STATE OF MAINE
DEPARTMENT OF AGRICULTURE, CONSERVATION AND FORESTRY
BOARD OF PESTICIDES CONTROL
28 STATE HOUSE STATION
AUGUSTA, MAINE 04333

PAUL R. LEPAGE
GOVERNOR

WALTER E. WHITCOMB
COMMISSIONER

To: Board of Pesticides Control Members
From: Mary Tomlinson, Pesticides Registrar/Water Quality Specialist
RE: *Bt* Corn Products with Pending Maine Registration Status
Date: July 19, 2017
****

Monsanto Company and Dow AgroSciences LLC have requested registration of several new *Bt* corn products. The new active ingredient (unique identifier 87411-9) is a dsRNA transcript comprising a DvSnf7 inverted repeat sequence which matches that from the Western corn rootworm.

> dsRNA transcript comprising a DvSnf7 inverted repeat sequence derived from *Diabrotica virgifera virgifera*, and the genetic material necessary for its production (vector PV-ZMIR10871) in MON 87411 corn (OECD Unique Identifier MON-87411-9)……..≤ 0.00000044%*

MON 87411 also contains CP4 EPSPS protein (5-enolpyruvylshikimate-3-phosphate synthase) and the genetic material (vector PV-ZMIR10871) necessary for its production in corn event MON 87411…...≤ 0.036%*. The EPSPS protein confers tolerance to glyphosate.

Products designed for the propagation of commercial seed have no spatial refuge which is typical of these types of products. SmartStax PRO Enlist requires a 5% non-*Bt* corn refuge, unless used for seed propagation, and SmartStax PRO Enlist Refuge Advanced contains a 5% interspersed refuge.

The 2015 EPA Registration Decision (RED) and USDA Draft Environmental Assessment for Mon 87411 are attached for your review.

The question posed to the Board is, are these products substantially different from currently registered *Bt* corn products? If so, what further review is recommended?

The labels for the products under consideration are attached for your review.

- EPA Reg. No. 524-631, MON 89034 x TC 1507 X MON 87411 X DAS-59122-7 RIB Complete
- EPA Reg. No. 524-632, MON 89034 x TC 1507 X MON 87411 X DAS-59122-7
- EPA Reg. No. 524-633, MON 87411 x DAS-59122-7
- EPA Reg. No. 524-635, MON 89034 x MIR162 x MON 87411
- EPA Reg. No. 62719-706 SmartStax PRO Enlist
- EPA Reg. No. 62719-707 SmartStax PRO Enlist Refuge Advanced
- EPA Reg. No. 62719-708, MON 89034 x MON 87411 x DAS-59122-7 Insect-Protected, Herbicide-Tolerant Corn
- EPA Reg. No. 62719-709, MON 87411 x DAS-59122-7 Insect-Protected, Herbicide-Tolerant Corn

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maine DEPARTMENT OF Agriculture Conservation & Forestry

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WWW.THINKFIRSTSPRAYLAST.ORG

- EPA Reg. No. 62719-710, TC 1507 x MON 87411 x DAS-59122-7 Insect-Protected, Herbicide-Tolerant Corn
- EPA Reg. No. 62719-711, TC 1507 x MON 87411 x DAS-59122-7 Insect-Protected, Herbicide-Tolerant Corn
- EPA Reg. No. 62719-712, MON 89034 x MON 87411 Insect-Protected, Herbicide-Tolerant Corn
- EPA Reg. No. 62719-713, MON 89034 x TC 1507 x MON 87411 x DAS-59122-7 Insect-Protected, Herbicide-Tolerant Corn
- EPA Reg. No. 62719-714, TC 1507 x MON 87411 Insect-Protected, Herbicide-Tolerant Corn

MON 89034 × TC1507 × MON 87411 × DAS-59122-7
RIB Complete®

Insect-Protected, Herbicide-Tolerant Corn
(OECD Unique Identifier: MON-89Ø34-3 × DAS-Ø15Ø7-1 × MON-87411-9 × DAS-59122-7)

(SmartStax® PRO RIB Complete® corn blend)‡

Active Ingredients:

dsRNA transcript comprising a DvSnf7 inverted repeat sequence derived from *Diabrotica virgifera virgifera*, and the genetic material necessary for its production (vector PV-ZMIR10871) in MON 87411 corn (OECD Unique Identifier MON-87411-9)……..≤ 0.00000044%*

Bacillus thuringiensis Cry1A.105 protein and the genetic material (vector PV-ZMIR245) necessary for its production in corn event MON 89034 (OECD Unique Identifier: MON-89Ø34-3) ……...……≤ 0.0088%*

Bacillus thuringiensis Cry2Ab2 protein and the genetic material (vector PV-ZMIR245) necessary for its production in corn event MON 89034 (OECD Unique Identifier: MON-89Ø34-3) ……≤ 0.0048%*

Bacillus thuringiensis Cry1F protein and the genetic material (vector PHP8999) necessary for its production in corn event TC1507 (OECD Unique Identifier: DAS- Ø15Ø7-1)……..≤ 0.00096%*

Bacillus thuringiensis Cry3Bb1 protein and the genetic material (vector PV-ZMIR10871) necessary for its production in corn event MON 87411 (OECD Unique Identifier: MON-87411-9) ……...≤ 0.0041%*

Bacillus thuringiensis Cry34Ab1 protein and the genetic material (vector PHP17662) necessary for its production in corn event DAS-59122-7 (OECD Unique Identifier: DAS-59122-7)…....≤ 0.012%*

Bacillus thuringiensis Cry35Ab1 protein and the genetic material (vector PHP17662) necessary for its production in corn event DAS-59122-7 (OECD Unique Identifier: DAS-59122-7)…..≤ 0.0026%*

Other Ingredients:

CP4 EPSPS protein (5-enolpyruvylshikimate-3-phosphate synthase) and the genetic material (vector PV-ZMIR10871) necessary for its production in corn event MON 87411…...≤ 0.036%*

PAT protein (phosphinothricin acetyl transferase) and the genetic material (vectors PHP17662 and PHP8999) necessary for its production in corn events TC1507 and DAS-59122-7 ……≤ 0.0001%*

*Percentage (wt/wt) on a dry weight basis for whole plant (forage)

‡ SmartStax® PRO RIB Complete™ corn blend with this refuge configuration contains 95% of the plant-incorporated protectant MON 89034 × TC1507 × MON 88017 × DAS-59122-7 mixed with at least 5% non-*Bt* corn within a single lot of seed.

KEEP OUT OF REACH OF CHILDREN

CAUTION

NET CONTENTS_____

EPA Registration No. 524-631
EPA Establishment No. 524-MO-002

Monsanto Company
800 North Lindbergh Blvd.
St. Louis, MO 63167

DIRECTIONS FOR USE

It is a violation of Federal law to use this product in any manner inconsistent with its labeling. This product must be used as specified in the terms and conditions of the registration.

This Plant-Incorporated Protectant (PIP) may be combined or produced through conventional breeding with other registered plant-incorporated protectants that are similarly approved for use in combination, through conventional breeding, with other registered plant-incorporated protectants to produce inbred corn lines and hybrid corn varieties with combined pesticidal traits.

MON 89034 × TC1507 × MON 87411 × DAS-59122-7 RIB Complete™ corn blend protects corn crops from leaf, stalk, and ear damage caused by lepidopteran corn pests listed on this label and root damage caused by corn rootworm larvae listed on this label. In order to minimize the risk of these pests developing resistance to MON 89034 × TC1507 × MON 87411 × DAS-59122-7 RIB Complete™ corn blend, an insect resistance management plan must be implemented as defined in the registration terms and conditions.

Grower agreements will specify that growers must adhere to the refuge requirements that will be described on the bag or bag/tag for MON 89034 × TC1507 × MON 87411 × DAS-59122-7 RIB Complete™ corn blend or other applicable product use documents.

Sales of corn hybrids that contain Monsanto's *Bt* corn plant-incorporated pesticide(s) must be accompanied by either an IRM/Grower Guide or information on the bag or bag-tag, on planting, production, and insect resistance management, and notes that routine applications of insecticides to control these insects are usually unnecessary when corn containing the *Bt* proteins is planted.

Corn seed bags or bag tags for products containing MON 89034 × TC1507 × MON 87411 × DAS-59122-7 RIB Complete™ corn blend must include the refuge size requirement in text and graphical format.

INSECT RESISTANCE MANAGEMENT

Growers are instructed to read information on insect resistance management in the bag and/or bag-tag.

For the sole purpose of manufacturing and small scale research trials for observation, these refuge requirements do not apply to seed increase/propagation of inbred and hybrid seed corn up to a total of 20,000 acres per county and up to a combined United States (U.S.) total of 250,000 acres per plant-incorporated protectant (PIP) active ingredient per registrant per year.

The seed producer must ensure a minimum of 5% non-PIP refuge seed is included with MON 89034 × TC1507 × MON 87411 × DAS-59122-7 in each lot of seed corn. The refuge seed in the seed mixture may not be treated with seed-applied insecticides for corn rootworm (CRW) control unless the MON 89034 × TC1507 × MON 87411 × DAS-59122-7 seed in the seed mixture receives the same treatment.

The IRM/Grower Guide for MON 89034 × TC1507 × MON 87411 × DAS-59122-7 RIB Complete™ corn blend or comparable information presented on the product bag or bag-tag, must contain the following information:

This product is a seed mixture containing MON 89034 × TC1507 × MON 87411 × DAS-59122-7 and a minimum of 5% non-Bt seed that when planted creates an interspersed refuge within the field. There are no requirements for a separate structured refuge for SmartStax®PRO RIB Complete™ corn blend when planted in the U.S. corn-growing region, including Alaska and Hawaii, because the refuge seed is contained within the bag/container.

The interspersed refuge can only be used by planting seed corn specifically generated by qualified seed producers/conditioners licensed by the registrant. Insecticidal treatments labeled for adult CRW control are discouraged during the time of adult CRW emergence.

The seed mix refuge option for SmartStax®PRO RIB Complete™ corn blend satisfies the refuge requirements in all regions other than in the cotton-growing region where corn earworm is a significant pest as defined below.

Additional refuge requirements in the cotton-growing region where corn earworm is a significant pest

In the cotton-growing region where corn earworm is a significant pest, as defined below, SmartStax®PRO RIB Complete™ corn blend requires the planting of an additional 20% structured refuge (i.e. 20 acres of non-Bt corn for every 80 acres of SmartStax®PRO RIB Complete™ corn blend planted).

The 20% refuge must be planted with corn hybrids that do not contain Bt technologies for the control of corn rootworms or corn borers. The refuge and the SmartStax®PRO RIB Complete™ corn blend should be sown on the same day, or with the shortest window possible between

planting dates to ensure that corn root development is similar among varieties. The structured refuge may be planted as an in-field or adjacent (e.g., across the road) refuge or planted as a separate block that is within ½ mile of the SmartStax®PRO RIB Complete™ corn blend field. In-field refuge options include blocks, perimeter strips (i.e., strips around the field), or in-field strips. If perimeter or in-field strips are implemented, the strips must be at least 4 consecutive rows wide. The refuge can be protected from lepidopteran damage by use of non-Bt insecticides if the population of one or more target lepidopteran pests of SmartStax®PRO RIB Complete™ corn blend in the refuge exceeds economic thresholds. In addition, the refuge can be protected from CRW damage by an appropriate seed treatment or soil insecticide; however, insecticides labeled for adult CRW control must be avoided in the refuge during the period of CRW adult emergence. Economic thresholds will be determined using methods recommended by local or regional professionals (e.g., Extension Service agents, crop consultants).

The cotton-growing region requiring the additional 20% refuge consists of the following states: Alabama, Arkansas, Georgia, Florida, Louisiana, North Carolina, Mississippi, South Carolina, Oklahoma (only the counties of Beckham, Caddo, Comanche, Custer, Greer, Harmon, Jackson, Kay, Kiowa, Tillman, and Washita), Tennessee (only the counties of Carroll, Chester, Crockett, Dyer, Fayette, Franklin, Gibson, Hardeman, Hardin, Haywood, Lake, Lauderdale, Lincoln, Madison, Obion, Rutherford, Shelby, and Tipton), Texas (except the counties of Carson, Dallam, Hansford, Hartley, Hutchinson, Lipscomb, Moore, Ochiltree, Roberts, and Sherman), Virginia (only the counties of Dinwiddie, Franklin City, Greensville, Isle of Wight, Northampton, Southampton, Suffolk City, Surrey, and Sussex) and Missouri (only the counties of Dunklin, New Madrid, Pemiscot, Scott, and Stoddard).

Corn Insects Controlled or Suppressed

European corn borer (ECB)	*Ostrinia nubilalis*
Southwestern corn borer (SWCB)	*Diatraea grandiosella*
Southern cornstalk borer (SCSB)	*Diatraea crambidoides*
Corn earworm (CEW)	*Helicoverpa zea*
Fall armyworm (FAW)	*Spodoptera frugiperda*
Stalk borer	*Papaipema nebris*
Lesser corn stalk borer	*Elasmopalpus lignosellus*
Sugarcane borer (SCB)	*Diatraea saccharalis*
Western bean cutworm (WBC)	*Richia albicosta*
Black cutworm	*Agrotis ipsilon*
Western corn rootworm (WCRW)	*Diabrotica virgifera virgifera*
Northern corn rootworm (NCRW)	*Diabrotica barberi*
Mexican corn rootworm (MCRW)	*Diabrotica virgifera zeae*

MON 89034 × TC1507 × MON 87411 × DAS-59122-7 seed blend is a product of Monsanto's research program offering unique genetic characteristics for specific grower needs and may be protected by one or more of the following U.S. patents that can be found at http://www.monsantotechnology.com

MON 89034 × TC1507 × MON 87411 × DAS-59122-7

Insect-Protected, Herbicide-Tolerant Corn
(OECD Unique Identifier: MON-89Ø34-3 × DAS-Ø15Ø7-1 × MON-87411-9 × DAS-59122-7)

Active Ingredients:
dsRNA transcript comprising a DvSnf7 inverted repeat sequence derived from *Diabrotica virgifera virgifera*, and the genetic material necessary for its production (vector PV-ZMIR10871) in MON 87411 corn (OECD Unique Identifier MON-87411-9)……..≤ 0.00000044%*

Bacillus thuringiensis Cry1A.105 protein and the genetic material (vector PV-ZMIR245) necessary for its production in corn event MON 89034 (OECD Unique Identifier: MON-89Ø34-3) ……...……≤ 0.0088%*

Bacillus thuringiensis Cry2Ab2 protein and the genetic material (vector PV-ZMIR245) necessary for its production in corn event MON 89034 (OECD Unique Identifier: MON-89Ø34-3) ……≤ 0.0048%*

Bacillus thuringiensis Cry1F protein and the genetic material (vector PHP8999) necessary for its production in corn event TC1507 (OECD Unique Identifier: DAS- Ø15Ø7-1)……..≤ 0.00096%*

Bacillus thuringiensis Cry3Bb1 protein and the genetic material (vector PV-ZMIR10871) necessary for its production in corn event MON 87411 (OECD Unique Identifier: MON-87411-9) ……...≤ 0.0041%*

Bacillus thuringiensis Cry34Ab1 protein and the genetic material (vector PHP17662) necessary for its production in corn event DAS-59122-7 (OECD Unique Identifier: DAS-59122-7)…....≤ 0.012%*

Bacillus thuringiensis Cry35Ab1 protein and the genetic material (vector PHP17662) necessary for its production in corn event DAS-59122-7 (OECD Unique Identifier: DAS-59122-7)…...≤ 0.0026%*

Other Ingredients:
CP4 EPSPS protein (5-enolpyruvylshikimate-3-phosphate synthase) and the genetic material (vector PV-ZMIR10871) necessary for its production in corn event MON 87411…...≤ 0.036%*

PAT protein (phosphinothricin acetyl transferase) and the genetic material (vectors PHP17662 and PHP8999) necessary for its production in corn events TC1507 and DAS-59122-7 ……≤ 0.0001%*

*Percentage (wt/wt) on a dry weight basis for whole plant (forage)

KEEP OUT OF REACH OF CHILDREN

CAUTION

NET CONTENTS_____

EPA Registration No. 524-632
EPA Establishment No. 524-MO-002

Monsanto Company
800 North Lindbergh Blvd.
St. Louis, MO 63167

DIRECTIONS FOR USE

It is a violation of Federal law to use this product in any manner inconsistent with its labeling. Information regarding commercial production reflected here and in the terms and conditions of this registration must be included in the Technology Use Guide.

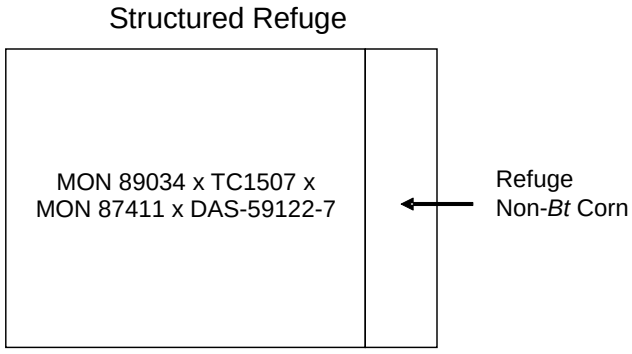
MON 89034 × TC1507 × MON 87411 × DAS-59122-7 protects corn crops from leaf, stalk, and ear damage caused by corn borers and root damage caused by corn rootworm larvae. In order to minimize the risk of these pests developing resistance to MON 89034 × TC1507 × MON 87411 × DAS-59122-7 corn, an insect resistance management plan must be implemented which includes planting of a structured refuge. Growers who fail to comply with the IRM requirements risk losing access to Monsanto's corn PIP products.

For the sole purpose of manufacturing and small scale research trials for observation, these refuge requirements do not apply to seed increase/propagation of inbred and hybrid seed corn up to a total of 20,000 acres per county and up to a combined United States (U.S.) total of 250,000 acres per plant-incorporated protectant (PIP) active ingredient per registrant per year.

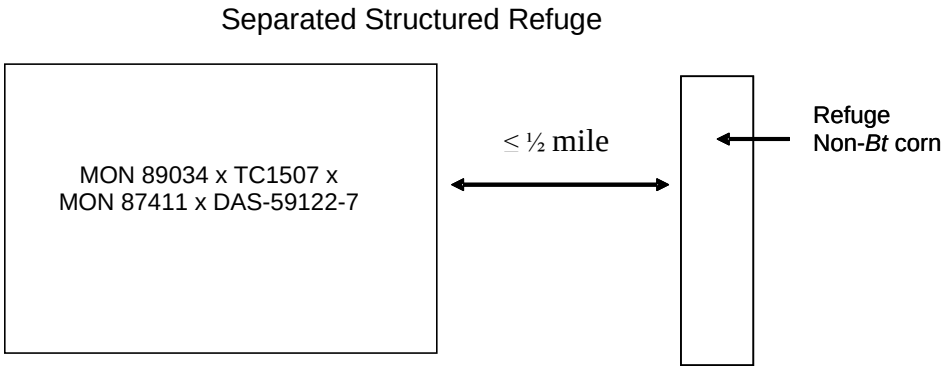
Several options for deployment of the refuge for MON 89034 × TC1507 × MON 87411 × DAS-59122-7 are available to growers. These options are based on the planting of MON 89034 × TC1507 × MON 87411 × DAS-59122-7 in cotton or non-cotton growing regions and the insect pressure present in those locations. The refuge sizes for these regions are either 5% (i.e. 5 acres of non-*Bt* corn for every 95 acres MON 89034 × TC1507 × MON 87411 × DAS-59122-7 planted) or 20% (20 acres of non-*Bt* corn for every 80 acres of MON 89034 × TC1507 × MON 87411 × DAS-59122-7 planted), and are presented in the table below:

Region	Refuge size	In-field or adjacent refuge	Refuge separated by up to ½ mile
Cotton belt where CEW is a significant pest and WCRW, NCRW and MCRW are not significant: NC, SC, GA, FL, TN, AL, MS, LA, AR, northern TX	20% non-*Bt* corn	Yes	Yes
Cotton belt where CEW is a significant pest and MCRW is significant: southern TX	20% non-*Bt* corn	Yes	No
Cotton belt where CEW is not a significant pest and WCRW, NCRW and MCRW are not significant: NM, AZ, CA, NV Non-cotton states where WCRW, NCRW and MCRW are not significant: OR, WA, ID, MT, WY, UT, CO, OK, VA, WV, PA, MD, DE, CT, RI, NJ, NY, ME, MA, NH, VT, HI, AK	5% non-*Bt* corn	Yes	Yes
Non-cotton-growing where WCRW, NCRW and/or MCRW are significant: KS, NE, SD, ND, MN, IA, MO, IL, WI, MI, IN, OH, KY	5% non-*Bt* corn	Yes	No

If corn rootworms are significant within a region, the structured refuge must be planted as an in-field or adjacent refuge using corn hybrids that do not contain *Bt* technologies for the control of corn borers or corn rootworms. It can be planted as a block within or adjacent (e.g., across the road) to the MON 89034 × TC1507 × MON 87411 × DAS-59122-7, perimeter strips (i.e., strips around the field), or in-field strips. If perimeter or in-field strips are implemented, the strips must be at least 4 consecutive rows wide. The refuge can be protected from lepidopteran damage by use of non-*Bt* insecticides if the population of one or more target lepidopteran pests of MON 89034 × TC1507 × MON 87411 × DAS-59122-7 in the refuge exceeds economic threshold. In addition, the refuge can be protected from CRW damage by an appropriate seed treatment or soil insecticide; however, insecticides labeled for adult CRW control should be avoided in the refuge during the period of CRW adult emergence. Economic thresholds will be determined using methods recommended by local or regional professionals (e.g., Extension Service agents, crop consultants). A schematic of one common refuge deployment option is shown below:



If corn rootworms are not significant within a region, the structured refuge may be planted as an in-field or adjacent refuge, or as a separate block that is within ½ mile of the MON 89034 × TC1507 × MON 87411 × DAS-59122-7 field. The structured refuge must be planted with corn hybrids that do not contain *Bt* technologies for the control of corn borers or corn rootworms. Economic thresholds will be determined using methods recommended by local or regional professionals (e.g., Extension Service agents, crop consultants). A schematic of one refuge option with the refuge planted within a ½ mile of the MON 89034 × TC1507 × MON 87411 × DAS-59122-7 field is shown below:



Corn Insects Controlled or Suppressed

European corn borer (ECB)	*Ostrinia nubilalis*
Southwestern corn borer (SWCB)	*Diatraea grandiosella*
Southern cornstalk borer (SCSB)	*Diatraea crambidoides*
Corn earworm (CEW)	*Helicoverpa zea*
Fall armyworm (FAW)	*Spodoptera frugiperda*
Stalk borer	*Papaipema nebris*
Lesser corn stalk borer	*Elasmopalpus lignosellus*
Sugarcane borer (SCB)	*Diatraea saccharalis*
Western bean cutworm (WBC)	*Richia albicosta*
Black cutworm	*Agrotis ipsilon*
Western corn rootworm (WCRW)	*Diabrotica virgifera virgifera*
Northern corn rootworm (NCRW)	*Diabrotica barberi*
Mexican corn rootworm (MCRW)	*Diabrotica virgifera zeae*

MON 89034 × TC1507 × MON 87411 × DAS-59122-7 is a product of Monsanto's research program offering unique genetic characteristics for specific grower needs and may be protected by one or more of the following U.S. patents that can be found at http://www.monsantotechnology.com

MON 87411 × DAS-59122-7

(OECD Unique Identifier: MON-87411-9 × DAS-59122-7)

Active Ingredients:

dsRNA transcript comprising a DvSnf7 inverted repeat sequence derived from *Diabrotica virgifera virgifera*, and the genetic material (vector PV-ZMIR10871) necessary for its production in MON 87411 corn (OECD Unique Identifier MON-87411-9).....≤ 0.00000044%*

Bacillus thuringiensis Cry3Bb1 protein and the genetic material (vector PV-ZMIR10871) necessary for its production in MON 87411 corn (OECD Unique Identifier: MON-87411-9) .....≤ 0.0041%*

Bacillus thuringiensis Bacillus thuringiensis Cry34Ab1 protein and the genetic material (vector PHP17662) necessary for its production in DAS-59122-7 corn (OECD Unique Identifier: DAS-59122-7).....≤ 0.012%*

Bacillus thuringiensis Cry35Ab1 protein and the genetic material (vector PHP17662) necessary for its production in DAS-59122-7 corn (OECD Unique Identifier: DAS-59122-7).....≤ 0.0026%*

Other Ingredients:

CP4 EPSPS protein (5-enolpyruvylshikimate-3-phosphate synthase) and the genetic material (vector PV-ZMIR10871) necessary for its production in MON 87411 corn .....≤ 0.036%*

PAT protein (phosphinothricin acetyl transferase) and the genetic material (vectors PHP17662 and PHP8999) necessary for its production in TC1507 and DAS-59122-7 corn .....≤ 0.0001%*

**Percentage (wt/wt) on a dry weight basis for whole plant (forage)

KEEP OUT OF REACH OF CHILDREN

CAUTION

NET CONTENTS_____

EPA Registration No. 524- 633
EPA Establishment No. 524-MO-002

Monsanto Company
800 North Lindbergh Blvd.
St. Louis, MO 63167

DIRECTIONS FOR USE

It is a violation of Federal law to use this product in any manner inconsistent with its labeling.

This plant-incorporated protectant (PIP) may be combined through conventional breeding with other registered plant-incorporated protectants that are similarly approved for use in combination, through conventional breeding, with other registered plant-incorporated protectants to produce inbred corn lines and hybrid corn varieties with combined pesticidal traits.

This product may be used for breeding purposes, agronomic testing, increasing inbred seed stocks, and producing hybrid seed on up to a combined United States (US) total of 5,000 acres per plant incorporated protectant (PIP) active ingredient per registrant per year. Commercial plantings of this product, for the purposes of grain production and controlling corn insect pests, are prohibited.

Harvested seed are not allowed for sale as a commercial seed in the US under the current conditions of this registration.

There are no refuge requirements for planting MON 87411 x DAS-59122-7 Corn.

MON 89034 × MIR162 × MON 87411

(OECD Unique Identifier: MON-89Ø34-3 × SYN-IR162-4 × MON-87411-9)

Active Ingredients:

Bacillus thuringiensis Cry1A.105 protein and the genetic material necessary for its production (vector PV-ZMIR245) in event MON 89034 corn (Unique Identifier MON-89Ø34-3) ..... ≤0.0027%*

Bacillus thuringiensis Cry2Ab2 protein and the genetic material necessary for its production (vector PV-ZMIR245) in event MON 89034 corn (Unique Identifier MON-89Ø34-3) ..... ≤0.0071%*

Bacillus thuringiensis Vip3Aa20 protein and the genetic material necessary for its production (vector pNOV1300) in event MIR162 corn ((Unique Identifier SYN-IR162-4) ..... ≤0.014%*

Bacillus thuringiensis Cry3Bb1 protein and the genetic material (vector PV-ZMIR10871) necessary for its production in corn event MON 87411 (OECD Unique Identifier: MON-87411-9)..... ...≤ 0.0086%*

dsRNA transcript comprising a *DvSnf7* inverted repeat sequence derived from *Diabrotica virgifera virgifera*, and the genetic material necessary for its production (vector PV-ZMIR10871) in MON 87411 corn (OECD Unique Identifier MON-87411-9)..... ≤ 0.00000037%*

Other Ingredients:

Phosphomannose isomerase (PMI) marker protein and the genetic material necessary (vector pNOV1300) for its production in the event MIR162 corn ..... ≤0.00085%*

CP4 EPSPS protein (5-enolpyruvylshikimate-3-phosphate synthase) and the genetic material (vector PV-ZMIR10871) necessary for its production in corn event MON 87411..... ≤ 0.029%*

*Percentage (wt/wt) on a dry weight basis for whole plant (forage)

KEEP OUT OF REACH OF CHILDREN

CAUTION

NET CONTENTS_____

EPA Registration No. 524-635
EPA Establishment No. 524-MO-002

Monsanto Company
800 North Lindbergh Blvd.
St Louis, MO 63167

DIRECTIONS FOR USE

It is a violation of Federal law to use this product in any manner inconsistent with its labeling.

This plant-incorporated protectant (PIP) may be combined through conventional breeding with other registered plant-incorporated protectants that are similarly approved for use in combination, through conventional breeding, with other registered plant-incorporated protectants to produce inbred corn lines and hybrid corn varieties with combined pesticidal traits.

This product may be used for breeding purposes, agronomic testing, increasing inbred seed stocks, and producing hybrid seed on up to a combined United States (US) total of 5,000 acres per plant incorporated protectant (PIP) active ingredient per registrant per year. Commercial plantings of this product, for the purposes of grain production and controlling corn insect pests, are prohibited.

Harvested seed are not allowed for sale as a commercial seed in the US under the current conditions of this registration.

There are no refuge requirements for planting MON 89034 × MIR162 × MON 87411 Corn.

Plant-Incorporated Protectant Label

SmartStax®PRO Enlist™

(Alternate Brand Name: SmartStax®PRO)
(Alternate Brand Name: MON 89034 × TC1507 × MON 87411 × DAS-59122-7 Insect-Protected, Herbicide-Tolerant Corn)
(OECD Unique Identifier: MON-89Ø34-3 × DAS-Ø15Ø7-1 × MON-87411-9 × DAS-59122-7)

(Alternate Brand Name: MON 87427 × MON 89034 × TC1507 × MON 87411 × DAS-59122-7 Insect-Protected, Herbicide-Tolerant Corn)
(OECD Unique Identifier : MON-87427-7 × MON-89Ø34-3 × DAS-Ø15Ø7-1 × MON-87411-9 × DAS-59122-7)

(Alternate Brand Name: MON 87427 × MON 89034 × TC1507 × MON 87411 × DAS-59122-7 × DAS-40278-9 Insect-Protected, Herbicide-Tolerant Corn)
(OECD Unique Identifier: MON-87427-7 × MON-89Ø34-3 × DAS-Ø15Ø7-1 × MON-87411-9 × DAS-59122-7 × DAS-4Ø278-9)

Active Ingredients:
dsRNA transcript comprising a DvSnf7 inverted repeat sequence derived from *Diabrotica virgifera virgifera*, and the genetic material necessary for its production (vector PV-ZMIR10871) in MON 87411 corn (OECD Unique Identifier MON-87411-9)……..≤ 0.00000044%*

Bacillus thuringiensis Cry1A.105 protein and the genetic material (vector PV-ZMIR245) necessary for its production in corn event MON 89034 (OECD Unique Identifier: MON-89Ø34-3)
……...……≤ 0.0088%*

Bacillus thuringiensis Cry2Ab2 protein and the genetic material (vector PV-ZMIR245) necessary for its production in corn event MON 89034 (OECD Unique Identifier: MON-89Ø34-3)
……≤ 0.0048%*

Bacillus thuringiensis Cry1F protein and the genetic material (vector PHP8999) necessary for its production in corn event TC1507 (OECD Unique Identifier: DAS- Ø15Ø7-1)……..≤ 0.00096%*

Bacillus thuringiensis Cry3Bb1 protein and the genetic material (vector PV-ZMIR10871) necessary for its production in corn event MON 87411 (OECD Unique Identifier: MON-87411-9) .....
……...≤ 0.0041%*

Bacillus thuringiensis Cry34Ab1 protein and the genetic material (vector PHP17662) necessary for its production in corn event DAS-59122-7 (OECD Unique Identifier: DAS-59122-7) ..... …....≤ 0.012%*

Bacillus thuringiensis Cry35Ab1 protein and the genetic material (vector PHP17662) necessary for its production in corn event DAS-59122-7 (OECD Unique Identifier: DAS-59122-7) ..... …...≤ 0.0026%*

Other Ingredients:
CP4 EPSPS protein (5-enolpyruvylshikimate-3-phosphate synthase) and the genetic material (vector PV-ZMIR10871) necessary for its production in corn event MON 87411…...≤ 0.036%*

PAT protein (phosphinothricin acetyl transferase) and the genetic material (vectors PHP17662 and PHP8999) necessary for its production in corn events TC1507 and DAS-59122-7
……≤ 0.0001%*

*Maximum percent (%) dry weight basis for whole plant (forage)

SmartStax® multi-event technology developed by Dow AgroSciences LLC and Monsanto
SmartStax® is a trademark of Monsanto Technology LLC
™ Enlist is a trademark of Dow AgroSciences LLC

KEEP OUT OF REACH OF CHILDREN NET CONTENTS _____

CAUTION

EPA Registration No. 62719-706

EPA Establishment No. 62719-IN-1

Dow AgroSciences LLC
9330 Zionsville Road
Indianapolis, IN 46268

DIRECTIONS FOR USE

It is a violation of Federal law to use this product in any manner inconsistent with its labeling. Information regarding commercial production reflected here and in the terms and conditions of this registration must be included in the Technology Use Guide.

SmartStax®PRO Enlist™ protects corn crops from leaf, stalk, and ear damage caused by corn borers and root damage caused by corn rootworm larvae. In order to minimize the risk of these pests developing resistance to SmartStax®PRO Enlist™ corn, an insect resistance management plan must be implemented which includes planting of a structured refuge. Growers who fail to comply with the IRM requirements risk losing access to Dow AgroSciences's corn PIP products.

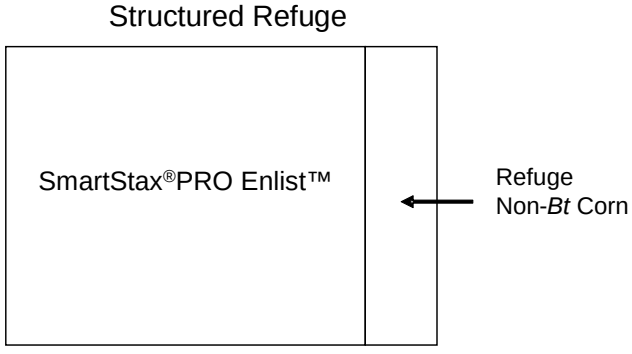
These refuge requirements do not apply to seed increase/propagation of inbred and hybrid seed corn and small scale research trials for observation.

Several options for deployment of the refuge for SmartStax®PRO Enlist™ are available to growers. These options are based on the planting of SmartStax®PRO Enlist™ in cotton or non-cotton growing regions and the insect pressure present in those locations. The refuge sizes for these regions are either 5% (i.e. 5 acres of non-*Bt* corn for every 95 acres SmartStax®PRO Enlist™ planted) or 20% (20 acres of non-*Bt* corn for every 80 acres of SmartStax®PRO Enlist™ planted), and are presented in the table below:

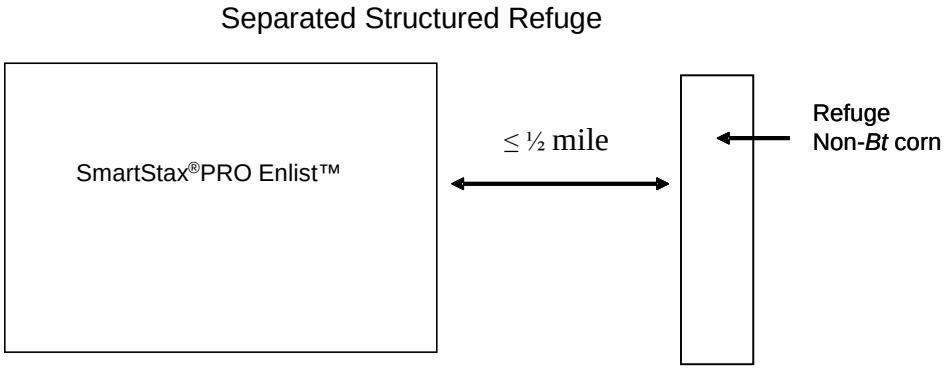
Region	Refuge size	In-field or adjacent refuge	Refuge separated by up to ½ mile
Cotton belt where CEW is a significant pest and WCRW, NCRW and MCRW are not significant: NC, SC, GA, FL, TN, AL, MS, LA, AR, northern TX	20% non-*Bt* corn	Yes	Yes

Cotton belt where CEW is a significant pest and MCRW is significant: southern TX	20% non-*Bt* corn	Yes	No
Cotton belt where CEW is not a significant pest and WCRW, NCRW and MCRW are not significant: NM, AZ, CA, NV Non-cotton states where WCRW, NCRW and MCRW are not significant: OR, WA, ID, MT, WY, UT, CO, OK, VA, WV, PA, MD, DE, CT, RI, NJ, NY, ME, MA, NH, VT, HI, AK	5% non-*Bt* corn	Yes	Yes
Non-cotton-growing where WCRW, NCRW and/or MCRW are significant: KS, NE, SD, ND, MN, IA, MO, IL, WI, MI, IN, OH, KY	5% non-*Bt* corn	Yes	No

If corn rootworms are significant within a region, the structured refuge must be planted as an in-field or adjacent refuge using corn hybrids that do not contain *Bt* technologies for the control of corn borers or corn rootworms. It can be planted as a block within or adjacent (e.g., across the road) to the SmartStax®PRO Enlist™, perimeter strips (i.e., strips around the field), or in-field strips. If perimeter or in-field strips are implemented, the strips must be at least 4 consecutive rows wide. The refuge can be protected from lepidopteran damage by use of non-*Bt* insecticides if the population of one or more target lepidopteran pests of SmartStax®PRO Enlist™ in the refuge exceeds economic threshold. In addition, the refuge can be protected from CRW damage by an appropriate seed treatment or soil insecticide; however, insecticides labeled for adult CRW control should be avoided in the refuge during the period of CRW adult emergence. Economic thresholds will be determined using methods recommended by local or regional professionals (e.g., Extension Service agents, crop consultants). A schematic of one common refuge deployment option is shown below:



If corn rootworms are not significant within a region, the structured refuge may be planted as an in-field or adjacent refuge, or as a separate block that is within ½ mile of the SmartStax®PRO Enlist™ field. The structured refuge must be planted with corn hybrids that do not contain *Bt* technologies for the control of corn borers or corn rootworms. Economic thresholds will be determined using methods recommended by local or regional professionals (e.g., Extension Service agents, crop consultants). A schematic of one refuge option with the refuge planted within a ½ mile of the SmartStax®PRO Enlist™ field is shown below:



Corn Insects Controlled or Suppressed

European corn borer (ECB)	*Ostrinia nubilalis*
Southwestern corn borer (SWCB)	*Diatraea grandiosella*
Southern cornstalk borer (SCSB)	*Diatraea crambidoides*
Corn earworm (CEW)	*Helicoverpa zea*
Fall armyworm (FAW)	*Spodoptera frugiperda*
Stalk borer	*Papaipema nebris*
Lesser corn stalk borer	*Elasmopalpus lignosellus*
Sugarcane borer (SCB)	*Diatraea saccharalis*
Western bean cutworm (WBC)	*Richia albicosta*
Black cutworm	*Agrotis ipsilon*
Western corn rootworm (WCRW)	*Diabrotica virgifera virgifera*
Northern corn rootworm (NCRW)	*Diabrotica barberi*
Mexican corn rootworm (MCRW)	*Diabrotica virgifera zeae*

EPA Accepted: 6/8/2017

Plant-Incorporated Protectant Label

SmartStax®PRO Enlist™ Refuge Advanced®‡

(Alternate Brand Name: SmartStax®PRO Refuge Advanced®)‡
(Alternate Brand Name: MON 89034 × TC1507 × MON 87411 × DAS-59122-7
Insect-Protected, Herbicide-Tolerant Corn with interspersed refuge)
(OECD Unique Identifier: MON-89Ø34-3 × DAS-Ø15Ø7-1 × MON-87411-9 × DAS-59122-7)

(Alternate Brand Name: MON 87427 × MON 89034 × TC1507 × MON 87411 × DAS-59122-7
Insect-Protected, Herbicide-Tolerant Corn with interspersed refuge)
(OECD Unique Identifier : MON-87427-7 × MON-89Ø34-3 × DAS-Ø15Ø7-1 × MON-87411-9 × DAS-59122-7)

(Alternate Brand Name: MON 87427 × MON 89034 × TC1507 × MON 87411 × DAS-59122-7 × DAS-40278-9
Insect-Protected, Herbicide-Tolerant Corn with interspersed refuge)
(OECD Unique Identifier: MON-87427-7 × MON-89Ø34-3 × DAS-Ø15Ø7-1 × MON-87411-9 × DAS-59122-7 × DAS-4Ø278-9)

Active Ingredients:

dsRNA transcript comprising a DvSnf7 inverted repeat sequence derived from *Diabrotica virgifera virgifera*, and the genetic material necessary (vector PV-ZMIR10871) for its production in corn event MON 87411 (OECD Unique Identifier MON-87411-9)…….……....≤ 0.00000044%*

Bacillus thuringiensis Cry1A.105 protein and the genetic material (vector PV-ZMIR245) necessary for its production in corn event MON 89034 (OECD Unique Identifier: MON-89Ø34-3) ……...……≤ 0.0088%*

Bacillus thuringiensis Cry2Ab2 protein and the genetic material (vector PV-ZMIR245) necessary for its production in corn event MON 89034 (OECD Unique Identifier: MON-89Ø34-3) .……≤ 0.0048%*

Bacillus thuringiensis Cry1F protein and the genetic material (vector PHP8999) necessary for its production in corn event TC1507 (OECD Unique Identifier: DAS- Ø15Ø7-1)……..≤ 0.00096%*

Bacillus thuringiensis Cry3Bb1 protein and the genetic material (vector PV-ZMIR10871) necessary for its production in corn event MON 87411 (OECD Unique Identifier: MON-87411-9) ……...≤ 0.0041%*

Bacillus thuringiensis Cry34Ab1 protein and the genetic material (vector PHP17662) necessary for its production in corn event DAS-59122-7 (OECD Unique Identifier: DAS-59122-7) …….....≤ 0.012%*

Bacillus thuringiensis Cry35Ab1 protein and the genetic material (vector PHP17662) necessary for its production in corn event DAS-59122-7 (OECD Unique Identifier: DAS-59122-7) ……...≤ 0.0026%*

Other Ingredients:
CP4 EPSPS protein (5-enolpyruvylshikimate-3-phosphate synthase) and the genetic material (vector PV-ZMIR10871) necessary for its production in corn event MON 87411……...≤ 0.036%*

The marker protein, PAT (phosphinothricin acetyl transferase) and the genetic material (vectors PHP17662 and PHP8999) necessary for its production in corn events TC1507 and DAS-59122-7 ……≤ 0.0001%*

*Maximum percent (%) dry weight basis for whole plant (forage)

‡ SmartStax®PRO Enlist™ Refuge Advanced® and SmartStax®PRO Refuge Advanced® seed with this refuge configuration contains 95% MON 89034 × TC1507 × MON 88017 × DAS-59122-7 mixed with at least 5% non-*Bt* corn within a single lot of seed.

KEEP OUT OF REACH OF CHILDREN NET CONTENTS _____

CAUTION

EPA Registration No. 62719-707

EPA Establishment No. 62719-IN-1

Dow AgroSciences LLC
9330 Zionsville Road
Indianapolis, IN 46268

DIRECTIONS FOR USE

It is a violation of Federal law to use this product in any manner inconsistent with its labeling. This product must be used as specified in the terms and conditions of the registration.

This Plant-Incorporated Protectant (PIP) may be combined or produced through conventional breeding with other registered plant-incorporated protectants that are similarly approved for use in combination, through conventional breeding, with other registered plant-incorporated protectants to produce inbred corn lines and hybrid corn varieties with combined pesticidal traits.

SmartStax®PRO Enlist™ Refuge Advanced® corn blend protects corn crops from leaf, stalk, and ear damage caused by lepidopteran corn pests listed on this label and root damage caused by corn rootworm larvae listed on this label. In order to minimize the risk of these pests developing resistance to SmartStax®PRO Enlist™ Refuge Advanced® corn blend, an insect resistance management plan must be implemented as defined in the registration terms and conditions.

Grower agreements will specify that growers must adhere to the refuge requirements that will be described on the bag or bag/tag for SmartStax®PRO Enlist™ Refuge Advanced® corn blend or other applicable product use documents.

Sales of corn hybrids that contain Dow AgroSciences's *Bt* corn plant-incorporated pesticide(s) must be accompanied by either an IRM/Grower Guide or information on the bag or bag-tag, on planting, production, and insect resistance management, and notes that routine applications of insecticides to control these insects are usually unnecessary when corn containing the *Bt* proteins is planted.

Corn seed bags or bag tags for products containing SmartStax®PRO Enlist™ Refuge Advanced® corn blend must include the refuge size requirement in text and graphical format.

INSECT RESISTANCE MANAGEMENT

Growers are instructed to read information on insect resistance management in the bag and/or bag-tag.

These refuge requirements do not apply to seed increase/propagation of inbred and hybrid seed corn up to a total of 20,000 acres per county and up to a combined United States (U.S.) total of 250,000 acres per plant-incorporated protectant (PIP) active ingredient per registrant per year.

The seed producer must ensure a minimum of 5% non-PIP refuge seed is included with SmartStax®PRO Enlist™ in each lot of seed corn. The refuge seed in the seed mixture may not be treated with seed-applied insecticides for corn rootworm (CRW) control unless the SmartStax®PRO Enlist™ seed in the seed mixture receives the same treatment.

The IRM/Grower Guide for SmartStax®PRO Enlist™ Refuge Advanced® corn blend or comparable information presented on the product bag or bag-tag, must contain the following information:

This product is a seed mixture containing SmartStax®PRO Enlist™ and a minimum of 5% non-Bt seed that when planted creates an interspersed refuge within the field. There are no requirements for a separate structured refuge for SmartStax®PRO Enlist™ Refuge Advanced® corn blend when planted in the U.S. corn-growing region, including Alaska and Hawaii, because the refuge seed is contained within the bag/container.

The interspersed refuge can only be used by planting seed corn specifically generated by qualified seed producers/conditioners licensed by the registrant. Insecticidal treatments labeled for adult CRW control are discouraged during the time of adult CRW emergence.

The seed mix refuge option for SmartStax®PRO Enlist™ Refuge Advanced® corn blend satisfies the refuge requirements in all regions other than in the cotton-growing region where corn earworm is a significant pest as defined below.

Additional refuge requirements in the cotton-growing region where corn earworm is a significant pest
In the cotton-growing region where corn earworm is a significant pest, as defined below, SmartStax®PRO Enlist™ Refuge Advanced® corn blend requires the planting of an additional 20% structured refuge (i.e. 20 acres of non-Bt corn for every 80 acres of SmartStax®PRO Enlist™ Refuge Advanced® corn blend planted).

The 20% refuge must be planted with corn hybrids that do not contain *Bt* technologies for the control of corn rootworms or corn borers. The refuge and the SmartStax®PRO Enlist™ Refuge Advanced® corn blend should be sown on the same day, or with the shortest window possible between planting dates to ensure that corn root development is similar among varieties. The structured refuge may be planted as an in-field or adjacent (e.g., across the road) refuge or planted as a separate block that is within ½ mile of the SmartStax®PRO Enlist™ Refuge Advanced® corn blend field. In-field refuge options include blocks, perimeter strips (i.e., strips around the field), or in-field strips. If perimeter or in-field strips are implemented, the strips must be at least 4 consecutive rows wide. The refuge can be protected from lepidopteran damage by use of non-Bt insecticides if the population of one or more target lepidopteran pests of SmartStax®PRO Enlist™ Refuge Advanced® corn blend in the refuge exceeds economic thresholds. In addition, the refuge can be protected from CRW damage by an appropriate seed treatment or soil insecticide; however, insecticides labeled for adult CRW control must be avoided in the refuge during the period of CRW adult emergence. Economic thresholds will be determined using methods recommended by local or regional professionals (e.g., Extension Service agents, crop consultants).

The cotton-growing region requiring the additional 20% refuge consists of the following states: Alabama, Arkansas, Georgia, Florida, Louisiana, North Carolina, Mississippi, South Carolina, Oklahoma (only the counties of Beckham, Caddo, Comanche, Custer, Greer, Harmon, Jackson, Kay, Kiowa, Tillman, and Washita), Tennessee (only the counties of Carroll, Chester, Crockett, Dyer, Fayette, Franklin, Gibson, Hardeman, Hardin, Haywood, Lake, Lauderdale, Lincoln, Madison, Obion, Rutherford, Shelby, and Tipton), Texas (except the counties of Carson, Dallam, Hansford, Hartley, Hutchinson, Lipscomb, Moore, Ochiltree, Roberts, and Sherman), Virginia (only the counties of Dinwiddie, Franklin City, Greensville, Isle of Wight, Northampton, Southampton, Suffolk City, Surrey, and Sussex) and Missouri (only the counties of Dunklin, New Madrid, Pemiscot, Scott, and Stoddard).

Corn Insects Controlled or Suppressed

European corn borer (ECB)	*Ostrinia nubilalis*
Southwestern corn borer (SWCB)	*Diatraea grandiosella*
Southern cornstalk borer (SCSB)	*Diatraea crambidoides*
Corn earworm (CEW)	*Helicoverpa zea*
Fall armyworm (FAW)	*Spodoptera frugiperda*
Stalk borer	*Papaipema nebris*
Lesser corn stalk borer	*Elasmopalpus lignosellus*
Sugarcane borer (SCB)	*Diatraea saccharalis*
Western bean cutworm (WBC)	*Richia albicosta*
Black cutworm	*Agrotis ipsilon*
Western corn rootworm (WCRW)	*Diabrotica virgifera virgifera*
Northern corn rootworm (NCRW)	*Diabrotica barberi*
Mexican corn rootworm (MCRW)	*Diabrotica virgifera zeae*

EPA Accepted: 6/8/2017

Plant-Incorporated Protectant Label

MON 89034 × MON 87411 × DAS-59122-7

Insect-Protected, Herbicide-Tolerant Corn

(OECD Unique Identifier: MON-89Ø34-3 × MON-87411-9 × DAS-59122-7)

Active Ingredients:

dsRNA transcript comprising a DvSnf7 inverted repeat sequence derived from *Diabrotica virgifera virgifera*, and the genetic material necessary for its production (vector PV-ZMIR10871) in MON 87411 corn (OECD Unique Identifier MON-87411-9)……..≤ 0.00000044%*

Bacillus thuringiensis Cry1A.105 protein and the genetic material (vector PV-ZMIR245) necessary for its production in corn event MON 89034 (OECD Unique Identifier: MON-89Ø34-3) ……...……≤ 0.0088%*

Bacillus thuringiensis Cry2Ab2 protein and the genetic material (vector PV-ZMIR245) necessary for its production in corn event MON 89034 (OECD Unique Identifier: MON-89Ø34-3) ……≤ 0.0048%*

Bacillus thuringiensis Cry3Bb1 protein and the genetic material (vector PV-ZMIR10871) necessary for its production in corn event MON 87411 (OECD Unique Identifier: MON-87411-9) ……...≤ 0.0041%*

Bacillus thuringiensis Cry34Ab1 protein and the genetic material (vector PHP17662) necessary for its production in corn event DAS-59122-7 (OECD Unique Identifier: DAS-59122-7)…....≤ 0.012%*

Bacillus thuringiensis Cry35Ab1 protein and the genetic material (vector PHP17662) necessary for its production in corn event DAS-59122-7 (OECD Unique Identifier: DAS-59122-7)…...≤ 0.0026%*

Other Ingredients:

CP4 EPSPS protein (5-enolpyruvylshikimate-3-phosphate synthase) and the genetic material (vector PV-ZMIR10871) necessary for its production in corn event MON 87411…...≤ 0.036%*

PAT protein (phosphinothricin acetyl transferase) and the genetic material (vector PHP17662) necessary for its production in corn event DAS-59122-7 ……≤ 0.0001%*

*Percentage (wt/wt) on a dry weight basis for whole plant (forage)

KEEP OUT OF REACH OF CHILDREN NET CONTENTS _____

CAUTION

EPA Registration No. 62719-708

EPA Establishment No. 62719-IN-1

Dow AgroSciences LLC
9330 Zionsville Road
Indianapolis, IN 46268

DIRECTIONS FOR USE

It is a violation of Federal law to use this product in any manner inconsistent with its labeling.

This plant-incorporated protectant (PIP) may be combined through conventional breeding with other registered plant-incorporated protectants that are similarly approved for use in combination, through conventional breeding, with other registered plant-incorporated protectants to produce inbred corn lines and hybrid corn varieties with combined pesticidal traits.

This product may be used for breeding purposes, agronomic testing, increasing inbred seed stocks, and producing hybrid seed on up to a combined United States (US) total of 200 acres per plant incorporated protectant (PIP) active ingredient per registrant per year. Commercial plantings of this product, for the purposes of grain production and controlling corn insect pests, are prohibited.

EPA Accepted: 3/1/2017.

Plant-Incorporated Protectant Label

MON 87411 × DAS-59122-7

Insect-Protected, Herbicide-Tolerant Corn

(OECD Unique Identifier: MON-87411-9 × DAS-59122-7)

Active Ingredients:

dsRNA transcript comprising a DvSnf7 inverted repeat sequence derived from *Diabrotica virgifera virgifera*, and the genetic material necessary for its production (vector PV-ZMIR10871) in MON 87411 corn (OECD Unique Identifier MON-87411-9)……..≤ 0.00000044%*

Bacillus thuringiensis Cry3Bb1 protein and the genetic material (vector PV-ZMIR10871) necessary for its production in corn event MON 87411 (OECD Unique Identifier: MON-87411-9) ……...≤ 0.0041%*

Bacillus thuringiensis Cry34Ab1 protein and the genetic material (vector PHP17662) necessary for its production in corn event DAS-59122-7 (OECD Unique Identifier: DAS-59122-7)…....≤ 0.012%*

Bacillus thuringiensis Cry35Ab1 protein and the genetic material (vector PHP17662) necessary for its production in corn event DAS-59122-7 (OECD Unique Identifier: DAS-59122-7)…...≤ 0.0026%*

Other Ingredients:

CP4 EPSPS protein (5-enolpyruvylshikimate-3-phosphate synthase) and the genetic material (vector PV-ZMIR10871) necessary for its production in corn event MON 87411…...≤ 0.036%*

PAT protein (phosphinothricin acetyl transferase) and the genetic material (vector PHP17662) necessary for its production in corn event DAS-59122-7 ……≤ 0.0001%*

*Maximum percent (%) dry weight basis for whole plant (forage)

KEEP OUT OF REACH OF CHILDREN NET CONTENTS _____

CAUTION

EPA Registration No. 62719-709

EPA Establishment No. 62719-IN-1

Dow AgroSciences LLC
9330 Zionsville Road
Indianapolis, IN 46268

DIRECTIONS FOR USE

It is a violation of Federal law to use this product in any manner inconsistent with its labeling.

This plant-incorporated protectant (PIP) may be combined through conventional breeding with other registered plant-incorporated protectants that are similarly approved for use in combination, through conventional breeding, with other registered plant-incorporated protectants to produce inbred corn lines and hybrid corn varieties with combined pesticidal traits.

This product may be used for breeding purposes, agronomic testing, increasing inbred seed stocks, and producing hybrid seed on up to a combined United States (US) total of 750 acres per plant incorporated protectant (PIP) active ingredient per registrant per year. Commercial plantings of this product, for the purposes of grain production and controlling corn insect pests, are prohibited.

EPA Accepted: 3/1/2017.

Plant-Incorporated Protectant Label

TC1507 × MON 87411 × DAS-59122-7

Insect-Protected, Herbicide-Tolerant Corn

(OECD Unique Identifier: DAS-Ø15Ø7-1 × MON-87411-9 × DAS-59122-7)

Active Ingredients:

dsRNA transcript comprising a DvSnf7 inverted repeat sequence derived from *Diabrotica virgifera virgifera*, and the genetic material necessary for its production (vector PV-ZMIR10871) in MON 87411 corn (OECD Unique Identifier MON-87411-9)……..≤ 0.00000044%*

Bacillus thuringiensis Cry1F protein and the genetic material (vector PHP8999) necessary for its production in corn event TC1507 (OECD Unique Identifier: DAS- Ø15Ø7-1)……..≤ 0.00096%*

Bacillus thuringiensis Cry3Bb1 protein and the genetic material (vector PV-ZMIR10871) necessary for its production in corn event MON 87411 (OECD Unique Identifier: MON-87411-9) ……...≤ 0.0041%*

Bacillus thuringiensis Cry34Ab1 protein and the genetic material (vector PHP17662) necessary for its production in corn event DAS-59122-7 (OECD Unique Identifier: DAS-59122-7)…....≤ 0.012%*

Bacillus thuringiensis Cry35Ab1 protein and the genetic material (vector PHP17662) necessary for its production in corn event DAS-59122-7 (OECD Unique Identifier: DAS-59122-7)…...≤ 0.0026%*

Other Ingredients:

CP4 EPSPS protein (5-enolpyruvylshikimate-3-phosphate synthase) and the genetic material (vector PV-ZMIR10871) necessary for its production in corn event MON 87411…...≤ 0.036%*

PAT protein (phosphinothricin acetyl transferase) and the genetic material (vectors PHP17662 and PHP8999) necessary for its production in corn events TC1507 and DAS-59122-7 ……≤ 0.0001%*

*Percentage (wt/wt) on a dry weight basis for whole plant (forage)

KEEP OUT OF REACH OF CHILDREN NET CONTENTS _____

CAUTION

EPA Registration No. 62719-710

EPA Establishment No. 62719-IN-1

Dow AgroSciences LLC
9330 Zionsville Road
Indianapolis, IN 46268

DIRECTIONS FOR USE

It is a violation of Federal law to use this product in any manner inconsistent with its labeling.

This plant-incorporated protectant (PIP) may be combined through conventional breeding with other registered plant-incorporated protectants that are similarly approved for use in combination, through conventional breeding, with other registered plant-incorporated protectants to produce inbred corn lines and hybrid corn varieties with combined pesticidal traits.

This product may be used for breeding purposes, agronomic testing, increasing inbred seed stocks, and producing hybrid seed on up to a combined United States (US) total of 200 acres per plant incorporated protectant (PIP) active ingredient per registrant per year. Commercial plantings of this product, for the purposes of grain production and controlling corn insect pests, are prohibited.

EPA Accepted: 3/1/2017.

Plant-Incorporated Protectant Label

MON 89034 × TC1507 × MON 87411

Insect-Protected, Herbicide-Tolerant Corn

(OECD Unique Identifier: MON-89Ø34-3 × DAS-Ø15Ø7-1 × MON-87411-9)

Active Ingredients:

dsRNA transcript comprising a DvSnf7 inverted repeat sequence derived from *Diabrotica virgifera virgifera*, and the genetic material necessary for its production (vector PV-ZMIR10871) in MON 87411 corn (OECD Unique Identifier MON-87411-9)……..≤ 0.00000044%*

Bacillus thuringiensis Cry1A.105 protein and the genetic material (vector PV-ZMIR245) necessary for its production in corn event MON 89034 (OECD Unique Identifier: MON-89Ø34-3) ……...……≤ 0.0088%*

Bacillus thuringiensis Cry2Ab2 protein and the genetic material (vector PV-ZMIR245) necessary for its production in corn event MON 89034 (OECD Unique Identifier: MON-89Ø34-3) ……≤ 0.0048%*

Bacillus thuringiensis Cry1F protein and the genetic material (vector PHP8999) necessary for its production in corn event TC1507 (OECD Unique Identifier: DAS- Ø15Ø7-1)……..≤ 0.00096%*

Bacillus thuringiensis Cry3Bb1 protein and the genetic material (vector PV-ZMIR10871) necessary for its production in corn event MON 87411 (OECD Unique Identifier: MON-87411-9) ……...≤ 0.0041%*

Other Ingredients:

CP4 EPSPS protein (5-enolpyruvylshikimate-3-phosphate synthase) and the genetic material (vector PV-ZMIR10871) necessary for its production in corn event MON 87411…...≤ 0.036%*

PAT protein (phosphinothricin acetyl transferase) and the genetic material (vector PHP8999) necessary for its production in corn events TC1507 ……≤ 0.0001%*

*Percentage (wt/wt) on a dry weight basis for whole plant (forage)

KEEP OUT OF REACH OF CHILDREN NET CONTENTS _____

CAUTION

EPA Registration No. 62719-711

EPA Establishment No. 62719-IN-1

Dow AgroSciences LLC
9330 Zionsville Road
Indianapolis, IN 46268

DIRECTIONS FOR USE

It is a violation of Federal law to use this product in any manner inconsistent with its labeling.

This plant-incorporated protectant (PIP) may be combined through conventional breeding with other registered plant-incorporated protectants that are similarly approved for use in combination, through conventional breeding, with other registered plant-incorporated protectants to produce inbred corn lines and hybrid corn varieties with combined pesticidal traits.

This product may be used for breeding purposes, agronomic testing, increasing inbred seed stocks, and producing hybrid seed on up to a combined United States (US) total of 750 acres per plant incorporated protectant (PIP) active ingredient per registrant per year. Commercial plantings of this product, for the purposes of grain production and controlling corn insect pests, are prohibited.

EPA Accepted: 3/1/2017.

Plant-Incorporated Protectant Label

MON 89034 × MON 87411

Insect-Protected, Herbicide-Tolerant Corn

(OECD Unique Identifier: MON-89Ø34-3 × MON-87411-9)

Active Ingredients:

dsRNA transcript comprising a DvSnf7 inverted repeat sequence derived from *Diabrotica virgifera virgifera*, and the genetic material necessary for its production (vector PV-ZMIR10871) in MON 87411 corn (OECD Unique Identifier MON-87411-9)……..≤ 0.00000044%*

Bacillus thuringiensis Cry1A.105 protein and the genetic material (vector PV-ZMIR245) necessary for its production in corn event MON 89034 (OECD Unique Identifier: MON-89Ø34-3) ……...……≤ 0.0088%*

Bacillus thuringiensis Cry2Ab2 protein and the genetic material (vector PV-ZMIR245) necessary for its production in corn event MON 89034 (OECD Unique Identifier: MON-89Ø34-3) ……≤ 0.0048%*

Bacillus thuringiensis Cry3Bb1 protein and the genetic material (vector PV-ZMIR10871) necessary for its production in corn event MON 87411 (OECD Unique Identifier: MON-87411-9) ……...≤ 0.0041%*

Other Ingredients:

CP4 EPSPS protein (5-enolpyruvylshikimate-3-phosphate synthase) and the genetic material (vector PV-ZMIR10871) necessary for its production in corn event MON 87411…...≤ 0.036%*

*Percentage (wt/wt) on a dry weight basis for whole plant (forage)

KEEP OUT OF REACH OF CHILDREN NET CONTENTS _____

CAUTION

EPA Registration No. 62719-712

EPA Establishment No. 62719-IN-1

Dow AgroSciences LLC
9330 Zionsville Road
Indianapolis, IN 46268

DIRECTIONS FOR USE

It is a violation of Federal law to use this product in any manner inconsistent with its labeling.

This plant-incorporated protectant (PIP) may be combined through conventional breeding with other registered plant-incorporated protectants that are similarly approved for use in combination, through conventional breeding, with other registered plant-incorporated protectants to produce inbred corn lines and hybrid corn varieties with combined pesticidal traits.

This product may be used for breeding purposes, agronomic testing, increasing inbred seed stocks, and producing hybrid seed on up to a combined United States (US) total of 900 acres per plant incorporated protectant (PIP) active ingredient per registrant per year. Commercial plantings of this product, for the purposes of grain production and controlling corn insect pests, are prohibited.

EPA Accepted: 3/6/2017.

Plant-Incorporated Protectant Label

MON 89034 × TC1507 × MON 87411 × DAS-59122-7

Insect-Protected, Herbicide-Tolerant Corn

(OECD Unique Identifier: MON-89Ø34-3 × DAS-Ø15Ø7-1 × MON-87411-9 × DAS-59122-7)

Active Ingredients:

dsRNA transcript comprising a DvSnf7 inverted repeat sequence derived from *Diabrotica virgifera virgifera*, and the genetic material necessary for its production (vector PV-ZMIR10871) in MON 87411 corn (OECD Unique Identifier MON-87411-9)……..≤ 0.00000044%*

Bacillus thuringiensis Cry1A.105 protein and the genetic material (vector PV-ZMIR245) necessary for its production in corn event MON 89034 (OECD Unique Identifier: MON-89Ø34-3) ……...……≤ 0.0088%*

Bacillus thuringiensis Cry2Ab2 protein and the genetic material (vector PV-ZMIR245) necessary for its production in corn event MON 89034 (OECD Unique Identifier: MON-89Ø34-3) ……≤ 0.0048%*

Bacillus thuringiensis Cry1F protein and the genetic material (vector PHP8999) necessary for its production in corn event TC1507 (OECD Unique Identifier: DAS- Ø15Ø7-1)……..≤ 0.00096%*

Bacillus thuringiensis Cry3Bb1 protein and the genetic material (vector PV-ZMIR10871) necessary for its production in corn event MON 87411 (OECD Unique Identifier: MON-87411-9) ……...≤ 0.0041%*

Bacillus thuringiensis Cry34Ab1 protein and the genetic material (vector PHP17662) necessary for its production in corn event DAS-59122-7 (OECD Unique Identifier: DAS-59122-7)…....≤ 0.012%*

Bacillus thuringiensis Cry35Ab1 protein and the genetic material (vector PHP17662) necessary for its production in corn event DAS-59122-7 (OECD Unique Identifier: DAS-59122-7)…...≤ 0.0026%*

Other Ingredients:

CP4 EPSPS protein (5-enolpyruvylshikimate-3-phosphate synthase) and the genetic material (vector PV-ZMIR10871) necessary for its production in corn event MON 87411…...≤ 0.036%*

PAT protein (phosphinothricin acetyl transferase) and the genetic material (vectors PHP17662 and PHP8999) necessary for its production in corn events TC1507 and DAS-59122-7 ……≤ 0.0001%*

*Percentage (wt/wt) on a dry weight basis for whole plant (forage)

KEEP OUT OF REACH OF CHILDREN NET CONTENTS _____

CAUTION

EPA Registration No. 62719-713

EPA Establishment No. 62719-IN-1

Dow AgroSciences LLC
9330 Zionsville Road
Indianapolis, IN 46268

DIRECTIONS FOR USE

It is a violation of Federal law to use this product in any manner inconsistent with its labeling.

This plant-incorporated protectant (PIP) may be combined through conventional breeding with other registered plant-incorporated protectants that are similarly approved for use in combination, through conventional breeding, with other registered plant-incorporated protectants to produce inbred corn lines and hybrid corn varieties with combined pesticidal traits.

This product may be used for breeding purposes, agronomic testing, increasing inbred seed stocks, and producing hybrid seed on up to a combined United States (US) total of 200 acres per plant incorporated protectant (PIP) active ingredient per registrant per year. Commercial plantings of this product, for the purposes of grain production and controlling corn insect pests, are prohibited.

EPA Accepted: 3/1/2017.

Plant-Incorporated Protectant Label

TC1507 × MON 87411

Insect-Protected, Herbicide-Tolerant Corn

(OECD Unique Identifier: DAS-Ø15Ø7-1 × MON-87411-9)

Active Ingredients:

dsRNA transcript comprising a DvSnf7 inverted repeat sequence derived from *Diabrotica virgifera virgifera*, and the genetic material necessary for its production (vector PV-ZMIR10871) in MON 87411 corn (OECD Unique Identifier MON-87411-9)……..≤ 0.00000044%*

Bacillus thuringiensis Cry1F protein and the genetic material (vector PHP8999) necessary for its production in corn event TC1507 (OECD Unique Identifier: DAS- Ø15Ø7-1)……..≤ 0.00096%*

Bacillus thuringiensis Cry3Bb1 protein and the genetic material (vector PV-ZMIR10871) necessary for its production in corn event MON 87411 (OECD Unique Identifier: MON-87411-9) ……...≤ 0.0041%*

Other Ingredients:

CP4 EPSPS protein (5-enolpyruvylshikimate-3-phosphate synthase) and the genetic material (vector PV-ZMIR10871) necessary for its production in corn event MON 87411…...≤ 0.036%*

PAT protein (phosphinothricin acetyl transferase) and the genetic material (vector PHP8999) necessary for its production in corn event TC1507 ……≤ 0.0001%*

*Percentage (wt/wt) on a dry weight basis for whole plant (forage)

KEEP OUT OF REACH OF CHILDREN NET CONTENTS _____

CAUTION

EPA Registration No. 62719-714

EPA Establishment No. 62719-IN-1

Dow AgroSciences LLC
9330 Zionsville Road
Indianapolis, IN 46268

DIRECTIONS FOR USE

It is a violation of Federal law to use this product in any manner inconsistent with its labeling.

This plant-incorporated protectant (PIP) may be combined through conventional breeding with other registered plant-incorporated protectants that are similarly approved for use in combination, through conventional breeding, with other registered plant-incorporated protectants to produce inbred corn lines and hybrid corn varieties with combined pesticidal traits.

This product may be used for breeding purposes, agronomic testing, increasing inbred seed stocks, and producing hybrid seed on up to a combined United States (US) total of 750 acres per plant incorporated protectant (PIP) active ingredient per registrant per year. Commercial plantings of this product, for the purposes of grain production and controlling corn insect pests, are prohibited.

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Ecological risk assessment for DvSnf7 RNA: A plant-incorporated protectant with targeted activity against western corn rootworm

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ABSTRACT

MON 87411 maize, which expresses DvSnf7 RNA, was developed to provide an additional mode of action to confer protection against corn rootworm (*Diabrotica* spp.). A critical step in the registration of a genetically engineered crop with an insecticidal trait is performing an ecological risk assessment to evaluate the potential for adverse ecological effects. For MON 87411, an assessment plan was developed that met specific protection goals by characterizing the routes and levels of exposure, and testing representative functional taxa that would be directly or indirectly exposed in the environment. The potential for toxicity of DvSnf7 RNA was evaluated with a harmonized battery of non-target organisms (NTOs) that included invertebrate predators, parasitoids, pollinators, soil biota as well as aquatic and terrestrial vertebrate species. Laboratory tests evaluated ecologically relevant endpoints such as survival, growth, development, and reproduction and were of sufficient duration to assess the potential for adverse effects. No adverse effects were observed with any species tested at, or above, the maximum expected environmental concentration (MEEC). All margins of exposure for NTOs were >10-fold the MEEC. Therefore, it is reasonable to conclude that exposure to DvSnf7 RNA, both directly and indirectly, is safe for NTOs at the expected field exposure levels.

1. Introduction

Over the past decade, a number of food crops utilizing RNA interference (RNAi), have received regulatory approvals from United States agencies such as the Environmental Protection Agency (U.S. EPA) and Department of Agriculture (USDA), as well as approval in other countries such as Canada, Mexico, Australia, New Zealand, Japan, Korea, and Brazil (CERA, 2012). The RNA-based products approved thus far have conferred resistance to specific viruses (e.g. plum-pox virus), extended produce quality (e.g. Arctic Apple) or nutritional enhancement (e.g. alfalfa, soy) (Auer and Frederick, 2009; CERA, 2012). Recently, genetically engineered (GE) insect-protected plants that confer resistance via RNA-based gene regulation have been developed and reported in the scientific literature (Bachman et al., 2013; Baum et al., 2007; Bolognesi et al., 2012; Mao et al., 2007). These plants express double-stranded RNAs (dsRNAs) targeted to suppress mRNA levels in a specific species or a small group of closely related species by utilizing the RNAi pathway. The sequence specific nature of RNAi allows these products to target pest species with a high level of specificity, while mitigating risk to non-target organisms (NTOs) (Bachman et al., 2013; Burand and Hunter, 2013; Whyard et al., 2009). Monsanto Company has developed a GE maize, MON 87411, that confers protection against corn rootworm (CRW) (*Diabrotica* spp.) utilizing RNAi as the mechanism of insecticidal action (Bolognesi et al., 2012). The DvSnf7 RNA expressed in MON 87411 is composed of a 968 nucleotide sequence containing 240 base pair dsRNA component plus the addition of a poly A tail (Urquhart et al., 2015) designed to target the western corn rootworm (*Diabrotica virgifera virgifera;* WCR) *Snf7* gene (DvSnf7). Upon consumption, the plant-produced RNA in MON 87411 is recognized by the CRW's RNAi machinery, which results in a rapid decrease of DvSnf7 mRNA and protein levels leading to growth inhibition followed by mortality (Bolognesi et al., 2012; Levine et al., 2015). It has been established that after ingestion of DvSnf7 by WCR, suppression of the DvSnf7 mRNA occurs within 24 h, followed by suppression of DvSNF7 protein and onset of mortality by day 5 (Bolognesi et al.,

Abbreviations: dsRNA, double stranded RNA; RNA, ribonucleic acid; RNAi, RNA interference; NTO, non-target organism; ERA, ecological risk assessment.

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2012). MON 87411 also contains a *cry3Bb1* gene that produces a modified *Bacillus thuringiensis* (subsp. *kumamotoensis*) Cry3Bb1 protein to protect against CRW larval feeding. The spectrum of activity of the Cry3Bb1 protein has previously been reviewed by the U.S. EPA and, at the levels expressed in GE maize, activity was only evident in the family Chrysomelidae within the order Coleoptera (U.S. EPA, 2010a). Corn rootworm active *Bt*-technologies, such as the Cry3Bb1 and Cry34Ab1/Cry35Ab1 proteins, have been safely marketed for over a decade, and have provided significant value to farmers (Prasifka et al., 2013). In addition, incorporation of multiple modes of action against CRW by pyramiding *Bt* and RNA-based traits will offer increased efficacy and durability of a product while maintaining a high degree of specificity for the target pest and environmental safety (Baum and Roberts, 2014).

A critical step in the deregulation and/or registration of a GE plant incorporated protectant (PIP) is performing an ecological risk assessment (ERA) to evaluate the potential for adverse ecological effects from cultivation. Assessment of potential ecological impacts, associated with the introduction of a PIP, is based on the characteristics of the crop and the introduced trait. The approach for evaluating ecological risks from pesticides is a multi-step iterative process (Romeis et al., 2013; U.S. EPA, 1998). Key steps include problem formulation, analysis of exposure and potential effects, and risk characterization. During problem formulation, the assessor defines protection goals, prepares a conceptual model to aid in identification of the relevant assessment and measurement endpoints, and then develops an analysis plan that serves as the basis for a risk characterization. Important information that was used to inform the problem formulation step for MON 87411 included the biology and familiarity with the crop and the trait, the mode of action (MOA), the spectrum of activity, the tissue specific expression profile, routes of exposure for ecological receptors and an assessment of potential persistence in the environment. In general, the scope of the ecological safety assessment for a PIP can be reduced when the MOA is well characterized, there is a narrow spectrum of activity, and expression levels of the trait are well characterized (Romeis et al., 2013). The MOA of DvSnf7 RNA has been well characterized (Bolognesi et al., 2012; Ramaseshadri et al., 2013) and has been shown to have a narrow spectrum of activity with activity only evident within a narrow subset of beetles, the Galerucinae subfamily in the order Coleoptera (Bachman et al., 2013). This limited range of activity reduces the potential for non-target effects and can narrow the scope of ecological testing. Additionally, the DvSnf7 RNA and Cry3Bb1 protein have been shown to act independently which allowed for Cry3Bb1 and DvDnf7 RNA to be tested and assessed independently (Levine et al., 2015). Taken together, information on the MOA, spectrum of activity, expression profile, lack of interaction, and routes of potential exposure were used to help inform and define the scope of NTO testing used for this ERA.

For the MON 87411 assessment, the protection goals were identified as the maintenance of ecological functions of NTOs 'in-field' and biodiversity of species 'off-field' that contribute to the structure and function of the environment. Ecological functions to be protected include pollination, predation and parasitism (i.e., biological pest control, referred to herein as biocontrol), decomposition of soil organic material, and soil nutrient cycling. Additional confirmatory data was collected to address regulatory requirements and to provide empirical data for a broad range of taxa for this first in class insecticidal RNAi product. This included a broader range of avian and other non-target vertebrate populations where a plausible risk hypothesis would typically not require such data given barriers to exposure in these taxa (see section 4.1 in Discussion). An important assessment endpoint for PIPs is the abundance of taxa within functional groups of NTOs. Primary

Table 1
The relationships between protection goals, assessment endpoints, indicators of effect, and measurement endpoints utilized in the DvSnf7 RNA/MON 87411 ecological risk assessment.

Non-target organism		Protection goals	Assessment endpoints	Indicators of effect	Measurement endpoints
Honey bee adult and larvae	*Apis mellifera*	Pollinationservices and pollinator biodiversity	Population size and function, biodiversity	Laboratory larval & adult bee toxicity studies	Adults (worker) survival Larval survival and development to adult
Ladybird beetle	*Coleomegilla maculata*	Biocontrol by non-target arthropods	Population size and function, biodiversity	Laboratory toxicity studies initiated neonates or adults	*C. maculata, P. chalcites, and O. insidiosus* survival, growth and development to adult
Rove Beetle	*Aleochara bilineata*				*A. bilineata and C. carnea* adult survival and reproduction
Ground Beetle	*Poecilus chalcites*				*P. foveolatus* adult survival
Green Lacewing	*Chrysoperla carnea*				*E. andrei* survival and body weight
Insidious Flower Bug	*Orius insidiosus*				*Collembola* survival and reproduction
Parasitic Wasp	*Pediobius foveolatus*				Carbon and nitrogen transformation
Earthworm	*Eisenia andrei*	Nutrient cycling by soil biota	Population size and function, biodiversity of soil macro-organisms	Laboratory toxicity studies initiated with neonates or adults	
Collembola	*Folsomia candida*		Functionality of microbially-mediated soil processes	Laboratory toxicity studies with soil microorganisms	
Beneficial soil microorganisms					
Bobwhite quail	*Colinus virginianus*	Avian and wild mammal populations	Population size, biodiversity	Toxicity studies with bobwhite quail, chickens, and catfish	Survival and growth
Chicken	*Gallus domesticus*				
Catfish	*Ictalurus punctatus*				

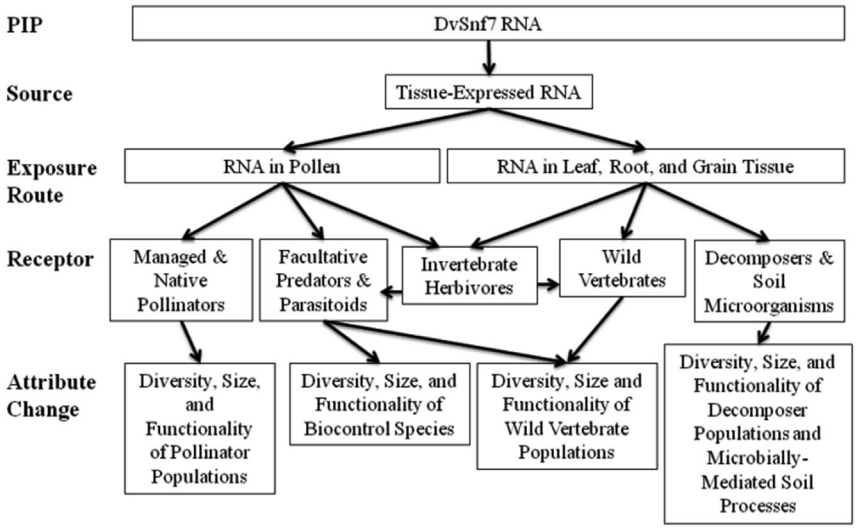

Fig. 1. Exposure-based conceptual model for MON 87411.

indicators of effect include impacts on survival, growth, development and reproduction. The relationship between protection goals, assessment endpoints, and indicators of effect (measurement endpoints) for DvSnf7 RNA are outlined in Table 1. Using an exposure-based conceptual model (Fig. 1), ecologically relevant routes of exposure for NTOs were identified and used to develop risk hypotheses. The over-arching risk hypothesis that was tested was that cultivation of MON 87411 will have no unacceptable adverse effects on NTOs resulting from environmental exposure to the DvSnf7 RNA. Testing this hypothesis required performing laboratory toxicity tests on individual species and placing the results into the context of an ERA. An *in silico* analysis, using publically available sequences for relevant NTOs associated with maize agriculture and/or key ecological functions, was conducted to assess potential effects to additional species.

2. Materials/methods

2.1. Conceptual model

An exposure-based conceptual model was developed for MON 87411 to illustrate routes of exposure to DvSnf7 RNA for ecological receptors (e.g. NTOs) that represent functional roles (Fig. 1). Key functional and measureable attribute changes were identified for the ecological receptors that were linked to the identified environmental protection goals (Table 1). Pollen was included as the route of exposure for pollinators, facultative predators and parasitoids, and invertebrate herbivores that could use pollen as a supplementary or life-stage specific food source. Leaf, root, and grain tissue were included as an exposure route for herbivorous invertebrates and wild vertebrates and senescent tissue was considered as the route of exposure for soil biota. The invertebrate herbivores feeding on leaf or root tissue were considered an indirect exposure route for biocontrol species (e.g. insect predators or parasitoids) and wild vertebrates. Aquatic exposures were considered but not included in the conceptual model because exposure of aquatic organisms to maize tissue after harvest is limited temporally and spatially; therefore potential exposure of aquatic organisms is low to negligible (U.S. EPA, 2010a). In addition, DvSnf7 RNA has been shown to rapidly degrade in aquatic systems (Fischer et al., 2016a,b). Measureable attribute changes (assessment endpoints) were identified for each ecological receptor including biodiversity, population size, and/or ecological functionality.

2.2. Effects testing

2.2.1. Test species selection and study design

Selection of test organisms was informed by the protection goals and conceptual model, and to meet the U.S. EPA's testing framework for PIPs (U.S. EPA, 2001). NTO testing included laboratory toxicity testing against a representative pollinator [honey bee (*Apis mellifera*)], six beneficial insect species that represent biocontrol species [parasitic wasp (*Pediobius foveolatus*), ladybird beetle (*Coleomegilla maculata*), carabid beetle (*Poecilus chalcites*), rove beetle (*Aleochara bilineata*), green lacewing (*Chrysoperla carnea*), and insidious flower bugs (*Orius insidiosus*)], representative soil biota [earthworm (*Eisenia andrei*), Collembola (*Folsomia candida*), and microbially-mediated soil processes], and representative wild vertebrates [bobwhite quail (*Colinus virginianus);* channel catfish *(Ictalurus punctatus);*and broiler chicken *(Gallus domesticus)*]. Survival, growth and/or developmental observations were examined in the ladybird beetle, carabid beetle, insidious flower bug, honey bee, and vertebrate studies; survival and reproduction with Collembola, rove beetle and green lacewing, and survival and biomass with earthworm. Carbon and nitrogen (C: N) transformation in soil mixed with root and shoot tissue derived from MON 87411 was measured to evaluate the functionality of soil nutrient cycling by microorganisms. In addition to the avian and catfish studies, the results of a 28-day (*Mus musculus*) repeat dose oral gavage study with the DvSnf7 RNA at doses up to 100 mg/kg/day (U.S. EPA, 2015; Petrick et al., 2016) was included as part of the ERA.

All NTO studies were conducted with diet-incorporation methodology and the organisms were fed *ad libitum*. Studies followed established regulatory guidelines or published methods from the authors' laboratory. Details for each test method are provided in Tables 2 and 3 and Supplementary Appendix A. Dietary exposures were initiated with the earliest life stages amenable to laboratory testing and consideration was given to selecting the life stage(s) with direct exposure to the PIP where applicable. The duration of each study was selected to exceed the known time to kill for DvSnf7 RNA to CRW (~5 days) as well as allow for the evaluation of ecologically relevant endpoints beyond mortality to adequately assess the potential for off-target effects. Where appropriate, positive control treatments were included to

Table 2
Non-target arthropod study design for DvSnf7_968 RNA laboratory studies.

Non-target organism	Guideline or method	Concentration of DvSnf7_968 RNA	Duration (days)	Environmental conditions	Sample size	Life stage at initiation
A. mellifera larvae	Tan et al., 2015	1000 ng/g diet; 11.3 ng/larvae	17[a]	Dosing: 24 °C; RH[b] 66%; Larval development: Ambient hive conditions; Adult emergence: 28 ± 2 °C; RH 60 ± 13%; 0L:24D	20 larvae × 4 replicates	2-3 day old larvae
A. mellifera adult	Tan et al., 2015	1000 ng/g diet	14	29 ± 1 °C; RH 50 ± 6%; 0L:24D	20 bees × 4 replicates	≤2-day old adults
C. maculata	Bachman et al., 2013	1000 ng/g diet	16–18	27 °C; RH 70%; 14L:10D	20 larvae × 3 replicates	≤32-h old larvae
P. chalcites	Bachman et al., 2013 and Duan et al., 2005	1000 ng/g diet	35	27 °C; RH 70%; 14L:10D	20 larvae × 3 replicates	≤24-h old larvae
A. bilineata	Grimm et al., 2000	1000 ng/g diet	70[c]	20±1 °C; RH 65 ± 10%; 16L:8D; 800–900 lux	20 (10 ♀ and 10 ♂) × 4 replicates	3-7 day old adults
C. carnea	Vogt et al., 2000	1001 ng/g diet	16–18	25±2 °C; RH 65 ± 16%; 16L:8D; 3600–4800 lux	20 (10 ♀ and 10 ♂) × 4 replicates	≤24-h old adults
P. foveolatus	Bachman et al., 2013	1000 ng/g diet	20	25 ± 5 °C; RH 70 ± 10%; 16L:8D	10 wasps × 4 replicates	24-hr old adults
O. insidiosus	Tan et al., 2011	1000 ng/g diet	10	25 ± 5 °C; RH 70 ± 10%; 16L:8D	40 nymphs/treatment	5-day old nymphs
E. andrei	OECD 207	5000 μg/kg soil	14	20 ± 1 °C; 24L:0D; 525–750 lux	10 worms × 4 replicates	~5-months old
F. candida	OECD 232	1000 ng/g diet	28	20 ± 2 °C; RH 71± 5%; 16L:8D; 470–540 lux	10 springtails × 4 replicates	9-10 day old juveniles

[a] Single exposure on Day 0.
[b] RH = relative humidity.
[c] 28day continuous dietary exposure followed by 42 day observation for emergence of F_1 generation.

Table 3
Beneficial soil microbe and non-target vertebrate study design or DvSnf7_968 RNA and/or tissue derived from MON 87411.

Non-target organism	Guideline or method	Concentration of DvSnf7_968 RNA	Duration (days)	Environmental conditions	Sample size	Life stage at initiation
Carbon Transformation	OECD 217	MON 87411 root & shoot tissue	28	22 ± 3 °C	5 replicate samples	
Nitrogen Transformation	OECD 216	MON 87411 root & shoot tissue	28	22 ± 3 °C	3 replicate samples	
C. virginianus	OPPTS 850.2200 U.S.EPA, 1996	1000 μg/kg diet	14	Days 0–6: 37.3 ± 4.0 °C; Days 7–14: 30.1 ± 1.2 °C; RH[a] 32± 12%; 16L:8D; 400 lux	5 quail × 6 replicates	14 day old
G. domesticus	Taylor et al., 2005	~57% MON 87411 grain	~42	Days 0–4: 24L:0D; 1.0–1.3 fc[b] Days 5–10: 10L:14D; 1.0–1.3 fc Days 11–18: 12L:12:D; 0.2–0.3 fc Days 19+: 16L:8D; 0.2–0.3 fc	100 birds/treatment (10 birds per pen × 5 pens ♀ and 5 pens ♂)	Approximately 1 day old chicks
I. punctatus	OECD 215; Hammond et al., 1996	30% MON 87411 grain	8 weeks	30 ± 2 °C; DO[c] 5.00–6.41 mg/L; 14L:10D; Flow 750–1667 mL/min.	20 catfish × 5 replicates	11 months old; mean wt 5.1–5.5 g

[a] RH = relative humidity.
[b] fc = footcandles.
[c] DO = Dissolved Oxygen.

Table 4
DvSnf7 RNA levels in selected maize tissues used to determine maximum expected environmental concentrations (MEEC) from MON 87411. The highest values in the range were used to determine MEECs.

Tissue type[a]	Developmental stage[b]	Mean (SD) Range μg/g
Pollen (fwt[c])	VT-R1	0.103×10^{-3} (0.069×10^{-3}) 0.056×10^{-3} - 0.224×10^{-3}
Leaf (fwt[c])	V14-R1	14.4×10^{-3} (6.71×10^{-3}) 5.40×10^{-3} - 33.8×10^{-3}
Root (fwt[c])	V3-V4	3.15×10^{-3} (1.79×10^{-3}) 1.74×10^{-3} – 8.00×10^{-3}
Whole Plant (dwt[d])	V6-V8	55.1×10^{-3} (23.1×10^{-3}) 33.0×10^{-3} – 106×10^{-3}
Grain (dwt[d])	R6	0..104×10^{-3} (0.033×10^{-3}) 0.056×10^{-3} - 0.175×10^{-3}

[a] For multiple over season tissue types (e.g. leaf) the tissue stage with the highest maximum expression is reported.
[b] The crop development stages at which each tissue was collected. The growth stages were described by Ritchie et al. (1997).
[c] The DvSnf7 RNA levels are calculated as microgram (μg) of DvSnf7 RNA per gram (g) of tissue on a fresh weight (fwt) basis. The sample means, SDs, and ranges (minimum and maximum values) were calculated for each tissue type across all 5 sites (n = 20), except for pollen n = 5 due to expressions from two pollen samples < LOD and from the rest of the samples for < LOQ).
[d] The DvSnf7 RNA levels are calculated as μg of DvSnf7 RNA per gram of tissue on a dry weight (dwt) basis. The sample means, SDs, and ranges (minimum and maximum values) were calculated for each tissue type across all 5 sites (n = 19).

demonstrate the effectiveness of the test system to detect an adverse effect as recommended by Romeis et al. (2011).

2.2.2. Test material

All terrestrial invertebrate NTO studies and the quail study were conducted using *in vitro* produced DvSnf7 RNA, referred to as DvSnf7_968 RNA that was prepared as described in Urquhart et al. (2015). *In vitro* synthesized DvSnf7_968 RNA was shown to be functionally equivalent to the DvSnf7 RNA produced *in planta* (Urquhart et al., 2015). This is critical information to support the risk assessment because it demonstrates that the DvSnf7 material used in testing was equipotent to DvSnf7 that non-target taxa would potentially be exposed to in the field. Soil microorganism testing was conducted using MON 87411 root and shoot tissue incorporated into a sandy loam soil and the catfish and broiler chicken studies were conducted using MON 87411 grain. With the exception of the earthworm study, all studies utilizing the *in vitro* produced test substance included a diet analysis. Diet analyses were performed using a sensitive insect (*Diabrotica undecimpunctata howardi*; Southern corn rootworm, SCR) to measure biological activity and/or concentration or a DvSnf7-specific Quantigene® assay to measure DvSnf7_968 RNA levels along with an insect bioassay to assess biological activity. Additionally, where appropriate based upon the diet matrix, the homogeneity of the test material and stability over the period of storage was also evaluated. A dose confirmation was not appropriate for the earthworm study due to the rapid degradation of RNA in the soil matrix (Dubelman et al., 2014; Fischer et al., 2016a,b).

2.2.3. Estimation of maximum expected environmental concentration

DvSnf7 RNA expression values from MON 87411 across a range of tissue types were used to determine the maximum expected environmental concentration (MEEC) for dietary or soil concentrations. Diet concentrations for specific studies were based on the highest expressing tissue type that the NTO would most likely be directly or indirectly exposed to in the maize agroecosystem and included pollen, leaf, senescent root and grain (Table 4) and concentrations were selected that represented a worst-case scenario exposure of greater than 10-times the MEEC (U.S. EPA, 2010a). DvSnf7 RNA expression levels were quantified using a validated QuantiGene® Plex 2.0 (Affymetrix Inc.) assay (Armstrong et al., 2013). Tissue samples were collected from MON 87411 plants produced at five sites during 2011–2012. The DvSnf7 RNA level in each tissue type was calculated on a microgram (μg) per gram (g) of fresh weight tissue (fwt) or dry weight tissue (dwt) basis.

Many of the invertebrate NTOs that were tested primarily feed upon pollen in the agroecosystem; therefore the maximum DvSnf7 RNA expression in pollen was used for the MEEC with honey bees, wasps, the ladybird beetle and the insidious flower bug. For predatory insects and insectivorous birds that consume herbivorous prey and have an indirect exposure to maize expressed DvSnf7 RNA, the maximum expression value from the leaf development stage with the highest expression (V14-R1) was used to represent worst-case scenario to calculate the margin of exposure (MOE). For other wild vertebrates, the most likely route of exposure to the DvSnf7 RNA is from grain produced by MON 87411 within the agroecosystem. The most ecologically relevant route of exposure for soil-dwelling organisms, such earthworms and Collembola, was considered primarily to be from root tissue with some addition of late season plant tissue that enters the soil environment. Of these tissue types the highest expressing tissue (root V3-V4) was used as a worst-case exposure scenario for these taxa. For the C: N transformation studies, lyophilized MON 87411 shoot and root tissues (V7) were incorporated into soil at 20 mg dwt tissue/g dwt soil. This concentration was used as a worst-case scenario and assumed the biomass of 1-acre of maize containing 25,000 plants at 650 g dry wt/plant (Sims and Holden, 1996) was incorporated into the top 6 inches of soil. Additionally, the use of lyophilized tissues provided a higher concentration of DvSnf7 for the respective tissue used in the ERA, therefore the maximum dry weight expression in V7 plants was used as the MEEC for soil microorganisms. Based upon knowledge of agronomics of maize, and that the amount of root or shoot tissue would be less than that for total plant tissue, it was concluded that this soil concentration would be in excess of the root and shoot tissue concentration occurring under normal cultivation of MON 87411.

2.3. In silico analysis

To provide additional data to evaluate the laboratory studies, bioinformatics analyses was conducted to evaluate whether non-target species have sufficient genomic match to the DvSnf7 sequence that would render them potentially susceptible to MON 87411 maize (Supplementary Appendix B). Twenty-three NTOs were selected based upon the following criteria: plausible exposure to MON 87411 maize, availability of public transcriptomes, and potential susceptibility based on current knowledge from laboratory bioassays (Supplementary Appendix B). The evaluation was conducted using STELLAR software (version 1.3, July 2012) and compared the DvSnf7 sequence with transcript (22 organisms) or EST (1 organism) sequences from the 23 organisms. The STELLAR searches were conducted to identify exact 21 or greater nucleotide (nt) matches between the DvSnf7 query and sequences contained in transcript or EST collections. The species selected included vertebrate (birds, fish and mammals) and invertebrate species (arthropods, insects, worms and crustaceans). Although bioinformatics were evaluated for several vertebrate species, direct feeding of dsRNA to induce RNAi has not been successful in vertebrates without the use of encapsulation to prevent degradation, or addition of chemical stabilization and penetration enhancers such as transfection agents (Petrick et al., 2013; Sifuentes-Romero et al., 2011; Ubuka et al., 2012). These species were included as part of the

Table 5
No significant ($p > 0.05$) adverse effects of DvSnf7_968 RNA in diet bioassays against a battery of non target arthropods demonstrates negligible risk to these taxa from exposure to MON 87411 maize.

Non-target organism	Endpoint	DvSnf7_968 RNA treatment	Assay control	Positive control	Statistical test	Analytical confimation[b]
A. mellifera adult	Mean Survival (%)	92.5	91.3	0[a]	T-test	SCR bioassay
A. mellifera larvae	Mean Survival (%)	100	100	0[a]	N/A	SCR bioassay
	Mean Capped Brood (%)	100	100	0[a]	N/A	
	Mean Time to 50% Adult Emergence (Days ± SE)	15.5 ± 0.3	15.6 ± 0.4	N/A	T-test	
C. maculata	Mean Survival (%)	91.7	90.0	16.7[a]	T-test	SCR bioassay
	Mean Development Time to Adult (Days ± SE)	14.9 ± 0.23	15.1 ± 0.32	N/A	T-test	
	Mean Adult Biomass (mg)	10.2 ± 0.19	10.2 ± 0.08	N/A	T-test	
P. chalcites	Mean Survival (%)	93.3	91.7	65[a]	T-test	SCR bioassay
	Mean Adult Emergence (%)	70.0	75.0	N/A	T-test	
	Mean Development Time to Adult (Days ± SE)	32.9 ± 0.38	32.9 ± 0.11	N/A	T-test	
	Mean Adult Biomass (mg)	31.9 ± 1.02	32.3 ± 0.99	N/A	T-test	
A. bilineata	Mean Survival (%)	88.7	92.5	95.0	Fischer's Exact Test	SCR bioassay and Quantigene
	Mean Number of F_1 Progeny per replicate	1028.0	991.8	39.0[a]	Dunnett's *t*-test	
C. carnea	Mean Survival (%)	93.3	81.7	70.0	Fisher's Exact test	SCR bioassay and Quantigene
	Mean Number of Viable eggs/female/day	20.3	18.2	1.0[a]	Dunnett's *t*-test	
P. foveolatus	Mean Survival (%)	100.0	100.0	0[a]	N/A	SCR bioassay
O. insidiosus	Mean Survival (%)	93.0	93.0	0.0[a]	T-test	SCR bioassay
	Mean Adult Emergence (%)	98.0	95.0	13.0[a]	T-test	
	Mean Development Time to Adult (Days ± SE)	10.9 ± 0.13	11.1 ± 0.15	10.6 ± 0.40	T-test	

[a] Significant difference from assay control at $\alpha = 0.05$.
[b] Confirmation of biological activity, concentration, stability and/or homogeneity of DvSnf7_968 in Diet.

assessment to provide a comprehensive approach to expand the range of NTOs that were evaluated.

3. Results

3.1. Effects testing

For all species tested, no statistically significant adverse effects from ingestion of or exposure to DvSnf7_968 RNA were detected when compared to the control for any of the measured endpoints (Table 5, Table 6, Table 7, and Supplementary Appendix C). It is important to recognize that all of the NTO studies, with one exception (wasp), conducted for MON 87411 assessed sub-lethal endpoints in addition to survival. Additionally, all studies met the prescribed validity or performance criteria for control survival, reproductive performance, and positive control response, and where applicable the stability, homogeneity and nominal concentration of DvSnf7 RNA was confirmed.

For the NTOs, MOEs were calculated based on the ratio of the no observed effect concentrations (NOECs) from the laboratory studies to the MEECs. The NOECs and MOEs determined for each of the species under a worst case exposure scenario are summarized in Table 8. Included in Table 8 is the no observed adverse effects level of 100 mg/kg as described in U.S. EPA (2015) and the calculated MOE for the 28-day repeat dose oral toxicity study with *M. musculus*. As no long-term adverse effects were observed in the C: N transformation studies with MON 87411 tissue, as well as the chicken and catfish feeding studies with MON 87411 grain at maximum incorporation rates, the MOEs for these organisms were considered to be ≥ 1.

3.2. In silico assessment

A comprehensive *in silico* evaluation with available genomes

Table 6
No significant ($p > 0.05$) adverse effects of DvSnf7_968 RNA or MON 87411 on non-target soil biota demonstrates negligible risk to these taxa from exposure to MON 87411 maize.

Non-target organism	Endpoint	DvSnf7_968 or MON 87411 treatment	Assay control	Positive control	Statistical test	Analytical confirmation [b]
F. candida	Mean Survival (%)	100.0	97.0	7.0[a]	Fisher's Exact test	SCR bioassay[c]
	Mean Number of Progeny	167	169	0.3[a]	Dunnett's T-test	
E. andrei	Mean Survival (%)	100.0	100.0	LC_{50} within reference range	N/A	No
	Mean Change in Biomass (% fwt)	8.4 ± 1.4 decrease	9.4 ± 2.4 decrease	N/A	T-test	
Carbon Transformation	CO_2 Production (% dev from control)	≤25%			≤25% dev from control	N/A
Nitrogen Transformation	NO_3–N Production (% dev from control)	≤25%			≤25% dev from control	N/A

[a] Significant difference from assay control at $\alpha = 0.05$.
[b] Confirmation of biological activity, concentration, stability and/or Homogeneity of DvSnf7_968 in Diet.
[c] Conducted as method development external to the definitive study.

Table 7
No significant ($p > 0.05$) adverse effects of DvSnf7_968 RNA or MON 87411 on non-target vertebrates demonstrates negligible risk to these taxa from exposure to MON 87411 maize.

Non-target organism	Endpoint	DvSnf7_968 or MON 87411 treatment	Assay control	Positive control	Statistical test	Analytical confirmation [a]
Colinus virginianus	Mean Survival (%)	100	100	N/A	N/A	SCR bioassay and Quantigene
	Mean Weight (g)	74.0 ± 9.0	75.0 ± 7.0	N/A	T-test	
	Mean Weight change (g)	43.0 ± 7.0	43.0 ± 6.0	N/A	T-Test	
G. domesticus	Mean Survival (%)	97.0	96.0	N/A	Fischer's Exact Test	Event specific PCR to verify identity of test substance and absence of test substance in control
	Mean Weight (g/bird ± SEM)	3004 ± 36.8	3011± 15.0[b]	N/A	ANOVA	
	Mean Weight Gain (g/bird ±SEM)	2963 ± 36.9	2970± 15.1[b]	N/A	ANOVA	
I. punctatus	Mean Survival (%)	100	100	N/A	N/A	N/A
	Mean Diet consumed (g/fish± SD)	30.6 ± 1.4	29.0 ± 1.8	N/A	ANOVA	
	Mean Weight Gain (g/fish± SD)	14.0 ± 2.2	14.1 ± 1.3	N/A	ANOVA	
	Diet conversion ratio (±SD)	2.3 ± 0.4	2.1 ± 0.1	N/A	ANOVA	

[a] Confirmation of biological activity, concentration, stability and/or homogeneity of DvSnf7_968 in Diet.
[b] Control and reference diets pooled.

and transcriptomes did not identify any ≥21 nt contiguous matches for the 23 species (Supplementary Appendix B). Therefore, no adverse effects of DvSnf7 RNA against these species are predicted. As mentioned above, honey bee adult and larvae were evaluated in dietary bioassays with DvSnf7_968 RNA and no adverse effects were observed (Tan et al., 2015). The results of that bioinformatics analysis confirm the results of the toxicity testing and provide an additional line of evidence to explain why no adverse effects were detected with larval and adult honey bees. Likewise, this bioinformatics analysis provides additional evidence for the lack of adverse effects to other NTOs (jewel wasp, *Nasonia vitripennis*) that also were evaluated in previous laboratory studies (Bachman et al., 2013).

4. Discussion

The ERA for MON 87411 has taken into consideration the MOA, the spectrum of insecticidal activity, routes and levels of exposure

Table 8
Maximum expected environmental concentrations (MEECs), no observed effect concentrations (NOECs) from non-target organism (NTO) studies and estimated margins of exposure (MOEs).

NTO	MEEC [a]	NOEC [b]	MOE [c]
A. mellifera larvae	0.000448 ng[d]	≥11.3 ng/larvae [e]	≥25,223
A. mellifera adult	0.224 ng/g fwt pollen	≥1000 ng/g	≥4464
C. maculata	0.224 ng/g fwt pollen	≥1000 ng/g	≥4464
P. chalcites	33.8 ng/g fwt leaf [f]	≥1000 ng/g	≥29
A. bilineata	33.8 ng/g fwt leaf [f]	≥1000 ng/g	≥29
C. carnea	33.8 ng/g fwt leaf [f]	≥1001 ng/g	≥29
P. foveolatus	0.224 ng/g fwt pollen	≥1000 ng/g	≥4464
O. insidiosus	0.224 ng/g fwt pollen	≥1000 ng/g	≥4464
E. andrei	8.0 ng/g fwt root [g]	≥5000 μg/kg dry soil	≥625
F. candida	8.0 ng/g fwt root [g]	≥1000 ng/g dry soil	≥125
Soil microorganisms (C:N Transformation)	106 ng/g dwt plant[h]	≥106 ng/g dwt plant	≥1
C. virginianus	33.8 ng/g fwt leaf [f]	≥1000 μg/kg [i]	≥29
G. domesticus	0.175 ng/g dwt grain	≥0.175 ng/g dwt grain	≥1
M. musculus	0.045 mg/kg/day[j]	≥100 mg/kg/day[k]	>2958
I. punctatus	0.175 ng/g dwt grain	≥0.175 ng/g dwt grain	≥1

[a] Maximum expression levels determined from MON 87411.
[b] NOECs reflect nominal test concentrations.
[c] MOE values were calculated based on the ratio of the NOEC to MEEC. The MOE was determined based on the maximum expression level of the DvSnf7 RNA in the tissue from MON 87411deemed most relevant to the NTO exposure.
[d] MEEC based upon mean quantity of DvSnf7 RNA expressed in 2 mg of MON 87411pollen (fwt). The average consumption of pollen by honey bee larvae is 2 mg during development (Babendreier et al., 2004). The MEEC was calculated as follows: (2 mg pollen × (0.224 ng DvSnf7 RNA/1000 mg pollen)).
[e] The NOEC represents a single dose of 10 μl of 1000 ng/g solution added to each larval cell. The total mass added and consumed in each larval cell was 11.3 ng DvSnf7/cell. The concentration of 1000 ng/g DvSnf7_968 RNA in the diet solution is calculated based on the density of the 30% sucrose/water (w/v) solution of 1.127 g/ml.
[f] The maximum expression value from the leaf development stage with the highest expression (V14-R1) was used to represent worst-case-scenario for a predator consuming a herbivorous prey.
[g] The maximum expression value from the root development stage with the highest expression (V3-V4) was used to represent worst-case-scenario for a soil dwelling invertebrates.
[h] For the C:N transformation studies, lyophilized MON 87411 80% shoot and 20% root tissues (V7) were incorporated into soil at 20 mg dwt tissue/g dwt soil. The highest expressing whole plant tissue dwt was used for the MEEC as this value exceeded all root expression values.
[i] The NOEC of ≥1000 μg/kg diet is equivalent to 190 μg DvSnf7 RNA/kg/day.
[j] The MEEC for *M. musculus* is based on a daily dietary dose (DDD). The DDD = Food Intake Rate (FIR)/body weight × dietary concentration, and was calculated for the grass eating herbivorous mammal with the highest FIR (1.33), the common vole that consumes 100% maize shoots. The highest leaf expression highest expression (V14-R1) was used to represent worst-case-scenario. Therefore, (1.33 × 0.0338 mg/kg fwt = 0.045 mg DvSnf7 RNA g body weight or mg/kg/body weight) following EFSA, 2009 and Crocker et al., 1998).
[k] U.S. EPA, 2015 and Petrick et al., 2016.

levels to DvSnf7 RNA produced by MON 87411 and the results from a taxonomically and functionally diverse group of NTO studies. NTO studies followed established methods and the tiered testing framework developed by the U.S. EPA to assess the environmental safety of PIPs. These studies evaluated ecologically relevant apical endpoints (survival, growth, development, and reproduction) to assess potential impacts to NTO populations and communities. Tier 1 NTO studies for PIPs are generally initiated with neonates, because they are typically thought to be the most sensitive life-stage, and the assays were run for a sufficient duration to evaluate developmental milestones (i.e. development to adult and/or reproduction). By evaluating a significant portion of the life cycle under conservative high dose exposure scenarios, it can be concluded with reasonable certainty that there is low likelihood of potential chronic adverse off-target effects at realistic field exposure levels. The Tier 1 studies for this ERA were conducted with concentrations (single limit dose) that far exceeded anticipated exposure of DvSnf7 RNA to maximize the potential for observing and documenting off-target effects. A limit dose is a treatment level that provides a high "worst-case" exposure level (i.e., 10 × anticipated field exposure level) and a large margin of exposure. Importantly, a lack of adverse effects in high dose testing has traditionally provided EPA with sufficient confidence to address uncertainties, conclude that there is no unacceptable risk to the environment, and conclude that no further data are required.

In an ecological assessment for PIPs, MOEs that are ≥10 are indicative of minimal risk in worst-case sub-chronic and chronic laboratory assays (U.S. EPA, 2010a). All of the MOEs calculated for the NTO species in this ERA were >10-times a high end predicted exposure level (Table 8). Of particular importance is the lack of adverse effects from exposure to DvSnf7 RNA in both adult and larval honey bees (*A. mellifera*). These results are consistent with (Velez et al., 2015), which found no adverse effects of adult or larvae honey bees fed high concentrations of a dsRNA with 100% sequence match to the honey bee. Additionally, no long-term adverse effects were observed on microbially-mediated soil nutrient cycling with MON 87411 tissues incorporated into soil at levels that exceed expected environmental concentrations. In vertebrate feeding studies at concentrations that approximate realistic field concentrations and at worst-case exposures, no adverse effects of MON 87411 or the DvSnf7 RNA were observed. In addition to the data reported herein, a 28-day mouse (*Mus musculus*) repeat dose oral gavage study with the DvSnf7 RNA at 100 mg/kg/day was performed and no adverse effects attributable to the DvSnf7 were observed (U.S. EPA, 2015; Petrick et al., 2016). An MOE for the mouse as a representative wild mammalian species can be calculated assuming a worst−case scenario for a herbivorous mammal consuming maize shoots (e.g. the common vole, *Microtus arvalis)* at a level of 133% of its body weight each day (Table 8) (Crocker et al., 1998; EFSA, 2009). This food intake rate of 1.33 exceeds a worst-case food intake rate corrected for body weight for an insectivorous mammal. In addition, insects would likely not accumulate DvSnf7 RNA to higher levels than what is expressed *in planta* because it is known that nucleic acids do not bioaccumulate. There is presently no evidence that the DvSnf7 RNA will persist or accumulate to levels higher than *in planta* expression in insects that feed on MON 87411 (Ivashuta et al., 2015). Therefore a worst-case assumption is that the concentration of DvSnf7 RNA in insects will equal that of the maximum expression in fresh weight MON 87411 plant tissue. Under these assumptions, given the NOEC for mice of 100 mg/kg/day and a maximum expression in leaf tissue of 33.8 ng/g fwt, the MOE for a herbivorous mammal is ≥ 2958 (Table 8).

Therefore, as with the previously assessed Cry3Bb1 protein, DvSnf7 RNA is not likely to produce adverse effects on terrestrial beneficial invertebrate and vertebrate species at field exposure levels. This conclusion is in agreement with prior published literature which reported that DvSnf7 activity is restricted to the Galerucinae subfamily within the Chrysomelidae family in the Order Coleoptera (Bachman et al., 2013). Further confirmation of results from laboratory studies were provided in a field study by Ahmad et al. (2015), where no adverse effects from MON 87411 maize were observed to non-target arthropod communities.

Recently, consideration has been given to whether the existing ERA framework is applicable to GE crops expressing RNA-based traits, especially insecticidal traits (Auer and Frederick, 2009; Lundgren and Duan, 2013; Scott et al., 2013). In their recent review of the risk assessment approach for GE plants containing RNA-based traits, Lundgren and Duan (2013) postulated that unintended off-target effects of insecticidal RNAs against NTOs could be widespread. This assertion was largely based upon data from pharmaceutical-specific publications that examined the effects of high concentrations of dsRNA in *in vitro* cell monolayers (Jackson and Linsley, 2010) and is not directly applicable to levels for the ecological assessment of MON 87411. Although off-target effects have been reported in *in vitro* systems in the pharmaceutical literature at high concentrations, these studies are not relevant to exposure scenarios for NTOs in agroecosystems. Only realistic routes and levels of exposure for NTOs, to a trait such as DvSnf7 RNA in MON 87411 maize, should be considered in the risk assessment (Fig. 1). Therefore, *in vitro* studies with RNA are not predictive of potential impacts to NTOs following dietary exposures due to much lower exposures in the environment and the absence of significant uptake afforded by use of transfection reagents in cultured cells. Additionally, pools of small RNAs, as would arise from dicing of a long dsRNA tend to eliminate off-target effects due to a dilution effect of a complex siRNA pool (Hannus et al., 2014). When off-target effects have been observed, gene suppression has been shown to be orders of magnitude less potent than that observed with small RNAs having full complementarity (Vaishnaw et al., 2010).

Lundgren and Duan (2013) also identified other reputed risks to NTOs based on the pharmaceutical literature such as immune stimulation and over-saturation of the RNAi machinery. The off-target effects observed in *in vivo* studies from the pharmaceutical literature result from exposure to large amounts of chemically stabilized dsRNA delivered specialized formulations via injection into the organism (Petrick et al., 2013). Therefore, these papers need to be interpreted with caution particularly in the context of low exposure scenarios to DvSnf7 RNA expressed by MON 87411. Under *in vitro* conditions, RNAi machinery saturation was shown to occur in a dose-dependent manner after transfection of relatively high doses of small RNAs into cells (Khan et al., 2009). This exposure condition in cell lines has limited or no relevance to risk an ERA for a PIP (Table 1). There are no reports to date suggesting that interferon or inflammatory responses occur following oral exposure (Petrick et al., 2013). Similar to humans and livestock, the diets of NTOs consist of plant or animal sources which naturally contain dsRNAs and there exists a long history of safe consumption of these endogenous dsRNA across eukaryotes. This has been illustrated specifically for grain from food and feed crops such as soybean, corn, and rice (Heisel et al., 2008; Ivashuta et al., 2009; Jensen et al., 2013), and as the result of viral infection in crops such as kidney bean, pepper, and barley (Fukuhara et al., 2006). With constant oral exposure to environmental dsRNA endogenously present in natural food sources, unintended effects in non-target organisms from immune stimulation and RNA machinery saturation are extremely unlikely to result from relatively low exposures to dsRNA resulting from cultivation of MON 87411.

Contrary to concerns regarding non-specific off-target effects, numerous studies have demonstrated that RNAi technology can

achieve sequence-specific gene silencing in some insects by feeding dsRNAs (Bachman et al., 2013; Baum et al., 2007; Whyard et al., 2009). Therefore, RNAi PIPs have the potential to selectively target economically important pest species and greatly reduce the likelihood of adverse effects on non-target organisms, including those beneficial to agriculture. The DvSnf7 RNA sequence in MON 87411 was carefully selected for its high degree of divergence between species to mitigate potential adverse effects on organism not closely related to the target pest species, WCR. This sequence has been shown to diverge rapidly within the subfamily level Galerucinae (Bachman et al., 2013), therefore, activity outside this subfamily is not predicted. The purposeful selection of the DvSnf7 sequence to reduce non-target effects is in alignment with recommendations from the 2014 Scientific Advisory Panel (SAP) on RNAi that recognized that targeting genes with a high degree of divergence will help "hone the specificity of RNAi to the target pest" (U.S. EPA, 2014). The SAP recommended that dsRNA sequences should be chosen that target a region of gene with no shared 21 nt sequences with other species (U.S. EPA, 2014). These recommendations are in alignment with previous studies by Baum et al. (2007), Whyard et al. (2009), and Bachman et al. (2013) that demonstrate how the insecticidal activity of ingested dsRNAs is directly related to the degree of sequence match to the target gene between species. Whyard et al. (2009) demonstrated that species-specific activity can be achieved in insects with dsRNAs that diverge at the species level. Bachman et al. (2013) demonstrated that for ingested dsRNAs, contiguous sequence matches of $\geq$21 nt to the target gene are necessary for biological activity to occur in insects, and that when no significant sequence match existed to the target gene then no adverse effects were observed in NTO testing. Finally, while a potential adverse effect from a dsRNA can be likely excluded when a 21 nt alignment is not present, it should be noted that NTO diets are continuously exposed to RNA that have 21 or greater bioinformatic alignments with the ingesting organism without evidence of a potential for hazard (Frizzi et al., 2014; Ivashuta et al., 2009).

The application of bioinformatics can have an important role in the selection and design of the dsRNAs and in informing the assessment process for NTOs. When bioinformatics data for non-target arthropods are available and indicate that the minimum sequence requirements for RNAi activity are not met, then the need for toxicity testing is diminished and the likelihood of detecting adverse effects is low. However, when the minimum sequence requirements are met, the converse is not true; these data cannot be reliably used to predict the presence of RNAi activity. Nevertheless, bioinformatics can assist with the developing a hypothesis-based taxonomic approach for characterizing the spectrum of activity for pest control, understanding the relationship between taxonomic relatedness and activity, and aid in the selection of test species for NTO testing (Bachman et al., 2013). This approach is in alignment with recommendations from RNAi –focused Scientific Advisory Panel (SAP) held in 2014 (U.S. EPA, 2014), which recommended that while a bioinformatics analysis is not an absolute predictor of effects, *in silico* searches of published genomes could be used to perform a screening level assessment to identify potential NTOs for further evaluation based upon the presence of sequence matches. While there are currently only a limited number of published complete and partial arthropod genomes publically available, additional genomes are being published at a rapid rate and are likely to become increasingly useful as a screening level tool for ERAs.

The confirmatory *in silico* analysis of NTO transcriptomes performed as part of this ERA support the findings of the laboratory bioassays, widen the scope of the NTO assessment, and provide further support to the conclusions of no adverse effects to NTOs from exposure to DvSnf7 RNA and cultivation of MON 87411 maize.

4.1. Further considerations for the ERA; exposure limitations of insecticidal RNA PIPs

In addition to sequence specificity, physical and biochemical barriers to the oral toxicity of dsRNAs exist in arthropods and other non-target taxa. As identified by the recent SAP on RNAi (U.S. EPA, 2014) these barriers vary across taxa and for insects include feeding behavior and diet, potential degradation of the dsRNA prior to ingestion, and the inherent sensitivity of the insect to ingested dsRNA based upon conservation and function of components of the RNAi machinery (Whyard et al., 2009).

4.1.1. Exposure/uptake

For a transgenic plant expressing an insecticidal trait, ingestion of the RNA via plant material is the most likely route of exposure. Induction of RNAi-mediated gene suppression in insects via an oral route of exposure requires efficient uptake of dsRNAs by midgut cells followed by suppression of the target mRNA leading to significant effects on growth, development and survival. In plants, nematodes and some basal arthropods (e.g. Acari), exogenous dsRNAs that enter the cell can be amplified via RNA-dependent RNA polymerases (RdRPs) to produce endogenous dsRNAs that supplement the RNAi pathway and prolong the RNAi effect (Grbic et al., 2011; Miller et al., 2012). However, in it important to note that insects, crustaceans and mammals have been shown to lack RdRPs (Grbic et al., 2011; Miller et al., 2012) and the ability of WCR and *Tribolium castaneum* to produce dose-dependent responses with RNAi is consistent with the absence of an endogenous amplification mechanism (Bolognesi et al., 2012; Miller et al., 2012). The lack of an endogenous amplification mechanism in insects suggests that exposure to dsRNA in higher trophic levels, via ingested prey species, will be limited because a mechanism for bioamplification is not evident. Other factors can also influence the efficiency of RNAi in insects, including concentration, potency and efficacy against the target, sequence and length, persistence of gene silencing and the insect life-stage (Baum et al., 2007; Huvenne and Smagghe, 2010; Whyard et al., 2009). In general, long dsRNAs that incorporate a high degree of sequence match to mRNAs in the target insect have greater potential for efficacy as a result of the number of siRNAs that can be produced from the sequence of each long dsRNA (Baum et al., 2007; Miller et al., 2012). Another mechanism that can affect RNAi efficiency in insects, and potentially limit environmental exposure, is the length of the dsRNA. Bolognesi et al. (2012) and Miller et al. (2012) demonstrated that a dsRNA must be of sufficient length (e.g. $\geq$ 60 bp) to result in efficacy against WCR and *T. castaneum*, respectively. Additionally, Bolognesi et al. (2012) demonstrated that a single 21 nt contiguous sequence match in a large carrier molecule was sufficient to induce biological activity in the southern corn rootworm (SCR, *Diabrotica undecimpunctata howardi*). Further, as demonstrated in Miller et al. (2012), the potency of a dsRNAs is positively related to the number of potential 21 nt matches contained in the sequence and therefore the number of 21 nt matches should be considered as part of the relevant environmental exposure necessary for biological activity under realistic exposure scenarios for NTOs in the agroecosystem.

4.1.2. Barriers

Physical and biochemical barriers to the oral toxicity of dsRNAs also exist in many arthropod taxa. These include potential degradation of the dsRNA prior to ingestion as well as the inherent sensitivity of the organism to ingested dsRNA (Whyard et al., 2009). For example, recent studies on the tarnished plant bug (*Lygus*

lineolaris, Hemiptera) demonstrated that endonucleases present in saliva rapidly degrade dsRNA creating a barrier to an RNAi effect in this species by oral delivery of dsRNA (Allen and Walker, 2012). In addition, as summarized in recent reviews (Baum and Roberts, 2014; Huvenne and Smagghe, 2010), insects display a wide range of sensitivities to ingested dsRNA, with the order Coleoptera demonstrating significantly greater sensitivity than other insect orders. For example, the order Lepidoptera has demonstrated variable sensitivity to ingested dsRNA and high concentrations are required to elicit a response in this order relative to coleopterans (Huvenne and Smagghe, 2010; Terenius et al., 2011). Additionally, rapid degradation of dsRNA in the hemolymph of *Manduca sexta* has been reported and attributed to nuclease activity, indicating that sensitivity to RNAi may be influenced by the instability of dsRNA within the insect (Garbutt et al., 2013). Successful induction of RNAi in aquatic invertebrates (shrimp, e.g. *Penaeus monodon*) via ingestion has been achieved, however all reported successful cases involved stabilization of the dsRNA in the diet either via of nanoparticle encapsulation or feed coated with bacteria expressing the dsRNA (Sarathi et al., 2008). Therefore, RNAi in aquatic invertebrates from ingestion of RNA-based PIPs or other unformulated dsRNAs is not expected.

Similar to the above barriers described for arthropods, all vertebrate digestive systems display commonalities in regards to structure and function such as enzymes that aid in digestion. The digestive systems of mammals and other vertebrates such as fish, reptiles and birds contain physical barriers such as the cellular membranes of the gut epithelium in addition to salivary endonucleases, harsh conditions in the stomach, and ribonucleases that hydrolyze nucleic acids in the gut lumen, and even nucleases in the blood (Houck, 1958; Park et al., 2006; Stevens and Hume, 1995). Therefore, the same digestive barriers that prevent oral activity of ingested RNA in insects are also applicable to other vertebrates.

To date, no successful feeding studies with naked (without transfection reagents) dsRNAs to induce an RNAi response have been achieved in vertebrate systems. Using mammal models (i.e. surrogate for non-target wild mammals), systemic delivery of RNA via the oral route has only been successful through the use of encapsulation to prevent degradation, or addition of chemical stabilization and penetration enhancers (Petrick et al., 2013). In avian species, successful RNAi has only been achieved with cell lines and/or embryos and has required the use of electroporation or other invasive techniques (Sifuentes-Romero et al., 2011; Ubuka et al., 2012). Likewise, successful RNAi with fish, amphibians and aquatic reptiles has only been achieved with cell lines and/or embryos and has required the use of transfection agents, direct injection, or other invasive techniques (Schyth, 2008; Sifuentes-Romero et al., 2011). In this ERA we evaluated a worst-case scenario exposure for an insectivorous avian species, *C. virginianus*. As would be predicted from the physiological barriers present in vertebrates and the selective activity of the DvSnf7 RNA, no adverse effects from 14-day of continuous exposure to DvSnf7 RNA were observed. As discussed previously, and consistent with these findings, no adverse effects were observed in a 28-day mouse repeat dose oral gavage study with the DvSnf7_968 RNA or a 42-day broiler chicken feeding study with MON 87411 grain containing the DvSnf7 RNA (U.S. EPA, 2015). Based on low exposure levels, physiological barriers to exposure, the likelihood of adverse effects to non-target terrestrial vertebrates from cultivation of MON 87411 is concluded to be extremely low.

Though aquatic habitats may be located near agricultural areas, the exposure of aquatic organisms to GE crops is limited temporally and spatially and the potential exposure of aquatic organisms is therefore low to negligible (U.S. EPA, 2010a). Additionally, DvSnf7 RNA has been shown to rapidly degrade in both terrestrial (Dubelman et al., 2014; Fischer et al., 2016b) and aquatic systems (Fischer et al., 2016a), further limiting the potential for exposure to aquatic taxa. Due to the aforementioned barriers, the lack of meaningful ecologically-relevant exposure to aquatic organisms from maize, other than through purposeful feeding of processed maize products, and the reported rapid degradation of DvSnf7 RNA in the environment, Tier 1 effects tests on aquatic species were not conducted for MON 87411. An 8-week channel catfish growth study has shown that no adverse effects are expected from feeding of processed maize products to with a diet consisting of 33% MON 87411 grain containing the DvSnf7 RNA (U.S. EPA, 2015).

In these studies, no adverse effects were observed in any NTO tested. Though barriers exist to systemic exposure in vertebrate species, the potential barriers to exposure in each invertebrate NTO was not characterized. Therefore, we cannot know which, if any, of these species (especially invertebrates) are recalcitrant to environmental/oral RNAi and hence cannot determine if the lack of adverse effects was related to the presence of barriers or lack of sequence match. In the absence of barriers, the bioinformatics assessment provided herein lends confidence to a conclusion that should exposure occur, significant sequence match does not exist between the DvSnf7 RNA and NTOs to elicit an adverse effect.

5. Conclusions

No adverse effects on NTOs were observed in a comprehensive battery of laboratory tests evaluating the potential adverse effects of DvSnf7 RNA/MON 87411 maize. These effects data, along with information on relevant exposure levels within the agroecosystem, were assessed with an approach that is consistent with EPA's current testing and assessment framework for genetically engineered plants (*e.g. Bt*-expressing plants). This ERA framework has enabled scientifically sound regulatory decisions with adequate certainty of acceptable risk and within the standards established by FIFRA (i.e., no unreasonable effects to the environment) (U.S. EPA, 2010b). Additionally, a tripartite group (government, industry and academia) evaluated this ERA approach and concluded that the current ERA framework and effects testing requirements for NTOs are applicable to plants engineered to express insecticidal RNA (ILSI-CERA, 2011).

As discussed, a key component of problem formulation is the identification of plausible risk hypotheses and evaluation of relevant routes of exposure through the conceptual model. In the case of MON 87411, based on the expected environmental exposure routes in the maize agroecosystem, the known environmental exposure concentrations, and the natural digestive barriers and physiological differences between NTOs, there is little probability of NTOs encountering DvSnf7 RNA in high enough concentrations to induce off-target effects as observed in the pharmaceutical literature and cautioned by Lundgren and Duan (2013). It is well established that RNAi is a sequence-specific mechanism, and activity is only possible when sufficient uptake and sequence complementarity to the target mRNA exists that leads to mRNA cleavage followed by gene silencing. There must be sufficient exposure to and uptake of the DvSnf7 RNA, sequence match, and sensitivity to RNAi in a given taxa for there to be a potential adverse effect.

Combining the lines of evidence from i) bioassays designed with appropriate duration and relevant endpoints to detect adverse and off-target effects specific to the known MOA of the DvSnf7 dsRNA in the target pest, ii) a spectrum of activity limited to within the Galerucinae, and iii) no adverse effects to NTOs from oral exposure to environmental dsRNA at MOE factors >10, and iv) rapid degradation in the environment, it can be concluded with reasonable certainty that there is low likelihood of MON 87411 maize adversely affecting NTOs at field exposure levels.

MON 87411 is the first commercial RNAi insecticidal PIP. As such, the studies incorporated in this ERA were not only designed to address specific risk hypotheses, but also intended to the lay the foundation for regulatory approvals of a new class of insecticides and provide data that will aid in communicating the environmental safety for an insecticidal RNA. For future RNAi products, consideration should be given to whether representatives of wild birds and mammals that have barriers to systemic exposure to RNA should be tested for an RNA-based product with low environmental exposures. Additionally, as a sequence based mechanism with a high potential for specificity, the selection of dsRNAs to have a narrow spectrum of activity can limit the potential for adverse effects beyond a select and closely related group of insects, thus building a case for the reduction of the number and types of invertebrate NTOs required for testing to make a sound and science-based conclusion on potential ecological risks. This opinion was expressed in the consensus points on the 2011 ILSI-CERA conference on "Problem Formulation for the Environmental Risk Assessment of RNAi Plants" where it was recognized that bioinformatic data coupled with activity spectra evaluations can be used to reduce the scope of NTO testing (ILSI-CERA, 2011).

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Transparency document

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Appendix A. Supplementary data

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NATIONAL ENVIRONMENTAL POLICY ACT DECISION
AND
FINDING OF NO SIGNIFICANT IMPACT

Monsanto Company
Corn Rootworm-Protected and Glyphosate-Tolerant MON 87411 Maize

United States Department of Agriculture
Animal and Plant Health Inspection Service
Biotechnology Regulatory Services

The United States Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS) has developed this decision document to comply with the requirements of the National Environmental Policy Act (NEPA) of 1969, as amended, the Council of Environmental Quality (CEQ) regulations implementing NEPA, and the USDA APHIS NEPA-implementing regulations and procedures (7 Code of Federal Regulations (CFR) part 372). This NEPA decision document, a Finding of No Significant Impact (FONSI), sets forth APHIS' NEPA decision and its rationale. Comments from the public involvement process were evaluated and considered in developing this NEPA decision.

In accordance with APHIS procedures implementing NEPA (7 CFR part 372), APHIS has prepared an Environmental Assessment (EA) to evaluate and determine if there are any potentially significant impacts to the human environment from a determination on the regulated status of a petition request (APHIS No. 13-290-01p) by Monsanto Company, St. Louis, Missouri (referred to as "Monsanto" in this document) for Monsanto 87411 Maize (referenced in this document as "MON 87411 Maize"), genetically engineered for resistance[1] to the herbicide, glyphosate, and to control corn rootworms.

[1] "Resistance" to herbicides is defined by the Herbicide Resistance Action Committee (HRAC) as the inherited ability of a plant population to survive and reproduce following repeated exposure to a dose of herbicide normally lethal to the wild type. Several technologies are available that can be used to develop herbicide resistance in plants including classical breeding, tissue culture, mutagenesis and genetic engineering. "Tolerance" is distinguished from resistance and defined by (HRAC. 2013. Guideline to the management of herbicide resistance. Herbicide Resistance Action Committee (HRAC) 2013. http://www.hracglobal.com/pages/ManagementofHerbicideResistance.aspx) as the inherent ability of a plant to survive and reproduce following exposure to an herbicide treatment. This implies that there was no selection or genetic manipulation to make the plant tolerant; it is naturally tolerant. Throughout the EA, and this FONSI, USDA-APHIS has used the terms "resistance" and "tolerance" consistent with the definitions of the HRAC. It should be noted however, that different terms for the same concept may be used interchangeably in some instances. In its petition to USDA-APHIS, Monsanto used the term "herbicide tolerant" throughout its documentation. This terminology can be considered synonymous with "herbicide-resistant" (HR) used in the EA and this FONSI.

MON 87411 Maize[2] contains three GE modes-of-actions (MOAs): two for insect pest protection; one for resistance to the herbicide, glyphosate. The insect protection mechanisms are designed to control corn rootworms (CRWs), a major pest of maize in the United States.

MON 87411 Maize contains two transgenes to control CRW. The *Cry3Bb1* gene protects against CRW larval feeding by promoting expression of an insecticidal crystalline (Cry) protein, Cry3Bb1. The *Cry3Bb1* gene is a modified form of a gene derived from the soil bacterium *Bacillus thuringiensis* subsp. *kumamotoensis*, also known as *Bt* (Monsanto, 2013). Crops producing Cry proteins are also known as *Bt* crops. Another transgene in MON 87411 Maize promotes expression of an interference RNA (RNAi). The RNAi expressed in MON 87411 Maize mediates a gene silencing mechanism that stops expression of a gene in western corn rootworm (WCR: *Diabrotica virgifera virgifera*) (Monsanto, 2013). When expression of the *Snf7* gene is suppressed by RNAi in WCR, production of the protein is suppressed. This results in WCR death (Bolognesi et al., 2012). This additional mechanism was developed and incorporated into MON 87411 Maize because some CRW populations, especially western corn rootworms (WCR) populations, have become resistant to the insecticidal Cry protein expressed by other *Bt* corn crops (Tabashnik et al., 2013; US-EPA, 2013; Gassmann et al., 2014).

MON 87411 Maize also contains the *epsps* gene coding sequence from an *Agrobacterium* sp. (strain CP4) that encodes the EPSPS (5-enolpyruvylshikimate-3-phosphate synthase) protein that confers resistance to glyphosate (Monsanto, 2013). The CP4 EPSPS protein in MON 87411 Maize is identical to the CP4 EPSPS protein present in several other commercially available crops that are no longer regulated following USDA reviews (e.g., glyphosate resistant [GR] varieties of soybean, maize, cotton, sugar beet, canola, and alfalfa). Expression of this glyphosate resistance trait in MON 87411 Maize allows growers to make post-emergent applications of herbicide products containing glyphosate as the active ingredient (a.i.) for broad-spectrum weed control.

The EA was prepared to specifically evaluate the impacts on the quality of the human environment[3] that may result from a determination of nonregulated status of MON 87411 Maize. The EA assessed alternatives related to a determination of nonregulated status of MON 87411 Maize, and analyzed the potential environmental and socioeconomic impacts that may result from the proposed action and the alternatives.

Regulatory Authority

[2] The terms, "maize" and "corn" are used interchangeably throughout this document for crops and products derived from *Zea mays.*

[3] Under NEPA regulations, the "human environment" includes "the natural and physical environment and the relationship of people with that environment" (40 CFR §508.14).

"Protecting American Agriculture" is the basic charge of APHIS. APHIS provides leadership in ensuring the health and care of plants and animals. The Agency improves agricultural productivity and competitiveness, and contributes to the national economy and public health. USDA asserts that all methods of agricultural production (conventional, organic, or the use of GE varieties) can increase farm income, and provide benefits to the environment and consumers.

Since 1986, the United States government has regulated GE organisms pursuant to a regulatory framework known as the Coordinated Framework for the Regulation of Biotechnology (Coordinated Framework) (51 FR 23302, 57 FR 22984). The Coordinated Framework, published by the Office of Science and Technology Policy, describes the comprehensive federal regulatory policy for ensuring the safety of biotechnology research and products and explains how federal agencies will use existing Federal statutes in a manner to ensure public health and environmental safety, while maintaining regulatory flexibility to avoid impeding the growth of the biotechnology industry. The Coordinated Framework is based on several important guiding principles: (1) agencies should define those transgenic organisms subject to review to the extent permitted by their respective statutory authorities; (2) agencies are required to focus on the characteristics and risks of the biotechnology product, not the process by which it is created; (3) agencies are required to exercise oversight of GE organisms only when there is evidence of "unreasonable" risk.

The Coordinated Framework explains the regulatory roles and authorities for the three major agencies involved in regulating GE organisms: USDA APHIS, the Food and Drug Administration (FDA), and the Environmental Protection Agency (EPA).

APHIS is authorized to regulate GE organisms that are potential plant pests under the plant pest provisions of the Plant Protection Act of 2000, as amended (7 USC §§ 7701 *et seq.*) to ensure that they do not pose a plant pest risk as defined in 7 CFR part 340.

The FDA regulates GE organisms under the authority of the Federal Food, Drug, and Cosmetic Act (FFDCA). The FDA is responsible for ensuring the safety and proper labeling of all foods for human consumption and animal feeds, including those that are genetically engineered or contain components and/or ingredients derived using genetic engineering. To help developers of food and feed derived from GE crops comply with their obligations under Federal food safety laws, FDA encourages them to participate in a voluntary consultation process. The FDA policy statement concerning regulation of products derived from new plant varieties, including those genetically engineered, was published in the Federal Register on May 29, 1992 (57 FR 22984). Under this policy, FDA uses consultation process to ensure that human food and animal feed safety issues or other regulatory issues (e.g., labeling) are resolved prior to commercial distribution of GE foods.

The EPA regulates plant-incorporated protectants (PIPs) under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA). EPA also sets tolerance limits for residues of pesticides on and in food and animal feed, or establishes an exemption from the requirement for a tolerance, under the Federal Food, Drug and Cosmetic Act (FFDCA) and regulates certain biological control organisms under the Toxic Substances Control Act (TSCA). The EPA is responsible for regulating the sale, distribution and use of pesticides, including pesticides that are produced by an organism through techniques of modern biotechnology.

Regulated Organisms

The mission of APHIS Biotechnology Regulatory Services (BRS) is to protect America's agriculture and environment using a dynamic, science-based regulatory framework that allows for the safe development and use of GE organisms. APHIS regulations at 7 CFR part 340 were promulgated pursuant to authority under the Federal Plant Pest Act. This authority has since been replaced by the plant pest provisions of the Plant Protection Act (PPA) of 2000, as amended (7 United States Code (U.S.C.) 7701-7772), which allows the Agency to regulate the introduction (importation, interstate movement, or release into the environment) of certain GE organisms and products. A GE organism is no longer subject to the plant pest provisions of the Plant Protection Act or to the regulatory requirements of 7 CFR part 340 when APHIS determines that it is unlikely to pose a plant pest risk. A GE organism is considered a regulated article under 7 CFR part 340 if the donor organism, recipient organism, vector, or vector agent used in engineering the organism belongs to one of the taxa listed in the regulation (7 CFR 340.2), and is also considered a plant pest. A GE organism is also regulated under 7 CFR part 340 if the Administrator determines the GE organism is a plant pest or has reason to believe is a plant pest. An individual may petition APHIS for a determination that a particular regulated article is unlikely to pose a plant pest risk, and therefore, is no longer regulated under the plant pest provisions of the Plant Protection Act or the regulations at 7 CFR part 340. Under §340.6(c) (4), petitioners are required to provide information related to plant pest risk that the agency may use to determine whether the regulated article is unlikely to present a greater plant pest risk than the unmodified organism. A GE organism is no longer subject to the regulatory requirements of 7 CFR part 340 or the plant pest provisions of the Plant Protection Act when APHIS determines that it is unlikely to pose a plant pest risk.

APHIS' Response to Petition for Nonregulated Status

Under the authority of the plant pest provisions of the Plant Protection Act and 7 CFR Part 340, APHIS has issued regulations for the safe development and use of GE organisms. As required by 7 CFR 340.6, APHIS must respond to petitioners who request a determination of the regulated status of GE organisms, including GE plants such as MON 87411 Maize. When a petition for nonregulated status is submitted, APHIS must determine if the GE organism of concern is likely to pose a plant pest risk. If APHIS determines, based on its Plant Pest Risk Assessment (PPRA), that the GE organism is unlikely to pose a plant pest risk, the GE organism is no longer subject to regulation under 7 CFR part 340.

MON 87411 Maize

MON 87411 Maize is currently regulated under 7 CFR part 340. Interstate movement and confined field releases of MON 87411 Maize were conducted under notifications acknowledged by APHIS, since 2010. These trials were conducted in diverse growing regions within the United States that include Arkansas, California, Colorado, Georgia, Hawaii, Iowa, Illinois, Indiana, Kansas, Louisiana, Michigan, Minnesota, Missouri, Mississippi, Nebraska, North Carolina, Ohio, Pennsylvania, Puerto Rico, South Dakota, Tennessee, Texas, and Wisconsin. Details about and data resulting from these field trials are described in the MON 87411 Maize petition (Monsanto, 2013), and were analyzed for plant pest risk in a preliminary Plant Pest Risk Assessment (PPRA) (USDA-APHIS, 2014).

Coordinated Framework Review

Food and Drug Administration

Mon 87411 Maize is within the scope of the FDA policy statement concerning regulation of products derived from new plant varieties, including those produced by genetic engineering. It is genetically engineered for resistance to glyphosate and control of corn rootworms. In June 2006, FDA published recommendations in "Guidance for Industry: Recommendations for the Early Food Safety Evaluation of New Non-Pesticidal Proteins Produced by New Plant Varieties Intended for Food Use" (US-FDA, 2011). These recommendation established voluntary food safety evaluations for new non-pesticidal proteins produced by new plant varieties, including GE plants, intended for use as food. Early food safety evaluations are designed to ensure that potential food safety issues related to a new protein in a new plant variety are addressed early in development.

Monsanto completed its submission of its safety and nutritional assessment of food and feed derived from MON 87411 Maize to FDA on March 14, 2014. All materials relevant to this notification were placed in a file designated as BNF 000145 by FDA. Based on the information Monsanto submitted, FDA acknowledged in a letter on October 17, 2014 that it had no further questions concerning food and feed derived from MON 87411 Maize.

Environmental Protection Agency

EPA has authority under FIFRA to establish pesticide use restrictions. These are listed on pesticide labels which are prepared during the pesticide registration process. Mon 87411 Maize is similar to currently available glyphosate-resistant (GR) maize varieties. Monsanto indicates that there will be no change in the use pattern for glyphosate on this glyphosate-resistant variety. APHIS used the current glyphosate labels relevant to applications for corn production as the basis for its evaluation of the potential impacts associated with the use of and exposure to glyphosate. EPA also regulates plants that express Bt proteins, as part of its authority to oversee plant-incorporated protectants (PIP), and the protein expressed by Mon 87411 has already been commercialized with extensive field use. Mon 87411 Maize incorporates an additional PIP DvSnf7 RNA that is expressed in the plant and specifically targets corn rootworm. EPA has produced an Environmental Risk Assessment for a FIFRA Section 3 Limited Seed Increase (US-EPA, 2015), based on Monsanto's submitted results required by provisions of the ecological impact assessment method used for other PIPSs. However, this will not necessarily be the same analysis used for future products. As EPA notes, "because of uncertainties associated with the potential for unexpected effects related to exposure to dsRNA, EPA raised questions to the SAP (Science Advisory Panel) regarding the applicability of the above approach to dsRNA PIPs. The SAP found that this approach was not sufficient to determine risks to nontarget organisms, and suggested an alternative framework (see pages 61-64 of the SAP minutes), which EPA is currently evaluating" (US-EPA, 2015). EPA plans to initially provide a limited acreage seed increase permit for two years to the product. EPA has also asked Monsanto for additional details about data and observations already supplied to EPA by Monsanto which will become part of the data package needed for the EPA permit for commercial use on Mon 87411 (US-EPA, 2015).Scope of the Environmental Analysis.

Although a determination of nonregulated status of Mon 87411 Maize would allow for new plantings of Mon 87411 Maize anywhere in the United States, APHIS primarily focused the environmental analysis on those geographic areas that currently support corn production. A

determination of nonregulated status of Mon 87411 Maize is not expected to increase corn production by its availability alone, or when accompanied by other factors, nor should it cause an increase in overall GE-corn acreage. To identify areas in the United States where corn is produced, APHIS used data from the National Agricultural Statistics Service (NASS, 2014).

Public Involvement

In a *Federal Register* notice (79 FR 13035-6) on March 7, 2014, APHIS announced the availability of the petition for public review and comment (Docket No. APHIS-2014-0007). The 60-day public comment period closed on May 7, 2014. APHIS received 423 comments during the period the petition was available for public review. Comments are available for public review in the docket file at:

http://www.regulations.gov/#!docketDetail;D=APHIS-2014-0007

On June 1, 2015, APHIS published a notice in the *Federal Register* (77 FR 13258-13260, Docket No. APHIS-2011-0129) announcing the availability of the MON 87411 Maize draft EA and preliminary PPRA for public review and comment. The comment period closed on July 1, 2015. APHIS received 12 comments during this review process. Responses to these comments are included in an addendum to this FONSI.

Major Issues Addressed in the EA

Issues discussed in the EA were identified by considering public concerns and issues described in public comments for the petition for nonregulated status of MON 87411 Maize and other environmental assessments of GE organisms. Issues identified in lawsuits, and those submitted by various stakeholders were also discussed. These issues, including those regarding the agricultural production of corn using various production methods, and the environmental food/feed safety of GE plants, were addressed to analyze the potential environmental impacts of MON 87411 Maize.

The EA describes the alternatives considered and evaluated using the issues identified. The alternatives encompassed the following topics that were identified as important to the scope of the analysis (40 CFR 1508.25):

Agricultural Production:

- Areas and Acreage of Maize Production
- Agronomic Practices
- Organic Maize Farming and Specialty Corn Production

Environmental Resources:

- Soil Quality
- Water Resources
- Air Quality

- Climate Change
- Animal Communities
- Plant Communities
- Soil Microorganisms
- Biological Diversity
- Gene Movement

Human Health:

- Public Health
- Worker Health and Safety

Animal Health:

- Animal Feed
- Livestock Health

Socioeconomics:

- Domestic Economic Environment
- Trade Economic Environment

Alternatives that were fully analyzed

The EA analyzes the potential environmental consequences of a determination of nonregulated status of MON 87477 Maize. To respond favorably to a petition for nonregulated status, USDA-APHIS must determine that MON 87411 Maize is unlikely to pose a plant pest risk. Based on its PPRA (USDA- APHIS, 2014), USDA-APHIS made a determination that MON 87411 Maize is unlikely to pose a plant pest risk. Therefore, APHIS must determine that MON 87411 Maize is no longer subject to 7 CFR part 340 or the plant pest provisions of the PPA. Two alternatives were evaluated in the EA: (1) no action and (2) determination of nonregulated status of MON 87411 Maize. APHIS has assessed the potential for environmental impacts for each alternative in the "Environmental Consequences" section of the EA.

<u>No Action</u>: Continuation as a Regulated Article

Under the No Action Alternative, USDA-APHIS would deny the petition. MON 87411 Maize and progeny derived from MON 87411 Maize would continue to be regulated articles under the regulations at 7 CFR part 340. Permits issued or notifications acknowledged by APHIS would still be required for introductions of MON 87411 Maize and measures to ensure physical and

reproductive confinement would continue to be applied. APHIS might choose this alternative if there were insufficient evidence to demonstrate the lack of plant pest risk from the unconfined cultivation of MON 87411 Maize.

This alternative is not the Preferred Alternative because APHIS concluded through its PPRA that MON 87411 Maize is unlikely to pose a plant pest risk (USDA- APHIS, 2014). Choosing this alternative would not satisfy the purpose and need of making a determination of plant pest risk status and responding to the petition for nonregulated status.

Preferred Alternative: Determination That Mon 87411 Maize Is No Longer a Regulated Article

Under this alternative, MON 87411 Maize and progeny derived from this event would no longer be regulated articles under the regulations at 7 CFR part 340. MON 87411 Maize is unlikely to pose a plant pest risk (USDA-APHIS, 2014b). Permits issued or notifications acknowledged by APHIS would no longer be required for introductions of MON 87411 Maize and progeny derived from this event.

This alternative best meets the purpose and need to respond appropriately to a petition for nonregulated status based on the requirements in 7 CFR part 340 and the Agency's authority under the plant pest provisions of the PPA. Based on the Agency's conclusion that MON 87411 Maize is unlikely to pose a plant pest risk, a determination of nonregulated status of MON 87411 Maize is a response that is consistent with the plant pest provisions of the PPA, the regulations codified in 7 CFR part 340, and the biotechnology regulatory policies of the Coordinated Framework. Under this alternative, growers may have future access to MON 87411 Maize and progeny derived from this event if the developer decides to commercialize MON 87411 Maize.

Alternatives Considered but Rejected From Further Consideration

APHIS assembled a list of alternatives that might be considered for MON 87411 Maize. APHIS evaluated these alternatives according to the Agency's authority under the plant pest provisions of the PPA, and the regulations at 7 CFR part 340, with respect to environmental safety, efficacy, and practicality to identify which alternatives would be further considered for MON 87411 Maize. Based on this evaluation, APHIS rejected several alternatives. These alternatives are discussed briefly below with the specific reasons for rejecting each.

Prohibit Any MON 87411 Maize from Being Released

In response to public comments that stated a preference that no GE organisms enter the marketplace, APHIS considered prohibiting the release of MON 87411 Maize, including denying any permits associated with the field testing. APHIS determined that this alternative is not appropriate because MON 87411 Maize is unlikely to pose a plant pest risk (USDA-APHIS, 2014).

In enacting the PPA, Congress found that:

> . . . "decisions affecting imports, exports, and interstate movement of products regulated under this title [i.e., the PPA] shall be based on sound science; . . . ."

On March 11, 2011, in a Memorandum for the Heads of Executive Departments and Agencies, the White House Emerging Technologies Interagency Policy Coordination Committee developed broad principles, consistent with Executive Order 13563, to guide the development and implementation of policies for oversight of emerging technologies (such as genetic engineering) at the agency level. In accordance with this memorandum, agencies should adhere to Executive Order 13563 and, consistent with that Executive Order, the following principle, among others, to the extent permitted by law, when regulating emerging technologies:

> "[D]ecisions should be based on the best reasonably obtainable scientific, technical, economic, and other information, within the boundaries of the authorities and mandates of each agency"

Based on its PPRA (USDA-APHIS, 2014) and the scientific data evaluated therein, USDA-APHIS concluded that MON 87411 Maize is not likely to pose a plant pest risk. Accordingly, there is no basis in science for prohibiting the release of MON 87411 Maize.

Approve the Petition in Part

The regulations at 7 CFR part 340.6(d)(3)(i) state that USDA-APHIS may "approve the petition in whole or in part." For example, a determination of nonregulated status in part may be appropriate if there is a plant pest risk associated with some, but not all events s described in a petition. Because USDA-APHIS has concluded that MON 87411 Maize is unlikely to pose a plant pest risk, there is no regulatory basis under the plant pest provisions of the PPA for considering approval of the petition only in part.

Isolation Distance between MON 87411 Maize and Non-GE Maize and Geographical Restrictions

In response to public concerns of gene movement between GE and non-GE plants, APHIS considered requiring an isolation distance separating MON 87411 Maize from non-GE maize production. However, because APHIS has concluded that MON 87411 Maize is unlikely to pose a plant pest risk (USDA-APHIS, 2014b), an alternative based on requiring isolation distances would be inconsistent with the statutory authority under the plant pest provisions of the PPA and regulations in 7 CFR part 340.

APHIS also considered geographically restricting the production of MON 87411 Maize based on the location of production of non-GE maize in organic production systems in response to public concerns regarding possible gene movement between GE and non-GE plants. However, as presented in the Agency's PPRA for MON 87411 Maize, there are no geographic differences associated with any identifiable plant pest risks for MON 87411 Maize (USDA- APHIS, 2014). Therefore, to be consistent with this determination, this alternative was rejected and not analyzed in detail. APHIS has concluded that MON 87411 Maize does not pose a plant pest risk, and will not exhibit a greater plant pest risk in any geographically restricted area (USDA-APHIS, 2014). Therefore, such an alternative would not be consistent with the APHIS statutory authority under the plant pest provisions of the PPA, the regulations in 7 CFR part 340 and the biotechnology regulatory policies described in the Coordinated Framework.

Based on the foregoing, the imposition of isolation distances or geographic restrictions would not meet APHIS' purpose and need to respond appropriately to a petition for nonregulated status

based on the requirements in 7 CFR Part 340 and the Agency's authority under the plant pest provisions of the Plant Protection Act. Nevertheless, APHIS is not expecting significant impacts. However, individuals might choose on their own to geographically isolate their non-GE maize productions systems from MON 87411 Maize or to use isolation distances and other management practices to minimize gene movement between cornfields. Information to assist growers in making informed management decisions for hybrid stacks based on MON 87411 Maize is available from Association of Official Seed Certifying Agencies (AOSCA, 2011).

Requirement of Testing for MON 87411 Maize

During comment periods for other petitions for nonregulated status, some commenters requested USDA to require and provide testing for GE products in non-GE production systems. USDA-APHIS notes that there are no nationally-established regulations involving testing, criteria, or limits of GE material in non-GE systems. Such a requirement would be extremely difficult to implement and maintain. Because MON 87411 Maize also does not pose a plant pest risk (USDA-APHIS, 2014), the imposition of any type of testing requirements is inconsistent with the plant pest provisions of the PPA, the regulations at 7 CFR part 340, and the biotechnology regulatory policies embodied in the Coordinated Framework. Therefore, imposing such a requirement for MON 87411 Maize would not meet the USDA-APHIS purpose and need to respond appropriately to the petition in accordance with its regulatory authorities.

Environmental Consequences of APHIS' Selected Action

The EA contains a full analysis of the alternatives to which we refer the reader for specific details. The following table briefly summarizes the results for each of the issues fully analyzed in the Environmental Consequences section of the EA.

Table 1. Summary of Potential Impacts and Consequences of Alternatives.

Attribute/ Measure	Alternative A: No Action	Alternative B: Determination of Nonregulated Status
Meets Purpose, Need and Objectives:	**No**	**Yes**
Unlikely to pose a plant pest risk:	Satisfied by regulated field trials.	Satisfied by risk assessment (USDA-APHIS, 2014)
Management Practices		
Areas and Acreage of Corn Production:	90% of U.S. corn is GE; 70% is stacked with HR and IR traits. Market economics is the primary factor influencing U.S. corn acreage and areas of production.	Areas and acreage devoted to corn production are not expected to change.
Herbicide Use and Weed Management Practices:	Weeds resistant to glyphosate and other herbicides will continue to increase. As HR weeds become more prevalent, growers are expected to shift to more costly weed control measures or other HR crops that are economically viable. Some potential exists for use of increased conventional tillage or reduced conservation tillage. Growers of corn not resistant to herbicides) are likely to continue the use of herbicides	Populations of weeds resistant to glyphosate and other herbicides will increase. Growers will continue to use herbicides in addition to glyphosate along with herbicide mixtures to control and avoid new resistant weed populations. Because MON 87411 is also resistant to glyphosate, it will be replacing other GR varieties and little or no change will accompany adoption of nonregulated MON 87411 Maize.
Insecticide Use:	EPA approves and labels uses of herbicides on corn and PIPs in GE corn. Chemical insecticide use has declined since the introduction of IR corn varieties.	Insecticide use likely to be unchanged or minimally changed (possibly reduced) compared to No Action Alternative (Coupe and Capel, 2015).

Attribute/ Measure	Alternative A: No Action	Alternative B: Determination of Nonregulated Status
Organic Farming:	An extremely small amount (0.25%) of corn production is certified organic and some may be grown outside major GE corn-growing sites.	Planting of organic corn is unlikely to change.
Specialty Corn Including Seed Production:	The U.S. specialty corn crop is small (5%) compared to total U.S. corn production.	Planting of specialty corn is unlikely to change.
Physical Environment		
Land Use:	Current trends in acreage and areas of production are likely to continue to be driven by market conditions for corn and corn products, by ethanol, animal feed needs and by Federal policy.	Current trends in acreage and production are likely to continue to be driven by market use and Federal policy.
Soil Quality:	Herbicide use in conjunction with HR corn has promoted conservation tillage; IR corn reduces reliance on chemical insecticides. Both tend to preserve or enhance soil quality.	Herbicide use with HR corn will continue to promote conservation tillage. MON 84711 is not expected to change the composition or structure of microbial communities.
Water Resources:	Agricultural NPS pollution sources (e.g., increased sedimentation from soil erosion; fertilizer and chemical pesticide residues) have declined as agronomic practices such as conservation tillage that mitigate runoff have been adopted for corn production.	Beneficial consequences of continued use of conservation tillage will remain the same as the No Action Alternative.

Attribute/ Measure	Alternative A: No Action	Alternative B: Determination of Nonregulated Status
Air Quality:	Pollution from agricultural sources (dust from tilling; drift/diffusion/volatilization of farm chemicals; exhaust emissions from mechanized farm equipment) have declined as mitigating agronomic practices such as conservation tillage have increased in conjunction with the introduction of GE corn	Pollution from agricultural sources will continue to decline.
Climate Change:	Agriculture-related activities that are sources of GHGs (e.g., exhaust from mechanized farm equipment; soil disturbance from tillage; fertilizer applications) have declined with the introduction of GE corn.	GHGs would continue to decline with determination of non-regulated status of MON84711.
Biological Resources		
Animal Communities:	Currently available insect resistant corn varieties do not impact populations of vertebrate and most invertebrate animals other than target pest species (e.g., European corn borer; CRWs). Non-target invertebrates are generally more abundant in *Bt*-corn fields than in fields of non- GE corn.	Expected to be the same as under the No Action Alternative. Studies have shown no adverse effects on vertebrate or invertebrate animals from diet containing the MON 84711 product or the dsRNA sequences that are produced by it. EPA regulates PIPs in IR corn and herbicides applied to HR corn, and determines whether specific PIPs including the RNAi PIP that is a subject of the EA, pose an unacceptable risk or impact on non-target organisms

Attribute/ Measure	Alternative A: No Action	Alternative B: Determination of Nonregulated Status
Plant Communities:	Corn growers will continue to use accepted practices to control weeds. Because glyphosate will continue to be used in corn production, increased populations of glyphosate resistant weeds are expected. High intensity agriculture will have some impact on plant communities near corn agricultural fields	MON 87411 is not a potential plant pest because it does not compete with native plant species, does not hybridize with relatives, and will not affect natural plant communities. Continued development of HR weeds is likely to continue, including the potential for development of weeds with resistance to multiple modes of action. Because MON 87411 is GR, replacing other GR varieties with this trait will have no new impacts. Corn growers use production practices to manage weeds in and around fields. EPA regulates herbicides applied to HR corn and PIPS, and determines whether they, including the RNAi PIP that is the subject of this final EA, pose an unacceptable risk or impact on non-target organisms including plants.
Soil Microorganisms:	Soil microbial communities will provide valuable resources to growers in the form of soil stability and quality, while responding to the transient impacts of common agricultural production practices.	Because MON 84711 has not been shown to impact soil microbial communities, determination of nonregulated status will not be expected to change microbial composition or structure.
Biological Diversity:	Currently available *Bt*-corn crops may increase non-target abundance compared to those treated with broad-spectrum insecticides. There is no evidence of landscape-level impacts from currently available IR HR corn varieties.	Field testing of MON 87411 in three countries has not shown any impacts on arthropod diversity when compared with fields planted to non-RNAi expressing varieties. MON 87411is not expected to alter biological diversity. EPA regulates impacts on biological diversity based on unacceptable risk or impact to non-target organisms

Attribute/ Measure	Alternative A: No Action	Alternative B: Determination of Nonregulated Status
Gene Movement:	Cultivated corn varieties can cross pollinate. Growers and seed-corn producers use various management practices to eliminate undesired cross pollination.	Current practices to maintain genetic purity of corn stocks are effective (Ireland, 2006). MON 84711 will not change these practices.
Public Health		
Human Health:	All corn varieties are associated with the same risks deriving from agricultural practices. Allergenicity to corn will continue to affect a small percentage of the population.	Neither the products of the RNAi mechanism associated with subject of this final EA (dsDvSnf7), nor the Cry proteins of *Bt*-corn products, nor the EPSPS protein are toxic to humans, and there are no known allergenic properties for humans.
Worker Safety:	EPA regulates herbicides applied to HR corn. Workers that routinely handle glyphosate may be exposed during spray operations. Because of low acute toxicity of glyphosate and absence of evidence of carcinogenicity and other toxicological concerns, occupational exposure data is not required for reregistration. However, EPA has classified	There are no effects of MON 87411 and its expressed RNAi dsDvSnf7 sequence on human health and no expectations of adverse worker exposure to the MON 87411 variety with its expressed Bt and EPSPS protein or exposure to the herbicide glyphosate.
Animal Feed:	Corn products will continue to be used in livestock feed.	Neither the products of the RNAi –based MON 87411 (expressing the dsDvSnf7) , the Cry proteins of this *Bt*-corn variety nor the EPSPS protein are known to be toxic to animal species fed corn products aside from targeted insects.
Socioeconomic Environment		

Attribute/ Measure	Alternative A: No Action	Alternative B: Determination of Nonregulated Status
Domestic Economic Environment:	The US will continue to produce both GE and conventional corn varieties.	Farm income is positively impacted by currently available *Bt* and HR corn by reducing production costs or increasing revenues. Pest-resistant corn generally has a positive impact on farm income because of cost savings from reduced pesticide use.
Trade Economic Environment:	The primary US corn export destinations are to the largest world importers of corn and do not have barriers for importing food or feed commodities produced from transgenic crops including those with insect resistance traits. Nevertheless, import of each specific trait	Export of MON 84711 will require applications and approvals by the importing country, and Monsanto has begun to seek those approvals.
Other Regulatory Approvals		
U.S. Agencies:	On March 31, 2004, the EPA established a permanent exemption from the requirement of a tolerance for the PIP, *Bacillus thuringiensis* Cry3Bb1 protein, and the genetic material necessary for its production in food and feed commodities of field corn, sweet corn and popcorn (40 CFR § 180.1214).	In a letter dated October 17, 2014 (Appendix A of this final EA), FDA confirmed completion of a consultation for a food/feed safety and nutritional assessment for Monsanto's 87411 corn. A summary of findings was submitted to FDA in November 2013.
Compliance with Other Laws		
CAA, CWA, EOs:	Fully compliant	Fully compliant

[1]Unchanged–the current conditions will not change as a result of the selection of this alternative;

[2]Minimal–the current conditions may change slightly as a result of the selection of this alternative, but the changes, if any, are negligible.

Finding of No Significant Impact

APHIS' analysis in the EA indicates that there will not be any significant impacts, individually or cumulatively, on the quality of the human environment as a result of this action. I agree with this conclusion and therefore find that an Environmental Impact Statement is not required. This NEPA determination is based on the following context and intensity factors as required by NEPA regulations (40 CFR 1508.27).

Context - The term "context" identifies potentially affected resources, the locations, and the specific circumstances and conditions in which the environmental impacts may occur. This action has potential to affect conventional and organic corn production systems, including surrounding environments and agricultural workers, human food and animal feed production systems, and foreign and domestic commodity markets.

Corn is grown in all 48 states of the conterminous continental United States. The highest concentration of production is located in the central United States (USDA-ERS, 2013a; USDA-NASS, 2013). The two states with the most production are Iowa and Illinois. They account for slightly more than a third of the United States (USDA-ERS, 2014c).

During the past two decades, corn acreage has increased. In 2000, 25% of U.S. corn production was from GE varieties (USDA-ERS, 2013b). In 2002, stacked hybrids were introduced. This led to a further increase in acreage of GE corn (Fernandez-Cornejo et al., 2014). By 2009, GE corn acreage exceeded 70% of the total in all major corn-growing states except Ohio (67%) (Fernandez-Cornejo et al., 2014). By 2013, 90% of the 87.6-million-acre U.S. crop was produced from GE corn.

In the period, 2006-2012, acreage of corn planted annually in the United States increased because market prices favored the planting of corn over alternative crops. In addition to the demand for feed grain, strong demand for ethanol production resulted in higher corn prices, which corresponded to an incentive to growers to increase acreage (USDA-ERS, 2013a). The increase in acreage involved all varieties of corn and occurred throughout the corn growing areas (USDA-ERS, 2010). In many cases, farmers increased corn acreage by adjusting crop rotations. Other sources of land for increased corn plantings were conversion from pasture and fallow land, acreage returned to production from expiring Conservation Reserve Program contracts, and shifts from other crops, such as soybean and cotton (USDA-ERS, 2014). A determination of nonregulated status of MON 87411 Maize is not expected to directly affect these influences on production trends, nor cause an increase in agricultural acreage devoted to corn production in general and that devoted to GE-corn cultivation. The availability of MON 87411 Maize will not change cultivation areas for corn production in the United States, and there are no anticipated changes to the availability of GE- and non-GE corn varieties on the market.

Intensity – Intensity is a measure of the degree or severity of an impact based upon ten factors. The following factors were used as a basis for this decision:

1. *Impacts that may be both beneficial and adverse.*

A determination of nonregulated status of MON 87411 Maize will have no significant environmental impact on the availability of GE, conventional or organic corn varieties. As discussed in Chapter 4 of the EA, a determination of nonregulated status of Mon 87411 Maize is expected to neither directly result in an increase in overall U.S. acreage of corn production, nor acreage of GE-corn. The availability of MON 87411 Maize will not change the areas of cultivation for corn production in the United States, and there are no anticipated changes in the availability of GE and non-GE corn varieties on the market. A determination of nonregulated status of Mon 87411 Maize will add another GE corn variety to the corn market, but is not expected to change the market demands for GE corn or corn produced using organic methods.

APHIS analyzed the data provided by Monsanto for MON 87411 (Monsanto 2013) andhas concluded in the EA that the availability of Mon 87411 Maize will not alter the agronomic practices, locations of corn production, nor the production methods and quality characteristics of conventional and GE corn seed production. The introduction of Mon 87411 Maize provides an alternative corn variety with traits that control CRW and the continuing sustainability of *Bt* proteins that are currently used for CRW control. The trait for resistance to glyphosate is similar to that of many current varieties of commercial corn, and would result in no new changes in development of weed resistance to glyphosate.

2. *The degree to which the proposed action affects public health or safety.*

A determination of nonregulated status of MON 87411 Maize would have no significant impacts on human or animal health. Compositional tests conducted by the petitioner indicate that MON 87411 Maize is compositionally similar to other commercially available GE corn varieties (Monsanto 2013). Monsanto initiated a consultation process with FDA for the commercial distribution of MON 87411 Maize and submitted a safety and nutritional assessment of food and feed derived from MON 87411 Maize to the FDA. In a letter dated October 17, 2014, FDA confirmed completion of this consultation. Based on the information Monsanto submitted, FDA confirmed that it had no further questions regarding MON 87411 Maize. Based on the FDA's consultation, laboratory data and scientific literature provided by Monsanto (Monsanto 2013), and safety data available on other Bt-expressing and herbicide-resistant products, APHIS has concluded that MON 87411 Maize would have no significant impacts on human or animal health.

3. *Unique characteristics of the geographic area such as proximity to historic or cultural resources, park lands, prime farmlands, wetlands, wild and scenic rivers, or ecologically critical areas.*

There are no unique characteristics of geographic areas such as park lands, prime farm lands, wetlands, wild and scenic areas, or ecologically critical areas that would be adversely impacted by a determination of nonregulated status for MON 87411 Maize. The common agricultural practices that would be carried out under the proposed action will not cause major ground disturbance, nor cause any physical destruction or damage to property, wildlife habitat, or landscapes, and do not involve the sale, lease, or transfer of ownership of any property. This action is limited to a determination of nonregulated status of MON 87411 Maize. The product will be planted on agricultural land currently suitable for production of corn, will replace existing varieties, and is not expected to increase the acreage of corn production. This action

would not convert nonagricultural land, and therefore would have no adverse impact on prime farm land. Standard agricultural practices for land preparation, planting, irrigation, and harvesting of plants would be used on agricultural lands planted to MON 87411 Maize including the use of EPA-registered pesticides. The applicant's adherence to EPA-label-use restrictions for all pesticides will mitigate potential impacts to the human environment. In the event of a determination of nonregulated status of MON 87411 Maize, the action is not likely to affect historic or cultural resources, park lands, prime farmlands, wetlands, wild and scenic rivers, or ecologically critical areas that may be in close proximity to corn production sites.

4. *The degree to which the effects on the quality of the human environment are likely to be highly controversial.*

   The effects on the quality of the human environment following a USDA determination of nonregulated status for MON 87411 Maize are not highly contested by scientists or those who may be in a position to supply substantive information. Although APHIS received public comments opposed to a determination of nonregulated status of MON 87411 Maize, this action is not likely to be highly controversial in terms of size, nature or effect on the natural or physical environment. As discussed in Chapter 4 of the EA, a determination of nonregulated status is not expected to directly cause an increase in agricultural acreage devoted to corn production in general, nor acreage devoted to GE corn cultivation. The availability of MON 87411 Maize will not change cultivation areas for corn production in the United States, and there are no anticipated changes to the availability of non-GE- and GE-corn varieties on the market. A determination of nonregulated status of MON 87411 Maize would add another GE-corn variety to the conventional corn market and is not expected to change the market demands for GE corn or corn produced using organic methods. A determination of nonregulated status of MON 87411 Maize will not change current practices for planting, tillage, fertilizer application or use, cultivation, pesticide application or use, or volunteer control. Management practices and seed standards for production of certified corn seed would not change. The effect of MON 87411 Maize on wildlife or biodiversity is no different than that of other GE corn currently used in agriculture, or other GE or non-GE corn produced in conventional agriculture in the United States. EPA will provide initially for only a seed increase permit for two years and on limited acreage, and has requested additional information about observations conducted to study arthropod biodiversity already supplied by Monsanto (US-EPA, 2015).

5. *The degree to which the possible effects on the human environment are highly uncertain or involve unique or unknown risks.*

   From the analysis documented in the EA, the possible effects on the human environment are understood, although as EPA acknowledged some "uncertainties associated with the potential for unexpected effects related to exposure to dsRNA" had to be considered by the Science Advisory Panel that offered advisement on the issues (US-EPA, 2015). However, EPA has produced an Environmental Risk Assessment for a FlFRA Section 3 Limited Seed Increase (US-EPA, 2015), based on the previous ecological impact assessment method used for other PIPSs (US-EPA, 2015). The effects of the proposed determination of nonregulated status are based on the preponderance of evidence provided by Monsanto and by USDA's assessment of potential risk through consideration of experimental evidence and factual information in the

scientific literature. USDA does not conclude that risks to the natural or physical environment are substantive ones.

As discussed in Chapter 4 of the EA, a determination of nonregulated status of MON 87411 Maize is expected to neither directly cause an increase in agricultural acreage devoted to corn production, nor increase acreage devoted to GE-corn cultivation. A determination of nonregulated status of MON 87411 Maize will not result in changes in the current practices of planting, tillage, fertilizer application/use, pesticide application/use or volunteer control. Management practices and seed standards for production of certified corn seed would not change. The effect of MON 87411 Maize on wildlife or biodiversity is neither different from that of other GE crops currently used in agriculture, nor that of other GE or non-GE corn produced in conventional agriculture in the United States. As described in Chapter 2 of the EA, well-established management practices, production controls, and production practices (GE, conventional, and organic) are currently being used in commercial corn crop and see production systems in the United States. Therefore, it is reasonable to assume that farmers who produce conventional corn (GE and non-GE varieties), or produce corn using organic methods, will continue to use these reasonable, commonly-accepted, best-management practices for their chosen systems and varieties during agricultural corn production. GE corn is also currently planted on the majority of U.S. corn acres. Based upon historic trends, conventional production practices that use GE varieties will likely continue to prevail in terms of acreage with or without a determination of nonregulated status of MON 87411 Maize. Given the extensive experience that APHIS, stakeholders, and growers have with the use of GE corn products, the possible effects to the human environment from the release of an additional GE-corn product are already well known and understood. Therefore, the impacts are not highly uncertain, and do not involve unique or unknown risks.

6. *The degree to which the action may establish a precedent for future actions with significant effects or represents a decision in principle about a future consideration.*

A determination of nonregulated status for MON 87411 Maize would not establish a precedent for future actions with significant effects, nor would it represent a decision in principle about a future decision. While the request to EPA for an Experimental Use Permit for MON 87411 represents a request for a new trait with a target dissimilar to any others already permitted (interference RNA to control an insect pest), EPA is using its current ecological risk assessment approach for PIPs that was developed primarily from experience with *Bt*-derived Cry and Vip proteins (US-EPA, 2015). However, this will not necessarily be the same analysis used for future products. As EPA notes, "because of uncertainties associated with the potential for unexpected effects related to exposure to dsRNA, EPA raised questions to the SAP [Science Advisory Panel) regarding the applicability of the above approach to dsRNA PIPs. The SAP found that this approach was not sufficient to determine risks to nontarget organisms, and suggested an alternative framework (see pages 61-64 of the SAP minutes), which EPA is currently evaluating" (US-EPA, 2015).

Similar to past regulatory requests reviewed and approved by APHIS, a determination of nonregulated status will be based on whether an organism is unlikely to pose a plant pest risk pursuant to the regulatory requirements of 7 CFR part 340. Each petition that APHIS receives is specific to a particular GE organism and undergoes this independent review to determine if

the regulated article poses a plant pest risk. Under the authority of the plant pest provisions of the PPA and 7 CFR part 340, APHIS has issued regulations for the safe development and use of GE organisms. As required by 7 CFR 340.6, APHIS must respond to petitioners who request a determination of the regulated status of GE organisms, including GE plants such as MON 87411 Maize. When a petition for nonregulated status is submitted, APHIS must determine if the GE organism is unlikely to pose a plant pest risk. If APHIS determines, based on its Plant Pest Risk Assessment, that the GE organism is unlikely to pose a plant pest risk, the GE organism is no longer subject to the plant pest provisions of the PPA and 7 CFR part 340.

7. *Whether the action is related to other actions with individually insignificant but cumulatively significant impacts.*

   No significant cumulative effects were identified during this assessment. The EA discussed cumulative effects on corn management practices, human and animal health, and the environment, and concluded that such impacts were not significant. A cumulative effects analysis is provided in Chapter 5 of the EA. In the event APHIS reaches a determination of nonregulated status of MON 87411 Maize, APHIS would no longer have regulatory authority over it and would no longer regulate it. In the event of a determination of nonregulated status of MON 87411 Maize, APHIS has not identified any significant impact on the environment that may result from the incremental impact of a determination of nonregulated status of MON 87411 Maize when added to past, present, and reasonably foreseeable future actions.

8. *The degree to which the action may adversely affect districts, sites, highways, structures, or objects listed in or eligible for listing in the National Register of Historic Places or may cause loss or destruction of significant scientific, cultural, or historic resources.*

   A determination of nonregulated status of MON 87411 Maize will not adversely impact cultural resources on tribal properties. Any farming activities that may be used by farmers on tribal lands are only conducted at the tribe's request. Thus, the tribes have control over any potential conflict with cultural resources on tribal properties. A determination of nonregulated status of MON 87411 Maize would not impact districts, sites, highways, structures, or objects listed in, or eligible for listing in the National Register of Historic Places, nor would they likely cause any loss or destruction of significant scientific, cultural, or historic resources. This action is limited to a determination of nonregulated status of MON 87411 Maize. Standard agricultural practices for land preparation, planting, irrigation, and harvesting of plants would be used on these agricultural lands including the use of EPA-registered pesticides. Adherence to EPA-label-use restrictions for all pesticides will mitigate impacts to the human environment. A determination of nonregulated status of MON 87411 Maize is a decision that will not directly or indirectly cause alteration in the character or use of historic properties protected under the National Historic Preservation Act (NHPA). In general, common agricultural activities conducted under this action do not have the potential to introduce visual, atmospheric, or audible elements to areas where they are used that could result in effects on the character or use of historic properties. For example, there is potential for audible effects on the use and enjoyment of a historic property when common agricultural practices, such as the operation of tractors and other mechanical equipment, are conducted close to such sites. A built-in mitigating factor for this issue is that virtually all of the methods involved would only have temporary effects on the audible nature of a site and can be ended at any time to restore the

audible qualities of such sites to their original condition with no further adverse effects. These cultivation practices are also being conducted currently throughout the corn production regions. The cultivation of MON 87411 Maize does not inherently change any of these agronomic practices in way that would cause any impact under the NHPA.

9. *The degree to which the action may adversely affect the endangered or threatened species or its habitat that has been determined to be critical under the Endangered Species Act of 1973.*

   As described in Chapter 6 of the EA, APHIS has analyzed the potential for effects from a determination of nonregulated status of MON 87411 Maize on federally-listed threatened and endangered species (TES), species proposed for listing, and designated critical habitat and habitat proposed for designation, as required under Section 7 of the Endangered Species Act. After reviewing possible effects of a determination of nonregulated status of MON 87411 Maize, APHIS has concluded that a determination of nonregulated status of MON 87411 Maize would have no effect on federally listed TES and species proposed for listing, or on designated critical habitat or habitat proposed for designation.

10. *Whether the action threatens a violation of Federal, State, or local law or requirements imposed for the protection of the environment.*

    The proposed action would be in compliance with all Federal, state, and local laws. EPA regulates all plant incorporated products, including both traits that express either the Bt protein, or the dsRNA for DvSnf7. EPA in an Environmental Risk Assessment has determined that "the activity of the Cry3Bbl protein expressed in MON 88017 was also determined to be biochemically and functionally equivalent to Cry3Bbl expressed in MON 863 maize, and both were determined to have no unreasonable adverse effects on nontarget organisms (US-EPA, 2015). EPA has also concluded, "Based on the data and rationale presented, adverse effects to nontarget organisms are not expected as a result of the proposed seed increase registration of DvSnf7 expressed in MON 874 11 corn.

    Because APHIS has concluded that MON 87411 Maize is unlikely to pose a plant pest risk, a determination of nonregulated status of MON 87411 Maize is a response that is consistent with the plant pest provisions of the PPA, the regulations codified in 7 CFR part 340, and the biotechnology regulatory policies in the Coordinated Framework. Monsanto initiated the consultation process with FDA for the commercial distribution of MON 87411 Maize and submitted a safety and nutritional assessment of food and feed derived from MON 87411 Maize to the FDA (Monsanto 2013). Based on the information Monsanto submitted, FDA confirmed on October 17, 2014 that it had no further questions regarding MON 87411 Maize. MON 87411 Maize is compositionally similar to currently available corn on the market. There are no other Federal, state, or local permits that are needed prior to the implementation of this action.

NEPA Decision and Rationale

I have carefully reviewed the EA prepared for this NEPA determination and the input from the public involvement process. I believe that the issues identified in the EA are best addressed by selecting Alternative 2 (Determination that MON 87411 Maize is No Longer a Regulated Article). This alternative meets the APHIS purpose and need to allow the safe development and use of GE organisms consistent with the plant pest provisions of the PPA.

As stated in the CEQ regulations, "the agency's preferred alternative is the alternative which the agency believes would fulfill its statutory mission and responsibilities, giving consideration to economic, environmental, technical and other factors." The preferred alternative has been selected for implementation based on consideration of a number of environmental, regulatory, and social factors. Based upon our evaluation and analysis, Alternative 2 is selected because (1) it allows APHIS to fulfill its statutory mission to protect America's agriculture and environment using a science-based regulatory framework that allows for the safe development and use of GE organisms; (2) it allows APHIS to fulfill its regulatory obligations. As APHIS has not identified any plant pest risks associated with Mon 87411 Maize, the continued regulated status of MON 87411 Maize would be inconsistent with the plant pest provisions of the PPA, the regulations codified at 7 CFR part 340, and the biotechnology regulatory policies in the Coordinated Framework. For the reasons stated above, I have determined that a determination of nonregulated status of MON 87411 Maize will not have any significant environmental effects.

10/23/2015

Michael J. Firko, Ph.D.

Date

APHIS Deputy Administrator

Biotechnology Regulatory Services

Animal and Plant Health Inspection Service

U.S. Department of Agriculture

Response to Public Comments on Monsanto 87411 Maize

In a *Federal Register* notice (79 FR 13035-6) on March 7, 2014, APHIS announced the availability of the petition for public review and comment (Docket No. APHIS-2014-0007). The 60-day public comment period closed on May 7, 2014. APHIS received 423 comments during the period the petition was available for public review. Comments are available for public review in the docket file:

http://www.regulations.gov/#!docketDetail;D=APHIS-2014-0007

Issues identified in comments submitted for the petition were considered by APHIS as part of its environmental analysis process and responses were incorporated into the EA.

On March 6, 2015, APHIS published a notice in the Federal Register (77 FR 13035-13036, Docket No. APHIS- 2014-0007) announcing the availability of the draft Environmental Assessment (EA) and preliminary plant pest risk assessment (PPRA) for a 60-day public review period. On June 1, 2015 the comment period was reopened for an additional 30 days (80 FR 30997-30998) Docket No. APHIS-2014-0007). APHIS received a total of 12 comments: two supported a decision of nonregulated status for MON 87411 Maize; nine were opposed. Comments can be reviewed in the docket file at:

http://www.regulations.gov/#!documentDetail;D=APHIS-2014-0007-0002.

Most of the comments expressing opposition to nonregulatory status for MON 87411 Maize expressed general opposition to genetically engineered (GE) food, the belief that GE crops harm the environment, or the belief that GE crops are not beneficial to farmers. Several specific issues related to the Monsanto CRW-protected and GR maize EA were identified by the public. All comments received were evaluated on the basis of whether they addressed the issues in question, whether they were based on valid science, and whether they were reasonable and practicable.

One opposing comment included 67 attachments of documents and published articles. APHIS has extensively reviewed the relevant articles submitted with this comment. Thirty-one of these attachments were relevant to Monsanto's petition and the EA; 63 either were not relevant to issues and topics considered in the EA, or were general review papers that did not provide any new information that had not been included in the EA.

Issues expressed in opposing comments related to Monsanto CRW-protected and GR maize EA were organized into categories. Substantive issues were identified and are addressed in the responses that follow. Some comments included more than one issue. Therefore, the number of issues that follow, and the Agency response to each of them, does not correspond to the number of opposing comments that were submitted for the EA.

Issue 1: The EA is based on incomplete and inadequate science and analyses, and lacks critical data and vital risk assessments.

Response 1: APHIS disagrees. The Agency reviewed all available information and performed a rigorous analysis of the consequences and uncertainties in its EA before making a decision. The CEQ requires that an EA must be based on the best-available information. It does not require that new studies be commissioned or that new data be developed to support a NEPA document and decision.

APHIS identified reasonable alternatives and analyzed them using available information from various sources, including the data provided by Monsanto (Monsanto, 2013) and that available in the peer-reviewed, scientific literature to make an informed regulatory decision regarding the possible plant pest risks that may be associated with MON 87411 CRW-protected and GR maize. The Agency concluded that this product is unlikely to pose a plant pest risk.

APHIS also carefully reviewed the information provided by the petitioner and all available other sources and considered the possible environmental effects of regulating MON 87411 Maize (no-action alternative) or not regulating it (preferred alternative). Using the best-available relevant scientific information, APHIS analyzed possible effects of MON 87411 Maize on the environment, and concluded in its EA that these effects would not cause significant impacts.

Issue 2: The broad geographical range and the widespread importance of corn as a major cropping system in the United States requires preparation of an environmental impact statement (EIS).

Response 2: APHIS notes that neither the geographical extent nor economic importance of a crop, such as corn, is primary requirements for initiating an EIS. NEPA regulations determined by CEQ clarify that the threshold establishing the need for an EIS is the identification of one or more significant environmental impacts by an agency during its analysis for completing an EA. APHIS prepared its EA to consider all possible environmental effects of the proposed action and the reasonable alternatives to that action, consistent with NEPA requirements (40 CFR parts 1500-1508, 7 CFR 1b, and 7 CFR part 372).

The EA specifically evaluated the possible effects on the quality of the human environment that may result from a determination of the regulated status of Monsanto CRW-protected and GR maize. APHIS assembled a list of alternatives and evaluated these alternatives consistent with the Agency's statutory authority under the plant pest provisions of the Plant Protection Act, the regulations at 7 CFR part 340, and NEPA requirements (40 CFR parts 1500-1508, 7 CFR 1b, and 7 CFR part 372). It considered environmental safety, efficacy, and practicality to identify which alternatives were the appropriate ones to evaluate before making its decision. As described in the EA, APHIS evaluated two alternatives; (1) no action and (2) a determination of nonregulated status for Monsanto's rootworm-protected and glyphosate resistance maize. APHIS concluded that the determination of nonregulated status would not cause significant impacts on the environment. Therefore, APHIS does not need to prepare an EIS before making a regulatory decision about to MON 87411 Maize.

Issue 3: MON 87411 Maize is unique because it incorporates an "animal" gene:

Response 3: The SvSbf7 gene in MON 87411 Maize is based on the partial coding sequence of the Snf7 gene from the corn rootworm, a Coleopteran insect (Monsanto, 2013). Data indicate

that the gene product is very specific, and is known to target only the corn rootworm, but not other insects even within the same family. There is no protein produced from the SvSbf7 gene, but rather only dsRNA. APHIS has experience evaluating numerous GE plants which utilize RNAi technology. In addition, APHIS has experience evaluating a variety of GE plants which contain genes from divergent sources including plants, bacteria, and viruses, and marine invertebrates. Thus, MON 87411 Maize utilizes familiar technology and does not present unique risks that have not been considered in other GE plants.

Issue 4: The commenter claims that potentially significant impacts on cultural development were not addressed in the petition, PPRA and EA.

Response 4: APHIS notes that the term, "cultural development" is not defined in the guidelines for responding to NEPA established by CEQ nor by the APHIS-implementing regulations for NEPA. The Agency also notes that the petitioner is not required to address "cultural development" in a petition for nonregulatory status.

If cultural development issues are included as components of the domestic economic environment and the trade economic environment, then APHIS addressed these in its EA, and determined that neither the preferred alternative nor the no action alternative will cause significant impacts on the domestic or trade economic environment.

Issue 5: A simple risk assessment based on safety to humans or the environment is inadequate to evaluate potentially significant cultural reactions to DvSnf7 RNA, such as not accepting the presence in food containing novel genes expressed in the plant.

Response 5: APHIS did not evaluate consumer preferences with regard to GE food in its EA because it is not within the scope of its NEPA requirement, APHIS regulatory authority, or the policies of the Federal government for products produced using recombinant DNA techniques as set forth in the Coordinated Framework for regulating biotechnology. FDA, not USDA, has authority over food safety and nutritional equivalencies of products derived from crops, whether genetically engineered or derived otherwise.

FDA responsibilities in reference to food products derived from GE crops are defined in the Coordinated Framework and Monsanto addressed those by engaging FDA in a consultation process.

Issue 6: "APHIS should consider all 'reasonably foreseeable' environmental impacts of the proposed deregulation of MON 84711, taking a programmatic approach to consider the use of RNAi technology on other crops and against other pests that will likely follow the deregulation of MON 84711."

Response 6: EPA, not USDA, has the authority to regulate the PIPs (plant-incorporated protectants: (*Bt* and RNAi) in MON 84711 Maize. EPA will continue to analyze environmental effects of this and other similar RNAi products which may impact nontarget animals beginning with a "White Paper," convening a Science Advisory Panel and following with a summary document of the Panel's findings. EPA has not fully completed a programmatic response to this type of product to allow for full registration. For MON 87411 Maize, EPA will require as

much data from Monsanto as needed to make a decision about the registration of this product and the required conditions for its use (pesticide labeling requirements), if any. In addition, current approaches for data needed to establish safety for other PIPs are being used in the EPA evaluation. Additional assessments will be made if new issues and risks are identified in the course of a proposed EPA permit for seed increase on limited acreage.

An EPA commitment for a programmatic approach to additional RNAi products has been made, which includes establishing new requirements for tests and observations. The requirements will be constructed after continued assessment of recent data requests of Monsanto, as well as any further field and lab data offered by Monsanto. An EPA Science Advisory Panel has already been convened in January 2015, and this has provided EPA with advice for the future development of regulation of future RNAi products. Finally, interactions with the company on an ongoing basis will also provide information sufficient for analysis of potential risks of future related products. When the regulatory protocols for these similar products are established, APHIS will use these to inform and confirm its future NEPA assessments.

Issue 7: **APHIS must assess the impacts associated with this novel technology which is in the early phases of its development in an EIS, and new information about host-induced gene silencing is only now being revealed.**

Response 7: Although RNAi technologies are not new, APHIS agrees new research continues to add to our understanding of the RNAi mechanism, however, much is known and APHIS has sufficient information on the phenotype and spectrum of activity of MON 87411 to perform a risk assessment. The commenter pointed out recent findings by Ivashuta et al. (2015), which show that long dsRNAs from corn may produce many 21 nucleotide (nt) siRNAs that correspond with western corn rootworm transcripts and are routinely formed in relatively high abundance. However, these do not affect the insect transcriptome, since most of these siRNAs derived from the host plant are formed from plant dsRNA by the insect in low copy number. While this is not a surprising finding, the conclusion that the authors could find no impact of plant-originated siRNAs on WCR RNA transcripts was important. The authors also found that while beetles were capable of cellular uptake and incorporation of environmental RNA (env-RNA), in a lepidopteran insect no plant-sourced siRNAs (one type of an env-RNA) were found. Feeding of whole animals with high concentrations of RNA isolated from corn or soy did not cause any changes of development or in weight gain. It appears that sequence identity of plant-produced RNAs is not alone sufficient to change transcription or host development, but that high copy numbers of the dsRNA are also required. This molecular analysis provides a mechanism for exclusion of potentially impacted nontarget organisms, confirming that such impacts on RNA insensitive insects such as Lepidoptera are unlikely. While new information about host induced gene silencing is valuable, the major issues about its mechanism are relevant, but the potential for impacts and under what circumstances they are important are

known from experimental observations and experience. Because EPA concludes that there are sufficient observations about these impacts, and that safety concerns have been satisfied by these observations, then EPA will provide conditional approval of two-year limited acreage seed increases. New details about underlying mechanisms elucidated by continuing research are also useful, and will be considered by USDA as future RNAi products are assessed for environmental impacts.

Issue 8: **APHIS cannot base claims of "no impact" for MON 87411 on previous examples of gene silencing in GE crops such as GE papaya, summer squash, plum or genes of the plant itself (GE potato, apple, altered oil soybean) because their targets are completely different.**

Response 8: While APHIS believes that the experience gained using other plants that use RNAi technology is relevant, we agree that each case is different and thus we continue to evaluate each on a case-by-case basis.

In this case, APHIS reviewed information which indicated that the activity spectrum of DvSnf7 RNA has been shown to be highly specific to corn rootworms. Bioassays were performed using representative insect species having close taxonomic relatedness to corn rootworm. In total 14 representative insect species from 10 Families and 4 Orders (Hemiptera, Hymenoptera, Lepidoptera, and Coleoptera) were tested. In these bioassays activity was found only in the subfamily Galerucinae in the family Chrysomelidae within the order Coleoptera. Specifically, only the western corn rootworm and the southern corn rootworm were affected. the Colorado potato beetle, which is in another subfamily (Chrysomelinae) of Chrysomelidae,is known to be sensitive to certain ingested dsRNAs; however, it was not affected by DvSnf7 RNA.

In addition, data indicated no effect of DvSnf7 RNA on any of the other nontarget species tested including the following which are often considered beneficial to agriculture: the spotted ladybird beetle, ground beetle, honeybee, insidious flower bug, and earthworm. This, together with the results from the study using the 14 species described above and the sequence specific nature of RNAi support a conclusion that it is unlikely that DvSnf7 RNA will have an effect on nontarget organisms.

APHIS also considered many other aspects of the observed phenotype in agronomic settings as described in the petition. . The totality of this information allowed APHIS to reach a Finding of No Significant Impact (FONSI).

Issue 9: Off-target effects of RNAi silencing are common – so common in fact that they constitute major obstacles to the use of gene silencing, for example in human therapy as noted by Haussecker and Kay, 2015, the production of RNAi pesticides as described by Palli, 2014, and the agronomic improvement of crops cited by Saurabh et al., 2014."

Response 9: The writer cites Saurabh et al., (2014) as suggesting that off-target effects are a "major obstacle" to commercial usages, but these authors note that one of the benefits of RNAi for gene silencing is that it is "precise—no off-target effects." While the issues for potential

human impacts of RNAi are noted by Haussecker and Kay (2015), these concerns are not directed towards environmental RNA, which would be the mode of human exposure to RNAi from the MON 87411 Maize product. Rather, additional but different modes of human exposure are the focus in this paper.

The first modality cited by Haussecker and Kay (2015) includes RNAi expressed by transformed human cells (that is, using a "genetic template") that produce dsRNA. The impacts of this usage would be on those internal cellular processes normally mediated by microRNAs. Second, these authors note the alternative strategy for providing an effective dosage of human RNAi is through administering oligonucleotides directly. This requires use of a specific 'delivery option' to protect introduced dsRNA from the mechanisms by which these RNAs are easily degraded in humans. As noted in the Environmental Consequences Human Impacts section, RNA is not stable in human digestive tracts or circulatory system and is rapidly degraded. The third author cited by the commenter, Palli (2014), recognizes the potential issue of off-target activity of either plant-expressed or applied (externally sprayed) RNAi, but he notes the study of Bachmann et al. (2013) which showed the specificity of the DvSnf7 and its lack of effects on the insects of 10 families. Spraying of RNAi in agricultural situations has potential for impacts but he notes that 90% of DvSnf7 is degraded in 36 hours (Dubelman et al., 2014), and was not detectable after two days. Palli (2014) cites the authors conclusion that DvSnf7 was not likely to accumulate in the environment, so is unavailable for uptake and thus unlikely to cause off-target effects.

Issue 10: Corn rootworms are likely to develop resistance to the RNA-interference-based mechanism for several likely reasons.

Issue 10 A. Several commenters addressed the possibility that corn rootworms would develop resistance to the RNAi component of MON 87411 Maize because each component, the Bt protein as well as the RNAi mechanism results in mortality consistent with a "low dose" strategy of plant protection.

Response 10 A: The development of resistance to any insecticidal mechanism should be managed and then averted if possible. However, given the available rootworm products such as various *Bts*, and now this product, the extremely high mortality that might be most desirable is not commercially available. Taking account of this limitation, multiple overlapping toxins are the best strategy to avoid a rapid selection for resistance (Storer et al., 2012). Critical to the usefulness of this is that first, the multiple toxins act independently of one another through different modes of action, so cross resistance isn't possible. As recently affirmed by Levine et al. (2015), the Cry protein, 3Bb1, currently used in field protection from damage caused by corn rootworm acts completely independently of DvSnf7 for toxicity to southern corn rootworm. Second, as noted by the commenter, the target insect should not be resistant to one of the multiple toxins used in the strategy. In the case of growers who have known or

suspected rootworm resistance to Cry3Bb1, these would be advised on Monsanto's and on independent websites, by field seed dealers and state extension personnel not to plant MON 87411 Maize combined with this *Bt* trait in their affected corn production fields. Grower perception of CRW resistance is considerable. About 23% of growers in Iowa in 2012 perceived that resistance to a *Bt* trait had occurred in their fields (Hodgson et al., 2013), and over half were able to confirm the suspicion with either direct root surveys or observations of corn plant goosenecking. APHIS concludes that growers will respond correctly to the advice of consultants to avoid planting the MON 87411 variety when a field location already is suggestive of susceptibility of CRW to Cry3bb1. Use of a seed combination of the MON 87411 trait along with *Bt*s to which the CRW were not previously resistant would be a robust strategy to protect the future use of *Bt*s, and also delay resistance to MON 87411.

Issue 10 B: Evidence for variable mortality responses to one RNAi-based pest control strategy are already described, and therefore resistance to the strategy may occur quickly (Chu et al., 2014).

Response 10 B: APHIS disagrees with Chu et al. (2014) who indicate that resistance to MON 87411 Maize will quickly appear in rootworm populations. From observations made by Chu et al. (2014) the authors conclude that RNAi silencing for insect control should be chosen so that the sequences used do not cause variable effects on different populations of the same species. APHIS agrees that differential susceptibility would potentially lead to early selection of populations for resistance to the introduced dsRNA sequence. In the case of the sequences that were assayed by Chu et al. (2014), the authors knew before beginning their observations that the genes were expressed at different levels in the three populations on which mortality would be assessed. Since it is known that pest populations with variable susceptibility to a particular RNAi based control method are likely to rapidly give rise to a largely resistant population. APHIS expects that any future products will be chosen which are broadly effective against the entire population when possible, thus delaying the possible selection of resistant pests.

Issue 10 C: Because the mechanism of cellular viral response to degrade virus impacts employs the same machinery as used by RNAi strategies for pest control, changes in viral susceptibility could alter the RNAi susceptibility as well.

Response 10 C: Multiple mechanisms are often involved in the development of insect resistance to external chemicals, and a mechanism that may change the RNAi machinery and allow susceptibility to the Snf7 dsRNAi sequence is possible (Swevers et al., 2013). As for this potential development in insects, and the consequences for other insect populations, APHIS does not disagree with the conclusion. No actual occurrences of this damage to silencing capacity in a cell have been demonstrated, neither to processing of an insect dsRNA, miRNA, nor siRNA. Some insect families may not have the capacity initially to process dsRNAs, but

these would be the native condition and for which an RNAi strategy would not be developed. APHIS estimates that if a population of pest insect became more susceptible to viruses (by inactivity of the Dicer/RISC) because they were tolerant of silencing dsRNA, populations of CRW could disappear. In contrast, if defenses against viruses were sharpened because the Dicer/RISC complex became more selective, discriminating between virus sequences for which siRNA was produced and which destroyed virus development and dsRNA against CRW which it failed to silence, infected insects as a source of a persist virus may be possible but of no consequence to insects other than the targeted pest species. It is clear that the capacity of many invertebrates to respond to virus infection is based on an RNAi mechanism, and may be indispensable for the protection afforded.

Issue 10 D: Defenses of insects against dsRNAs may be dispensable traits, and if so, this would allow new mechanisms of resistance to arise against the RNAi strategy.

Response 10 D: APHIS agrees that a variety of genetic adaptations could be used by CRW to overcome an RNAi-based defensive mechanism expressed by plants. As noted by Swevers et al. (2013), "as for every method for insect control, however, the rise of insecticide resistance is always a major issue." Speculation about these mechanisms is certainly justified as the commenter reports. Selective loss of the Dicer/RISC based defenses against viruses (the mechanism that is used by the RNAi expression) would be a highly tenuous insect strategy inasmuch as there would need to be simultaneous development of an alternative means to control viruses as noted by Shabalina and Koonin (2008). The most important issue is not that CRW may be unintentionally selected for susceptibility to RNAi, but that appropriate strategies should be developed and executed by growers to effectively delay the potential for new resistance. Increasingly corn growers recognize that they must detect and respond to new incidents of corn rootworm resistance in their managed fields (Hodgson et al., 2013). These growers are well aware of recently arising CRW resistance in corn with one of the available *Bt* traits (and possibly another), and are incorporating additional strategies beyond reliance on seed technology to protect current resources used to defend corn from rootworm-inflicted losses of yield (Hodgson et al., 2013). Growers will also likely defend future resources by choosing good insect management practices for MON 87411.

Issue 10 E: In the nematode *C. elegans*, persistent viral infections and deficiency of RNAi s are correlated in some existing strains, and the underlying mechanism for the observation although not known, could become a mechanism of resistance in CRW if environmental RNA never accumulated in some populations of insect pests.

Response 10 E: The potential for disruption of the siRNA mechanism by virus infection is suggested by Swevers et al. (2013) who find evidence for several such mechanisms, including

some in insects. These mechanisms are those directed by the virus to inhibit a component of the Dicer/RISC based system that responds to virus infections (to the detriment of the virus). The question posed by Swevers et al. (2013) is whether deployment of an RNAi based technique can demonstrate whether latent or chronic viral infections might be a successful mechanism for resistance to a commercial gene silencing mechanism. Again, APHIS asserts that mechanisms for resistance to any insect control strategy may well develop in an environment which exerts a consistent selecting pressure against an insect, but the focus for growers who plant this variety should be one of stewardship in which users attempt to delay that result by using appropriate pest management techniques.

Issue 10 F: APHIS concludes that the likelihood of CRW developing resistance to DvSnf7 RNAi is decreased by the presence of CRW-targeted Bt protein, but susceptibility of the insect to new mechanisms of resistance to the RNAi could reduce the ability of sustainable use of the Bt proteins which it would otherwise be supporting.

Response 10 F: As noted earlier in these Responses to Comments, APHIS does not disagree that resistance mechanisms to RNAi have been proposed, and that some may be potentially efficacious for developing resistance if selected for by exposure of CRW to RNAi. In the EA, APHIS has recognized the current status of corn rootworm resistance to *Bt*s (Section 5.3.1) and does not speculate on the future usefulness of those CRW *Bt*s to which resistance has not yet developed. However, APHIS asserts that the combination of multiple CRW toxins is a more effective strategy than either of these alone, either RNAi or specific *Bt* traits. Monsanto plans to stack commercial varieties with both MON 87411 toxins, and thus, seed production will not be pursued with the RNAi trait alone to resist CRW, which may not be a sustainable approach to provide sustained defenses against CRW.

Issue 11: APHIS ignored substantial uncertainties and data gaps in its EA analysis and based its analysis on very recent studies of Monsanto itself.

Response 11: The uncertainties about potential for impacts on the environment have been identified by EPA's Scientific Advisory Panel, convened in January, 2014, and acknowledged in EPA's summary of the record (US-EPA, 2014). Several authors who have reviewed the potential for impacts from RNAi use have also described some of the means by which these products might be assessed; these means may reduce the uncertainties of granting EPA permits and if adopted by EPA, further encourage their deployment on a commercial scale. EPA recently requested additional data from Monsanto supplementing the permit application, to further investigate the safety of MON 87411 Maize (personal communication, US-EPA). EPA subsequently received the data from Monsanto which EPA accepted but is also requesting clarification of some of the completed experiments and their conditions (US-EPA, 2015). The initial EPA and human effects and environmental effects analyses have been released for public

comment, and these represent the primary federal analysis of risks to the environment from MON 87411 (US-EPA, 2015). EPA has regulatory authority over pesticides and Plant-Incorporated Protectants (PIPs) and employs that authority to issue permits for this and other PIPs.

EPA has determined that it would primarily assess the potential impacts of DvSnf7 dsRNA using criteria and testing protocols developed for other plant incorporated protectants (US-EPA, 2015). EPA has concluded that the types of barriers within nontarget organisms for environmental RNA were sufficient to prevent environmental impacts (US-EPA, 2015), and although certain types of genomic and transcriptional details in these nontarget organisms might be of interest (US-EPA, 2014), an empirical approach was more likely to be adequate for analysis of the impact possibilities.

Although APHIS analyzed possible effects of MON 87411 Maize in its EA, USDA defers to the regulatory authority of EPA consistent with its findings and conclusions, regarding risks that may be associated with MON 87411 Maize. Some of these are uncertain because although possible impacts have been proposed, currently available data neither confirm nor refute these possibilities.

USDA used the best available data to prepare its EA, which is the requirement of NEPA, and made its conclusion based on the preponderance of evidence that MON 87411 Maize would not cause any significant environmental impacts if it were no longer regulated as a plant pest.

Issue 12: To carefully weigh the risks associated with RNAi to express a pesticide trait, USDA should work with the EPA to design a new risk assessment framework that can adequately capture the unintended consequences of the introduction of dsRNA molecules before any crops containing the technology are approved.

Response 12: As noted in previous responses, the approach that EPA is taking for future products is development of a risk assessment framework, which by following the pattern of previous permit processes, will prescribe specific types of tests and most likely, expected designs for field trials. As EPA announced for MON 87411 Maize, the assessment will include a permit for only a limited spatial release (15,000 acres) for the purpose of producing seed and potentially extending existing Monsanto observations and data, during a limited temporal release (for two years). Additional information about existing data will be used by EPA to make a final decision (US-EPA, 2015). This period of conditional and limited approval of a permit for the novel PIP (RNAi) in MON 87411 Maize will allow Monsanto to provide additional support for this RNAi product.

Issue 13: USDA must look at the literature surrounding this technology and evaluate the specific safety concerns for a method with so many associated risks.

Response 13: Since the publication of the Science Advisory Panel Minutes and discussion offered within the EPA white paper on RNA Interference (US-EPA, 2014), more details of the fate of dsRNA in the environment have been determined, and their conclusions published. Fate in agricultural soils established that the dsRNA from MON 87411 does not persist for any but a short time (Dubelman et al., 2014). As discussed in the EA, all evidence shows that persistence of RNAs in water is highly unlikely. No controversy exists to show that environmental persistence of DvSnf7 is at issue.

Issue 14: Many studies have shown that RNAi can actually suppress unintended genes that are similar to the target gene. These unintended effects may also be heritable through reproduction, which could have serious ramifications for plant and animal populations.

Response 14: APHIS agrees that silencing specific RNAi sequences of a target organism may also silence unintended sequences of nontarget organisms. Identical sequences in another organism which might be exposed, or possibly even some that were nearly identical or similar, may potentially be targeted. First, it is becoming clear that there are multiple reasons why environmental dsRNA might not be sufficient to silence genes. One is that the quantity of the environmental dsRNAi to which an organism is exposed is important. In those observations where copy number is low, such as transcripts produced by the natural RNA output of a host plant with similar 21nt sequences to those found in animal targets, recipients may take up these RNAs, but there still may be no impact at all on host incorporated transcripts; these observation have been made in honeybees (Snow et al., 2013). Two is that effective copy number may be insufficient for gene silencing because of inacessible subcellular location of the transcripts in addition to low sequence copy number (Wittwer and Hirschi, 2004). In fact, among miRNAs, only 60% of those detected in tissues may have any "discernable activity" (Mullokandov et al., 2012). Second, as noted earlier in previous responses to comments here, genomic repetition number of a 21 nt (nucleotide) sequence empirically distinguishes whether or not an organism will respond to exogenous dsRNAs (such as from diet or a plant). In beetles there must be a minimum number of three of these dvSnf7 sequences in sensitive species (Bachman et al., 2013). Clearly the frequency of these repeated sequences decreases with decreasing phylogenetic relationship of the target organism (Bachman et al., 2013). Too few repeats will not trigger an appropriate RNAi impact on target sequences.

As described, not all organisms are sensitive to environmental RNAi, degrading it before it can be taken up by cells; gene silencing following exposure to env-RNAi in humans and vertebrates is not likely, a consensus clear from the EPA's 2014 Science Advisory Panel (US-EPA, 2014). Off target effects, in which target sequences do not precisely correspond with the RNAi sequences silenced may also be a potential issue. Evidence of silencing of non-identical sequences from the insect *Plutella xylostella* is that these occur when the supplied environmental RNA populations are extremely high (see Section 5.4.1 of the EA and (Bautista

et al., 2009)), the nature of the host, the type of exposure, duration of exposure, the endogenous defensive mechanisms as well as the total cellular exposure are all relevant to any silencing response at all. Finally, it should be noted that the use of environmental RNA, through the use of dsRNA in insect diets, cannot be inherited because there is no cellular machinery in animal cells to form DNA from RNA sequences.

Issue 15: APHIS ignores impacts of glyphosate and makes outdated conclusions about herbicide use.

Response 15: The EA includes thorough documentation in support of the fact that MON 87411 Maize will only replace other corn varieties that express the GR trait and that this will not result in an increase /expansion of U.S. corn acreage planted in GR varieties. Therefore, the glyphosate use on corn in the United States is not expected to change, so any effects associated with its use will not change if MON Maize 87411 is no longer regulated as a plant pest.

The general uses of glyphosate are outside the scope of the EA. EPA is responsible for reviewing and analyzing the uses and toxicity of pesticides such as glyphosate, and establishing through its registration and labeling process restrictions on uses that have provide an acceptable margin of safety. While one organization (WHO) has made allegations of new hazards from exposure to glyphosate, US-EPA has no credible evidence to affirm the conclusion.

Issue 16: APHIS failed to consider impacts on monarch butterflies.

Response 16: Brower et al. (2012) analyzed the decrease in population abundance of monarch butterflies in Mexico, which is an overwintering area for some populations of monarchs. While the paper suggests that the potential decrease in habitat for the monarch's host plant, milkweed, may be due in part to the increased spraying of glyphosate on GR crops and, subsequently, may be responsible for decreased monarch population levels, the study showed a statistically significant difference in monarch population levels over a period of several years, but did not contain any data or present any experiments which demonstrated that GE crop adoption is, in fact, responsible for any decrease in population.

Brower et al. (2012) also mentioned other potential causes of monarch population decline, such as extreme weather occurrences, and forest degradation. Furthermore, Brower et al. (2012) has been questioned by other researchers, including Davis et al. (2012), who performed a statistical analysis of monarch population levels of colonies in New Jersey and Michigan, and found that that population levels were not decreasing, but were, in fact, stable over a long period of time.

Chapter 4 of the EA provides a general review of the possible effects of GE crops on nontarget organisms. There are many variables that may affect population levels of nontarget organisms. These include cropping practices (e.g., strip or contour cropping, crop rotation), soil conservation practices that maintain grass strips, windbreaks and shelterbelts and the like, tillage, and the application of agrochemicals. The rotation of crops and strip contour cropping provide varied habitat that can benefit biodiversity. Crop production in general impacts

biodiversity at the landscape scale by potentially converting natural lands that have greater animal and plant species diversity to more monocultural landscapes. Glyphosate was found by the EPA to be no more than slightly toxic to birds, moderately toxic to practically nontoxic to fish, and practically nontoxic to aquatic invertebrates and honeybees (US-EPA, 1993).

The EA also includes thorough documentation in support of the fact that MON 87411 Maize will only replace other corn varieties that express the GR trait and that this will not result in an increase or expansion of U.S. corn acreage planted in GR varieties. Therefore, current glyphosate use on corn in the United States is not expected to change, so any effects associated with it use on monarch butterflies or other non-target organism is unlikely to change. The general uses of glyphosate are outside the scope of the EA. EPA is responsible for reviewing and analyzing the uses and toxicity of pesticides such as glyphosate to non-target organisms, and establishing through its registration and labeling process restrictions on uses that mitigate effects on non-target organisms.

Issue 17: APHIS did not adequately assess potential migratory bird impacts or those on threatened and endangered (T&E) species. One commenter also stated that APHIS failed to consider that the novel trait of MON 87411 Maize combined with the BT trait will result in expansion of corn acreage into natural areas.

Response 17: APHIS disagrees. The EA contains a section that reviewed the Agency's obligations under EO 13186 (US-NARA, 2010), *"Responsibilities of Federal Agencies to Protect Migratory Birds*, and the potential for MON 87411 Maize to impact migratory birds. APHIS concluded that there is no reason to expect impacts to migratory birds.

As required under Section 7 of the ESA, APHIS considered the potential for effects from the proposed determination of nonregulated status for MON 87411 Maize on federally listed threatened and endangered species and species proposed for listing, as well as effects on designated critical habitat and habitat proposed for designation. APHIS considered possible effects on all listed species and on all species proposed for listing. It also considered all designated critical habitat and habitat proposed for designation in States where corn is commercially grown. Species information was obtained from the USFWS Environmental Conservation Online System (ECOS; as accessed January 20, 2015 at http://ecos.fws.gov/tess_public/pub/stateListingAndOccurrence.jsp) (USDA-APHIS, 2015a), (USDA-APHIS, 2015b). After analyzing the potential for any effect, APHIS could not identify any stressor that would affect the reproduction, numbers, or distribution of any species, or affect their critical habitat. Based on this analysis, APHIS concluded that the determination of nonregulated status for MON 87411 Maize will have no effect on any federally listed T&E species or species proposed for listing, nor will it affect any designated critical habitat or habitat proposed for designation. This no effect determination eliminates a need for a consultation with, or the concurrence of, the USFWS and/or NMFS, consistent with ESA requirements.

Prior to performing its effects analysis on T&E species, APHIS considered the potential for MON 87411 Maize to expand corn production into natural areas. As reported in the EA, the conclusion from this analysis was that MON 87411 Maize is only expected to replace existing GE corn varieties in areas where corn is currently grown. It is not expected to increase total

U.S. corn acreage, nor is it likely to shift any existing corn acreage from where it is now grown into natural areas.

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