



PAUL R. LEPAGE
GOVERNOR

STATE OF MAINE
MAINE DEPARTMENT OF AGRICULTURE, CONSERVATION AND FORESTRY
BOARD OF PESTICIDES CONTROL
28 STATE HOUSE STATION
AUGUSTA, MAINE 04333-0028

WALTER E. WHITCOMB
COMMISSIONER
HENRY S. JENNINGS
DIRECTOR

BOARD OF PESTICIDES CONTROL

February 21, 2014

AMHI Complex, 90 Blossom Lane, Deering Building, Room 319, Augusta, Maine

AGENDA

8:30 AM

1. Introductions of Board and Staff
2. Minutes of the January 8, 2014, Board Meeting

Presentation By: Henry Jennings
Director

Action Needed: Amend and/or Approve

3. Consideration of Complaint Filed by Donna Herczeg of Portland Concerning TruGreen Lawncare and Sterling Insect-Lawn Control

Chapter 90 of the Board's rules (attached) allows citizens and organizations to submit complaints to the Director for the purpose of having the complaint placed on a Board Meeting agenda. While most complaints are not handled in this manner, Chapter 90 provides an alternate avenue to the public to present concerns directly to the Board on matters in which the compliance staff is unable to address. The Board will review the complaint and determine if any action is warranted at this time.

Presentation By: Henry Jennings
Director

Action Needed: Determine whether any action is warranted

4. Review of Board Policy Relative to the Environmental Risk Advisory Committee

In 1999, the Board first created the Environmental Risk Advisory Committee (ERAC) as an analog to the Medical Advisory Committee (MAC), to assist the Board in evaluating and addressing state-specific environmental concerns. The ERAC has not been active since 2006, when it completed work relating to concerns about browntail moth spraying. Since the committee has no current membership, and it has not met in nearly eight years, the staff proposes that the Board review the ERAC policy to ensure that it best articulates the Board's goals, and decide whether the proposed membership still makes sense.

Presentation By: Henry Jennings Lebelle Hicks
Director Staff Toxicologist

Action Needed: Provide Feedback to the Staff about the ERAC Policy and the Proposed Committee Membership

5. Formation of an Environmental Risk Advisory Committee to Address Concerns about Potential Pesticide Impacts on Marine Invertebrates

At the January 8, 2014, meeting, the Board reviewed pesticide-related bills currently being considered by the Maine Legislature. In the course of discussing LD 1678, An Act To Protect Maine's Lobster Fishery, the staff highlighted some related emerging research which suggests that synthetic pyrethroids may have the potential to cause adverse effects on aquatic invertebrates. As a result of the discussion, the Board voted to direct the staff to form an Environmental Risk Advisory Committee (ERAC), intended to assess the potential impacts of insecticides on lobsters and other marine invertebrates. The staff will suggest members for the committee and seek Board input as well.

Presentation by: Henry Jennings Lebelle Hicks
 Director Staff Toxicologist

Action Needed: Provide Guidance to the Staff on the Scope and Membership of the ERAC

6. Review of Current Rulemaking Ideas

Over the past several months, the Board has discussed a number of policy areas for which some additional refining of rules may be desirable. The staff will summarize recent rulemaking ideas and seek Board guidance on whether and when to initiate any additional rulemaking.

Presentation By: Henry Jennings
 Director

Action Needed: Provide Guidance to the Staff

7. Consideration of a Consent Agreement with Atlantic Pest Solutions of Kennebunkport

On June 3, 1998, the Board amended its Enforcement Protocol to authorize staff to work with the Attorney General and negotiate consent agreements in advance on matters not involving substantial threats to the environment or public health. This procedure was designed for cases where there is no dispute of material facts or law, and the violator admits to the violation and acknowledges a willingness to pay a fine and resolve the matter. This case involved drift from a mosquito/tick control operation into a brook.

Presentation By: Raymond Connors
 Manager of Compliance

Action Needed: Approve/Disapprove the Consent Agreement Negotiated by Staff

8. Consideration of a Consent Agreement with Ramon Forestry Service, LLC, of Clinton

On June 3, 1998, the Board amended its Enforcement Protocol to authorize staff to work with the Attorney General and negotiate consent agreements in advance on matters not involving substantial threats to the environment or public health. This procedure was designed for cases where there is no dispute of material facts or law, and the violator admits to the violation and acknowledges a willingness to pay a fine and resolve the matter. This case involved drift to a residential property from an application to an abutting blueberry field.

Presentation By: Raymond Connors
 Manager of Compliance

Action Needed: Approve/Disapprove the Consent Agreement Negotiated by Staff

9. Consideration of a Consent Agreement with Gateway Inn of Medway

On June 3, 1998, the Board amended its Enforcement Protocol to authorize staff to work with the Attorney General and negotiate consent agreements in advance on matters not involving substantial threats to the environment or public health. This procedure was designed for cases where there is no dispute of material facts or law, and the violator admits to the violation and acknowledges a willingness to pay a fine and resolve the matter. This case involved applications by an unlicensed applicator to areas open to the public.

Presentation By: Raymond Connors
Manager of Compliance

Action Needed: Approve/Disapprove the Consent Agreement Negotiated by Staff

10. Consideration of a Consent Agreement with Olde English Village, LLC, of South Portland

On June 3, 1998, the Board amended its Enforcement Protocol to authorize staff to work with the Attorney General and negotiate consent agreements in advance on matters not involving substantial threats to the environment or public health. This procedure was designed for cases where there is no dispute of material facts or law, and the violator admits to the violation and acknowledges a willingness to pay a fine and resolve the matter. This case involved pesticide applications by an unlicensed applicator.

Presentation By: Raymond Connors
Manager of Compliance

Action Needed: Approve/Disapprove the Consent Agreement Negotiated by Staff

11. Consideration of a Consent Agreement with Jato Highlands Golf Course of Lincoln

On June 3, 1998, the Board amended its Enforcement Protocol to authorize staff to work with the Attorney General and negotiate consent agreements in advance on matters not involving substantial threats to the environment or public health. This procedure was designed for cases where there is no dispute of material facts or law, and the violator admits to the violation and acknowledges a willingness to pay a fine and resolve the matter. This case involved pesticide applications by an unlicensed applicator.

Presentation By: Raymond Connors
Manager of Compliance

Action Needed: Approve/Disapprove the Consent Agreement Negotiated by Staff

12. Other Old or New Business

- a. Friends of Penobscot Bay Offer to Assist with Coastal Sediment Sampling—H. Jennings
- b. Risk Assessment of Mosquito Adulticides—L. Hicks
- c. Report to the Joint Standing Committee on Agriculture, Conservation and Forestry Regarding Grants and the Adequacy of the Product Registration Fee—H. Jennings
- d. Legislative Update—H. Jennings
- e. The Woodland Club Chapter 29 Variance—H. Jennings

- f. Central Maine Power Transmission Right-of-Way Vegetation Management Plan for 2014—H. Jennings
- g. Beekeeper Petition to Discourage Large Retailers from Selling Neonicotinoids—H. Jennings
- h. Other?

13. Discussion About the Approval Process Relating to a Registration Request for a *Bt* Soybean Product

Dow AgroSciences LLC, has submitted a request to register a *Bt* soybean product that may be used only for seed increase, breeding, research, and seed production in breeding nurseries and research stations. Since the Board has never registered a soybean plant incorporated protectant (PIP), the staff is seeking guidance about what sort of review process—if any—the Board would like to undertake before considering the registration request.

Presentation by: Lebelle Hicks
 Staff Toxicologist

Action Needed: Provide Guidance to the Staff About the Review of the Registration Request

14. Schedule of Future Meetings

March 28, May 9, June 17, August 18, and September 12, 2014, are tentative Board meeting dates. The June 17 meeting is planned to be held in the Madison/Skowhegan area, following a tour of Backyard Farms. The Board will decide whether to change and/or add dates.

Adjustments and/or Additional Dates?

15. Adjourn

NOTES

- The Board Meeting Agenda and most supporting documents are posted one week before the meeting on the Board website at www.thinkfirstspraylast.org.
- Any person wishing to receive notices and agendas for meetings of the Board, Medical Advisory Committee, or Environmental Risk Advisory Committee must submit a request in writing to the Board's office. Any person with technical expertise who would like to volunteer for service on either committee is invited to submit their resume for future consideration.
- On November 16, 2007, the Board adopted the following policy for submission and distribution of comments and information when conducting routine business (product registration, variances, enforcement actions, etc.):
 - *For regular, non-rulemaking business*, the Board will accept pesticide-related letters, reports, and articles. Reports and articles must be from peer-reviewed journals. E-mail, hard copy, or fax should be sent to the attention of Anne Bills, at the Board's office or anne.bills@maine.gov. In order for the Board to receive this information in time for distribution and consideration at its next meeting, all communications must be received by 8:00 AM, three days prior to the Board meeting date (e.g., if the meeting is on a Friday, the deadline would be Tuesday at 8:00 AM). Any information received after the deadline will be held over for the next meeting.
- During rulemaking, when proposing new or amending old regulations, the Board is subject to the requirements of the APA (Administrative Procedures Act), and comments must be taken according to the rules established by the Legislature.



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BOARD OF PESTICIDES CONTROL

January 8, 2014

Augusta Civic Center, 76 Community Drive, Kennebec/Penobscot Room, Augusta, Maine

MINUTES

3:00–4:00 PM (BOARD MEETING)

4:00–5:00 PM OPEN FORUM (COSPONSORED BY THE IPM COUNCIL)

5:00–6:00 PM (BOARD MEETING CONTINUED)

Present: Flewelling, Jemison, Stevenson, Morrill, Granger, Eckert

1. Introductions of Board and Staff
 - The Board, staff, and Assistant Attorney General Randlett introduced themselves
 - Staff present: Jennings, Connors, Tomlinson, Fish, Bills
2. Minutes of the December 13, 2013, Board Meeting

Presentation By: Henry Jennings
Director

Action Needed: Amend and/or Approve

- On page 3, bullet 2, change “showed” to “should.”
- On page 6, last bullet, make “determination” plural.
 - **Granger/Eckert: Moved and seconded to accept the minutes as amended**
 - **In Favor: Unanimous**

3. Request from Maine Migrant Health Program and Eastern Maine Development Corporation to Help Support a Worker Safety Training Program for Summer 2014

Since 1995, the Board has supported a Migrant and Seasonal Farmworker Safety Education program. During 2013, 388 individuals received Worker Protection Standard training, 176 individuals received take-home exposure training and 260 received heat stress training. The Maine Migrant Health Program and Eastern Maine Development Corporation are proposing to provide one health and safety outreach worker during the 2014 agricultural season. Funding to support this effort is being requested in the same amount as last year, and funds have been budgeted in the Board’s FY’14 work plan.

Presentation By: Chris Huh, Program Manager, Farmworkers Jobs Program,
Eastern Maine Development Corporation
Elizabeth Charles, Enabling Services Coordinator, Maine Migrant Health
Program

Action Needed: Discussion and determination if the members wish to fund this request

- Charles explained that in 2013 the program transitioned from having two AmeriCorps members to having a single paid employee on the Maine Migrant Health Program staff. That person was able to meet with all the farms from previous years, and provided training for a total of 388 workers in the Midcoast, Aroostook and Downeast areas. There was a lot of concern about fruit flies in 2013 and the program was able to provide supplemental print material to answer questions. For 2014, the partnership has secured a grant of \$3,010 from farm worker opportunity programs, and, combined with the requested \$3,500 from the BPC, it would support the summer staff position.
- Huh explained that the plan for 2014 is similar to 2013: a goal of 350 workers trained on the WPS, and 175 individuals trained on family pesticide exposure. The partnership would also like to do some outreach to new farms and growers. Some of the grant money also goes for assistance for transportation which is needed, as well.
 - **Eckert/Flewelling: Moved and Seconded to Approve the Request**
 - **In Favor: Unanimous**

4. Continuing Discussion of Planning Session Topics

The Board discussed a variety of topics during its annual planning session as part of the September 6, 2013 Board meeting. Several topics were also discussed at the October 18 and December 13 meetings, and some decisions were made at the December 13 meeting. The Board will now review the status of the planning session topics and determine whether additional discussion and/or action is appropriate.

Presentation By: Henry Jennings
Director

Action Needed: Provide Guidance to the Staff about Planning Session Topics

- Jennings explained that the memo for this agenda item included a new column listing outcomes and discussions to date. He thought that in reviewing the memo a few things would jump out that still needed addressing, but that was not the case. Some items did not get discussed at the planning session.
- Morrill said that there had been tremendous progress on the streamlining of licensing. Stevenson noted that though there is a lot of online training available, people don't know about it. Jennings said that since the last meeting the staff had made efforts to make it more visible on the website and that it would be mentioned at training seminars.
- Eckert remarked that she would like presentations on Maine-grown commodities to be available, if we could get Cooperative Extension to make them.
- Granger noted that Board members could request a topic to be added to an agenda at any time, so if people aren't prepared to talk about specific topics today, they could bring them back later.
- Jennings suggested that the Board review potential rulemaking at the February meeting and draft concepts in preparation for rulemaking.

5. Water Quality Monitoring

The Board has a 20-plus year history of monitoring both ground and surface waters for pesticide residues. In 2005, the Maine Legislature reinforced the importance of the Board efforts by codifying the requirement for water residue surveys under 7 M.R.S. § 607-A (2-A). However, recently, sampling efforts have been curtailed due to difficulty contracting for competent laboratory services. The staff recently entered into an agreement with the Montana State Laboratory which utilizes cutting-edge pesticide analytical methodology. Consequently, plans are being made to resume water quality

monitoring. The staff will update the Board on the laboratory issues and seek Board input on water quality priorities.

Presentation by: Mary Tomlinson
Water Quality Specialist

Action Needed: Provide Guidance to Staff on Water Quality Priorities

- Jemison remarked that the state has a history of doing a lot of water quality monitoring and that he finds the data very useful in his classes.
- Tomlinson explained that the Board now has a contract with the Montana Department of Agriculture lab for enforcement as well as water quality samples. It is a temporary arrangement until the Maine Health and Environmental Testing Lab (HETL) is able to handle the work. The Montana lab is on the cutting edge, they have developed a screening process for over 90 pesticides to subparts per billion. There will be only a two to three week turnaround instead of having to wait months for results. They can screen for newer pesticides that come on the market. We are also working with the Maine HETL lab so they can eventually take over the work; we need to have a Quality Assurance Project Plan (QAPP) and a memorandum of understanding, then we can transfer money from EPA for equipment.
- Jemison asked whether the Maine lab would eventually be able to do what the Montana lab is doing, and whether they have equipment the Maine lab doesn't have. Tomlinson said that the Maine lab is getting equipment from other grants. We are encouraging them to get EPA training, and one of their chemists did attend last year.
- Tomlinson explained that this year's testing is planned for late winter/early spring. Sediment sampling in the past was centered on Back Cove, but the staff will probably be redirecting the focus based on concerns across the country of pesticides affecting marine organisms; therefore, sampling will focus on the Maine coast.
- Granger asked if anything of concern had been found in Maine samples. Tomlinson said that the last sediment sampling in streams was done in 2010, and all results were substantially below human health limits, but close to aquatic limits. The methodology was not sensitive enough to detect compounds at ultra-low concentrations.
- Granger asked if other states are doing similar testing and if anyone is compiling the findings. Tomlinson said that USGS has a database and that EPA requires states to submit monitoring data and plans to use water and sediment data in risk assessments for re-registrations.
- Jemison asked what the role of the Board would be. Jennings said that in the past decisions had to be made about what to test for, but with the new lab in Montana they test for everything. A group will have to decide where to test, but there probably isn't time for the Board to approve a plan, since the testing should be done before the ground thaws. Tomlinson said the plan is to do 60 this year and 60 next year and that they would try to redo sites that have been done in the past.

6. Review of Pesticide Bills Before the Legislature

There are three bills concerning pesticides under considerations by the Agriculture, Conservation and Forestry Committee of the Maine Legislature: LD 1587 An Act To Temporarily Ban the Use of Neonicotinoid Pesticides; LD 1678 An Act To Protect Maine's Lobster Fishery; and LD 1674 An Act To Further Ensure the Provision of Safe Medical Marijuana to Maine Patients. The Board will discuss the bills and determine whether to take an official position and/or provide testimony on any of them.

Presentation By: Henry Jennings
Director

Action Needed: Determine Whether to Take a Position on any of the Three Bills

- Jennings summarized LD 1587, An Act To Temporarily Ban the Use of Neonicotinoid Pesticides, and explained that, in his opinion, science doesn't point to neonicotinoids as the cause of Colony Collapse Disorder. A discussion ensued about the stresses on bees, and concern was raised about what would be used in place of the banned products, as it might be more risky. This was expressed as a concern in both the landscape and the agricultural areas.
 - **Granger/Morrill: Moved and seconded to oppose the bill and direct the staff to testify against it**
 - **In favor: Unanimous**

[4:00—BREAK FOR LISTENING SESSION; 5:00—RECONVENE]

- Jennings summarized LD 1674, An Act To Further Ensure the Provision of Safe Medical Marijuana to Maine Patients. Last year a bill was passed allowing growers to use 25(b) pesticides, provided the label was broad enough; this bill expands the pesticides that would be allowed. The concern is that as written it allows pesticides that aren't registered in Maine, nor is there any language about whether the use is allowed by the label. A discussion ensued about the various products listed in the bill. It was pointed out that if the bill passes as written it would allow the use of certain pesticides under DHHS rules that would be illegal to use under pesticide rules.
 - **Morrill/Flewelling: Moved and seconded to oppose the bill as written and direct the staff to testify against it**
 - **In favor: Unanimous**
- Jennings summarized LD 1678 An Act To Protect Maine's Lobster Fishery, and explained that methoprene is used in some states to control mosquito larvae in catch basins, but not in Maine. Resmethrin is also not used for mosquito control in Maine. Methoprene is mostly used in flea and tick products for pets. The way the bill is written, it would be difficult to enforce; technically any product applied on a pet could end up in the water. If there were an outbreak of a mosquito-borne disease in Maine, government agencies might want to have these products available. There is a potential for any pesticide to affect lobsters, so why not look at the wider issue? Jennings suggested convening an Environmental Risk Advisory Committee.
 - **Eckert/Granger: Moved and seconded to oppose the bill as written and direct the staff to testify against it**
 - **In favor: Unanimous**

7. Other Old or New Business

- a. Other?

8. Schedule of Future Meetings

February 21, March 28, May 9, and June 27, 2014, are tentative Board meeting dates. The June 27 meeting includes a tour of Backyard Farms in Madison in the morning, with a Board meeting at Madison High School after lunch. The Board will decide whether to change and/or add dates.

Adjustments and/or Additional Dates?

- The Board added August 18 and September 12 as meeting dates.

9. Adjourn

- **Granger/Morrill: Moved and seconded to adjourn at 5:30 PM**
- **In favor: Unanimous**

01 DEPARTMENT OF AGRICULTURE, FOOD AND RURAL RESOURCES

026 BOARD OF PESTICIDES CONTROL

Chapter 90: COMPLAINTS

SUMMARY: These regulations describe the procedure a person must follow in bringing a complaint to the Board and outline the steps the Board may take in response.

Section 1. Purpose

The purpose of this section is to provide a formal procedure which assures that the Board of Pesticides Control will consider all complaints regarding uses of pesticides.

Section 2. Complaint

Any person, individual, corporation, unincorporated association, group of individuals or government agency may submit a complaint regarding any person, known or unknown, relative to the use of pesticides.

Section 3. Address to Director

All complaints shall be sent to the Director, Board of Pesticides Control, Department of Agriculture, 28 State House Station, Augusta, Maine 04333-0028. Anyone who cannot submit a complaint in writing to the Director may make arrangements with the Director or staff to record the content of the complaint in a manner and time frame convenient to the Director, staff and complainant. The Director may, at his/her discretion, investigate the complaint prior to Board action.

Section 4. Placed on Board's Agenda

The complaint shall be placed on the Board's agenda, the Board shall give reasonable notice to the complainant, the person who is the subject of the complaint, if known, and any other party the Director believes is interested in the complaint.

Section 5. Considered

The Board shall consider the complaint along with any information which the Director may have available, and take whatever action it deems necessary to protect the public's interest. Action could include taking no action, requesting the Board's staff to investigate the complaint, scheduling an informal hearing between the affected parties or instituting formal adjudicatory

proceedings. Any such consideration shall not constitute an adjudicatory proceeding within the meaning of chapter 70 of the Board's regulations.

STATUTORY AUTHORITY: 22 M.R.S.A., Chapter 258-A

EFFECTIVE DATE:

July 6, 1979 - filing 79-338

AMENDED:

October 2, 1996 - filing 96-410

EFFECTIVE DATE (ELECTRONIC CONVERSION):

March 1, 1997

CONVERTED TO MS WORD:

March 11, 2003

NOV 21 2013

November 19th, 2013

State of Maine
Dept of Agriculture, Conservation & Forestry
Board of Pesticides Control
28 State House Station
Augusta, Maine 043330028

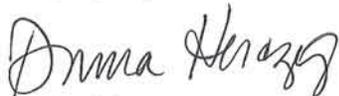
Re: Complaint-Trugreen

Dear Henry Jennings, Director

This letter is a formal request to have the attached set of concerns placed on the Board of Pesticides agenda for review. As I would like to be present at that meeting, please notify me as soon as possible the date.

I have also included pictures of Sterling's Pesticide Application signage which I would like to present at this meeting. From the street the sign just looks like marketing signage and on the back is the pesticide caution sign. From the street there is no way to know that pesticides have been applied and from the back it is so small you can barely read the dates.

Thank you,



Donna Herczeg
173 Longfellow St.
Portland, ME 04103

207-879-6366
donnaph@maine.rr.com

Donna Herczeg

From: Donna Herczeg <donnaph@maine.rr.com>
Sent: Tuesday, September 17, 2013 9:57 AM
To: 'raymond.g.connors@maine.gov'
Subject: Trugreen Complaint

Hi Raymond,

I am writing to you today to let you know about a conversation I had on September 12th with Anthony Terramagra, the Westbrook Service Manager at Trugreen.

As a neighbor who was called because I am on the Pesticide Information Registry, I wanted to know what was being sprayed that day and also discuss the weather conditions that were calling for heavy rainfall. This is what he told me after I requested the Material Data Sheet:

- 1) You can't go by what the MDS sheet says because that is the concentrated amount. After dilution "the sprays are less harmful than Windex".
- 2) He also said "the sprayed areas are safe to walk on after 2 hours and that he allows his children, dogs and cats to walk on the sprayed areas and they have never had an allergic reaction".
- 3) After my concerns about heavy rainfall being predicted the same day as spraying he said "only granular products leach from water penetration and that liquid sprays will not after 1 hour of application".
- 4) Said OSHA and the EPA have certified these products as safe.
- 5) Also informed me that after our discussion he called the Maine Board and spoke to Jan who said he was correct and that he could spray that day and that "they know who I am".
- 6) He also said I had better watch it or I would be facing litigation from my neighbors for harassment.

This same company told another neighbor that their products were organic and she asked that question every time they sprayed. It was not until I got the MDS sheets and showed her that she realized toxic chemicals were being sprayed on her lawn and discontinued the service.

Trugreen's marketing brochures states they are an "environmentally responsible lawn care" company, when in fact they are using toxic herbicides and pesticides. Their "Earthcare Program" states they use "organic-based" fertilizer treatments (a dubious claim at best), including pre and post crabgrass control, broadleaf weed control, and surface insect control, making it look like these products are environmentally safe as well. Even the front of one of these brochures says that dandelion's are a "harmful weed to a healthy lawn".

As a member of Beyond Pesticides and having personally done extensive research on lawn chemicals, I am extremely frustrated and concerned about the blatant disregard of the dangers these chemicals pose and the misleading negligent information this company is providing. These chemicals are proven to be toxic to wildlife, children and pets and are a major threat to aquatic wildlife and waterways.

I appreciate your help in this matter and hope you will take this letter seriously and investigate the claims that are being made by this company and employees.

Donna Herczeg
173 Longfellow St.
Portland 879-6366

REFERRAL SAVINGS

\$25 OFF FOR YOU	\$25 OFF FOR YOUR FRIEND
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STERLING
SINCE 1914
SterlingTheSolution.com

APPLICATOR: [blank] DATE: 12-10
ADDRESS: 05-11 CITY: [blank]
PHONE: [blank] ZIP: 05-29

CAUTION
PESTICIDE APPLICATION
KEEP OFF 
KEEP OFF THE GRASS AND DRIVEWAY

207-767-5555



STERLING
207-767-5555



STERLING
207-767-5555

From: Terry Shoemaker [<mailto:terry@sterlingthesolution.com>]
Sent: Tuesday, February 11, 2014 12:04 PM
To: Jennings, Henry
Subject: Posting sign

To the board of pesticides:

I am aware of the complaint on our posting signs. I will make sure when applications are made that the applicators face the warning symbol in the right direction

Terry Shoemaker

Sent from my iPhone

From: TERRAMAGRA, ANTHONY [<mailto:ANTHONYTERRAMAGRA@Servicemaster.com>]

Sent: Tuesday, February 11, 2014 10:26 AM

To: Jennings, Henry

Cc: Dufault, Ed A

Subject: RE: Trugreen Complaint

Hi Henry,

I am writing in response to the complaint filed by Donna Herczeg's. Our conversation was on September 12th 2013 as she said. We were scheduled to do an application on the neighbors lawn that day. We did inform here that we would be coming out. At the time she called into the branch to see what application would be applied. I let her know what would be applied and that if it did in fact rain that day that we would do a natural treatment. In response to her complaints read as follows,

1. I did explain that the MSDS sheets only give information on concentrates and not the diluted forms. It is company policy and approved that our pesticides are diluted 100% from their lethal toxicity, and once diluted they are much safer than a lot of the products used on the market for consumers.
2. Also it states in the pesticide manuals and on the MSDS sheets that reentry to grounds that are sprayed is safe after the pesticide has dried on the surface which takes one to two hours(it takes a half hour to an hour for the dust to settle on a granular applications). I also did state that I have pets and kids that have had no health issues from pesticide use on my property when following these guidelines. She then stated that I was a horrible person for letting my kids and pets on the lawn. I told her I am following all guidelines of the pesticides I am using that are set by the federal and state government, at which point she told me our company is lying and so is the government.
3. I did explain that if the liquid application dries on the surface before the rainfall that there is less chance of leaching and that granular has a tendency to leach more because it does not react until hit with water, which is also stated in the pesticide manuals.
4. I did say that the government agency's have approved the pesticides for use in the correct manor at which point she said the EPA and OSHA are wrong.
5. I called the pesticide board that day to make sure we were doing everything right and I was assured that I was following all state and federal laws.
6. I also did inform her that customers have complained about her harassing them and she should take up her concerns with them because it is their property and we are doing the job that we were contracted to do. Also we are not breaking any laws.

To some up I did not make any claims that were not backed by the federal and state government. All of my employees are well informed of what they are using and any dangers they might pose to the environment. Donna has called in several times and tries to pull people into debating with her on the subject. I myself made the mistake of letting her draw me into the conversation with her. In the future I will follow all guidelines approved by the state and federal government as I always have and I will make sure that all people on the pesticide registry are well informed.

Also my company strives to make sure that we use the safest pesticides possible and does not make any claims that are not true. If we made false claims then we would be facing serious charges. If you need any more information from me please call me at 207-245-7254 or e-mail me.

Sincerely,

Anthony Terramagra



JOHN ELIAS BALDACCI
GOVERNOR

STATE OF MAINE
DEPARTMENT OF AGRICULTURE, FOOD AND RURAL RESOURCES
BOARD OF PESTICIDES CONTROL
28 STATE HOUSE STATION
AUGUSTA, MAINE 04333-0028

SETH H. BRADSTREET III
COMMISSIONER
HENRY JENNINGS
DIRECTOR

MAINE BOARD OF PESTICIDES CONTROL POLICY RELATING TO THE ENVIRONMENTAL RISK ADVISORY COMMITTEE (ERAC)

Adopted June 25, 1999
Amended September 29, 2000

Background

The Maine BPC recognizes the potential impact of some pesticides on the environment from their federally approved label uses. Evaluation of these products with regard to specific situations and local Maine conditions is critical to reducing potential adverse effects on the environment. The Board needs expert advisors, knowledgeable in the field of environmental toxicology and ecology research, who can add their assessments to the medical, economic and benefit recommendations of others prior to the Board initiating and ruling on pesticide restrictions.

These persons will be established as a volunteer Environmental Risk Advisory Committee (ERAC) to the Board of Pesticides Control.

Membership

The ERAC will be composed of four standing members and two ad hoc members. One standing member will be one of the Board members appointed to represent the public with a demonstrated interest in environmental protection. This member will also chair the committee. The other three standing members will be qualified professionals in related environmental or ecological research disciplines such as an aquatic or terrestrial biologist, aquatic or terrestrial entomologist and environmental toxicologist. In addition, up to six members will be chosen ad hoc with expertise specific to the potential environmental impact in question. The Board will solicit and review resumes for any vacancy on the ERAC. The Board should appoint persons whose disciplines in aggregate are suitable for identifying potential environmental problems and recommending courses of action that would prevent their occurrence.

Term

The standing committee members of the ERAC will be appointed by the Board for three years of service, with terms to be staggered. The ad hoc members will serve for the duration of a specific issue but not longer than a three year term, unless re-appointed.

Meetings

The Committee will meet on an as needed basis at the invitation of the ERAC chairman.

Compensation

The ERAC is voluntary and no compensation for services is available. However, all reasonable travel expenses will be reimbursed, subject to the approval of the staff director, in a manner consistent with State Travel Policy.

DRAFT PROPOSED ERAC COMMITTEE MEMBERS 2/20/14

1. Chair

Curtis C. Bohlen, Board of Pesticides Control Member
Director,
Casco Bay Estuary Partnership
University of Maine Muskie School of Public Service

2. Other Board members if they are interested

3. Environmental toxicologist

John Wise Ph.D
Wise Laboratory CIAET
USM PO Box 9300
96 Falmouth St
Portland, ME 04104-9300
207-228-8050

NOTE: If John is not available, maybe another member of his group

4. Aquatic Entomologist

Leon Tsomides
ME DEP Land and Water Quality
State House Station #17
Augusta, ME 04333
207-287-3901

5. Terrestrial Entomologist

James Dill, PhD, IPM Entomologist
University of Maine Cooperative Extension, Pest Management
491 College Avenue
Orono, Maine

6. Lobster Biologist from Department of Marine Resources

Carl Wilson
DMR Marine Fisheries Laboratory P O Box 8
West Boothbay Harbor Me 04575
(207) 633-9539

7. Expert on pyrethroid residues in sediment and pyrethroid analytical chemistry

Lawrence LeBlanc Ph.D
School of Marine Sciences
5741 Libby Hall Room 215
University of Maine
Orono Me 04469-5714
207-581-4376

DRAFT PROPOSED ERAC COMMITTEE MEMBERS 2/20/14

8. Lobster development and mosquito insecticides

Michael N. Horst PhD

Formerly of the School of Medicine, Mercer University Macon GA 31207 will be at the Darling Marine Center on his retirement. Dr. Horst participated in the evaluation of the health of lobster in Long Island Sound in 2003 to 2005 and presented at the lobster forum at UMO in 2005.

9. Others?

Kohl Kanwit, Director of Public Health, Department of Marine Resources?

Staff

Lebelle Hicks, PhD DABT Pesticides Toxicologist

Mary Tomlinson Water Quality Specialist

Henry Jennings, Director

Potential Rulemaking Items for Board Consideration

BPC Rule	Potential Change	Reason for Change
20	Incorporate Positive Identification of Proper Treatment Site by Commercial Applicators into rule (see policy)	Clarity; policies are not enforceable
22 Section 2D	Exempt “linear” (ROW) projects from the Identifying and Recording Sensitive Areas requirement.	Because it is impractical to identify all sensitive areas within 500 feet of a ROW, the staff routinely grants variances from this requirement. Since the Board always grants variances with the same conditions, does it make sense to codify the de facto standard in rule?
22 Section 2D	Exempt the requirement for Identifying and Recording Sensitive Areas for category 7E (Biting Fly and other Arthropod Vectors (ticks)) as it is for 3B (turf), 3A (ornamental tree and plant) and 7A (structural)	Since all areas in a residential area are technically sensitive areas, there is no point in mapping them. Requiring signs serves a more useful purpose of alerting people entering a treated area.
22 Section 2D	Exempt the requirement for Identifying and Recording Sensitive Areas for category 6B (Industrial/Commercial/Municipal Vegetation Management) as it is for 3B (turf), 3A (ornamental tree and plant) and 7A (structural)	Since all areas in a residential area are technically sensitive areas, there is no point in mapping them. Requiring signs serves a more useful purpose of alerting people entering a treated area.
28 Section 3	Add category 7E to those required to post signs.	see above
28 Section 3	Add category 6B to those required to post signs.	see above
26 Section 1	Change the definition of “occupied buildings” to mean fully enclosed indoor spaces inside buildings	To clarify the intent of the rule and eliminate the need for the policy which states that open air structures are not buildings for the purpose of the rule.
27 Section 2B(4)ii	Add the words “in school buildings” to make it clear that all application records are required to be maintained	Fix a mistake from the last rulemaking and clarify the requirement
29 Section 6	Incorporate the policies around plants with a dermal toxicity hazard and invasive plants into rule.	Clarity; policies are not enforceable; eliminate the need for variances
31 Section 1E	Exempt employees and volunteers who supervise children from licensing requirements for the use of insect repellents to those children	Clarity
31 Section 4	Allow for reciprocal licenses for aerial applicators in the event of a vector-borne disease threat or other emergency	Eliminate the bottleneck of getting aerial applicators licensed in an emergency situation.
31 Section 5A(V)a,b	Revise the waiting periods for re-taking exams after failing	Some Board members questioned the propriety of the 15 and then 30 day (after failing twice) wait periods
32 Section 2A(4)a,b	Revise the waiting periods for re-taking exams after failing	Some Board members questioned the propriety of the 15 and then 30 day (after failing twice) wait periods

33 Section 2A(4)a,b	Revise the waiting periods for re-taking exams after failing	Some Board members questioned the propriety of the 15 and then 30 day (after failing twice) wait periods
41 Section 3	Remove hexazinone from Chapter	Was originally included so that only licensed applicators would have access to it; because farmers are now required to have an AgBasic License, there is no need for the special requirements.
New chapter	Create licensing and certification requirements for those who make pesticide recommendations as part of their job	To ensure that people making pesticide recommendations are aware of key laws about proper pesticide use.

**Proposed Administrative Consent Agreement
Background Summary**

Subject: Ted St. Amand
Atlantic Pest Solutions
PO Box F
Kennebunkport, ME 04046-1695

Date of Incident(s): July 12, 2013

Background Narrative: The Board received a call from a Wayne resident alleging that an Atlantic Pest Solutions's employee, in the process spraying an abutting property for mosquitoes and ticks, caused pesticide to enter a small brook that drains into Androscoggin Lake. Board staff interviewed the caller and employees of Atlantic Pest Solutions and also collected foliage samples beside the brook as well as a foliage sample from plants growing in the brook. Lab results were positive for bifenthrin, the active ingredient in Talstar P, the insecticide applied by Atlantic Pest Solutions's employees to their customer's property.

Summary of Violation(s):

- 7 U.S.C. § 136j (a)(2)(G), 7 M.R.S.A. § 606 (2)(B) and 22 M.R.S.A § 1471-D(8)(F), use of a pesticide inconsistent with the product labeling
- 22MRSA §1471-D(8)(C), used or supervised the use of pesticides applied in a careless, negligent or faulty manner or in a manner which is potentially harmful to the public health, safety or welfare or the environment

Rationale for Settlement: Evidence indicated that the application was made without taking sufficient precautions to keep the pesticide out of water. Allowing pesticide to enter surface water is a violation of the pesticide label as well as laws and regulations.

Attachments: Proposed Consent Agreement

JAN 27 2014

STATE OF MAINE
DEPARTMENT OF AGRICULTURE, FOOD AND RURAL RESOURCES
BOARD OF PESTICIDES CONTROL

Ted St. Amand)
Atlantic Pest Solutions) ADMINISTRATIVE CONSENT AGREEMENT
PO Box F) AND
Kennebunkport, ME 04046-1695) FINDINGS OF FACT

This Agreement, by and between Atlantic Pest Solutions (hereinafter called the "Company") and the State of Maine Board of Pesticides Control (hereinafter called the "Board"), is entered into pursuant to 22 M.R.S. §1471-M (2)(D) and in accordance with the Enforcement Protocol amended by the Board on June 3, 1998.

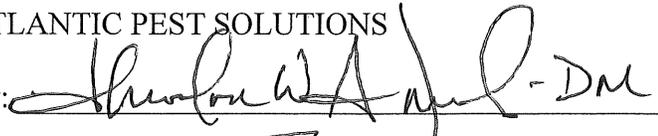
The parties to this Agreement agree as follows:

1. That the Company is in the business of applying pesticides including outdoor applications to control mosquitoes and ticks.
2. That the Board received a call from an abutter to a Company customer in Wayne. The caller said he thought a Company applicator crossed the property line and pesticide spray went into a small brook that drains into Androscoggin Lake.
3. That on July 12, 2013, a Board inspector met with the abutting property owner who called the Board and the inspector collected a foliage sample from plants growing in the brook and a soil sample from the edge of the brook.
4. That on July 16, 2013, the same Board inspector met at the Wayne application site with Company personnel Garrett Bissonnette (operations mgr.), Ralph Blumenthal (general mgr.), and Tyler Gagnon (licensed applicator /supervisor on the job). Also present were the Company customer and abutting property owner. The inspector pointed out the flags he placed in the ground to mark the places he sampled earlier. Gagnon pointed out where and how he directed his unlicensed co-worker, Benjamin Chick-Reny, who made the application to spray. Gagnon confirmed the company made a mosquito/tick application to the customer's property at 22 Island View Drive in Wayne on July 12, 2013.
5. That at the meeting described in paragraph four, Gagnon stated that Chick-Reny stood within two feet of standing water, his back towards the brook and sprayed inwards towards the customer's property using a hose connected to powered equipment in the back of a pickup truck. Gagnon acknowledged both he and Chick-Reny were involved in a confrontation with the abutting property owner during the application. The abutting owner took the position that spray was directed towards the standing water and flowing brook.
6. That at the meeting described in paragraph four, the inspector collected a specimen label for Talstar P Professional insecticide, the insecticide applied by Chick-Reny at the Wayne site on July 12, 2013, a photograph of the invoice/work order for that job, and a photo of the map of the job site. Gagnon also completed a written statement
7. That on July 17, 2013, the Board inspector met with Chick-Reny at the Company's Brunswick office. The inspector used the map collected at the meeting described in paragraph six to review the application practices of Chick-Reny for the Wayne job. Chick-Reny explained that he kept his back to any standing water or runoff areas and kept a three foot buffer from these areas when making his application. Chick-Reny also summarized his comments about the application in a written statement. The inspector collected an original Talstar P professional insecticide label and marked in blue on the map where the stream was, the applicator position when spraying, and an area not sprayed across the road.

8. That the lab results from the samples taken as described in paragraph two were positive for bifentrin, the active ingredient in Talstar P Professional insecticide. The foliage sample was positive at 0.820 ppm and the soil sample was positive at 0.069 ppm.
9. That the Talstar P Professional insecticide label (EPA reg. #279-3206) states "To protect the environment, do not allow pesticide to enter or run off into storm drains, drainage ditches, gutters or surface waters".
10. That the circumstances described in paragraphs one through nine, establish that the insecticide was applied in such a way as to allow it to enter surface water.
11. That the circumstances described in paragraphs one through ten constitute the use of a pesticide inconsistent with the product labeling in violation of 7 U.S.C. § 136j (a)(2)(G), 7 M.R.S.A. § 606 (2)(B) and 22 M.R.S. § 1471-D(8)(F).
12. That the circumstances described in paragraphs one through ten were in violation of the following, 22M.R.S. § 1471-D(8)(C) which reads, "used or supervised the use of pesticides applied in a careless, negligent or faulty manner or in a manner which is potentially harmful to the public health, safety or welfare or the environment".
13. That the Board has regulatory authority over the activities described herein.
14. That the Company expressly waives:
 - a. Notice of or opportunity for hearing;
 - b. Any and all further procedural steps before the Board; and
 - c. The making of any further findings of fact before the Board.
15. That this Agreement shall not become effective unless and until the Board accepts it.
16. That, in consideration for the release by the Board of the causes of action which the Board has against the Company resulting from the violations referred to in paragraphs eleven and twelve, the Company agrees to pay to the State of Maine the sum of \$750. (Please make checks payable to Treasurer, State of Maine).

IN WITNESS WHEREOF, the parties have executed this Agreement of two pages.

ATLANTIC PEST SOLUTIONS

By:  -DM Date: 1/24/17

Type or Print Name: THEODORE W. ST. AMANT. DM.

BOARD OF PESTICIDES CONTROL

By: _____ Date: _____

Henry Jennings, Director

APPROVED

By: _____ Date: _____

Mark Randlett, Assistant Attorney General

Proposed Administrative Consent Agreement Background Summary

Subject: Baldemar Ramon
Ramon Forestry Service LLC
765 Hill Road
Clinton, Maine 04927

Date of Incident(s): April 28, 2013

Background Narrative: Board staff responded to a drift complaint in Palermo alleging that drift occurred to a residential property when a pesticide application was made to an abutting blueberry field. The owner / commercial applicator of Ramon Forestry Service LLC, Baldemar Ramon applied Fitness Fungicide (propiconazole) to the blueberry field. Two separate foliage samples collected from turf near the house on the abutting property tested positive for propiconazole.

Summary of Violation(s):
CMR 01-026 Chapter 22 section 4 (B) I

Standards for Unconsented, Off-Target Drift of Pesticides

- I. General Standard. Pesticide applications shall be undertaken in a manner which minimizes pesticide drift to the maximum extent practicable, having due regard for prevailing weather conditions, toxicity and propensity to drift of the pesticide, presence of Sensitive Areas in the vicinity, type of application equipment and other pertinent factors.

Rationale for Settlement: The staff took into consideration the levels of residue detected, the precautions the applicator took, and the conditions on site at the time of the application.

Attachments: Proposed Consent Agreement

**STATE OF MAINE
DEPARTMENT OF AGRICULTURE, CONSERVATION AND FORESTRY
BOARD OF PESTICIDES CONTROL**

In the Matter of:)
Baldemar Ramon) ADMINISTRATIVE CONSENT AGREEMENT
Ramon Forestry Service LLC) AND
765 Hill Road) FINDINGS OF FACT
Clinton, Maine 04927)

This Agreement by and between Baldemar Ramon (hereinafter called the "Owner") and the State of Maine Board of Pesticides Control (hereinafter called the "Board") is entered into pursuant to 22 M.R.S.A. §1471-M (2)(D) and in accordance with the Enforcement Protocol amended by the Board on June 3, 1998.

The parties to this Agreement agree as follows:

1. That the owner works both as an employee of RT Allen & Sons Inc. and an independent pesticide applicator contractor (Ramon Forestry Service LLC.) hired by RT Allen & Sons Inc.
2. That on April 28, 2013, the owner, working in the capacity of a hired licensed commercial pesticide applicator, made a pesticide application to RT Allen & Sons Inc.'s 47 acre blueberry field (Sanborn Lot) on the Level Hill Road in Palermo.
3. That the following day the Board received a call from an abutting property owner who thought that based on prevailing winds during the application described in paragraph two, it was possible that drift came onto their property. They have two young children and had concerns about their family's potential exposure to the pesticide applied.
4. That on April 29, 2013, a Board inspector met with the abutting property owner and took two foliage samples and one wipe sample on their property. The foliage samples were taken at a distance of 10 feet from the southeast corner of the abutting property owner's house and 15 feet from the northeast corner of the house, respectively.
5. That the abutting property is a Sensitive Area Likely to be Occupied as that term is defined in CMR 01-026 Chapter 10 section 2(CCC).8.
6. That on the same day the Board inspector collected a foliage sample from the Sanborn Lot.
7. That on April 30, 2013, a Board inspector did an inspection with the Owner who stated he applied Fitness Fungicide to the Sanborn Lot using a mist blower on April 28, 2013.
8. That all samples collected as described in paragraphs four and six were sent to a lab for analyses.
9. That the lab results for the foliage sample collected ten feet from the southeast corner of the house was positive for propiconazole at 0.036 ppm (16% of target) and the foliage sample collected 15 feet from the northeast corner of the house was positive for propiconazole at 0.155 ppm (67% of target). The foliage sample collected from the Sanborn lot was positive for propiconazole at 0.252 ppm. Propiconazole is the active ingredient in Fitness Fungicide.
10. That CMR 01-026 Chapter 22 section 4(B).I requires applicators to undertake applications in a manner that minimizes pesticide drift to the maximum extent practicable.

JAN 21 2014

- 11. That CMR 01-026 Chapter 22 section 4(B).II provides that pesticide residues in or on any off-target Sensitive Area Likely to be Occupied resulting from off-target drift of pesticides from a nearby application that are 1% or greater of the residue in the target area are considered prima facie evidence that the application was not conducted in a manner to minimize drift to the maximum extent practicable.
- 12. That the circumstances described in paragraphs one through eleven establish that sufficient precautions were not taken to minimize drift to the maximum extent practicable.
- 13. That the circumstances described in paragraphs one through twelve constitute a violation of CMR 01-026 Chapter 22 section 4(B).I.
- 14. That the Board has regulatory authority over the activities described herein.
- 15. That the Owner expressly waives:
 - a. Notice of or opportunity for hearing;
 - b. Any and all further procedural steps before the Board; and
 - c. The making of any further findings of fact before the Board.
- 16. That this Agreement shall not become effective unless and until the Board accepts it.
- 17. That, in consideration for the release by the Board of the causes of action which the Board has against the Owner resulting from the violations referred to in paragraph thirteen, the Owner agrees to pay to the State of Maine the sum of \$400. (Please make checks payable to Treasurer, State of Maine.)

IN WITNESS WHEREOF, the parties have executed this Agreement of two pages.

RAMON FORESTRY SERVICE LLC

By: Baldemar Ramon Date: 1-16-2014

Type or Print Name: Baldemar Ramon

BOARD OF PESTICIDES CONTROL

By: _____ Date: _____
Henry Jennings, Director

APPROVED:

By: _____ Date: _____
Mark Randlett, Assistant Attorney General

check number 1738
check amount 400

check date 1/16/14

Proposed Administrative Consent Agreement Background Summary

Subject: Ellen McLaughlin
Gateway Inn
1963 Medway Road
Medway, Maine 04460

Date of Incident(s): Throughout 2012

Background Narrative: Through inspection work by a Board inspector, it was determined that the owner of the Gateway Inn made unlicensed indoor applications of pesticides to the facility. Pesticide applications were made to common areas as well as to rented rooms. In addition, the owner did not post the application information so that employees were informed about the pesticide applications.

Summary of Violation(s):

- Any person making a pesticide application that is a custom application, as defined under 22 M.R.S. § 1471-C(5-A), must be a certified commercial applicator or under the direct supervision of a certified applicator in accordance with 22 M.R.S. § 1471-D(1)(A) and CMR 01-026 Chapter 31 Section 1(A) III.
- CMR 01-026 Chapter 26 Section 3(B) requires that at least 24 hours before a non-exempt pesticide application is made, a business must post or cause to be posted a Board approved written notice to employees about the applications.

Rationale for Settlement: The staff compared the violation to similar cases settled by the Board, the extent of the unlicensed applications and the applicator's lack of candor in formulating the penalty proposal.

Attachments: Proposed Consent Agreement

FEB 3 2014

STATE OF MAINE
DEPARTMENT OF AGRICULTURE, CONSERVATION AND FORESTRY
BOARD OF PESTICIDES CONTROL

CK#16953
Date: 1-29-14
Amt: \$500.00
PL

Ellen McLaughlin)
Gateway Inn) ADMINISTRATIVE CONSENT AGREEMENT
1963 Medway Road) AND
Medway, Maine 04460) FINDINGS OF FACT

This Agreement, by and between Gateway Inn (hereinafter called the "Company") and the State of Maine Board of Pesticides Control (hereinafter called the "Board"), is entered into pursuant to 22 M.R.S. §1471-M (2)(D) and in accordance with the Enforcement Protocol amended by the Board on June 3, 1998.

The parties to this Agreement agree as follows:

1. That the Company is located in Medway, Maine and rents rooms to the public for overnight accommodations. The Company is owned and managed by Ellen McLaughlin.
2. That from a market place inspection on January 17, 2013, and sales records collected by a Board inspector during a follow up marketplace inspection at Maine Paper and Janitorial Products in Hermon on February 19, 2013, it was determined that the Company received a case (12 x 16 oz.) of Bed Bug, Lice and Dust Mite Spray (EPA reg. no. 706-110) on the following dates: June 10, 2011; August 19, 2011; August 31, 2011; September 18, 2011; September 30, 2011; October 15, 2011; October 31, 2011; March 3, 2012; March 17, 2012; April 16, 2012; August 18, 2012; September 3, 2012; and September 15, 2012. On July 19, 2011, the Company received two cases of this same product.
3. That Maine Paper and Janitorial Products invoice # 079751 indicates on January 23, 2013, the Company returned and was credited for one case (12 x 16 oz.) and four cans of Bed Bug, Lice and Dust Mite Spray.
4. That on January 17, 2013, a Board inspector contacted McLaughlin at the Company to ask about her use of the Bed Bug, Lice and Dust Mite Spray in 2012. McLaughlin stated she used it throughout the building throughout 2012 and that she would treat rooms for fleas and ticks when the renters had dogs with them while those guests were out on errands and activities. She said that a Dept. of Health and Human Services employee later told her she could not use the product. When the Board inspector asked McLaughlin for specific information on where and when she used the pesticide she asked him to leave the premises.
5. That on January 22, 2013, the Board inspector again met with McLaughlin at the Company's Medway site to do an inspection on her use of pesticides. McLaughlin stated that she applied Bed Bug, Lice and Dust Mite Spray at the facility sometime in August of 2012 when Modern Pest Control was there to treat a bed bug problem in room 104. McLaughlin said she sprayed the two mattresses, box springs and bed frames in the room. She also sprayed the hallway as a precaution because the articles she treated were carried out from the room and put on her truck for later disposal.
6. That during the inspection described in paragraph five, McLaughlin told the inspector she did not post notification information about the August 2012 application anywhere in the building for her employees.
7. That from the inspection described in paragraph five the inspector collected McLaughlin's typed statement about her use of Bed Bug, Lice and Dust Mite Spray as outlined in that same paragraph. McLaughlin wrote in part that she purchased the spray from Maine Paper.

8. That during the inspection described in paragraph five, the inspector collected signed written statements that in part stated McLaughlin did not know the disposition of 63 cans of the Bed Bug, Lice and Dust Mite Spray, that she started ordering sprays in 2012, that in the fall of 2012, around October, a DHS inspector told her to stop using sprays, and that she applied the spray as a flea treatment to unoccupied rooms.
9. That during the inspection described in paragraph five, McLaughlin showed the inspector 16 (13 oz.) cans of Bed Bug, Lice and Dust Mite Spray that were on her desk. McLaughlin stated these were the only cans of this product she could find on the premises. The inspector placed a stop sale, use, removal order on these products.
10. That CMR 01-026 Chapter 26 Section 3(B) requires that at least 24 hours before a non-exempt pesticide application is made, a business must post or cause to be posted a Board approved written notice to employees about the applications.
11. That the circumstances in paragraphs one, two, four, five, six, and ten constitute a violation of CMR 01-026 Chapter 26 Section 3(B).
12. That any person making a pesticide application that is a custom application, as defined under 22 M.R.S. § 1471-C(5-A), must be a certified commercial applicator or under the direct supervision of a certified applicator in accordance with 22 M.R.S. § 1471-D(1)(A) and CMR 01-026 Chapter 31 Section 1(A)III.
13. That a custom application is defined in 22 M.R.S. § 1471-C(5-A) as any application of any pesticide under contract or for which compensation is received or any application of a pesticide to a property open to use by the public. The applications described in paragraphs four and five were applications made to areas that are open to use by the public.
14. That the pesticide applications made to the Company as described in that paragraphs four and five above constitute custom applications under 22 M.R.S. § 1471-C(5-A) and, therefore, a commercial applicator's license was required for those applications.
15. That no one from the Company had a commercial pesticide applicator's license at the time of the pesticide applications described in paragraphs four and five were made.
16. That the circumstances described in paragraphs one, two, four, five, twelve, thirteen, fourteen, and fifteen constitute multiple violations of 22 M.R.S. § 1471-D(1)(A) and CMR 01-026 Chapter 31 Section 1(A)III.
17. That the Board has regulatory authority over the activities described herein.
18. That the Company expressly waives:
 - a. Notice of or opportunity for hearing;
 - b. Any and all further procedural steps before the Board; and
 - c. The making of any further findings of fact before the Board.
19. That this Agreement shall not become effective unless and until the Board accepts it.
20. That, in consideration for the release by the Board of the causes of action which the Board has against the Company resulting from the violations referred to in paragraphs eleven and sixteen, the Company agrees to pay to the State of Maine the sum of \$500. (Please make checks payable to Treasurer, State of Maine.)

IN WITNESS WHEREOF, the parties have executed this Agreement of three pages.

GATEWAY INN

By: Ellen McLaughlin Date: 12-9-14

Type or Print Name: Ellen McLaughlin

BOARD OF PESTICIDES CONTROL

By: _____ Date: _____
Henry Jennings, Director

APPROVED

By: _____ Date: _____
Mark Randlett, Assistant Attorney General

Proposed Administrative Consent Agreement Background Summary

Subject: Donald and Christopher McCarthy
Olde English Village, LLC
503 Westbrook Street
South Portland, ME 04106

Date of Incident(s): Various dates in 2011, 2012 and 2013

Background Narrative: A Board inspector followed up on a call received on January 15, 2013. The caller said that employees at the Olde English Village apartment building complex were making unlicensed pesticide applications. The inspector interviewed employees, including the manager of the facility. Based on those interviews, on-site observations of pesticides in inventory, application equipment seen and documentation of a specific, representative pesticide application, it was confirmed that employees of Olde English Village were making unlicensed commercial pesticide applications. In addition, while on site the inspector saw two employees with an unmarked, partially filled pesticide container.

Summary of Violation(s):

- Any person making a pesticide application that is a custom application, as defined under 22 M.R.S. § 1471-C(5-A), must be a certified commercial applicator or under the direct supervision of a certified applicator in accordance with 22 M.R.S. § 1471-D(1)(A) and CMR 01-026 Chapter 31 Section 1(A) III.
- 7 M.R.S. §606 2D, prohibits handling, transporting or otherwise distributing pesticides in a careless, faulty, or negligent manner.

Rationale for Settlement: The staff compared the violations to similar cases settled by the Board factoring in storing pesticides in an unmarked container.

Attachments: Proposed Consent Agreement

FEB 3 2014

STATE OF MAINE
DEPARTMENT OF AGRICULTURE, CONSERVATION AND FORESTRY
BOARD OF PESTICIDES CONTROL

CK# 5222
Date: 1-30-14
Amt \$ 500.00
PL

Olde English Village, LLC) ADMINISTRATIVE CONSENT AGREEMENT
503 Westbrook Street) AND
South Portland, ME 04106) FINDINGS OF FACT

This Agreement, by and between Olde English Village, LLC (hereinafter called the "Company"), and the State of Maine Board of Pesticides Control (hereinafter called the "Board"), is entered into pursuant to 22 M.R.S. §1471-M (2)(D) and in accordance with the Enforcement Protocol amended by the Board on June 3, 1998.

The parties to this Agreement agree as follows:

1. That the Company owns and manages the Olde English Village apartment complex at 503 Westbrook Street in South Portland, Maine.
2. That on January 15, 2013, the Board received a call alleging employees of the Company had performed unlicensed pesticide applications at the apartment building described in paragraph one.
3. That in response to the call in paragraph two, a Board inspector met with Company employees Leah Hennigar (building mgr.), Ronald Garland (maintenance mgr.), and Chris Anderson (worker) on January 29, 2013, to conduct an inspection on pesticide use at this facility.
4. That during the inspection described in paragraph three, Garland acknowledged that Company employees applied Bed Bugs No More insecticide to rented Company apartments. The inspector documented one such application made by Company employees Chris Anderson and Jeremy Creamer to the interior of rented apartment number 307 in B building on January 8, 2013.
5. That during the inspection described in paragraphs three and four, Company employees Chris Anderson and Jeremy Creamer arrived on site with an unmarked partially filled one gallon container they told the inspector contained Bed Bugs No More insecticide. Garland stated that the container probably got wet and the label fell off.
6. That during the inspection described in paragraphs three and four, the inspector saw a pump-up type hand sprayer with a Roundup herbicide logo on it, and asked what it was used for. Anderson stated it was used by Company employees to apply Roundup to Company sidewalks in the summer.
7. That the inspector asked Garland if there were any other pesticides stored at the Company. Garland said no.
8. That the inspector asked to see the boiler rooms for units A and F. No pesticides were found stored in unit A's boiler room. In the boiler room for unit F, the following stored pesticides were found: three cans of Raid Fogger, EPA reg. no. 4822-452; five cans of Bed Bug and Flea Fogger, EPA reg. no. 1021-1674-8845; and approximately twenty- four containers of JT Eaton Kills Bed Bugs and Crawling Insects, EPA reg. no. 56-67.
9. That a box that held full JT Eaton Kills Bed Bugs and Crawling Insects containers had a shipping label with Garland's name on it and was addressed to the Company. The date on the label was 6-18-10.
10. That Anderson, Garland, and Creamer said the JT Eaton Kills Bed Bugs and Crawling Insects insecticide had not been used at the Company in more than two years, but the Raid Fogger had been used within six months, and the Bed Bug and Flea Fogger within three months.

11. That the inspector saw an empty can of Bed Bug and Flea Fogger in the trash can directly under the shelf where the pesticides described in paragraph eight were stored.
12. That any person making a pesticide application that is a custom application, as defined under 22 M.R.S. § 1471-C(5-A), must be a certified commercial applicator or under the direct supervision of a certified applicator in accordance with 22 M.R.S. § 1471-D(1) (A) and CMR 01-026 Chapter 31 Section 1(A) III.
13. That a custom application is defined in 22 M.R.S. § 1471-C(5-A) as any application of any pesticide under contract, or for which compensation is received or any application of a pesticide to a property open to use by the public. Applications made to rented apartments are considered applications for which compensation is received, and applications made to the halls of the apartment building are considered as applications made to areas that are open to the public.
14. That neither the Company, nor anyone employed by the Company, had a commercial pesticide applicator's license at the time of the applications described in paragraph four, six, and ten were made.
15. That the circumstances described in paragraphs one through fourteen constitute violations of 22 M.R.S. § 1471-D(1)(A) and CMR 01-026 Chapter 31 Section 1(A) III.
16. That 7 M.R.S. §606 2D, prohibits handling, transporting or otherwise distributing pesticides in a careless, faulty, or negligent manner.
17. That the circumstances in paragraphs three, four and five, constitutes a violation of 7 M.R.S. §606 2D. Company employees used a pesticide in an unmarked container.
18. That the Board has regulatory authority over the activities described herein.
19. That the Company expressly waives:
 - a. Notice of or opportunity for hearing;
 - b. Any and all further procedural steps before the Board; and
 - c. The making of any further findings of fact before the Board.
20. That this Agreement shall not become effective unless and until the Board accepts it.
21. That, in consideration for the release by the Board of the causes of action which the Board has against the Company resulting from the violations referred to in paragraphs fifteen and seventeen, the Company agrees to pay to the State of Maine the sum of \$500. (Please make checks payable to Treasurer, State of Maine).

IN WITNESS WHEREOF, the parties have executed this Agreement of three pages.

OLDE ENGLISH VILLAGE LLC

By: 

Date: 1/30/14

Print name: CHRISTOPHER MCCARTHY

Its: GENERAL MANAGER

BOARD OF PESTICIDES CONTROL

By: _____
Henry Jennings, Director

Date: _____

APPROVED

By: _____
Mark Randlett, Assistant Attorney General

Date: _____

Proposed Administrative Consent Agreement Background Summary

Subject: Barry Webster
Jato Highlands Golf Course
175 Town Farm Road
Lincoln, ME 04457

Date of Incident(s): April 22, 2012 and May 15, 2012

Background Narrative: Jato Highlands Golf Course is a public golf course. Because the golf course is open to the public, pesticide applications at the course must be made or supervised by a licensed commercial pesticide applicator. In addition, each commercial pesticide application company, including golf courses that make their own pesticide applications, must employ at least one Master Applicator. The facility's golf course superintendent and master pesticide applicator terminated in September of 2011. A Board inspector documented that a Jato Highlands Golf Course employee made two unlicensed commercial pesticide applications in 2012.

Summary of Violation(s): CMR 01-026 Chapter 31, Section 1(A) Commercial pesticide applications can only be made or supervised by licensed commercial pesticide applicators.

Rationale for Settlement: The staff compared the violation to similar cases settled by the Board in formulating a penalty proposal.

Attachments: Proposed Consent Agreement

JAN 31 2014

STATE OF MAINE
DEPARTMENT OF AGRICULTURE, CONSERVATION AND FORESTRY
BOARD OF PESTICIDES CONTROL

CK# 5543

Date: 1-28-14

\$250.00

Barry Webster) ADMINISTRATIVE CONSENT
Jato Highlands Golf Course) AGREEMENT
175 Town Farm Road) AND
Lincoln, ME 04457) FINDINGS OF FACT

This Agreement, by and between Jato Highlands Golf Course (hereinafter called the "Company") and the State of Maine Board of Pesticides Control (hereinafter called the "Board"), is entered into pursuant to 22 M.R.S. §1471-M (2)(D) and in accordance with the Enforcement Protocol amended by the Board on June 3, 1998.

The parties to this Agreement agree as follows:

1. That the Company operates a public golf course in Lincoln, Maine.
2. That on June 18, 2012, a Board inspector conducted a pesticide inspection and determined that on April 22, 2012, and again on May 15, 2012, Company employee Barry Webster applied Andersons Systemic Fungicide to the turf at the golf course with a granular spreader.
3. That the golf course is considered open to use by the public in accordance with 22 M.R.S. § 1471-C(5-A).
4. That the use of any pesticide in an area open to use by the public constitutes a commercial pesticide application in accordance with 22 M.R.S. § 1471-C(5).
5. That commercial pesticide applications can only be made or supervised by licensed commercial applicators pursuant to CMR 01-026 Chapter 31, Section 1(A).
6. That neither Webster nor anyone employed by the Company was a licensed commercial applicator at the time of the pesticide applications described in paragraph two.
7. That the circumstances described in paragraphs one through six constitutes violations of CMR 01-026 Chapter 31, Section 1(A).
8. That the Board has regulatory authority over the activities described herein.
9. That the Company expressly waives:
 - a. Notice of or opportunity for hearing;
 - b. Any and all further procedural steps before the Board; and
 - c. The making of any further findings of fact before the Board.
10. That this Agreement shall not become effective unless and until the Board accepts it.
11. That the Board has regulatory authority over the activities described herein.

12. That the Company expressly waives:
- d. Notice of or opportunity for hearing;
 - e. Any and all further procedural steps before the Board; and
 - f. The making of any further findings of fact before the Board.
13. That this Agreement shall not become effective unless and until the Board accepts it.
14. That, in consideration for the release by the Board of the causes of action which the Board has against the Company resulting from the violations referred to in paragraph seven, the Company agrees to pay to the State of Maine the sum of \$250. (Please make checks payable to Treasurer, State of Maine).

IN WITNESS WHEREOF, the parties have executed this Agreement of two pages.

JATO HIGHLANDS GOLF COURSE

By:  Date: 01/28/14

Type or Print Name: THOMAS W. GARDNER owner

BOARD OF PESTICIDES CONTROL

By: _____ Date: _____
Henry Jennings, Director

APPROVED

By: _____ Date: _____
Mark Randlett, Assistant Attorney General



Dr. Henry Jennings, PhD, director
Maine Board of Pesticides Control
28 State House Station
Augusta, ME 04333-0028

February 11, 2014

Dear Dr. Jennings

Friends of Penobscot Bay is a nonprofit citizens association dedicated to stewardship of Maine's biggest bay. Our membership includes leaders of Penobscot Bay's lobstering community and representatives of Penobscot Bay's other fisheries

We were glad to learn from your testimony on Thursday that the Maine Board of Pesticides Control is committing resources to sampling Maine's coastal sediments for their pesticide loads, and that the Board's Environmental Risk Advisory Committee will direct this effort.

Over the past year the Friends of Penobscot Bay has worked with researchers from University of Maine, Unity College and St Joseph's College, as well as the concerned public, on sampling and testing our bay's sediments and intertidal organisms for acidity, metals and other wastes.

Please let us know how we can best work with the Pesticides Control Board's Environmental Risk Advisory Committee to help make your testing initiative as thorough and successful as possible. We understand that volunteers from Friends of Casco Bay have been helpful in sample gathering for pesticides in Casco Bay, and we are pleased to offer the same volunteer deployment services in Penobscot Bay.

Learning about the level of pesticides in our lobsters and other seafood species, and what strategies to take to limit pesticide entry into our bay is very important to us! In summary we'd like to work with the Pesticides Control Board's Environmental Risk Advisory Committee to help make the Penobscot Bay portion of your coastwide testing initiative as thorough and successful as possible.

We look forward to hearing from you!

Sincerely

Harlan McLaughlin

Harlan McLaughlin, president
Friends of Penobscot Bay

Report on the Status of Products Registered
for use as Wide Area Public Health
Mosquito Adulticides in Maine-2013

And

Summary of EPA's Most Recent Public
Health and Environmental Risk Assessments

Lebelle Hicks PhD DABT
Pesticides Toxicologist
Maine Board of Pesticides Control
December 20, 2013

MOSQUITO WIDE AREA PUBLIC HEALTH ADULTICIDES IN MAINE 2013

BACKGROUND

The pesticides registered for use for mosquito control in Maine include:

Adulticides, products which kill adult mosquitoes, ten of which are discussed below

Repellents, products used on human skin, human gear and animals to repel adult mosquitoes

Aquatic larvicides, products added to water at breeding sites to prevent the development of the mosquitoes, these include the biological insecticides, the insect growth regulator methoprene and monomolecular films which mechanically control the larvae

Non-aquatic larvicides, insect growth regulators which are labelled for use indoors, outdoors and on animals

Of the 1,322 products registered for use on mosquitoes in Maine -2013, 1,125 of these products contain at least one adulticide and approximately 30 have specific directions for use in wide area public health uses (NSPIRS 2013). This review is limited to a subset of these products which are registered for use in public health wide area mosquito control projects used to address an outbreak of either Eastern Equine Encephalitis (EEE) or West Nile Virus (WNV). Since the labels are legal documents and are approved by EPA in accordance with their risk assessments, human health and environmental, the label statements limiting the areas of use and specifics of applications go a long way to limiting exposure while providing efficacy in control of adult mosquitoes.

There are two chemical classes of insecticides, pyrethrins-pyrethroids-PBO (including etofenprox, permethrin, piperonyl butoxide (PBO) (synergist), permethrin, phenothrin, prallethrin, pyrethrins and resmethrin) and the organophosphates (chlorpyrifos, malathion and naled). The synergist PBO is found in all but two of the pyrethroid-pyrethrin products and is not in the organophosphate products. A synergist increases the activity of the pyrethroid-pyrethrin insecticides while having no insecticidal efficacy of its own.

HUMAN RISK ASSESSMENT

The human health risks are evaluated by comparing the most sensitive endpoint in lab animals, to expected environmental exposures. The standard measure of human health risk is the 'margin of exposure' (MOE). The MOE is the ratio of the most sensitive toxicity result from the animal study to the expected exposure dose resulting from the use in question. A pesticide product with a higher calculated MOE has a lower risk to humans. EPA has established chemical specific 'levels of concern' (LOC) for short (1 to 7 days) and intermediate (1 to 6 months) term exposures. Risks higher than the LOC are deemed acceptable. Human health risks are evaluated for toddlers for exposure following an application via incidental oral route (putting hands or objects in mouth after playing on grass, or eating grass) and dermal (skin) exposure and inhalation, and for adults via skin and inhalation routes (EPA 2012c).

With regard to the pyrethrins-pyrethroids and piperonyl butoxide (PBO), with the exception of prallethrin (a component of Duet EPA# 1021-1795-8329) the MOE exceed EPA's LOC by approximately ten to over a million times for both aerial and ground applications at the maximum use rate for public health adult mosquito control. EPA has yet to finalize the human health risk assessment for prallethrin. The human health risk associated with the use of these materials is exceedingly low. Mosquito adulticides are applied by ultra-low-volume equipment by air or by ground. For the adulticide products containing pyrethrins-pyrethroids-PBO, risks from aerial applications by ultra-low-volume are lower and efficacy against mosquitoes is better than those made by ground ultra-low-volume.

Given the low risks from exposure to the pyrethrins- pyrethroids-PBO, any could be used in a wide area public health adulticiding program. The phenothrin-PBO containing product, Anvil 10+10 (EPA# 1021-1688-8329) has been used in other states, because of its very low application rate (0.0036lbs ai/A), its low risk to humans, its allowed use over agricultural areas (40 CFR 180.647) and the tolerances in all raw agricultural commodities as a result of mosquito adulticiding.

The three organophosphates, chlorpyrifos, malathion and naled, registered for wide area adult mosquito control have lower margins of exposure (higher risk to people) than do the pyrethrins-pyrethroids-PBO compounds. However, with the exception of chlorpyrifos at 0.01 lb ai/A, the risk of inhalation exposure in both toddlers and adults is higher (the MOE is lower) than EPA's levels of concern for these applications. For air applications of the organophosphate pesticide naled, the calculated risks to toddlers range from 54 times higher than the level of concern for oral exposure to approximately 240 times higher for dermal exposure (EPA 2002a, EPA 2006a). Similar to phenothrin, there is a universal tolerance on agricultural products intended for human consumption for naled residues following wide area mosquito adulticiding applications (40CFR180.215). Among organophosphates, naled and malathion, are considered the lowest risk, effective pesticides and are often used in the southern and mid-western U.S. for wide area mosquito control.

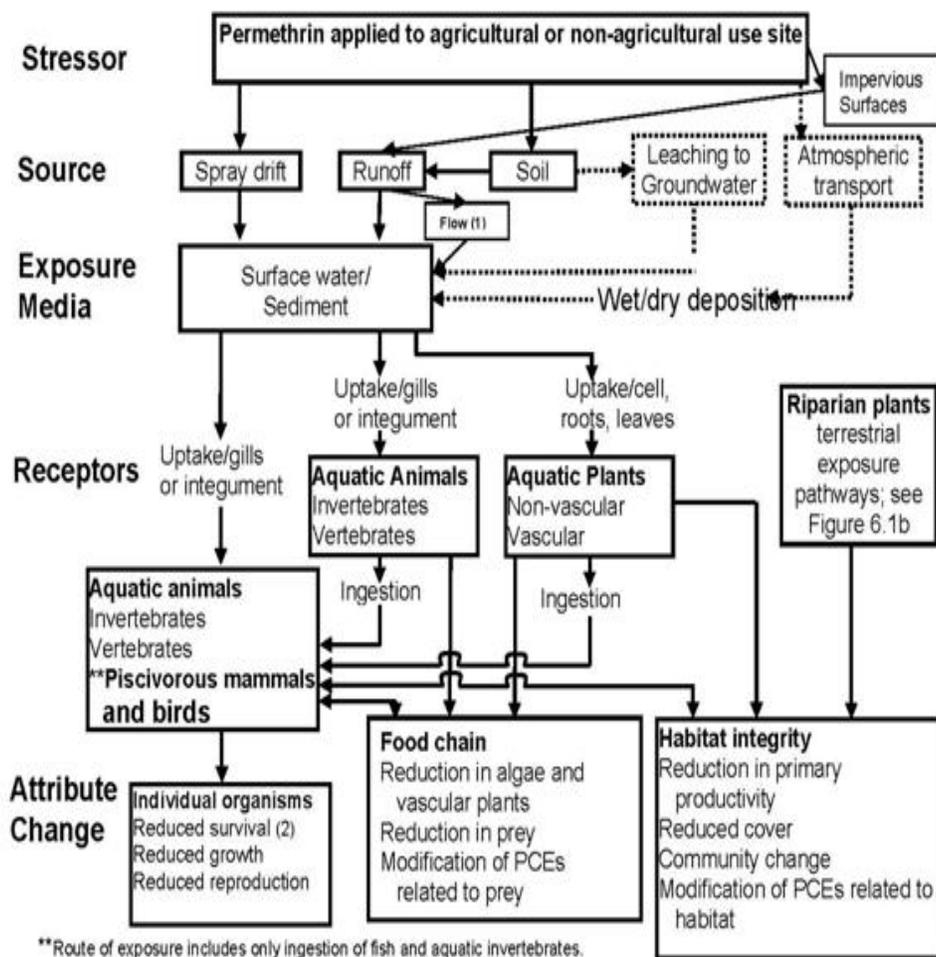
The potential for pesticides to cause an increase in cancer rates in the human population is considered in EPA risk assessments. The cancer potentials for the adulticides are categorized as "not likely" or "no evidence" for phenothrin, and naled, "not likely at low doses" for etofenprox and pyrethrins, suggestive or possible for PBO and malathion, and likely for permethrin and resmethrin (EPA 2012a). However, the cancer risks from exposure to permethrin following ultra-low-volume ULV applications is 3 orders of magnitude (1,000 times) lower than EPA's acceptable risk level of 1 in a million by ground and eleven orders of magnitude lower, when the application is done by air (EPA 2009d). The residential cancer risks following mosquito adulticiding with permethrin both by air and ground are lower than EPA's acceptable risk level 1 in a million (EPA 2006f).

Allergy reactions as a result of insecticide exposure, including asthma exacerbations are difficult to predict. Because of this, the message to the public if a municipal adulticiding application were to occur, would include, persons with allergies, take extra care (stay inside, close windows etc.) to reduce exposure.

Environmental Risk Assessment

Because of the wide variety of ecological niches and species occupying those niches, assessing risks to organisms in the environment is much more complicated (Figure 1) than human health assessments.

Figure 1 Aquatic Conceptual Model of Exposure pathways for Permethrin (EPA 2011h)



Laboratory species are used to determine the critical toxicology value and exposure is estimated using a combination of modeling and environmental sampling. Unlike the human health process, the environmental risks are evaluated using the risk quotient method; estimated environmental concentration divided by the toxicity factor. In this case the lower the risk quotient, the lower the risks. The levels of concern (LOC) used by EPA have been established for acute (short term exposure, LOC = 0.5), chronic (long term exposure, LOC = 1).

Fish and aquatic invertebrates lack the metabolic capability of the mammalian liver and lack the protective barrier found in humans or other mammals, therefore they are generally more sensitive to insecticides. This is reflected in both the toxicity of the insecticides as well as the risks. Exposure to birds and wild mammals is estimated using the T-REX model (EPA 2012b). The risks to birds and

wild-mammals parallels the risks to humans. Because there was no toxicity seen in the animal studies, EPA did not perform risk assessments for etofenprox (EPA 2009a) and phenothrin (d-phenothrin; Sumithrintm) (EPA 2008f). The other pyrethrins-pyrethroids and PBO risks are within EPA's level of concern of acute and chronic exposures at rates used for mosquito control (EPA 2005g, EPA 2006i, EPA 2006b, EPA 2006d, EPA 2010b, EPA 2011h, EPA 2011i, EPA 2012h, EPA 2012i). The risk quotients for the organophosphates for birds and mammals are generally higher (more risky) than the pyrethrins-pyrethroids-PBO compounds (EPA 2008d, EPA 2008e, EPA 2008g, EPA 2009g). They are still within EPA's level of concern for acute and chronic exposure.

The data currently in the EPA reviews indicate that the highest risks from ultra-low-volume mosquito adulticiding applications are to freshwater and marine invertebrates living in the water column and to those dwelling in the sediment. The toxicity of the pyrethrins and pyrethroids to sediment dwelling invertebrates is an area of active research. EPA has issued data-call-ins for the pyrethrins and most of the pyrethroids.

EPA's aquatic risk assessments rely on modeling for estimating environmental exposure. The assumptions are for multiple aerial applications 25 to 50 per year with intervals ranging from 1 day (EPA 2011h) to 7 days (EPA 2012h). They also assume that temperature is 85° F and the relative humidity is 90%. Most of the ultra-low-volume mosquito adulticide labels require a temperature of above 50° F. Given the climate in Maine and our relatively short warm season permitting mosquito development, and the fact that EEE and WNV are often not detected in mosquitoes until late in the season, the likelihood of more than one or two applications per year is low.

CONCLUSIONS

Adult mosquito control is only one part of a comprehensive IPM approach that includes education to promote the use of repellents and staying indoors when risk is high, and when possible, eliminating standing water where mosquitoes breed, or treating mosquito breeding habitats with lower risk larvicides. However, the use of adulticides can be a lower risk and necessary means for protecting communities when the risk of WNV or EEE reaches critical levels. When risks of mosquito borne illness are high and mosquito habitat reduction and larval control are infeasible and/or insufficient to reduce adult mosquito populations, aerial or ground-based applications of insecticides are often a necessary component of an integrated mosquito management program (CDC 2003).

The overview of mosquito products and the label review are appended for consultation. The risk assessment information (100+ pages) is compiled and will be made available at your request.

SECTION 1. SCOPE; UNIVERSE OF PESTICIDE PRODUCTS REGISTERED FOR USE ON MOSQUITOES IN MAINE 2013 AND PESTICIDE PRODUCTS LABELED FOR USE AS PUBLIC HEALTH MOSQUITO ADULTICIDES

The 53 active ingredients in the 1,322 products currently registered in Maine with mosquito control on their labels. The active ingredients are summarized in Table 1.1. These products have been grouped as to function: adulticide, aquatic larvicides, insect growth regulators, repellents, and products with multiple uses. When a product has two or more active ingredients in the same group, adulticide, larvicide or repellent, that is consider a single group. For example a product with two pyrethroids would be considered an adulticide, a product with one pyrethroid and an insect growth regulator would be considered a multi-use-product. One thousand one hundred and twenty five of the mosquito products registered in Maine-2013 contain at least one adulticide, 206 products contain at least one insect growth regulator (for purposes this classification products containing methoprene with non-aquatic uses are grouped with the IGRs and aquatic uses are grouped with the aquatic larvicides), 163 contain at least one repellent and 47 are aquatic larvicides. Three hundred and sixty five of these products contain one of two synergists, either PBO (piperonyl butoxide) or MGK 264 (N-Octyl bicycloheptene dicarboximide).

In addition to the active ingredients, pesticide products contain “inert” or “other” ingredients. These ingredients are present to increase the activity of the active ingredient, but they have no pesticidal action against the target pest. A review of the inert ingredients in the public health adulticides, could be undertaken, but was beyond the scope of the current project.

The products included in the current review were limited to the adulticide products with specific directions for wide area public health uses and include pyrethrins, five synthetic pyrethroids (etofenprox, permethrin, phenothrin, prallethrin and resmethrin) and three organophosphates (chlorpyrifos, malathion and naled) (Table 2.1). Future reviews of the other types of mosquito products may be done.

The most common active ingredients in mosquito products are: permethrin is also found in over 300 products, the synergist, PBO (over 300 products) and pyrethrins (over 200 products). These three active ingredients are found in the public health products listed in Table 2.1. Permethrin has uses on human gear, indoor, outdoor and direct uses on animals. PBO and pyrethrins have a variety of indoor, outdoor and direct uses on animal (NSPIRS 2013).

Table 1.1 Overview of Mosquito Products Registered in Maine in 2013; The Active Ingredients in Bold are found in the Public Health Wide Area Mosquito Products

Type	# Products	Active Ingredients	Notes
Biological larvicides	32	Bti-Bs	Microbial disruptors of insect midgut membranes (IRAC 2013)
Repellents	179	DEET	These repellents are registered for use on human skin and are recommended by the federal CDC as mosquito repellents. MGK 326 Repellent (Dipropyl isocinchomeronate) is registered for use on human gear in products with indoor and outdoor uses. BPG (Butoxypolypropylene glycol) is found in combination with other repellents pyrethroids and synergist. Registered for agricultural use on livestock. Linalool is registered in impregnated materials (candles torches etc.) to repel mosquitoes outdoors. The linalool products also have indoor uses. Other repellents: Oil of Eucalyptus (can be used on skin), Metofluthrin, Oil of Citronella
		IR3535	
		Oil of Lemon Eucalyptus	
		Picaridin	
		PMD	
Synergists	455	PBO (piperonyl butoxide)	PBO used in most of the pyrethrin-pyrethroid products used in public health wide area projects.
		MGK 264 (N-Octyl bicycloheptene dicarboximide)	MGK 264 is found in a dozen products with human skin and gear on their labels and numerous indoor outdoor and animals use products.
Insect Growth Regulators	258	Methoprene	Methoprene is a juvenile hormone analogue (IRAC 2013) and is found in aquatic larvicide 12 products; the non-aquatic uses of methoprene are on cats and dogs for flea and tick control
		Pyriproxyfen	Pyriproxyfen is a juvenile hormone analogue (IRAC 2013). The primary uses of pyriproxyfen are on cats and dogs for flea and tick control

Table 1.1 Overview of Mosquito Products Registered in Maine in 2013; The Active Ingredients in Bold are found in the Public Health Wide Area Mosquito Products

Type	# Products	Active Ingredients	Notes
Neonicotinoids	38	Acetamiprid, Dinotefuran, Imidacloprid	These compounds activate the insect nicotinic acetylcholine receptor (nAChR) (IRAC 2013).
Organophosphates	39	Chlorpyrifos, Malathion, Naled	Organophosphate insecticides act by irreversibly inhibiting the enzyme acetylcholinesterase in the nervous system (IRAC 2013).. These may be used in public health wide area projects.
		DDVP, Tetrachlorvinphos	Six impregnated strips containing 18.6% DDVP.and one DDVP/ tetrachlorvinphos are registered for agricultural uses. DDVP is also found as a metabolite of naled
		Temephos	Temephos is an aquatic larvicide.
Carbamates	10	Carbaryl	Carbamate insecticides act by reversibly inhibiting the enzyme acetylcholinesterase in the nervous system (IRAC 2013)
Pyrethrins - Pyrethroids	1181	Ethofenprox, Permethrin, Phenothrin, Prallethrin, Pyrethrins, Resmethrin	Pyrethrins and pyrethroids act by modulating the sodium channels in neurons (IRAC 2013). Ethofenprox, Permethrin, Phenothrin, Prallethrin, Pyrethrins, or Resmethrin may be used in public health wide area projects. All of the public health products contain the synergist PBO except for the etofenprox products.
		Other pyrethroids: Allethrin-d and d-trans, Bifenthrin, Bioallethrin-s, Cyfluthrins, Cyhalothrins, Cypermethrins, Deltamethrin, Esfenvalerate, Fluvalinate, Tetramethrin	
Others	148	2-Phenylethyl propionate, d-Limonene, Fipronil, Mineral oil, NEEM, POE isooctadecanol, Soap, Spinosad, Triethylene glycol	Includes two aquatic larvicides with mechanical means of control; mineral oil and POE isooctadecanol. Fipronil acts by blocking the GABA gated chloride channels in nerves. Spinosad acts as a nACh allosteric activator (IRAC 2013)

SECTION 2. TYPICAL ADULTICIDE PRODUCTS LABELED FOR WIDE AREA PUBLIC HEALTH ULV USES

In an effort to summarize the potential for human and environmental hazards associated with public health mosquito abatement programs, a product search was conducted for Maine 2013 registration, followed by a search for active federal registrations for public health mosquito adulticide products. The search terms included: adult mosquito, and aerial or ultra-low volume (ULV) (NSPIR 2013). There were approximately 30 products identified by the search, with the language on their labels specifying:

“For use only by federal, state, tribal, or local government officials responsible for public health or vector control, or by persons certified in the appropriate category or otherwise authorized by the state or tribal lead pesticide regulatory agency to perform adult mosquito control applications, or by persons under their direct supervision”

The EPA registration numbers (EPA#) for the selected public health wide area mosquito adulticide products registered in Maine in 2013 containing synthetic pyrethroids, pyrethrins and PBO, their diluents, are found in Table 2.1. Similar information for the organophosphate containing products is found in Table 2.2.

The review is based on selected products because the number of products could change, with the Maine registration of a federally registered product. The federal search identified 108 products, 27 of which are currently registered Maine. Of the remaining 84 products, 78 have the same mosquito adulticide active ingredients and similar formulations as those registered in Maine-2013. The other six products, may be registered in Maine -2013, but do not have public health mosquito control uses on their labels. Four of these contain the active ingredients carbaryl (one home owner; three agricultural products), 2 contain the synthetic pyrethroid, lambda cyhalothrin. Wide area mosquito adulticiding public health uses are not on these federal labels (Bayer 2009, Tessendro-Kerley 2012, Tessendro-Kerley 2013, Loveland Chemical 2011, Syngenta 2010, LG Lifesciences 2009).

The maximum use rates in pounds pyrethroid-pyrethrins and PBO active ingredient per acre (lbs ai/A) are presented in Table 2.3. The organophosphate active ingredient maximum use rates are found in Table 2.4. The use rates for malathion are 0.23 lbs ai/A by air and 0.11 lbs ai/A by ground (Table 2.4.). Use rates for the synthetic pyrethroids, pyrethrins and the organophosphates chlorpyrifos and naled are the same for both aerial and ground ultra-low volume (ULV) applications.

Table 2.1 Typical Public Health Adult Mosquito Products Containing Pyrethroids-Pyrethrins-Piperonyl Butoxide (PBO) Registered in Maine for 2013 sorted by Active Ingredient (NSPIRS 2013) ^(a)

Active ingredients	Percent Active Ingredients	Diluent	EPA REG #	References
Etofenprox	4% Etofenprox	Ready to use	2724-807	Wellmark 2010a, Wellmark 2010b,
	20% Etofenprox	Oil	2724-791	Wellmark 2009a, Wellmark 2009b,
Permethrin-PBO	2% Permethrin, 2% PBO ^(b)	Ready to use	73748-3	Univar 2013a, Univar 2013b
	< 5% Permethrin, < 5% PBO	Oil	655-898	Prentiss 2012a, Prentiss 2012b
	20% Permethrin, 20% PBO	Water	432-796	Bayer ^(c) 2013a, Bayer 2013b
	20.6% Permethrin, 20.6% PBO	Oil or Water	53883-274	Control Solutions 2010a, Control Solutions 2010b,
	> 30 % Permethrin, > 30% PBO	Oil	73748-5	Univar 2013g, Univar 2013h
Phenothrin-PBO	10% Phenothrin ^(d) , 10% PBO	Oil	1021-1688-8329 ^(h)	Clarke ^(e) 2013a, Clarke 2009
Phenothrin-Prallethrin-PBO	5% Phenothrin ^(d) , 1% Prallethrin, 5% PBO	Oil	1021-1795-8329 ^(h)	Clarke 2013b, Clarke 2008
Pyrethrins-PBO	5 to 12% Pyrethrins, 25 to 60% PBO	Oil	1021-1199	MGK ^(f) 2013a, MGK 2013b
Resmethrin-PBO	4.14 to 18% Resmethrin, 12.42 to 54% PBO	Oil	432-716	Bayer 2012a, Bayer 2012b

a) Selection of a product for label review does not constitute an endorsement

b) PBO = Piperonyl butoxide, pesticide synergist

c) Bayer = Bayer Environmental EPA Company number 432

d) Phenothrin = Sumithrin

- e) The company number for these products is McLaughlin Gormley King (MGK) company number, 1021, the product number varies with the product and 8329 is the company number for the distributor, Clarke Mosquito Products
- f) MGK = McLaughlin Gormley King

EPA REG #	Active Ingredients	Diluent	lbs ai/gal	References
53883-251	19.36% Chlorpyrifos ^(b)	Oil	1.5	Control Solutions 2009a, Control Solutions 2010d
67760-34	96.5% Malathion	Oil	9.9	Cheminova 2011a, Cheminova 2011b,
5481-479	62% Naled	Water	7.5	AMVAC 20012a, AMVAC 20012b
5481-481	78% Naled	None	10.8	AMVAC 2010a, AMVAC 2010b
5481-480	87.4% Naled	Oil	13.2	AMVAC 2009a, AMVAC 2009b

- a) Selection of a product for label review does not constitute an endorsement
- b) There are a number of other chlorpyrifos containing products registered for public health mosquito adulticide use (NSPIRS 2013)

Active Ingredients	Rate (lbs ai/A)	Annual Rate (lbs ai/A/year)	Reference
Etofenprox	0.007	0.18	Wellmark2010a, EPA 2009a
Permethrin	0.007	0.18	Bayer 2011f, EPA 2009c
Phenothrin (Sumithrin)	0.0036	1	MGK 2012a, EPA 2007, EPA 2008
PBO	0.08	2	EPA 2004b
Prallethrin	0.0008	0.02	Clarke Mosquito 2013b
Pyrethrins	0.008	0.2	MGK 2013a, EPA 2006b
Resmethrin	0.007	0.2	Bayer 2012a

Table 2.4 Use Rates for Active Ingredients (lbs ai/A and lbs ai/A/year) for Public Health Adult Mosquito Products Containing Pyrethroids-Pyrethrins and PBO			
Active Ingredients	Rate (lbs ai/A)	Annual Rate (lbs ai/A/year)	Reference
Chlorpyrifos	0.01	0.26	Control Solutions 2009a, Control Solutions 2009b
Malathion (air)	0.23	Not more than 3 times in any one week. More frequent treatments may be to control mosquito-borne diseases in animals or humans	Cheminova 2011a, EPA 2004a, EPA 2009b
Malathion (ground)	0.11		
Naled (air and ground)	0.1	10.73	AMVAC 20012a, AMVAC 20012b

SECTION 3. LABEL REVIEW

Pesticide labels are legal documents. The statement “**It is a violation of Federal Law to use this product in a manner inconsistent with its labeling**” is required on all pesticide labels (EPA 2007 to 2012). The pesticide product label language requirements are spelled out in the EPA Label Review Manual found at: <http://www.epa.gov/oppfead1/labeling/lrm/> (EPA 2007 to 2012). These statements are required based on the toxicity databases for the technical grade active ingredient and the pesticide end use product (active and inert ingredients).

For the public health mosquito adulticide the label sections summarized below are signal words, hazards to humans and domestic animals and personal protective equipment. EPA assigns mammalian toxicity categories for the technical grade active ingredients (TGAI) and the end use products offered for sale and use based on acute toxicity data. The criteria for EPA’s toxicity categories are set in 40CFR156.62 and the relationship with required label language are found in Appendix II.

SIGNAL WORDS, HAZARDS TO HUMANS AND DOMESTIC ANIMALS

PYRETHROIDS- PYRETHRINS-PBO PRODUCTS

Signal Words

Etofenprox, Permethrin-PBO, Phenothrin (Sumithrin[™])-PBO, Phenothrin (Sumithrin[™])-PBO-Prallethrin, Pyrethrins-PBO, Resmethrin-PBO

All of the wide area public health mosquito adulticide products containing pyrethrins, pyrethroids and PBO have “caution” signal words indicating low risks to mammals from acute exposure.

Hazards to humans and domestic animal

Etofenprox, Permethrin-PBO, Phenothrin-PBO, (Anvil 10 +10-oil based), Pyrethrins-PBO, Resmethrin-PBO, have warnings for moderate eye irritation. Anvil 10 + 10 (EPA# 1021-1688-8239) also has a warning for moderate eye irritation

Phenothrin-PBO (Aqua Anvil-water based), Phenothrin (Sumithrin[™])-PBO-Prallethrin (Duet-oil based and Aqua Duet-water based) have no eye warnings.

Personal Protective Equipment

In Table 2.1, the Pyrethrins-Pyrethroids-PBO containing products are primarily permethrin-BPO at a variety of concentrations. There are two products with etofenprox as the sole active ingredient, two phenothrin (Sumithrin[™])-PBO products, two phenothrin (Sumithrin[™])-PBO-prallethrin products, three pyrethrins-PBO products and two Resmethrin-PBO containing products. The personal protective equipment statements are found below.

Etofenprox containing products have no personal protective equipment requirements on the labels of the two mosquito adulticide product labels.

Ten of the eleven permethrin-PBO containing products registered for use in Maine 2013 have labels approved by EPA in 2011, 2012 and 2013 with the following personal protective equipment requirements:

“Mixers, loaders, applicators and other handlers must wear:

- Long-sleeved shirt and long pants,
- Shoes plus socks,
- Chemical-resistant gloves for all handlers except for applicators using motorized ground equipment, pilots, and flaggers
- Chemical-resistant apron for mixers/loaders, persons cleaning equipment, and persons exposed to the concentrate”

The other permethrin product, PBO/Permethrin 20:20, (EPA# 53883-274), has no PPE requirements and the label was approved in 2010. Since the RED for permethrin was issued in 2009 (EPA 2009c), most likely the next iteration of this label would incorporate the PPE requirements from the RED.

Anvil 10 + 10 (EPA# 1021-1688-8329), hydrocarbon based, Multicide® Mosquito Adulticiding Concentrate 2705 (EPA# 1021-1688) requires applicators, mixers and loaders to wear: long-sleeve shirt and pants, shoes and socks, and chemical resistant gloves made of barrier laminate nitrile rubber, neoprene rubber or viton.

Aqua Anvil, water based (EPA# 1021-1807-8329): Multicide® Mosquito Adulticiding Concentrate 2807 (EPA# 1021-1807) labels require applicators mixers and loaders wear: long-sleeve shirt and pants and shoes and socks.

Duet (EPA#1021-1795-8329) petroleum base, Multicide Fogging Concentrate 2798 (EPA# 1021-1795) and Aqua Duet (EPA#1021-2562-8329), Multicide Fogging Concentrate 2922 (EPA# 1021-2562) labels require applicators mixers and loaders wear: long-sleeve shirt and pants and shoes and socks.

Two resmethrin products registered in Maine 2013 for adult mosquito control in public health settings are SCOURGE® Insecticide with resmethrin/piperonyl butoxide 18% + 54% MF FORMULA II (EPA# 432-667) and SCOURGE® Insecticide with SBP-1382/Piperonyl Butoxide 4%+12% MF FII (EPA# 432-716).

The personal protective equipment requirements from both labels are:

- Long-sleeved shirt and long pants
- Shoes plus socks
- Chemical-resistant gloves for all handlers except applicators.

The Scourge product label for product with the higher concentrations, (EPA# 432-667), chemical resistant gloves are required for all applicators except applicators using motorized ground equipment pilots and flaggers.

Organophosphates

Signal Words

The organophosphate products containing chlorpyrifos and malathion also have “caution” signal word. The naled containing products have “danger” signal words due to irreversible corrosive effects on the skin and eyes.

Hazards to humans and domestic animal

Chlorpyrifos and Malathion

Technical grade chlorpyrifos is more acutely toxic than technical grade malathion (Table B). The adulticide products are a soluble concentrate containing 19.36% chlorpyrifos (1.5 lbs/gal) product and a ready to use 96.5% malathion (9.9 lbs/gal) product. Both the chlorpyrifos product and the malathion product labels have “caution” as the signal word. The different human and domestic animal hazard sections reflect the differences in potency.

Chlorpyrifos

CSI 1.5 (EPA# 53883-251) human and domestic animal hazard section reads:

“Harmful if swallowed. Avoid contact with skin or clothing. Wash thoroughly with soap and water after handling and before eating, drinking, chewing gum, using tobacco, or using the toilet. Prolonged or frequently repeated skin contact may cause allergic reactions in some individuals (Control Solutions 2009a, Control Solutions 2009b).”

The Fyfanon (EPA# 67760-34) malathion containing product label states:

“Harmful by swallowing, inhalation or skin contact. Avoid contact with skin. Avoid breathing spray mist” (Cheminova 2011a, Cheminova 2011b.)”

Naled

All of the naled containing products registered for use as public health mosquito adulticides are classified **RESTRICTED USE PESTICIDE DUE TO EYE AND SKIN CORROSIVITY HAZARD** and have **DANGER** signal words because of corrosiveness to eyes and skin.

Human health hazard statements include:

- “Causes irreversible eye and skin damage.

- Causes skin burns.
- May be fatal if swallowed.
- Harmful if inhaled or absorbed through the skin.
- Do not get in eyes, on skin, or on clothing.
- Do not breathe vapor or spray mist.
- Prolonged or frequently repeated skin contact may cause allergic reactions in some individuals (AMVAC 2009a, AMVAC 2010a, AMVAC 20012a.)”

Personal Protective Equipment Requirements

The organophosphate containing products include one chlorpyrifos, one malathion and three naled products. The personal protective equipment statements are found below.

Chlorpyrifos

CFI 1.5 containing 19.36% chlorpyrifos (1.5 lbs/gal) (EPA# 53883-251) has the following directions for personal protective equipment:

“Personal Protective Equipment (PPE): All mixers and loaders involved in ground application must wear coveralls over long-sleeved shirt and long pants, shoes plus socks, chemical-resistant gloves, and a NIOSH-approved dust mist filtering respirator with MSHAINIOSH approval number prefix TC21C or a NIOSH-approved respirator with any R, P, or HE filter. Applicators involved in ground ULV application must use an enclosed cab as described in the

Engineering Controls Section of this label and must wear long-sleeved shirt and long pants, shoes plus socks, and chemical-resistant gloves. Aerial applicators and pilots must use an enclosed cockpit and wear long-sleeved shirt, long pants, shoes, and socks (Control Solutions 2009a, Control Solutions 2009b.)”

Malathion

Fyfanon ULV containing 96.5% malathion (9.9 lbs/gal) (EPA# 53883-34) label directions for personal protective equipment are:

“For all formulations and use patterns - mixers, loaders, applicators, flaggers, and other handlers must wear:

- Long-sleeved shirt and long pants
- Chemical-resistant gloves
- Shoes plus socks (Cheminova 2011a, Cheminova 2011b)”

Naled

Personal protective equipment from the naled product labels read:

“If engineering controls are in use:

- Protective eye wear (goggles, face shield, or safety glasses)
- Long-sleeved shirt and long pants
- Socks plus shoes
- Chemical-resistant gloves (barrier laminate, butyl rubber, nitrile rubber, or viton, selection category E) and apron when mixing or loading. See engineering controls for additional requirements

In the absence of engineering controls:

- Protective eye wear (goggles, face shield, or safety glasses)
- Coveralls over long-sleeve shirt and long pants
- Chemical-resistant gloves
- Chemical-resistant footwear plus socks
- Chemical-resistant apron if exposed to the concentrate • Chemical-resistant headgear for overhead exposure
- A respirator with an organic-vapor removing cartridge with a prefilter approved for pesticides (AMVAC 2009a, AMVAC 2010a, AMVAC 20012a.)”

ENVIRONMENTAL HAZARD STATEMENTS

PYRETHROIDS- PYRETHRINS-PBO CONTAINING PRODUCTS

The environmental hazard statement from Zenivex E20 (EPA#2724-791) containing 20% etofenprox label states:

“This pesticide is toxic to aquatic organisms, including fish and aquatic invertebrates. Runoff from treated areas or deposition into bodies of water may be hazardous to fish and other aquatic organisms. Do not apply over bodies (of water (lakes, rivers, permanent streams, natural ponds, commercial fish ponds, swamps, marshes or estuaries), **except when necessary to target areas where adult mosquitoes are present**, and weather conditions will facilitate movement of applied material away from water in order to minimize incidental deposition into the water body. Do not contaminate bodies of water when disposing of equipment rinsate or washwasters. [Emphasis added].

This product is highly toxic to bees exposed to direct treatment on blooming crops or weeds. Time applications to provide the maximum possible interval between treatment and the next period of bee activity. Do not apply to blooming crops or weeds when bees are visiting the treatment area, **except when applications are 'made to prevent or control a threat to public and/or animal health determined by a state, tribal, or local health or vector control agency on the basis of documented evidence of disease-causing agents in vector mosquitoes or the occurrence of mosquito-borne disease in animal or human populations**, or if specifically approved by the state or tribe during a natural disaster recovery effort (Wellmark 2010c, Wellmark 2010d.)” [emphasis added].

Similar extensive environmental hazard warnings are found on all of the pyrethrins-pyrethroid-PBO have warnings similar or identical to the Zenivex E20 (EPA# 2724-791) (Wellmark 2010c, Wellmark 2010d.)”

In addition, the two Scourge products containing resmethrin and PBO are classified as restricted use products because of acute toxicity to fish (Bayer 2012a, Bayer 2012b, Bayer 2012c, Bayer 2012d). The restricted use classification means that certification and licensing are needed to purchase and use the products.

ORGANOPHOSPHATE CONTAINING PRODUCTS

Pyrofos 1.5 ULV Vector Control Insecticide containing 19.36% chlorpyrifos (1.5 lbs/gal) (EPA# 53883-251) has the following environmental hazard statements:

“This pesticide is toxic to fish, aquatic invertebrates, small mammals and birds. Runoff from treated areas or deposition of spray droplets into a body of water may be hazardous to fish and aquatic invertebrates. Do not apply over bodies of water (lakes, rivers, permanent streams, natural ponds, commercial fish ponds, swamps, marshes or estuaries) ~ **except when necessary to target areas where adult mosquitoes are present, (emphasis added)** and weather conditions weather facilitate movement of applied material beyond the body of water in order to minimize incidental deposition into the water body. Do not contaminate bodies of water when disposing of equipment rinsate or wash waters.

This product is highly toxic to bees exposed to direct treatment or residues on blooming crops or weeds Do not apply this product or allow it to drift to blooming crops or weeds if bees are visiting the treated area, **except 'When applications are made to prevent or control a threat to public and/or animal health determined by a state, or local health or vector control agency on the basis of documented evidence of disease causing agents in vector mosquitoes, or the occurrence of mosquito-borne disease in animal or human populations, or if specifically approved by the state or tribe during a natural disaster recovery effort (emphasis added)** (Control Solutions 2009a, Control Solutions 2009b).”

The environmental hazard section of the Fyfanon ULV containing malathion read much the same as the synthetic pyrethroids:

“This pesticide is toxic to aquatic organisms, including fish and invertebrates. Use care when applying in or to an area which is adjacent to any body of water, and do not apply when weather conditions favor drift from target area. Poorly draining soils and soils with shallow water tables are more prone to produce runoff that contains this product. When applying as a wide area mosquito adulticide, before making the first application in a season, it is advisable to consult with the state or tribal agency charged with primary responsibility for pesticide regulation to determine if other regulatory requirements exist.

This product is highly toxic to bees exposed to direct treatment on blooming crops or weeds. Do not apply or allow to drift onto blooming crops or weeds while bees are actively visiting the treatment area, **except when applications are made to prevent or control a threat to public**

and/or animal health determined by a state, tribal or local public health or vector control agency on the basis of documented evidence of disease causing agents in vector mosquitoes or the occurrence of mosquito-borne disease in animal or human populations, or if specifically approved by the state or tribe during a natural disaster recovery effort (emphasis added).

When applying as a wide area mosquito adulticide, do not apply over bodies of water (lakes, rivers, permanent streams, natural ponds, commercial fish ponds, swamps, marshes or estuaries), except when necessary to target areas where adult mosquitoes are present, and weather conditions will facilitate movement of applied material away from the water in order to minimize incidental deposition into the water body. Do not discharge effluent containing this product into lakes, streams, ponds, estuaries, oceans, or other waters unless in accordance with the requirements of a National Pollutant Discharge Elimination System (NPDES) permit and the permitting authority has been notified in writing prior to discharge. Do not discharge effluent containing this product to sewer systems without previously notifying the local sewage treatment plant authority. For guidance contact your State Water Board or Regional Office of the EPA (Cheminova 2011a, Cheminova 2011b.)”

Another consideration not found on other public health mosquito products is: “undiluted spray droplets of Fyfanon ULV Mosquito will permanently damage vehicle paint finishes unless the aircraft used for the ultra-low volume application meets all of the specifications listed under AERIAL APPLICATION (Cheminova 2011a, Cheminova 2011b).

Regarding non-target toxicity the naled labels read:

“This pesticide is toxic to fish, aquatic invertebrates, and wildlife. Runoff from treated areas or deposition of spray droplets into a body of water may be hazardous to fish and aquatic invertebrates. Before making the first application in a season, consult with the primary State agency responsible for regulating the pesticides to determine if permits are required or regulatory mandates exist. Do not apply over bodies of water (e.g., lakes, swamps, rivers, permanent streams, natural ponds, commercial fish ponds, marshes or estuaries), **except when necessary to target areas where adult mosquitoes are present (emphasis added)**, and weather conditions will facilitate movement of applied material away from the water in order to minimize incidental deposition into the water body. Do not contaminate bodies of water when disposing of equipment washwaters or rinsate (AMVAC 2009a, AMVAC 2010a, AMVAC 20012a).

This product is highly toxic to bees exposed to direct treatment on blooming crops or weeds. To minimize hazard to bees, it is recommended that the product is not applied more than two hours after sunrise or two hours before sunset, limiting application to times when bees are least active. Do not apply this product or allow it to drift to blooming crops or weeds while bees are visiting the treatment area, except when applications are made to prevent or control a threat to public and/or animal health determined by a state, tribal or local health or vector control agency on the basis of documented evidence of disease causing agents in vector mosquitoes or the occurrence of mosquito-borne disease in animal or human populations, or if specifically approved by the state or the tribe during a: natural disaster recovery effort (AMVAC 2009a, AMVAC 2010a, AMVAC 20012a).

LABEL LANGUAGE FOR USE OVER FARMS AND AGRICULTURAL AREAS

PYRETHROIDS- PYRETHRINS-PBO PRODUCTS

Depending on the existence of US food or feed tolerances (Appendix III), the label language for the pyrethrins-pyrethroid containing adulticides is different.

Piperonyl butoxide (PBO), is present in all of the pyrethrins-pyrethroid products with the exception of the etofenprox products. PBO is exempt from tolerance on raw agricultural commodities when used according to good agricultural practice (40CFR180.905).

There are no tolerances for etofenprox in raw agricultural commodities with the exception of rice (40CFR180.620). Etofenprox containing products have label directions to “Cover exposed drinking water in corrals, feedlots, swine lots cropland or any exposed drinking water” and “do not spray or allow drift onto pastureland, cropland or potable water sources. Given the “cover drinking water” sources for livestock and “do not spray or allow drift” statements on the etofenprox labels, food residues resulting from public health mosquito applications should not be an issue.

Permethrin has many tolerances in raw agricultural commodities (40 CFR180.378) these are for the commodities listed on the permethrin product labels. Permethrin-PBO products, in one form or another have the following label language, “Do not spray this product on or allow it to drift onto cropland (other than crops listed) or potable water supplies (followed by the list of commodities which have tolerances for permethrin and PBO residues). In the treatment of corrals feedlots animal confinements/houses swine lots poultry ranges and zoos cover any exposed drinking water drinking fountains and animal feed before application.

Phenothrin has a universal tolerance 0.01 ppm for raw agricultural commodities (40CFR180.647) and PBO is exempt from tolerance (40CFR180.905). Prallethrin only has a universal tolerance for uses in food and feed establishments and no tolerances on raw agricultural commodities (40CFR180.545). Anvil 10 + 10, oil based and Aqua Anvil, water-based, have the following statement regarding use over agricultural areas: “May be applied over agricultural areas for the control of adult mosquitoes within or adjacent to the treatment areas” Because of the presence of prallethrin and the lack of tolerances, the Duet and Aqua Duet, Phenothrin-PBO-Prallethrin have the following statement regard agricultural areas: “Do not spray this product on or allow it to drift onto rangeland cropland poultry ranges or potable water supplies In treatment of corrals feed lots swine lots and zoos cover any exposed drinking water drinking water fountains and animal feed before application”

Pyrethrins are exempt from tolerance on raw agricultural commodities (40CFR180.905).

Pyrethrins-PBO product labels state: “This concentrate may be diluted or used as supplied for mosquito control programs involving residential, industrial, recreational and agricultural areas where adult mosquitoes are present in annoying numbers in vegetation surrounding swamps, marshes, overgrown waste areas, roadsides and pastures. Use in agricultural areas should be in such a manner as to avoid residues in excess of established tolerances for pyrethrins and PBO on crops or commodities”

Similar to prallethrin, resmethrin has a universal tolerance for uses in food and feed establishments and no tolerances on raw agricultural commodities (40CFR180.525.). Given the site limitations on the resmethrin containing product labels, food residues resulting from public health mosquito applications should not be an issue. The two Scourge products containing resmethrin and PBO labels state: “Scourge is designed for application as an Ultra-Low Volume (ULV) aerosol to control adult mosquitoes and flies in residential industrial urban recreational areas and other areas where the labeled pests are a problem.

ORGANOPHOSPHATE CONTAINING PRODUCTS

There are at least 80 tolerances (40CFR180.342) for chlorpyrifos, given the non-crop-land statement on the chlorpyrifos label, food residues resulting from public health mosquito applications should not be an issue. Chlorpyrifos containing product, CSI 1.5 ULV (EPA# 53883-251) is designed for application either as a thermal fog or as an ultra-low volume (ULV) non-thermal aerosol (cold fog) to control adult mosquitoes in: “Outdoor residential and recreational areas and other non-cropland areas where these insects are a problem”

Malathion has tolerances in over 150 commodities (40CFR180.111). Given the site limitations on the malathion containing product label, food residues resulting from public health mosquito applications should not be an issue. Aerial Applications for Fyfanon ULV are limited to “Rangeland, Pasture, and Other Uncultivated Non-Agricultural Areas (Wastelands, Roadsides). There are no such limits on ground applications.

There are 38 tolerances for naled. In addition, a universal tolerance of 0.5 part per million is established for the pesticide naled in or on all raw agricultural commodities, except those otherwise listed in this section, from use of the pesticide for area pest (mosquito and fly) control (40CFR180.215). Two of the three products containing naled have mosquito (and nuisance fly) uses only, Dibrom Concentrate (EPA# 5481-480) and Trumpet EC (EPA# 5481-481). The third product, Dibrom 8 Emulsive (EPA# 5481-479) has the mosquito, nuisance fly and agricultural uses on its label. The two products with no agricultural uses on their labels have the following directions regarding use over agricultural areas:

“It is not necessary to avoid farm buildings, dairy barns, pastures, feed or forage areas. Use in agricultural areas must be in a manner as to ensure that residues do not exceed the established federal tolerance for the active ingredient in or on raw agricultural commodities resulting from use for wide area pest control. Treat shrubbery and vegetation where mosquitoes may be present. Shrubby and vegetation around stagnant pools, marshy areas, swamps, residential areas, municipalities, woodlands, pastures, farm buildings and feedlots may be treated.”

The product with both agricultural and mosquito/ nuisance fly uses, Dibrom 8 Emulsive (EPA# 5481-479) in the section on controlling mosquitos reads:

“It is not necessary to avoid farm buildings. Make applications during peak of infestation and repeat as necessary. See crop recommendation for use limitations near harvest. Treat shrubbery and

vegetation where mosquitoes may rest. Shrubbery and vegetation around stagnant pools, marshy areas, ponds and shorelines may be treated.

References

40CRF180.215, 2013, Tolerances for Naled

40CRF180.647, 2013, Tolerances for Phenothrin (Sumithrin)

AMVAC 2009a, Dibrom Concentrate, EPA# 5481-480, containing 87.4% naled, EPA Label

AMVAC 2009b, Dibrom Concentrate, EPA# 5481-480, containing 87.4% naled, ME-2013 Label

AMVAC 2010a, Trumpet EC Insecticide, EPA# 5481-481, containing 78% naled, EPA Label

AMVAC 2010b, Trumpet EC Insecticide, EPA# 5481-481, containing 78% naled, ME-2013 Label

AMVAC 2012a, Dibrom 8 Emulsive, EPA# 5481-479, containing 62%, naled, EPA Label

AMVAC 2012b, Dibrom 8 Emulsive, EPA# 5481-479, containing 62%, naled, ME-2013 Label

Bayer CropSciences 2009, Sevin Brand RP4 Carbaryl Insecticide, EPA# 264-335, containing 43% Carbaryl EPA Label

Bayer Environmental Services 2011a, Aqua-Permanone, EPA# 432-796, containing 20% permethrin-20% PBO, EPA Label

Bayer Environmental Services 2011b, Aqua-Reslin, EPA# 432-796, containing 20% permethrin-20% PBO, ME-2013 Label

Bayer Environmental Services 2011c, Omen 30-30 ULV, EPA# 432-1235, containing 30% permethrin-30% PBO, EPA Label

Bayer Environmental Services 2011d, Permanone 30-30, EPA# 432-1235, containing 30% permethrin-30% PBO, ME-2013 Label

Bayer Environmental Services 2011e, Permanone Insecticide Concentrate, EPA# 432-1250, containing 31.28% permethrin-66% PBO, EPA Label

Bayer Environmental Services 2011f, Permanone 31-66, EPA# 432-1250, containing 31.28% permethrin-66% PBO, ME-2013 Label

Bayer Environmental Services 2011g, Pyrenone Crop Spray, EPA# 432-1033, EPA Label

Bayer Environmental Services 2012a, Scourge Insecticide w/ Resmethrin/Piperonyl Butoxide 4%+12% MF FII, EPA# 432-716, containing 4.14% resmethrin-12.42% PBO, EPA Label

Bayer Environmental Services 2012b, Scourge Insecticide w/ Resmethrin/Piperonyl Butoxide 4%+12% MF FII, EPA# 432-716, containing 4.14% resmethrin-12.42% PBO ME-2013 Label

Bayer Environmental Services 2012c, Scourge Insecticide w/ Resmethrin/Piperonyl Butoxide 18% + 54% MF FII, EPA# 432-667, containing 18% resmethrin-54% PBO, EPA Label

Bayer Environmental Services 2012d, Scourge Insecticide w/ Resmethrin/Piperonyl Butoxide 18% + 54% MF FII, EPA# 432-667, containing 18% resmethrin-54% PBO, ME-2013 Label

Centers for Disease Control and Prevention (CDC) 2003, Epidemic/Epizootic West Nile Virus in the United States: Guidelines for Surveillance, Prevention and Control

Cheminova 2011a, Fyfanon ULV Mosquito Insecticide, EPA# 67760-34, containing 96.5% malathion, EPA Label

Cheminova 2011b, Fyfanon ULV Mosquito Insecticide, EPA# 67760-34, containing 96.5% malathion, ME-2013 Label

Clarke Mosquito Control 2013a, Anvil 10+10 ULV, EPA# 1021-1688-8329, containing 10% sumithrin (phenothrin)-10% PBO, ME-2013 Label

Clarke Mosquito Control 2013b, Duet EPA# 1021-1795-8329, containing 1% Prallethrin 5% sumithrin (phenothrin)-5% PBO, ME-2013 label

Clarke Mosquito Control 2013c, Aqua Anvil Water Based Adulticide, EPA# 1021-1807-8329, containing 10% sumithrin (phenothrin)-10% PBO, Label from Clarke mosquito Website:
http://www.clarke.com/index.php?option=com_content&view=category&layout=blog&id=47&Itemid=126

Clarke Mosquito Control 2013d, Aqua Duet, EPA# 1021-2562, containing 1% Prallethrin 5% sumithrin (phenothrin)-5% PBO, Label from Clarke mosquito Website:
http://www.clarke.com/index.php?option=com_content&view=category&layout=blog&id=47&Itemid=126

Control Solutions 2009a, Pyrofos, EPA# 53883-251, containing 19.36% chlorpyrifos (1.5 lbs/gal) EPA Label

Control Solutions 2010e Pyrofos, EPA# 53883-251, containing 19.36% chlorpyrifos (1.5 lbs/gal) ME-2013 Label

Control Solutions 2010a, PBO/Permethrin 20:20, EPA# 53883-274, containing 20.6% permethrin-20.6% PBO, EPA Label

Control Solutions 2010b, Vector-Flex 20:20, EPA# 53883-274, containing 20.6% permethrin,-20.6% PBO, ME-2013 Label

Direct AG Source 2013, Permethrin 3.2 AG, EPA# 83222-3, containing 36.8% Permethrin [3.2 lbs/gal] EPA Label

Dow AgroSciences 2012, Dursban 50W in Water Soluble Packet,s EPA# 62719-72, Wettable Powder in Water Soluble bags Containing 50% Chlorpyrifos EPA Label

EPA 2002a, 2006a, Interim Re-registration Eligibility Decision for Naled; Finalized in 2006

EPA 2005g, Screening Ecological Risk Assessment for the Re-registration of Piperonyl Butoxide Insecticide Synergist

EPA 2006b, Revised Pyrethrins RED Chapter after Additional 60-Day Comment Period Phase 5

EPA 2006d, Re-registration Eligibility Decision (RED) for Resmethrin

EPA 2006f, Revised Occupational and Residential Exposure Assessment and Recommendations for the Re-registration Eligibility Decision (RED) for Resmethrin

EPA 2006i, The Agency Revised Risk Assessment for the Registration Eligibility Decision for Permethrin Following Public comments, Phase III

EPA 2006j, Glyphosate Human Health Risk Assessment for Proposed Use on Indian Mulberry and mended Use on Pea, Dry. PC Code: 417300, Petition No: 5E6987, DP Num: 321992, Decision No. 360557.

EPA 2008d, EFED Registration Review-Preliminary Problem Formulation for the Ecological Risk Assessment of Naled

EPA 2008e, EFED Registration Review – Preliminary Problem Formulation for Ecological Risk and Environmental Fate, Endangered Species and Drinking Water Assessments Chlorpyrifos (PC Code 059101; DP Barcode D355212)

EPA 2008f, EFED Preliminary Environmental Fate And Effects Assessment Science Chapter for the Re-registration Eligibility Decision of D-phenothrin (Sumithrin)

EPA 2008g, Risks of Naled Use to Federally Threatened California Red Legged Frog (*Rana aurora drayonii*)

EPA 2009a, Environmental Fate and Ecological Risk Assessment for Etofenprox New Uses on Rice and Vector Control

EPA 2009d, Permethrin: Sixth Revision of the HED Chapter of the Re-registration Eligibility Decision Document (RED)

EPA 2009g, Registration Review Preliminary Problem Formulation for the Ecological Risk, Environmental Fate and Endangered Species Assessments for Malathion (PC code 057701; DP Barcode D359863)

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EPA 2011h, EFED Registration Review Preliminary Problem Formulation for Permethrin

EPA 2011i, EFED Registration Review Preliminary Problem Formulation for Pyrethrins

EPA 2012a, Chemicals Evaluated for Carcinogenic Potential, Office of Pesticides Programs 2012

EPA 2012b, Use's Guide to T-REX Version 1.5

EPA 2012c, Standard Operating Procedures for Residential Pesticide Exposure Assessment

EPA 2012h, EFED Registration Review: Preliminary Problem Formulation for Environmental Fate, Ecological Risk, Endangered Species, and Drinking Water Exposure Assessment for Prallethrin

EPA 2012i, EFED Registration Review: Preliminary Problem Formulation for Resmethrin

LG Lifesciences 2009, Lamdastar 1 CS-PCO, EPA# 71532-27, containing 12% lambda cyhalothrin Fed Label

Loveland Chemical 2011, Carbaryl 4L, EPA# 34704-447, containing 43% Carbaryl EPA-Label

McLaughlin Gromley King 2012a, Pyroicide Mosquito Adulticiding Concentrate for ULV Fogging 7395, EPA# 1021-1570, containing 12% pyrethrins-60% PBO, ME-2013 Label

McLaughlin Gromley King 2012b, Pyroicide Mosquito Adulticiding Concentrate for ULV Fogging 7395, EPA# 1021-1570, containing 12% pyrethrins-60% PBO, EPA Label 2012

McLaughlin Gromley King 2012c, Multicide Mosquito Adulticiding Concentrate for ULV Fogging 2705, EPA# 1021-1688, containing 10% sumithrin (phenothrin)-10% PBO, EPA-2012 Label

McLaughlin Gromley King 2012d, Multicide Mosquito Adulticiding Concentrate for ULV Fogging 2795, EPA# 1021-1795, containing 1% Prallethrin 5% sumithrin (phenothrin)-5% PBO, EPA-2012 Label

McLaughlin Gromley King 2012c, Multicide Mosquito Adulticiding Concentrate for ULV Fogging 2705, EPA# 1021-1807, containing 10% sumithrin (phenothrin)-10% PBO, EPA-2012 Label

McLaughlin Gromley King 2012d, Multicide Mosquito Adulticiding Concentrate for ULV Fogging 2795, EPA# 1021-2562, containing 1% Prallethrin 5% sumithrin (phenothrin)-5% PBO, EPA-2012 Label

McLaughlin Gromley King 2013a, Pyrocide Fogging Formula 7067, EPA# 1021-1199, containing 5% pyrethrins-25% PBO, EPA Label

McLaughlin Gromley King 2013b, Pyrocide Fogging Formula 7067, EPA# 1021-1199, containing 5% pyrethrins -25% PBO, ME-2013 Label

McLaughlin Gromley King 2013c, Pyrocide Mosquito Adulticiding Concentrate for ULV Fogging 7396, EPA# 1021-1569, containing 5% pyrethrins-25% PBO, EPA Label

McLaughlin Gromley King 2013d, Pyrocide Mosquito Adulticiding Concentrate for ULV Fogging 7396, EPA# 1021-1569, containing 5-pyrethrins-,25% PBO, ME-2013 Label

NuFarm Americas 2012, ATERA GC 2+1 SC Insecticide, EPA# 228-557, containing 21.99% [2 lbs/gal] imidacloprid and bifenthrin 10.654% [1 lb./gal]

Prentiss 2012a, Prentox Perm-X UL 4-4, EPA# 655-898, containing 4% permethrin-4% PBO, EPA Label

Prentiss 2012b, Prentox Perm-X UL 4-4, EPA# 655-898, containing 4% permethrin-4% PBO, ME-2013 Label

Prentiss 2012c, Prentox Perm-X UL 30-30, EPA# 655-811, containing 30% permethrin, 30% PBO, EPA Label

Prentiss 2012d, Prentox Perm-X UL 30-30, EPA# 655-811, containing 30% permethrin-30% PBO, ME-2013 Label

Prentiss 2012e, Prentox Perm-X UL 31-66, EPA# 655-812, containing 31% permethrin-66% PBO, EPA Label

Prentiss 2012f, Prentox Perm-X UL 31-66, EPA# 655-812, containing 31% permethrin-66% PBO, ME-2013 Label

Syngenta 2010, Demand Pest Tabs, EPA# 100-1082, containing 10% lambda-cyhalothrin, EPA Label

Tessendro-Kerley 2012 Sevin Brand 4F Carbaryl Insecticide, PA# 61842-38, containing 43% Carbaryl, EPA-Label

Tessendro-Kerley 2013 Sevin Brand 85 Sprayable Carbaryl Insecticide, EPA# 61842-33, containing 85% Carbaryl, EPA-Label

United Phosphorous 2012, Up-Cyde Pro 2 0 EC Termiticide/Insecticide (EPA # 70506-19) EPA Label

Univar Environmental Services 2013a, Masterline Kontrol 2-2, EPA# 73748-3, containing 2% permethrin-2% PBO, EPA Label

Univar Environmental Services 2013b, Masterline Kontrol 2-2, EPA# 73748-3, containing 2% permethrin-2% PBO, ME-2013 Label

Univar Environmental Services 2013c, Masterline Kontrol 4-4, EPA# 73748-4, containing 4.6% permethrin-4.6% PBO, EPA Label

Univar Environmental Services 2013d, Masterline Kontrol 4-4, EPA# 73748-4, containing 4.6% permethrin-4.6% PBO, EPA Label

Univar Environmental Services 2013e, Masterline Aqua Kontrol Concentrate, EPA# 73748-1, containing 20% permethrin-20% PBO, ME-2103 Label

Univar Environmental Services 2013f, Masterline Aqua Kontrol Concentrate, EPA# 73748-1, containing 20% permethrin-20% PBO, EPA Label

Univar Environmental Services 2013g, Masterline 30-30, EPA# 73748-5, containing 30% permethrin-30% PBO, ME-2103 Label

Univar Environmental Services 2013f, Masterline 30-30, EPA# 73748-5, containing 30% permethrin-30% PBO, EPA Label

Wellmark International 2010c, Zenivex E20, EPA# 2724-791, containing 20% etofenprox, EPA Label

Wellmark International 2010d, Zenivex E20, EPA# 2724-791, containing 20% etofenprox, ME-2013 Label

Wellmark International 2010a, Zenivex E4 RTU, EPA# 2724-807, containing 4% etofenprox, EPA Label

Wellmark International 2010b, Zenivex E4 RTU, EPA# 2724-807, containing 4% etofenprox, ME-2013 Label

Report to the Joint Standing Committee on Agriculture, Conservation and Forestry—126th Maine State Legislature

Pursuant to 7 M.R.S.A. § 607(6), Grants Funded, Adequacy of the Product Registration Fee

Submitted by the Maine Board of Pesticides Control, February 15, 2014

In 2013, the Maine Legislature revised 7 M.R.S. § 607(6) by enacting Public Law 2013, Chapter 290. The new amendments require the Board to:

- increase the pesticide product registration fee from \$150 to \$160 per product per year;
- make an annual grant to the University of Maine Cooperative Extension of no less than \$135,000;
- provide grants for other programs within certain guidelines if funding is available; and
- annually submit a report to the joint standing committee of the Legislature having jurisdiction over agriculture, conservation and forestry matters on grants funded and recommendations on the adequacy of the fee to fund the specified programs.

Since the amendments to 7 M.R.S.A. § 607 became effective on January 1, 2014, there have not been any grants issued pursuant to the statute. Funding appears adequate to provide the annual grant to the University of Maine Cooperative Extension by April 1, 2014. Whether any additional grants may be funded during the 2014 calendar year has yet to be determined.

At this time, the \$160 annual pesticide product fee appears adequate to fund both the Board and related Department programs, and the annual grant to the University for both 2014 and 2015. A more careful assessment of the adequacy of the fee for these purposes is advisable at this time in 2015.



126th MAINE LEGISLATURE

SECOND REGULAR SESSION-2014

Legislative Document

No. 1744

H.P. 1250

House of Representatives, January 23, 2014

An Act To Protect Maine Lakes

Approved for introduction by a majority of the Legislative Council pursuant to Joint Rule 203.

Reference to the Committee on Environment and Natural Resources suggested and ordered printed.

Millicent M. MacFarland
MILLICENT M. MacFARLAND
Clerk

Presented by Representative McCABE of Skowhegan.
Cosponsored by Senator GRATWICK of Penobscot and
Representatives: BLACK of Wilton, CHIPMAN of Portland, GRAHAM of North Yarmouth,
HAMANN of South Portland, HICKMAN of Winthrop, McLEAN of Gorham, POWERS of
Naples, Senator: JOHNSON of Lincoln.

1 **Be it enacted by the People of the State of Maine as follows:**

2 **Sec. 1. 38 MRSA §410-L, first ¶**, as enacted by PL 1997, c. 643, Pt. YY, §1, is
3 amended to read:

4 The Lakes Assessment and Protection Program is established within the department
5 to monitor and protect the health and integrity of the State's lakes through activities
6 identified in section 410-M.

7 **Sec. 2. 38 MRSA §410-M**, as amended by PL 2011, c. 655, Pt. EE, §22 and
8 affected by §30, is repealed and the following enacted in its place:

9 **§410-M. Lakes assessment and protection**

10 In implementing the Lakes Assessment and Protection Program, the commissioner
11 shall ensure that the department:

12 **1. Education.** Develops:

13 A. Educational materials that inform the public about the health and functions of
14 lakes in the State; the value of lakes to the residents, communities and economy of
15 the State and wildlife in the State; the sources of risk posed to the health and integrity
16 of lakes; and actions that individuals can take to help preserve the health and water
17 quality of lakes. The department shall make the educational materials readily
18 available on its publicly accessible website and through other outreach resources; and

19 B. Educational materials designed for classroom instruction relating to the health and
20 integrity of lakes in the State. To the extent possible, the department shall serve as a
21 resource to schools and teachers. The department shall make the educational
22 materials readily available to schools;

23 **2. Monitoring lakes and conducting research.** Monitors lakes and conducts
24 research relating to the ecology and health of lakes, the vulnerability of and risks to lakes,
25 the relationship between lake water quality and development, the design and effectiveness
26 of best management practices and the effectiveness of efforts to protect lakes. The
27 department shall integrate the use of water quality monitors, academic institutions and
28 other lake monitoring resources in monitoring pursuant to this subsection. The
29 department shall make data collected pursuant to this subsection and the department's
30 analysis of the data regularly available on its publicly accessible website and through
31 other outreach resources. The department shall include the data and analysis in the report
32 submitted to the Legislature pursuant to section 464, subsection 3, paragraph A;

33 **3. Compliance monitoring and enforcement.** Promotes and monitors compliance
34 with and enforcement of the natural resources protection laws, the mandatory shoreland
35 zoning laws, the storm water management laws, the erosion and sedimentation control
36 laws and other state and local laws providing standards for the protection of lakes;

37 **4. Water quality and habitat protection, restoration and maintenance.** Directs
38 and assists with activities that protect, restore and maintain lake water quality and the
39 quality of habitat in lakes and on land surrounding lakes that affect the health and

1 integrity of lakes. The department shall develop partnerships pursuant to subsection 5 to
2 assist with these activities; and

3 **5. Partnerships.** Develops partnerships with lake associations, municipalities,
4 businesses, academic institutions, water quality monitors and other interested individuals
5 to increase public understanding about risks posed to the health and integrity of lakes and
6 actions that can be taken to reduce those risks and sustain lake water quality. To the
7 extent possible, the department shall provide technical and financial assistance to partners
8 pursuant to this subsection. A partnership developed pursuant to this subsection may
9 assist the department in water quality and habitat protection, restoration and maintenance
10 activities pursuant to subsection 4.

11 **Sec. 3. 38 MRSA §418-B** is enacted to read:

12 **§418-B. Restrictions on application of fertilizers, herbicides, pesticides and soil**
13 **amendments**

14 **1. Definitions.** As used in this section, unless the context otherwise indicates, the
15 following terms have the following meanings.

16 A. "Fertilizer" means a substance containing one or more recognized plant nutrients
17 that is used for its plant nutrient content and designed for use or claimed to have
18 value in promoting plant growth. "Fertilizer" does not include animal and vegetable
19 manures that are not manipulated, marl, lime, limestone or topsoil.

20 B. "Herbicide" means a substance or mixture of substances used to destroy,
21 desiccate, defoliate or prevent the growth of unwanted vegetation.

22 C. "Pesticide" means any substance or mixture of substances intended for preventing,
23 destroying, repelling or mitigating any pest and any substance or mixture of
24 substances intended for use as a plant regulator, defoliant or desiccant.

25 D. "Soil-amending ingredient" means any substance that is intended to improve the
26 chemical, biological or physical characteristics of the soil.

27 E. "Soil amendment" means any product consisting of a soil-amending ingredient
28 and other ingredients.

29 **2. Prohibition.** Notwithstanding any other provision of law, a person may not apply
30 a fertilizer, herbicide, pesticide, soil-amending ingredient or soil amendment within 25
31 feet of fresh surface waters, except that a person may apply a fertilizer, herbicide,
32 pesticide, soil-amending ingredient or soil amendment within 25 feet of fresh surface
33 waters for agricultural production from April 1st to October 15th on ground that is not
34 frozen.

35 **Sec. 4. 38 MRSA §444-B** is enacted to read:

36 **§444-B. Photographic record of shorelines to assist enforcement**

37 To aid in enforcing shoreland zoning ordinances, the following goals and
38 requirements relating to establishing a photographic record of the shorelines of great
39 ponds are established.

1 **1. State's goals.** The State's goals for establishing a photographic record of the
2 shorelines of great ponds are as follows.

3 A. By December 31, 2016, it is the goal of the State to have a photographic record of
4 the shorelines of 50% of great ponds bordered by at least 10 developed lots.

5 B. By December 31, 2018, it is the goal of the State to have a photographic record of
6 the shorelines of 70% of great ponds bordered by at least 10 developed lots.

7 C. By December 31, 2020, it is the goal of the State to have a photographic record of
8 the shorelines of 90% of great ponds bordered by at least 10 developed lots.

9 **2. Shoreline inventories.** The department, municipalities and the Maine Land Use
10 Planning Commission shall provide leadership in achieving the State's goals in subsection
11 1. To minimize costs, the department, municipalities and the Maine Land Use Planning
12 Commission shall work with lake associations, land trusts, community groups, colleges
13 and universities and volunteers to create photographic records of the shorelines of
14 developed great ponds and shall work to update the records in 2020 and every 5 years
15 thereafter.

16 **3. Priority great ponds.** The department shall develop and make available to the
17 public biennially a list of priority great ponds for developing a photographic record of the
18 shorelines of great ponds. Priority must be based on water quality conditions, density of
19 shoreline development, projections of future development and the absence of an existing
20 photographic record of the complete shoreline.

21 **4. Rules.** The department shall adopt rules to implement this section. Rules adopted
22 pursuant to this subsection are routine technical rules as defined in Title 5, chapter 375,
23 subchapter 2-A. By January 15, 2015, the department shall adopt rules requiring:

24 A. An applicant for a permit for development within a shoreland zone to provide to
25 the permitting authority a preconstruction photograph and a postconstruction
26 photograph of the shoreline vegetation and development site; and

27 B. A municipal permitting authority to visit a proposed development site prior to
28 final approval of a permit for development within a shoreland zone.

29 **Sec. 5. 38 MRSA §450** is enacted to read:

30 **§450. Training for municipalities**

31 The department and the Department of Agriculture, Conservation and Forestry shall
32 develop and make available to municipal officials and code enforcement officers training
33 relating to the provisions of this article including the importance of the law in protecting
34 the quality of surface waters of the State, changes in department rules relating to
35 shoreland zoning and municipal enforcement obligations. The training must be provided
36 in multiple locations in the State and may be provided in conjunction with other training
37 programs.

38 **Sec. 6. Landscape contractor certification program.** By December 1, 2015,
39 the Department of Environmental Protection shall develop an environmental leader

1 certification program for landscape contractors that provide landscape services to
2 properties adjacent to surface waters of the State. The certification program must focus
3 on low-maintenance landscape design and landscaping methods that are protective of
4 water quality.

5 **Sec. 7. Vacancies.** By December 31, 2014, the Department of Environmental
6 Protection shall hire qualified personnel for vacant staff positions that have been
7 authorized by the Legislature for the purpose of education, monitoring, research and
8 enforcement activities related to the protection of the health and integrity of the State's
9 lakes.

10 **Sec. 8. Reducing water quality impacts of camp roads, logging roads,**
11 **driveways and boat launches.** The Department of Environmental Protection shall
12 evaluate options and develop a strategy for reducing risks to the water quality of lakes of
13 the State from camp roads, logging roads, driveways and boat launches. In conducting
14 the evaluation, the department shall seek input from the Maine Land Use Planning
15 Commission, lake associations, municipalities, conservation organizations and other
16 stakeholders. By December 1, 2015, the department shall submit its recommendations to
17 the joint standing committee of the Legislature having jurisdiction over environmental
18 and natural resources matters, and the committee may report out a bill relating to the
19 recommendations to the Second Regular Session of the 127th Legislature.

20 **Sec. 9. Promoting voluntary certification for pollution reduction**
21 **measures by lakefront property owners.** By December 1, 2014, the Department of
22 Environmental Protection shall evaluate the status of the LakeSmart program, which was
23 transferred from the department to the Maine Lakes Society. The evaluation must include
24 the following information for a period beginning on the date management of the program
25 was transferred:

- 26 1. The number of property owners who received LakeSmart Awards;
- 27 2. The number of lake associations involved in helping promote the program;
- 28 3. The number of lakes for which property owners received LakeSmart Awards; and
- 29 4. Implementation challenges experienced by the Maine Lakes Society.

30 The evaluation must also include information relating to the financial sustainability of
31 the LakeSmart program. The department shall solicit information necessary for the
32 evaluation from the Maine Lakes Society and shall evaluate whether additional funding
33 or technical resources from the department would help ensure the success of the program.
34 The department shall make a report of its evaluation available for public comment. By
35 January 15, 2015, the department shall submit the report and public comments to the joint
36 standing committee of the Legislature having jurisdiction over environmental and natural
37 resources matters. The committee may report out a bill relating to the report to the First
38 Regular Session of the 127th Legislature.

1 **SUMMARY**

2 This bill amends the laws governing the Lakes Assessment and Protection Program.
3 It prohibits the application of fertilizers, herbicides, pesticides and soil amendments
4 within 25 feet of fresh surface waters. It establishes goals for developing a photographic
5 record of the shorelines of lakes. It directs the Department of Environmental Protection
6 and the Department of Agriculture, Conservation and Forestry to develop training for
7 municipalities relating to the laws regulating shoreland zoning. It also directs the
8 Department of Environmental Protection to:

- 9 1. Develop an environmental leader certification program for landscape contractors;
10 2. Fill vacant staff positions;
11 3. Evaluate options and develop a strategy for reducing risks to lake water quality
12 from camp roads, logging roads, driveways and boat launches; and
13 4. Evaluate the LakeSmart program.



PAUL R. LEPAGE
GOVERNOR

STATE OF MAINE
DEPARTMENT OF AGRICULTURE, CONSERVATION AND FORESTRY
BOARD OF PESTICIDES CONTROL
28 STATE HOUSE STATION
AUGUSTA, MAINE 04333-0028

WALTER E. WHITCOMB
COMMISSIONER

HENRY S. JENNINGS
DIRECTOR

February 11, 2014

Ryan Minzner
The Woodlands Club
39 Woods Road
Falmouth, Maine 04105

Re: 2014 Variance Permit

Dear Mr. Minzner:

This letter will serve as The Woodlands Club's Chapter 29 variance permit for your 2014 pest management program. Please bear in mind that this variance permit is dependent upon following the measures outlined in the variance application, particularly Section IX: Method to assure equivalent protection.

We will alert the Board at its February 21, 2014 meeting that the variance permit has been issued. If you have any questions concerning this matter, please feel free to contact me at 287-2731.

Sincerely,

Henry Jennings
Director
Maine Board of Pesticides Control



**CENTRAL MAINE
POWER**

January 3, 2014

Henry Jennings, Director
Maine Board of Pesticide Control
State House Station 28
Augusta, ME 04333

Dear Mr. Jennings:

Enclosed is a copy of Central Maine Power Company's Transmission Right-of-Way Vegetation Management Plan for 2014. If you have any questions, I can be reached at 621-3942.

Sincerely,

Nicholas Hahn
Vegetation Management



DRIFT MANAGEMENT PLAN FOR CENTRAL MAINE POWER TRANSMISSION LINE RIGHTS-OF-WAY

During the 2014 calendar year, Central Maine Power Company (CMP) will be treating approximately 10,000 acres as part of our regular vegetation management program. Some of this acreage is comprised of agricultural and industrial uses, and only needs to be patrolled. Integrated vegetation management techniques are employed on the remaining acreage to minimize the use of herbicides.

The first phase of the program requires that a contract crew patrol each right-of-way cutting all hardwood species over 8 feet tall and most of the softwood species. The stumps of trees capable of resprouting are treated with a herbicide. This reduces the amount of foliage that must be treated each cycle. Areas not suitable for foliar herbicide application during the summer are to be entirely cut at this time, and stump treatment to be used where appropriate.

The second phase of this year's program requires that the contract crew patrol each transmission line a second time, treating all remaining tree species capable of growing into the conductors or that block access to the right-of-way. The herbicides are applied with a backpack, hand pressurized spray tank. The tank pressure is low, so the potential for off target movement of the mix is minimized. A contract crew composed of 5 to 8 people will selectively treat the capable species.

A no spray zone is maintained around wells, municipal water supplies or any open water. The buffer zone will vary depending on the topography, a minimum of 25 feet is maintained on all water and a minimum 100-foot buffer is maintained on drinking water supplies. These buffers provide an additional margin of safety.

A low-pressure foliar application technique will be used on the majority of right-of-way scheduled this year. The herbicides and adjuvants, including a drift control agent, are mixed in water at rates of 1/8% - 5%. A hand-pressurized backpack sprayer is used to selectively apply the mix directly to the leaves of the undesirable species. The large droplet size, low tank pressure, and drift control agents, combined with the selective application technique, reduces the potential for drift to a very minimal level. The following is a list of herbicides CMP may use depending on species composition, density and environmental factors:

Garlon 4 Ultra	Arsenal Powerline	Milestone VM
Rodeo	Stalker	Krenite

Before a treatment technique or herbicide is selected, a review of the right-of-way is conducted including a list of landowner maintenance agreements, known municipal water supplies, and brush densities. This information helps CMP personnel select the herbicides and determine the mix rates.

A form is given to each crew foreman before the job starts listing all special arrangements, herbicides, and mix rates. All the work is performed by licensed contract

crews. The contract crews will post a sign on the first structure on each side of all public roads stating the date and herbicide used. If herbicides are not applied near the road crossing structure, the first structure where herbicides are used will be posted.

Each town that has a transmission right-of-way scheduled for herbicide work in 2014 will be notified in advance. A landowner maintenance agreement is available to any landowner or municipality objecting to the use of herbicides. The landowner agrees to keep brush to a height less than 10 feet and a CMP inspector looks over each area annually. CMP personnel will notify the staff of the Board of Pesticide Control at the start of the season of general work locations. Daily locations are available at CMP's General Office.

The following list identifies the CMP transmission section numbers and general locations for 2014 scheduled work. Plan and profile maps for each right-of-way are on file at the General Office in Augusta.

2014 CMP TRANSMISSION VEGETATION MANAGEMENT SCHEDULE

<u>Section</u>	<u>Location</u>
5	Detroit - Parkman
5A	Corinna
17	Rockport - Camden
21	Rockport - Rockland
21A	Rockport
41	Farmingdale - Lewiston
41A	Gardiner
49	Windsor - Rockport
60	Windsor - Farmingdale
62	Lewiston - Pownal
64	Lewiston - Pownal
66	Moscow - Detroit
67	Detroit - Windsor
67A	Benton
72	Lewiston
72A	Lewiston
82	Athens - Detroit
83	Moscow - Benton
83C	Skowhegan
84	Winslow - Windsor
85	Detroit - Parkman
105	Old Orchard Beach
106	Old Orchard Beach
201	Lewiston
203	Detroit - Bucksport
211	Rumford - Woodstock
212	Farmingdale - Lewiston Indian Stream Twp. -
222	Moscow
222A	Moscow
241	Benton - Oakland
241A	Benton - Oakland
241B	Cornville - Madison
242	Benton - Winslow
374	Buxton - Pownal
385	Sanford - Buxton
388	Windsor - Winterport
388BHE	Bucksport - Orrington
391	Sanford - Buxton

From: Jadczyk, Anthony M
Sent: Tuesday, February 11, 2014 2:03 PM
To: Jennings, Henry; Fish, Gary
Subject: FW: [MSBA Board:1250] petition to remove neonicotinoids from plants at Lowes and Home Depot

The latest neonic activities.

From: msba-board@googlegroups.com [<mailto:msba-board@googlegroups.com>] **On Behalf Of** Erin MacGregor-Forbes
Sent: Monday, February 10, 2014 9:07 PM
To: cbeekkeepers@googlegroups.com; york-county-beekeeper@googlegroups.com; maine-swarm@googlegroups.com; msba-board@googlegroups.com
Subject: [MSBA Board:1250] petition to remove neonicotinoids from plants at Lowes and Home Depot

Hello Beekeepers –

There is a printable valentine towards the bottom of this email that you can print and bring to your local home depot or Lowes, asking them not to sell plants that have been treated with neonicotinoids.

With the help of beekeepers around the country, this campaign could really make a difference in the quality of plant material that our friends, neighbors, and community members are buying, and planting where our colonies forage. Much of the plant material (if not all) that is purchased at these stores has been treated with systemic neonicotinoids which express themselves in the nectar and pollen of the plants, causing a number of problems when the nectar and pollen are brought back to the honey bee (or native bee) nest.

Please take a moment to read this email, communicate with our local retailer, and share with your friends.

Best to you and your bees,
-Erin

Friends,

Big news: On top of the 203,000 people nationwide who signed our petition telling Home Depot and Lowe's to stop selling bee-killing pesticides, hundreds of thousands of other people nationwide have signed similar petitions with other organizations. **In total, more than half a million people are now calling on the stores to take responsibility and stop selling neonic pesticides.**

Now its time to turn up the pressure on Lowe's and Home Depot. There are a number of petition delivery events in the next few days where activists will be delivering signatures and bee-themed valentines to local stores. If you live in or near one of the following cities, click the appropriate link below to RSVP for the petition delivery event. These are peaceful events so we ask that you're courteous to store employees, managers and shoppers if you choose to attend.

- [Eugene, Oregon: Saturday February 15 at 11 a.m.](#)
- [Emeryville, California: Wednesday, February 12 at 11 a.m.](#)
- [Washington, DC: Wednesday February 12 at 11 a.m.](#)
- [Minneapolis, MN: Wednesday February 12 at 11:15 a.m.](#)

You can also deliver a valentine to your local Home Depot or Lowe's. Click here to [download a printable valentine](#) to deliver, and [click here for instructions on how-to deliver it](#).

Or [click here to share the graphic below on Facebook](#).

Show Bees Some



TELL HOME DEPOT
AND LOWE'S TO
STOP SELLING BEE
KILLING PESTICIDES!

valentine.beeaction.org #beelove

Join the National Swarm February 10th-16th



In addition, we encourage everyone who plants their own pollinator-friendly plants from seeds, or purchases already potted plants, to use only organic or 'non-neonicotinoid treated' soils. This should assure that pollinators will not be assimilating 'neonics' through nectar or pollen. We thank you again for your continuing concern and involvement in the important work of saving the pollinators.

Thanks,
Philip Smith

P.S. You can also forward the petition to your friends and family with this link: <http://www.credomobilize.com/petitions/home-depot-and-lowes-you-must-stop-selling-bee-killing-pesticides>

You received this email because you signed the petition 'Home Depot and Lowe's: You must stop selling bee-killing pesticides!'. If you don't want to receive emails from the 'Home Depot and Lowe's: You must stop selling bee-killing pesticides!' campaign in the future, please [unsubscribe](#).

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You received this message because you are subscribed to the Google Groups "MSBA Board" group. To unsubscribe from this group and stop receiving emails from it, send an email to msba-board+unsubscribe@googlegroups.com.

To post to this group, send email to msba-board@googlegroups.com.

Visit this group at <http://groups.google.com/group/msba-board>.

For more options, visit https://groups.google.com/groups/opt_out.

DAS-81419-2 Soybean

Plant-incorporated protectant *Bacillus thuringiensis* subsp. *kurstaki* Cry1Ac protein and *Bacillus thuringiensis* subsp. *aizawai* Cry1F protein as produced in insect-protected soybean cells (OECD Unique Identifier: DAS-81419-2).

Active Ingredients:

Bacillus thuringiensis Cry1Ac protein and the genetic material (vector pDAB9582) necessary for its production in DAS-81419-2 soybean 0.000079 - 0.00014%*

Bacillus thuringiensis Cry1F protein and the genetic material (vector pDAB9582) necessary for its production in DAS-81419-2 soybean 0.001041 - 0.00169%*

Other Ingredient:

The marker protein, PAT (phosphinothricin *N*-acetyltransferase), and the genetic material (vector pDAB9582) necessary for its production in DAS-81419-2 soybean 0.000063 - 0.00011%*

*Maximum percent (wt/wt) of dry grain.

KEEP OUT OF REACH OF CHILDREN

CAUTION

NET CONTENTS _____

EPA Registration No. 68467-20

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 plant-incorporated protectant in a manner inconsistent with its labeling.

DAS-81419-2 Soybean was transformed to express *Bacillus thuringiensis* Cry1Ac and Cry1F insecticidal proteins. The insect-protected DAS-81419-2 Soybean may be used only for seed increase, breeding, research, and seed production in breeding nurseries and research stations as specified in the terms of this registration and on this label.

The insect-protected soybean may be grown on up to a total of 250,000 acres per year with no more than 20,000 acres per county (in non-cotton growing regions); 10,000 acres per county (in cotton-growing counties with at least 25,000 acres of soybean); or 1,000 acres per county (in cotton-growing counties with less than 25,000 acres of soybean) per year in the United States and the Commonwealth of Puerto Rico. Cotton growing regions are defined as follows: Alabama, Arkansas, Florida, Georgia, 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).

Equipment used for planting, harvesting, and handling of this insect-protected soybean must be thoroughly cleaned before further use. All plant propagation materials produced by Dow AgroSciences LLC and its cooperators that contain the insect-protected soybean must be securely stored for export, future planting, research, or use for additional plant propagation materials pursuant to the terms of this registration. Harvested seeds are not allowed for sale as commercial seed in the U.S.

Maine Board of Pesticides Control

**Miscellaneous Pesticides Articles
February 2014**

(identified by Google alerts or submitted by individuals)

BANGOR DAILY NEWS

Are there alien genes in your food?

By Nancy Oden, Special to the BDN

Posted Jan. 01, 2014, at 3:16 p.m.

“Alien genes” are genes from one species that are forced into a different species, creating a mutant life form that would never occur in nature. They’re called genetically modified organisms, or GMOs, and are created by chemical and pharmaceutical corporations.

They include, for example:

- The bacterium *Bacillus thuringiensis* spliced into [some potatoes and corn](#), causing the plants to produce a toxin to ward off insects;
- Fish genes into tomatoes (a product that [didn’t go on the market](#));
- Genes from antibiotics and pesticides inserted into corn, soy, canola, sugar beets — in your food today, unless you’re eating organic.

Creating GMOs is not selective breeding within one species. Selective breeding cross-pollinates, for example, one tomato with another, leading to a somewhat different tomato. This happens in nature all the time. It would never lead to a tomato with fish genes in it.

Forcing potentially toxic material into the plants humans and animals eat is not meant to “improve” the plants; it’s meant to sell more product — such as agriculture giant Monsanto’s Roundup Ready soybeans, alfalfa, corn, cotton, canola and sugar beets, which contain tolerance to Roundup herbicides.

Don’t wonder why the government allows these materials in your food: A former Monsanto executive [is heading up](#) the Food and Drug Administration. Surprised? Likely not.

When you eat GMO-containing food, you force your body to accept foreign, potentially toxic genes into every cell of your body as “food.” Since few studies of GMOs have been done on humans, the bio-tech corporations can say, “GMOs aren’t proven to harm humans.” But there are good reasons to wonder.

Monsanto, Bayer, Syngenta, Dow and DuPont tell people food would cost more if they had to label GMO products. Nonsense. We need to know whether our foods contain GMOs. If there’s nothing wrong with GMOs, why do they object so strenuously?

Truth: They’re afraid that if you know what’s in these GMO plants, you might not want to buy or eat them.

For instance:

- Pigs fed a GMO corn and soy diet [contracted severe stomach inflammation](#). Animals fed GMOs also have [shown](#) disrupted liver, pancreas, kidneys and testes function.
- [Residues of Roundup herbicide](#) have been shown to remain within and on the corn, soy, canola, sugar beets, etc., that are heavily sprayed during their growth period. It cannot be washed off, so you’re eating that herbicide in the GMOs.
- *Agrobacterium tumefaciens* and cauliflower mosaic virus are [commonly used to breach plants’ cellular walls](#). Mutations of these foreign genes are taking place within these GMO plants; is this also happening to people who eat them?

Unfortunately, the so-called GMO labeling bill, [LD 718](#), that passed the Legislature this year, and which Gov. Paul

LePage has said he'll sign, is a very weak bill. GMOs in Maine will not be labeled until other contiguous states decide to label.

The bill also excludes products from animals that have been fed GMOs — even though the GMOs the animals eat are in their cells. Thus, when you drink milk from GMO-fed cows, or eat butter, cheese or yogurt developed from these cows, you are eating the GMO from their bodies, too.

An honest label would tell you if the animals' flesh, eggs and milk are from GMO-fed animals.

But we can call it a beginning while we work toward a serious labeling bill, so people can know what's really in their food. Only then can we make good decisions about what to buy and feed our families.

Nancy Oden is an organic farmer who has lived in Washington County for 35 years. She may be reached at cleanearth@acadia.net.

<http://bangordailynews.com/2014/01/01/opinion/are-there-alien-genes-in-your-food/> printed on January 2, 2014

Oregon home gardeners could face pesticide restrictions under proposed legislation

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on January 01, 2014 at 6:00 AM, updated January 01, 2014 at 4:06 PM

A Portland lawmaker plans to introduce legislation in February that would effectively ban home gardeners from using some pesticides implicated in **mass bee die-offs last summer** in Wilsonville, Portland and other cities.

Rep. **Jeff Reardon**, D-Portland, is crafting legislation to add four pesticides to a restricted use list, which would effectively force home gardeners to hire professional pesticide applicators or use less effective alternatives.

The legislation is necessary to protect bees that provide crucial pollination for flowers and crops, said Reardon, a home gardener. Bee populations have also declined in recent years from colony collapse disorder, which **some scientists say is linked to pesticide usage**.

"These are dangerous chemicals," Reardon said. "People who aren't willing to take the time and effort to become fully educated, then they should look for alternatives."

Reardon seeks to restrict the use of dinotefuran, imidacloprid, clothianidin and thiamethoxam, which belong to a class of pesticides known as neonicotinoids. **The four pesticides are used in some Bayer, Ortho, and other garden products.** The pesticide Safari, which contains dinotefuran, was used in a **Wilsonville incident that killed 50,000 bees** last June after pesticide applicators **failed to follow label instructions**.

Environmental groups support the proposed legislation, but the bill's chances are unclear, particularly in the Senate, where environmental bills have faltered in the past year.

The bill will face opposition from groups like **Oregonians for Food & Shelter**. Scott Dahlman, the group's executive director, says there's no evidence that home gardeners' usage of neonicotinoids has caused mass bee deaths. The Wilsonville incident resulted from "blatant misapplications" from licensed pesticide applicators, he said.

"Pesticide regulation should be based on science," Dahlman said. "When we're not seeing a connection to a problem here, yet we want to restrict something, that raises a lot of red flags for us."

Neonicotinoids are also much safer for pesticide applicators than the alternatives, he said.

Gardeners like neonicotinoids because they're absorbed through the roots, protecting plants from the inside out, and can last for a year or more, said Jimmy Mack, a manager at **Portland Nursery**.

"It's a real easy application, and it lasts for a long time," he said. "That's why consumers love it, and growers, too."

More
Continuing coverage of the deaths of an estimated 50,000 bumblebees.

Neonicotinoids like imidacloprid are the most effective pesticides against some bugs, such as the **lace bug that has infected azalea and rhododendron shrubs** since it arrived in Oregon in 2009. Alternatives are less effective and would require early protection, Mack said.

"Consumers are going to be upset they're losing their plants," Mack said. "The onus becomes: How do you educate people on what to do next? It'd be tough, but I think we'd adjust."

The **Oregon Department of Agriculture** in November **announced additional education and testing requirements** for licensed pesticide applicators, but Reardon wants home gardeners to meet similar requirements before they can use the pesticides.

Reardon wants the state to develop an online course that home gardeners would need to take before passing a test to obtain a pesticide license -- requirements that most hobby gardeners would be loath to meet. Computerized pesticide applicator tests cost about \$58.

Oregon pesticide licenses are designed for commercial and agricultural uses, and the state currently doesn't require licenses for private backyard uses of neonicotinoids, said Dale Mitchell, manager of the pesticides program at the state Department of Agriculture.

The Washington State Department of Agriculture last year **declined a request to restrict neonicotinoids** and said there is no evidence that use of the pesticides is a significant contributor to the decline of bee colonies.

Meanwhile, the **European Union on Dec. 1 implemented a two-year ban** on three types of neonicotinoids, citing "high acute risks" to bees in certain circumstances. Authorized uses are restricted to professionals.

On a federal level, Congressman **Earl Blumenauer, D-Portland, has proposed legislation** to restrict four pesticides until the **Environmental Protection Agency finishes its ongoing review of all neonicotinoids**. Blumenauer acknowledged the **bill is unlikely to pass**.

-- Yuxing Zheng

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Posted: 1:40 PM
Updated: 2:30 PM

LePage signs Maine bill to label genetically modified food

Maine becomes second state to pass a law requiring food producers to label GMO food, but other states must follow before it goes into effect.

By [Steve Mistlersmistler@pressherald.com](mailto:Steve.Mistlersmistler@pressherald.com)
Staff Writer

Gov. Paul LePage has signed a bill that would require food producers to label foods that contain genetically modified ingredients. The law makes Maine the second state in the country to pass such a measure. However, other states must adopt similar legislation before Maine's labeling provision goes into effect.



click image to enlarge

A label on a snack item at a Portland supermarket indicates it is certified organic and does not contain any genetically modified ingredients.

2013 Staff File Photo/Gordon Chibroski/Staff Photographer

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The governor promised last year to sign the bill, which was sponsored by Rep. Lance Harvell, R-Farmington. His signature is symbolic because legislative rules don't allow the law to go into effect until the Legislature adjourns later this year. However, advocates of the bill hailed the law's eventual passage as a victory for advocates of laws to label genetically modified foods. Such proposals have been introduced in nearly 30 states as part of a national effort to compel Congress to enact a comprehensive labeling law.

Previous GMO labeling efforts have been staunchly opposed by agribusiness and the biotech food products industry, which have also spent millions to defeat ballot measures and state legislation.

Industry argues that labeling genetically engineered products unfairly stigmatizes modified foods despite a dearth of scientific research proving that they are any less healthful than those that are grown conventionally.

The U.S. Department of Agriculture estimates 70 percent of the products sold in American supermarkets contain genetically modified ingredients. The Food and Drug Administration regulates genetically modified foods, but regulators have left testing to the industry that is producing them.

Maine Conservation Voters' Executive Director Maureen Drouin said in a news statement that the new law "will give Maine people the information they need to make informed decisions about the food they and their families eat."

She added, "We thank Gov. LePage for recently signing the bill into law and thank Maine's Legislature for passing the bill with overwhelming support last year. We are thrilled that Gov. LePage has signed the GMO labeling bill," said Maine Organic Farmers and Gardeners Association Executive Director Ted Quaday. "MOFGA supporters have worked tirelessly, organizing five different legislative campaigns on this issue since the early 1990s. The time was right for a diverse and collaborative effort to take hold and move the discussion forward. People want and have the right to know what's in their food."

Still, the Maine GMO labeling law faces another challenge. The law doesn't go into effect unless five contiguous states, including New Hampshire, pass labeling laws. Late last year, the prospects of a New Hampshire law dimmed when a committee broke along party lines to oppose a labeling measure there.

The New Hampshire Legislature will take up the bill this winter.

The national battle over labeling laws has pitted activists in the organic food movement against a consortium led by the biotech industry and corporate food producers such as General Mills, Nestle USA and Monsanto.

It appeared that industry heavyweights were initially taken aback by activists who introduced labeling legislation this year in at least 30 states, according to the National Conference of State Legislatures. However, reports from New Hampshire indicate that the industry has rallied and become more effective.

The Maine bill brought together such factions as libertarian Republicans and liberal Democrats, creating strong support. So far, that hasn't happened in New Hampshire and the bill has become more partisan there.

The provision requiring passage in contiguous states was added to the Maine bill to help build broad support.

Proponents of the bill said the provision would quell concerns about an almost-certain lawsuit by industry groups and Monsanto, which vowed to challenge the laws in Maine and Connecticut on the basis that they violate the free speech and interstate commerce provisions of the U.S. Constitution.

Maine Attorney General Janet Mills told lawmakers last year that the bill was almost certain to face a legal challenge, and said she could not guarantee that her office could defend its constitutionality.

Already 64 countries around the world label foods that contain genetically modified ingredients, including all of Europe, Russia, China, Brazil, India and Saudi Arabia.

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Research for the Environment

» Press » Press releases » Press archive » Press releases 2013 » Biodegradable or not?

Press Release, 10th December 2013:

Biodegradable or not?

Scientists are developing classifications in order to better differentiate readily-biodegradable from long-lasting pesticides

Leipzig. In order to improve the evaluation process for the long-term consequences of pesticides, scientists have developed a new detection method and a model that can enable determinations regarding whether and how readily biodegradable the residues of pesticides are. The study, conducted by scientists at the Helmholtz-Centre for Environmental Research (UFZ), the Rhine-Westphalian Technical University Aachen (RWTH) and the Technical University of Denmark has recently appeared in the scientific journal "Critical Reviews in Environmental Science and Technology".



Photo: Kara-Fotolia.de

Pesticides have a bad reputation: they harm the environment, have negative effects on the diversity of species and pollute the soil. "This is partially correct, but also partially incorrect. Pesticides are important for the efficacy of our modern agriculture methods. And pesticides are not necessarily pesticides – differentiation is necessary in this context. Generally speaking, biodegradability is supposed to be the top priority when deploying pesticides", says Prof. Dr. Matthias Kästner, Director of the Department Environmental Biotechnology at the Helmholtz-

Centre for Environmental Research – UFZ in Leipzig.

Worldwide, today approximately 5,000 pesticides are utilized as substances for plant protection and for pest control. As varied as their respective effectiveness is, their effects on the environment are equally varied. Some pesticides are quickly biodegraded, while others take longer. And some of them create chemical bonds with components in the soil and form the so-called bound residues. One has always previously assumed that these residues were, per se, toxic. This is why pesticides that form more than 70% bound residues are no longer in compliance today. Kästner: "But what exactly is concealed behind these bound residues, i.e. whether or not they really are toxic or what chemical structures they have hidden, could not yet be evaluated."

By applying the so-called ¹³C-method, Kästner and his team applied pesticides onto various reference soils and examined them thoroughly regarding their fate. For this purpose, they initially marked the pesticide to be examined with the non-radioactive, heavy carbon [isotope](#) ¹³C – and tracked it in various bio-molecules with the aid of a mass spectrometer after completion of the experiment timeframe. In this manner the scientists were able to determine the residues, the changes in the pesticide, and its breakdown products in the soil.

The most significant result from the study states – there are various groups of bound residues. In the current issue of the technical journal "Critical Reviews in Environmental

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Science and Technology", the UFZ research scientists compile their results and introduce a classification system and a modelling approach for bound residues. As regards Type 1, the pesticide itself or its breakdown products of organic materials are deposited in the soil (humus) or trapped within, and can in principle be released at any time. If the pesticide has undergone a chemical bond with the humus, bound residues are allocated to the Type 2, which can only be released with difficulty. Residues from both Type 1 and Type 2 are to be categorised as toxicologically relevant. "At this juncture a precise examination must be carried out regarding whether or not approval of a pesticide that forms such residues in the soil is possible and defensible," says Matthias Kästner. As regards residues of the Type 3, the pesticide was decomposed by bacteria, and the carbon contained therein was transported into the microbial bio-mass. "For these kinds of residues, we can give the "all-clear" signal and confirm that there is no further risk", Kästner states. Pesticides, from which the bound residues in the soil are allocated to Type 3, could thus be approved without risk in the future. Conversely, pesticides, which heretofore were considered to be risk-free, could possibly be classified as critical using this method. Kästner says "Only when we are capable of differentiating between biodegradable and high-risk pesticide residues we can act accordingly. This is why we hope that the ¹³C-method will be included in the dossiers of the approval procedure in the future. This is what we suggested to the German Federal Environmental Agency as well."

The initial findings from the UFZ study have already been accepted into the assessment processes of the officials involved in the approval procedure. Thus, for the residues of the approved pesticides 2,4 dichlorophenoxyacetic acid (2,4-D for short) and 2 methyl 4 chlorphenoxyacetic acid (MCPA for short), they were able to give the all-clear. "In order to better control the deployment of pesticides and their environmental consequences, we still have a lot of work to do", says Kästner. "The problems that we had with DDT (dichlorodiphenyltrichloroethane) and atrazine must not be repeated. Therefore, it is very important to understand what actually happens with pesticides after application." *Nicole Silbermann*

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Publication:

Matthias Kästner, Karolina M. Nowak, Anja Miltner, Stefan Trapp, Andreas Schäffer (2013): Classification and modelling of non-extractable residue (NER) formation of [xenobiotics](#) in soil – a synthesis. Critical Reviews in Environmental Science and Technology. DOI: 10.1080/10643389.2013.828270

↳ <http://dx.doi.org/10.1080/10643389.2013.828270>

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Links

Risk Assessment and Environmental Safety Affected by Compound Bioavailability in Multiphase Environments (RAISEBIO):

→ <http://www.ufz.de/index.php?de=10757>

Molecular Approaches and MetaGenomic Investigations for optimizing Clean-up of PAH contaminated sites (MAGICPAH):

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At the **Helmholtz Centre for Environmental Research (UFZ)** scientists are interested in the wide-ranging causes and impacts of environmental change. They conduct research on water resources, [biodiversity](#), the impacts of [climate change](#) and adaptation strategies, environmental and biotechnologies, bioenergy, the behaviour of chemicals in the environment and their effects on health, modelling and sociological issues. Their guiding motto: our research serves the sustainable use of natural resources and helps towards long-term food and livelihood security in the face of global change. The UFZ has over 1,100 employees working in Leipzig, Halle und Magdeburg. It is funded by the federal government, as well as by the State of Saxony and Saxony Anhalt.

The **Helmholtz Association** contributes to finding solutions for large and pressing issues in society, science and the economy through excellence in the following six areas of research: energy, earth and the environment, health, key technologies, structure of matter, transport and aerospace. With almost 35,000 employees and coworkers in 18 research centres and an annual budget of approx. 3.8 billion Euros the Helmholtz Association is the largest scientific organization in Germany. Work is conducted in the tradition of the renowned natural scientist Hermann von Helmholtz (1821-1894).

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Beekeepers call for pesticide ban

North Bay Nipissing News

Editor's note: The following is an open letter to Ontario Premier Kathleen Wynne, a copy of which was shared with our publication.

Dear Premier Wynne,

In the next 60 days we expect both the Federal and Ontario governments to announce actions on bee health. The choice is clear: take real action and protect Ontario's bees from neonicotinoid pesticides or allow another year of irreversible damage to our bee population and our beekeepers.

Health Canada, for whom we rely on for protection against harmful pesticides, has confirmed the linkage between neonicotinoid pesticides used to treat corn and soybeans and extensive bee-kills in both 2012 and 2013. With more than four million acres planted in soy and corn in Ontario, we have a situation that beekeepers cannot avoid.

Pesticide lobbyists have tried to create a divide between grain farmers and beekeepers. All farmers aim to be good stewards of their land. No farmer wants to put beekeepers - their fellow farmers - out of business. But if the status quo continues, pesticide companies will continue to profit at the cost of destroying our bees and our livelihoods.

Independent study after study shows the immediate and lasting effects of these pesticides on pollinators, birds and on our soil and water. A thorough scientific review convinced the European Union to ban neonicotinoids this year. Health Canada, itself, has concluded that, "current agricultural practices related to the use of neonicotinoid treated corn and soy are unsustainable."

In the words of Dr. Peter Kevan, professor of biology at the University of Guelph, "We have a situation with neonicotinoids now which is an exact parallel with the previous situation with DDTs ... where there was broad scale use over the entire landscape, and where this poison was everywhere."

When announcing your Local Food Act this year, you proclaimed: "Supporting local food does so much for Ontario." Clearly, without pollinators it will be impossible to realize the economic and health benefits of local Ontario foods.

Ontario must lead Canada. The public good of Ontarians is at stake. The OBA, its supporters and the 65,000 Ontarians who signed our petition, urge you to suspend the use of neonicotinoid pesticides now until we can determine if, or how they can be used safely.

Dan Davidson President, Ontario Beekeepers' Associatio

Environmental Leader

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- EPA Funds Aim to Reduce Pesticides, Improve Bee Health

January 9, 2014

EPA Funds Aim to Reduce Pesticides, Improve Bee Health

The EPA has awarded almost half a million in funding to three universities — Louisiana State University, University of Vermont and Pennsylvania State University — for projects to reduce [pesticide](#) use and lower risks to [honeybees](#).

The [agricultural grants for Integrated Pest Management](#) (IPM) practices also aim to control pests and save money, the agency says.

The Louisiana State University (\$167,874) project aims to minimize impacts to bees from insecticides used in mosquito control. Practices and guidelines resulting from the project will be distributed to mosquito control districts and beekeepers throughout the US.

The University of Vermont project (\$131,758) aims to reduce pesticide use and improve pest control while increasing crop yields on 75 acres of hops in the Northeast. The awardees will also develop and distribute outreach materials to help farmers adopt these practices. The project's goal is to reduce herbicide and fungicide applications by 50 percent while decreasing downy mildew, a plant disease.

The Pennsylvania State University project (\$159,632) aims to protect bees and crops by reducing reliance on neonicotinoid pesticide seed treatments and exploring the benefits of growing crops without them. IPM in no-till grain fields will be used to control slugs and other pests that damage corn and soybeans. Researchers will share their findings with mid-Atlantic growers and agricultural professionals.

The EPA says protection of bee populations is among its top priorities. Some of the factors that contribute to the decline in pollinators include: loss of habitat, parasites and disease, genetics, poor nutrition and pesticide exposure. The agency is working with beekeepers, growers, pesticide manufacturers, the US Department of Agriculture and states to apply technologies to reduce pesticide exposure to bees.

Last month the Pollinator Stewardship Council, the National Honey Bee Advisory Board,

American Honey Producers Association, the American Beekeeping Federation and individual beekeepers sued the EPA over the agency's approval of sulfoxaflor, a [neonicotinoid pesticide made by Dow Chemical](#).

Monsanto, Bayer and Syngenta are among the [agrichemical companies funding honeybee research](#) in the US as scientists investigate pesticides as a potential cause of honeybee decline. Bayer and Syngenta, which produce neonicotinoids, a class of [pesticides linked to bee decline](#), are helping fund research at Iowa State University and Ohio State University to study the affect of insecticidal seed treatment dust on bee losses.

Monsanto, which uses the pesticides to coat its seeds, hosted a [June 2013 summit to discuss potential bee solutions](#) and says it's focusing its bee health research efforts on finding a way to control the varroa mite.

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Supreme Court hands Monsanto victory over farmers on GMO seed patents, ability to sue

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Reuters / Darren Hauck

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The US Supreme Court upheld biotech giant Monsanto's claims on genetically-engineered seed patents and the company's ability to sue farmers whose fields are inadvertently contaminated with Monsanto materials.

Tags Ecology, Food, GMO, Law, Monsanto, USA

The high court left intact Monday a federal appeals court [decision](#) that threw out a 2011 lawsuit from the Organic Seed Growers and Trade Association and over 80 other plaintiffs against Monsanto that sought to challenge the agrochemical company's aggressive claims on patents of

genetically-modified seeds. The suit also aimed to curb Monsanto from suing anyone whose field is contaminated by such seeds.

The group of plaintiffs, which included many individual American and Canadian family farmers, independent seed companies and agricultural organizations, were seeking preemptive protections against Monsanto's patents. The biotech leviathan has filed over 140 lawsuits against farmers for planting the company's genetically-engineered seeds without permission, while settling around 700 other cases without suing.

None of the plaintiffs are customers of Monsanto and none have licensing agreements with the company. The group argued that they do not want Monsanto's genetically-modified organisms (GMOs) and want legal protection in case of inadvertent contact with the company's products.

The appeals court decision was based on Monsanto's supposed promise not to sue farmers whose crops - including corn, soybeans, cotton, canola and others - contained traces of the company's biotechnology products.

In a June 2013 ruling, the US Court of Appeals for the Federal Circuit in Washington, DC said it was inevitable, as the farmers' argued, that contamination from Monsanto's products would occur. Yet the appeals panel also said the plaintiffs do not have standing to prohibit Monsanto from suing them should the company's genetic traits end up on their holdings *"because Monsanto has made binding assurances that it will not take legal action against growers whose crops might inadvertently contain traces of Monsanto biotech genes (because, for example, some transgenic seed or pollen blew onto the grower's land)."*

The panel's reference to *"traces"* of Monsanto's patented genes means farms that are affected by less than 1 percent.

The plaintiffs asked Monsanto to pledge not to sue, but the company rebuffed the request, saying, *"A blanket covenant not to sue any present or future member of petitioners' organizations would enable virtually anyone to commit intentional infringement."*

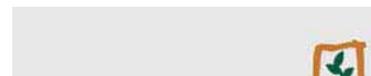
Monsanto's GMO seeds are designed to withstand the company's own ubiquitous herbicide, Roundup. Recently, questions have begun to arise from the bioengineered seed's resistance to pestilence, which has caused some farmers to increase their use of traditional pesticides.

"Monsanto never has and has committed it never will sue if our patented seed or traits are found in a farmer's field as a result of inadvertent means," said Kyle McClain, the Monsanto's chief litigation counsel, according to Reuters.

Vigsq q irhih



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Man creates brain-dead s system to cut your grocery bi WATCH

US farmers challenging Monsanto patent claims appeal to Supreme Court 25

"The lower courts agreed there was no controversy between the parties," McClain added, "and the Supreme Court's decision not to review the case brings closure on this matter."

Organic Seed Growers and Trade Association President Jim Gerritsen expressed disappointment that the Supreme Court reaffirmed the previous ruling, refusing to hear the case.

"The Supreme Court failed to grasp the extreme predicament family farmers find themselves in," said Gerritsen, an organic seed farmer in Maine. "The Court of Appeals agreed our case had merit. However ... safeguards they ordered are insufficient to protect our farms and our families."

In addition to Monday's news and the appeals court decision against them, the plaintiffs - many of them non-GMO farmers and who make up over 25 percent of North America's certified organic farmers - also lost a district court case.

"If Monsanto can patent seeds for financial gain, they should be forced to pay for contaminating a farmer's field, not be allowed to sue them," said Dave Murphy, founder and executive director of Food Democracy Now!, in a statement "Once again, America's farmers have been denied justice, while Monsanto's reign of intimidation is allowed to continue in rural America."

"Monsanto has effectively gotten away with stealing the world's seed heritage and abusing farmers for the flawed nature of their patented seed technology," said Murphy. "This is an outrage of historic proportions and will not stand."

The case is *Organic Seed Growers and Trade Association, et al., v. Monsanto Company, et al.* Supreme Court Case No. 13-303.

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free-world-logic 14.01.2014 15:07

It would be interesting if the critics of GMO's actually took the time to learn the BASICS of bio-chemistry and genetic engineering as well as what the scientific method is, the difference in correlation and causation is and started with a mind open to the possibility that scientific proof could alter



EPA's fast-track approval process for pesticides raises health concerns

Jan 15, 2014



[Katia Savchuk](#)

Reporting Intern

Topic(s):

- [Business and Technology](#)
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The EPA, headquartered in the William Jefferson Clinton Federal Building, says it has enough data on pesticide Nanosilva to know that it's safe while the manufacturer finishes testing.

Credit: [c_nilsen/Flickr](#)

Tiny particles of silver could appear soon in children's toys and clothing, embedded inside plastics and fabrics to fight stains and odors.

No one knows how the germ-killing particles, part of a new pesticide called Nanosilva, affect human health or the environment in the long run. But regulators have proposed letting Nanosilva on the market for up to four years before the manufacturer has to submit studies on whether the particles pose certain dangers.

That's because the U.S. Environmental Protection Agency has [backed approving Nanosilva](#) through conditional registration, a fast-track process that recently has drawn criticism for oversight problems. Unlike regular registration, it allows a pesticide to be sold before all required safety studies are in. In this case, manufacturer Nanosilva LLC can move ahead even though it hasn't explored fully the potential health risks if the product were to seep out of plastic or be inhaled.

Nanosilva's approval, which could be finalized early this year, has renewed focus on the loophole, designed mainly to help the EPA speed up approvals of pesticides nearly identical to those already being sold.

Recent reviews have found vast problems with the EPA's oversight of [conditional registration](#). An internal audit showed in 2011 that 70 percent of all active pesticides had been conditionally approved. The audit also concluded that the agency used the label too broadly. Since then, its use has increased. Figures the EPA provided in December put the number at 80 percent.

Thousands of pesticides kept conditional status for more than 20 years, the Natural Resources Defense Council, a nonprofit environmental advocacy group, found in 2010. The EPA says studies typically are due within four years.

And last year, federal auditors found the agency couldn't reliably track how many products were conditionally registered or whether safety studies were submitted. As a result, pesticides could linger on the market for years without critical tests, the [Government Accountability Office](#) warned in August.

These aren't new problems. At least seven independent reviews dating back to 1980 have noted flaws with the agency's systems for tracking pesticide registrations.

The EPA said it has enough data on Nanosilva to know that it's safe while the manufacturer finishes testing, as the law requires. But some scientists and environmentalists say the agency is taking a risk on products that are hardly essential, like sports clothing that doesn't stain or stink or toys that last longer.

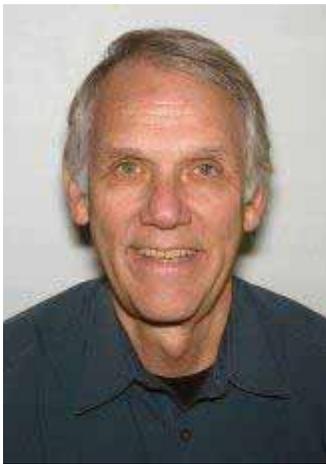
"You could allow some uses that are justified based on human well-being, such as medical implements, but to allow the possibility that nanosilver would be released in plastic on children's toys, and your kid could chew on it and ingest that material before we understand its toxicity – that's unnecessary risk," said Samuel Luoma, a research ecologist at the University of California, Davis. "It doesn't make any sense."

Conventional silver has been used as an antibacterial product for centuries. It releases ions that are deadly for many bacteria and fungi.

Recently, scientists have broken down silver into particles more than 1,000 times smaller than the width of a human hair – some not much wider than a DNA strand. They're called nanosilver. Nanosilva is just one brand that contains them.

Nanosilver can be embedded directly into plastics, fabrics and other materials. Companies say this helps products last longer. It also allows them to call items antibacterial and attract germ-conscious consumers. Nanosilver needs to be registered as a pesticide because it claims to kill bacteria and other live organisms.

Regular silver is highly toxic to fish and other aquatic life but isn't usually dangerous for humans. But scientists say nanosilver could pose unique hazards, and they know little about its long-term risks.



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Samuel Luoma, a research ecologist at the University of California, Davis, says the EPA is taking a risk by allowing nanosilver to be used in nonessential products before scientific testing is complete.

Credit: Courtesy of Samuel Luoma

Animal studies show that nanosilver can slip into cells and build up in the brain, heart and other organs. The EPA doesn't know whether nanosilver causes reproductive harm or cancer because there are no valid studies. Research on animals suggests that it can collect in the male reproductive system, potentially harming fertility, and may cause genetic mutations, which sometimes are linked to cancer.

Scientists have warned that nanosilver may be more toxic than regular silver and act as a carrier for other poisonous chemicals. Besides human health risks, researchers worry that nanosilver could kill fish and disrupt food chains if it makes its way into the environment.

The EPA argues that approving Nanosilva promotes innovation and lets consumers enjoy better products. The agency also says it didn't give the manufacturer enough time to do safety tests. The EPA didn't ask for those tests until nearly four years after an independent scientific advisory panel counseled the EPA on how it should evaluate nanosilver in 2009.

And, in evaluating Nanosilva, the EPA ignored some of that panel's advice.

The scientists told the agency to evaluate every nanosilver product separately. Just because one is safe doesn't mean others will be, they said. The agency instead figured out many health and environment risks based on studies on particles that were different from those in Nanosilva. The EPA said the tests were "scientifically appropriate."

Nanosilva officials couldn't be reached for comment.

This isn't the first time the EPA has conditionally approved pesticides containing nanosilver.

In November, a federal appeals court overturned the approval of two nanosilver products, ruling that the EPA had incorrectly found they posed no risks to toddlers. That decision didn't affect Nanosilva because the EPA used different calculations in each case.

Regulators still are grappling with how to deal with nanomaterials. While only two companies have asked for EPA approval, hundreds of products containing nanosilver already are on the market, according to an [inventory](#) by The Project on Emerging Nanotechnologies.

The EPA also has fast-tracked other controversial pesticides, including ones linked to the collapse of honeybee colonies and tree deaths.

All conditionally registered pesticides meet legal safety standards, the EPA said. The agency said it's taken steps to prevent staff from coding registrations incorrectly, which it said was the main reason numbers appeared high.

The EPA also has reviewed some conditionally approved products to look for missing data and other problems. But it hasn't traced the paper trail for all pesticides, as it told federal auditors it would do by last fall.

The EPA also doesn't have a concrete plan for the main fix auditors prescribed, an automated tracking system to guarantee that studies arrive and get reviewed. Currently, pesticide managers sometimes rely on handwritten notes and memory to keep track.

"Until they figure out the system, they shouldn't be using conditional registration," said Mae Wu, an attorney for the Natural Resources Defense Council.

The EPA first told federal auditors it would develop an automated tracking system more than 25 years ago.

This story was edited by Andrew Donohue and copy edited by Nikki Frick and Christine Lee.

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Scientists fit honey bees with tiny sensors to study decline of colonies

CSIRO-led team will use data they collect to investigate role of pesticides in collapse of 10m beehives worldwide

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Thousands of Australian honey bees have been fitted with tiny sensors in a study to help understand what is causing the precipitous collapse of colonies around the world.

About 5,000 bees will carry the 2.5mm x 2.5mm sensors, like hi-tech backpacks, for the next two months at the study site in Hobart.

The CSIRO-led research will build data on the movements and habits of several generations of bees to

shed light on the causes of colony collapse disorder, which causes the rapid loss of bees and has led to more than 10m beehives [being wiped out](#) worldwide in the past six years.

Australia is so far free of this phenomenon, as well as the deadly varroa mite, which has wreaked havoc on bee populations in almost every other country.

Researchers will place bees in a fridge set to 5C, which will send the insects to sleep. The sensors will then be carefully placed, under a microscope, onto the bees' backs before they are returned to the hive.

CSIRO will study four hives, each with about 50 tagged bees. Two of the hives will be provided a feeder with normal nectar and pollen while the other two will feed on nectar and pollen that contains a small amount of pesticide, which is thought to cause colony collapse.

Scientists will then be able to study the impact of pesticide on the bees' ability to complete their tasks and honey production. Bees are routine-based insects and any deviation will be observable.

CSIRO said the information would provide farmers and fruit growers with greater knowledge of bees. About a third of the food regularly eaten by humans requires pollination.

The results could also lead to government action on certain types of pesticides. Since 1 December farmers in the European Union [have been banned](#) from using three types of pesticide suspected of decimating bee populations.

"The sensors are basically a tag which lets us know how long the bees go for, how many follow them and so on," Paulo de Souza, lead scientist at CSIRO, told Guardian Australia. "We will monitor the hives for changes, such as whether the bees are slower to come back or go to other hives.

"This will be the largest study ever done of this kind, given that there will be 5,000 sensors. Two months is quite a long time to be studying them, too."

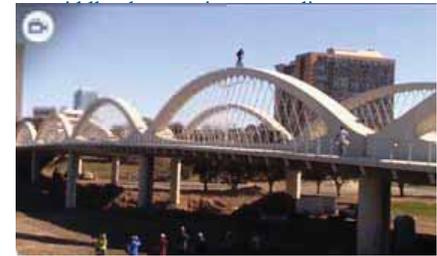
De Souza said that while Australian bees were in "pretty good shape" and are often exported overseas for pollination purposes, they still face looming threats.

"We don't know how long they will stay in this condition for – pesticides are one risk as well as monocultures of pollen that bees don't do well in," he said. "We are doing some things that might contribute to a future

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collapse, so it's important we study this area."

CSIRO will study other potential causes of colony collapse, such as hive management, after the pesticide research has finished.

It also plans to reduce the size of the sensors to fit onto flies, mosquitos and even smaller winged insects.

"We want to go smaller, maybe to 100 micrometres, although it can be hard to manipulate the sensors at that stage," de Souza said.

"The bees are amazing – they learn very quickly how to fly with the extra weight. They are very focused on their work. Fruit flies take a little longer."



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Health

3:30 AM MON JANUARY 13, 2014

Pitt Researchers Make Discovery About Deadly Mosquito-Borne Virus

By JESSICA NATH

You might not have ever heard of eastern equine encephalitis virus (EEEV) — it only affects five to 30 people in the United States annually — but it kills about half the people it infects, doesn't have a cure and is becoming more common.

That's according to William Klimstra, who, along with other University of Pittsburgh researchers, has made a major discovery that could lead to possible treatment for and prevention against the mosquito-borne virus.

The researchers have discovered that EEEV uses a never-before-documented mechanism to "hijack" one of its host's cellular regulatory systems in an effort to suppress immunity.

Unlike other viruses, which continually replicate themselves, EEEV restricts its own replication using a microRNA-binding site, which can fool the human body.

Klimstra said the technique prevents the immune system from detecting and attacking the virus, so it travels to the brain without warning.

"The advantage that the virus gains from this is that when it gets into the brain, the brain hasn't had any signal from the peripheral tissues that they are infected," Klimstra said. "So the brain is essentially unprotected when the virus gets there and that's why the disease is so severe."

The researchers created a mutant version of the virus without the microRNA-binding site and found that the host's -- in this case mice -- immune system was able to attack the mutant version.

The virus might be rare now, but Klimstra said that could not be the case for long.

"Typically the virus is maintained in a cycle between birds and a swamp mosquito that people aren't very commonly exposed to, but in the last few years it's been found more and more in mosquitos that inhabit urban environments and prefer to feed on people," Klimstra said. "So there's concerns from a number of different areas in terms of the potential for increased cases."

EEEV causes inflammation of the brain, resulting in the sudden onset of headache, high fever, chills and vomiting. It can progress quickly to disorientation, seizures and coma.

The virus has a 30 to 70 percent fatality rate, but those who do survive usually suffer substantial brain damage.

Klimstra said they hope this discovery will help researchers develop vaccines and treatments for the virus.

“The virus where we’ve deleted the binding sites for the microRNA, that virus is actually a natural vaccine vector because it’s naturally attenuated, it stimulates a much better immune response than the wild-type virus does, and something that we haven’t mentioned yet is that it is also incapable of infecting a mosquito,” Klimstra said.

He said this is important because it shows that the vaccine won’t spread from an immunized person to someone else.

He said there is also potential to create a treatment by purifying high levels of the microRNA and administering them to infected people to block virus replication.

TAGS: [University Of Pittsburgh \(/term/university-pittsburgh\)](/term/university-pittsburgh) [University of Pittsburgh Center for Vaccine Research \(/term/university-pittsburgh-center-vaccine-research\)](#)

Exposure to pesticides results in smaller worker bees



A honey bee robs a comb. Photo by Lynn Ketchum

Exposure to a widely used pesticide causes worker bumblebees to grow less and then hatch out at a smaller size, according to a new study by Royal Holloway University of London.

The research, published today in the *Journal of Applied Ecology*, reveals that prolonged exposure to a pyrethroid pesticide, which is used on flowering crops to prevent insect damage, reduces the size of individual bees produced by a colony.

The researchers, Gemma Baron, Dr Nigel Raine and Professor Mark Brown from the School of Biological Sciences at Royal Holloway worked with colonies of bumblebees in their laboratory and exposed half of them to the pesticide.

The scientists tracked how the bee colonies grew over a four month period, recording their size and weighing bees on micro-scales, as well as monitoring the number of queens and male bees produced by the colony.

"We already know that larger bumblebees are more effective at foraging. Our result, revealing that this pesticide causes bees to hatch out at a smaller size, is of concern as the size of workers produced in the field is likely to be a key component of colony success, with smaller bees being less efficient at collecting nectar and pollen from flowers," says researcher Gemma Baron from Royal Holloway.

The study is the first to examine the impact of pyrethroid pesticides across the entire lifecycle of bumblebees. The topical research is at the heart of a national Bee Health Conference running in London from Wednesday to Friday this week (22-24 January 2014).

Professor Mark Brown said: "Bumblebees are essential to our food chain so it's critical we understand how wild bees might be impacted by the chemicals we are putting into the environment. We know we have to protect plants from insect damage but we need to find a balance and ensure we are not harming our bees in the process."

Given the current EU moratorium on the use of three neonicotinoid pesticides, the use of other classes of pesticide, including pyrethroids, is likely to increase.

Dr Nigel Raine, who is an Invited Speaker at this week's bee conference, said: "Our work provides a significant step forward in understanding the detrimental impact of pesticides other than neonicotinoids on wild bees. Further studies using colonies placed in the field are essential to understand the full impacts, and

conducting such studies needs to be a priority for scientists and governments."

Provided by Royal Holloway, University of London

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NEWS

Genetically modified mosquito plans draw opposition

Posted by TERRENCE SMITH on Fri, Jan 17, 2014 at 3:51 PM

Genetically modified organisms were the topic Thursday night at the University of South Florida's Oval Theatre, but this time the focus wasn't only on what we eat.



Jeffrey M. Smith

The biotech company Oxitec has been pushing to release

genetically modified mosquitoes in the Florida Keys, as part of an experiment aimed at curbing the spread of Dengue Fever. The hope is that the genetically modified mosquitoes, which would be sterilized males, breed and eventually kill off the species. In response, the GMO Free Florida, Food and Water Watch, and the Florida Keys Environmental Coalition have organized a statewide tour intended to inform the public and voice opposition.

Tampa served as the tour's first stop, drawing more than 100 people. The presentation, which was organized locally by Going Green Tampa, featured Dr. Carlos Garcia and prominent consumer advocate Jeffrey M. Smith, author of the worldwide bestseller *Seeds of Deception*.

Smith devoted the first half of his hour-long speech to the risks of consuming GMOs and familiar foe Monsanto, but pulled no punches in his criticisms of Oxitec's plan, citing the potential for risks and a lack of transparency by the company on the scientific side.

"The idea is to create mosquitoes, to release the males which don't bite, then they create sterile offspring. So you reduce the population of the type of mosquito that may carry Dengue Fever. They didn't tell us that actually 3% are not sterile. Millions will end up in the environment forever. And sometimes it's not actually the males, there's some female

that get in there too, but it's okay, they've done it before. And they didn't actually reduce Dengue Fever, but it's in the plans. Their numbers keep changing and most of it's hidden. ... The technology is leaky, i've talked to one of the top insect GMO scientists from Florida recently. She created the first genetically modified trial of insects in a lab and she had to talk to all these different agencies. They all showed up. And she said this is what we want. In science, we want transparency, thoroughness, care. What we're seeing with Oxitec and the FDA is hidden information and shoddy science."

Oxitec has previously released these mosquitoes in both the Cayman Islands and Malaysia in an attempt to curb Dengue Fever, which according to Smith prompted local outrage and ignores a potential solution that was actually found in Florida.

"In Malaysia where they released the genetically engineered mosquito, the people were very angry, but in another location they released a predatory mosquito that kills the type of mosquito that carries Dengue Fever. It was so successful, no outbreaks of Dengue Fever occurred afterwards, the community wanted more. Do you know where this was developed? In Florida! That's where this predatory mosquito is from. You can do this whole thing without genetically engineering anything new, without introducing a new mosquito that's never before been on this planet."

Smith even pointed out a possible risk to Florida's tourist economy, with even a rumor of "Frankenbugs" spreading diseases turning the state into a no-go area for international visitors.

"Imagine if someone was bit by a vampire mosquito in the keys and gets sick from something and dies. Imagine the person who thinks it's a genetically modified mosquito, whether it is or isn't, and imagine some paper of standing does a story about Frankenbugs in South Florida. Imagine if it gets picked up at a time of great anti-GMO sentiment or someone with a problem with GMOs dropped it somewhere else. Think about the risk to Florida tourism off a story. There was a rumor that genetically modified papaya was stolen from a field trial in Thailand and Europe cancelled all orders of papaya in Thailand. Imagine the risk that's being taken in Florida, so that a company can experiment on a population of mosquitos and Floridians."

Smith advised all in the audience to get in contact with the governor and tell him the idea was stupid. He ended his speech on a hopeful note, encouraging the audience and looking back on how far the anti-GMO movement has come. Major strides have been made recently, with both Cheerios and Post's Grape Nuts going GMO free.

"This is an amazing time, and we may take this information and feel burdened by it, but I'm going to give you another angle. Who else in history, what other generation, has had the opportunity to protect everyone who eats and all living beings and all future generations? This is unprecedented. This is more power to do good than our ancestors ever had in the history of the human race. ... It turns out this window is the most important window of opportunity in the history of GMO activism. In the history of any GMO opportunity, and because the GMO words are buzzing around the internet, it's happening. We're getting coverage for the first time. The other side is pulling out all the stops. They're trying to discredit me like crazy, so it must be working. This is the time now where a little energy and a little attention has such a leveraged, powerful response. ... But please, let us take this time together, on behalf of all living beings, that we can support and celebrate the nature of nature for all living beings and together we can claim a non-GMO food supply for all living beings and all future generations will celebrate us."

The tour continues, with a stop next week in Coconut Creek before ending in Key West.

Tags: [GMOs](#), [Oxitec](#), [Mosquitos](#), [Jeffrey M. Smith](#), [Image](#)

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Thank you for bringing awareness on these important issues so that we may make



informed consumer choices - At least with food! As-far-as these mosquitoes are concerned, if they get released, how does one avoid being bitten? What if you have a reaction? There are too many unanswered questions if we rush ahead with this experiment.

Thanks for covering some of the most important issues facing our State.

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Systemic Spread and Propagation of a Plant-Pathogenic Virus in European Honeybees, *Apis mellifera*

Ji Lian Li, R. Scott Cornman, Jay D. Evans, et al.
2014. Systemic Spread and Propagation of a Plant-Pathogenic Virus in European Honeybees, *Apis mellifera*. mBio 5(1): .
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Systemic Spread and Propagation of a Plant-Pathogenic Virus in European Honeybees, *Apis mellifera*

Ji Lian Li,^a R. Scott Cornman,^b Jay D. Evans,^b Jeffery S. Pettis,^b Yan Zhao,^c Charles Murphy,^d Wen Jun Peng,^a Jie Wu,^a Michele Hamilton,^b Humberto F. Boncristiani Jr.,^e Liang Zhou,^f John Hammond,^g Yan Ping Chen^b

Key Laboratory of Pollinating Insect Biology of the Ministry of Agriculture, Institute of Apicultural Research, Chinese Academy of Agricultural Science, Beijing, China^a; Department of Agriculture, ARS, Bee Research Laboratory, Beltsville, Maryland, USA^b; Department of Agriculture, ARS Molecular Plant Pathology Laboratory, Beltsville, Maryland, USA^c; Department of Agriculture, ARS, Soybean Genomic & Improvement Laboratory, Beltsville, Maryland, USA^d; Department of Biology, University North Carolina at Greensboro, Greensboro, North Carolina, USA^e; Department of Pediatrics, Emory University School of Medicine, Atlanta, Georgia, USA^f; Department of Agriculture, ARS, Floral and Nursery Plants Research Unit, Beltsville, Maryland, USA^g

ABSTRACT Emerging and reemerging diseases that result from pathogen host shifts are a threat to the health of humans and their domesticates. RNA viruses have extremely high mutation rates and thus represent a significant source of these infectious diseases. In the present study, we showed that a plant-pathogenic RNA virus, tobacco ringspot virus (TRSV), could replicate and produce virions in honeybees, *Apis mellifera*, resulting in infections that were found throughout the entire body. Additionally, we showed that TRSV-infected individuals were continually present in some monitored colonies. While intracellular life cycle, species-level genetic variation, and pathogenesis of the virus in honeybee hosts remain to be determined, the increasing prevalence of TRSV in conjunction with other bee viruses from spring toward winter in infected colonies was associated with gradual decline of host populations and winter colony collapse, suggesting the negative impact of the virus on colony survival. Furthermore, we showed that TRSV was also found in ectoparasitic *Varroa* mites that feed on bee hemolymph, but in those instances the virus was restricted to the gastric cecum of *Varroa* mites, suggesting that *Varroa* mites may facilitate the spread of TRSV in bees but do not experience systemic invasion. Finally, our phylogenetic analysis revealed that TRSV isolates from bees, bee pollen, and *Varroa* mites clustered together, forming a monophyletic clade. The tree topology indicated that the TRSVs from arthropod hosts shared a common ancestor with those from plant hosts and subsequently evolved as a distinct lineage after transkingdom host alteration. This study represents a unique example of viruses with host ranges spanning both the plant and animal kingdoms.

IMPORTANCE Pathogen host shifts represent a major source of new infectious diseases. Here we provide evidence that a pollen-borne plant virus, tobacco ringspot virus (TRSV), also replicates in honeybees and that the virus systemically invades and replicates in different body parts. In addition, the virus was detected inside the body of parasitic *Varroa* mites, which consume bee hemolymph, suggesting that *Varroa* mites may play a role in facilitating the spread of the virus in bee colonies. This study represents the first evidence that honeybees exposed to virus-contaminated pollen could also be infected and raises awareness of potential risks of new viral disease emergence due to host shift events. About 5% of known plant viruses are pollen transmitted, and these are potential sources of future host-jumping viruses. The findings from this study showcase the need for increased surveillance for potential host-jumping events as an integrated part of insect pollinator management programs.

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Editor Anne Vidaver, University of Nebraska

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The European honeybee (*Apis mellifera*) provides pollination services to 90 commercial crops worldwide. In the United States alone, honeybee pollination is valued at \$14.6 billion annually (1). However, over the past several decades, there has been much concern throughout the world over the steep decline in populations of honeybees (2). Colony collapse disorder (CCD), a mysterious malady that abruptly wiped out entire hives of honeybees across the United States, was first reported in 2006 (3, 4) and has since spread around the world (5), exacerbating the already dire situation for honeybees. RNA viruses, alone or in conjunction with other pathogens, have frequently been implicated in colony losses (3, 6, 7).

Previous studies have shown that viruses that cause common infections in managed honeybees, *A. mellifera*, also infect other hymenopteran pollinators, including the bumblebee, which has also been declining worldwide (8–11). A study conducted by Singh et al. (11) reported that deformed wing virus (DWV), sacbrood virus (SBV), and black queen cell virus (BQCV), which are common in *A. mellifera*, were detected in eleven species of native bees and wasps as well as in pollen pellets collected directly from healthy foraging bees. Furthermore, the study by Singh et al. (11) showed that viruses in the pollen were infective, as illustrated by the fact that queens became infected and laid infected eggs after

virus-negative colonies consumed virus-contaminated foods. This discovery raised concerns about a possible role of pollen in spreading viruses and suggested that viruses could possibly contribute to the observed pollinator decline around the world. In order to advance our understanding of the role of pollen in virus transmission of honeybees, we carried out a study to screen bees and pollen loads of bee colonies for the presence of frequent and rare viruses. Our study resulted in the serendipitous detection of a plant virus, tobacco ringspot virus (TRSV), in honeybees and prompted us to investigate whether this plant-infecting virus could cause systemic infection in exposed honeybees.

Generally, the majority of plant viruses are dependent upon herbivorous insects for their spread from one host plant to another in nature but cause infection only in plants that the insect vectors feed upon. To date, only a few plant viruses are known that also infect their insect vectors. *Rhabdoviridae*, a family of arboviruses carried by arthropods, has long been recognized to have a broad range of hosts throughout the animal and plant kingdoms (12). Flock house virus (FHV), a positive-stranded RNA virus of insect origin belonging to the family *Nodaviridae*, has been shown to replicate in plants as well as in yeast (*Saccharomyces cerevisiae*) and mammalian cells (13, 14). A recent study (15) showed that a plant-pathogenic virus, tomato spotted wilt virus (TSWV), which is a member of the family *Bunyaviridae*, could directly alter the behavior of thrips that vector it. The phenomenon of viral host range spanning the plant and animal kingdoms adds an additional layer to the already complex plant-pathogen-pollinator interactions and could have important epidemiological consequences.

TRSV is a type species of the genus *Nepovirus* within the family *Secoviridae* (16). TRSV infects a wide range of herbaceous crops and woody plants, some of considerable economic importance. The infected plants show discoloration, malformation, and stunted growth, accompanied by reduced seed yield or almost total seed loss due to flower and pod abortion. Of a number of plant diseases caused by TRSV, bud blight disease of soybean (*Glycine max* L.) is the most severe. It is characterized by necrotic ring spots on the foliage, curving of the terminal bud, and rapid wilting and eventual death of the entire plant, resulting in a yield loss of 25 to 100% (17). Like other members of the genus, TRSV has a bipartite genome of positive-sense, single-stranded polyadenylated RNA molecules, RNA-1 and RNA-2, which are encapsidated in separate virions of similar size. Both RNA molecules possess a genome-linked protein (Vpg) covalently bound at their 5' ends. RNA-1 encodes a large polyprotein precursor that is proteolytically processed into protease cofactor (P1A), putative ATP-dependent helicase (Hel), picornain 3C-like protease (Pro), and RNA-directed RNA polymerase (Pol). RNA2 encodes a virion capsid protein (CP), a putative movement protein (MP), and an N-terminal domain involved in RNA-2 replication (P2A). Proteins encoded by RNA-1 are required for RNA replication, while proteins encoded by RNA-2 function in cell-to-cell movement and viral RNA encapsulation. RNA-1 is capable of replication independently of RNA-2, but both are required for systemic infection. Transmission of TRSV can occur in several ways. The numerous vectors include a dagger nematode (18), aphids, thrips, grasshoppers, and tobacco flea beetle (19–21); however, vertical transmission through seeds is important for long-distance dispersal of the virus (22). It has also been shown that honeybees transmit TRSV when they move between flowers and transfer virus-borne pollen from infected plants to healthy ones (23–26). It was,

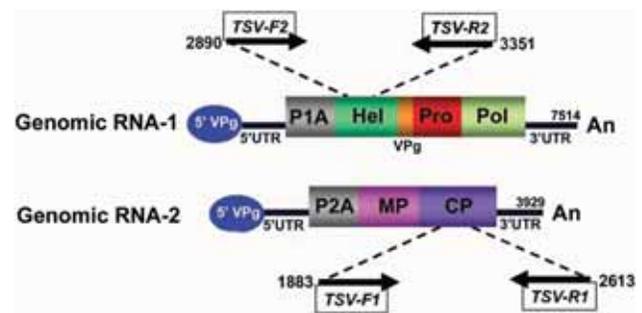


FIG 1 Schematic diagram showing the genome organization of TRSV and locations of primer sets used for virus distribution and replication studies. Open reading frames encoding proteins are boxed and labeled. Positions of primers utilized for amplification of the flanks are marked by black arrows for both RNA segments.

however, unknown prior to our study whether honeybees could become infected by plant viruses they physically encounter or consume.

In the present study, we provide evidence that TRSV is present in honeybees and the infection can be widespread through the body of honeybees. TRSV in honeybees does not fit a circulative-propagative model of insect-vectored plant viruses, in which virions are ingested by an insect vector, replicate, and disperse to salivary glands for reinfection of the plant host. Instead, our data indicate that the replication of TRSV occurs widely in the honeybee body but not in the gut or salivary gland and that TRSV in conjunction with other bee viruses is correlated with winter colony level declines. Further, virus was found in a common ectoparasite mite of honeybees, *Varroa destructor*, but was restricted to the gastric cecum. This study presents a unique example of viruses that cause infection in both plants and animals.

RESULTS

Sequence identity of TRSV genomic segments and morphology of the virus isolates. Sequence analysis of cDNA libraries from purified virus preparation revealed overlapping and nonoverlapping clones of different lengths. About 75% of the clones ($n = 40$) matched the genome sequences of common honeybee viruses, including BQCV, DWV, and Israeli acute paralysis virus (IAPV). Unexpectedly, about 20% of the clones ($n = 10$) matched the sequences of TRSV for two genomic segments in the NCBI database. By assembling sequence fragments from different cDNA clones, we obtained a 1,545-bp length of nucleotide sequences encoding the RNA helicase and covering ~21% of the coding region of the polyprotein gene of genomic RNA-1. We also obtained a 2,024-bp long sequence encoding the complete capsid protein. A BLAST search of the helicase sequence showed highest identity with a TRSV strain isolated from bud blight disease of soybean (GenBank accession no. U50869), with 88% homology at the nucleotide level and 96% homology at the amino acid level. A BLAST search of the DNA fragment encoding the capsid protein showed strongest similarity to a TRSV strain from bean (GenBank accession no. L09205), with 96% homology at the nucleotide level and 99% homology at the amino acid level. The cDNA sequences were used to design two primer sets, TRSV-F1/R1 and TRSV-F2/R2 (Fig. 1), for the subsequent studies of TRSV replication and distribution in honeybees and *Varroa* mites.

Electron microscopy showed no obvious contamination from

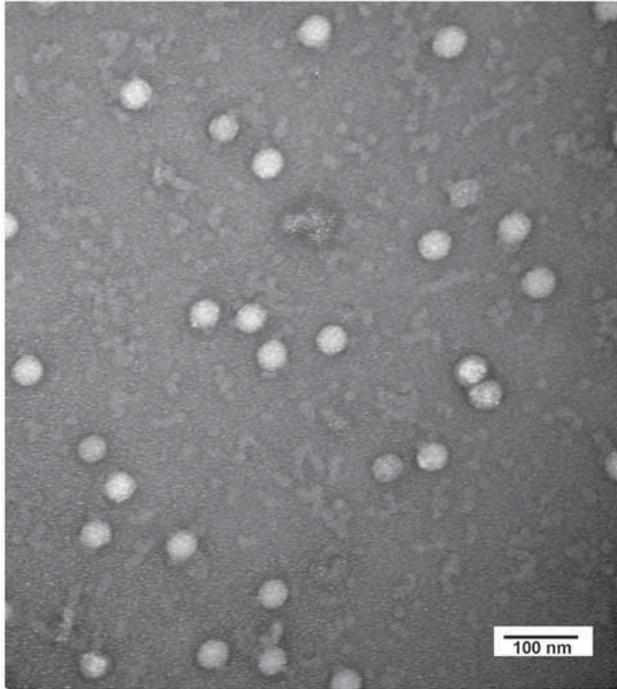


FIG 2 Electron microscopy of TRSV particles from infected honeybees. The presence of TRSV particles in viral preparation was confirmed by RT-PCR assay. Bar, 100 nm.

host cellular material. Negatively stained viral particles had a diameter of 25 to 30 nm and an icosahedral shape, typical morphological features of secoviruses (Fig. 2), and RT-PCR assay confirmed the presence of TRSV in the viral preparation for EM analysis.

The purity of the virus preparation in our study was confirmed by electron microscopy. Electron microscopy showed no obvious contamination from host cellular material. Negatively stained viral particles had a diameter of 25 to 30 nm and an icosahedral shape, typical morphological features of secoviruses (Fig. 2). However, the viral preparation was determined by RT-PCR to contain not only TRSV but other bee viruses as well, including BQCV, DWV, and IAPV. It was not possible to definitely distinguish TRSV viral particles morphologically from these other bee viruses.

Distribution and replication of TRSV in infected honeybees.

Although no apparent disease symptoms were observed in examined bees, TRSV was widespread in honeybee tissues, which was confirmed by the amplification of a 731-bp PCR fragment with the TRSV-F2/R2 primer set. Except for the compound eyes, TRSV was found in all tissues examined, including hemolymph, wings, legs, antennae, brain, fat bodies, salivary gland, gut, nerves, tracheae, and hypopharyngeal gland. Although there was the same amount of input cDNA, the intensity of the PCR signals varied between samples. Tissues of the gut and muscle had weaker PCR bands than other tissues, indicating a relatively lower level of TRSV infection (Fig. 3). It is unclear if the absence of PCR amplification in the compound eye was due to PCR inhibition previously reported for that tissue (27).

TRSV is a positive-stranded RNA virus replicating through the production of a negative-stranded intermediate; therefore, the

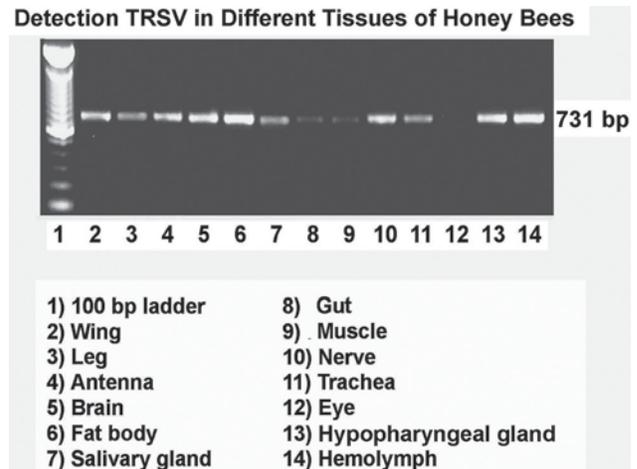


FIG 3 Detection of tobacco ringspot virus (TRSV) in different tissues of honeybees by conventional RT-PCR. The 731-bp bands on the right side of the gels indicate the presence of positive signal for TRSV.

presence of negative-stranded RNA constitutes proof of active viral replication. To investigate the replication of TRSV in bees, negative-stranded RT-qPCR was performed using a tagged primer system (28). Amplification and sequence analysis of a 462-bp negative-strand-specific product in different tissues showed that active replication of TRSV occurs in most tissues (Fig. 4). A single peak on the melting curve analysis corroborated the specificity of the amplicon. The lack of amplification following RT-qPCR of total RNA without primers in the reverse transcription reaction mixture ruled out any nonspecific effect from self priming due to the secondary structure of viral RNA or false priming by antigenomic viral RNA or cellular RNAs. Among tissues with detectable levels, the relative abundance of negative-stranded TRSV varied significantly ($P < 0.001$; one-way analysis of variance [ANOVA]). The brain had the lowest detectable level of negative-stranded TRSV and was chosen as the calibrator. The abundance of TRSV in other tissues relative to the brain ranged from 56-fold to 957-fold. The concentration of TRSV in additional body tissues showed the following ranking: muscle > hypopharyngeal gland > leg > fat body > trachea > hemolymph > antenna > nerve > wing. The replication of TRSV was not evident in the salivary gland, gut or compound eye (Fig. 5), although the presence of PCR inhibitors in the latter is a possibility (27).

Localization of TRSV in the ectoparasitic *Varroa* mite of honeybees. *In situ* hybridization showed that TRSV could also be detected in the ectoparasitic mite, *V. destructor*, collected from the same TRSV-infected bee colonies. Sections hybridized with a digoxigenin (DIG)-labeled TRSV RNA probe had strong staining within the storage organs of the mite, the upper and lower gastric ceca (Fig. 6A), although histopathological signs were not evident in these areas. No positive signal of TRSV was observed in other mite tissues, and no signal was observed with the negative-control probe (Fig. 6B).

Prevalence of TRSV infection in honeybee colonies. Of ten bee colonies included in this study, six were classified as described in Materials and Methods as strong colonies and four were classified as weak colonies. Both TRSV and IAPV were absent in bees from strong colonies in any month, but both were found in bees from weak colonies. As with other detected viruses, TRSV showed

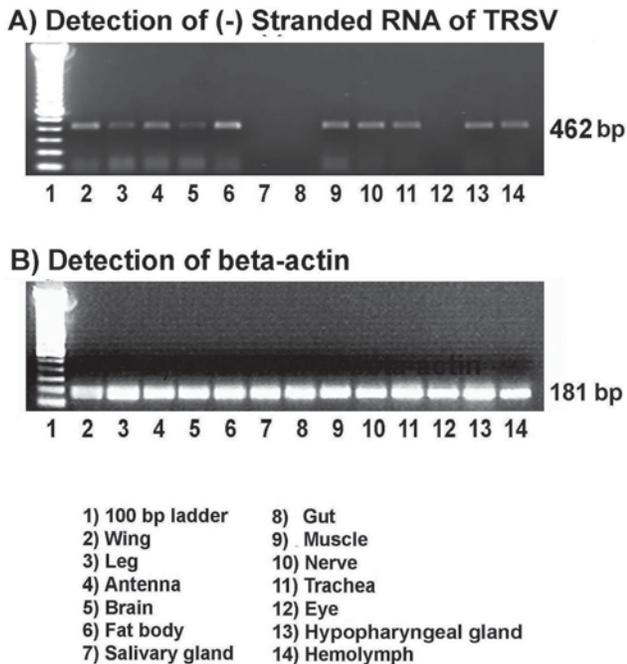


FIG 4 Detection of negative-stranded RNA of TRSV and housekeeping gene for β -actin in different tissues of honeybees by strand-specific RT-qPCR. The 462-bp bands on the right side of the gels indicate the presence of a positive signal for negative-stranded RNA of TRSV. The similar signal intensity of β -actin indicates the same amount of starting material in each tissue sample.

a significant seasonality. The infection rate of TRSV increased from spring (7%) to summer (16.3%) and autumn (18.3%) and peaked in winter (22.5%) before colony collapse. Of viruses detected in weak colonies, DWV was the most commonly detected, with an average annual infection rate of 44%, followed by BQCV, IAPV, and TRSV. Additionally, a low incidence of SBV and chronic bee paralysis virus (CBPV) infections was also detected in bees from weak colonies. While DWV and BQCV were detected in both healthy and weak colonies all year round, the prevalence of DWV and BQCV in weak colonies was significantly higher than that in strong colonies. The bee populations in weak colonies that had a high level of multiple virus infections began falling rapidly in late fall. All colonies that were classified as strong in this study survived through the cold winter months, while weak colonies perished before February. In Fig. 7A and B, the seasonal prevalence of TRSV along with other bee viruses in both weak and strong colonies is presented.

Phylogenetic characterization of TRSV isolates. Figure 8 illustrates the phylogenetic relationship among our TRSV isolates and viruses with existing GenBank TRSV sequence records, based on the partial capsid protein sequence amplified with primers. TRSV isolates infecting plants constitute the early lineages of the phylogenetic tree, and TRSV isolates from honeybees, bee pollen, and *Varroa* mites clustered together, branching next from the early lineage. There is no obvious sequence divergence among TRSV isolates from bees, mites, and bee pollen.

DISCUSSION

Among major pathogen groups, RNA viruses have the highest rate of mutation, because the virus-encoded RNA polymerases lack

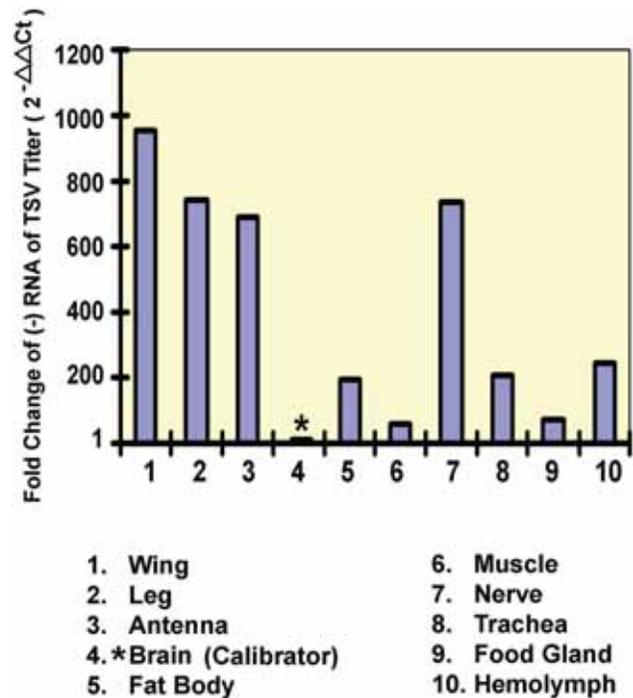
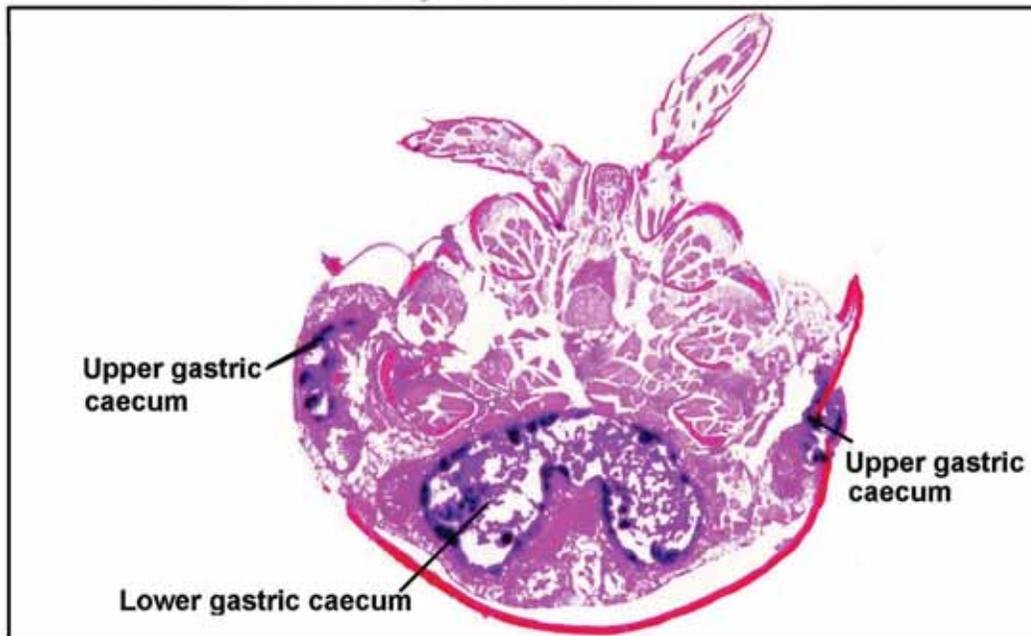


FIG 5 Relative abundance of negative-stranded RNA of TRSV in different tissues of honeybees. Brain tissue had the minimal level of TRSV and therefore was chosen as a calibrator. The concentration of negative-stranded RNA of TRSV in other tissues was compared with the calibrator and expressed as n -fold change. The y axis depicts fold change relative to the calibrator.

3'→5' exonuclease proofreading activity (29). The consequence of such high mutation rates is that populations of RNA viruses exist as "quasispecies," clouds of genetically related variants that might work cooperatively to determine pathological characteristics of the population (30). These sources of genetic diversity coupled with large population sizes facilitate the adaptation of RNA viruses to new selective conditions, such as those imposed by a novel host. RNA viruses therefore are the most likely source of emerging and reemerging infectious diseases, such as human immunodeficiency virus (HIV), severe acute respiratory syndrome (SARS), type A avian influenza A (H5N1), and swine origin influenza A (H1N1), that have engendered worldwide public health concern because of their invasiveness and ability to spread among different species (31–35).

Honeybees carry a strong electrostatic charge that ensures the adherence of pollen to their bodies, and they also actively store pollen in specialized pollen baskets on their hind legs. It is therefore not unexpected that the foraging behavior of honeybees could move virus-contaminated pollen to the flowers of healthy plants (26, 36). However, this study represents the first evidence that honeybees exposed to virus-contaminated pollen could also be subsequently infected and that the infection could be systemic and spread throughout the entire body of honeybees. About 5% of known plant viruses are pollen transmitted, and the genomes of the majority of plant viruses are made of RNA (37, 38), providing a large set of potential host-jumping viruses. The finding from this study illustrates the complexity of relationships between plant pathogens and the pollinating insects and emphasizes the need for surveillance for potential host-jumping events as an integrated part of insect pollinator conservation.

A. Hybridized With Dig-Labeled TRSV Probe



B. Hybridized Without Dig-labeled TRSV Probe

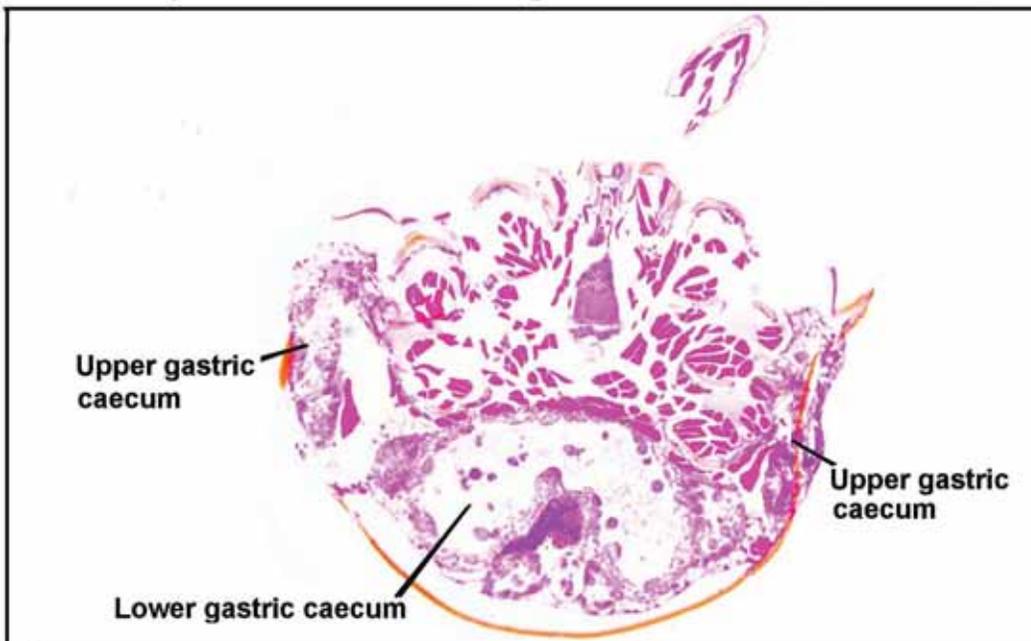
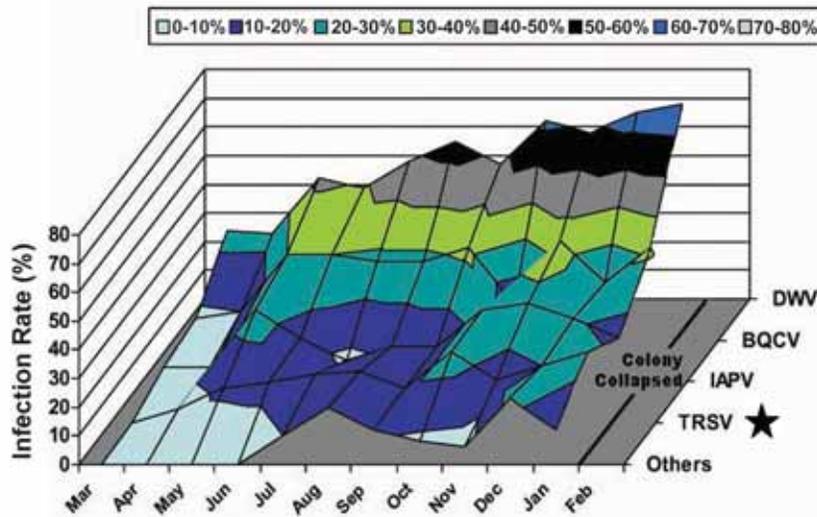


FIG 6 In situ hybridization analysis of *Varroa* mites. (A) The slides were hybridized with DIG-labeled TRSV probe. (B) The slides were not hybridized with DIG-labeled TRSV probe. The positive signal is dark blue, and the negative areas are pink. The infected tissues of the upper and lower gastric caeca are shown in dark blue.

For a virus to successfully establish infection in a novel host, the virus must overcome three major hurdles. First, it must have the opportunity to come into contact with a prospective host for the viral particles to gain entry into the host cells. Second, the virus must undergo genetic changes that mediate the entry of virus into

host cells, typically through host receptors on the cell surface. The virus must also undergo genetic changes that can lead to the ability to bypass the host's immune defense and replicate its genome using the host's cellular machinery. Finally, the virus must gain the ability to spread horizontally between individuals of the same gen-

A. Weak Colonies



B. Strong Colonies

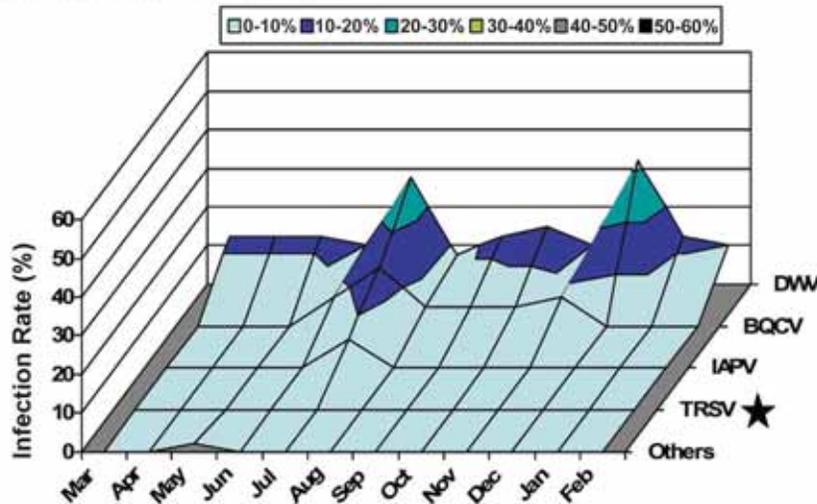


FIG 7 Seasonal prevalence of TRSV and other honeybee viruses in honeybee colonies. (A) Weak colonies. The prevalence of TRSV along with deformed wing virus (DWV), black queen cell virus (BQCV), Israeli acute paralysis virus (IAPV), and two rarely detected viruses, sacbrood virus (SBV) and chronic bee paralysis virus (CBPV) was found in all season. The viral infections reached their peaks in winter before the colony collapsed. Of viruses detected in weak colonies, DWV was the most prevalent, followed by BQCV, IAPV, TRSV, and others (SBV and CBPV). (B) Strong colonies. Only DWV and BQCV were detected in healthy colonies all year round, but the prevalence of the viruses in strong colonies was significantly lower in weak colonies. All strong colonies survived through the cold winter months.

eration within new host populations. The detection of replicate intermediates of TRSV in different tissues of honeybees and the prevalence of TRSV in bee populations provide strong evidence that TRSV has overcome these key hurdles. The presence of a TRSV-positive signal in parasitic *Varroa* mites suggests that *Varroa* could serve as a vector to facilitate the horizontal transmission of TRSV between bees in the colonies.

Food-borne transmission is one of the most important routes for virus transmission in honeybees. Infections of several honeybee viruses occur through ingestion of virus-contaminated food followed by dissemination of the viruses from the midgut into other tissues through the hemolymph (39). Since TRSV is a

known pollen-borne plant virus, we initially believed that the presence of TRSV was restricted to the bees' digestive tract. However, titers of TRSV in our study were unexpectedly low in the gut. Viral replication was not detected in either the gut or the salivary gland. Instead, high titers of negative-stranded virus were found in the wing, nerve, antenna, trachea, hemolymph, and fat body, indicating replication in those tissues. The absence of virus replication in the tissues of the gut and salivary gland excludes the possibility of TRSV as a persistent-propagative virus which must first replicate in epithelial cells of the midgut and then migrate to the salivary glands to be ejected together with saliva. Our quantitative analysis suggests that TRSV is neurotropic in honeybees, with

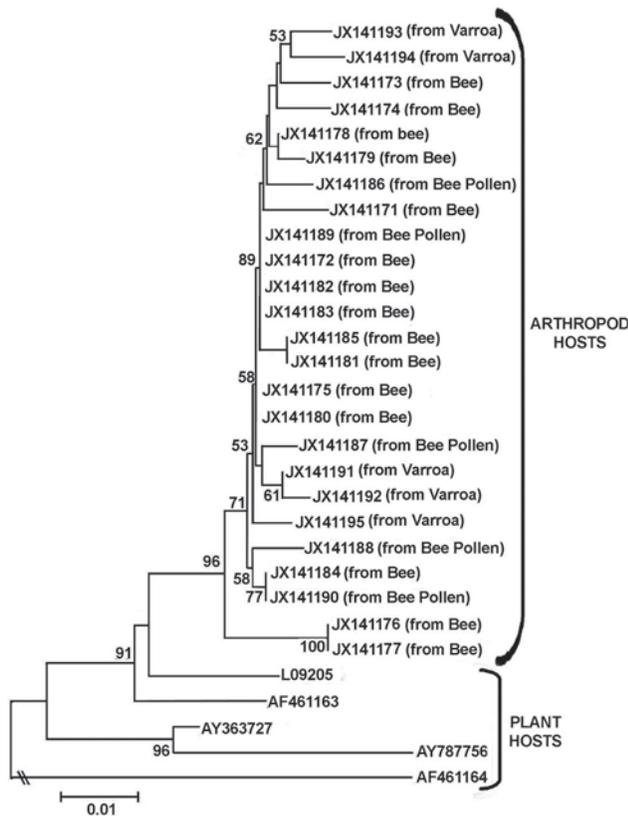


FIG 8 Phylogenetic tree showing the relationship of TRSV isolates from arthropod and plant hosts. The partial sequences of capsid protein of TRSV amplified from honeybees, *Varroa* mites, and bee pollen were compared with the corresponding regions of TRSV that were isolated from plants and retrieved from GenBank. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test are shown next to the branches. Numbers at each node represent bootstrap values as percentages of 500, and only bootstrap values of >50% are shown. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree and in the units of the number of base substitutions per site. Individual sequences are labeled by their GenBank accession numbers.

more extensive infection detected in the nervous system than in other internal tissues, and therefore it is conceivable that severe TRSV infection can cause functional impairment of the nerve and muscle in honeybees. The low levels of TRSV in the gut suggests a possible result of sloughing off of infected epithelial cells from midgut as a host defensive mechanism or the possibility that TRSV might utilize some alternative invasion routes such as the neural or tracheal route. Further investigation of the virus transmission and pathogenicity is warranted.

The circulation of TRSV in bee hemolymph was further proven by the presence of TRSV in *Varroa* mites. *Varroa* is an obligate parasite of the honeybee and has been catastrophic for the bee-keeping industry. Both adult mites and nymphs use their piercing mouth parts to penetrate the body wall of the bees and suck out the hemolymph. In addition to its direct detrimental effects on host life span and colony vigor (40–45), the feeding of mites on bees provides an entry for microbial pathogens (44). Indeed, the roles played by *Varroa* mites in acquiring and transmitting honeybee viruses have been experimentally demonstrated in several studies

(46–49). The observation of the positive signal of TRSV within the storage organ of the mites suggests that the *Varroa* mite is not merely a mechanical vector that physically transports viruses from host to host with its mouthparts. More work is needed to confirm whether *Varroa* mites can act as a biological vector to support TRSV replication.

TRSV isolates from honeybees, *Varroa* mites, and bee pollen clustered together phylogenetically, indicating that they descended from a common ancestor. It is likely that *Varroa* mites obtained the virus from their hosts during the blood feeding and that the virus-infected bees contaminated the bee pollen when they mix plant pollen with their glandular secretions and honey to produce “bee bread.” The finding that TRSV isolates from honeybees appeared to be derived more recently on the evolutionary timeline than TRSV from plants suggests that life cycles of the virus involving arthropod hosts evolved after host expansion. However, it remains to be determined whether TRSV possesses the ability to maintain persistent infection in honeybee colonies in the absence of newly inoculated viruses from visited plants or whether infected bees can subsequently inoculate healthy plants. It will similarly be helpful to screen other pollinator species for the presence of TRSV, since it is known that honeybees and other pollinators share some viral species (8–11, 50).

Sequence comparison of the TRSV isolates from this study with isolates with other accession numbers suggests that the capsid protein region is much more conserved than the RNA helicase region at the nucleotide level. The relatively high level of sequence similarity at the amino acid level for both capsid protein and helicase indicates a high level of structural and functional conservation. Nevertheless, substitution of a single or a few amino acids at the surface of virus particles can be sufficient to alter receptor recognition and thereby alter host range (51). Thus, the few amino acid polymorphisms observed in TRSV strains infecting honeybees may still be associated with cell tropism and host adaptation. It would be helpful to further characterize the complete genome of TRSV isolates from honeybees as well as from *Varroa* mites to deepen our understanding of genetic diversity of this virus. More work is needed to elucidate the molecular basis of cell tropism and host range modifications and to investigate the roles of the honeybee as a newly identified host in the epidemiology of TRSV.

The evidence of systemic spread and propagation of a plant-pathogenic virus in honeybees raises awareness of the potential impact of new viral disease emergence on bee health. While findings from this study have important implications for understanding TRSV transmission and pathogenesis, much remains to be learned about the intracellular life cycle, species-level genetic variation, and pathogenesis of the virus in honeybee hosts. Although the cause(s) of CCD and the decline in the worldwide bee population is not yet fully understood (52), a growing body of evidence has indicated that parasites and pathogens are key culprits involved in widespread disappearance/death and population declines of honeybees (3, 5, 53–57). The observation that increasing prevalence of TRSV in conjunction with other bee viruses in infected colonies is associated with gradual decline of host populations and winter colony collapse supports the argument that virus infections could have a significant negative impact on colony survival. While the simultaneous presence of multiple viruses and asymptomatic viral infections in honeybees as well as lack of a cell culture system for virus production (58, 59) makes Koch’s postulates of disease causality difficult to fulfill, the observed negative

correlation between the level of TRSV infections and size of host populations suggests that TRSV, in combination with other viruses, is likely a contributing factor to poor survivorship of honeybee colonies.

MATERIALS AND METHODS

Honeybee colonies and sample collection. Honeybee colonies used for this study were maintained in the research apiaries of the USDA-ARS Bee Research Laboratory in Beltsville, MD. For viral particle purification and tissue dissection, fifty adult worker bees were collected by removing a central frame filled with brood and covered with adult bees from a hive and gently scraping worker bees into a 50-ml conical tube. In addition, bee pollen that was processed by bees and stored in combs around the brood was collected using a spatula and transferred into 15-ml conical tubes. Individual *Varroa* mites that had crawled from brood cells onto the tops of brood frames were collected with forceps and transferred into 1.5-ml microcentrifuge tubes.

For assessing the effects of viruses on honeybees, the seasonal prevalence of virus infections was determined in ten colonies for a period of 1 year starting in March and finishing in February of the following year. Bee colonies were classified as strong or weak based on the size of adult populations, amount of sealed brood, and presence of food stores, as previously described (60). Bee colonies that had more than ten frames covered with adult workers and more than six frames filled with brood and food stores were defined as strong colonies, while bee colonies that had a small number of foraging bees flying in and out, fewer than ten frames of adult bees, fewer than six combs with brood, and small patches of food stores were defined as weak colonies. For each colony, samples of 20 adult workers were collected every month and stored at -80°C until subsequent RNA isolation for virus analysis.

Virus purification and electron microscopy. Thirty worker bees were frozen in liquid nitrogen, ground to a fine powder, and homogenized in 10-ml extraction buffer (0.1 M potassium phosphate buffer [pH 7.5], 0.2% diethyldithiocarbamate, 1/5 volume of diethyl ether). The mixture was emulsified with 5 ml carbon tetrachloride and centrifuged at $5,000 \times g$ at 4°C for 30 min to remove tissue debris. Supernatant containing viruses was centrifuged once more at $5,000 \times g$ at 4°C for 30 min and then filtered through a $45\text{-}\mu\text{m}$ filter to remove small tissue debris. The filtrate was then centrifuged at $10,187 \times g$ for 6 h at 4°C to pellet the viral particles. The pellet was resuspended in 2 ml of 0.2 M phosphate-buffered saline (PBS) buffer. A $15\text{-}\mu\text{l}$ portion of viral solution was examined for the presence of virus particles in an electron microscope. The rest of the viral solution was saved for subsequent viral RNA isolation and cDNA library construction.

Virus particles were negatively stained with 2% uranyl acetate on a Formvar-coated Ni grid and viewed in a Hitachi H-7000 electron microscope at magnifications between $\times 33,000$ and $\times 100,000$.

cDNA library construction and virus-specific primer design. Total RNA was extracted by homogenizing the viral solution with TRIzol LS reagent (Invitrogen), a solution of phenol and guanidine isothiocyanate used for isolating total RNA from liquid samples according to the manufacturer's instructions. The resultant RNA pellets were resuspended in DNase- and RNase-free water (Invitrogen) in the presence of ribonuclease inhibitor (Invitrogen). The quantity and purity of RNA were measured with a NanoDrop spectrophotometer (NanoDrop Technologies). The cDNA library was constructed using a CloneMiner cDNA library construction kit (Invitrogen) per the manufacturer's protocol. First-strand cDNA was synthesized from extracted RNA using Superscript II reverse transcriptase with a biotin-conjugated *attB2* oligo(dT) primer. After cDNA synthesis, the products were size fractionated by column chromatography to remove excess primers, adapters, and small cDNAs and cloned into an *attP*-containing donor vector, pDONR 222. The BP (recombination between *attB* and *attP* sites) reaction products were transformed into ElectroMAX DH10B T1 phage-resistant cells, and the transformed cells were plated onto LB agar medium supplemented with

kanamycin ($50\ \mu\text{g/ml}$). The positive clones were purified using the Wizard Plus miniprep DNA purification system (Promega). A total of 50 cDNA clones were randomly selected and sequence analyzed to confirm the presence of the insert.

Primers specific for TRSV RNA segments 1 and 2 were designed based on the nucleotide sequences obtained from cDNA clones of this study. The sequences of primers for amplifying a 462-bp region of helicase (Hel) of RNA segment 1 were TRSV-F1 (5'-CATGAATGTTGTTATCCAAT-3') and TRSV-R1 (5'-TCCTCAGTAAATTTTCATTTG-3'). The sequences of primers for amplifying a 731-bp region of capsid protein (CP) region of RNA segment 2 were TRSV-F2 (5'-GTGTGCTGTGACGGTTGTCC-3') and TRSV-R2 (5'-TGCCAGACCACCCAAGATTCC-3'). Figure 1 illustrates the positions of primers.

Bee tissue dissection. Twenty adult worker bees were individually fixed on the wax top of a dissecting dish with steel insect pins. Under a dissecting microscope, about $10\ \mu\text{l}$ of hemolymph was collected from each bee with a micropipette tip by making a small hole on the roof of the bee's thorax with a needle to make it bleed. Following hemolymph collection, the legs, wings, antennae, and compound eyes were cut off with a pair of fine scissors. The body was opened by cutting along the dorsal midline from the tip of the abdomen to the head with scissors. Tissues of the brain, fat body, salivary gland, gut, muscle, nerve, trachea, and hypopharyngeal gland were individually removed using a pair of fine forceps under a dissecting microscope. In total, thirteen tissues were collected from each bee, and a total of thirty bees were dissected. The scissors and forceps were wiped between tissues once with a cotton pad soaked with 10% bleach and once with a cotton pad soaked with 70% alcohol followed by a final rinse in sterile water. To prevent possible contamination with hemolymph, all tissues were rinsed once in $1 \times$ phosphate-buffered saline (PBS) and twice in nuclease-free water. The washing solution was changed every time for each tissue to prevent cross-contamination. All freshly dissected tissues were subjected to subsequent RNA extraction immediately.

Total RNA extraction and conventional RT-PCR. Total RNA was isolated from dissected tissues, adult bees, bee pollen, and *Varroa* mites using Invitrogen Trizol reagent according to the manufacturer's instructions. Conventional RT-PCR was performed on RNA samples extracted from adult bees, *Varroa* mites, different tissues, and bee bread collected from the same colony for the presence and distribution of TRSV. The Promega one-step Access RT-PCR system was used for virus detection as previously described (58). PCR products were purified and sequenced to confirm the specificity of the primers.

To determine the seasonal prevalence of TRSV in honeybee colonies, bee samples collected every month were subject to RT-PCR analysis individually for TRSV as well as other seven common honeybee viruses, including acute bee paralysis virus (ABPV), BQCV, chronic bee paralysis virus (CBPV), DWV, Israeli acute paralysis virus (IAPV), Kashmir bee virus (KBV), and SBV. The primer pair TRSV-F2/TRSV-R2 was used for RT-PCR amplification of TRSV. The primer sets used for RT-PCR amplification of common honeybee viruses have been reported previously (49, 58). Putative TRSV amplification products were purified and sequenced to confirm the specificity of the RT-PCR assay. The infection rate of each virus (20 workers) and strength of individual colonies were recorded every month throughout the year.

Strand-specific RT-qPCR. In order to determine the ability of TRSV to replicate in different tissues of honeybees, RNA samples were further analyzed for the presence and abundance of negative-stranded RNA, a replicative intermediate, using strand-specific reverse transcription coupled with quantitative PCR (RT-qPCR). For each tissue sample, the first-strand cDNA was synthesized from total RNA using Superscript III reverse transcriptase (Invitrogen) with Tag-TRSV-F1 (5'-AGCCTGCGCA CGTGGcatgaatgtgttatccaat-3'), where the capitalized sequence corresponding to Tag was published by Yue and Genersch (61). The synthesized cDNAs were then purified using a MinElute PCR purification kit (Qiagen) followed by a MinElute Reaction Clean kit (Qiagen) to remove short fragments of oligonucleotides and residue of enzymatic reagents to

prevent amplification of non-strand-specific products (28). cDNA derived from negative-stranded RNA was amplified using the Brilliant SYBR green qPCR master mix (Stratagene) with a 0.4 μ M concentration each of the Tag (3'-AGCCTGCGCACCGTGG-5') and TRSV-R1 primers in a 25- μ l volume according to the manufacturer's protocol. To normalize the qPCR result, amplification of a housekeeping gene, the β -actin gene, was performed for each sample with a previously reported primer set (62).

The amplification for both TRSV and β -actin was carried out following the manufacturer's recommended protocol for thermal profile parameters for three-step PCR. After amplification, a melting curve analysis was performed to determine the specificity of the PCR products. Each sample was run in triplicate, and the qPCR assay was repeated twice. The amplification efficiencies of the SYBR green real-time RT-qPCR assay for both TRSV and β -actin were proved to be approximately equal (data not shown). The output of RT-qPCR assays for TRSV in different tissues was interpreted by using the comparative cycle threshold method ($\Delta\Delta C_T$ method). The average C_T value (ΔC_T) of TRSV in each tissue was normalized using the C_T value corresponding to the endogenous control, β -actin, with the following formula: $\Delta C_T = \text{average } C_T(\text{TRSV}) - \text{average } C_T(\beta\text{-actin})$. The tissue that had the lowest level of TRSV was chosen as a calibrator. The ΔC_T value of each tissue was subtracted from the ΔC_T value of the calibrator to yield $\Delta\Delta C_T$. The concentration of TRSV in each tissue was calculated using the formula $2^{-\Delta\Delta C_T}$ and expressed as n -fold difference relative to the calibrator.

In situ hybridization. Purified amplicons corresponding to the region flanked by the TRSV-F2 and TRSV-R2 primer set were incorporated into a pCR2.1 TA cloning vector upstream of a T7 promoter (Invitrogen, Carlsbad, CA) following the manufacturer's protocol. Recombinant plasmid DNAs with the TRSV insert were linearized by restriction enzyme BamHI (New England Biolabs, Ipswich, MA) at 37°C for 2 h. The linearized DNAs were extracted once with an equal volume of phenol-chloroform-isoamyl alcohol (25:24:1), precipitated by ethanol, and dissolved in nuclease water. The DIG-labeled RNA probe complementary to TRSV genomic RNA was synthesized using a DIG-RNA labeling kit (T7) (Roche Applied Science, Indianapolis, IN) following the manufacturer's protocol.

Live *Varroa* mites were fixed in 4% paraformaldehyde in 100 mM PBS (pH 7.0) overnight at 4°C, rinsed in nuclease-free water three times, and then stored in 70% ethanol (200 proof) at 4°C until used. Tissue dehydration was carried out by successive incubations in ethanol (70%, 95%, and 100%) and xylol (twice for 5 min each) and embedded in paraffin. Paraffin sections were cut 2 to 5 micrometers thick and mounted on poly-L-lysinated slides and stored at 4°C overnight. The sections were then rehydrated through a descending concentration of ethanol (100%, 95%, and 70%), dewaxed in xylol, treated with proteinase K (10 μ g/ml) for 30 min, and acetylated with 0.33% (vol/vol) acetic anhydride in 0.1 M triethanolamine-HCl (pH 8.0) for 10 min prior to hybridization.

The sections were prehybridized in prehybridization solution (50% formamide, 5 \times SSC [1 \times SSC is 0.15 M NaCl plus 0.015 M sodium citrate], 40 μ g/ml salmon sperm) at 58°C for 2 h and incubated in hybridization buffer with DIG-labeled TRSV probe solution to a concentration of 100 to 200 ng/ml probe in prehybridization solution at 58°C overnight. After hybridization, the sections were washed twice in low-stringency wash solution (2 \times SSC, 0.1% SDS) at room temperature for 5 min and washed twice in high-stringency wash solution (0.1 \times SSC, 0.1% SDS) at 52°C for 15 min. The hybridization signals were detected with alkaline phosphatase (AP)-labeled sheep anti-DIG antibody conjugate (Roche Applied Science). The conjugate solution was added to the dry sections and incubated at 4°C for 2 h in a humid chamber. The slides were rinsed three times with washing buffers. The color development was performed by adding the buffer solution containing nitroblue tetrazolium (NBT) and 5-bromo-4-chloro-3-indolyl phosphate (BCIP) to the tissue sections and incubating for 3 to 6 h at room temperature with protection from light. The color reaction was stopped by a 5-min wash in Tris-EDTA (0.1 mM, pH 8.0). The nonspecific staining was removed in 95% ethanol

overnight. The sections were rehydrated through successive incubation in ethanol (70%, 95%, and 100%) and xylol (twice for 15 min each) and mounted in Eukitt resin. Negative control reactions included regular dUTP instead of DIG-labeled TRSV probe. *In situ* hybridization slides were observed under a light microscope (Eclipse TE 300; Nikon) and photographed with a Nikon digital camera (DXM 1200). Dark blue coloring indicates where the DIG-labeled probe bound directly to the viral RNA. The section hybridized with the negative control showed pink staining only from the application of nuclear fast red.

Phylogenetic analysis. The sequences of the 731-bp TRSV fragment amplified from the region encoding the capsid protein by the primer pair TRSV-F2 and TRSV-R2 from honeybees, bee pollen, and *Varroa* mites were compared with existing GenBank sequences isolated from plants. Phylogenetic analysis was conducted in MEGA4 (63). The sequences were aligned using ClustalW, and the sequences that could not be aligned unambiguously at both 3' and 5' ends were truncated. A tree was built using the neighbor-joining method (64) with distances computed using the maximum composite likelihood method (65). The reliability of the phylogenies was assessed by bootstrap replication (500 replicates) (66). Node labels correspond to bootstrap support, and values of >50% were regarded as evidence for the phylogenetic grouping.

Nucleotide sequence accession numbers. The cDNA sequence data have been submitted to the GenBank sequence database and assigned the accession numbers JQ710729 and JQ710730 for the helicase and capsid protein coding regions, respectively.

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NEWS

Studies highlight differences between banned pesticides

21 January 2014, by Alex Peel

Two separate studies have added new weight to the idea that some varieties of controversial neonicotinoid pesticides are less harmful to bees than others.

The research tested the effects of feeding two different neonicotinoid compounds, thiamethoxam and imidacloprid, to bumblebees. The EU recently banned both pesticides over concerns about their effects on bee health.

Ian Laycock, from the University of Exeter, led the studies.

'We found that both thiamethoxam and imidacloprid were capable of repressing feeding and brood production in bumblebees,' he says. 'But with thiamethoxam this only happened with very large concentrations of the pesticide – concentrations that bees are unlikely to encounter in the environment.'

In contrast, imidacloprid reduced both egg production and pollen consumption by half even at the very low concentrations that bees are likely to encounter when they forage on treated crops.

'What our study shows is that we cannot simply apply the conclusions about the safety of imidacloprid to other neonicotinoids'
Ian Laycock,
University of Exeter

well.

This kind of 'pulsed' exposure is designed to replicate the scenario in the wild, where bees feed on mass-flowering crops, like oil-seed rape, for a window of just a few weeks while they're in flower. When the bloom is over, wild bees often switch back to pesticide-free wildflowers.

While the scientists tested imidacloprid on regular colonies, the research on thiamethoxam was carried out on micro-colonies, each made up of four worker bees kept apart from the queen. This allowed the scientists to monitor responses such as feeding, egg-laying and brood survival in precise detail.

In natural colonies, the queen bee does most of the brood production herself, and Laycock acknowledges that the lack of queens in the micro-colonies does limit the study's ability to replicate the bees' real-world environment.

But he believes the research still gives a useful insight into the different effects of neonicotinoid compounds. And he maintains that lab studies have an important role to play in gathering evidence on pesticides.

'It's impossible to perfectly replicate the real world in the lab, and for that reason some people will always question the



'We think this occurs because imidacloprid produces a stronger repression of feeding in bumble bees than thiamethoxam, and this imposes a greater limitation on the bees' ability to produce eggs,' says Laycock.

'Brood production is particularly important in bumble bee colonies because the number of eggs and larvae a colony produces can dictate how many workers it has - and colonies with more workers tend to be more successful.'

'In particular they produce more queens that go on to found new colonies and keep the colony cycle going the following year. So our results raise further concern about the threat of imidacloprid to bumblebees.'

But the research also had some better news for bees. After two weeks' exposure, the bees were allowed to recuperate, and seemed to bounce back



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'But carefully designed lab studies are vital because, unlike field studies, we can precisely control variables such as the concentration or dose of the pesticide that the bee receives.'



In December last year, the EU introduced a two-year moratorium on neonicotinoids. The UK voted against the ban, citing a lack of evidence of harm to bees in field studies.

Laycock believes that the evidence against imidacloprid is probably strong enough to justify a temporary ban, giving researchers more time to look into its effects. But he says we shouldn't tar all neonicotinoids with the same brush.

'What our study shows is that we cannot simply apply the conclusions about the safety of imidacloprid to other neonicotinoids,' he says.

He is also concerned that the moratorium could encourage farmers to use alternative pesticides on their crops, whose effects on bees are unstudied, and may prove to be even worse.

These thoughts are echoed by Dr Chris Connolly, from the University of Dundee, who was not involved with either study.

'Whether or not these pesticides are responsible for bee decline, there is clear evidence that they have an effect, and so the moratorium seems like a wise precaution.'

'But it would be totally unwise if they are just replaced with other compounds, which we know very little about - it's irrational to set the bar higher for one pesticide than all the others. The whole thing needs to be looked at a bit more scientifically.'

'It's irrational to set the bar higher for one pesticide than all the others'
Dr Chris Connolly,
University of Dundee

One potential alternative, belonging to a group of chemicals called pyrethroids, was the subject of a separate lab study at Royal Holloway, University of London. Colony exposure to the pesticide was shown to reduce the size of bumblebees' offspring, posing a potential risk to colony success.

Keywords: [Biodiversity](#), [Biology](#), [Ecology](#), [Europe](#), [Farming](#), [Insects](#), [Natural resources](#), [Pollinators](#), [UK](#),

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Ian Laycock, Exeter

Tuesday, 21 January 2014 - 13:12

Common crop pesticides kill honeybee larvae in the hive



Honeybee. Credit: Adam Siegel

Four pesticides commonly used on crops to kill insects and fungi also kill honeybee larvae within their hives, according to Penn State and University of Florida researchers. The team also found that N-methyl-2-pyrrolidone (NMP)—an inert, or inactive, chemical commonly used as a pesticide additive—is highly toxic to honeybee larvae.

"We found that four of the pesticides most commonly found in beehives kill bee larvae," said Jim Frazier, professor of entomology, Penn State. "We also found that the negative effects of these pesticides are sometimes greater when the pesticides occur in combinations within the hive. Since pesticide safety is judged almost entirely on adult honeybee sensitivity to individual pesticides and also does not consider mixtures of pesticides, the risk assessment process that the Environmental Protection Agency uses should be changed."

According to Frazier, the team's previous research demonstrated that forager bees bring back to the hive an average of six different pesticides on the pollen they collect. Nurse bees use this pollen to make beebread, which they then feed to honeybee larvae.

To examine the effects of four common pesticides—fluralinate, coumaphos, chlorothalonil and chlorpyrifos—on bee larvae, the researchers reared honeybee larvae in their laboratory. They then applied the pesticides alone and in all combinations to the beebread to determine whether these insecticides and fungicides act alone or in concert to create a toxic environment for honeybee growth and development.

The researchers also investigated the effects of NMP on honeybee larvae by adding seven concentrations of the chemical to a pollen-derived, royal jelly diet. NMP is used to dissolve pesticides into formulations that then allow the active ingredients to spread and penetrate the plant or animal surfaces onto which they are applied. The team fed their treated diet, containing various types and concentrations of chemicals, to the laboratory-raised bee larvae.

The team's results are reported in the current issue of *PLoS ONE*.

"We found that mixtures of pesticides can have greater consequences for larval toxicity than one would expect from individual pesticides," Frazier said.

Among the four pesticides, honeybee larvae were most sensitive to chlorothalonil. They also were

negatively affected by a mixture of chlorothalonil with fluvalinate. In addition, the larvae were sensitive to the combination of chlorothalonil with the miticide coumaphos. In contrast, the addition of coumaphos significantly reduced the toxicity of the fluvalinate and chlorothalonil mixture.

According to Chris Mullin, professor of entomology, Penn State, these pesticides may directly poison honeybee larvae or they may indirectly kill them by disrupting the beneficial fungi that are essential for nurse bees to process pollen into beebread.

"Chronic exposure to pesticides during the early life stage of honeybees may contribute to their inadequate nutrition or direct poisoning with a resulting impact on the survival and development of the entire bee brood," he said.

The researchers note that fluvalinate and coumaphos are commonly used by beekeepers on crops to control Varroa mites, and are found to persist within beehives for about five years. Chlorothalonil is a broad-spectrum agricultural fungicide that is often applied to crops in bloom when honeybees are present for pollination because it is currently deemed safe to bees. Chlorpyrifos is a widely used organophosphate in crop management.

"Our findings suggest that the common pesticides chlorothalonil, fluvalinate, coumaphos and chlorpyrifos, individually or in mixtures, have statistically significant impacts on honeybee larval survivorship," Mullin said. "This is the first study to report serious toxic effects on developing honeybee larvae of dietary pesticides at concentrations that currently occur in hives."

The team also found that increasing amounts of NMP corresponded to increased larval mortality, even at the lowest concentration tested.

"There is a growing body of research that has reported a wide range of adverse effects of inactive ingredients to human health, including enhancing pesticide toxicities across the nervous, cardiovascular, respiratory and hormone systems," Mullin said. "The bulk of synthetic organic chemicals used and released into U.S. environments are formulation ingredients like NMP, which are generally recognized as safe. They have no mandated limits on their use and their residues remain unmonitored.

"Multi-billion pounds of these inactive ingredients overwhelm the total chemical burden from the active pesticide, drug and personal-care ingredients with which they are formulated. Among these co-formulants are surfactants and solvents of known high toxicity to fish, amphibians, honey bees and other non-target organisms. While we have found that NMP contributes to honeybee larvae mortality, the overall role of these inactive ingredients in pollinator decline remains to be determined."

Other authors on the paper include Wanyi Zhu, graduate research assistant in entomology, Penn State, and Daniel Schmehl, postdoctoral associate in entomology and nematology, University of Florida.

Provided by Pennsylvania State University

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New Hampshire House kills GMO labeling bill

By LAURA McCRYSTAL

Monitor staff

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The House killed a bill yesterday that would have required labels on foods that are the product of genetic engineering.

Several House members said yesterday that they heard from many constituents who supported the bill because they fear that there are health risks associated with genetically modified organisms, or GMOs. But House members debated those health concerns yesterday, with the bill's opponents arguing that the risks have been exaggerated.

"There's a lot of hysterical momentum behind this anti-GMO movement," said Rep. Jim Parison, a New Ipswich Republican. "It's sort of like an angry mob seeking justice for a crime just by lynching the first possible suspect."

Parison encouraged the House to kill the bill, even if it would be unpopular with some constituents. He said the labeling would not necessarily protect consumers, and it would hurt business owners. The bill would hold retailers – not manufacturers or food processors – responsible for labeling products.

The House voted, 185-162, to kill the bill.

Supporters said the bill would have allowed consumers to know what is in their food and make their own decisions about the potential risks associated with GMOs.

"Our constituents have spoken about safety concerns," said Rep. Peter Bixby, a Dover Democrat. "Mandatory labeling would empower these individuals to make their own decisions."

Rep. Tim Smith, a Manchester Democrat, said he heard from many constituents who are in favor of GMO labeling. He read a letter from one woman who said, "I just want to know what goes into the food I give my

children, ages 3 and 8.”

Other opponents of GMO labeling said the state did not have the ability to enforce it. Rep. Bob Haefner, a Hudson Republican, said New Hampshire cannot regulate food that comes from other states and suggested the bill could be challenged in court as unconstitutional.

“I will never argue about a right to know,” Haefner said. “But . . . this is a federal issue. It is not a state issue.”

(Laura McCrystal can be reached at 369-3312 or lmccrystal@cmonitor.com () or on Twitter [@lmccrystal](https://twitter.com/lmccrystal) (Q).)

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Four Common Pesticides, Their Mixtures and a Formulation Solvent in the Hive Environment Have High Oral Toxicity to Honey Bee Larvae

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Abstract

Recently, the widespread distribution of pesticides detected in the hive has raised serious concerns about pesticide exposure on honey bee (*Apis mellifera* L.) health. A larval rearing method was adapted to assess the chronic oral toxicity to honey bee larvae of the four most common pesticides detected in pollen and wax - fluvalinate, coumaphos, chlorothalonil, and chlorpyrifos - tested alone and in all combinations. All pesticides at hive-residue levels triggered a significant increase in larval mortality compared to untreated larvae by over two fold, with a strong increase after 3 days of exposure. Among these four pesticides, honey bee larvae were most sensitive to chlorothalonil compared to adults. Synergistic toxicity was observed in the binary mixture of chlorothalonil with fluvalinate at the concentrations of 34 mg/L and 3 mg/L, respectively; whereas, when diluted by 10 fold, the interaction switched to antagonism. Chlorothalonil at 34 mg/L was also found to synergize the miticide coumaphos at 8 mg/L. The addition of coumaphos significantly reduced the toxicity of the fluvalinate and chlorothalonil mixture, the only significant non-additive effect in all tested ternary mixtures. We also tested the common 'inert' ingredient N-methyl-2-pyrrolidone at seven concentrations, and documented its high toxicity to larval bees. We have shown that chronic dietary exposure to a fungicide, pesticide mixtures, and a formulation solvent have the potential to impact honey bee populations, and warrants further investigation. We suggest that pesticide mixtures in pollen be evaluated by adding their toxicities together, until complete data on interactions can be accumulated.

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Introduction

Recently, one hundred and twenty one different pesticides and metabolites were identified in the hive with an average of seven pesticides per pollen sample, including miticides, insecticides, fungicides, herbicides, and insect growth regulators [1,2]. Feeding on pollen and nectar in the larval diet directly exposes honey bee larvae transdermally, orally and internally [3]; therefore, the potential for chronic toxicity and synergistic interactions at the brood stage seems likely to occur, especially considering the fact that early life stages might be much more sensitive to certain contaminants relative to the adult stage. Several studies have demonstrated that insecticides ranging from insect growth regulators and encapsulated organophosphate formulations to systemic insecticides are more toxic to larvae than to adult bees [4–8]. Moreover, because beebread serves as an absolute requirement for developing bee larvae, pesticide disruption of the beneficial mycofloral community in the colony may thwart the processing of pollen into beebread and allow undesirable pathogens to thrive, therefore indirectly impacting the brood health [9,10]. Indeed, chronic exposure to pesticides during the early life stage of honey bees may thus contribute to inadequate nutrition and/or direct poisoning with a resulting impact on the survival and development

of bee brood [11]. Conceivably, these impacts on the larval phase could lead to weakening of the colony structure over time. To date, only a few peer-reviewed pesticide toxicity studies assess the risks of oral toxicity of pesticides to honey bee larvae. Therefore, a goal of our study was to assess the chronic and mixture effects of common pesticides at realistic exposure concentrations on larval honey bee survival. In order to mimic realistic exposure scenarios of honey bee larvae to contaminated pollen food, we chose the four most frequently detected pesticides in the hive - fluvalinate, coumaphos, chlorothalonil, and chlorpyrifos, and tested them alone and in all combinations via chronic dietary exposure, at concentrations found in pollen and beebread.

The pyrethroid *tau*-fluvalinate and the organophosphate coumaphos have been used widely for Varroa mite control, and found highly persistent in the hive with an estimated half-life in beeswax of about 5 years [12]. These compounds have shown evidence of synergistic toxicity on adult honey bees at the level of cytochrome P450-mediated detoxification [13]. Chlorothalonil, a broad-spectrum agricultural fungicide with an unclear mode of action [14], is often applied to crops in bloom when honey bees are present for pollination, because it is currently deemed safe to bees. However, some fungicides have shown direct toxicity to honey bees or solitary bees at field use rates [15] and fungicides in stored

pollen are known to inhibit the growth of beneficial fungi thereby reducing the nutritional value of the pollen to bees [10]. Chlorpyrifos is a widely employed organophosphate in crop management [16] and its residues were frequently found in honey, propolis and dead bees. These in-hive (beekeeper applied) varroacides and out-of-hive (farmer applied) insecticides and fungicides may act alone or in concert, in ways currently unknown, to create a toxic environment for honey bee growth and development.

Another goal of this study was to examine the effect of an 'inert' ingredient on brood survival. Little data exist concerning the toxicity of 'inert' ingredients on honey bees, likely because bee toxicity information for pesticide formulations is not currently required by the U.S. Environmental Protection Agency as part of the pesticide registration process in contrast to the European Union where toxicity for representative formulations is mandatory [17]. Pesticide risk assessment is largely stymied by lack of public access to product-specific information of 'inerts' or co-formulants [18]. Some 'inert' ingredients such as those in formulations of the herbicide glyphosate are more toxic than active ingredients when tested on aquatic organisms [19]. That 'inert' more than active ingredients dominate pesticide formulations and spray tank adjuvants so to increase efficacy and stability of the pesticide makes it important to examine the role of 'inerts' on honey bee toxicity. Here, we studied the chronic toxicity of N-methyl-2-pyrrolidone (NMP, CAS 872-50-4) to bee brood development. The co-solvent NMP is used extensively in chemical processing and agricultural chemical formulations [20,21]. The NMP tested alone or in formulations has demonstrated developmental toxicity in rats by various routes of administration [22] and also has shown high toxicity potential for aquatic invertebrates [23]. There is presently no information in the published literature regarding toxic effects of NMP to honey bees. Our study will be the first to test if this common 'inert' ingredient is toxic to honey bee larvae by continuous dietary exposure, and will serve as a foundation for future studies exploring 'inert' toxicity.

Specific objectives of the present study using the standardized *in vitro* larval feeding method developed by Aupinel et al. [24] are to: (i) assess possible toxic effects of single pesticides on the survival of individual *A. mellifera* larva during a 6-d continuous feeding with contaminated diet; (ii) compare the sensitivity difference between larval and adult bees to the same pesticide exposure; (iii) determine whether the selected pesticides in all combinations at realistic concentrations have any synergistic effects; and (iv) examine the toxicity of environmentally realistic levels of the formulation ingredient NMP on larval survival. Measurable impacts on larvae should demonstrate the need to extend pesticide risk assessment for honey bees from primarily acute effects on adults to chronic impacts on brood survival and development, and of the need to consider both active and 'inert' ingredients in formulations, so that more informed decisions can be made by governments, beekeepers and growers about pesticide application inside and outside the hive.

Materials and Methods

Acquisition of 1st instar larvae

Honey bee (*A. mellifera*) 1st instar larvae were collected from two colonies of *A. m. ligustica* strain reared in our experimental apiary (GPS Coordinates: 40°49'20"N, 77°51'33"W). In order to collect newly emerged larvae, a honey bee queen was confined in the queen excluder cage and placed in the 2nd super from the bottom of the hive and positioned in the center of the super to allow for proper incubation of the newly laid eggs. After being caged for

30 h, the queen was released from the cage and eggs were incubated in the hive for 3.5 days. Frames of newly-hatched 1st instar larvae were taken to the laboratory in a pre-warmed chamber (~35°C).

Diet preparation

Honey bee larval diet (adaptation of [24]) was prepared using 50% royal jelly (Beenatura.com), 12% D-glucose (Fischer Chemical, Fair Lawn, NJ, USA), 12% D-fructose (Fischer Chemical, Fair Lawn, NJ, USA), 2% yeast extract (Bacto™, Sparks, MD, USA), and distilled water (24%). Royal jelly was preserved at -80°C until use. Ingredients minus royal jelly were completely dissolved and filtered through a 0.2 µm membrane (Corning) to remove particulate matter and bacteria. This solution was poured onto royal jelly that was free of wax particles, and mixed thoroughly at room temperature using a spatula. Diet was stored at 4°C for a maximum of three days prior to use.

Pesticide application

The concentrations of applied pesticides were selected based on our previous laboratory findings of commonly found pesticides in pollen [1]. According to the survey of pesticide residues conducted on bee-related product samples from migratory and other beekeepers during the 2007–08 growing seasons, the most prevalent detections at 95th percentile values (levels at which only 5% of detections are higher) in trapped pollen samples were 0.3 mg/L (0.3 ppm) fluvalinate, 0.8 mg/L coumaphos, 0.15 mg/L chlorpyrifos, and 3.4 mg/L chlorothalonil (unpublished data up to 2009). Foraging bees may avoid and dilute contaminated pollen with that from alternative hosts; therefore, the level of contamination found in the trapped pollen pellets varies in relation to the foraging environment of the colony [1,2,25]. We have observed that apple pollen contributes approximately 10% of overall trapped pollen samples from hives placed in apple orchards during a 10-d pollination event (unpublished data). In addition, these pesticides have also been detected in other hive products at even higher levels including beebread, wax comb, foundation, and more rarely in bees. Developing bees are exposed to pesticide residues by contact with the wax, beebread and contaminated bees, so the level found in trapped pollen or royal jelly is not fully representative of actual exposure of larval bees to pesticides. For example, pollen residues of fluvalinate and coumaphos primarily originate by transfer from the contaminated comb wax, which contains much higher levels (e.g. 100-times) of these miticide residues [1,2]. Therefore, in the absence of exact measures of pollen residues in larval foods, we chose to test at 10 times the levels of these four pesticides found in pollen samples. We mixed fluvalinate (purity, 95%), coumaphos (purity, 99%), chlorpyrifos (purity, 99%), and chlorothalonil (purity, 98%) purchased from Chem Service (West Chester, PA, USA) in the larval diet at nominal concentrations of 3, 8, 1.5, and 34 mg/L, respectively. Our calculated concentrations are in accordance with the maximal levels of pesticides detected in both trapped pollen and beebread samples and within the range of 95 percentile values of four selected pesticides detected in hive samples [1]. Therefore, we believe that applying a factor of 10 can give a rough but realistic estimation of the actual exposure of larval bees through contaminated diet or direct transfer from much higher residues in the comb.

Pesticide treatments included four pesticides tested alone and in two, three, and four-component mixtures. To prepare stock solutions, each technical grade pesticide was individually dissolved in acetone and methanol, respectively. Each test solution was mixed thoroughly into the artificial diet at specific concentrations

and stored in 2 ml sterile glass vials (Corning, USA). We monitored three control groups in the study: untreated diet, one solvent-treated diet containing 1% methanol and another solvent control containing 1% acetone. We also tested the dietary toxicity of a range of N-methyl-2-pyrrolidone concentrations on larval survival. NMP can be used to 100% of the solvent in pesticide formulations [26]. Table S1 lists the percentage of the solvent NMP in some pesticide formulations that disclose it in MSDS. Here, we tested seven nominal concentrations including 0.01% (100 mg/L in diet), 0.02%, 0.05%, 0.1%, 0.2%, 0.5% and 1% (10,000 mg/L).

Each experiment was repeated twice including control (3 groups), single (6 treatment groups), mixture (binary mixtures: 6 treatment groups; ternary mixtures: 6 treatment groups; four-component mixtures: 2 treatment groups), and 'inert' toxicity tests (seven concentrations of NMP). Sample size for each treatment starting from the same experimental day is 3 replicates with 24 larvae per replicate.

In vitro larval rearing technique

Newly hatched 1st instar larvae were transferred from hive frames into sterile, 48-well culture plates (Corning, USA) for the *in vitro* rearing technique with 24 larvae per plate. Larval transfers were done in the lab without the use of a sterile hood. The sterile, push-in queen cups (B&B Honey Farm, USA) were placed in every other well. Diet was warmed to ~34°C in a heating block prior to larval transfer. Using an Eppendorf 10–100 µl variable volume pipette, 10 µl of each diet treatment was placed per queen cup. A 00 camel hair paintbrush was used to transfer each larva from the cell on the frame to the cup. The paintbrush was dipped into distilled water between each larval transfer to aid in a smooth transfer, and was sanitized by dipping in 95% ethanol after every four to five transferred larvae. Larvae were placed directly on top of the diet and inspected for mobility to ensure a quality transfer. Four additional queen cups were equally spaced in four of the remaining open wells before placing the lid on the culture plate, allowing for adequate ventilation of the larvae throughout the experiment. Each plate was placed in a humidity chamber and kept at 95% relative humidity with a 10% aqueous solution of sulfuric acid being used at the base of the chamber to maintain humidity. Humidity chambers were placed in an incubator at 34°C in the dark and were not disturbed throughout the experiment, except when replacing the diet for ~15 min/d.

For this study, only the survivorship of honey bees during the larval stage was monitored to evaluate the impacts of selected pesticides. Larval mortality was recorded daily by probing the larvae with sanitized forceps. The dead larvae were removed daily. Diet for each larval bee was replaced daily. Old diet was removed using a glass disposable pipette and new diet was immediately placed in each queen cup according to the following schedule to account for larval growth: day 1- 10 µl, day 2- 10 µl, day 3- 20 µl, day 4- 30 µl, day 5- 40 µl, and day 6- 50 µl.

Kaplan-Meier survival analysis

The 6-d larval survival data were segregated by pesticide treatment and analyzed using Kaplan-Meier survival analysis [27]. This estimate generally assumes independence among the individual death events and randomization within the treatment group. The hazard rate $h(t)$ is the conditional probability of failure or death in a small time period given that the subject has survived up until a specified time t . The greater the value of the hazard rate, the greater the probability of impending death. The null hypothesis of no difference between survival curves of treatment and control groups was tested by the Log-rank test that weights

each death by the square root of the total number of individuals at risk per time interval, placing less emphasis on deaths occurring later in the experiment. All the survival analyses were implemented in SAS survival program (SAS/STAT® 9.2 User's Guide).

Comparison between adult and larval sensitivity

The difference in sensitivity to the same pesticide between adult bees and larvae can be quantitatively evaluated by comparing the actual larval mortality per day from the *in vitro* test with the predicted mortality for adult bees if exposed to the same concentrations of pesticides. The larval mortality data were corrected with Abbott's formula beforehand. Here, the impacts of pesticide treatments on adult bees were estimated from the adult acute topical LD₅₀ data converted to whole-bee LC₅₀ values [1], because neither the chronic nor acute oral toxicity data of adult bees are currently available for all pesticides selected for this study. Predicted adult toxicity can be estimated as a function of the magnitude of toxicant exposure and the individual's sensitivity to a toxicant, which is generally characterized by the probit model [28]. The predicted proportion of insects killed (\hat{p}), in probit transformed units, calculated as $\hat{p} = a + bx$ where a = intercept and b = slope from the regression of the transformed data and x is the log-transformed concentration or time. Results of probit analyses are reported typically as a concentration or time required to kill a certain proportion of the test insects (e.g., LC₅₀). Table 1 shows the average LC₅₀ values from the literature [1] and probit slopes from other sources [28]. One exception is chlorothalonil, which is estimated using the default probit slope of 4.5 because its mortality levels under topical or oral applications to honey bees are found to be insufficient to establish a dose-response relationship. Therefore, the probit function for each pesticide to adult honey bees can be inferred from the LC₅₀ values (x), probit mortality ($\hat{p} = 5$) and probit slope (b) [13,28]. Then, the probit model can be extrapolated to predict the probability of an impact of each pesticide on adult bee survival for a specified concentration. Using the Probit program in SAS 9.2 (SAS/STAT® 9.2 User's Guide), the predicted probit-type mortality can be transformed to the original percent units and compared with the actual larval percent mortality data. Using the compilation of acute data from different sources may complicate the accurate estimation of the adult toxicity because of the heterogeneity introduced by differences among the studies; however, given the limitations we felt this was a reasonable approach to obtain a first approximation of the differences in adult and larval sensitivity to the same pesticide exposure.

Pesticide interaction determination

We used significant departures from additive toxicity to define antagonistic and synergistic interactions between pesticides in mixtures [29]. The expected additive toxicity for the chemical mixture is the sum of each chemical's toxicity to larval survival, calculated as f chemical components in the pesticide mixture and h_i is the hazard rate for a specific component estimated from the laboratory bioassay data. The sum of the responses (Eh_n) to the individual components is estimated based on the assumption that the selected pesticide mixtures are the combination of substances with independent modes of action or similar modes of action. The mixture toxicity can be predicted as follows: *Additive interactions*—Simultaneous action of components in which the observed response of honey bee larvae to a mixture (h_n) is equal to the sum of the responses (Eh_n) to the individual components; *Synergistic interactions*—Simultaneous action of components in which h_n is significantly higher than Eh_n ; *Antagonistic interactions*—Simultaneous action of components in which h_n is significantly less than Eh_n .

Table 1. Comparison between the predicted adult mortality rate (PM, %) for each tested concentration (Conc., mg/L) of four pesticides using a probabilistic toxicity model and the observed brood mortality rate (AOM, %) for bee larva from the 6-d *in-vitro* rearing experiments.

Pesticide	Adult honey bee				Honey bee larva						
	Inverse probit prediction			PM ^c	<i>In-vitro</i> brood test						
	β^a	LC ₅₀ ^b	Conc.		1-d ^d	2-d ^d	3-d ^d	4-d ^d	5-d ^d	6-d ^d	AOM ^e
Fluvalinate	2.5	15.86	3	3.6	3.13*	8.06	12.28	10.00	11.11	68.85**	11.72
Coumaphos	2.9	46.3	8	1.4	6.25*	1.67	8.47	5.56	3.92	53.73**	8.60
Chlorothalonil	4.5	1110	34	4 E-10	0.00	8.93	7.84	12.77	7.32	56.60**	9.82
Chlorpyrifos	10	1.22	1.5	82	0.00	4.17	8.70	33.33**	32.14**	0.00	10.07

^a β is the slope of the probit function for different pesticides [13,28].

^bLC₅₀ is the median lethal concentrations of each pesticide to adult honeybees [1].

^cPM = predicted adult mortality rate (%) for each pesticide at the tested concentrations using inverse prediction of the probit function.

^d1,2,3,4,5,6-d is the observed conditional mortality rate (%) for larval bees at each age (in day) in the *in vitro* rearing process.

^eAOM = average daily mortality rate (%) for larval bees in the *in vitro* rearing process.

*Significant at $p < 0.05$;

**significant at $p < 0.001$. (Statistical differences in larval survival were assessed between pesticide-treated and solvent control groups.)

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We did not test different concentrations of each pesticide component and of the combinations to fit dose-response curves. Neither food intake nor concentrations of pesticides consumed by each larva were measured during the oral feeding. Therefore, this method does not allow exact quantification of the level of interaction but makes only an initial qualitative assessment of synergism or antagonism.

Results

Control toxicity

No significant differences in larval mortality were observed when larvae were reared on untreated artificial diet or diet mixed with 1% methanol or 1% acetone (Log-rank test, $p > 0.05$) (data not shown). These three control groups showed an accumulative 6-d percent mortality of approximately 17.2% (Fig. 1), which is within the normal range observed for control mortality using the *in-vitro* larval rearing protocol [24,30]. Because control mortality exceeds 10%, the larval mortality data from treatment groups were corrected with Abbott's formula.

Single pesticide toxicity

Chronic exposure of bee larvae to each of the four pesticides at tested concentrations showed significant toxic effects on larval survival (Log-rank test, $p < 0.0001$), resulting in an overall 2- to 4-fold reduction in the total 6-d percentage survival compared to the control mortality (Fig. 1A). Based on age-specific toxicity data, mortality rates for each pesticide were uneven across different larval stages (Fig. 1B). For 1-day-old larvae, 8 mg/L coumaphos and 3 mg/L fluvalinate were more toxic than the other two pesticides. The 2 and 3-day-old larvae showed similar sensitivity to different pesticide exposures, approximately 10% mortality per day. The 4 and 5-day-old larvae were most sensitive to 1.5 mg/L chlorpyrifos, causing more than 32% larval death each day (Table 1). A dramatic increase in larval mortality for 6-day-old larvae was observed in 34 mg/L chlorothalonil and the two miticide groups, ranging from 53.73% to 68.85%. Using the probit model, notable differences were found in pesticide sensitivity between the adult bee and larvae (Table 1). Among the four pesticides tested, 1.5 mg/L chlorpyrifos was the only treatment that adult bees were more susceptible to than the larvae. For the

other pesticides, the larvae showed increased sensitivity over that of adult bees. Notably, chlorothalonil at the sublethal concentration of 34 mg/L was least toxic to adult bees, however most toxic to larvae followed by 8 mg/L coumaphos and 3 mg/L fluvalinate. On average, coumaphos was the least toxic to larval bees among the four pesticides.

Synergistic interactions

I. Chronic toxicity of chlorothalonil and coumaphos. The effects of chlorothalonil (34 mg/L), coumaphos (8 mg/L), and their mixture on larval survival through the 6-d development are shown in Fig. 2A. In the first 3 days of larval rearing, these three groups exhibited similar survival curves ($p = 0.1988$, Log-rank test). Subsequently, the larvae reared on the diet contaminated with the chlorothalonil/coumaphos mixture died most quickly. The risk of 4-day-old larvae being killed by the mixture was higher than for the other stages of larvae and the single pesticide groups. The hazard rate of the combination group ($\ln(4) = 0.523$) was 9-times higher than the coumaphos group ($\ln(4) = 0.057$) and 3-times higher than the chlorothalonil group ($\ln(4) = 0.136$). The conditional probability of 4-day-old larvae being killed by the mixture treatment was 5-times higher than that of expected additive toxicity (Fig. 2B, $\text{Ehn}(4) = 0.0965$, $p < 0.0001$, Mann-Whitney test). Therefore, the pairing of chlorothalonil and coumaphos produced a significant synergism on mortality of larvae older than 4 days.

II. Chronic toxicity of chlorothalonil and fluvalinate. For the 4-day-old larvae, the hazard rate of the mixture ($\ln(4) = 0.78$) was the highest during the 6-d larval development, which was 7-times higher than the fluvalinate (3 mg/L) group ($\ln(4) = 0.105$) and 5-times higher than the chlorothalonil (34 mg/L) group ($\ln(4) = 0.136$) (Fig. 2C). The chlorothalonil/fluvalinate mixture at the tested concentrations gave a synergistic interaction, which significantly magnified the hazard rate by 7 fold over the sum of the individual effects (Fig. 2D, $\text{Ehn}(4) = 0.121$, $p < 0.0001$, Mann-Whitney test).

Additive interactions

I. Chronic toxicity of fluvalinate and chlorpyrifos. Larval survival on fluvalinate (3 mg/L) and chlorpyrifos (1.5 mg/L) declined the fastest among pesticide mixture treatments, ranging

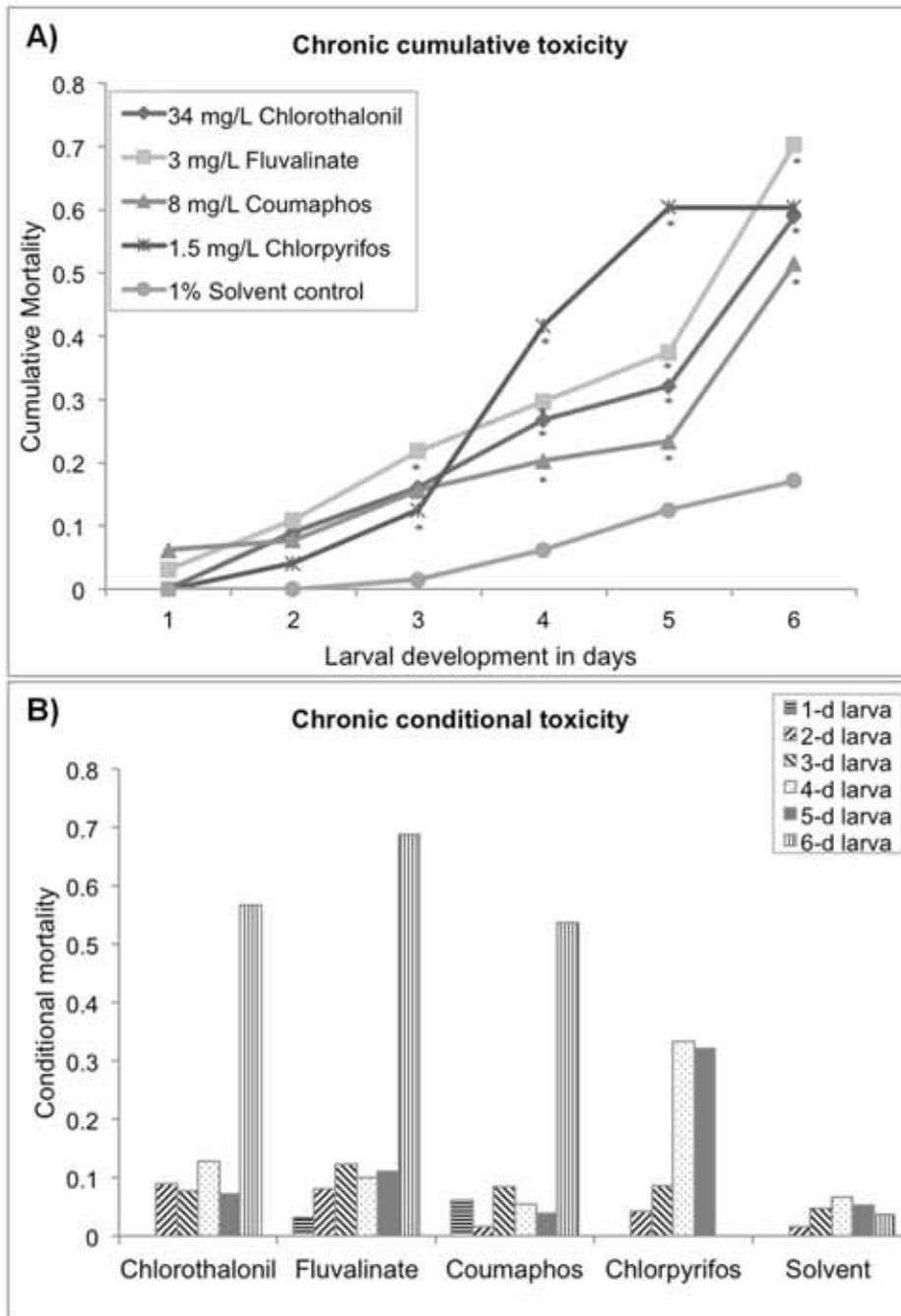


Figure 1. Larval survival during the 6-d development stage reared on artificial diet contaminated with four pesticides at the selected concentrations and a 1% solvent control. (A) shows the cumulative mortality of honey bee larvae through 6-d development continually exposed to 34 mg/L Chlorothalonil, 3 mg/L Fluvalinate, 8 mg/L Coumaphos, 1.5 mg/L Chlorpyrifos and 1% solvent; (B) illustrates the conditional mortality for different development stages of bee larva. Asterisks denote significant difference from the respective solvent controls (analysis of variance, Log-rank test, $p < 0.0001$). doi:10.1371/journal.pone.0077547.g001

from 4.17% to 70.83% (Fig. 3). No significant differences were found in larval survival between single component groups through the 6-d development (Fig. 3A, Log-rank test, $p = 0.1711$). This binary combination produced additive toxicity. The 6-d cumulative percent mortality caused by this mixture ($h_n = 71\%$) was slightly higher than the sum of the response to single components, but not at a significant level (Fig. 3B, $E_h = 48.96\%$, $p = 0.171$, Mann-Whitney test).

II. Chronic toxicity of chlorpyrifos and coumaphos. The larval chronic toxicity of this combination treatment was the highest among tested pesticide mixtures causing from 10.4% to 79.2% mortality during the 6 days. Survival was least affected by the diet with 8 mg/L coumaphos (Fig. 3C). The interaction between these pesticides showed an additive effect. The 6-d cumulative percent mortality of larvae reared on the mixture

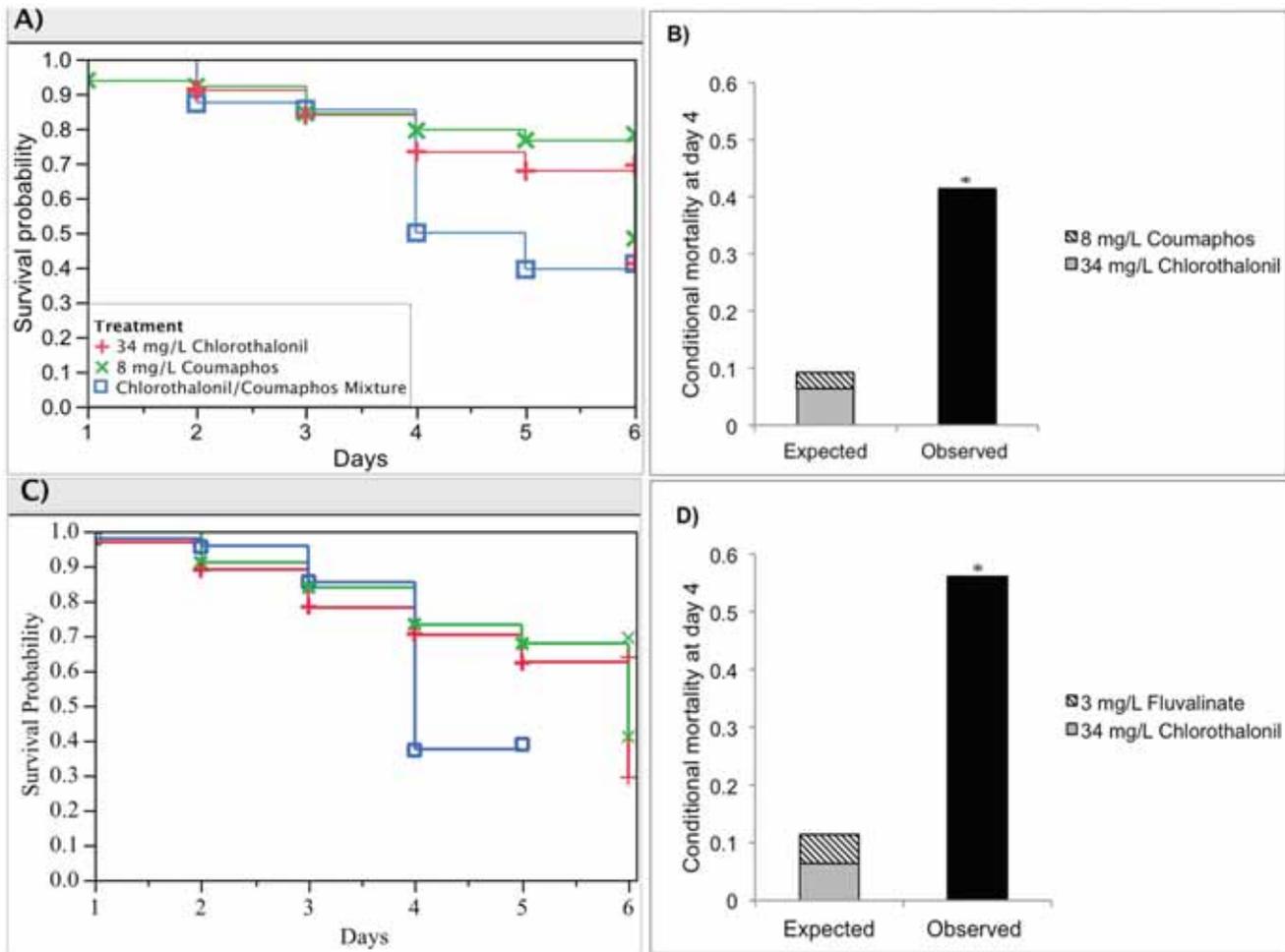


Figure 2. Synergistic interactions for two pairs of pesticide mixtures: 8 mg/L Coumaphos, 34 mg/L Chlorothalonil and the mixture; 3 mg/L Fluvalinate, 34 mg/L Chlorothalonil and the mixture. (A) and (C) show the respective Kaplan-Meier survival plots for honey bee larvae reared for each pair of pesticide mixture; (B) and (D) illustrate the interaction determination based on the deviation of observed mixture toxicity (black bar) from the expected additive toxicity (stacked bar). Asterisks denote significant difference from the expected additive toxicity (Mann-Whitney test, $p < 0.0001$). doi:10.1371/journal.pone.0077547.g002

($h_n = 79.2\%$) did not differ significantly from expected additive toxicity (Fig. 3D, $Eh_n = 56\%$, $p = 0.558$, Mann-Whitney test).

III. Chronic toxicity of fluvalinate and coumaphos. The survivorship of larval bees on the combination and fluvalinate alone treatments exhibited a similar gradual declining trend, achieving the highest cumulative mortality at the end of the 6-d development (Fig. 3E). Both showed more toxicity to larval bees than coumaphos alone (Fig. 3E, $p = 0.0425$, Log-rank test). Fluvalinate and coumaphos, mixed at 3 mg/L and 8 mg/L respectively, showed an additive effect. The accumulative percent mortality in the mixture group ($h_n = 68.75\%$) did not vary significantly from the expected additive toxicity (Fig. 3F, $Eh_n = 60.94\%$, $p = 0.052$, Mann-Whitney test).

Antagonistic interactions

I. Chronic toxicity of fluvalinate and chlorothalonil at low concentrations. The 3.4 mg/L chlorothalonil and 0.3 mg/L fluvalinate mixture showed the least toxicity to larval development among pesticide combinations tested (Fig. 4A). Especially, for the 4-day-old larva, the hazard rate of individual component groups ($h_{CL}(4) = 0.214$, $h_{Flu}(4) = 0.259$) was greater than twice the mixture treatment ($h_n(4) = 0.088$). This mixture showed antago-

nistic interaction, significantly reducing the hazard rate of 4-day-old larvae by three-fold from the expected additive toxicity (Fig. 4B, $Eh_n(4) = 0.2365$, $p < 0.0001$, Mann-Whitney Test).

Three-component mixture toxicity

All six possible pairings were selected to determine the toxicity for three-component mixtures including chlorothalonil/fluvalinate/coumaphos and fluvalinate/coumaphos/chlorpyrifos. The only significant difference found was when coumaphos (8 mg/L) was added to the two-component mixture of fluvalinate (3 mg/L) and chlorothalonil (34 mg/L), giving a 3% reduction in the 6-d accumulative larval mortality ($h_n = 38\%$) from the expected additive effect (Fig. 4C and 4D; $Eh_n = 41.41\%$, $p = 0.006$, Mann-Whitney Test). The other five pairings did not yield significant changes in larval survival when adding one component into the existing binary mixtures.

Four-component mixture toxicity

Two pairings of mixtures including chlorothalonil added to fluvalinate/coumaphos/chlorpyrifos and chlorpyrifos added to chlorothalonil/fluvalinate/coumaphos were tested at the same concentrations as before to determine toxicity interactions in going

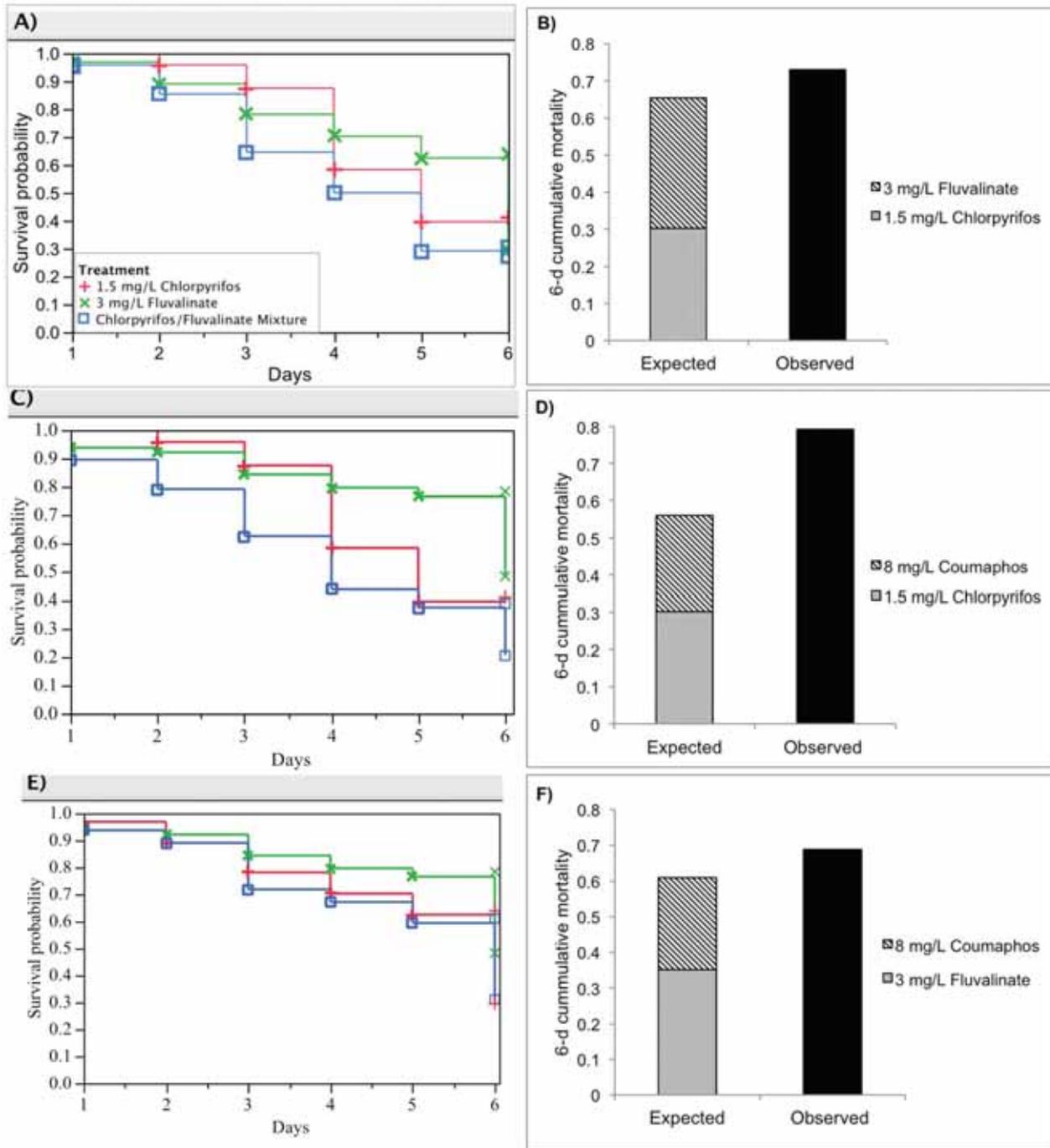


Figure 3. Additive effects for three pairs of pesticide mixtures: 3 mg/L Fluvalinate, 1.5 mg/L Chlorpyrifos and the mixture; 8 mg/L Coumaphos, 1.5 mg/L Chlorpyrifos and the mixture; 8 mg/L Coumaphos, 3 mg/L Fluvalinate and the mixture. (A), (C) and (E) show the respective Kaplan-Meier survival plots for honey bee larvae reared for each pair of pesticide mixture; (B), (D) and (F) illustrate the interaction determination based on the deviation of observed mixture toxicity (black bar) from the expected additive toxicity (stacked bar). doi:10.1371/journal.pone.0077547.g003

from three- to four-component mixtures. There were no significant changes in larval survival when integrating a fourth component into these three-component mixtures. The four-component mixture caused 54.17% larval mortality at the end of the 6-d larval development.

‘Inert’ ingredient toxicity

Chronic exposure of bee larvae to the ‘inert’ ingredient NMP at seven different concentrations ranging from 0.01% to 1% greatly impacted larval survival (Fig. 5). Increasing amounts of NMP correspondingly increased larval mortality. A 1% concentration (10,000 mg/L) of NMP was the most acutely toxic, generating 100% mortality within 24 h after treatment. Even for the lowest

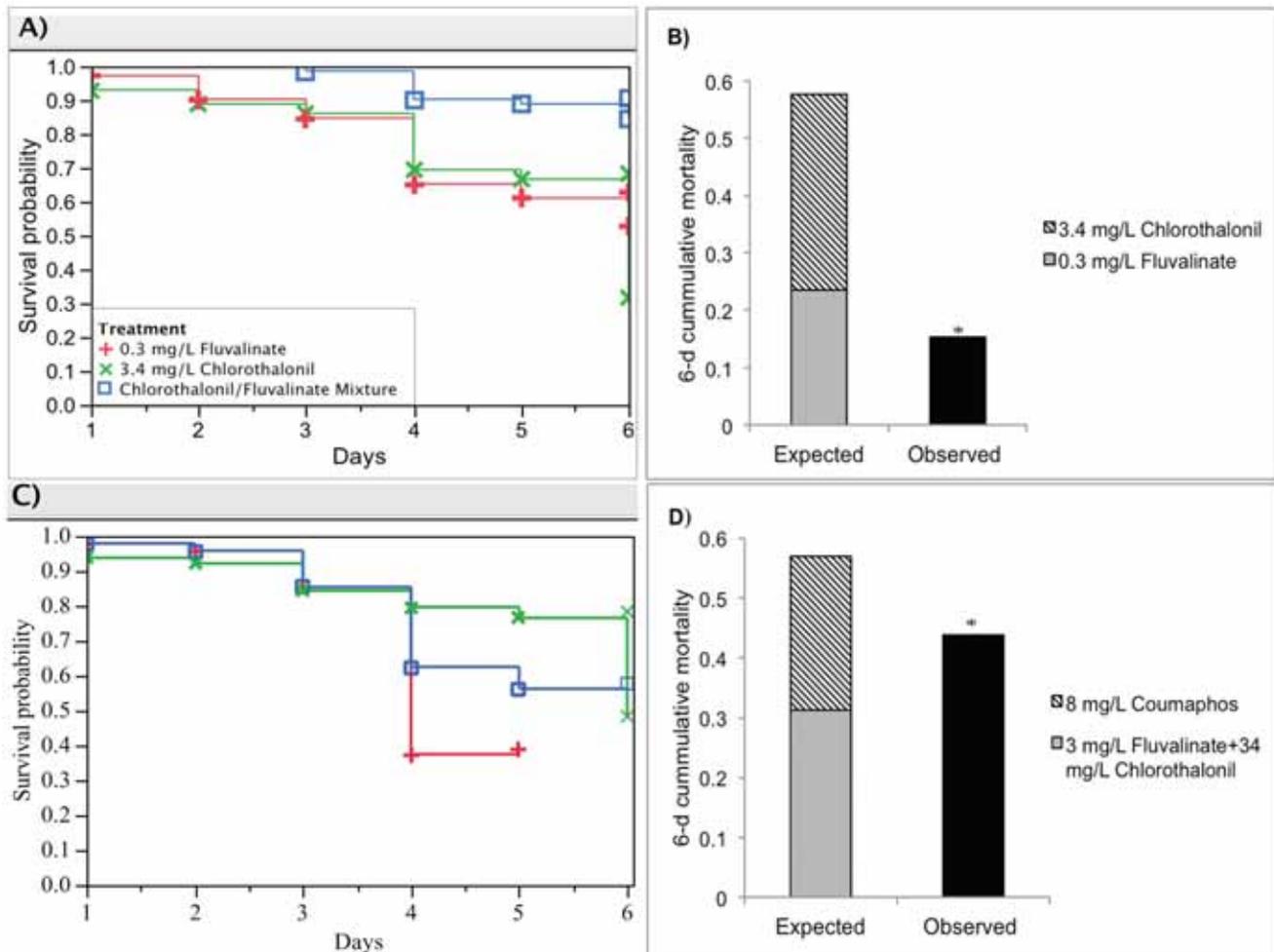


Figure 4. Antagonistic interactions for two pairs of pesticide mixtures: 0.3 mg/L Fluvalinate, 3.4 mg/L Chlorothalonil and the mixture; 3 mg/L Fluvalinate+34 mg/L Chlorothalonil mixture, 8 mg/L Coumaphos and the three-component mixture. (A) and (C) show the respective Kaplan-Meier survival plots for honey bee larvae reared for each pair of pesticide mixture; (B) and (D) illustrate the interaction determination based on the deviation of observed mixture toxicity (black bar) from the expected additive toxicity (stacked bar). Asterisks denote significant difference from the expected additive toxicity (Mann-Whitney test, $p < 0.0001$). doi:10.1371/journal.pone.0077547.g004

concentration of 0.01% (100 mg/L), the estimated time to cause 50% larval mortality was 4 days.

Discussion

Chronic toxicity

Our findings suggest that chronic dietary feeding at hive levels of common pesticide ingredients including the fungicide chlorothalonil, miticides fluvalinate and coumaphos, and insecticide chlorpyrifos, individually or in mixtures, have statistically significant impacts on honey bee larval survivorship. A significant increase in larval mortality was found at or beyond 4-d of feeding. This is the first study to report serious toxic effects on developing honey bee larvae of dietary pesticides at measured hive residue concentrations. The maximum concentrations of fluvalinate, coumaphos, chlorothalonil, and chlorpyrifos found in our hive samples are 204 mg/L, 94.1 mg/L, 98.9 mg/L, and 0.9 mg/L, respectively (Table S2), which are much higher for the miticides and fungicide, or similar for the insecticide, to those levels tested here (Table 1). This chronic (6-d) toxicity is likely to be undetected in a conventional acute (24/48 h) toxicity study, resulting in

potential underestimation of pesticidal effects. The lethal effects on honey bee larvae appearing after 4-d continuous exposure to pesticides at low concentrations are also observed in adult honey bees. The accumulated dose of the organophosphorus insecticides acephate, methamidophos or dimethoate resulting in 50% adult bee mortality was over 100-fold lower than the respective acute 24 h oral LD₅₀ [31]. For these organophosphates and also the pyrethroids tested, their toxicity to worker bees was significantly increased by continuous versus single ingestion of the contaminated food. At low doses of imidacloprid, adult bee mortality was observed only 72 h after onset of feeding in contrast to immediate effects at much higher doses [32].

The causes for chronic larval bee toxicity for 6-d dietary subacute pesticide exposures remain unknown. It may be associated with the extended time needed to accumulate sufficient insecticide concentrations internally to exert nerve action at central target sites, which is consistent with the pharmacological receptor theory; or may reflect variation in honey bee detoxification capacities from the more peripheral to internal tissue sites. For instance, the results of high toxicity of low doses of all imidacloprid metabolites suggest the existence of binding sites with different

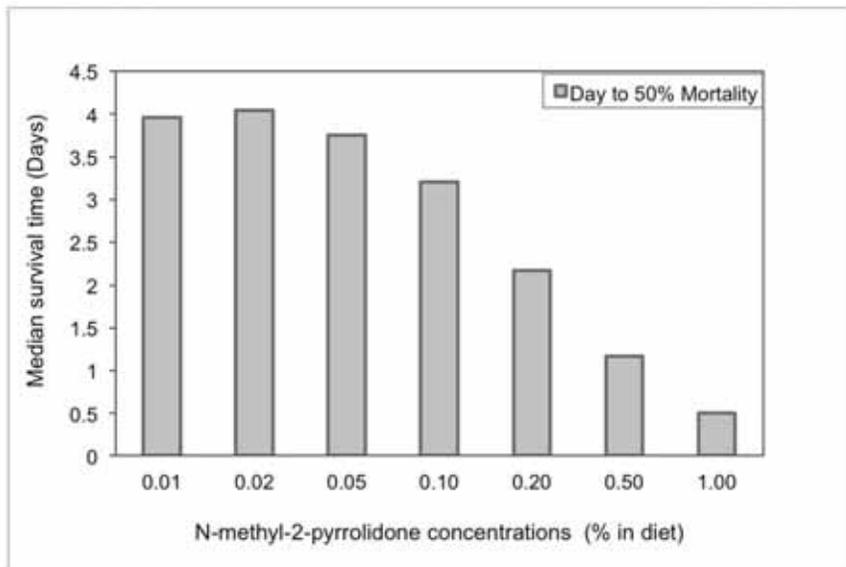


Figure 5. The estimated time to cause 50% larval mortality by seven nominal concentrations of N-methyl-2-pyrrolidone mixed in larval diet.

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affinities in honey bees [32]. Another explanation may be that honey bee detoxification mechanisms are not induced by chronic exposure of low concentrations of active substances, but require higher more acute concentrations to impact honey bee susceptibilities. In the former case, bee mortality would be latent due to the time needed for pesticide bioaccumulation, further favored by the more lipophilic pesticides fluvalinate, coumaphos, chlorpyrifos and chlorothalonil tested here. The latter case of acute higher concentrations driving induction of detoxification enzymes can result in both antagonistic and synergistic effects on the target-effective insecticide concentration depending on if the induced cytochrome P450 first activates (e.g., chlorpyrifos, coumaphos to respective oxons) or detoxifies (e.g., fluvalinate) the insecticide [33,34]. Other induced enzymes (e.g., hydrolases, glutathione transferases) will further degrade and detoxify the primary metabolites.

It is also plausible that more general stress mechanisms (e.g., altered feeding, suppressed growth) dominate the chronic response. For example, exposures of some repellent pesticides such as pyrethroids at sublethal levels have been demonstrated to impair feeding behaviors of honey bees and bumble bees [3,8]. In the case of honey bee larvae, they retain internally all metabolic wastes throughout the larval stage up to the pupal molt after which they defecate a waste pellet called the meconium [25]. Concentrations of pesticides and metabolites within brood tissues may result in continuous pesticide stress [35], which differs from the adult honey bee and most other insects where excretion of toxic wastes regularly occurs. Little information is available on the distribution of fluvalinate [36] and coumaphos [37] and their degradates in honey bee adults and brood. Further studies to examine the distribution and accumulation of fluvalinate, coumaphos, chlorpyrifos and chlorothalonil and their metabolites, in honey bees at different developmental stages are needed. Meanwhile, how honey bees at different life stages withstand chronic exposure need more detailed study of metabolic regulation in this social insect.

Remarkably, among the four pesticides tested in the present study, immature honey bees are highly vulnerable to the common

fungicide chlorothalonil (Figs. 1 and 2). Dietary chlorothalonil killed more than 50% of larvae in 6 days at a level of 34 mg/L, a nontoxic dose to adult bees in acute bioassays (Table 1). This difference in larval to adult susceptibility was the largest among the four pesticides tested. It is unclear why, larval bees exhibited much greater sensitivity to chlorothalonil compared to adult bees; however, the present results demonstrate that investigating fungicide impacts on honey bees is particularly necessary for a realistic evaluation of pesticide impacts on colony health, given the frequent detections of chlorothalonil in pollen and wax samples. Hence, considering that honey bees are experiencing a diverse array of agrochemicals in the hive, the chronic toxicity test may better assess pesticide exposure for a honey bee colony.

Mixture toxicity

Currently, studies of mixture toxicity between different classes of pesticides at concentrations of environmental relevance are rarely available for honey bees [34]. The present study of four pesticides in all combinations is the first study to investigate the potential synergism of common pesticides at realistic exposure levels to larval bees. The present results showed interactions between binary combinations of synthetic pesticides tested were mostly additive, which can be attributed to the same or independent mode of actions of the pesticides involved [33,34]. For instance, additivity of the coumaphos/chlorpyrifos mixture may be explained by their identical action as organophosphate inhibitors of acetylcholinesterase. The additive toxicity of the pyrethroid fluvalinate with either coumaphos or chlorpyrifos is probably due to the independent primary action of the former on nerve sodium channels. Our result with larvae is not consistent with the adult honey bee study of Johnson et al., where the combination of fluvalinate and coumaphos was synergistic [13]. This discrepancy may be explained by the different life stage, lower insecticide concentration levels, and longer length of exposure used here.

The three and four component mixtures of tested pesticides have mostly demonstrated additive effects in larval bees. This finding is in general agreement with the Funnel Hypothesis [38], which states that the toxicity will tend towards concentration

additivity as the number of components in equitoxic mixtures increases. One exception was the significantly less than additive response when coumaphos was integrated into the fluvalinate and chlorothalonil mixture. That coumaphos antagonizes the synergistic effect of fluvalinate and chlorothalonil may be related to its possible induction of the detoxification of one or both of the other pesticides. This anomaly may be related to the observation that elevated coumaphos levels in brood had the highest discriminatory value with regard to healthy bee colonies whereas higher levels of this miticide in the pollen food correlated with colony collapse [39], again indicating that pesticide susceptibilities differ across honey bee developmental stages.

Remarkably, binary mixtures of chlorothalonil with the miticides fluvalinate or coumaphos were synergistically toxic to 4-day-old bee larvae. This is the first demonstration for honey bee brood of a synergistic interaction between dominant in-hive miticides and the frequently-encountered fungicide chlorothalonil at environmentally relevant concentrations. Synergism with chlorothalonil and fluvalinate but not coumaphos for adult honey bee toxicity has been noted previously [40,41].

Surprisingly, a significant antagonism was found for larval toxicity from the fluvalinate-chlorothalonil combination at one-tenth of the concentrations (Fig. 4) that otherwise exhibited a five-fold synergism (Fig. 2). One rationale behind this latter interaction, beyond the fact that the very diverging pyrethroid-multi-site chlorothalonil mechanisms of action may alone elicit synergistic effects, is that the high concentrations may directly inhibit detoxification enzymes. For example, the competitive inhibition of cytochrome P450 monooxygenase enzymes has been suggested to explain the synergistic interactions among pesticides for adult honey bees such as pyrethroid insecticides or mixtures of organophosphate insecticides and ergosterol biosynthesis inhibiting fungicides [42,43]. Also, synergism between chlorothalonil and the herbicide atrazine has been documented in aquatic species [44]. Modes of action for chlorothalonil range from inhibiting glutathione and other thiol-dependent enzymes or protein receptors, to disrupting or degrading cell membranes causing lysis that can enhance penetration of other pesticides [14]. The tendency toward antagonism of brood toxicity at the lower dietary chlorothalonil-fluvalinate concentration may be associated with alternative peripheral mechanisms such as gut microbial detoxification that may be overwhelmed at higher dosage where more internal neurotoxic effects of the pyrethroid can prevail. The consequence is that biphasic low and high dose response relationships may result depending on the extent of multiple peripheral and internal sites of action that diverge in sensitivity to the toxicants as well as to the available detoxification pathways that differ in a tissue-dependent manner to the concentrations required for their induction.

While the mechanisms of interactions among pesticides with diverse modes of action and their dynamics in the developing honey bee larvae are not known, application of the concentration-addition model combined with chronic feeding tests represents a starting point for investigation of mixture effects at realistic levels and their risks for this pollinator. Considering that the diverse arrays of chemicals [1,2,45] and general additivity exist in the hive environment, examining the toxicity of chemical mixtures in addition to single toxicants is critical for a realistic assessment of pesticide hazards experienced by honey bees and other non-target organisms. In today's agriculture dominated by mass monocultures, adults and larvae of *A. mellifera* are inevitably exposed to transgenic material via pollen consumption of GM-crops [46], which might be another confounding factor for bee health. Although minor evidence showed adverse effects of Bt-crops on *A.*

mellifera, the risk assessment of combined effects of Bt-crops and pesticides are completely lacking [47–49]. Hence, the dose dependency of the synergy, the multitude of compounds, the differences in adult bees and larvae, the possibility of continuous exposures, and the interaction with GM pollen should be taken into account in the environmental risk assessment.

'Inert' toxicity

Another important health issue that involves pesticide formulations and bees is the consequence of the additives or so-called non-active ingredients. The commonly-used 'inert' solvent N-methyl-2-pyrrolidone was found here to be highly toxic to larval honey bees (Fig. 5). Unfortunately, despite the potential toxicity of 'inert' ingredients and their widespread use in pesticide products, their testing and risk assessment seems to be inadequate. There is a growing body of research that has reported a wide range of adverse effects of 'inert' ingredients to human health, including enhancing pesticide toxicities across the nervous, cardio-vascular, respiratory, and hormonal systems [18,50,51]. However, limited data exist on the potential impacts of 'inerts' on non-target pollinators, although recent studies implicate formulation additives or adjuvants as key risk factors [52]. As one example, the toxicity of the fungicide captan to honey bee brood development was attributed to formulation ingredients other than the active ingredient alone [53]. The lack of detailed information of the usage of formulation ingredients greatly impedes appropriate risk assessment of 'inert' ingredient toxicity; therefore, label disclosure of the composition of pesticide formulations would facilitate this much-needed evaluation.

Conclusions

The current study demonstrates the chronic oral and mixture toxicity of common pesticides at hive levels to honey bees at the larval stage. Most notable are the chronic larval toxicities of the fungicide chlorothalonil and its synergistic combinations with frequently used in-hive miticides, and the unexpected high toxicity of the formulation ingredient N-methyl-2-pyrrolidone. Considering the extensive detection of chlorothalonil and its coexistence with other pesticides in diverse combinations especially in hive pollen and wax, and its substantial larval toxicity alone and in mixtures shown here, the application of this and other fungicides during crop bloom cannot be presumed innocuous to pollinating honey bees. Given the critical sensitivity of larvae to chlorothalonil and its complex interactions with other pesticides, the potential impacts of fungicides on colony survival and development need further investigation. In the more complex milieu of this social insect and its aging hive environment, pesticides, formulation additives and their resulting mixtures may have greater long-term impacts on colony health than previously considered. Consequently, the scope of pesticide risk assessment for non-target honey bees should be expanded from the present emphasis on acute toxicity of individual pesticides to a priority for assessment of chronic and mixture toxicities that incorporate fungicides, other pesticide pollutants and their 'inert' ingredients.

Supporting Information

Table S1 Some pesticide formulations that disclose in msds the percentage of the solvent NMP.

(DOCX)

Table S2 Pesticide detections in 329 wax and 496 pollen samples collected 2007–12 from North American honey bee colonies.

(DOCX)

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Author Contributions

Conceived and designed the experiments: WYZ DRS CAM JLF. Performed the experiments: WYZ DRS. Analyzed the data: WYZ CAM. Contributed reagents/materials/analysis tools: WYZ DRS CAM JLF. Wrote the paper: WYZ. Provided suggestions and comments on the manuscript: CAM.

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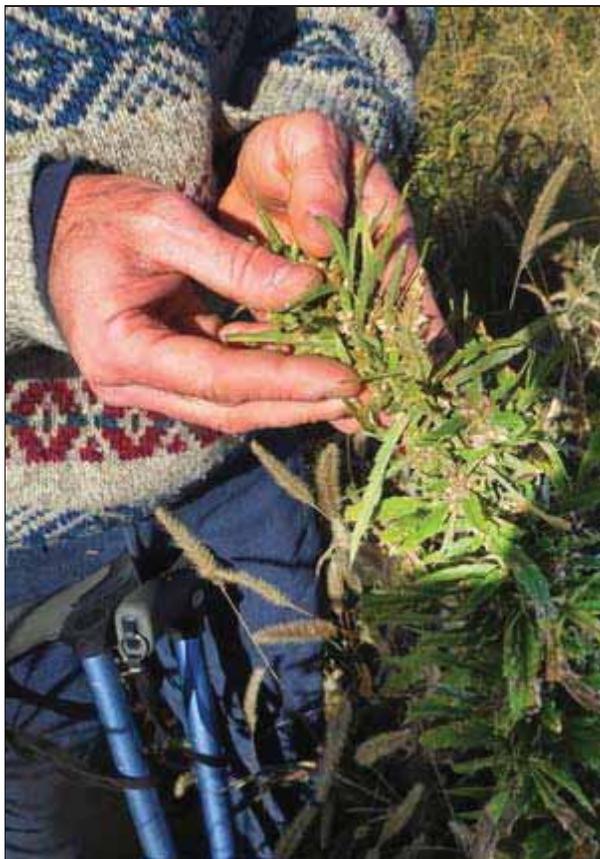
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Growing hemp nears legality in Maine, but just for research

The U.S. farm bill heading for final passage would allow it in Maine, but since industrial crops are still illegal, little study of it is expected.

By J. Craig Anderson canderson@pressherald.com
Staff Writer

Maine is one of a dozen states in which hemp could be grown for research purposes if the farm bill passed Wednesday in the U.S. House of Representatives becomes law.



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In this Oct. 5, 2013 photo, Jason Lauve, executive director of Hemp Cleans, looks at hemp seeds at a farm in Springfield, Colo. during the first known harvest of industrial hemp in the U.S. since the 1950s. Hemp and marijuana are the same species, *Cannabis sativa*, just cultivated differently to enhance or reduce marijuana's psychoactive chemical, THC. (AP Photo/Kristen Wyatt)

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But as long as commercial hemp production remains a violation of federal law, it's unlikely that research institutions in Maine would have much interest in studying it, said an official at one of the state's leading agricultural research centers.

John Rebar, executive director of the University of Maine Cooperative Extension, said the potential market for hemp remains unknown because it is illegal to grow commercially in the United States, and the farm bill wouldn't change that.

"Nobody has looked at hemp as a viable crop for Maine," he said. "Why would you commit to doing industrial hemp research?"

Industrial hemp, which contains only trace amounts of THC, the psychoactive compound found in its close cousin marijuana, has thousands of commercial uses including rope, fabrics, paper, wax, food-grade seeds and oil, and even fuel.

A state law passed in 2009 makes it legal to grow hemp commercially in Maine, if and when it becomes legal nationwide.

If the farm bill passes next week in the Senate and becomes law, as expected, hemp cultivation for research purposes will be allowed in states with their own laws permitting it.

Those states are Maine, California, Colorado, Hawaii, Kentucky, Maryland, Montana, North Dakota, Oregon, Vermont, Washington and West Virginia.

Advocates for the legalization of industrial hemp say it could become a hugely profitable crop for the United States, but Rebar said the point is irrelevant to farmers in Maine until hemp production is legalized.

Agricultural research in Maine is now focused on products with strong market demand, he said, such as hops for brewing beer, wheat for making bread, and cheeses.

"Agriculture is just as much a business as anything else," he said.

In 2003, the Maine Legislature commissioned the Maine Agricultural and Forest Experiment Station to assess the commercial viability of cultivating industrial hemp.

In response, the station's director produced a report that said Maine's soils and climate are adequate to produce it.

The report identified potential benefits to hemp production.

As a crop, hemp is highly pest-resistant and naturally suppresses weeds, which would reduce the cost to farmers and pollution associated with pesticides and weed killers, it said.

Paper made from hemp would reduce deforestation and make paper mills run cleaner, it said, because hemp requires fewer chemicals than wood for processing.

But those benefits are irrelevant as long as commercial hemp production remains a federal crime, the report said.

"As long as this is the case, new products and new uses for hemp will not develop," it said.

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Posted: Today

Updated: 5:46 AM

Protecting Maine lobsters by banning pesticides gets tepid support

The Maine Lobstermen's Association says the state needs a comprehensive assessment of pesticide threat.

By Steve Mistler smistler@pressherald.com
Staff Writer

AUGUSTA — A bill designed to protect Maine's \$340 million lobster industry by banning two pesticides that have been partially blamed for decimating lobster populations in New York and Connecticut is facing a headwind in the Legislature.

L.D. 1678 is sponsored by Rep. Walter Kumiega, D-Deer Isle. It would prohibit the use of methoprene and resmethrin, chemicals that were used during a massive mosquito spraying operation in 1999 to combat an outbreak of West Nile in areas along Long Island Sound.

Shortly thereafter, a severe die-off of lobsters wiped out the fishery there, although warming ocean temperatures and other factors are also believed to have played a role.

Rep. Michael Devin, D-Newcastle, told lawmakers on the Agriculture Conservation and Forestry Committee on Thursday that Maine should join Connecticut and ban the two chemicals, traces of which were found in dead lobsters studied in the sound.

"Whatever we apply in the terrestrial environment eventually makes its way to the coast and out to sea," Devin said.

"The cigarette butt you saw this morning on the sidewalk will end up in the Kennebec River and then flow down to the ocean. Insecticides ... all end up in our ocean."

The proposal, however, lacks the support of the LePage administration and the Maine Lobstermen's Association, a trade group representing the industry. Patrice McCarron, the association's executive director, told lawmakers Thursday that lobstermen are concerned about pesticides, but worry that banning methoprene and resmethrin could give a "false sense of security" while ignoring other chemicals that could be more harmful to lobsters.

McCarron said the association supports a more comprehensive analysis to determine which pesticides, if any, are affecting a fishery that pumps \$1.7 billion into the state economy, according to estimates by the Lobster Institute at the University of Maine.

Such an analysis may be on the horizon.

The Department of Marine Resources, which oversees the lobster fishery, and other stakeholders are discussing whether to conduct a sediment survey of Casco Bay.

The study could be part of an assessment of the risk of all pesticides, not just methoprene and resmethrin.

The assessment would be overseen by the Maine Board of Pesticides Control, which regulates chemical use and helps set policy.

Henry Jennings, director of the pesticide control board, told lawmakers Thursday that the two chemicals have not been used by government agencies in Maine.

But the state would want the chemicals available for use if there is an outbreak of a mosquito-borne disease such as West Nile, he said.

Jennings also said that recent studies have concluded that linking the two pesticides to the Long Island Sound lobster die-off was “fundamentally flawed.”

He warned that banning the chemicals in Maine could have unintended consequences.

“Banning chemicals without a careful assessment of what products will take their place is never sound public policy,” he said. “It generally leads to the use of higher-risk products in their place.”

He added that although Connecticut instituted a similar ban, the study lawmakers there used to justify it has since been invalidated.

No environmental groups testified Thursday.

Methoprene and resmethrin are commonly used in flea and tick control medicine for pets. The Maine Veterinary Medical Association opposes the bill.

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Continued GAP certification

To schedule time for an audit to get GAP certification you may contact the Maine Department of Agriculture, Conservation and Forestry inspectors based in Aroostook County, at 800-292-6552 to schedule a GAP audit later in the spring or summer. Hourly travel costs along with inspection time expenses are included in the final bill for this service. In 2013 the costs were about \$300 to \$500. If the proposed increase goes through please keep in mind that this will almost double the cost for the certification. AgMatters, LLC can work with groups or one on one

with Maine growers to help create their food safety plan in preparation for GAP and Good Handling Practice (GHP) Certification.

Growers who want to learn more about food safety practices for storage or processing facilities may want to attend a Sanitation Course offered by the University of Maine Cooperative Extension for a \$65 fee (including course materials and lunch) on Wednesday, March 5th from 8 to 5 at 203 Hitchner Hall, the University of Maine, Orono. On March 12 and 13, Hazard Analysis Critical Control Point (HACCP) training is also available for food processors, including meat

and poultry, for a \$150 course fee. The training also held at 203 Hitchner Hall, the University of Maine in Orono from 8 to 5pm, includes course materials and lunch. At the end of this training you will have a full HACCP plan for your business.

For further questions, contact Theresa Tilton at 207.942.7396, or 800.287.1485 (toll free in Maine) or theresa.tilton@maine.edu. You may also contact Dr. Jason Bolton at Jason.Bolton@maine.edu or call 207.581.1366 if you have additional food safety questions or for consultation about setting up food processing facilities. 🌱

Researchers Conduct Important Spotted Wing Drosophila IPM Studies on Wild Blueberries

The Wild Blueberry Commission of Maine is collaborating with University of Maine Cooperative Extension faculty to develop studies in support of Integrated Pest Management for Spotted Wing Drosophila (SWD). The IPM research on the Spotted Wing Drosophila, an invasive species that infests small soft fruits such as wild blueberries, strawberries, and raspberries is known to reproduce exponentially while in soft fruit and berry crops. Drs. Frank Drummond, David Handley, and James Dill focused on evaluating preliminary trap and bait method for early SWD detection from which they determined that traps suspended above the crop with yeast and sugar mix seemed to have the best capture rate during August



Tractor demonstration at Field Day at Blueberry Hill Farm in Jonesboro, Maine.

and September. Researchers want to determine what the relationship is between first and subsequent SWD trap captures and occurrence and severity of fruit damage in raspberries, strawberries; and highbush, and wild blueberry. Additional studies

in the coming year will help determine:

- What are the most effective insecticides for SWD management and trap thresholds?
- Field edge or perimeter insecticide application strategies including, the most

continued on page 6

Continued SWD studies

effective crop sanitation tactics, and whether mass trapping of adult SWD and netting of small planting areas will reduce pest pressure

Look for more information about the results of 2013 studies by linking to the Extension website at <http://umaine.edu/blueberries/factsheets/insects/> and review Spotted Wing Drosophila fact sheets. Also over the next year, the University of Maine SWD research studies are projected to determine: the overwintering success of the pest; the field movement inside of perimeter treatments that have occurred; and spring distribution in and around wild blueberry fields. Entomologists and IPM experts will also assess the possible use of biocontrol agents including parasitic insects. Extension presentations of results to growers are expected to occur at the late winter and spring



Commissioner Walt Whitcomb (left) and Dr. David Yarborough, UMCE Wild Blueberry Specialist, at Field Day at Blueberry Hill Farm in Jonesboro, Maine.

education sessions around wild blueberry growing areas, as well as research education during Field Day at the Blueberry Hill Farm in July. As is the case with wild blueberry research, 4-6 years of IPM research is necessary

to prove a practice works consistently. The Commission will continue to work with the UMaine wild blueberry research and extension team on conducting much needed research and ensuring IPM research funds. 🌿

Eastern States Exposition Wrap Up – The Big E 2013

With the good weather occurring over the course of the 17 day Big E in September 2013, the overall attendance record was broken again as the gate hit almost 1.5 million people during the fair! Maine Day at the Fair on the last Saturday was one of the record breaking attendance days during 2013. If you are interested in the vast entertainment offerings and exhibits at the Fair, visit their

website at www.thebigE.com

The Wild Blueberry Commission of Maine wrapped up another successful Big E in the Maine building on the Avenue of States with 25 volunteer growers and their friends and family staffing the Public Education information booth at the Fair. As always we appreciate growers taking time out to come to the Fair, and talk to people about the special Maine wild blueberry.

In the coming year the Wild Blueberry information and food booths will receive booth design upgrades to present a colorful professional exhibit in the Maine Building. We thank those who made 2013 such a successful year! We invite veteran and new volunteers to join us at the Big E in the Fall of 2014, a notice will be distributed in this spring! 🌿

In Spring of 2014, look for WBANA promotional materials that showcase a new brand identity!
