comments provided December 15, 2008 by
Maryland Pesticide Network
Ruth Berlin, LCSW-C, Executive Director
544 Epping Forest Road
Annapolis, MD 21401
410.849.3909, ext. 1
berlinmpn@aol.com

BACKGROUND

The Maryland Pesticide Network (MPN) is a grassroots coalition of 25 organizations in Maryland dedicated to protecting the public and the environment from the hazards of pesticides and promoting safer alternatives. The impact of pesticides is a complex issue about which we will never have perfect knowledge. Therefore, the coalition's work is based on the precautionary principal that,

> *"When an activity raises threats of harm to human health or their environment, precautionary measures should be taken even if some cause and effect relationships are not fully established scientifically."*

MPN (see list of coalition members below) includes health care provider, health-affected, public health, children's health, educational, environmental, labor and agricultural organizations. MPN is the only such coalition in Maryland. MPN's steering committee includes three physicians and a nurse with expertise on the impact of pesticides on public health. The following comments are provided by MPN's steering committee on behalf of the coalition.

The Maryland Pesticide Network Membership

- American Academy of Pediatrics, MD Chapter
- AFL-CIO, MD
- American Lung Association, MD Chapter
- Audubon Naturalist Society
- Assateague Coastal Trust
- Baltimore Physicians for Social Responsibility
- Rachel Carson Council
- Chemical Sensitivity Disorders Association
- Chesapeake Bay Foundation
- Federated Garden Clubs of MD
- Leukemia and Lymphoma Society-MD Chapter
- MD Clean Water Action
- MD Organic Food and Farmers Association
- MCS Referral and Resources
- MD Nurses Association
- MD Association of School Health Nurses
- MD Interfaith Coalition for the Environment
- MD League of Women Voters
- MD League of Conservation Voters
- MD Public Interest Research Group
- MD State Teachers Association
- MD Parent Teacher Association (MD PTA)
- Beyond Pesticides
- Sierra Club, MD Chapter

ENVIRONMENTAL EFFECTS

Certain pesticides present a real and urgent environmental threat to the Chesapeake watershed and aquatic life. Because of diflubenzuron/Dimilin's toxicity to crab, shrimp, and other aquatic invertebrates, it is a restricted pesticide and the product label warns of hazards to aquatic invertebrates.

Washington State's Department of Health notes that by disrupting the normal molting process, diflubenzuron/Dimilin, used for gypsy moth eradication, prevents insects and other invertebrates from becoming adults and breeding. The Department also notes that Dimilin may have broader ecosystem effects than the more selective Btk and NVP products.

In 2007, the New Jersey Department of Environmental Protection (DEP) concluded a month-long review of the Department of Agriculture's (NJDA) petition to waive the state's ban on aerial-spraying of broad-spectrum pesticides. The state upheld the ban, effectively blocking widespread use of the chemical Dimilin.

The state of Rhode Island declined to use Dimiln in response to its lethal impact on lobsters. **EXHIBIT A** provides three abstracts of studies that underscore the danger of Dimiln to aquatic life - specifically blue crabs.

Maryland's and neighboring states' blue crab populations have diminished to the point of serious economic impact on the watermen in those states. As noted in a September 24, 2008 article in the Washington Post [**EXHIBIT B** "Blue Crab 'Fishery Failure' Declared"], "The crabs' numbers have fallen by more than 70 percent since the 1990s.." and "the Commerce Department said the value of the bay's crab harvest, including hard- and soft-shell crabs, had declined 41 percents since the late 1990s." In the last 15 years Maryland has sprayed Btk and Dimilin on an average of 30,557 acres annually with 1991 seeing the highest acreage sprayed, 150,000 acres and 2008 being the second highest year with 99,222 acres sprayed (reported by the MDA to Maryland Gypsy Moth Task Force at the October 8, 2008 meeting).

According to the US Environmental Protection Agency, all the agency's "levels of concern" were exceeded when the agency looked at Dimilin's impacts on freshwater invertebrates (insects and related animals). EPA concluded that "Use of diflubenzuron is expected to cause adverse acute and chronic effects" to these animals. Similar effects were found for estuary fish. In turn, Dimilin "may cause adverse effects" on the animals that feed on the aquatic invertebrates such as game fish, waterfowl, shorebirds, small mammals, reptiles, and amphibians. Additionally, the USDA GYPSY MOTH MANAGEMENT IN THE US: DRAFT Supplemental Environmental Impact Statement June 2008 (DRAFT EIS 2008), states that both diflubenzuron and tebufenozide "will interfere with growth and development" of "both terrestrial and aquatic arthropods" (refer to DRAFT EIS 2008 Vol. IV appendix M page xiii).

We recommend that use of insecticides for gypsy moth eradication should be limited until the full impact on aquatic life is studied.

HUMAN HEALTH EFFECTS OF GYPSY MOTH SPRAY PROGRAM

In addition, MPN is concerned about the health and environmental impacts related to pesticides used for gypsy moth eradication. For example, the DRAFT EIS 2008 raises concern about the occurrence of super-infections when individuals with reparatory infections are co-exposed to Bt. Additionally, the Environmental Protection Agency (EPA) classifies Dimilin as "moderately toxic" to humans. Two breakdown products of diflubenzuron are classified as probable carcinogens according to EPA, p-chloroaniline (PCA) and p-chlorophenylurea (CPU). CPU is the major breakdown product found in water and therefore could be widely distributed in certain waterways following aerial application of Dimilin. Also, the persistence in the environment of the product CONFIRM, also used for gypsy moth eradication, has been shown to result in contamination of food sources for over 90 days.

Animal studies show that diflubenzuron and tebufenozide can cause methemoglobinemia. Although cats and dogs are especially at risk for developing methemoglobinemia, humans are also susceptible (refer to Draft EIS 2008 Vol. IV Appendix J P.3-23). The molecular effects in marine species suggests that other human effects may be possible. Anecdotal reports of human symptoms following exposure in sensitive individuals have been made although no published studies make this link.

Bacillus thurigensis (Bt) is also used for gypsy moth control. Infection of wounds in susceptible individuals has been reported though such reports are rare, nonetheless the USDA cannot dismiss the evidence that such exposures could result in fatalities (refer: **EXHIBIT C** – DRAFT EIS 2008 Vol 3 Appendix F p. 3-31, 3-32). Aerial spraying of pesticides can cause adverse health effects in humans because the small particle size of the spray can penetrate into the lungs. Also, according to DRAFT EIS 2008, “broadcast applications mean that the potential for exposure is high and, in many cases, unavoidable” (refer to DRAFT EIS 2008 Vol. IV Appendix M page xiii).

Some states such as the New England States and New York have decided not to spray pesticides as a means of controlling gypsy moths, yet the Draft EIS 2008 report does not discuss how these states are faring. Other states and countries have also responded to concerns about the impacts of pesticides used for moth eradication:

On June 19, 2008, California announced it would abandon aerial spraying over urban areas in an attempt to eradicate the light brown apple moth. The decision followed months of protests and lawsuits from residents in California after chemicals were sprayed in 2007. Proposed spraying of microcapsules containing synthetic pheromones elicited concerns about potential lung damage from inhalation of the capsules themselves [**EXHIBIT D** – article *Victory in California: Moth Spraying Cancelled* June 2008 and link to lawsuit text]. The lawsuit raised several issues regarding dietary exposure, exposure of sensitive populations (be they human or animal liable to be at risk), dermal and inhalation exposures, spray drifts, impact on ecologically sensitive habitats, and use of alternative environmental controls that must be addressed before aerial spraying continues for moth suppression.

From 2002 to 2004 the New Zealand Ministry of Agriculture and Forestry (MAF) sprayed Foray 48B (a Bt also used in Maryland to control gypsy moths) over Auckland and Hamilton City suburbs. At the height of the spray campaigns over 400 people reported having several symptoms including: headaches, wheezing, diarrhea, gas discomfort, chest pain, problems concentrating and double vision [**EXHIBIT E** – The New Zealand Medical Journal, 14 March 2004, *Symptom complaints following aerial spraying with biological insecticide Foray 48B ...* medical journal article cited in DRAFT EIS 2008 Vol 3 Appendix F Table 8].

When the MAF dismissed these symptoms as psychosomatic, the people convened a “People’s Inquiry” [**EXHIBIT F** - magazine article *Residents Publish People’s Inquiry Report, October 2007*] which was presided over by four international experts. The Inquiry discovered that one of Foray 48B’s inert adjuvants was propylene glycol, a solvent that can harm the kidneys, nervous system, lungs and heart. Propylene glycol breaks down very quickly in the body and is very difficult to detect even when symptoms are present. As a result of the inquiry, an Australian Minister acknowledged that officials were wrong to tell the public there were no health risks.

Many of the 25 symptoms reported in New Zealand track closely with the symptoms of dysautonomia, a condition that occurs when the sympathetic and parasympathic nervous systems do not operate together properly. These systems, which make up the autonomic nervous system, are responsible for involuntary, unconscious functions of the body, such as breathing, digestion, sleeping, heart beat and blood pressure. Chronic autonomic failure can occur as a consequence of exposure to toxic agents [**EXHIBIT G** – Annals of Internal Medicine, 5 November 2002, *Dysautonomias: Clinical Disorders of the Autonomic Nervous System*]

USDA GYPSY MOTH MANAGEMENT IN THE US: DRAFT Supplemental Environmental Impact Statement June 2008

MPN is particularly concerned about the following human health impacts documented in the DRAFT EIS 2008 report.

BACILLUS thuringiensis var. Kurstaki (B.t.k.)

Irritants such as volatile organic compounds (VOCs) found in pesticides can disrupt the autonomic nervous system and manifest as multiple chemical sensitivity (MCS). Among the adverse human health effects associated with B.t.k. exposure, irritant effects are the most common" (DRAFT EIS 2008 Vol 3 Appendix F p. 3-28).

A 2000 Study [**EXHIBIT H** – FEMS Immunol Med Microbiol, **November 2000; 29(3):177-81,** *Super-infection by Bacillus thuringiensis H34 or 3a3b can lead to death in mice infected with the influenza A virus*, Hernandez, et al] cited in June 2008 Impact Statement raises serious concerns about the susceptibility of individuals with influenza or other viral respiratory infections (e.g. the common cold, AIDS, etc.) to developing a super-infection when co-exposed to Bt. Such a super-infection increased mortality in mice significantly [**EXHIBIT C** – DRAFT EIS 2008 Vol 3 Appendix F p. 3-31, 3-32]. Since the DFAFT EIS 2008 acknowledges that "viral enhancement of bacterial infections is not uncommon and the enhancement of B.t.k. toxicity by a viral infection seems plausible" [**EXHIBIT C** - DRAFT EIS 2008 Vol 3 Appendix F p. 3-32], then further use of B.t.k. should be suspended, until studies confirm that it will not result in super-infections in susceptible hosts.

The Swadener 1994 study also cited on page 3-31 noted that some B.t.k. formulations contain sodium sulfite, which may cause adverse effects in asthmatics taking steroid treatments. Furthermore, Swadener noted that the B.t.k. formulation contained sodium hydroxide, "a severe corrosive that can be extremely hazardous." [**EXHIBIT C** - DRAFT EIS 2008 Vol 3 Appendix F p. 3-31 Swadener 1994 Study].

Finally the DRAFT EIS 2008 states that "B.t.k. concentrations in indoor air may remain higher than those in outdoor air" [refer to Vol 3 Appendix F p. 3-18]. This is confirmed in the personal testimonies, where sensitive people seeking to avoid B.t.k. by staying indoors may actually have made themselves sicker by doing so. This population is therefore challenged to find or create safe havens for themselves anywhere near a sprayed area.

GYPCHEK

The June 2008 Impact Statement delineates several concerns about Gypchek, the naturally occurring virus formulated as a control agent for gypsy moths. According to the label, Gypchek is a severe eye irritant (refer to DRAFT EIS 2008 Vol 3 Appendix G p. 3-10). "The toxicity data base on Gypchek is extremely limited for...immunotoxicity, endocrine effects and neurotoxicity" (refer to DRAFT EIS 2008 Vol 3 Appendix G p. vii).

"The toxicity data on Gypchek adjuvants are extremely limited as well" (refer to DRAFT EIS 2008 Vol 3 Appendix G p. viii), but the surfactants used "can have a spectrum of toxic effects, most of which involve irritation to biological membranes" (p. viii). Gypchek contains gypsy moth parts and these constituents have irritant effects on the skin and in the respiratory tract of humans (refer to DRAFT EIS 2008 Vol 3 Appendix G p. 3-1). Gypchek can persist in the lungs with a halftime of about 5 days (p. 3-2).

Gypchek has not been tested for activity as an agonist or antagonist of the major hormone systems, so no definitive hazard identification is possible (refer to DRAFT EIS 2008 Vol 3 Appendix G p. 3-4).

DIMILIN and CONFIRM

According to the latest USDA risk assessment (i.e. refer to DRAFT EIS 2008 Volumes 3 and 4), the greatest health concern for Dimilin and Confirm is methemoglobinemia, a condition that decreases the blood's ability to carry oxygen. This impairment can result in serious adverse effects equivalent to suffocation (refer to DRAFT EIS 2008 Vol 3 Appendix I p. 3-1). It is possible that infants less than 3 months old could be more sensitive to the hematologic effects of Dimilin [refer to DRAFT EIS 2008 Vol 3 Appendix I p. 3-33] and Confirm [refer to DRAFT EIS 2008 Vol 3 Appendix J p. 3-28] than adults. These pages additionally note that infants with an intolerance to cow's milk or soy milk would be at an increased risk of methemoglobinemia if exposed to Dimilin or Confirm. Dimilin and Confirm are likely to have an additive effect on methemoglobinemia (refer to DRAFT EIS 2008 Vol 3 Appendix J p 3-28), so people who travel through or near areas sprayed with these two pesticides are at an increased risk for methemoglobinemia.

Likewise exposure to other compounds in the environment that induce methemoglobinemia may also lead to an additive effect (refer to DRAFT EIS 2008 Vol 3 Appendix J p. 3-28). For example individuals exposed to combustion smoke, carbon monoxide or high levels of nitrates may be at increased risk if exposed to Dimilin or Confirm (p 3-28). There is no apparent dose duration relationship for Confirm. So short-term exposures are likely to lead to changes in the blood comparable to those observed after longer-term exposures (refer to DRAFT EIS 2008 Vol 3 Appendix J p. 4-2). Finally, Dimilin can be absorbed from the skin in sufficient amounts to cause hemotologic effects (refer to DRAFT EIS 2008 Vol 3 Appendix I p 3-1).

A metabolite of Dimilin, 4-chloroaniline, is of particular concern. In addition to inducing methemoglobinemia, 4-chloroaniline, which is formed by the biodegradation of Dimilin in the soil, is a Group B-2 carcinogen (refer to DRAFT EIS 2008 Vol 3 Appendix I p 3-2 and p 3-10). Concentrations of 4-chloroaniline peak in 30 to 70 days. The consumption of water that is contaminated with 4-chloroaniline is "the greatest source of concern for members of the general public in the application of Dimilin to control Gypsy Moths" (refer to DRAFT EIS 2008 Vol 3 Appendix I p. 3-33). The other cancer concern is the consumption of vegetation sprayed with Dimilin (refer to DRAFT EIS 2008 Vol 3 Appendix I p. 3-24)

One formulation of Dimilin contains petroleum oil. At sufficiently high doses, some petroleum oils can cause gastrointestinal, central nervous system and renal effects (refer to DRAFT EIS 2008 Vol 3 Appendix I p 3-9). In an acute inhalation study involving Dimilin, excessive salivation and labored breathing were observed both during and after exposure (p. 3-5). Dimilin can cause slight to moderate eye irritation (p. 3-14).

With regard to Confirm, the greatest threat to human health involves the longer term consumption of contaminated vegetation (refer to DRAFT EIS 2008 Vol 3 Appendix J p. xi). Consumption of contaminated vegetation as for example, berries sprayed with Confirm, can result in an unacceptable risk for over 90 days after Confirm is applied (p 3-27) Dermal absorption is the primary route of exposure for workers. If Confirm is inhaled at very high exposure levels, it will induce irritation. Confirm may cause skin or eye irritation (p 3-1). At the upper range of plausible exposures, the hazard quotient for ground spray workers reaches a level of concern (p xiv). Confirm is associated with adverse reproductive effects in experimental mammals (p 3-1). The EPA has failed to derive an acute RFD for Confirm. This limits the ability to quantitatively characterize risks associated with acute exposures. Confirm is relatively persistent in the environment and may be subject to bio-concentration (p 2-1).

MARYLAND PHYSICIAN LETTERS

A letter [**EXHIBIT I** - Letter Dr. Ziem Sept 22, 2008] from a doctor, who specializes in Occupational and Environmental Health in Emmetsburg, MD, Dr. Ziem, cites the Hazardous Substance Database which states that the half life for Confirm can be 3 to 6 months. Moreover, Confirm has an agent designed to cling to contact surfaces for prolonged periods of time. The result is that people living near spray zones are likely to track the confirm into the home where it persists for up to 6 months even if people take all possible measures to avoid exposure. Consequently Dr. Ziem's patients who are sensitive to Confirm have experienced serious neurological, respiratory and systemic symptoms for up to 6 months.

Dr. Layton, a physician in Towson, MD who has treated a number of patients who react to pesticide exposure states in his letter of September 11, 2008 (**EXHIBIT J**) that a number of individuals when exposed to gypsy moth spraying develop a host of medical symptoms and that these chemicals are putting the citizens of Maryland at significant risk.

SYNERGISTIC EFFECTS

A final concern about the use of pesticides in the environment involves mixtures. According to the Draft EIS 2008 Vol. III appendix F page 3-32, "exposure to other chemicals in the environment may impact the sensitivity of individuals to B.t.k. or other agents; however, the available data are not useful for assessing the significance of such interactions." Very little is known about the effects of pesticides in complex mixtures. No testing is required for pesticide mixtures. Consequently the current system of pesticide regulation does not adequately address pesticide synergy, putting public health and the environment at risk. Pesticides are simply tested in isolation. We know that pesticide mixtures, even at levels that are 10 to 100 times below the EPA standards for ingredients can be harmful. Moreover scientists have found that with some chemicals, traces as minute as mere parts per trillion, have adverse biological effects. By ignoring the real-world interactions between different chemicals, the safety reports may be grossly underestimating the danger these chemicals cause. [Refer to Environmental Health Perspectives, April 2006, *Pesticide Mixtures, Endocrine Disruption, and Amphibian Decline: Are we understanding the impact,* Tyrone Hayes, et al and January 23, 2007 subsequent study in same publication]

IMPACT OF NOT SPRAYING

Finally, the Draft EIS 2008 mentions speculation in Vol. IV Appendix L page 3-5, that heavy infestations of gypsy moths may actually slow the spread of Lyme disease. The primary vector in the Northeastern and Central US for Lyme disease is a tick carried by the white-footed mouse. Since the white-footed mouse eats acorns produced by Oak trees, a decrease in acorns caused by gypsy moth infestation in Oak trees may in turn cause a decrease in the white-footed mouse population and consequently limit the transmission of Lyme disease to humans.

WEAKNESSES OF THE DRAFT EIS 2008 STATEMENT

The Draft EIS 2008 has several weaknesses. It fails to mention the outcomes in states that have chosen to suspend pesticide applications to control gypsy moths. As reported by the Maryland Department of Agriculture to the Maryland Gypsy Moth Task Force on December 10, 2008, these states are Maine, Vermont, New Hampshire, Massachusetts, Rhode Island, Connecticut, New York and Delaware. The Connecticut Agricultural Experiment Station has documented that spraying makes the problem worse because "egg masses in plots previously treated with chemical insecticides were larger than those in untreated plots" [**EXHIBIT K** - p.21 of June 1974 Bulletin Number 744 of the Connecticut Agricultural Experiment Station B744 (1974) *Gypsy Moth Aerial Tests with Bacillus thuringiensis and Pyrethroids*. Kaya, H.K.; Dunbar, D.M.; Doane,C.C.; Weseloh, R.M.; Anderson, J.F.], but it is not discussed in the Draft EIS 2008 . The Draft EIS 2008 mentions an outbreak of dermal irritation in Massachusetts as a result of gypsy moth hair exposure, but fails to say how often these outbreaks have occurred.

There is little discussion of spray drift for any of the pesticides. How far away does a sensitive person have to be to avoid symptoms? The Draft EIS 2008 is often contradictory. On one page it discounts adverse health effects and on other pages it lists several. One such example is the statement in Appendix F p. 3-30 that "there remains no basis for asserting that the use of B.t.k. to control the gypsy moth is likely to have adverse toxic effects on any group" and then goes on to discuss several groups at special risk in Section 3.4.4 of p. 3-31. References need to be double checked throughout the document. Some mentioned in the text are omitted in the list of studies consulted. For example Swandener's 1994 study and Van Netten's 2000 study mentioned in Appendix F are omitted in the bibliography.

More studies on the effect of the program's pesticides on bees and cats need to be done. For example only one study on the toxicity of Confirm to bees, and none for cats, is cited. No studies involving the Chesapeake Bay were cited in spite of the fact that Maryland has been in the program for over 15 years and the Chesapeake Bay is on the EPA's dirty waters list. The Draft EIS 2008 should include monitoring reports for states in the program on possible adverse effects on animal (i.e. domestic, wildlife and aquatic life) populations at risk such as bees, wasps, butterflies, crabs, racehorses, cats and dogs.

Finally, the Draft EIS 2008 fails by and large to study the impact of pesticides used in the program on sensitive groups like people with multiple chemical sensitivities, cystic fibrosis, AIDS, celiac disease, and other compromised immune system illnesses. For example, Appendix F p. 3-15 of the Draft EIS 2008 states that the B.t.k. formulation contains a variety of flours, but there is no discussion of how such an exposure would impact people with gluten intolerance. Therefore MPN recommends that states in the program monitor all use of these pesticides through mandatory reporting to facilitate the discernment of any health effects.

CLOSING STATEMENT

Given the potential and significant adverse environmental and public health effects noted in this document, we request that the Forestry Service and the state of Maryland amend their protocol for Gypsy Moth suppression in order to protect public health and the environment. by focusing on non-toxic measures to control gypsy moth infestations and stop reliance on chemical interventions. MPN recommends the Forestry Service further investigate the work of states identified in this document that have suspended the use of chemical means of suppression.

EXHIBITS

A Three Abstracts referenced in ENVIRONMENTAL EFFECTS

B Washington Post, September 24, 2008, "*Blue Crab 'Fishery Failure' Declared";* David A. Fahrenthold

C USDA GYPSY MOTH MANAGEMENT IN THE US: DRAFT Supplemental Environmental Impact Statement, June 2008, Vol 3 Appendix F p. 3-31, 3-32 Hernandez, et al

D PAN North American Magazine, 19 June 2008, *Victory in California: Moth Spraying Cancelled,*
URL: http://www.panna.org/mag/summer2008/news/victory-in-california-lbam / and link to lawsuit text

E The New Zealand Medical Journal, 14 March 2004, *Symptom complaints following aerial spraying with biological insecticide Foray 48B ,* URL: http://www.nzma.org.nz/journal/116-1170/354/ also referenced in USDA Impact Statement June 2008, Vol 3 Appendix F Table 8

F PAN North American Magazine, Spring 2008, *Spray from the Air, Ignored by the State – Auckland Residents Publish People's Inquiry Report, October 2007*
URL: http://www.panna.org/mag/spring2008/features/sprayed-from-the-air-ignored-by-the-state

G Annals of Internal Medicine, 5 November 2002, *Dysautonomias: Clinical Disorders of the Autonomic Nervous System*, page 755 www.annals.org

H FEMS Immunol Med Microbiol, **November 2000; 29(3):177-81,** *Super-infection by Bacillus thuringiensis H34 or 3a3b can lead to death in mice infected with the influenza A virus*, Hernandez, et al

I Letter Dr. Ziem Sept 22, 2008

J Letter Dr. Layton Sept 11, 2008

K p.21 of June 1974 Bulletin Number 744 of the Connecticut Agricultural Experiment Station B744 (1974) *Gypsy Moth Aerial Tests with Bacillus thuringiensis and Pyrethroids*. Kaya, H.K.; Dunbar, D.M.; Doane,C.C.; Weseloh, R.M.; Anderson, J.F.

Journal of Crustacean Biology, Vol. 12, No. 3 (Aug., 1992), pp. 354-360, ***Effects of Diflubenzuron on Chitin Synthesis in the Postmolt Blue Crab Callinectes sapidus: A Morphologic Study Using an in Vitro Explant Culture System***; Anna N. Walker and Michael N. Horst

Abstract

Diflubenzuron is an insect larvicide that inhibits chitin synthesis. The effects of diflubenzuron were investigated on a nontarget organism, the postmolt adult blue crab Callinectes sapidus. Sections (explants) of the cuticle and epithelium were cut from the dorsal carapace of freshly molted blue crabs and maintained for 6 h in a buffered Ringer's solution containing diflubenzuron and sup3/supH-glucosamine. Control sections were taken from the same animals and were maintained in a like fashion but without exposure to diflubenzuron. The sections were subsequently fixed, processed and examined by electron microscopy, or probed with a chitin oligosaccharide-binding lectin, or studied for sup3/supH-glucosamine incorporation by autoradiography. Ultrastructurally, diflubenzuron-treated tissues showed coarse clumping of nuclear chromatin, dilation of the rough endoplasmic reticulum, and vesiculation of the apical cytoplasm of the cuticular epithelial cells. Decrease in nascent chitin in the treated tissues was demonstrated at the light microscopic level by scant binding of the lectin and minimal radiolabeling in the endocuticular region. The results offer morphologic evidence that diflubenzuron can interfere with crustacean chitin synthesis.

Journal of Crustacean Biology, Vol. 15, No. 3 (Aug., 1995), pp. 401-408, ***Biochemical Effects of Diflubenzuron on Chitin Synthesis in the Postmolt Blue Crab Callinectes sapidus;*** Michael N. Horst and Anna N. Walker

Abstract

In vivo and in vitro metabolic studies were conducted on the effects of the insect growth regulator diflubenzuron (DFB) on chitin synthesis in the postmolt blue crab Callinectes sapidus. The effects of the pesticide on the incorporation of either sup3/supH-glucosamine or sup3/supH-N-acetylglucosamine into sodium dodecyl sulfate (SDS) insoluble cuticular residue were examined. The radiolabeled product formed was identified as chitin by chemical and enzymatic criteria. sup3/supH-N-acetylglucosamine was found to be incorporated to a greater extent than sup3/supH-glucosamine during metabolic studies. During in vitro explant culture experiments, the highest concentration of DFB tested (1 ppm) caused 98% inhibition of chitin synthesis; 64% inhibition was observed at concentrations as low as 0.7 parts per billion. The results indicate that diflubenzuron inhibits the incorporation of sup3/supH-N-acetylglucosamine into cuticular chitin in postmolt blue crabs. The data are consistent with our previous autoradiographic and ultrastructural studies on the effects of DFB in the blue crab; i.e., that the Golgi complex is disrupted by DFB treatment.

Journal of Crustacean Biology, Volume 15(3), 1995 August: 401-408***, Biochemical Effects of Diflubenzuron [Dimilin] on Chitin Synthesis in the Postmolt Blue Crab Callinectes sapidus***; Michael N. Horst and Anna N. Walker. School of Medicine, Mercer University, Macon, Georgia 31207, USA.

Abstract

In vivo and in vitro metabolic studies were conducted on the effects of the insect growth regulator diflubenzuron (DFB) on chitin synthesis in the postmolt blue crab Callinectes sapidus. The effects of the pesticide on the incorporation of either ^{3}H-glucosamine or ^{3}H-N-acetylglucosamine into sodium dodecyl sulfate (SDS) insoluble cuticular residue were examined. The radiolabeled product formed was identified as chitin by chemical and enzymatic criteria. ^{3}H-N-acetylglucosamine was found to be incorporated to a greater extent than ^{3}H-glucosamine during metabolic studies. During in vitro explant culture experiments, the highest concentration of DFB tested (1 ppm) caused 98% inhibition of chitin synthesis; 64% inhibition was observed at concentrations as low as 0.7 parts per billion. The results indicate that diflubenzuron inhibits the incorporation of ^{3}H-N-acetylglucosamine into cuticular chitin in postmolt blue crabs. The data are consistent with our previous autoradiographic and ultrastructural studies on the effects of DFB in the blue crab; i.e., that the Golgi complex is disrupted by DFB treatment.

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washingtonpost.com

Blue Crab 'Fishery Failure' Declared

By David A. Fahrenthold
Washington Post Staff Writer
Wednesday, September 24, 2008; B03

A major and extremely important uncertainty in this risk characterization concerns the use of a toxicity study involving nasal instillation and the attendant uncertainties in extrapolating this type of study to inhalation exposures in humans. An inhalation study similar in general design to the study by Hernandez et al. (2000) – i.e., using mice challenged with an influenza virus as well as appropriate controls – would be necessary for assessing more fully and improving the quality of the risk characterization.

3.4.4. Groups at Special Risk

The previous USDA risk assessment (Durkin 1994; USDA 1995) notes a weakly positive relationship in the incidence of irritant effects in ground workers with and without a history of asthma, seasonal allergies, or eczema (Cook 1994). Swadener (1994) also notes that some formulations of *B.t.k.* contain sodium sulfite, which may cause adverse effects in asthmatics taking steroid treatments. As discussed in Section 3.1.2, Pearce et al. (2002) conducted an epidemiology study designed specifically to address the potential increased risk for young asthmatics exposed to *B.t.k.*. The results of the study indicate that there were no significant differences among individuals present inside or outside the treated area. The study, which involved subjective reports of health as well as clinical measurements of peak expiratory flow rates has limitations. Specifically, the treated and control areas were close to one another, and the monitoring data indicate that individuals in the treated and control areas were exposed to *B.t.k.* Nonetheless, there was no detectable adverse effects in either population (Pearce et al. 2002).

Swadener (1994) summarizes an incident in which a carbohydrate inert in Foray 48B may have caused an allergic response in one woman. As discussed in Section 3.1.7, the incident is not well documented and the interpretation remains uncertain. Commercial formulations of *B.t.k.* are complex mixtures of many different carbohydrates and other materials to which certain members of the general population may be allergic (Oregon Health Services 2003). There is, however, no documented case of a severe allergic response in the epidemiology studies conducted on *B.t.k.* (Table 3-1).

Hernandez et al. (2000) demonstrate a substantial increase in mortality in mice pre-treated with an influenza virus and exposed to various doses of *B.t.k.* The study raises concern regarding the susceptibility of individuals with influenza or other viral respiratory infections to the toxicity of *B.t.k.*. As illustrated in Figure 3-2, increased mortality was observed at a very low dose—i.e., 100 cfu/mouse —which is one-million times lower than the lethal dose in non-viral treated mice—i.e., 1×10^8 cfu/mice. Based on an extra risk of 0.1, the estimated lower limit on the benchmark dose is 30 cfu/mouse (see Section 3.3.4). Following the conversion approach used in Table 3-7, this value corresponds to a human exposure level of 42,000 cfu/m^3. The use of the LD_{10} is not to suggest that such a risk is acceptable but rather to illustrate an exposure level for which the response rate would be readily detected in most epidemiology studies.

The potential significance of the Hernandez et al. (2000) study to public health is difficult to assess. As noted in Table 3-3, most human exposure levels are well below 42,000 cfu/m^3. On the other hand, cumulative exposure levels for the general public, based on the conservative estimates used for this risk assessment, could range up to 360,000 cfu/m^3 × hours. More plausible estimates, based on only a 2-hour rather than a 24-hour duration, range from 1200 to 30,000 hours × cfu/m^3 for members of the general public. Consequently, it is not clear whether the human experience with *B.t.k.*—i.e., the epidemiology studies summarized in Table 3-3—can be used as evidence to preclude the possible association between viral infections and the enhanced toxicity of *B.t.k.* or to establish that the viral enhancement of *B.t.k.* toxicity is not of plausible concern regarding human exposure. Such effects were not observed in ground workers, who clearly are exposed to *B.t.k.* concentrations far greater than 42,000

cfu/m^3 × hours. Nonetheless, the viral enhancement of bacterial infections is not uncommon and the enhancement of *B.t.k.* toxicity by a viral infection seems plausible. This issue is likely to the subject of further study in the coming years and should be monitored by groups involved in the use of *B.t.k.*

3.4.5. Cumulative Effects and Connected Actions

The cumulative effects associated with the application of *B.t.k.* formulations must consider the normal background exposure to *B.t.k.*, residual exposure to *B.t.k.* and formulation products after a single application, and the effects of multiple applications in a single season and over several years. Since the dose-response assessment is based on measures of cumulative exposure —i.e., hours × cfu/m^3—and is supported by epidemiology studies, this type of cumulative effect is implicitly considered in the dose-response assessment. Given the reversible nature of the irritant effects of *B.t.k.* and the low risks for serious health effects, cumulative effects from spray programs conducted over several years are not expected.

Workers or members of the general public who are exposed to aerial or ground sprays of *B.t.k.* also will be exposed to the gypsy moth and may be exposed to other control agents. There are no data indicating that risks posed by these other agents will affect the response, if any, to *B.t.k.* formulations. Similarly, exposure to other chemicals in the environment may impact the sensitivity of individuals to *B.t.k.* or other agents; however, the available data are not useful for assessing the significance of such interactions.

N O T E

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PAN North American Magazine, 19 June 2008, *Victory in California: Moth Spraying Cancelled,*

URL: http://www.panna.org/mag/summer2008/news/victory-in-california-lbam /

also please note that lawsuit text can be found at

URL: http://www.volkerlaw.com/httpdocs/cases/COMPLAINT%20[FINAL]%2011-25-08.pdf

THE NEW ZEALAND MEDICAL JOURNAL

Vol 116 No 1170 ISSN 1175 8716
NZMJ 14 March 2003, Vol 116 No 1170 Page 1 of 7
URL: http://www.nzma.org.nz/journal/116-1170/354/ © NZMA

Symptom complaints following aerial spraying with biological insecticide Foray 48B

Keith Petrie, Mark Thomas and Elizabeth Broadbent

N O T E

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PAN North American Magazine, Spring 2008,

Spray from the Air, Ignored by the State – Auckland Residents Publish People's Inquiry Report, October 2007

URL: http://www.panna.org/mag/spring2008/features/sprayed-from-the-air-ignored-by-the-state

N O T E

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Annals of Internal Medicine, 5 November 2002,

Dysautonomias: Clinical Disorders of the Autonomic Nervous System, page 755

www.annals.org

FEMS Immunol Med Microbiol. 2000 Nov;29(3):177-81.

Super-infection by Bacillus thuringiensis H34 or 3a3b can lead to death in mice infected with the influenza A virus.

Hernandez E, **Ramisse F**, **Gros P**, **Cavallo J**.
Service de Biologie M□dicale, H□pital d'Instruction des Arm□es Begin, 69 Avenue de Paris, 94160 Saint-Mand□, France. hnz.eric@freesurf.fr

Bacterial super-infections are the main cause of complication and mortality after influenza virus (IAV) infection. Since Bacillus thuringiensis (Bt) is considered non-pathogenic for humans and is widely sprayed in urban areas, the aim of this work was to evaluate the potential pathogenicity of a combined infection Bt-IAV in a mouse model of pneumonia. Bacteria used for super-infections were Bt serotype H34 isolated from human infection and the insecticidal strain 3a3b obtained from a commercial source. Virus strain was A/Scotland/20/74 (H3N2) adapted to BALB/c mice by serial lung passage. Combined infection with 4% of the viral lethal dose 50% (LD(50)) and 10(2) spores of Bt H34 killed 40% of the mice. Mortality rates increased up to 55% and 100% when combined infections were done with respectively 10(4) and 10(7) spores. The insecticidal strain Bt 3a3b was less pathogenic than Bt H34. A dose of 10(4) spores associated with 4% of IAV LD(50) killed 50% of the mice. This inoculum must be compared with the doses usually sprayed in agriculture. 10(11) spores m(-2). Total protection against super-infection was obtained when mice were treated with amantadine. Even if only a few cases of Bt human infection have been reported, these results suggest a possible risk for workers spraying Bt-based biopesticides during flu outbreaks.

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Cited in EPA risk assessment

Grace Ziem, M.D., Dr. P.H.
Occupational and Environmental Health
16926 Eyler's Valley Road
Emmitsburg, MD 21727-9729
Phone 301-241-4346 Fax 301-241-4343
Website: chemicalinjury.net

September 22, 2008

Dr. Richard Humphries, Member
Gypsy Moth Task Force
State of Maryland

Dear Dr. Humphries:

I am a physician who has been practicing medicine for 41 years, specializing in evaluation and care of patients with chemically-induced illness. I have a Master of Science and Doctor of Public Health from Harvard as well as a Master of Public Health from Johns Hopkins, where I also had extensive study in toxicology and related subjects. I have also been a consultant and/or guest speaker for numerous federal agencies including US EPA, Maryland Dept. of the Environment, California Dept. of Health Services, US Agency for Toxic Substances and Disease Registry, National Academy of Sciences, US Congress, World Health Organization, State of New Jersey Dept. of Health, American Lung Association and others.

For many years I have had patients who had very significant neurologic, respiratory and systemic symptom exacerbation from the use of Dimilin, typically used for gypsy moth control. I have also had numerous patients over the years with respiratory exacerbation following use of BT for gypsy moth control.

This is the first year I am aware of the use of Dow's aerial Confirm pesticide for gypsy moth control. I have had several patients experience serious neurologic, respiratory and systemic symptoms lasting for many months despite taking all possible measures to avoid exposure.

Confirm, according to the Hazardous Substance Database information, has a half-life persistence on debris of the woods and forest floor lasting for 3 to 6 months. Furthermore, it contains an agent designed to cling to contact surfaces for prolonged periods of time. This clinging effect can occur in ways that jeopardize health.

For example, children, adults and animals walk on the ground of areas that have been sprayed. The sticky residue containing Confirm is readily capable of adhering to their shoes, clothing, skin and pet fur. It can then be readily transported into the home, creating ongoing potential for skin and other exposure. Persons living near sprayed areas are likely to have repeated transfer of this pesticide into the home environment, on carpets, household items, food surfaces and other materials. This allows further exposure in the home, given its long half-life.

I feel it is medically dangerous to rely upon these chemical approaches to suppress a pest that we have not seriously tried to control with less toxic measures. "Advanced warning" has not worked effectively in my experience, and would be unlikely to protect individuals from Confirm, because of the transfer within the home as discussed above.

Sincerely

G Ziem MD

Grace Ziem, M.D., Dr. P.H.

AllergyConnection
Integrated Medicine, Allergy and Specialized Pediatrics

Dr. Richard E. Layton
901 Dulaney Valley Road
Dulaney Center II, Suite 101
Towson, MD 21204
Phone: 410.337.2707
Toll Free: 888.337.2707

09/11/08

Subject: ***Gypsy Moth Risk Assessment***

As a physician who has treated a number of patients who react to pesticides, I am concerned with the recent risk assessment of gypsy moth spraying by the EPA. The reality is that a number of individuals when exposed to gypsy moth spraying develop a host of medical symptoms.

This includes: cognitive impairment, fatigue, headaches, and muscle/joint pain. The main cognitive involvement is short-term memory deficit.

I certainly understand that there is a need to control gypsy moths; however, the chemicals currently used for gypsy moth spraying are putting the citizens of Maryland's health at significant risk.

Sincerely,

Richard E. Layton, M.D.

Richard E. Layton, M.D.

REL/lds

Effect on nontarget insects — Drop cloth counts

The most common nontarget insects found on the drop cloths are shown by orders in Table 10. Adult insects in the orders Mecoptera, Plecoptera, Orthoptera, Psocoptera and Odonata, and 2 spiders were also collected in the pyrethroid plots.

Table 10. Total numbers of nontarget insects found on the drop cloths after spray application of *B. thuringiensis* and pyrethroids.

	B. thuringiensis[1]			Pyrethroids[1]		
Order	Thuricide-16B	Dipel	Untreated[2]	Resmethrin	Bioethanomethrin	Untreated[2]
Lepidoptera	32 a	35 a	3 b	227 a	236 a	2 b
Hymenoptera	6	4	6	33 a	50 a	2 b
Hymenoptera (Parasitoids)	1	0	2	10 a	15 a	2 a
Diptera	9	9	4	48 a	45 a	3 b
Diptera (Parasitoids)	0	1	1	5	3	1
Hemiptera	2	1	0	36 a	45 a	0 b
Coleoptera	17 ab	21 a	6 b	56 a	72 a	6 b

[1]Numbers in the same row followed by the same letter are not significantly different at the 5% level (Duncan's multiple range test). *B. thuringiensis* and pyrethroid treatments were analyzed separately.
[2]Total for untreated plots for *B. thuringiensis* comparisons are for a 10-day period and for the pyrethroid comparisons are for a 4-day period.

As with the gypsy moth, most of these were knocked down within 5 hr after application. Although none of these insects was moving, it is possible that some of them would have recovered had they been left in place. For example, observers in the plots reported that *C. sycophanta* adults were knocked down from trees but recovered without any apparent adverse effects within 2 hr after treatment. In the *Bt* plots, the numbers found were more or less uniformly distributed over the collection dates.

If more than 10 insects belonging to a single order were knocked down in the plots, analyses of variance were performed. In the *Bt* plots, Lepidoptera and Coleoptera were more abundant than in the untreated ones while in the pyrethroid plots, Lepidoptera, Coleoptera, Hymenoptera, Diptera, and Hemiptera were more abundant than in the untreated ones. More species of insects were recovered from the resmethrin plots than from the bioethanomethrin plots.

Eleven adult specimens of 4 species of gypsy moth parasitoids were found on the drop cloths. These were *A. melanoscelus*, *B. intermedia*, *P. agilis*, and *B. scutellata*. Two ichneumonid cocoons identified as *Phobocampe* sp. and a tachinid puparium close to *Compsilura* sp. were also found on the drop cloths. Because of the low numbers no analysis was made.

Discussion

Pyrethroids did not protect the foliage. Net defoliation was between 29-33% in the treated plots and 33% in the untreated plots. These data contrast with those of Dunbar and Doane (1973) who reported good foliage protection from ground spraying with a mistblower. Although the knock down rate from aerial application was high, many gypsy moth larvae recovered from the pyrethroid treatments and continued their feeding. Terminal counts of larvae, defoliation estimates, and direct observations verified that a large proportion of larvae survived the treatments. The low rate, low residual activity and large droplet size of the spray may account for the lack of control. With contact insecticides, Himel (1969) indicated that the optimum size for spray droplets is in the range of 20μ diameter, while droplets of 50-100μ diameter provide marginal efficiency. The droplet sizes of the pyrethroids sprayed with the D-2 hollow cone nozzles were 5-7 times the optimum size. In future aerial tests, a higher rate and smaller droplet size may provide effective control.

A number of formulations of *Bt* have been aerially tested in the northeastern United States with variable results (Lewis *et al.* 1962, Doane and Hitchcock 1964, Lewis and Connola 1966, Secrest and McLane 1971, and Dunbar *et al.* 1973). Recent improvements in formulations and use of a different strain of *Bt* (HD-1 strain) have increased the effectiveness of this microbial insecticide. In tests conducted in 1972, Dunbar *et al.* (1973) reported that net defoliation for oaks in plots treated with Thuricide HPC or Thuricide-16B was between 26-39% compared with 52% in the untreated plots. In 1973, net defoliation of oaks

was 21% in the Dipel plots and 26% in the Thuricide-16B plots. Net defoliation in the untreated plots was 48%. These results show that some foliage protection can be achieved with aerial application of *Bt*; however, the degree of protection did not compare with aerial application of conventional chemical insecticides (Doane and Schaefer 1971).

Small droplets are optimum with contact insecticides (Himel 1969), but droplet size must be kept large to minimize drift to prevent contamination of nontarget areas (Lofgren 1971). Paradoxes such as these make aerial application of insecticides difficult. The spray coverage test with Thuricide-16B-dye mixture showed that better leaf coverage in the canopy was obtained with larger droplets. Because only 1 pass was made, the difference in coverage may have resulted from the smaller droplets drifting away from the plot. Since this difference may be an artificial one, more critical studies of spray coverage in forest situations are needed.

Application of *Bt* from the air may not reduce the next generation of gypsy moth. Dunbar *et al.* (1973) reported that gypsy moth egg mass counts increased markedly in plots treated with *Bt* as well as in the untreated plots. Conversely, Doane and Hitchcock (1964) showed that egg mass counts decreased in all plots whether they were treated with *Bt* or not. Our data also showed a decrease in egg mass counts in all plots. These varied results indicate that population increase or decrease in the next generation is not dependent on aerial application of *Bt*.

Egg masses in plots previously treated with chemical insecticides were larger than those in untreated plots (Doane 1968). Similar results were obtained in plots treated with *Bt*. The difference between *Bt* and untreated plots is probably related both to reduced competition and to an epizootic of nuclear-polyhedrosis virus which occurred in late June toward the end of larval feeding. Presumably, larval populations in plots treated with *Bt* were thinned before the onset of the epizootic. In contrast to larvae in untreated plots, survivors in the *Bt* plots had ample foliage on which to feed and were less likely to contact virus-infected larvae. Adults emerging in the *Bt* plots were healthy and deposited relatively large egg masses. In the untreated plots the population remained dense until the epizootic decimated the population. Undoubtedly under these conditions, crowding, effect of sublethal dosages of nuclear-polyhedrosis virus and the shortage of suitable foliage resulted in small larvae, small adults and small egg masses. Inasmuch as pyrethroids

were ineffective, small egg masses were produced in these plots. Thus, it appears that both chemical and biological insecticides influence egg mass size if they are effectively applied to populations that are beginning to collapse from density-dependent factors such as the nuclear-polyhedrosis virus. Both the rate of disease transmission and intraspecific competition are greatly reduced resulting in healthier, larger individuals which lay more eggs.

The results obtained on percent parasitism of *A. melanoscelus* and the counts of *A. melanoscelus* cocoons under burlap seem to indicate that *Bt* had beneficial effects on this parasitoid. However, the percent parasitism data may be misleading because fewer numbers of gypsy moth larvae were present in the *Bt* plots than in the untreated ones after spraying. With fewer gypsy moth larvae available, percent parasitism should increase as suggested by Dunbar *et al.* (1973). It is possible that differential mortality of gypsy moth larvae caused by nuclear-polyhydrosis virus affected these percentages.

On the other hand, numbers of cocoons counted under burlap were highest in the *Bt* plots. Cocoon counts are themselves an estimate of numbers of parasitoids rather than relative proportions and cannot be explained in the same manner as percent parasitism unless differential virus mortality occurred between the *Bt* and untreated plots. The burlap counts suggest that *Bt* sprays benefited *A. melanoscelus*. It is possible that additives such as molasses in the Dipel mixture attracted adult parasitoids into the plots or extended the lives of the parasitoids. These different interpretations of the results between percent parasitism and burlap cocoon counts mean that any conclusion regarding the effects of *Bt* on adult *A. melanoscelus* activity needs further investigation.

As expected, pyrethroids had a greater effect on nontarget insects than *Bt*. A fairly high number of Coleoptera in the families Scarabeidae, Elateridae, Lampyridae, Cleridae and Cantharidae were found on the drop cloths in the *Bt* plots. No explanation is offered for these findings.