**COMMENTS ON USDA GYPSY MOTH MANAGEMENT DRAFT**

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The major change in the new draft is the addition of tebufenozide (TBF), a second generation synthetic pesticide sold as Mimic for forestry use. This chemical has the potential for serious human health problems among sensitive individuals and for adverse environmental impacts on sensitive species and their developmental stages, as do some of the other proposed methods of gypsy moth control.

TEBUFENOZIDE

Although TBF is targeted towards Lepidoptera, it has effects on other species, is relatively persistent in the environment, transported by water, and bioconcentrates. Significant levels have been measured in lagoons downstream from TBF applications

In humans, it stimulates methemoglobin production, which reduces the oxygen-carrying capacity of the blood and can lead to breakdown of blood cells. Individuals exposed to diflubenzuron or nitrates in air, water, or food show increased levels of methemoglobin and are at increased risk to TBF exposure. Many drugs and sprays such as benzocaine cause methemoglobinemia and produce an additive effect which can be serious (headache, dypsnea, dizziness, and heart palpitations) or fatal (Ash-Bernal et al., 2004), especially in anemic patients. In this study of hospital patients with methemoglobinemia (methemoglobin >1.5%), 94-5 % (male, female) were anemic, and methemoglobin levels as low as 4.7% produced symptoms severe enough to be treated with methylene blue.
While healthy persons may not become symptomatic until methemoglobin levels reach 15%, persons with hematologic (e.g. anemia), cardiovascular or pulmonary disease have symptoms at much lower levels. This condition produces organ damage and may be even become fatal. Persons with these conditions have elevated sensitivity to methemoglobin and sulfhemoglobin producing drugs and chemicals and are at greater risk from TBF exposure. With their greater sensitivity they may have difficulty finding diagnosis and treatment because of diagnostic assay difficulties and inadequate information about increased hazards for people with tissue oxygenation issues. The Dow Agrochemical MIMIC MSDS ( 2007 ) lists human and animal health effects but does not warn that anemia can result in pathology at methemoglobin levels as low as <5%. (Ash-Bernal et al, 2004). No warning appears in the MSDS about the dangers of using methylene blue to treat methemoglobinemia in cases of G-6-PD deficiency (As-Bernal et al 2004) or severe renal insufficiency (Clifton et al., 2003). The Hazardous Substance Data Base does not give any human health effects, nor treatment that is specific to the TBF toxic methemoglobinemia. Calls to Poison control centers are evaluated relative to the general population, not sensitive individuals. Also the effects of TBF on blood from people with sickle cell disease needs to be studied before it is used around people with this disease.

The draft report states that there are no sensitive human populations. This is clearly not true. In addition to the people with oxygen delivery issues, infants have low methemoglobin reductase and therefore are

much more likely to develop methemoglobinemia if exposed to TBF (USDA 2008 IV:3-28). Some adults have congenital methemoglobinemia (Das Gupta et al. 1980), some children and adults have reduced ability to detoxify pesticides in general, and children and adults with chemical hypersensitivity and chemical induced asthma react to pesticides and the solvents and adjuvants in the mixtures at much lower levels than the general population. Among the solvents are alkyl aryl polyether alcohol. Spray exposure of children and sensitive individuals seems likely in populated areas and recreation areas, including parks.

Without knowing the contents of the mixture used in Mimic, there is no way to judge the hazard based on "active " ingredients only. Most pesticide mixtures include, for example, ingredients that increase permeability into organisms and cells, increase the half life, and/or inhibit the pumps that transport the pesticide out of cells.

Mimic can cause eye, skin, and respiratory irritation (USDA, 2008 IV: xii). Data regarding dermal absorption kinetics of TBF are not available, and inhalation toxicity, though the most toxic route of exposure for sprayed insecticides, is not considered "of substantial concern" (USDA 2008 II 4:22). However, rats exposed to 1.33 mg/L Mimic showed irritation of the respiratory tract and redness in the lungs of all 12 animals examined and hemorrhagic foci in the lungs of 5 of 6 males (USDA 2008 IV J 3-8).

Effects on other non-target animals are also of concern. In contrast to the summary statement that TBF "should not affect any aquatic species" (USDA 2008 IV M viii), low concentrations of TBF can cause reproductive effects in aquatic invertebrates and fish (USDA 2008 IV: 4-1). A recent study of TBF-treated lake trout showed changes in immune response and white blood cells, with a decrease in thrombocytes and an increase in lymphocytes compared to controls (Hamontene et al., 2008). Reproductive effects were also seen in birds and mammals (USDA 2008 IV: 4-3).

Toxicity studies of adult bees with mortality as the end point is not as relevant as studies that test the emergence of workers, which is more likely to be affected by an insect growth regulator like TBF, in light of colony collapse disorder, which depends on the number of workers.

Another major concern is the effect on crabs and smaller crustaceans such as grass shrimp, on which many other aquatic organisms feed. Diflubenzuron, another inhibitor of arthropod molting, causes mortality at crab molting (Cardiinal et al. 1980). In Maryland, reported environmental concentrations of diflubenzuron (1.5 ug/L) exceeds the LD50 =1.11 ug/L for the premolt stage of the grass shrimp (Fischer and Hall,1992). Since TBF acts similarly and has a longer half life, washes off in water, and bioacumulates, the effects of TBF should be tested on such economically vital organisms as blue crabs and grass shrimp before TBF is used in the Chesapeake Bay watershed. The blue crab population in the Chesapeake Bay is already down 70% from 1990 levels , and Maryland and Virginia have requested disaster status for crabs (Associated Press, 2008).

## DIFLUBENZURON

Diflubenzuron (DFB – trade name Dimilin) is an inhibitor of arthropod molting. Like tebufenozide, it activates formation of methemoglobin and lysis of red blood cells. Coexposure to the two pesticides may produce a cumulative effect on methemoglobin production as does smoke, carbon monoxide, and

nitrates in air, water, or food (USDA 2008 I:8). Also, DFB is synergistic with the widely used pyrethroid, permethrin (Sfara et al., 2007).

DFB inhibits chitin synthesis in arthropods and thus was thought not to act on animals like vertebrates, which lack chitin. But DFB does not act directly on any of the enzymes that synthesize chitin. Instead, DFB acts by inhibiting Ca2+ uptake (Nakagawa & Matsumura, 1994), and its site of action is on an ABC (ATP Binding Cassette) transporter called the sulfonylurea receptor (Abo-Elgher et al, 2004). Sulfonylureas bind to similar receptors in vertebrates and are drugs used to treat type II diabetes. Altered intracellular calcium concentrations make excitable cells such as neurons, muscle, and secretory cells dysfunctional. So other non-target effects of DFB can be expected.

A DFB breakdown product 4-chloroanilin has been classified by the EPA (2006) as a probable human carcinogen (not "possible carcinogen" as stated in the draft (USDA 2008 I: 8)). A major concern for humans is drinking or eating it in contaminated water or food.
Also, DFB induces mouse CYP3A1/2, which activates the carcinogens/toxins aflatoxins and nitropyrenes in lung and kidney, and produces a marked reduction of testosterone metabolism (Sapone et al., 2005).

In the human liver cell line HepG2, DFB competes with the dioxin TCDD and displaces it from the aryl hydrocarbon binding site. As a result, DFB inhibits TCDD induction of cytochrome p450 CYP1A1 expression (Ledirac, 2000) and thus dioxin and other neurotoxic and carcinogenic molecules may not be metabolized as fast by this cytochrome p450 system.

A recent study by Maduenho and Marinez (2008) on fish found that DFB at sublethal doses is an inhibitor of acetylcholinesterase activity in muscle, which can lead to hyperactivity, axon withdrawal and excitotoxicity.

As discussed earlier, DFB inhibits crab molting and can be lethal (Cardinal et al., 1980).
If blue crabs are exposed to Dimilin on the day of molt and subsequently exposed to repeated doses, the LC50 = 18.5 ug/L (Rebach and French, 1996). Effects were age and molt-stage sensitive. Blue crab reproduction is impaired at 0.5 ppb (Beyond Pesticides 2008). Fiddler crabs exhibited limb regeneration effects at low DFB levels (Weis et al. 1987). DFB inhibited their molting and escape behavior at 2 ug/L and thus may influence the ability of juvenile crabs to avoid predation and feed (Cunningham and Myers, 1987). DFP also inhibits reproduction of parasitic wasps and thus inhibits a natural control of gypsy moths (Schneider et al. 2003).

BACILLUS THURINGIENSIS k (Btk)

Btk use is given lower hazard quotients than DFB or TBF (USDA 2008 I: 12), but we are concerned about the lethal effects of Btk on virus-infected mice (USDA 2008 III: 3-23 to 32). That viral infections enhance bacterial infections is well known. Hernandez et al (2000) gave mice three different doses of Btk, with or without influenza virus at 4% of the LD50, and observed mortality of 4/20, 8/20, and 14/20 mice in the groups receiving virus and Btk, a nearly linear dose relationship (USDA 2008 III: Fig 3-2). This "clearly suggests that otherwise non-lethal doses of Btk can be associated with pronounced lethality in mice infected with otherwise non-lethal doses of influenza virus" (USDA 2008 III: 3-25). Influenza A virus produces a protein that specifically targets and destroys lung alveolar macrophages (Coleman, 2007), leaving the lung exposed to bacterial infection, the primary cause of death in influenza patients.

Btk has been demonstrated to be a human pathogen. Btk infection was reported in burn wounds (Damgaard et al, 1997). Also, a war-wounded immuno-competent soldier developed Btk-infected abscesses that resolved after 15 days of intensive care with the proper antibiotics (Hernandez et al., 1998). Btk is difficult to diagnose, because it is often mistaken for B. cereus or other strains of B.t.

Similar pathology was found in Btk treated uninjured immuno-supressed mice. Immuno-suppressed mice became infected with Btk, whereas control Btk mice did not (Hernandez et al, 1998). This raises the possibility that immuno-suppressed (e.g. transplant patients) or immuno-compromised (e.g. AIDS patients) humans can be infected more easily by exposure to Btk.

SUMMARY AND PROPOSED ACTIONS

Tebufenozide has sufficient risks to sensitive human populations that it should not be applied in populated areas and recreation areas, including parks. TBF, like DFB, caused methemoglobin formation, reduced O2 carrying capacity, and red blood cell hemolysis. Many aspects of TBF uptake and toxicity have not been adequately studied. It should not be used in the Chesapeake Bay watershed until its effects on blue crabs and grass shrimp have been studied and found acceptable. The only reason to add TBF to the list is if it is a less-toxic replacement for DFB and only if it is used in non-populated, unoccupied areas. TBF should not be sprayed on farm animals, domestic pets, and organic and non-organic food production areas. Dogs are especially sensitive to TBF, and cats are sensitive to DFB but apparently have not been tested for TBF sensitivity (no evidence was found in the literature), making both chemicals an inappropriate choice in urban areas, near kennels or catteries, or in hunting areas. A dead duck is one thing; a dead hunting dog would please no hunter. Since essentially all of the eastern U. S. is populated or a recreation area with hiking trails, TBF should not be approved for use on gypsy moths.

Whether or not TBF is added to the approved treatment list, we believe that diflubenzuron should be removed from the list of approved treatments. Its metabolite, 4-chloroanaline, is a <u>probable</u> human carcinogen, its hazard quotients are higher than TBF for nontarget terrestrial (32 vs 4) and aquatic (5 vs 0.4) species. And many individuals with chemical hypersensitivity, chemical-induced asthma, or chronic fatigue syndrome have experienced serious reactions to aerially sprayed Dimilin (DFB-containing mix).

Recent evidence that Btk is a human pathogen and can be lethal if a human is co-infected with influenza virus, requires reconsideration of its use in populated areas and recreational areas, including parks.

Gypchek seems like a more appropriate alternative, although it contains gypsy moth parts that may induce allergic reactions. Together with the naturally occurring fungus, <u>Entomophaga</u> <u>maimaiga</u>, Gypchek provides “outstanding” gypsy moth control (Webb et al., 2005).

Parasitic wasps should be part of the solution when used with the fungus and virus and not killed by TBF or DFB. Several eastern states report adequate control of the gypsy moth without using these chemicals.

Also, we are opposed to Alternative 3. We believe that public input is necessary before approval to use any new pesticide for such widespread use as spraying for gypsy moth management. Poisoning and contamination of one’s home is much more stressful than the loss of trees. Physicians, scientists and

people with chronic or inherited conditions that confer greater sensitivity deserve consideration by the forestry community in the process of developing strategies for gypsy moth control. Also, we note that much molecular biology is becoming available for these chemicals that should be considered where there is analogy with humans, which is usually the case. Other tools such as proteomic microarrays are now available to measure chemically-induced changes in protein levels and should become a part of risk assessment and part of the manufacturers' responsibility in developing and marketing these chemicals. Gypsy moths can be reduced without poisoning our fellow citizens or their companion or business animals.

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