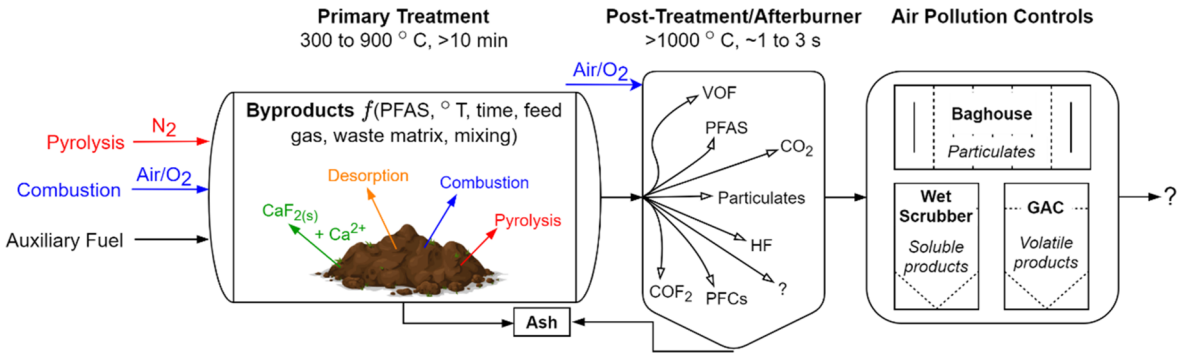


Figure 9. Schematic of a general two-stage thermal treatment system with air pollution control devices installed to treat the produced gases. Potential products and their potential to be captured in typical air pollution control devices are shown. Products formed will depend on temperature, gas mixture (e.g., pyrolysis, combustion), waste components (e.g., calcium), gas turbulence, and residence time.

compounds (e.g., oxygen, catalyst), to bring forth a chemical change in the contaminant. The primary variables that control the efficiency of thermal treatment methods are temperature, gas content, gas- and solid-phase mixing rates, and residence time. For PFASs, thermal degradation has been shown to lead to numerous complete and incomplete combustion products, whose formation depends on the operating conditions and type of PFAS (Table S2).

There are four major thermal degradation technologies: combustion, pyrolysis, gasification, and hydrothermal. Combustion is conducted at stoichiometric or excess oxygen ratios (i.e., excess air). Pyrolysis is performed in the complete absence of oxygen, and gasification is a starved air process performed below stoichiometric oxygen requirements. Hydrothermal treatment processes use water and heat to degrade solid waste at relatively low temperature (<375 °C) but higher pressure (4−22 MPa) and treatment times (>30 min) compared to other thermal processes.[134] It is typically used to treat wastes with a high liquid content, and the temperature and pressure limitations are related to the vapor−liquid critical point of water. Other thermal processes include plasma and microwave treatment. Plasma is an emerging heating technology for extremely high temperature pyrolysis and gasification,[135] but its use has not been specifically applied to PFAS treatment. Microwave heating is an emerging technology for generating rapid temperature changes internally.[136] Gagliano et al. demonstrated that relatively low power microwaves (<500 W) can be used to directly regenerate PFAS-spent GAC, reaching temperatures greater than 600 °C.[137] Combustion is the most commonly used thermal treatment process, and we depict a typical combustion or pyrolysis thermal treatment process treating PFASs in Figure 9, as well as the potential products and the likelihood of products to be captured by typical air pollution control measures.

For PFASs, most of the published combustion studies have involved controlled, laboratory-scale experiments involving the use of simple tube or muffle furnaces.[50,66,81,83,93,138] Lab-scale smoldering has also been investigated. Smoldering, which is a self-sustained combustion, was shown to remove at least 98% of six PFASs from GAC or soil when operated at temperatures above 900 °C.[139] The general consensus across these lab-scale studies is that even the most stable PFASs (e.g., long-chain sulfonates) desorb at temperatures less than 1000 °C, and they are destroyed in the gas phase at temperatures greater than 1000 °C. The critical questions that remain involve the effect of operating parameters (e.g., residence times, oxygen content), the effect of waste type/matrix, the identification of all fluorinated byproducts, and the ability to complete a fluorine mass balance on the system. These questions can be mostly answered at the lab scale with carefully planned experiments, but correlation of the outcomes to a larger scale is also critical. While some studies have confirmed the partial or full removal of PFASs from wastes in a full-scale thermal treatment process,[140−143] there is still a need for more detailed, specific, peer-reviewed studies that probe the impact of operating conditions and completely analyze inorganic and organic volatile fluorine compounds (including PFASs) and HF in the effluent stream.[144]

Further, there are no direct comparative studies on how differing types of full-scale incinerators impact the treatment of PFASs. The incinerator type is defined by the injection, mixing, and heating methods (e.g., fixed or open hearth, rotary film or kiln, fluidized bed, starved air modular). Complete combustion of PFASs will likely be most successful in incinerators that employ a two-stage process. In these, the waste is first fed into the primary combustion chamber where PFASs desorb and partially degrade. The gaseous byproducts are sent to a secondary chamber (the afterburner) that operates in excess air (stoichiometric excess of oxygen) at high temperature (>950 °C) and short residence times (1−3 s). These two-stage incinerators are likely to provide ample residence time to desorb PFASs in the primary chamber and then potentially sufficient temperature and time to mineralize gaseous fluorine products in the secondary chamber.

Although pyrolysis is a common alternative to combustion processes for treating solid waste,[145] there is limited full-scale data on the use of pyrolysis and gasification systems for treating PFASs. Pyrolysis of PFASs forms perfluoroalkanes, olefins, and VOF. Gasification (and combustion) will additionally form oxygenated fluorine compounds (e.g., COF_2). One pilot-plant study treating biosolids containing PFASs confirmed that pyrolysis coupled with combustion was potentially an effective treatment approach, achieving greater than 90% removal of PFOS and PFOA; although, complete defluorination and F mass balance were not achieved.[146]

Hydrothermal treatment is an emerging technology that could be useful for removing PFASs from solid wastes because of the ability of the high-pressure environment to promote defluorination.[134] Yu et al. investigated hydrothermal treatment for destroying PFOA, PFOS, 7:3 FTCA, 8:2 fluorotelomer sulfonate (FTS), and 8:2 fluorotelomer unsaturated carboxylic acid (FTUCA) from wastewater sludge.[147] At a reaction temperature of 350 °C for 90 min, greater than 99% of the carboxylic PFASs were decomposed, but only 34% and 67%

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degradation occurred for PFOS and 8:2 FTS, respectively. Although complete mineralization was not achieved, these results were obtained at a temperature much lower than required for combustion (>700 °C).

One potential solution for the more recalcitrant sulfonates is to amend the liquid phase with acids/bases to catalyze the reactions. Wu et al.[110] showed that conducting hydrothermal treatment at basic conditions at 350 °C for 90 min achieved ∼80% defluorination of PFOS. This approach was confirmed with AFFF waste in a reactor amended with 5 N NaOH.[126] A similar behavior was observed for the hydrothermal treatment of plant biomass. At 300 °C, PFCAs were removed from the biomass to below the detection limit (50% defluorination), and less than 20% removal was observed for PFSAs.[148] The addition of 1 N KOH increased the removal efficiency of PFOS from the biomass beyond 80%, but the shorter-chain PFSAs were less susceptible to destruction (e.g., removal of PFBS was less than 50% observed). This aligns with other destructive technologies, where PFSAs and short-chain PFASs were more recalcitrant.[45,149]

4.2. Co-Occurring Constituents in Wastes. Solid wastes entering thermal treatment systems can be extremely complex, containing myriad co-occurring constituents that may affect the thermal behavior of PFASs. For example, simply changing the PFAS counterion influences both the degradation temperature and the resulting products (Section 3.2.1).[76] The presence of $Ca(OH)_2$ or CaO in the matrix has been shown to affect both the required desorption/degradation temperature and the fate of PFASs during thermal treatment.[83,94,150] In general, for PFAAs, the presence of Ca improved mineralization efficiency and reduced the temperature required for degradation,[83,150] and it prevented the formation of terminal PFAAs when PFAA precursors were treated.[94] In a smoldering column, Duchesne et al.[139] showed that the defluorination efficiency of PFASs was more effective for GAC (44%) compared to a soil (16%). The reason is unknown, but possible mechanisms may be linked to the high specific surface area and porosity of GAC, differing moisture conditions of the solids, differing surface chemistry of the solids, or effects of other constituents in the matrix. Given the complexity of solid waste, attempts to model the thermal behavior PFASs in real systems may prove to be too challenging. Even so, there is a critical need to study the specific effect of co-occurring waste constituents on the thermal behavior of PFASs.

From an operational perspective, the classification of the incinerator will also play a role in deciding what co-occurring constituents will be present during treatment. Municipal, hazardous, and medical waste incinerators are classified as either mass burn or refuse-derived fuel (RDF). For mass burn incinerators, the waste is processed as received, whereas the waste sent to an RDF incinerator is first processed to remove poorly combustible material (e.g., glass, metal), and combustible items are shredded to a uniform size. The removal of glass (i.e., silica) and calcium may alter PFAS degradation pathways because the combustion products (e.g., HF) will react directly with Si and Ca (i.e., Sections 3.3.3.3 and 3.3.3.4).[150] Thus, the thermal behavior of a PFAS is likely to be different in an RDF compared to a mass burn even under similar operating conditions (e.g., temperature).

4.3. Incinerator Solid and Gaseous Byproduct Control Measures. Waste byproducts of incineration include bottom ash, which contain noncombusted products, and gas, containing small particulate and volatile products. Ash, which contains mineralized fluorine but also some PFASs that remain bound to inorganic compounds such as calcium,[150] is typically sent to a landfill or repurposed (e.g., fly ash for concrete). For the gas, there are numerous air pollution control methods available. Particulates that remains in the gas are captured with electrostatic precipitators or baghouses. Numerous methods exist for removing acid gases, including HF that is formed from PFAS thermal conversion. All involve the use of a calcium or sodium salts, such as calcium hydroxide or sodium bicarbonate, forming a corresponding salt (e.g., CaF_2). Escaped HF or volatiles products are presumably captured in the alkaline wet scrubbers, although there is a substantial gap in our understanding of the gas/liquid partitioning (Henry's Law constants) for nearly all PFASs and their combustion products.[53,54] In general, an abundance of information is known about how to capture HF in the gas stream, but very little knowledge exists for removing VOF.

5. IDENTIFYING ANALYTICAL NEEDS TO EVALUATE THERMAL TREATMENT SYSTEMS

The anticipated goal of thermal treatment processes is to completely remove PFASs from the solid/liquid waste and/or mineralize PFASs to CO_2 and inorganic fluorine. Many publications to date report the removal of PFASs from solid or liquid waste matrices but fall short in quantitatively accounting for all fluorine in the system. Reporting a complete F mass balance on thermal treatment systems is important to assess the relative risk of the released products but is challenging because of the following: (1) Numerous fluorinated byproducts are formed (i.e., Table S2). (2) There is no robust analytical method for their quantification. (3) Analysis of multiple phases (gas, surfaces, ash, scrubber liquids) is required.

For targeted PFAS analysis, liquid and gas chromatography−tandem mass spectrometry (LC-MS/MS and GC-MS/MS) can be used to quantify specific PFASs that are present in the produced gas or extractable from the ash.[12,58] However, targeted analysis of PFASs is incapable of quantifying all products, and it has been shown that greater than 50% of postcombustion fluorine is not captured by the sum of LC-MS/MS and HF measurements.[139] Additional compounds can be identified using nontarget analysis, which typically employs high-resolution mass spectrometry (HRMS).[2,151,152] However, this technique is laborious and poorly or not quantitative and does not capture all fluorinated or nonfluorinated products. Achieving a complete mass balance in thermal treatment processes requires total F methods beyond mass spectrometric techniques and measurements of HF.

Common total F analytical approaches include combustion ion chromatography (CIC), particle-induced gamma-ray emission (PIGE), instrumental neutron activation analysis (INAA), and ^{19}F nuclear magnetic resonance (^{19}F-NMR). Schultes et al.[153] showed that CIC, PIGE, and INAA yield similar results when measuring homogeneously packaged samples, but each has advantages and disadvantages. CIC and PIGE have emerged as the most reported methods because of their ease of use, but CIC is more readily available than PIGE.[125] One critical issue for CIC is that it cannot be used to quantify F in solids remaining after combustion (i.e., ash) because CIC requires F to be combusted to HF gas, and ash has already been through a combustion process. PIGE has been used to regularly quantify total F in numerous matrices,[6,153−156] and because it is a direct solid-phase analysis, quantification of total F in all matrices of thermal treatment processes is possible. ^{19}F nuclear magnetic resonance spectroscopy (^{19}F-NMR) has been used to

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measure total F and distinguishes between organofluorine and inorganic F in solids,[157] but poor detection limits (ppm levels) impede its broad application.

Gas-phase compounds may be captured by activated carbon, hydrophobic−lipophilic balance media, or polyurethane foam/XAD samplers[58,158] and then analyzed by LC-MS/MS or GC-MS/MS for targeted compounds or by various total F methods. These methods are validated for well-studied PFASs. However, the capture and recovery of PFAS thermal decomposition products is unknown, and there is a lack of knowledge regarding how sampling procedures may bias the results (i.e., formation of dioxins during cooling of combustion gases).[159] Additionally, GC-MS/MS measurements of gas-phase PFASs using electron impact ionization results in fragmentation spectra that are similar for most PFASs, with prominent product ions representative of the fluoroalkyl chain (e.g., 69 m/z CF_3^+, 119 m/z $CF_2CF_3^+$, 169 m/z $CF_2CF_2CF_3^+$).[58] Chemical ionization reduces fragmentation, but not all GC-MS are equipped with chemical ionization sources.[160,161] Volatile fluorine products can also be measured by Fourier transform infrared spectroscopy (FTIR), and it has been used to quantify COF_2, HF,[162] and CF_4, and C_2F_6.[163] The greatest challenge of employing FTIR to measure such compounds in the gas phase is calibrating the instrument, as pure gas-phase standards used for generating calibration curves are not widely available.

Applying these various capture and analytical schemes is challenging but represents a meaningful first step toward closing the fluorine mass balance. Knowledge of important gaseous products is essential to design and operate next-generation incineration processes that truly achieve complete mineralization of PFASs.

6. KNOWLEDGE GAPS AND RESEARCH NEEDS

The thermal decomposition of only a limited group of mostly pure-phase PFASs under pyrolysis conditions have been thoroughly investigated to date. The implications of such a limited range of studies are relevant to industries, municipalities, and regulators interested in using thermal treatment processes to manage PFAS wastes. Past research focused primarily on determining temperatures that initiate desorption and degradation, as measured by mass loss of the PFASs from the waste. Other studies, to a more limited extent, include measurements or computational predictions of likely decomposition mechanisms and expected products. There are significantly more studies that used computational chemistry to make such conclusions than there are those that used experimental measurements. Some of the coinciding experimental and computational studies do not agree, and several differ significantly. Thus, there is a critical need to conduct studies that incorporate both computation and experimental measures.

A major weakness of existing mechanistic studies is that they do not mimic full-scale thermal treatment systems, which have operational variations that are significantly different than lab-scale systems. There is also a lack of knowledge about the efficiency of full-scale effluent treatment processes for the thermal degradation products of PFASs. While these processes should capture the numerous products, additional studies are required to confirm this hypothesis. Closing the fluorine mass balance on ash, scrubber solutions, and gaseous emissions should be a priority in the near term to understand the fate of PFASs in incinerators having been operated for the past several decades and to understand how to modify incinerator processes in the future.

To adequately assess the fate of PFASs in thermal treatment systems requires analytical techniques that can close the F mass balance. Key to this will be total fluorine measurements because mass spectrometry-based methods do not have readily available standards for the numerous products that may form. Further, measurements of PFASs in the produced ash is a significant challenge, as the detection limits for total fluorine instrumentation (CIC, PIGE, NMR, INAA) are high, and thus, sample preparation and concentration methods are needed if trace levels are to be reached.

To improve upon the understanding of the fate of PFASs during thermal treatment, we propose the following ranked research needs:

(1) Experimental studies of relevant mixtures of PFASs during combustion on different types of surfaces (soils, activated carbon, ion exchange, liquid brines). Although some PFAS decomposition pathways in oxidizing atmospheres are provided in this review, most of the experimental literature focuses on pyrolysis, and therefore, more knowledge relevant to commercial incinerators is needed.
(2) Measurements of Henry's Law constants of organofluorine compounds. Henry's Law constants include interactions with water and better represent environmental systems and incinerator air pollution control measures than vapor pressure (pure compound). These measurements will lead to an understanding of which PFASs are the most likely to elude air pollution control measures. Such constants are not generally available, and combined with the first research need, will provide knowledge of which PFASs are the most likely to be released in flue gases during thermal treatment.
(3) Experimental confirmation of computational studies. Select studies have focused on experimentally derived decomposition, and there is some divergence published between computation and experimental results. Robust studies are needed that incorporate experiments used to validate computational models.
(4) Development and validation of instrumentation and sampling and analytical methods capable of measuring products identified in research needs 1, 2, and 3. These methods will help to close the mass balance on fluorine during thermal treatment and better understand the fate of PFASs.
(5) Experimental research of PFAS decomposition products and temperature-dependent kinetics and pathways of formation of less studied PFASs (e.g., fluorotelomers, iodinated PFASs, PFPAs, perfluorophosphinic acids).

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.est.2c02251.

Additional figures and a table related to vapor pressure of PFASs and a table containing measured and reported thermal decomposition products from published literature (PDF)

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Notes

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Environmental exposure to perfluoroalkyl substances in early pregnancy, maternal glucose homeostasis and the risk of gestational diabetes: A prospective cohort study

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ABSTRACT

Background: Humans are widely exposed to environmental perfluoroalkyl substances (PFAS), which may affect glucose homeostasis. However, research linking PFAS exposure to glucose homeostasis during pregnancy is limited and the results were inconsistent. We aimed to investigate the association between PFAS exposure and glucose homeostasis in pregnancy in a large prospective cohort.

Methods: A total of 2747 pregnant women who participated in the Shanghai Birth Cohort, had blood samples in early pregnancy and completed a 75 g oral glucose tolerance test (OGTT) at 24–28 gestational weeks were included. 10 PFAS were determined by high-performance liquid chromatography/tandem mass spectrometry (HPLC/MS-MS) in the plasma samples in early pregnancy. Logistic regression was used to explore the associations between PFAS concentrations and gestational diabetes mellitus (GDM), while multiple linear regression was used to model the associations between PFAS and OGTT fasting, 1-h and 2-h glucose levels. Potential confounders were adjusted. Bayesian kernel machine regression (BKMR) and a quantile-based g-computation approach (qgcomp) were employed to explore the joint and independent effects of PFAS on glucose homeostasis.

Results: The incidence of GDM was 11.8%. One log-unit increment in plasma concentrations in early pregnancy was associated with an increased risk of GDM for perfluorobutane sulfonate (PFBS) (adjusted odd ratio (aOR) = 1.23, 95% confidence interval (95% CI): 1.05, 1.44) and perfluoroheptanoic acid (PFHpA) (aOR = 1.25, 95% CI: 1.07, 1.46). Perfluorooctane sulfonic acid (PFOS), perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluorohexanesulfonate (PFHxS) and PFHpA were positively correlated with 1-h and 2-h glucose levels. Results of the mixed exposure model showed that the joint effects of PFAS were significantly associated with abnormal glucose homeostasis; In the BKMR model, PFAS mixture exposure was positively associated with the GDM incidence, 1-h and 2-h glucose levels and negatively correlated with FBG level. A similar trend could be observed in qgcomp and the positive correlation between PFAS and 2-h glucose level was significant ($\beta = 0.12$, 95% CI: 0.04, 0.20). PFOS, PFNA and PFHpA may be the main contributors after controlling for other PFAS congeners. PFOS was significantly correlated with GDM incidence and 2-h glucose level, and PFHpA was significantly associated with FBG and 2-h glucose levels. The above associations were more prominent among women with a normal prepregnant BMI.

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Conclusions: Environmental exposure to PFAS may affect glucose homeostasis in pregnancy and increase the risk of GDM, especially in normal weight women.

1. Introduction

Gestational diabetes mellitus (GDM) is a common pregnancy complication. The reported prevalence varies substantially worldwide, ranging from 5% to >30% (Chiefari et al. 2017). Developing countries tend to have higher GDM incidence than developed countries. For instance, the incidence is around 15% in the Middle East, North Africa, South-East Asia and China (Gao et al. 2019; McIntyre et al. 2019). GDM increases the risk of adverse birth outcomes (Johns et al. 2018). The offspring of GDM pregnancy are at a higher risk of metabolic diseases in adulthood (Blotsky et al. 2019; Lowe et al. 2019).

Per- and polyfluoroalkyl substances (PFAS) are a group of synthetic chemicals and have been used extensively in industrial and consumer products due to their thermal stability, hydrophobic and oleophobic properties (Wang et al., 2017b). They are commonly used as additives in oil- and water-resistant textiles, nonstick cookware, greaseproof food packaging and personal care products. They may be absorbed by human body through foods and drinking water (Domingo and Nadal 2019). Even though perfluorooctane sulfonic acid (PFOS) and perfluorooctanoic acid (PFOA) were listed in the Stockholm Convention as persistent organic pollutants for restricted use or elimination in 2009 and 2019, respectively, they are still universally detectable in humans worldwide due to their persistence and bioaccumulation (Lee et al. 2020). Moreover, it was reported that the total production of PFAS has been increasing rapidly in Asian countries, especially in China, due to the transfer of production line from North America and Europe to Asia (Wang et al. 2019; Zhang et al. 2012). China is currently the main producer of PFOS and PFOA in the world. It is estimated that about 250 tons of PFOA and its salts were discharged into the environment between 2004 and 2012 (Wang et al. 2020). Coupled with the persistence in the environment, the exposure concentration of PFOA and PFOS has been maintained at a high level. In addition, the replacements for PFOS and PFOA have been increasingly produced in recent years, despite that the toxicity of many PFAS remains unclear. As PFAS have been linked to a variety of health outcomes (Pelch et al. 2019), the potential effects on human metabolism are gaining more attention (Timmermann et al. 2014; Wang et al., 2017a; Yang et al. 2018).

Some epidemiologic studies showed that exposure to PFAS may increase the risk of type 2 diabetes mellites (T2DM) (Conway et al. 2016; Donat-Vargas et al. 2019; Lin et al. 2009). Animal experiments demonstrated that PFAS may cause metabolic disorders and insulin resistance through inflammatory response and endocrine disruption, and increase the risk of diabetes (Fang et al. 2012; Yan et al., 2015). Peroxisome proliferator-activated receptor (PPAR) activation is considered to be an important pathway (Ammazzalorso et al. 2019; Corrales et al. 2018; Liu et al. 2018). However, it is unclear whether and how PFAS may affect glucose metabolism in pregnant women who have high metabolic rates. The limited number of epidemiologic studies have generated inconsistent results. Null or even negative associations were also reported, which may be attributable to variations in sample size, study design and inadequate information of potential confounders in previous studies (Shapiro et al. 2016; Valvi et al. 2017; Wang et al., 2018b; Zhang et al. 2015).

In addition, high pre-pregnant BMI is a risk factor for GDM (Giannakou et al. 2019). PFAS exposure may be associated with abnormal gestational weight gain during pregnancy and excessive weight gain could increase the GDM incidence (Marks et al. 2019; Mitro et al. 2020). PFAS exposure may induce child obesity through disturbing the metabolic processes (Braun 2017). These evidence indicate that the adiposity status of pregnant women may modify the health effects caused by PFAS exposure. More evidence is needed.

We aimed to examine the association between PFAS levels in early pregnancy and glucose homeostasis and the risk of GDM in later pregnancy in a large prospective cohort in China. The effects of the joint exposure and the independent effect of PFAS congeners were further explored. We also sought to evaluate whether the associations vary by pre-pregnancy weight status.

2. Material and methods

2.1. Study population

The present study used data from the longitudinal Shanghai Birth Cohort (SBC). SBC recruited women in early pregnancy from six participating hospitals in Shanghai, China during 2013–2016. Detailed description of the cohort was provided elsewhere (Zhang et al. 2019). In brief, eligible women were those who were at least 20 years old, registered Shanghai resident or married to a Shanghai resident; planned to seek prenatal care and give birth at the collaborative hospitals; were willing to sign a consent form and have follow-up visits regularly in the following 2 years. The participant provided the following information in questionnaire survey: demographic characteristics (e.g. maternal age, race/ethnicity, height, pre-pregnancy weight, education, family economic status), behavior and lifestyle (e.g. smoking and drinking status in early pregnancy, physical activities in early pregnancy) and reproductive characteristics (e.g. parity), disease history (e.g. diabetes). Medical records were retrieved and reviewed by trained research staff for information on the pregnancy.

A total of 3046 pregnant women had both PFAS measurements and pregnancy outcomes of interest. 67 subjects were excluded due to pre-pregnancy diabetes mellitus. We further excluded women who had previous GDM (n = 7) or missing data on GDM diagnosis (n = 225), leaving 2747 subjects in the final analysis (see Fig S1). GDM was diagnosed based on a 75 g oral glucose tolerance test (OGTT) between 24 and 28 gestational weeks according to the International Association of Diabetes and Pregnancy Study Groups (IADPSG) criteria (Basri et al. 2018): the glucose concentration met any of the following 3 thresholds: a) fast plasma glucose (FPG) $\geq$ 5.1 mmol/L; b) 1-h plasma glucose $\geq$ 10.0 mmol/L; or c) 2-h plasma glucose $\geq$ 8.5 mmol/L. The blood glucose detection is carried out by an automatic blood glucose meter. The detection mechanism is that the glucose dehydrogenase on the test paper will convert the blood glucose into glucose lactone and then generate changed current. The accuracy is above 95% and the variation coefficient of repeated measurements is less than 5%.

2.2. Plasma PFAS assessment

Blood samples were collected in early pregnancy at a median gestational age of 15 weeks [interquartile range (IQR): 13–17 weeks] and centrifuged at 4000 rpm for 10 min. Plasma and blood cells were separated and stored at −80 °C until assays. A 100 μl plasma was used for PFAS quantification using high-performance liquid chromatography / tandem mass spectrometry (HPLC/MS-MS; Agilent1290–6490, Agilent Technologies Inc., USA). Detailed methods of PFAS measurement have been described elsewhere (Zhou et al. 2017). Both intra- and inter-assay coefficients of variation (CV) were below 10% for all PFAS congeners. The 10 measured PFAS congeners were PFOS, PFOA, perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUnDA), perfluorohexanesulfonate (PFHxS), perfluorododecanoic acid (PFDoA), perfluorobutane sulfonate (PFBS), perfluoroheptanoic

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acid (PFHpA), perfluorooctane sulfonamide (PFOSA). The limits of detection (LOD) were 0.09 ng / mL for PFOA and PFOS, 0.02 ng / mL for PFDA, PFNA, PFUnDA and PFHxS, 0.03 ng / mL for PFHpA, 0.05 ng / mL for PFDoA, 0.009 ng / mL for PFBS and 0.12 ng / mL for PFOSA, respectively. PFAS concentrations below the LOD were replaced by the LOD/ $\sqrt{2}$ (Hornung 1990).

2.3. Covariates

Data on maternal age, weight, height, education, smoking, alcohol consumption, physical activity, economic status, parity and disease history were collected using a standardized questionnaire. Pre-pregnant body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared. Maternal education was grouped into high school or below, college and postgraduate. In this study, only 3 women were current smokers. Thus, we combined active with passive smoking into a single category. Since only a few women drank alcohol (1.3%) in pregnancy, alcohol consumption was thus not included in the final analyses. Parity was classified into nulliparous and parous. Maternal economic status was grouped into four levels based on the question "How do you feel about your family financial situation currently": very good, fairly good, fairly poor and very poor. Physical activity was measured using the short version of the International Physical Activity Questionnaire (IPAQ) (Patterson 2005). We calculated each individual's weekly physical activity level (MET-min/w), combined the frequency of activities and further divided the physical activity intensity into low, middle and high groups. Potential confounders were selected based on previous studies and a directed acyclic graph (DAG) (see Supplementary Fig S2) (Chen et al. 2015; Shrier and Platt 2008; Zheng et al. 2017).

2.4. Statistical analysis

We assessed the distribution of all PFAS and relevant covariates. PFAS were reported as medians and interquartile range. Other continuous and categorical covariates were reported as mean and standard deviation, number and percentage, respectively. All PFAS concentrations were natural logarithm (ln) transformed for further model analyses. Differences in PFAS, characteristics and glucose parameters between GDM and non-GDM groups were tested using Wilcoxon's rank sum test (skewed distribution) or Chi squared test (categorical variables) where appropriate. Spearman rank correlation analysis was conducted to explore the pairwise correlation among PFAS original value. Multiple imputation with fully conditional specification (FCS-MI) method was used to impute missing values of the covariates before regression analyses. This method can specify the multivariate imputation model on a variable-by-variable basis and offers a principled yet flexible method to address missing data (Liu and De 2015). Ten datasets with imputations for missing data were created and modeled separately. Results were pooled to obtain the final effect parameters in single pollutant analyses and PFAS mixture exposure analyses.

We applied logistic regression and multiple linear regression models to assess the associations of PFAS with GDM and continuous outcomes (FBG, 1-h and 2-h glucose levels), respectively. PFAS congeners were analyzed one at a time in relation to glucose homeostasis. Tolerance and variance inflation factor (VIF) values were calculated to evaluate multicollinearity between covariates, with tolerance <0.1 and VIF > 10 being considered indicative of multicollinearity. All models adjusted for maternal age, pre-pregnant BMI, maternal education, smoking status, parity, physical activity and economic status. PFOSA was not included in our regression model due to the low detection rate. Restricted cubic spline (RCS) models with knots at the 5th, 27.5th, 50th, 72.5th and 95th percentiles of each PFAS were fitted to further identify potential nonlinearities between plasma PFAS and glucose homeostasis. Odds ratios (ORs) with 95% confidence intervals (CIs) were used to report the relative increase in the risk of GDM for every log unit increase in PFAS, while β-coefficients with 95% CIs were used to report the change in glucose concentration.

Bayesian kernel machine regression (BKMR) was used to estimate individual and overall effects of PFAS on GDM incidence, FBG, 1-h and 2-h glucose levels. BKMR is a semi-parametric method that can be used to estimate the effects of individual mixture components, the overall mixture effect and interactions between mixture components (Bobb et al. 2015). A Gaussian kernel was adopted to support flexible exposure–response shapes for continuous outcomes. A probit link function was used to fit the exposure–response relationship for binary outcome (i.e., GDM). For all BKMR analyses, PFAS were ln-transformed and centered to a mean of 0 and scaled to a standard deviation of 1 to account for skewness and to reduce the influence of extreme values and different value scales in the variables. Each of the BKMR models was adjusted for the corresponding covariates as described above. The overall mixture effects of PFAS were estimated by subtracting the mean value of the outcome when the PFAS concentrations were at their 25th percentile with the mean value of the outcome when all the PFAS concentrations were at their 75th percentile. Similarly, individual effects of each PFAS mixture component were estimated by subtracting the mean outcome value when the specified PFAS was at its 25th percentile with the mean value of the outcome when the specified PFAS was at the 75th percentile and. Other PFAS congeners were fixed at their medians and all covariates held constant. Plots were used to evaluate the overall associations of PFAS mixture with different glucose homeostasis indicators and visualize the exposure–response relationship for each PFAS isomer while holding the other exposures to their 50th percentile and covariates constant (Lebeaux et al. 2020). The group posterior inclusion probability (groupPIP) and the conditional posterior inclusion probability (condPIP) were calculated, representing the probability of a mixture group and a particular PFAS within the group, respectively. All BKMR models were fit 50,000 iterations using the Markov Chain Monte Carlo (MCMC) sampler.

Even though BKMR benefits from the flexibility of modeling the exposure–response function and allowing for the nonlinear and non-additive association between multiple PFASs and glucose levels, it cannot provide parsimonious parametric inference (e.g., a single coefficient corresponding to the change in the outcome per unit of joint exposure). We further applied a recently developed quantile-based g-computation method to explore the joint effects based on parameter inference. This approach combines the inferential simplicity of weighted quantile regression (WQS) with the flexibility of g-computation and also allows for nonlinearity and non-additivity of BKMR, which appears to be less biased and more robust (Keil et al. 2020). We used this method to model the joint and individual effects of exposure to all nine PFAS on the categorical GDM outcome and continuous glucose levels. Binomial and gaussian distributions were specified as link functions, respectively. Parameter q represents the specified quantile and was set to 4 as most of the dose–response relationships did not show a significant nonlinear pattern. 500 bootstrap iterations were performed to calculate the CIs. In addition, stratified analyses were conducted in BKMR and qgcomp models by pre-pregnant weight status (BMI). We grouped the study subjects into normal weight and overweight or obese with the BMI cutoff at 24 kg/m^2, since this cut-off value is appropriate for Chinese (Xia et al. 2019).

Several sensitivity analyses were performed to test the robustness of our results. 1) In our pre-analysis, only a few PFAS showed non-linear relationships with glucose related outcomes. In order to better evaluate the accuracy and stability of the linear model, PFAS were categorized by tertiles and then included in the binary logistic or multiple linear model one PFAS at a time. 2) Multicollinearity due to high correlation among PFAS congeners may bias the parameter estimates between exposure and outcome. We applied the elastic net regression (ENR) model to address this issue and to evaluate the robust and stability of BKMR and qgcomp modeling results. This method combines the properties of least absolute shrinkage, selection operator and ridge penalty and has a better performance in addressing multiple-collinearity

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(Friedman et al. 2010). α value was set from 0 to 1 by 0.01 and λ value equal to 100. A 10-fold cross-validation was performed to obtain the optimal combination of α and λ (Huo et al. 2020). We further modeled the PFAS selected by ENR to estimate the joint and individual effect in qgcomp and obtained the estimate parameter. 3) We added the interaction analysis between PFAS exposure and pre-pregnant BMI to the single pollutant models and mixed exposure models to quantitatively evaluate their possible relationship. 4) Considering that there is substantial missingness in some of the covariates, e.g., physical activity. We performed an additional analysis among those with complete information. We further created dummy variables for those with missing information. As for values below the limit of detection, we further adopted a left censored log-normal regression model to impute them and the results across different datasets were combined using Rubin's rule. All of these analyses helped to assess the stability and reliability of our results. 5) When assessing the outcomes with high prevalence, odds ratio may not be an appropriate measure as it may overestimate the possible effects. Therefore, we also calculated the relative risk (RR) of PFAS exposure on GDM by using the marginal parameters to yield estimates of the population average exposure effect. 6) GDM group and normal women significantly differ in glucose homeostasis. After modeling the pooling data, we further conducted an analyses using data stratified by GDM status and explored whether there existed metabolic vulnerability in pregnant women. 7) We restricted the study subjects to nulliparous women to eliminate the influence of excluding women who had previous GDM on parity.

All statistical analyses were conducted in SAS V9.4 (SAS Institute Inc., Cary, NC, USA) and R (version 3.6.1, R Development Core Team 2019). The BKMR model, qgcomp and ENR model were implemented using the R package "bkmr", "qgcomp" and "glmnet", respectively (Bobb et al. 2015; Friedman et al. 2010; Keil et al. 2020). An α level of 5% (two-sided) was considered statistically significant. This project was approved by the Research Ethics Committees of Xinhua Hospital affiliated to Shanghai Jiao Tong University School of Medicine.

3. Results

3.1. Population characteristics

Table 1 presents the demographic characteristics of the study cohort. The mean maternal age was 29.1 years. The average pre-pregnant BMI was 21.40 kg/m^2. 15.8% of the subjects were overweight or obese. The distribution difference in BMI status between GDM and non-GDM groups is statistically significant ($P < 0.01$). The majority of subjects had university education. Nearly 36% of women were passive smokers. Over 80% were nulliparous. Most pregnant women had low to mid intensity physical activity (73%). 63% of pregnant women had fairly good family economic status. The incidence of GDM was 11.8%. The average FBG, 1-h and 2-h glucose levels were 4.45 (SD: 0.48), 7.55 (SD: 1.63) and 6.52 (SD: 1.35) mmol/l, respectively. The incidence of GDM increased with maternal age ($P < 0.01$). The differences in other variables beside maternal age, pre-pregnant BMI and glucose measurements between GDM and non-GDM groups were statistically non-significant.

3.2. Maternal plasma PFAS

Table 2 describes the LOD and detection rate of each PFAS congener, the median and interquartile range (IQR). PFOSA had a low detection rate (21.6%) and, therefore, was not analyzed further. PFOA was the dominant compound detected with the median concentration at 11.56 ng/ml, followed by PFOS at 9.40 ng/ml. PFBS and PFHpA concentrations were significantly higher in GDM group compared to non-GDM group, while PFUnDA showed an inverse trend. Pairwise correlation analysis shows significant correlations between PFAS congeners. The correlation coefficients of different PFAS ranged from −0.02 to 0.94 and there were strong correlations among several long-chain PFAS (PFNA, PFDA and PFUnDA), with r ≥ 0.6 (Fig S1).

Table 1
Demographic characteristics and oral glucose tolerance test results by gestational diabetes status in Shanghai Birth Cohort (N = 2747).

Characteristics	All subjects (n = 2747)	Non-GDM (n = 2422)	GDM (n = 325)	*p*-value*
Maternal age (years) [Mean (SD)]	29.1 (3.7)	28.91 (3.7)	30.2 (4.0)	<0.01
Pre-pregnant BMI [n (%)]				<0.01
<18.5	409 (14.9)	380 (15.7)	29 (8.9)	
18.5–23.9	1751 (63.7)	1573 (65.0)	178 (54.8)	
24.0–27.9	336 (12.2)	264 (10.9)	72 (22.2)	
≥28.0	97 (3.5)	72 (3.0)	25 (7.7)	
Missing	154 (5.6)	133 (5.5)	21 (6.5)	
Maternal educational level [n (%)]				0.06
High school and below	260 (9.5)	221 (9.1)	39 (12.0)	
College	2042 (74.3)	1818 (75.1)	224 (68.9)	
Postgraduate	325 (11.8)	279 (11.5)	46 (14.2)	
Missing	120 (4.4)	104 (4.3)	16 (4.9)	
Smoking status [n (%)]				0.43
Never	1436 (52.3)	1276 (52.7)	160 (49.2)	
Active/passive smoking	1088 (39.6)	955 (39.4)	133 (40.9)	
Missing	223 (8.1)	191 (7.9)	32 (9.9)	
Parity [n (%)]				0.42
0	2332 (84.9)	2061 (85.1)	271 (83.4)	
1+	383 (13.9)	335 (13.8)	48 (14.8)	
Missing	32 (1.2)	26 (1.1)	6 (1.9)	
Level of physical activity [n (%)]				0.80
Low	970 (35.3)	851 (35.1)	119 (36.6)	
Middle	1026 (37.4)	910 (37.6)	116 (35.7)	
High	32 (1.2)	28 (1.2)	4 (1.2)	
Missing	719 (26.2)	633 (26.1)	86 (26.5)	
Economic status [n (%)]				0.69
Very good	443 (16.1)	396 (16.4)	47 (14.5)	
Fairly good	1735 (63.2)	1525 (63.0)	210 (64.6)	
Fairly poor	269 (9.8)	241 (10.0)	30 (9.2)	
Very poor	28 (1.0)	26 (1.1)	2 (0.6)	
Missing	270 (9.8)	234 (9.7)	36 (11.1)	
Fasting blood glucose [Mean (SD)]	4.45 (0.48)	4.38 (0.40)	4.88 (0.73)	<0.01
1-h glucose [Mean (SD)]	7.55 (1.63)	7.24 (1.37)	9.63 (1.74)	<0.01
2-h glucose [Mean (SD)]	6.52 (1.35)	6.25 (1.06)	8.31 (1.70)	<0.01

GDM, gestational diabetes mellitus; SD, standard deviation.
* Wilcoxon rank sum test for continuous variables and Chi-square test for categorical variables.

3.3. PFAS, GDM and blood glucose levels

Table 3 illustrates the associations between individual PFAS, GDM risk and glucose levels in different models after controlling for potential confounders. Significant positive associations were observed between PFBS and PFHpA exposure and GDM risk (OR = 1.23; 95% CI: 1.05, 1.44 for PFBS; and OR = 1.25; 95% CI: 1.07, 1.46 for PFHpA), which were consistent with the significant difference in their concentrations between the GDM and non-GDM groups. PFOS, PFNA, PFDA and PFHxS were significantly associated with increased 1-h glucose concentrations (by 0.11–0.22 mmol/l per log unit increment). Similar results were observed for the 2-h glucose level.

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Table 2
Perfluoroalkyl substances (PFAS) levels (ng/mL) in early pregnancy by gestational diabetes status (N = 2747).

PFAS	All subjects (N = 2747)			Non-GDM (N = 2422)	GDM (N = 325)	
	LOD	<LOD (%)	Median (IQR)	Median (IQR)	Median (IQR)	*p*-value
PFOA	0.09	0.00	11.56 (5.86)	11.55 (5.93)	11.62 (5.60)	0.57
PFOS	0.09	0.00	9.40 (6.93)	9.40 (6.90)	9.41 (7.32)	0.56
PFNA	0.02	0.00	1.65 (1.07)	1.65 (1.07)	1.61 (1.08)	0.48
PFDA	0.02	0.00	1.70 (1.36)	1.71 (1.38)	1.56 (1.30)	0.13
PFUnDA	0.02	0.04	1.39 (1.10)	1.40 (1.10)	1.27 (1.03)	0.01
PFHxS	0.02	0.00	0.53 (0.26)	0.53 (0.26)	0.54 (0.25)	0.26
PFDoA	0.05	17.99	0.15 (0.13)	0.15 (0.13)	0.14 (0.11)	0.31
PFBS	0.009	19.26	0.036 (0.05)	0.035 (0.05)	0.045 (0.05)	<0.01
PFHpA	0.03	19.18	0.055 (0.04)	0.054 (0.04)	0.061 (0.06)	0.01
PFOSA	0.12	78.41				

GDM, gestational diabetes mellitus; LOD, the limit of detection; IQR: interquartile range; PFOA, perfluorooctanate; PFOS, perfluorooctane sulfonate; PFNA, perfluorononanoic acid; PFDA, perfluorodecanoic acid; PFUnDA, perfluoroundecanoic acid; PFHxS, perfluorohexanesulfonate; PFDoA, perfluorododecanoic acid; PFBS, perfluorobutane sulfonate; PFHpA, perfluoroheptanoic acid; PFOSA, perfluorooctane sulfonamide.

Table 3
Associations of log-transformed PFAS concentration in early pregnancy with GDM, fasting blood glucose, 1-h and 2-h glucose levels at 24–28 weeks of gestation (N = 2747).

PFAS	GDM [OR (95% CI)][a]	FBG [β (95% CI)][b]	1-h glucose [β (95% CI)][b]	2-h glucose [β (95% CI)][b]
PFOA	1.11 (0.83, 1.50)	0.01 (−0.04, 0.06)	0.11 (−0.05, 0.26)	0.10 (−0.03, 0.23)
PFOS	1.10 (0.88, 1.36)	−0.01 (−0.04, 0.03)	**0.15 (0.04, 0.26)**	**0.13 (0.03, 0.23)**
PFNA	1.03 (0.81, 1.30)	0.03 (−0.01, 0.07)	**0.18 (0.06, 0.30)**	**0.15 (0.04, 0.25)**
PFDA	0.95 (0.78, 1.16)	0.01 (−0.02, 0.04)	**0.12 (0.01, 0.22)**	0.08 (−0.002, 0.17)
PFUnDA	0.91 (0.74, 1.12)	−0.001 (−0.03, 0.03)	0.08 (−0.03, 0.18)	0.06 (−0.03, 0.15)
PFHxS	1.15 (0.86, 1.54)	0.003 (−0.04, 0.05)	**0.22 (0.06, 0.37)**	0.08 (−0.06, 0.21)
PFDoA	0.99 (0.78, 1.26)	**−0.05 (−0.09, −0.01)**	−0.03 (−0.16, 0.10)	0.03 (−0.09, 0.14)
PFBS	**1.23 (1.05, 1.44)**	−0.01 (−0.04, 0.02)	0.04 (−0.04, 0.13)	0.05 (−0.02, 0.13)
PFHpA	**1.25 (1.07, 1.46)**	0.02 (−0.01, 0.05)	**0.11 (0.02, 0.20)**	**0.13 (0.05, 0.20)**

Note: All models adjusted for maternal age, pre-pregnant BMI, maternal education, smoking status, parity, averaged physical activity and economic status.
[a] Binary logistic regression was used to estimate the associations. Effect estimate was expressed as odds risk (OR) and 95% confidence interval (95% CI).
[b] Multiple linear regression was used to estimate the linear associations. Effect change was expressed as Beta coefficient (β) and 95% confidence interval (95% CI).

3.4. PFAS joint and individual exposure with glucose homeostasis based on BKMR

The overall associations between the PFAS mixture and glucose homeostasis are shown in Fig. 1. The risk of GDM increased significantly with increasing total PFAS mixture levels. Similar results were observed between PFAS exposure and 1-h and 2-h glucose levels but not with FBG level. We further examined individual PFAS exposure–response functions of early pregnancy exposure and glucose homeostasis (Fig S3). Positive relationships between PFNA exposure and FBG, 1-h and 2-h glucose levels were observed. PFHpA exposure was positively associated with GDM and 1-h glucose level, while PFDA exposure was positively associated with FBG level. The condPIP of PFHpA was relatively high within this group and stable with outcomes (GDM, FBG, 1-h and 2-h glucose levels), whereas the groupPIPs of PFDA for FBG and PFNA for 2-h glucose level were all above 0.5 (Table S2).

When the results were stratified by pre-pregnant BMI in BKMR, PFAS mixture was positively associated with 1-h and 2-h glucose levels and negatively associated with FBG level, while the trend weakened in overweight/obese subjects (Fig S4). The condPIP of PFHpA was relatively stable with an extremely high value at 0.98 in normal weight pregnant women. The condPIPs of PFNA, PFBS and PFDoA were >0.5 whereas in the overweight/obese group, only PFHpA showed relative more importance.

3.5. Mixture analyses by quantile-based g-computation method

Table 4 presents the quantile-based g-computation analyses for GDM, FBG, 1-h and 2-h glucose levels. PFAS as a whole was significantly associated with increased 2-h glucose level, with β = 0.12 (95% CI: 0.04, 0.20) for every quantile increase in PFAS concentrations. PFOS and PFHpA had a larger association with glucose homeostasis while PFDoA exposure was negatively associated with FBG and 1-h glucose levels. PFAS with significant associations had higher weights than the others.

Furthermore, we observed that the joint effects of nine PFAS on GDM risk were more pronounced in normal weight women. A similar pattern was observed for independent effects of PFOS, PFHpA and PFDoA on glucose homeostasis. PFNA was associated with higher 1-h and 2-h glucose levels among women with normal weight.

3.6. Sensitivity analyses

Table S3 shows the results of logistic regression and multiple linear regression with PFAS included as an ordinal variable. Overall, the results were consistent with Table 3. The highest tertiles of PFBS and PFHpA exposure were positively associated with GDM risk (OR = 1.54; 95% CI: 1.15, 2.07 for PFBS and OR = 1.44; 95% CI: 1.09, 1.95 for PFHpA, respectively). The highest tertiles of PFOS, PFDA and PFHpA exposure were also significantly associated increased 1-h and 2-h glucose levels. High exposure of PFHxS and PFUnDA were associated with increased 1-h and 2-h glucose levels, respectively. In addition, PFUnDA and PFHpA were found to be significantly associated with increased FBG level. Meanwhile, negative correlations were observed between PFDoA or PFBS exposure and FBG level.

To validate the results of mixed exposure models, ENR was used (Table S4). The mixed effects of the selected PFAS were associated with increased GDM risk and increased 2-h glucose level. PFNA had independent effects on FBG and 1-h glucose levels. PFHpA exposure was significantly correlated with increased FBG and 2-h glucose levels, while PFDoA showed an inverse association. In the interaction analysis, the product terms of PFHpA and pre-pregnant BMI were statistically significant for GDM and 1-h glucose level in single pollutant analyses and significant for 2-h glucose level in mixed exposure analyses (Table S5 and S6).

Results of completed data analyses showed that PFAS mixture exposure was positively associated with 2-h glucose level. PFHpA, PFBS, PFUnDA and PFDoA have significant effects on glucose homeostasis after controlling for other PFAS (Table S7). In the analyses that including missing information as a dummy variable, the PFAS mixture was significantly associated with GDM incidence, 1-h and 2-h blood glucose levels (Table S8). PFNA, PFBS, PFHpA, PFHxS and PFDoA showed significant associations with various outcomes. PFAS mixture

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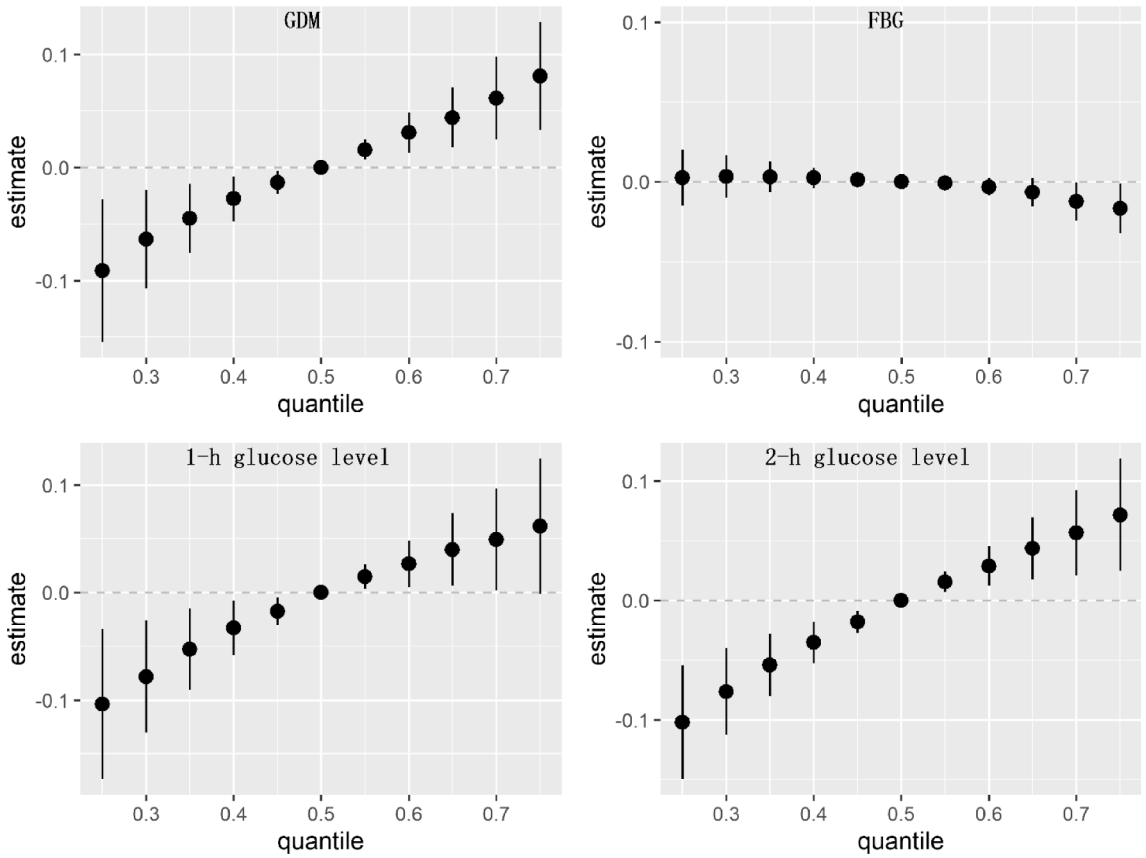


Fig. 1. Joint effect estimates with 95% CI for the PFAS mixture total exposure in relation to glucose homeostasis in the hierarchical BKMR models; all the chemicals at particular percentiles (from 0.25 to 0.75 increment by 0.05) were compared to all the chemicals at their 50th percentile.

exposure were associated with increased 2-h glucose level. PFOS, PFDoA and PFHpA were the identified PFAS with significant effects on glucose homeostasis (Table S9). Although the results in Table S7, Table S8 and Table S9 were somewhat different, the specific PFAS with significant effect may vary, the overall trend and direction of the effect of PFAS on glucose outcomes were basically consistent. Results of estimated RR values showed that PFOS exposure may increase the GDM incidence after controlling other PFAS exposures (OR = 1.24; 95% CI: 1.02, 1.51), which is more pronounced in normal weight group (OR = 1.30; 95% CI: 1.03, 1.63) compared to overweight/obese group (OR = 1.23; 95% CI: 0.86, 1.77) (Table S10). The associations estimated by the RR value were consistent with which by the OR value. In the stratified analysis by pre-pregnant BMI, although there were certain differences, they had the same trend. PFAS mixed exposure may affect the 2-h glucose level in the non-GDM group, among which PFNA, PFHpA and PFDoA were the significant pollutants (Table S11). These effects were attenuated in GDM group, which indicated that non-GDM pregnant women may be more sensitive to PFAS exposure. Table S12 shows that PFAS mixture exposure may increase the risk of GDM incidence (OR = 1.22, 95%CI: 1.01, 1.46) and the effects of PFOS and PFBS are significant. PFHpA exposure is positively correlated with 1-h and 2-h glucose levels.

The RCS analysis indicates that the association between PFAS exposure, GDM and 2-h glucose level was linear, while some of the PFAS congeners exposure, FBG and 1-h glucose levels show some evidence of non-linear associations (Fig S5-S8).

4. Discussion

In this large prospective cohort, we explored the associations between PFAS exposure and glucose homeostasis during late pregnancy using multiple statistical models. Overall, PFAS mixture exposure may affect the glucose homeostasis by increasing 2-h glucose level. The joint effects of multiple PFAS congeners were more pronounced in women with normal weight. As for specific PFAS congeners, PFOS may increase the risk of GDM while PFNA and PFHpA may increase the glucose levels, especially for FBG and 2-h glucose levels.

A number of studies have reported a positive link between PFAS exposure and T2DM in non-pregnant individuals (Domazet et al. 2016; Donat-Vargas et al. 2019; Duan et al. 2020). For example, a nested case-control study in the USA and another cross-sectional study conducted in elderly Swedish population showed that higher PFAS exposure was associated with an elevated risk of T2DM, insulin resistance and may damage beta cell function (Cardenas et al. 2017; Lind et al. 2014; Sun et al. 2018). So did two other epidemiologic studies (Christensen et al. 2016; Mancini et al. 2018).

Compared to healthy adults, pregnancy leads to a series of physiological changes in order to support the demands of the growing fetus, including adaptations to the cardiovascular, renal, hematologic, respiratory and metabolic systems (Plows et al. 2018). Insulin sensitivity is one important metabolic adaptation during this period and may promote a state of insulin resistance together with a series of local and placental hormones (Catalano et al. 1991). The glucose homeostasis fluctuation during pregnancy makes it more sensitive to the interference of environmental pollutants, especially pollutants with estrogen-like effects (Rahman et al. 2019; Zhang et al. 2020).

A total of 9 studies so far have examined the association between PFAS exposure and GDM in pregnant women, but only 3 of them were conducted in developing countries. Seven of them reported positive associations between specific PFAS congeners and glucose homeostasis, which is consistent with our findings. For example, both our study and the Odense Child Cohort study conducted in Denmark found that PFNA was associated with glucose homeostasis (Jensen et al. 2018). A study

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Table 4
The total effect of exposure mixture and independent effect of individual PFAS after controlling for other PFAS congeners on GDM, FBG, 1-h and 2-h glucose levels, stratified by pre-pregnancy weight status (N = 2747).

	GDM		FBG		1-h glucose		2-h glucose	
Items	Weight	[OR (95% CI)]	Weight	[β (95% CI)]	Weight	[β (95% CI)]	Weight	[β (95% CI)]
All subjects[a] (N = 2747)								
PFAS mixture		1.17 (0.99, 1.38)		−0.01 (−0.03, 0.02)		0.09 (−0.004, 0.19)		**0.12 (0.04, 0.20)**
PFOA	−0.18	0.94 (0.82, 1.08)	0.002	0.0001 (−0.02, 0.02)	−0.26	−0.07 (−0.14, 0.01)	−0.22	−0.04 (−0.10, 0.03)
PFOS	0.41	**1.24 (1.02, 1.51)**	−0.19	−0.01 (−0.04, 0.02)	0.24	0.08 (−0.02, 0.18)	0.33	**0.09 (0.01, 0.18)**
PFNA	−0.005	1.00 (0.81, 1.23)	0.41	0.03 (−0.01, 0.06)	0.29	0.10 (−0.01, 0.21)	0.32	0.09 (−0.01, 0.18)
PFDA	−0.76	0.77 (0.59, 1.01)	−0.01	−0.001 (−0.04, 0.04)	0.06	0.02 (−0.12, 0.16)	−0.41	−0.06 (−0.18, 0.06)
PFUnDA	0.06	1.03 (0.81, 1.32)	0.23	0.02 (−0.02, 0.05)	−0.27	−0.07 (−0.20, 0.06)	−0.06	−0.01 (−0.12, 0.10)
PFHxS	0.14	1.08 (0.95, 1.23)	−0.03	−0.003 (−0.02, 0.02)	0.19	0.06 (−0.003, 0.13)	−0.04	−0.01 (−0.06, 0.05)
PFDoA	−0.04	0.99 (0.85, 1.15)	−0.60	**−0.04 (−0.07, −0.02)**	−0.47	**−0.12 (−0.19, −0.05)**	−0.27	−0.04 (−0.11, 0.02)
PFBS	0.22	1.12 (0.97, 1.30)	−0.15	−0.01 (−0.03, 0.01)	0.09	0.03 (−0.04, 0.10)	0.05	0.01 (−0.05, 0.07)
PFHpA	0.15	1.08 (0.96, 1.22)	0.34	**0.02 (0.003, 0.04)**	0.13	0.05 (−0.02, 0.11)	0.30	**0.08 (0.03, 0.14)**
Normal weight[b] (N = 2160)								
PFAS mixture		**1.24 (1.02, 1.51)**		−0.01 (−0.04, 0.02)		0.06 (−0.05, 0.16)		**0.11 (0.02, 0.20)**
PFOA	−0.08	0.97 (0.83, 1.13)	0.10	0.01 (−0.02, 0.03)	−0.13	−0.04 (−0.11, 0.05)	−0.11	−0.02 (−0.08, 0.05)
PFOS	0.45	**1.33 (1.07, 1.65)**	−0.05	−0.004 (−0.04, 0.03)	0.29	0.09 (−0.02, 0.20)	0.32	0.09 (−0.01, 0.18)
PFNA	0.11	1.07 (0.85, 1.36)	0.42	0.03 (−0.01, 0.06)	0.40	**0.12 (0.004, 0.24)**	0.42	**0.11 (0.01, 0.21)**
PFDA	−0.75	0.74 (0.55, 1.01)	0.21	0.01 (−0.03, 0.06)	−0.22	−0.06 (−0.21, 0.10)	−0.54	−0.08 (−0.21, 0.05)
PFUnDA	−0.07	0.97 (0.74, 1.28)	−0.10	−0.01 (−0.05, 0.03)	−0.03	−0.01 (−0.15, 0.13)	−0.03	−0.004 (−0.12, 0.11)
PFHxS	0.07	1.04 (0.90, 1.21)	−0.08	−0.01 (−0.03, 0.01)	0.18	0.06 (−0.02, 0.13)	−0.05	−0.01 (−0.07, 0.05)
PFDoA	−0.10	0.96 (0.81, 1.14)	−0.59	**−0.05 (−0.07, −0.02)**	−0.61	**−0.16 (−0.24, −0.08)**	−0.24	−0.04 (−0.11, 0.03)
PFBS	0.20	1.14 (0.96, 1.35)	−0.18	−0.01 (−0.04, 0.01)	0.09	0.03 (−0.04, 0.10)	−0.01	0.00004 (−0.06, 0.06)
PFHpA	0.17	1.11 (0.97, 1.27)	0.27	0.02 (−0.001, 0.04)	0.04	0.01 (−0.06, 0.08)	0.23	**0.06 (0.01, 0.12)**
Overweight or obese[b] (N = 433)								
PFAS mixture		0.81 (0.56, 1.18)		0.01 (−0.08, 0.10)		0.23 (−0.05, 0.50)		0.13 (−0.09, 0.36)
PFOA	−0.32	0.78 (0.57, 1.08)	−0.06	−0.01 (−0.09, 0.06)	−0.28	−0.11 (−0.31, 0.09)	−0.16	−0.05 (−0.21, 0.12)
PFOS	−0.05	0.95 (0.60, 1.50)	−0.27	−0.05 (−0.16, 0.05)	0.21	0.13 (−0.14, 0.40)	0.29	0.13 (−0.11, 0.37)
PFNA	0.18	1.09 (0.66, 1.80)	0.13	0.03 (−0.08, 0.14)	0.09	0.06 (−0.24, 0.35)	−0.05	−0.01 (−0.26, 0.24)
PFDA	0.08	1.04 (0.56, 1.90)	0.54	0.12 (−0.05, 0.28)	0.12	0.08 (−0.31, 0.47)	−0.56	−0.17 (−0.51, 0.18)
PFUnDA	−0.41	0.72 (0.40, 1.31)	−0.37	−0.08 (−0.21, 0.06)	−0.66	−0.26 (−0.58, 0.07)	0.12	0.06 (−0.23, 0.35)
PFHxS	0.51	1.26 (0.92, 1.74)	0.08	0.02 (−0.06, 0.09)	0.19	0.12 (−0.07, 0.31)	0.02	0.01 (−0.16, 0.18)
PFDoA	−0.05	0.96 (0.69, 1.34)	−0.27	−0.05 (−0.13, 0.02)	−0.01	0.002 (−0.21, 0.22)	−0.17	−0.05 (−0.23, 0.13)
PFBS	0.11	1.05 (0.77, 1.44)	0.02	0.004 (−0.07, 0.08)	0.06	0.04 (−0.14, 0.21)	0.14	0.06 (−0.10, 0.22)
PFHpA	−0.05	0.96 (0.72, 1.29)	0.21	0.04 (−0.03, 0.11)	0.29	0.18 (−0.003, 0.36)	0.38	**0.16 (0.01, 0.32)**

[a] Quantile-based g-computation model adjusting for maternal age, pre-pregnant BMI, maternal educational level, smoking status, parity, averaged physical activity and economic status.

[b] Quantile-based g-computation model adjusting for maternal age, maternal educational level, smoking status, parity, averaged physical activity and economic status.

from the Spanish INMA Birth Cohort and a cross-sectional study in China found that PFOS and PFHxS were positively associated with impaired glucose tolerance and GDM (Matilla-Santander et al. 2017; Wang et al., 2018b). Another cross-sectional study in the USA found that certain PFAS such as PFOS may alter glucose homeostasis (Preston et al. 2020). Both of these two studies can support our findings on the effect of PFOS. A positive effect of PFHxS was also observed in the MIREC study in Canada (Shapiro et al. 2016). The LIFE study in the USA and another prospective study with repeat measurements in China found that PFOA was positively associated with increased GDM risk and impaired insulin sensibility, which was not repeated in our study (Wang et al., 2018b; Zhang et al. 2015).

In addition, our study suggests that short-chain PFAS such as PFHpA might affect glucose homeostasis during pregnancy. This is supported by a recent nested case-control study showing that PFBS level in early pregnancy was associated with a substantially higher GDM risk (Xu et al. 2020). Given the increasing use of short-chain PFAS as a replacement for long-chain ones, further research on this issue is urgently needed. Besides, no obvious association between PFAS and GDM was observed in another longitudinal study in Denmark (Valvi et al. 2017). Incidentally, we found that PFDoA seemed inversely associated with glucose metabolic outcomes, which was inconsistent with results of previous studies (Rahman et al. 2019; Seo et al. 2018). This finding may be due to chance as many comparisons have been made in the analysis. But the possibility that the disturbance of PFAS on glucose homeostasis might vary by PFAS congeners cannot be ruled out. The inconsistent results may also be attributable to differences in regional variation in PFAS concentrations (higher PFOA in China and higher PFOS in Europe and North America), study design (longitudinal and cross-sectional), sample size, study population and control of confounding factors. More population-based studies, especially from developing countries with high PFAS exposure, are needed to verify these associations.

Our study showed that prepregnant BMI may be an important modifier in the effects of PFAS. A similar modification was also observed in two previous studies (Radesky et al. 2008; Wang et al., 2018a). Both of them treated prepregnant BMI as an important modifier in the exploration of factors affecting the pathogenesis of GDM. In our study, pre-pregnant BMI was associated with GDM and PFAS exposure, but the effect of PFAS on GDM was attenuated in overweight or obese women. This is in contrast to a previous study in which the effects of PFAS were more pronounced among obese children than in normal weight children (Timmermann et al. 2014). Evidence from the non-obese diabetic mice model found that PFUnDA exposure could increase apoptosis in pancreas and reduce macrophage function as well as accelerate the development of insulitis, thus promoting the development of type 1 diabetes (Bodin et al. 2016). PFAS seems more likely to directly interfere with the glucose homeostasis. As obesity is a risk factor for diabetes, the chronic inflammation state in adipose tissue may induce insulin resistance (Roden and Shulman 2019). The effects of environmental factors, therefore, may be masked in overweight and obese pregnant women. Consequently, these associations appeared to be more pronounced in normal weight women. Although PFHpA and pre-pregnant BMI have a significant interaction effect on glucose homeostasis in this study, more evidence is needed to clarify its role in the PFAS exposure- outcome

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relationship.

How PFAS might affect glucose homeostasis remains unclear. PPARs are the most interested metabolic pathway. They are a subfamily of three ligand-inducible transcription factors, belonging to the superfamily of nuclear hormone receptors with three different isoforms: PPAR-α, PPAR-β/δ and PPAR-γ (Botta et al. 2018). *In vivo* and *in vitro* studies showed that PPAR could control a group of genes involved in adipogenesis, lipid metabolism, inflammation and maintenance of metabolic homeostasis. Among them, PPAR-α is mainly expressed in energy-demanding tissues that show high rates of β-oxidation and ketogenesis. Likewise, PPAR-β/δ are ubiquitously expressed in humans and found to be associated with an increase in fatty acid β-oxidation and antioxidant factors and a decrease in nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB). PPAR-γ is expressed at high levels in the adipose tissue (Chen and Yang 2014; Dubois et al. 2017; Saluja et al. 2001).

Some PFAS were found to be able to activate human PPAR-α and weakly bind to human PPAR-γ (Behr et al. 2020). The expression of PPAR-α and PPAR-β may lower glucose uptake, suppress pyruvate transition to acetyl-CoA and promote gluconeogenesis, specifically from glycerol (Botta et al. 2018; Takacs and Abbott 2007). Besides, PFAS may affect insulin resistance by inhibiting phosphorylation and activating protein kinase B (AKT) (Qiu et al. 2016). The AKT pathway involving cellular insulin signaling may be another important mechanism to enhance insulin sensitivity and inhibit hepatic glycogen synthesis and, thus, alter glucose homeostasis (Yan et al., 2015).

A recent study showed that PFOS could stimulate insulin secretion and intracellular calcium level via GPR40. Six compounds (PFHxS, PFOA, PFNA, PFDA, PFUnA and PFDoA) could bind with human GPR40; PFOA and PFDoA could enhance the insulin secretion by β-TC-6 cells (Qin et al. 2020). GPR40-mediated increase in intracellular calcium levels could promote the fusion of insulin-containing vesicles to plasma, resulting in the secretion of insulin, disturb glucose homeostasis and, finally, aggravate insulin resistance (Ghislain and Poitout 2017). However, more studies are needed to elucidate the possible mechanisms.

In addition, previous studies on the associations between environmental contaminants and GDM were mostly cross-sectional in design and the contaminants were evaluated one by one, which may introduce bias by reverse causation and miss the correlations between pollutants (Bobb et al. 2015; Preston et al. 2020; Tanner et al. 2020). Furthermore, the effects of different pollutants on health outcomes may show directional heterogeneity. This calls for a mixed exposure analysis to assess the effect of multiple PFAS exposures. Mixture effects of 30 environmental contaminants on incident metabolic syndrome were assessed in the Prospective Study of the Vasculature in Uppsala Seniors (PIVUS) and found that a mixture of contaminants were associated with future development of the metabolic syndrome in an additive fashion (Lind et al. 2017). To our knowledge, only the Viva Project assessed the joint effects of PFAS on GDM using the BKMR and found that certain PFAS may alter glucose homeostasis during pregnancy, but may not be associated with clinically diagnosed GDM (Preston et al. 2020).

Our study used BKMR and the novel quantile-based g-computation, which allows estimation of parsimonious mixture dose–response parameters by a joint marginal structural model (Keil et al. 2020). The results of these two models showed consistent findings. Compared to the Viva Project, we included a larger sample size and adding stronger evidence to the effect of PFAS on glucose metabolism. The mixed exposure analysis also took into account the non-linear associations between pollutants and health outcomes and the estimation error caused by the insufficient sample size. These methods helped to better assess the overall health effects of specific types of environmental pollutants.

In this study, multiple statistical models provided mutual validation on our findings. The BKMR model fits the nonlinear relationship between PFAS mixture exposure and glucose homeostasis and identify key pollutants better while the qgcomp model can generate the parameter estimate of mixed exposure effects. Both the weight of individual PFAS calculated in the qgcomp model and the specific PFAS congener selected by the ENR can help to assess the main contributors that affect glucose homeostasis. Generally, the results of BKMR and qgcomp were consistent and can be mutually verified. In addition to the robustness of the models, a series of sensitivity analyses such as completed data analysis, data analysis that includes missing information as dummy variables, data analysis using multiple imputation for small values that below the LOD and stratified analysis based on GDM status can help us to assess the stability and reliability of the results and the rationality of the modeling data. The stratified analysis can quantitatively test the possible interaction between PFAS and pre-pregnancy BMI.

Our study has several strengths. First, to our best knowledge, this is the largest study on this topic in the literature, particularly in a developing country with high PFAS exposure level and GDM incidence. Second, our study was a prospective cohort study with a clear temporal relationship between exposure and outcome. Still, our study has some limitations. First, although we have adjusted for as many potential confounders as possible, other important factors such as diet and nutritional supplements have not been included. We could not completely rule out the possibility of residual confounding. Second, only a single estimate of plasma PFAS concentrations in early pregnancy was determined in our study. However, previous studies demonstrated that PFAS concentration during pregnancy was relatively stable due to its long half-life (Papadopoulou et al. 2015). Last, the lack of information on some important metabolic factors such as insulin and C-peptide makes it difficult to evaluate the insulin resistance and pancreatic function.

5. Conclusions

Our study indicates that exposure to PFAS was associated with disturbance of glucose homeostasis in pregnancy and an increased risk of GDM. PFOS, PFNA and PFHpA may be the major contributors. The effect of PFAS on glucose homeostasis in pregnancy is more pronounced in women with normal weight. As PFAS are ubiquitous and GDM not only is common but also has long-term impact on women and their offspring, our findings may have important public health implications.

Credit authorship contribution statement

G-Q Y and J Z did the background research and conceptualization. G-Q Y, Y H and M-F J contributed to the data curation. G-Q Y conducted the formal analysis. M-F J and Y H helped to validate and interpret the results. R A and M N did the PFAS detection, and took responsibility for the integrity and accuracy of the exposure data. T Z, Y T, W-Y W, Z-C L, L-S S and X-P W helped to the data collection. G-Q Y drafted the manuscript. Q D, W-P X and J Z supervised the project, carefully reviewed and edited the manuscript. Each author contributed important intellectual content during manuscript drafting or revision. All authors accept accountability for the overall work.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

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