AN ECOLOGICAL RISK ASSESSMENT FOR MOSQUITO INSECTICIDES

by

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A thesis submitted in partial fulfillment
of the requirements for the degree

of

Master of Science

in

Entomology

MONTANA STATE UNIVERSITY
Bozeman, Montana

April 2007

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of a thesis submitted by

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This thesis has been read by each member of the thesis committee and has been found to be satisfactory regarding content, English usage, format, citations, bibliographic style, and consistency, and is ready for submission to the Division of Graduate Education.

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April 2007

ACKNOWLEDGMENTS

A special thank you to my advisor Dr. Robert K.D. Peterson for welcoming me into his laboratory. Throughout this thesis, he helped me realize my potential as a student and a researcher. Without his guidance this project would not have been successful. Thanks to my committee members, Dr. Gregory Johnson, Dr. Patricia Denke, and Dr. Billie Kerans who helped give focus and direction especially at the beginning of the project. Thanks to past and present members of Team CoBRA for all of their support and ideas. Team members include Leslie Shama, Marjolein Schat, Jerome Shleier, Dr. Frank Antwi, Courtney Pariera Dinkins, Tim Bennett, Jessie Weise, Norma Irish, and Jenny Marquez. Dr. Paula Macedo was very important to this study. Her insight was invaluable, along with her support and enthusiasm. My family has given continued encouragement throughout my schooling. A special thanks to my wife Michelle Davis, my parents Dr. Scott E. Davis and Janet Davis as well as my brother Benjamin Davis for supporting me throughout the preparation of this thesis. The field work for this project could not have been done without the generosity of the Cascade County Mosquito and Vector Control District and their director Doug Johnson; the Northwest Mosquito and Vector Control Association was supportive with encouragement and finances. Nor would it have been possible without the help of Bob Johnson and the staff at Benton Lake National Wildlife Refuge who gave us a place to conduct our experiments. This project was funded by the U.S. Armed Services Pest Management Board and the Montana Agricultural Experiment Station. A special thanks to Captain Gary Breeden, Captain Stan Cope, and Dr. Graham White who encouraged the growth of this project.

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ABSTRACT

West Nile Virus (WNV) has been a concern for people across the North America since the disease was observed in the summer of 1999. WNV has caused the largest arboviral encephalitis epidemic in U.S. history. In response, vector management programs have been implemented. Concerns have been raised about these programs My ecological risk assessments focused on six common mosquito adulticides used in vector management, including 3 pyrethroids, pyrethrins, 2 organophosphates, a synergist, and 4 larvicides. Both aquatic and terrestrial non-target organisms were considered for acute and chronic exposures to the adulticides and larvicides. Tier I exposure estimates for adulticides were derived using modeling software. A probabilistic assessment was conducted for the adulticides to account for variability within the exposure models. The larvicide risk assessment included an even settling model into a standard farm pond. Risk quotients (RQ) were obtained by comparing exposure to toxic endpoints. Organophosphates breached a risk quotient (RQ) level of concern (LOC) for amphipods. All of the but one RQ calculated in the deterministic model were greater than the 95th percentile of possible outcomes. *Daphnia magna's* RQ when exposed to monomolecular films breached a USEPA RQ LOC. Two studies were conducted during the summers of 2004 through 2006 near Great Falls, Montana. In 2004 and 2005, a study was conducted to assess acute impacts of mosquito insecticides on non-target organisms after one application. The second experiment was conducted in 2005 and 2006 to assess chronic impacts of a mosquito adulticide on non-target terrestrial arthropods after multiple applications. For aquatic samples in the first study no overall treatment effects were observed. During the same study 1 of 54 responses had a significant overall treatment effect for terrestrial samples. Many of the responses for terrestrial samples suggested significant time effects and time by treatment effects. For the multiple spray study conducted in 2005 and 2006, six of the response variables collected via terrestrial samples exhibited significant overall treatment effects; none had fewer individuals in the treatment plots. Time and time by treatment effects were prevalent in 2005 but no discernable pattern was evident.

CHAPTER 1

INTRODUCTION

Mosquito borne diseases like malaria and Yellow fever have been cause for the development of mosquito management plans throughout the world. The latest in North America, West Nile Virus (WNV), is a current concern for people across the United States. Many are concerned with the risks associated with managing the vector mosquitoes using insecticides. This concern is related to the risk that ecological and human exposure to the insecticides will lead to negative effects that are more severe than the disease itself. WNV was first observed in the U.S. in the late summer of 1999 in and around New York City (Lanciotti et al. 1999). It was first characterized by an unexplained outbreak of human encephalitis. Lanciotti et al. (1999) identified the virus as a flavivirus that is closely related to a WNV strain isolated in Israel in 1998. This research was prompted in part by WNV's emergence throughout North America. The current project seeks to quantify the ecological risks associated with commonly used mosquito management agents that are used to manage mosquitoes that vector WNV and other infectious diseases.

West Nile Virus

Opportunistic blood feeders like mosquitoes and ticks will attack vertebrate hosts. Those that feed on a variety of hosts have potential to infect humans with pathogens, like WNV, from other vertebrates that act as a disease reservoir. In some cases, humans infected with WNV can develop encephalitis (CDC 2007). Since the WNV outbreak

began, there have been 23,962 human cases reported including 950 deaths. In 2003 human cases of WNV appeared in 46 states with 9,862 cases reported to the U.S. Centers for Disease Control; this has been the year with most WNV cases to date. These reports included 264 fatalities attributed to the disease (CDC 2007).

West Nile Virus has even a greater effect on horses, causing 24,816 reported cases throughout the 1999-2006 period. The largest number of infections occurred in 2002 where 15,257 cases were reported; 2003 and 2004 were also significant years where 5,181 and 1,406 cases were reported, respectively (APHIS 2007).

Birds are the major hosts for the disease with many thousands of cases reported since the 1999 outbreak. Other vertebrate clinical cases seem to be infrequent with only a few reported in various species since the outbreak, including a small number of bats, one chipmunk, one squirrel, and one rabbit. Dogs and cats, although commonly infected, seem unaffected by the disease (CDC 2007).

West Nile Virus poses a particular concern for human health as it is spread quickly and many species of birds and mosquitoes are susceptible to infection (CDC 2007; Reisen et al. 2005; Turrel et al. 2005). Over 200 bird species have been reported to the CDC with infected individuals; the virus has been found in 60 mosquito species (CDC 2007). The disease is amplified by a multitude of mosquito species but as yet only a few have been identified as the main vectors including: *Culex tarsalis* Coquillett, *Culex pipiens* L., and *Culex quinquefasciatus* Say (Turell et al. 2005). Ticks are able to carry the disease although there role in transmission is unlikely and not described (Lawrie et al. 2004). Natural persistence of the virus lies in an avian reservoir (Lanciotti et al. 1999).

Horizontal transmission including an avian reservoir can occur in two ways: viremic and non-viremic. In viremic transmission, the virus incubates in the avian host and viremia develops in the host's system (Higgs et al. 2005). The viremic host is fed on by a female mosquito and she is infected with the virus. The female mosquito then transmits the virus to a new host when she feeds upon the blood of an uninfected bird or mammal. Mammals are dead end hosts for viremic transmission of WNV. Viremia levels are not high enough to transfer the virus to a feeding mosquito. In non-viremic transmission, a small percentage of vectors that feed on the host will be infected with the virus. In this case, the host has not yet developed an illness because the virus has not had time to reproduce in the host's system (Higgs et al. 2005).

Transmission can occur vertically as identified by Baqar et al. (1993). In this type of transmission (filial transmission) the disease reservoir is the mosquito. The female mosquito lays eggs that are infected with the virus. *Aedes* species overwinter unhatched; the virus can reenter the vertebrate reservoir; if a particular *Aedes* species is a competent vector its offspring can re-infect the avian reservoir, upon emergence in the spring. North American *Culex* species can vertically transmit the disease (Goddard et al. 2003). These species overwinter as adults. The females from the most recent generation generally do not take a blood meal before overwintering, but can carry the disease that was transferred from mother to offspring. Current vertical transmission data suggests that these transmission events happen infrequently. In a study done in California with *Culex pipiens pipiens*, *Culex pipiens quinquefasciatus*, and *Culex tarsalis* the minimum filial

infection rates were 0/1000, 3/1000, and 6.9/1000 mosquitoes for each respective species (Goddard et al. 2003).

Mosquito Management

Mosquito management is important to contain vector-borne illnesses like WNV. Managing mosquito habitats, using repellents, and targeting the insects using insecticides are ways to manage mosquitoes. Ecological concerns over mosquito insecticides include mortality of species in aquatic and terrestrial interaction webs, harmful impacts on bees and other beneficial arthropods, and negative effects on terrestrial and aquatic vegetation.

Adulticides

Common mosquito adulticides, those insecticides that target adult mosquitoes, are pyrethroids such as d-phenothrin, resmethrin, and permethrin, as well as organophosphates such as malathion and naled. Natural pyrethrins are also available as mosquito management agents.

Adulticides are applied via ultra low volume (ULV) space sprays that target adult mosquitoes as they are flying through the air. Adulticides used as space sprays are released in the atmosphere as extremely small liquid droplets that range from 8-30 microns in ULV formulations (Rose 2001). Droplets this small have a large surface area available to contact the target mosquitoes. The insecticide is absorbed through the cuticle and takes effect soon after contact. Most are sprayed in the evening, or early morning when female mosquitoes are seeking a blood meal, and many other arthropods, particularly pollinators, are inactive. The nature of the aerosol has a few drawbacks. The

small droplet size increases the probability that the treatment will drift out of the spray zone and deposit in areas that might affect non-target organisms. Drift due to air turbulence and wind lowers the efficacy of ultra low volume sprays because a large percentage of the insecticide is lost from the treatment area.

Surrogate animals are used in screening level risk assessments and are model organisms used for the toxicological data available for them.

Pyrethrins are common chemicals used in adulticiding. They are derived from 6 essential oils derived from chrysanthemum flowers. They are contact insecticides that affect an insect's nervous system via sodium channel disruption (USEPA 2006g). These extracts are valuable for insect management, but in many cases are metabolized and rendered nontoxic by the insect before the chemical causes morbidity or mortality. The chemical has low toxicity to birds and mammals with an acute LC_{50}, lethal concentration of food products that causes a 50% reduction in a given population, greater than 5000 mg/kg body weight for bobwhite quail (*Colinus virginianus* L.), mallard duck (*Anas platyrhynchos* L.), and ring-necked pheasant (*Phasianus colchicus* L.) (USEPA 1997a, 2005f) and an acute LD_{50}, the lethal dose corresponding to body weight that reduces a given population by 50%, of 700 mg/kg body weight for rats (USEPA 2005f). The chronic NOEL, the highest tested dose based on body weight where no effect was observed, is 100 mg/kg body weight. Aquatic organisms are more susceptible with acute LC_{50}s, lethal chemical concentrations in water that cause a 50% reduction in a given population, ranging from 0.0032 ppm for rainbow trout (*Oncorhynchus mykiss* Walbaum) (USEPA 2005f) and 0.0187 ppm for bluegill sunfish (*Lepomis macrochirus* Rafinesque)

(USEPA 2006a) and a chronic NOEC, the highest concentration tested that did not correspond to any observed physiological or behavioral change within a population group, for *D. magna* (*Daphnia magna Straus)* of $9.6x10^{-5}$ ppm (USEPA 1997a).

Pyrethroids are a synthetic versions of pyrethrins. Synthetic pyrethroids were developed to harness the insecticidal properties of pyrethrins and limit their toxicity to non-target species. Like pyrethrins, these chemicals are broad-spectrum insecticides.

Permethrin is one of the older pyrethroid chemicals and is moderately toxic to mammals, non-toxic to birds, and toxic to organisms in aquatic ecosystems. Anderson (1982) found that chronic exposure of permethrin causes behavioral changes and mortality in both caddisflies (Trichoptera) and stoneflies (Plecotera) after 21 days of exposure at 0.029 µg/L and 0.52 µg/L, and that 60% of black flies (Diptera:Simulidae) in the trial died after 6 hours when exposed to 1 µg/L for 1-hour. In some areas of the world, permethrin is applied directly to water as a larvicide. Crosa et al. (2001) completed a field evaluation that examined permethrin's effect on aquatic communities when applied directly to two Guinean rivers. The researchers concluded that gatherer-collector invertebrates showed a marked reduction in relative abundance at the end of a treatment year, but a resurgence in the following season. Under most conditions permethrin degrades rapidly in the environment with dissipation half-lives ranging from 6-106 days as described by the U.S. National Library of Medicine Hazardous Substance Database (USNLM) (2005).

D-Phenothrin is a more recently developed synthetic pyrethroid characterized by low toxicity to birds and mammals. There is little publicly available information on

efficacy and its chronic toxicity to surrogate organisms. Acute LC_{50}s for birds are greater than 5000 mg/kg body weight (USEPA 1997a). The rat LD_{50} is greater >5000 mg/kg body weight with a chronic NOEL of greater than 1000 ppm (WHO/FAO 1994). Fish are more acutely susceptible to d-phenothrin with LC_{50}s of about 0.001 ppm and a chronic NOEC of 1.1 ppm for rainbow trout (USEPA 1997a, 2006a). *Daphnia magna* is robust to exposure with an acute LC_{50} of 30,000 ppm. D-Phenothrin is extremely insoluble in water and immediately begins bonding to suspended organic materials upon application (USNLM 2005).

Piperonyl butoxide (PBO) is a synergist that is used with pyrethroids to improve their efficacy. PBO has low toxicity to birds and mammals with acute LC_{50}s greater than 5620 ppm for birds and chronic NOECs of greater than 300 ppm . The rat LD_{50} is greater than 4570 mg/kg body weight and the chronic NOEL is greater than 1000 mg/kg body weight (USEPA 2006f). *Daphnia magna* are more susceptible to exposure than are fish with an acute LD_{50} of 0.51 ppm and a chronic NOEL of 0.07 ppm (USEPA 1997a, 2005h). Fish have a higher tolerance with acute LD_{50}s greater than 1.5 ppm (USEPA 2005h).

Resmethrin, another pyrethroid, is almost non toxic to humans and other mammals. It has little negative impact on birds with an acute LC_{50} greater than 5000 ppm and chronic NOECs greater than 60 ppm for common surrogates, which are model species used in screening level risk assessments. The acute LD_{50} for rats is 4639 mg/kg body weight with a chronic NOEL of 500 mg/kg body weight (USEPA 2005g). It is highly toxic to aquatic organisms with LC_{50}s less than 1 ppb for *D. magna,* bluegill

sunfish and rainbow trout (USEPA 1997a, 2005g, 2006a). It is also highly toxic to bees. The persistence of resmethrin in the field is comparable to other pyrethroids with an estimated soil half-life of 30 days and a water half-life of 36.5 days. It has a very short photolytic half-life of 22-47 minutes when exposed to sunlight (Rand 2002).

Malathion is a broad spectrum organophosphate insecticide used to manage many types of arthropods. It is one of the earliest developed organophosphate insecticides and was first registered in 1956 (USEPA 2006e). The insecticide is moderately toxic to mammals and birds with low acute and chronic toxicities. LC_{50}s for surrogate birds are greater than 2500 ppm. Chronic NOECs range from 110 ppm to 1200 ppm (USEPA 2005d). The rat LD_{50} is 390 mg/kg body weight and the chronic NOEL is 100 ppm (USEPA 2005d). Its toxicity to aquatic organisms varies. It is highly toxic to some species like walleye (*Sander vitreus* Mitchill) and cutthroat trout *(Oncorhynchus clarki* Richardson), and only moderately toxic to fathead minnows (*Pimephales promelas* Rafinesque). *Daphnia magna*, bluegill sunfish, and rainbow trout are susceptible to malathion exposure. Acute LC_{50}s for these surrogates are less than 0.02 ppm; chronic NOECs are less than 0.002 ppm (USEPA 2006e). Because malathion is a broad spectrum insecticide, its toxicity to beneficial arthropods, such as honey bees is high with an LD_{50} of 0.2 µg ai/bee (USEPA 2006a). Pankiw and Jay (1992) found that bee colonies exposed to aerial ULV applications of malathion sprayed directly onto hives slowed colony weight gain, population growth, foraging, and increased bee mortality close to the spray dates. Aquatic insects were unaffected by malathion after one spray event. Jensen et al. (1999) conducted a study of seasonal wetlands sprayed with malathion. No

reduction in macro-invertebrate biomass was detected. However, the EPA claims that malathion is highly toxic to most sentinel terrestrial arthropods and their aquatic larvae. Some stonefly nymphs have an LC_{50} of less than 1 ppb. Malathion breaks down quickly in soil, air, and water with half-lives of 1-25 days, 1.5 days, and less than 7 days, respectively (USNLM 2005).

Naled is a widely registered organophosphate insecticide. Like malathion, it is a broad-spectrum insecticide used to kill a variety of arthropods (USEPA 2006b). Some toxicological data support that naled is highly to moderately toxic to mammals, birds, and fish (USEPA 1997a, 2002). Acute LC_{50}s are greater than 2000 ppm and chronic NOECs are greater than 100 ppm for surrogate birds (USEPA 2006b). The rat LD_{50} is 235 mg/kg body weight and the chronic NOEL is 100 mg/kg body weight (USEPA 1997c). *Daphnia magna*, rainbow trout, and bluegill sunfish are very susceptible to negative effects of naled with acute LC_{50}s ranging form 0.0003 ppm for *D. magna* to 2.2 ppm for bluegill sunfish (USEPA 1997b), and a chonic NOEL for *D. magna* listed as $4.5x10^{-5}$ ppm. Zhong et al. (2003) found that aerially applied naled had a negative effect on honey bees and reduced their honey production over a season. In a later study the same researchers found that non-target effects on bees could be minimized by using a different type of spraying system (Zhong et al. 2004). Naled has a very low environmental persistence with a half-life of less than 2 days under field conditions (USEPA 2002).

Larvicides

In addition to adulticides, some insecticides called larvicides kill mosquito immatures. Methoprene, *Bacillus thuringiensis* var. *israelensis*, *Bacillus sphaericus,* and monomolecular films are common larvicides with diverse modes of action.

Methoprene is a chemical that mimics the growth hormone of certain insects. The agent hinders regular maturation of early mosquito instars (USEPA 1991). The larvae that consume methoprene are unable to reach adulthood. Methoprene is somewhat toxic to fish and birds as well as some aquatic invertebrates. *Daphnia magna* is especially sensitive with an acute LC_{50} of 0.07 ppm and a chronic NOEC of 0.03 ppm. Rainbow trout are also sensitive, with an acute LC_{50} of 1.01 ppm (USEPA 1997a). Methoprene has adverse effects on the scud (*Gammarus* sp.) (Breaud et al. 1977), the mayfly species *Callibaetis pacificus* Seemann, chironomids (Diptera:Chironomidae) and a dytiscid beetle (Coleoptera:Dyticidae), *Laccophilus* sp. (Norland and Mulla 1975). In a long-term study, in which each site was treated at three week intervals six times over the season, Hershey et al. (1998) concluded that methoprene had a negative impact on aquatic insect predators at treated sites. These impacts were considered to be both direct and indirect as the chemical acted to cause mortality to the predator populations and lessened the availability of prey. The environmental degradation of methoprene occurs quickly in soil, groundwater, exposed water, and vegetation. Methoprene degrades rapidly in water; 80% will degrade within 13 days after application (USEPA 1991).

Bacillus thuringiensis (Bt) is a soil bacterium. Its insecticidal property resides in a crystalline by-product of spore production (endotoxin) that attacks an insect's stomach

lining when consumed (Mittal 2003). The bacterium is a highly regarded insecticide because it has many strains that target receptors of specific insect species or groups. The endotoxins are practically non-toxic to mammals, fish, and birds (Mittal 2003). LC_{50}s for fish are greater than 0.66 ppm. *Daphnia magna* is less susceptible with an acute LC_{50} greater than 13 ppm (USEPA 1997a). *Bacillus thuringiensis israelensis* (Bti) is the strain of Bt that is used for mosquito management. In addition to causing mosquito mortality, it may have negative effects on non-target black flies and midges (Hershey et al. 1998). Ali (1981) found that treatments in 4x6-m experimental ponds significantly lowered non-target chironomid numbers. At the highest treatment rate of 4 kg ai/ha there was 54-92% reduction in chironomid numbers. But the same product used in golf course ponds had no significant effect on chironomid numbers; a treatment of 3 kg/ha, only 30-67% reduction in chironomid numbers was observed. Normal numbers returned 14 days after treatment. Charbonneau et al. (1994) found that although Bti caused high mortality of chironomids in a lab environment, a reduced mortality was observed in the field. They concluded that Bti did not significantly reduce chironomid numbers in the field. Hershey et al. (1998) conducted the most rigorous study on larviciding. Throughout a full season of treatment they found that Bti was effective at killing target dipterans, and that the food chain was disrupted. Predator numbers dropped as prey became less abundant implying that perhaps the effect was indirect. It breaks down quickly in the environment.

Bacillus sphaericus is another soil bacterium that has a similar insecticidal action to Bti (Mittal 2003). In this case the insecticidal agent is included in the spore cell wall and as a byproduct of spore production. When the agent is consumed by the larvae, it

attacks the lining of the mosquito gut. The insecticide has been more effective against *Anopheles* and *Culex* species than *Aedes* species (Mittal 2003). Brown et al. (2004) measured the effects of *Bacillus sphaericus* on nontarget species. They found no negative non-target effects and no effects on water quality.

Monomolecular films are insecticides that form a thin film over the water in which they are applied. Their main mode of action is to add a buffer between the water air interface so that the surface tension of the water is decreased. The immature mosquito cannot maintain proper orientation to the air water interface and drowns (Levy et al. 1980). Mulla (1983) found that Arosurf® (dimethyldioctadecylammonium chloride) treatments had no effect on sentinel aquatic arthropods. Monomolecular films are relatively non-toxic to mammals with an oral LD_{50} of >11,300 mg/kg in rats (USNLM 2005). Acute LC50s range from 1.9 ppm for *D. magna* to greater than 98 ppm for fish (USEPA 1997a) When applied as a larvicide in water, Arosurf® has a short half-life ranging from 5 to 15 days (USNLM 2005).

Larvicide application differs from adulticide application as the larvicides are added directly to water where the larvae live. Methoprene is applied as a direct application insecticide either over water or into the water as briquets. Bt and Bs are applied at higher rates as briquets directly into water , or as a liquids over water. Larvicides can come in time-release capsules that release the toxin for a period of a month in some cases (Hershey et al. 1998).

Milam et al. (2000) found that treatments of common larvicides were much more damaging to mosquitoes than non-arthropod sentinel species. In another study,

methoprene and Bti were much less toxic than temephos and pirimiphos-methyl to non-target shrimp (Brown et al. 2000). In a long-term project in Minnesota wetlands with methoprene and Bti, no treatment related effects were found on zooplankton taxa. The wetlands were treated for three years. Plots assigned methoprene and Bti treatments were sprayed six times each of the three years. Each insecticide affected insect species richness. Chironomid as well as other nematocerans abundance was reduced by one-third compared to the control sites (Hershey et al. 1998). This project also examined possible vertebrate effects as a separate experiment. No impact was found on migratory birds (Niemi et al. 1999).

Mosquito Management Efficacy

The ability for each insecticide to cause high mortality in mosquito populations depends on a number of factors, including ambient temperature, time of day, wind speed, mosquito susceptibility, and application rate.

Knepper et al. (1982) found that the efficacy of malathion increased when applied on warmer days. Conversely, resmethrin had increased efficacy when the temperature was cooler. Hodjati and Curtis (1999) found that permethrin caused a much higher mortality in *Anopheles gambiae* Giles, and *Anopheles stephensi* Liston at higher temperatures.

There are instances where mosquitoes have developed resistance to insecticides. Over years of treatment with organophosphate compounds, primarily malathion, mosquitoes in some areas of the world have developed resistance.. In Sri Lanka continued treatments of malathion as both an adulticide and a broad-spectrum insecticide

has led to the resistance of mosquitoes in the genera *Anopheles* and *Culex* (Karunaratne and Hemingway 2001). Mosquitoes in some study areas are highly susceptible to adulticides. Lab tests conducted on species that vector dengue in India found that *Aedes aegypti* L. was highly susceptible to malathion and permethrin with the treatment causing 100% mortality in laboratory tests (Katyal et al. 2001). A group of researchers in Florida conducted an experiment to test the efficacy of larvicides against *Aedes taeniorhynchus* Wiedemann immatures. In this study, both methoprene and Bti had high efficacy on sentinel mosquitoes caged in the field, and temporary rain pools that were the mosquitoes' normal habitat (Lawler et al. 1999).

Woodrow (1995) found that methoprene had a 80% inhibitory rate over five weeks when tested for control of *Culiseta melanura* Coquillett in NY. Methoprene in this case was not applied as an ULV treatment but as time release pellets at 5 lbs/acre.

Risk Assessment

Risk assessment is a formal discipline that provides an objective evaluation of risk. The discipline has become widely used to make decisions about new technologies. Scientific data as well as societal considerations are made to describe the nature of the risk and communicate risk with decision makers. Within the risk assessment, assumptions and uncertainties are clearly presented. Ecological risk can be quantified as a function of hazard and exposure (NRC 1983). The approach uses a tiered modeling system that moves from deterministic models based on conservative assumption erring on the side of environmental safety, to refined probabilistic models using more accurate

assumptions (SETAC 1994). Risk assessment follows a logical framework. The process proceeds in stepwise fashion starting with 1) problem formulation, 2) hazard identification, 3) dose response relationships, 4) exposure assessment, and 5) risk characterization. These steps allow one to compare an estimated environmental concentration with a reference dose derived from a toxic endpoint (NRC 1983).

Objective

The purpose of this project is to determine the risks associated with current recommendations for management of the disease vectors. These recommendations include the following: public education, surveillance, and mosquito management (Shapiro and Micucci 2003). Obviously there is little risk involved with public education and surveillance. However, decision makers need data to make effective judgments about public health when confronting issues with mosquito management. The best program is one that will ensure the preservation of good public health, low environmental impact, and effective mosquito management. A tier I risk assessment will be done to identify any potential negative ecological effects of each chemical. The specific chemicals that will be examined are the adulticides malathion, naled, permethrin, resmethrin, d-phenothrin, pyrethrins, and the synergist, piperonyl butoxide (PBO), as well as the larvicides methoprene, Bti, Bs, and monomolecular films.

CHAPTER 2

AN ECOLOGICAL RISK ASSESSMENT FOR ADULTICIDES USED IN MOQUITO MANAGEMENT

Abstract

Since 1999, North America has seen the largest arboviral encephalitis epidemic in its history as a result of West Nile Virus (WNV). Vector management programs have been intensively implemented to manage mosquitoes that carry WNV. My ecological risk assessment focused on six common mosquito adulticides used in vector management, including four pyrethroids (d-phenothrin, resmethrin, and permethrin), pyrethrins, and two organophosphates (malathion and naled). Piperonyl butoxide, a synergist for the pyrethroids, was also included. Both aquatic and terrestrial non-target organisms were considered for acute and chronic exposures to the adulticides. Tier I exposure estimates were derived from ISCST3 and AERMOD (Industrial Source Complex models) for deposition and air concentrations affecting terrestrial organisms and PRZM-EXAMS (Pesticide Root Zone Model-Exposure Analysis Modeling System) for standard pond concentrations affecting aquatic organisms. A probabilistic assessment was also conducted to account for variability within the exposure models. Non-targets included for adulticides were small mammals, birds, as well as aquatic vertebrates and invertebrates in a pond receiving the chemical via drift and runoff. Risk quotients were obtained by comparing exposure with toxic endpoints. These risk quotients were compared to the probability of obtaining the deterministic values given the models used. Neither the

deterministic nor the probabilistic assessment calculated risk quotients that exceeded a regulatory level of concern except for amphipods exposed to organophosphates. All of the risk quotients calculated in the deterministic model had risk quotients greater than the 95th percentile of the possible outcomes given the probabilistic model.

Introduction

West Nile Virus (WNV) has been a concern for people across North America since the disease was initially observed in the U.S. in the summer of 1999. Since that year, WNV has caused the largest arboviral encephalitis epidemic in U.S. history (Huhn et al. 2003). However, the disease has resulted in thousands of morbidity cases and hundreds of deaths in humans; many people are also concerned about the risks associated with managing mosquitoes using insecticides (Peterson et al. 2006). This concern is related to the perception that ecological and human exposure to the insecticides will lead to risks that are more severe than WNV itself.

West Nile Virus is vectored by several mosquito species, mostly from the *Culex* genus (Turrel et al. 2005). Mosquito management plans have been implemented in the U.S. and globally to manage mosquito vectors of WNV, malaria, dengue, and other diseases. Common mosquito adulticides are pyrethroids such as d-phenothrin, resmethrin, and permethrin, as well as organophosphates such as malathion and naled. Natural pyrethrins are also available as mosquito management agents.

The ultra-low-volume (ULV) space sprays target adult mosquitoes as they are flying through the air. Adulticides used as space sprays become effective when they are

released in the atmosphere as extremely small liquid droplets ranging in size from 8-30 microns (Rose 2001). The small droplet size increases the surface area available to contact the target. The insecticide is absorbed through an insect's cuticle and takes effect soon after contact (Rose 2001; USEPA 2006d). Each adulticide is a broad spectrum insecticide that is toxic to many arthropods. Pyrethrins, pyrethroids, and organophosphates are all neurotoxins. Pyrethrins and pyrethroids act via sodium channel disruption in the insect nervous system; organophosphates act as acetocholinesterase inhibitors. Other insects in the spray zone may be deleteriously affected by the application of adulticides. Mosquito management programs often aggressively apply adulticides in the midst of a disease outbreak. Most are sprayed in the evening, or early morning when female mosquitoes are seeking a blood meal, and many other arthropods, particularly pollinators, are inactive .

For most mosquito insecticides, ecological incident reports (i.e., adverse effects) have been reported, but have been typically associated with pest management programs in crops. Despite the much lower use rates and ULV delivery methods, it is plausible that adult mosquito management programs may pose similar ecological risks.

Risks from mosquito management plans implemented to manage WNV vectors can be quantified using a risk assessment framework. Risk assessment is a formal discipline that provides an objective methodology to evaluate risk. The discipline has become widely used to make decisions about new or controversial technologies that may pose a risk to the public or the environment. Scientific data as well as societal considerations are used to describe the nature of the risk and communicate risk with

decision makers. Within a risk assessment, assumptions and uncertainties are clearly presented. Ecological risk can be quantified as a function of hazard and exposure (NRC 1983). The approach uses a tiered modeling system that moves from deterministic models based on conservative assumptions erring on the side of environmental safety, to refined probabilistic models using more realistic assumptions (SETAC 1994). Risk assessment follows a logical framework. The process proceeds in stepwise fashion including: (1) problem formulation, (2) hazard identification, (3) dose response relationships, (4) exposure assessment, and (5) risk characterization. These steps allow for the comparison of an estimated environmental exposure with a reference dose derived from a toxic effect (NRC 1983). Probabilistic risk assessment uses a stochastic approach to verify the risks predicted by the screening level model and shed some light on probable risks outside of a reasonable worst-case scenario. My risk assessment includes an exclusive relationship between the tier-1 deterministic assessment and the more realistic probabilistic assessment where the probabilistic assessment is an expansion of plausible outcomes given the model used in the deterministic assessment.

To my knowledge, ecological risk assessments for insecticides used in adult mosquito management have not been published in the scientific literature. Therefore, the objective of this study was to quantify the ecological risks to warm- and cold-water vertebrates and aquatic invertebrates that may be in a generic watersheds where a mosquito management programs are implemented, as well as birds and mammals that are in the spray zone.

Materials and Methods

Problem Formulation

Mosquito management programs are important to effectively manage vector-borne illnesses such as WNV. Limiting mosquito habitat, using repellents, and targeting the insects using synthetic and natural chemicals are tactics for integrated mosquito management. In the case of pesticides, each active ingredient may pose an unacceptable risk to non-target aquatic and terrestrial organisms where the chemicals are applied to manage mosquitoes. Therefore, protecting the public and the environment from deleterious exposures to chemicals is a major concern when managers consider implementing a mosquito management program.

Applying adulticides via a ULV sprayer spreads small aerosol particles that target flying mosquitoes as they are moving through the air (CDC 2003). My conservative (lower tiered) assessment considered acute and chronic exposure and risk of ULV insecticides to aquatic vertebrates and aquatic invertebrates after a truck-mounted sprayer treated an area where part of the spray drifted into the pond, and a portion of the insecticide that deposited on the ground was transported to the pond as runoff. The assessment also estimated risk to terrestrial vertebrates from exposure to the insecticides from deposition on fur and feathers followed by grooming, inhalation for 24 hours after the spray event, and ingestion of food items that the insecticides settled on. A probabilistic risk assessment was conducted in tandem to verify the conservatism of the reasonable worst-case scenario.

Hazard Identification

The ecological risk assessment focused on six common mosquito adulticides, including three broad spectrum pyrethroids (d-phenothrin, resmethrin, and permethrin), pyrethrins, and two broad spectrum organophosphates (malathion and naled). Piperonyl butoxide (PBO), a synergist for the pyrethroids and pyrethrins, also was assessed on its own. These chemicals are applied in the evening or early morning when female mosquitoes are seeking a blood meal and most other flying insects are inactive.

Dose-Response Relationships and Toxic Endpoints

Toxicity endpoints were gathered for surrogate organisms, model organisms used in screening level risk assessments, where consistent previously published data were available including three birds, bobwhite quail (*Colinus virginianus*), mallard duck (*Anas platyrhynchos*), and ring-necked pheasant (*Phasianus colchicus*), four mammals common in the many settings, rat (*Rattus rattus* L.), shrew (*Blarina brevicauda* Say), vole (*Microtus pennsylvanicus* Ord), and mouse (*Peromyscus maniculatus* Wagner), and four freshwater aquatic organisms, rainbow trout (*Oncorhynchus mykiss*), bluegill sunfish (*Lepomis macrochirus*), *Daphnia magna*, and amphipods (*Gammarus sp*.).

Each of these insecticides is classified by the United States Environmental Protection Agency (USEPA) as slightly to moderately toxic to birds with acute LC_{50} values (insecticide concentrations in food that cause mortality in half of the test animal population) ranging from 2,538 to 21,117 mg/kg dry weight of food, with naled being the most toxic to ring-necked pheasants. Chronic no-observed-effect-concentrations

(NOECs), the highest doses tested within a toxicology study where no effects were observed, were generally collected from five month reproductive studies for all of the chemicals except malathion's toxicity to mallard duck, which was a growth and viability study, and resmethrin's toxicity, which considered reduced weight of adult males both for mallard duck, and bobwhite quail. NOECs ranged from 60 to 1,200 mg/kg dry weight of food; of the five insecticides for which chronic NOECs were available, resmethrin had the lowest NOEC (Table 1).

Each mosquito adulticide is considered by USEPA as slightly to moderately toxic to mammals. Most mammalian testing is done on rats and calculated for other small mammal species by using dose-related rat toxic endpoints and converting them to dietary endpoints for other small mammals by implementing the following (USEPA 1998b):

$$TE_m = TE_r * BW \div C \qquad [1]$$

where TE_m is the estimated dietary toxic endpoint for other small mammals (mg/kg dry weight food), TE_r is the dose related dietary endpoint for rats (mg/kg body weight), BW is the body weight of the small mammal (kg), and C is the food consumption in one day (kg). Consumption data for each of the small mammals evaluated in the risk assessment were obtained from the USEPA Wildlife Exposure Factors Handbook (USEPA 1993) or appropriate allometric equations found within. I used the following assumptions from the handbook: rats weighed 390 g, and consumed 18 g/day, shrews weighed 15 g and consumed 8 g/day, mice weighed 21 g and consumed 4 g/day, and voles weighed 32 g and consumed 7 g/d. Acute LC_{50} values ranged from 443 mg/kg dry weight of naled in food for shrews to 73,500 mg/kg dry weight of pyrethrins in food for mice. Chronic

NOECs collected from five month reproductive studies, except for malathion for which the NOEC was based on cholinesterase reduction, ranged from 17 mg/kg dry weight of naled in food for mice to 530 mg/kg dry weight of d-phenothrin or PBO in food for shrews (Table 2).

Each adulticide is classified as almost non-toxic to highly toxic to fish and aquatic invertebrates, with acute endpoints ranging from 0.000075 ppm (mg/L of water) of resmethrin to bluegill sunfish, to 30,000 ppm of d-phenothrin to *D. magna*. Chronic lifecycle NOECs ranged from 0.000039 ppm permethrin for bluegill sunfish to 1.31 ppm PBO for rainbow trout (Table 3).

Exposure Assessment

Terrestrial Exposures In addition to standard dietary consumption exposure routes, exposure through inhalation and grooming was included in the exposure pathways. Adulticides were assumed to be sprayed a total of 10 times (days 1, 4, 14, 17, 27, 30, 33, 43, 46, and 56) at the maximum label application rates. Application rates were 39 g/ha for PBO, 4 g/ha for d-phenothrin, 8 g/ha for permethrin and resmethrin, 64 g/ha for malathion, 22.5 g/ha for naled, and 9 g/ha for pyrethrins. We used a total of 10 sprays to represent a reasonable worst-case application regime during an outbreak of WNV in humans (NYCDOH 2005; Peterson et al. 2006). AERMOD v. 1.0 (USEPA 1999) was used to predict the air concentrations of each insecticide 7.62 m (25 ft) from the path of the spray within 1- and 12-hour time ranges for each adulticide and PBO at the ground level for mammals and at an altitude of 7 m for birds. AERMOD is an industrial source

plume model developed to predict air concentrations of pollutants from industrial sources and has been adopted as a tier-I model for this project. The reasonable worst-case exposure scenario had the following assumptions: (1) each chemical had a 24 hour half-life in the environment except for naled, which was given an 18 hour half-life, (2) the ULV applications had 3% of the emitted particles greater than the allowable particle size, (3) the insecticides were applied at the maximum application rate as stated on each label, (4) all of the insecticides were susceptible to the same weather conditions using standardized weather data from Albany, NY in 1988, (5) all spray events occurred at 9 pm, and (6) each spray release occurred 1.5 m above ground level.

Modeled receptors were set up on a Cartesian grid at five intervals of 25 m at 7.62 m from each side of the spray emission area. The receptors were at ground level for mammals and 7 m above ground for birds, as many bird species are likely to be exposed above ground. Each receptor recorded the 1- and 12-hour average air concentrations for each insecticide. An average was taken of readings from the 8 receptors at 7.62 m that were not at the edges of the spray zone. The following data were obtained using this network of receptors: the 1-hour average concentration at 7.62 m, and the 12-hour average at 7.62 m.

The industrial source complex dispersion model (ISCST3) (USEPA 1995) was used to model particle deposition at 7.62 m from the spray area at the 1-hour average. ISCST3 is AERMOD's predecessor and was developed first for prediction of deposition and aerial concentrations of industrial pollutants. This model was used to estimate deposition because AERMOD did not have a deposition parameter at the start of this

project. The same assumptions were used with this program as with AERMOD except that the default meteorological data were from Salem, MA. The following assumptions were made in addition to those from AERMOD: (1) the ULV applications had 3% of the emitted particles greater than the allowable particle size as stated on the label, and (2) the particles were assigned a density in accordance with the specific gravity of each formulated product.

A similar Cartesian Grid was used for ISCST3 that was used in AERMOD above. Receptors were at 7.62 m from the spray source. The receptors were at ground level and 7 m in accordance with the grid used for AERMOD. All of the same methods were used to calculate the average deposition at 7.62 m. AERMOD and ISCT3 did not consider deposition onto foliar surfaces or lateral impingement by vegetation. This serves as a reasonable worst-case scenario as lateral impingement would limit deposition and inhalation exposure to birds.

The Kenaga nomogram was used to predict concentrations on food such as long grass, short rangegrass, fruits, seeds, and insects, and broadleaf plants (Fletcher et al. 1994). The nomogram is a linear model that uses application rate to predict concentrations of the insecticide on different types of food. For this exercise, estimated environmental concentrations (EEC) were calculated for short rangegrass (high value) as well as fruits, seeds, and insects (low value) because these two food type groups were at the extremes of predicted concentrations.

Sprays were followed through the 90th day to estimate chronic exposures to surrogate birds and mammals that would be likely to eat food in the spray area, using the following degradation model (USEPA 2004):

$$D=\sum_{j=i}^{n} Pe^{r_1 t} \qquad [2]$$

where D is the sum of the deposition over one spray, P is the peak deposition after a spray event, r_1 is the rate of decay calculated by using each active ingredient's aerobic soil half-life, t is the time in hours, i is the spray day, and n is the decay period. The average daily deposited concentration (DD) within n days can be calculated by the following:

$$DD = (\sum_{j=1}^{n} Pe^{r_1 t} + \sum_{j=4}^{n} Pe^{r_1 t} + \ldots\ldots + \sum_{j=56}^{n} Pe^{r_1 t}) \div n \qquad [3]$$

Total acute exposures for the terrestrial wildlife were assumed to be a summation of exposure to the animal through its diet, cleaning and preening of fur or feathers, and inhalation on the evening of the spray event. Chronic exposures were evaluated in a similar way, although the grooming and inhalation doses were assumed to correspond with 1-day pulse events each spray day. Deposition on the surface of the animal was assumed to cover the entire body at the concentrations calculated from ISCST3. Inhalation doses corresponded with the values predicted from AERMOD. Animals were assumed to breathe the peak average concentration for 2 hours after the spray event, and to breathe the 12-hour average concentration for an additional 22 hours to develop a

reasonably conservative estimated inhalation exposure on the spray date. Additionally, all exposures were standardized for dietary ingestion, which is congruous with the assumptions specified in equation 1. Equation 4 outlines the acute exposure calculation for terrestrial wildlife.

$$EE_A = (DD^* C + EEC_d * SA + EEC_a * IR) \div C \quad [4]$$

where EE_A is the estimated acute exposure, DD is the estimated environmental concentration on food (mg/kg dry weight) from Equation 3, C is the consumption rate of the animal (kg dry weight/day), EEC_d is the estimated environmental concentration on at the given receptor height for deposition (mg/m^2), SA is the animal's total surface area (m^2) as estimated by allometric equations or outlined in USEPA (1993), EEC_a is the estimated aerial concentration of the adulticide (mg/m^3), and IR is the inhalation rate (m^3) (Table 4). Chronic exposures were assessed by Equation 5:

$$EE_C = \sum_{n=1}^{90} EE_A \div 90 \quad [5]$$

where EE_C is the estimated chronic exposure and n is the date within the 90 day model. For this summation, EEC_d and EEC_a are zero within Equation 4 on non-spray days to mimic pulse events.

As a refinement to the tier I assessment for terrestrial receptors, a probabilistic assessment was conducted to include variability within the Kenaga nomogram, ISCST3, and AERMOD. A frequency related approach utilizing Monte Carlo sampling was used to predict a range of exposure, and their likelihood given the models used. This assessment included receptors within 91.44 m on either side of the spray source to

represent the width of recommended spray swaths, and spray times that ranged from 9 pm to 3 am. Variation in food sources as identified by the Kenaga nomogram for fruits and seeds, short rangegrass, long rangegrass, and broadleaf plants were assigned a uniform distribution. Food intake rates and their associated standard errors based on those reported in USEPA (1993) were also used. Non-estimated data were assigned a normal distribution centered at the mean. Data estimated by allometric equations were assigned normal distributions accounting for variability within the model used to predict food intake. Those data where distibutional information was not available for food intake were assigned normal distributions with standard deviations that were proportional to those reported for like groups. Inhalation and deposition exposures were assigned distributions based on the chi-square goodness of fit statistic. The family of distributions assigned to inhalation and deposition exposures included Pareto, Weibull, exponential, and gamma (Decisioneering 1998). Monte Carlo simulations included 5,000 iterations to determine the most likely exposure distributions given the deterministic model. A sensitivity analysis was included to determine which variables in the model contributed the most variability to the distribution. Risk quotients based on those predicted by both the deterministic and probabilistic model and the sensitivity analysis are presented in the results.

Aquatic Exposures The USEPA water quality software EXPRESS v. 1.00.00.012 (USEPA 2005c) was used to obtain EEC's in the standard farm-pond, 2 m deep with a 1 ha surface area, scenario through its interface with the Pesticide Root Zone Model v. 3.12.3 (PRZM) (USEPA 2005e), and the Exposure Analysis Modeling System v.

2.98.04.06 (EXAMS) (USEPA 2005b) for aquatic organism exposures. The input parameters used for all adulticides were: (1) a 45-m buffer as the distance of the spray from the pond as the chemicals are not to be sprayed into permanent water bodies , (2) spray drift into the pond was 1%, reflecting the default drift percentage from a high boom, fine particle, ground sprayer, (3) applications were made on Florida turf grass, a conservative, minimal vegetative cover that would be in a mosquito management area, (4) applications began on July 1st and ended August 25th with 3-and 10-day intervals between applications, and (5) applications were made at the maximum use rate listed on the label for mosquito management. PRZM-EXAMS input parameters for most of the mosquito insecticides were gathered from their respective USEPA Reregistration Eligibility Document or other sources (Table 5). Acute exposures for fish and *D. magna* were predicted as the peak estimated environmental concentration. Chronic exposures for fish were defined as the 60- or 90-day estimated average environmental concentration depending on the length of the study associated with the appropriate toxic endpoint. Chronic exposures for *D. magna* were defined as the 21-day estimated average environmental concentration. PRZM-EXAMS has a probabilistic component native to the program. Exposure concentrations extracted from PRZM-EXAMS were at the 95th percentile of possible exposures over a 30-year period.

None of the models used in this study specifically incorporates ULV applications. In ISCST3 a range of aerosol particle diameter values is incorporated into the model. PRZM-EXAMS was developed to mimic broad spectrum crop pest management programs and only has a “very-fine” spray parameter. These models that do not use ULV

spray application modeling are most likely very conservative because the particle size definitions would tend to concentrate the particle in an area of interest. Despite not having a "ULV" parameter, PRZM-EXAMS lets the modeler specify the amount of drift into a pond regardless of spray type.

Risk Characterization

Ecological risks for the adulticides were assessed by dividing the EEC determined by PRZM-EXAMS for aquatic organisms, or a combination of AERMOD, ISCST3, and the Kenaga nomogram for terrestrial organisms by the toxic endpoints for each organism to obtain a risk quotient (RQ). Exposure periods that corresponded to the period of each chronic endpoint study were used for aquatic organisms, and terrestrial organisms were assumed to be exposed for 90 consecutive days after the first spray date.

Results

Acute risks to birds exposed to one spray application ranged from an RQ of <0.001 for permethrin to 0.02 for malathion (Table 6). Chronic RQs were calculated only for bobwhite quail and mallard duck because there were limited data on chronic toxicity endpoints for ring-necked pheasant. Chronic RQs ranged from 0.01 for permethrin, malathion, and naled to 0.2 for resmethrin. Chronic endpoints were not available for d-phenothrin or pyrethrins (Table 7). Ingestion from preening contributed most of the acute exposure to birds, ranging from 69-99% of total exposure. Dietary or preening ingestion contributed most of the chronic exposure depending on the persistence of the chemical. If the chemical was deposited and ingested on fruits and seeds, the major contributing

exposure route was preening, with a 61-97% contribution to total exposure. If it was applied to short rangegrass the major contributor varied between dietary intake and preening. Preening contributed 9-65% if the dietary exposure was calculated from short rangegrass. The probabilistic assessment revealed that the overall exposure estimates were most likely conservative given the models. Each RQ estimate in the reasonable worst case model exceeded the 95th percentile of the probabilistic model.

Acute RQs were calculated for surrogate species that would represent the most common mammals in an area. Risk quotients ranged from <0.001 for permethrin, PBO, resmethrin, d-phenothrin, and pyrethrins to 0.07 for malathion (Table 8). Chronic RQs ranged from 0.003 for d-phenothrin to 0.5 for malathion (Table 9). Contributing exposure for mammals was similar to birds, with ingestion from grooming contributing the largest portion of the acute exposure in most cases, contributing 28-99% of total exposure. Grooming or diet contributed the largest portion of chronic exposure depending on the persistence of the chemical and the organism. Shrew exposure estimates were a particular anomaly because they consume over half of their body weight in food per day. Grooming ranged from almost no contribution to 99% of the contribution for non-persistent chemicals.

Daphnia magna and amphipods (scuds) were the only aquatic invertebrates evaluated in the assessment. Acute RQs for *D. magna* ranged from <0.001 for PBO to 0.04 for naled, malathion, and permethrin. Chronic risks for *D. magna* were <0.001 for PBO to 0.04 for permethrin. Available information for amphipods was limited. Acute RQs for scuds exposed to organophosphates ranged from 0.08 to 0.09 (Table 10).

Acute RQs for both warm- and cold-water fish ranged from <0.001 for PBO, permethrin, and naled to 0.02 for d-phenothrin. Chronic RQs for rainbow trout ranged from <0.001 for PBO and d-phenothrin to 0.004 for malathion (Table 10).

A probabilistic assessment was conducted for terrestrial receptors to include model variability. Inhalation, grooming and preening, food intake, and deposition onto food were varied to include distance from receptor within 91.44 m, spray date, and food type. Probability distributions indicate that all of the acute and chronic RQs estimated for birds and mammals were above the 95th percentile except for rats exposed to pyrethrins. The estimated RQs in the deterministic model exceeded 95 % of the possible outcomes given the models used. (Tables 6-9). Sensitivity analyses indicated that the exposure distribution from deposition onto feathers accounted for the majority of the acute estimated exposures to birds (54.4-90.7%); deposition onto food accounted for the rest of the variability in the acute model (9.3-44.6%). Sensitivity analyses for the chronic 10 spray scenario indicated that deposition onto feathers (0.4-61.8%) or deposition onto food (7-99.5%) accounted for most of the variability in the model (Table 11). For acute exposure to mammals, deposition onto food (4.4-99.9%) or food intake per day (0-95.4%) accounted for most of the variability in the model; for chronic exposures, most of the variability in the models were accounted for by deposition onto food (73.9-~100%) (Table 12).

Discussion

RQ levels of concern (LOCs) are regulatory tools used as aides in the risk management process (USEPA 2006h). Managers compare the RQ and LOC to determine if regulatory action is needed. The acute regulatory LOCs used by the USEPA are 0.5 for non-endangered birds and mammals, 0.2 for non-endangered terrestrial vertebrates exposed to restricted use insecticides, 0.1 for endangered birds and mammals, 0.5 for aquatic species, 0.1 for aquatic species exposed to restricted use insecticides, and 0.05 for endangered aquatic species. Chronic LOCs are 1.0 for all animals. If a RQ breaches a regulatory LOC, then the manager decides to either restrict the product's use or progress to a higher tiered assessment which utilizes a probabilistic or field-verified model (USEPA 2006h).

Only amphipods exposed to organophosphates exceeded USEPA RQ LOCs for any of the chemicals within any LOC category. No other chosen sentinel organisms had risk quotients that breached a RQ LOC due to the minimal exposure to the insecticide released in the environment.

Mammalian toxicity endpoints derived from studies done on rats did not account for interspecific variation within the mammalian surrogates considered. Although these extrapolations are common practice within USEPA risk assessments for insecticide reregistration, safety factors could be applied to the mammalian RQs to preserve reasonable worst case scenario conservatism. My probabilistic study predicted that RQs predicted in the reasonable worst case scenario were unlikely given the model. Insecticide exposures were mitigated by including model variance.

The results from my risk assessment can be compared to a few other studies. Pearce and Balcom (2005) summarized a worst-case impact of pyrethroid insecticides sprayed around Long Island Sound, New York that may have been responsible for a lobster kill within the fishery. Tier II modeling approaches concluded that insecticide concentrations in Long Island Sound of malathion ranged from 1 ppm to 5 ppm; the highest estimated environmental concentrations were 0.099 ppm for d-phenothrin and 0.034 ppm for resmethrin (Landeck-Miller et al. 2005). Therefore, Pearce and Balcom (2005) concluded that the relative risk of the pesticides was minimal compared to other circumstances, such as persistent disease and warm water that were already affecting the lobster population.

Jensen et al. (1999) found no treatment effects on non-target aquatic invertebrates downwind from ULV sprays of pyrethrins, malathion, and permethrin. Furthermore, they found no impacts on species diversity within the seasonally impounded ponds and detected chemical concentrations that would be non-toxic to vertebrate receptors.

The New York City Department of Health (NYCDOH) conducted a geographically specific environmental impact statement (EIS) published online in 2005 in which risk outcomes from adulticiding are reasonably consistent with my findings. Many of the chemicals evaluated in this risk assessment were evaluated by NYCDOH. Ecological risks for avian and mammalian receptors were below LOCs for all surrogates after a Tier II risk assessment. Potential non-target impacts on terrestrial and aquatic invertebrates that exceeded an LOC were addressed by citing studies where evidence suggests that non-target impacts on these communities would be negligible. NYCDOH

(2005) used different predictive modeling resources to conduct their risk assessment. I used the PRZM-EXAMS model in my study and provided screening level/Tier II aquatic concentration outcomes within a long-term scenario where consistent adulticiding was assumed to occur every summer. These predictive outcomes were of lower magnitude than the NYCDOH model, but are considered robust by USEPA (NYCDOH 2005).

An EIS similar to NYCDOH (2005) is currently being conducted by the Suffolk County Department of Public Works and Department of Health Services in Suffolk County, New York (Suffolk-County 2006). The draft Suffolk County EIS is consistent with my results and predicts negligible acute and long-term ecological effects of adulticiding at both the individual and community level. Terrestrial dietary RQs from the terrestrial screening level assessment did not exceed 1.0 for any of the species examined. The screening level of the aquatic assessment identified risks that breached levels of concern in aquatic environments for malathion and permethrin synergized by PBO. A Tier II risk assessment conducted to mitigate potential risks reduced RQs below LOCs.

Complete reregistration eligibility documents have been prepared by USEPA for naled, malathion, and PBO. No regulatory issues were identified for mosquito management where the application rate parameter was 111 g/ha for naled. This estimate was made using an application rate five times of that used in my assessment (USEPA 1997c, 2000). Malathion exceeded regulatory LOCs for freshwater fish and invertebrates at the maximum application rate for mosquito management of 706 g/ha assuming 20% drift from a truck-mounted ULV application (USEPA 2000). USEPA (2005h) found that PBO used in mosquito abatement did not breach acute or chronic LOCs for aquatic

organisms in a scenario similar to my assessment, nor were any chronic LOCs breached for mammals when PBO was assumed to be applied at 560 g/ha, however there was some chronic risk to birds at that application rate. The lowest application rate in the USEPA reregistration eligibility document is 14 times greater than the rate suggested for use in mosquito management. The application rates we used were gathered from insecticide labeling and represent rates commonly used in mosquito management.

Many terrestrial arthropods will be exposed in the same way as flying or resting mosquitoes. Spray applicators generally apply mosquito insecticides when mosquitoes are active at dawn and dusk. Spray events that follow the mosquitoes' behavior are likely to limit exposure to honey bees and other terrestrial arthropods in the spray zone. Arthropods that are active at dawn and dusk, like mosquitoes, may be exposed to the spray with potentially negative effects. However, because of the nature of the applications and their frequency, it is unclear how arthropod communities will be affected in the field (Caron 1979).

Coldburn and Langford (1970) found high bee mortality from applications of naled, malathion, and pyrethrum with PBO. These field tests did not use a ULV sprayer, but the study suggests that chemicals commonly used in mosquito management may lead to non-target arthropod mortality. Caron (1979) exposed caged honey bees and hives to ULV sprays of malathion, naled, and pyrethrum. This study showed decreasing mortality as distance increased from the insecticide release. Pankiw and Jay (1992) found that honey bees in cages experienced significant mortality from malathion spray drift. Hester et al. (2001) observed significant bee mortality in hives that were exposed to malathion

spray events both in open fields and in a forested environment. However, there was no effect on beehive weight or health of the colony over a season. Tietze et al. (1996) used sentinel crickets to measure spray deposition in a peri-domestic environment. Cricket mortality varied between 12.5 and 48.7% depending on where crickets were put in residential yards in relationship to the spray zone.

There is variability and uncertainty associated with environmental modeling approaches. None of the models has a scenario for ULV spray applications, which limits their ability to predict EECs in mosquito adulticiding scenarios. A robust series of insecticide and aerosol studies that focus on environmental fate and the insecticide's action in the environment would greatly benefit future modeling applications. The physico-chemical input parameters for the environmental fate models also are uncertain. The USEPA uses conservative estimates in their reregistration eligibility documents on insecticide persistence depending on how many studies have been conducted on the degradation of each insecticide. Their input parameters are inconsistent with the properties listed in the USDA-ARS pesticide properties database, and, in many cases, are multiples of these values. The nature of the environmental fate models used in my study, also used by the USEPA, represent reasonable worst-case scenarios and consistently over-predict both acute and chronic exposures to insecticides. The probabilistic assessment incorporated much of the uncertainty and variability associated with the data and models used in this study. Sensitivity analyses identify environmental fate of the insecticide in the environment as the largest contributor to uncertainty. In light of the

sensitivity analysis, more data is needed when incorporating environmental fate and persistence of the adulticides evaluated in this risk assessment.

Limited studies on downwind deposition and drift, specifically related to acute exposures, highlight the probable conservative outcomes from AERMOD and ISCST3. Moore et al. (1993) found that deposition of malathion on ground-level patches between 15.2 and 91.2 m ranged from $8.4x10^{-4}$ to $4.2x10^{-5}$ g/m^2. Tietze et al. (1996) measured a immediate-peak deposition of malathion in a peridomestic environment of $4.73x10^{-3}$ g/m^2. Average depositions in the same study were three times less, ranging from $2.99x10^{-4}$ to $8.8x10^{-4}$ g/m^2. Knepper et al. (1996) observed 0.1439 g/m^2 and 0.0922 g/m^2 concentrations of permethrin and malathion, respectively, in turfgrass after a ULV spray event in a suburban environment. Although Knepper et al. (1996) observed higher concentrations of malathion than Tietze et al. (1996) and Moore et al. (1993) immediately after spraying, Knepper et al. (1996) showed malathion and permethrin had very low persistence in the environment with almost no trace of either after 36 hours. The deposition values modeled in my assessment were 1.7 to 4.9 times greater than the averages from Tietze et al. (1996) and Moore et al. (1993), but less than Knepper et al. (1996). However, my chronic models used chemical half lives much greater than those observed by Knepper et al. (1996).

We also conducted similar simulations using Agdrift v. 2.00.05 (Spray-Drift-Task-Force 2000), which resulted in much lower estimates of insecticide deposition at the ground level compared to ISCST3. Indeed, predicted ranges in the tier-I Agdrift model were 1-3% of the predicted surface deposition concentrations used in my assessment.

Agdrift is currently the industry standard for predicting both on and off target spray drift deposition and is considered a conservative model.

Including bootstrapping or Monte Carlo simulations that allow for uncertainty in the modeling process is an available technique until more studies are conducted on the degradation of these chemicals. Model variability can be varied within AERMOD and ISCST3 in the form of deposition and air concentration characteristics from topography, spray time, spray date, geographical location, and a variety of other factors. Crop type and spray date could be varied in PRZM-EXAMS. In some cases, different studies come to different conclusions about the persistence of chemicals given different substrates and varied environmental conditions. Variation in the inhalation, preening, and ingestion rates and food items in birds and mammals also add to the variation in RQ outcomes. Varying characteristics and entering them as distributions into stochastic models that develop a range of exposure predictions related to the variability of each input provides a range of risk values.

Synergized pyrethroids (containing PBO) have been shown to be more toxic to trout than technical grade pyrethroids (Paul et al. 2005). Based on my results, the RQs when PBO is added to the pyrethroids and pyrethrins would still be below LOCs for aquatic vertebrates. If toxicity was increased 10-fold for aquatic invertebrates, RQs would still be below LOCs for non-endangered species (Table 10). However, a large scale management response to WNV where PBO is applied with pyrethrins or pyrethroids has been shown to synergize with more persistent pyrethroids in aquatic systems. Weston et al. (2006) found supplemental concentrations of PBO in creeks around Sacramento,

California that increased residual pyrethroid efficacy, particularly bifenthrin, on non-target amphipods. However, none of the 11 water samples collected in this study caused significant mortality to *Ceriodaphnia dubia* Richard.

Several receptors that may be sensitive to the insecticides evaluated in this assessment were not included as data is inconsistent both within each insecticide and between insecticides. Amphibians, benthic invertebrates, and estuarine species may be particularly important when assessing the risks of each insecticide in the environment.

Results from my conservative and probabilistic ecological risk assessments and the weight of scientific evidence suggest that risks to ecological receptors most likely are small from ULV insecticides applied numerous times within a mosquito management program. Because we used several conservative exposure assumptions, more realistic assessments most likely would result in RQ values much lower than reported here. Further, environmental exposures from adulticide applications are not likely to add appreciably to background levels of the same active ingredients from agricultural and urban uses.

Table 1. Toxic endpoints of adulticides for birds

Chemical	Species	Acute LC_{50} (mg/kg dry weight)	Chronic NOEC (ppm)
Malathion	Bobwhite Quail	3497[b]	110[b]
	Mallard Duck	5000[b]	1200[b]
	Pheasant	2639[b]	NA
Naled	Bobwhite Quail	2117[c]	130[a]
	Mallard Duck	2724[c]	260[c]
	Pheasant	2538[c]	NA
Permethrin	Bobwhite Quail	5200[d]	500[d]
	Mallard Duck	10000[d]	125[d]
	Pheasant	23000[d]	NA
Resmethrin	Bobwhite Quail	5000[e]	60[e]
	Mallard Duck	5000[e]	60[e]
	Pheasant	NA	NA
PBO	Bobwhite Quail	5620[f]	300[f]
	Mallard Duck	5620[f]	300[f]
	Pheasant	NA	NA
d-Phenothrin	Bobwhite Quail	5000[a]	NA
	Mallard Duck	5620[a]	NA
	Pheasant	NA	NA
Pyrethrins	Bobwhite Quail	5620[g]	NA
	Mallard Duck	5000[a]	NA
	Pheasant	5000[a]	NA

[a] (USEPA 1997a)
[b] (USEPA 2005d)
[c] (USEPA 2006b)
[d] (USEPA 2005a)
[e] (USEPA 2005g)
[f] (USEPA 2005h)
[g] (USEPA 2005f)

Table 2. Toxic endpoints of adulticides for mammals

Chemical	Species	Acute LC_{50} (mg/kg dry weight)	Chronic NOEC (ppm)
Malathion	Shrew	736	53
	Mouse	2048	19
	Vole	1857	21
	Rat	(LD_{50} 390[a])	(NOEL 100[a])
Naled	Shrew	443	48
	Mouse	1234	17
	Vole	1119	19
	Rat	(LD_{50} 235[b])	(NOEL 90[b])
Permethrin	Shrew	2791	53
	Mouse	7765	19
	Vole	7043	21
	Rat	(LD_{50} 1479[c])	(NOEL 100[c])
Resmethrin	Shrew	8753	265
	Mouse	24355	95
	Vole	22090	105
	Rat	(LD_{50} 4639[d])	(NOEL 500[d])
PBO	Shrew	8623	530
	Mouse	23992	190
	Vole	21761	210
	Rat	(LD_{50} 4570[e])	(NOEL 1000[e])
d-phenothrin	Shrew	9434	530
	Mouse	26250	190
	Vole	23810	210
	Rat	(LD_{50} 5000[f])	(NOEL 1000[f])
Pyrethrins	Shrew	1,321	53
	Mouse	3,675	19
	Vole	3,333	21
	Rat	(LD_{50} 700[g])	(NOEL 100[g])

[a](USEPA 2005d)
[b](USEPA 2006b)
[c](USEPA 2005a)
[d](USEPA 2005g)
[e](USEPA 2006f)
[f](WHO/FAO 1994)
[g](USEPA 2006g)

Table 3. Toxic endpoints of adulticides for aquatic organisms

Chemical	Species	Acute LC_{50} (ppm)	Chronic NOEC (ppm)
Malathion	*Daphnia magna*	0.001 [b]	0.00059 [b]
	Bluegill Sunfish	0.02 [b]	NA
	Rainbow Trout	0.004 [b]	0.002 [b]
Naled	*Daphnia magna*	0.0003 [c]	4.5E-05 [a]
	Bluegill Sunfish	2.2 [c]	NA
	Rainbow Trout	0.16 [c]	NA
Permethrin	*Daphnia magna*	0.00011 [d]	3.9E-05 [e]
	Bluegill Sunfish	0.00079 [e]	0.0003 [a]
	Rainbow Trout	0.0084 [a]	.09 [a]
Resmethrin	*Daphnia magna*	0.00022 [a]	NA
	Bluegill Sunfish	0.000075 [d]	NA
	Rainbow Trout	0.00028 [f]	NA
PBO	*Daphnia magna*	0.51 [g]	0.066 [a]
	Bluegill Sunfish	4 [g]	NA
	Rainbow Trout	1.8 [g]	1.31 [a]
d-phenothrin	*Daphnia magna*	30000 [d]	NA
	Bluegill Sunfish	0.001 [a]	NA
	Rainbow Trout	0.0014 [d]	1.1 [a]
Pyrethrins	*Daphnia magna*	0.0116 [h]	9.6E-05 [a]
	Bluegill Sunfish	0.0187 [d]	NA
	Rainbow Trout	0.0032 [h]	NA

[a](USEPA 1997a)
[b](USEPA 2005d)
[c](USEPA 2006b)
[d](USEPA 2006a)
[e](USEPA 2005a)
[f](USEPA 2005g)
[g](USEPA 2005h)
[h](USEPA 2005f)

Table 4. Descriptive statistics for terrestrial animal exposure

	Food Intake (kg/day)	Surface Are (m^2)	Inhalation Rate (m^3/day)
Bobwhite Quail	0.0177	0.0331	0.1
Ring-neck Pheasant	0.0582	0.1002	0.41
Mallard Duck	0.0619	0.1132	0.45
Rat	0.018	0.0594	0.1002
Shrew	0.008	0.0054	0.026
Mouse	0.004	0.0089	0.024
Vole	0.0068	0.0139	0.043

Table 5. Input parameters for PRZM-EXAMS

Chemical	Molecular Weight (grams/mole)	Koc	Vapor Pressure (mPa)	Solubility (ppm)	Aerobic Soil Half Life (days)	Foliar Half Life (days)	Aerobic Biolysis (days)	Anaerobic Biolysis (days)	Aqueous Photolysis (days)	Hydrolysis (days)		
										ph 5	ph 7	ph 9
Malathion	330.4[a]	1200[a]	0.45[a]	130[a]	1[a]	5.5[b]	3.5[b]	7.64[b]	42[b]	107	6.3	0.05[a]
Naled	381[a]	157[a]	26[a]	1.5[a]	4[a]	NA	1.5[c]	4.5[c]	4.4[c]	4	0.642	0.067[c]
Resmethrin	338.4[a]	100000[a]	0.01[a]	0.0379[a]	197.5[d]	NA	36.5[d]	682[d]	0.033[d]	89	168	127[d]
d-phenothrin	350.5[e]	56000[e]	1.43E-7[e]	0.0097[e]	26[f]	NA	7.2[f]	61.9[f]	5[f]	301	495	120[e]
Pyrethrins	328.4[a]	100000[a]	0.001[a]	0.001[a]	9.5[g]	NA	10.5[g]	86[g]	0.5[g]	stable	stable	17[g]
Permethrin	391.3[a]	39300[a]	0.0029[a]	0.006[a]	30[a]	NA	38[h]	175[h]	30[a]	stable	stable	49.87[a]
PBO	338.5[i]	399[i]	5E-13[i]	14.3[i]	14[i]	NA	75[i]	181[i]	0.35[i]	stable	stable	stable

[a](USDA-ARS 2005) [d](USEPA 2005g) [g](USEPA 2005f)

[b](USEPA 2000) [e](USNLM 2005) [h](USEPA 2005a)

[c](USEPA 1997c) [f](WHO/FAO 1994) [i](USEPA 2005h)

Table 6. Acute risk quotients for surrogate birds after 1 adulticide application

	Bobwhite quail	Probabilistic percentile		Ring-necked pheasant	Probabilistic percentile		Mallard duck	Probabilistic percentile	
Chemical	Deterministic	50th	95th	Deterministic	50th	95th	Deterministic	50th	95th
Malathion	≤0.02	0.01	0.01	≤0.02	0.01	0.01	≤0.01	0.005	0.007
Naled	≤0.01	0.005	0.006	≤0.009	0.004	0.005	≤0.008	0.003	0.004
Permethrin	≤0.002	<0.001	0.001	<0.001	<0.001	<0.001	≤0.001	<0.001	<0.001
PBO	≤0.01	0.004	0.005	NA	NA	NA	≤0.009	0.003	0.004
Resmethrin	≤0.002	0.001	0.001	≤0.002	0.001	0.001	NA	NA	NA
d-phenothrin	≤0.002	<0.001	<0.001	NA	NA	NA	≤0.002	<0.001	<0.001
Pyrethrins	≤0.001	<0.001	0.001	≤0.001	0.001	0.001	≤0.001	0.001	0.001

Table 7. Chronic risk quotients for surrogate birds after 10 adulticide applications

Chemical	Bobwhite quail Deterministic	Probabilistic Percentile 50th	95th	Mallard duck Deterministic	Probabilistic Percentile 50th	95th
Malathion	≤0.08	0.04	0.05	≤0.01	0.003	0.004
Naled	≤0.03	0.02	0.03	≤0.01	0.008	0.01
Permethrin	≤0.01	0.007	0.01	≤0.06	0.03	0.05
PBO	≤0.08	0.04	0.06	≤0.08	0.06	0.08
Resmethrin	≤0.2	0.1	0.2	≤0.2	0.1	0.2
d-phenothrin	NA	NA	NA	NA	NA	NA
Pyrethrins	NA	NA	NA	NA	NA	NA

Table 8. Acute risk quotients for surrogate mammals after 1 adulticide application

Chemical	Shrew Deterministic	Probabilistic Percentile 50th	Probabilistic Percentile 95th	Mouse Deterministic	Probabilistic Percentile 50th	Probabilistic Percentile 95th	Vole Deterministic	Probabilistic Percentile 50th	Probabilistic Percentile 95th
Malathion	≤0.07	0.04	0.06	≤0.04	0.03	0.04	≤0.04	0.007	0.01
Naled	≤0.03	0.03	0.03	≤0.03	0.03	0.03	≤0.006	0.002	0.004
Permethrin	≤0.001	<0.001	0.001	<0.001	<0.001	<0.001	≤0.002	0.002	0.002
PBO	≤0.02	0.003	0.003	0.004	0.003	0.003	≤0.01	<0.001	<0.001
Resmethrin	<0.001	0.001	0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
d-phenothrin	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Pyrethrins	≤0.003	0.003	0.003	≤0.003	0.003	0.003	<0.001	<0.001	<0.001

Table 9. Chronic risk quotients for surrogate mammals after 10 adulticide applications

Chemical	Rat Deterministic	Probabilistic Percentile 50th	Probabilistic Percentile 95th	Shrew Deterministic	Probabilistic Percentile 50th	Probabilistic Percentile 95th	Mouse Deterministic	Probabilistic Percentile 50th	Probabilistic Percentile 95th	Vole Deterministic	Probabilistic Percentile 50th	Probabilistic Percentile 95th
Malathion	≤0.06	0.04	0.06	≤0.5	0.5	0.5	≤0.5	0.5	0.5	≤0.5	0.1	0.2
Naled	≤0.04	0.02	0.04	≤0.09	0.08	0.5	≤0.3	0.2	0.3	≤0.3	0.1	0.2
Permethrin	≤0.06	0.03	0.06	≤0.1	0.07	0.1	≤0.3	0.2	0.4	≤0.3	0.2	0.3
PBO	≤0.02	0.01	0.02	≤0.03	0.02	0.04	≤0.09	0.05	0.08	≤0.09	0.05	0.08
Resmethrin	≤0.02	0.009	0.02	≤0.04	0.02	0.04	≤0.1	0.07	0.1	≤0.1	0.05	0.1
d-Phenothrin	≤0.003	0.002	0.003	≤0.03	0.007	0.01	≤0.07	0.03	0.07	≤0.01	0.004	0.01
Pyrethrins	≤0.01	0.01	0.02	≤0.03	0.03	0.03	≤0.1	0.1	0.1	≤0.1	0.01	0.01

Table 10. Acute and chronic risk quotients for surrogate aquatic receptors exposed to adulticides

Species	PBO	Pyrethrins	Permethrin	Resmethrin	Naled	Malathion	d-phenothrin
Acute one adulticide application							
Daphnia magna	<0.001	0.001	0.04	0.01	0.04	0.04	NA
Bluegill Sunfish	<0.001	0.001	0.005	0.04	<0.001	0.002	0.02
Rainbow Trout	<0.001	0.006	<0.001	0.01	<0.001	0.01	0.01
Amphipod	NA	NA	0.002	NA	0.08	0.09	NA
Chronic ten adulticide applications							
Daphnia magna	<0.001	0.04	0.04	0.01	0.04	0.03	NA
Rainbow Trout	<0.001	NA	NA	0.002	NA	0.004	<0.001

Table 11. Percent contribution to variance of exposure parameters to total adulticide exposure for birds

Chemical	Bird	Deposition onto feathers		Deposition onto Food		Food Intake		1-hour concentration in air		12-hour concentration in air	
		Acute	Chronic	Acute	Chronic	Acute	Chronic	Acute	Chronic	Acute	Chronic
Malathion	quail	82.9	61.8	17.1	38.2	0	0	0	0	0	0
	pheasant	80.5	59	18.8	40.5	0.7	0.5	0	0	0	0
	mallard	76.1	52	23.5	47.8	0.4	0.2	0	0	0	0
Naled	quail	80.5	0.8	19	99.1	0.2	0	0.2	0	0	0
	pheasant	77.4	0.7	21.3	99.3	0.9	0	0.2	0	0.2	0
	mallard	72.3	0.5	26.4	99.5	0.8	0	0.2	0	0.2	0
PBO	quail	90.7	4.1	9.3	95.9	0	0	0	0	0	0
	pheasant	89.1	3.5	10.3	96.4	0.6	0.1	0	0	0	0
	mallard	85.6	2.6	13.5	97.4	1	0	0	0	0	0
Permethrin	quail	85.5	4.9	14.4	95.1	0	0	0	0	0	0
	pheasant	84	4.4	15.4	95.6	0.6	0	0	0	0	0
	mallard	80	3.6	19.2	96.4	0.8	0	0	0	0	0
Pyrethrins	quail	89.9	28.9	10.1	60.8	0	8.7	0	0.1	0	1.5
	pheasant	88	1.6	11.4	10.1	0.6	88.3	0	0	0	0
	mallard	84.8	9.8	14.7	68.6	0.5	20.4	0	0.1	0	1
Resmethrin	quail	88.4	1.9	11.6	98	0	0	0	0	0	0
	pheasant	86.7	1.7	12.3	98.2	1	0.1	0	0	0	0
	mallard	82.8	1.3	16	98.5	1.2	0.2	0	0	0	0
d-phenothrin	quail	89.4	2.1	10.6	13.9	0	69.6	0	7.1	0	7.3
	pheasant	54.4	8	44.6	34.9	0.9	12.4	0	22	0	22.7
	mallard	56.1	0.4	43.8	7	0.1	91.3	0	0.7	0	0.6

Table 12. Percent contribution to variance of exposure parameters to total adulticide exposure for mammals

Chemical	Mammal	Deposition onto fur		Deposition onto Food		Food Intake		1-hour concentration in air		12-hour concentration in air	
		Acute	Chronic	Acute	Chronic	Acute	Chronic	Acute	Chronic	Acute	Chronic
Malathion	Rat	0	0	4.4	73.9	95.4	25.9	0	0	0	0.2
	Shrew	0	0	42.9	74	0.6	22.5	0	0	56.5	3.5
	Mouse	0	0	83.6	95.3	12.6	3.5	0	0	3.7	1.1
	Vole	0	0	91.5	94.5	8.5	5.5	0	0	0	0
Naled	Rat	0	0	84.3	99.7	15.2	0	0	0	0.4	0.3
	Shrew	0	0	96.2	99.7	2.7	0.1	0	0	1.1	0.2
	Mouse	0	0	72	99.4	22.3	0.3	0	0	5.7	0.3
	Vole	0	0	99.5	99.5	0	0.3	0	0	0.5	0.1
PBO	Rat	0	0	80.4	99.9	18.8	0	0	0	0.8	0.1
	Shrew	0	0	94.2	99.9	3	0	0	0	2.8	0
	Mouse	0	0	60.5	99.8	26.8	0.1	0	0	12.6	0.1
	Vole	0	0	98.9	99.9	0.1	0.1	0	0	1	0
Permethrin	Rat	0	0	79.9	99.9	18.9	0	0	0	1.2	0.1
	Shrew	0	0	93.6	100	2.9	0	0	0	3.5	0
	Mouse	0	0	60	99.8	26.4	0.1	0	0	13.6	0.1
	Vole	0	0	98.5	100	0	0	0	0	1.5	0
d-phenothrin	Rat	0.1	0.1	91.5	99.8	8.3	0	0	0	0.1	0.1
	Shrew	0	0.1	98.3	99.4	1.5	0.4	0	0	0.1	0.1
	Mouse	0	0.1	99.8	99.5	0	0.3	0	0	0.1	0.1
	Vole	0	0	99.9	100	0.1	0	0	0	0	0
Resmethrin	Rat	0.8	0	69	100	30.2	0	0	0	0	0
	Shrew	0	0	91.5	100	5.1	0	0	0	3.4	0
	Mouse	0	0	48.1	100	40.5	0	0	0	11.4	0
	Vole	0	0	98.7	100	0	0	0	0	1.2	0
Pyrethrins	Rat	0	0	71.4	100	28.6	0	0	0	0	0
	Shrew	0	0	94.2	99.9	5.8	0	0	0	0	0
	Mouse	0	0	57.6	99.5	42.4	0.4	0	0	0	0
	Vole	0	0	99.9	99.9	0	0.1	0	0	0	0

CHAPTER 3

A DETERMINISTIC RISK ASSESSMENT FOR LARVICIDES USED IN MOSQUITO MANAGEMENT

Abstract

Larviciding, the management of immature mosquitoes, has been implemented in management plans to suppress mosquito vectors. Concerns have been raised that chemicals used in larviciding may be more harmful to people and the environment than vector borne diseases like West Nile Virus. A deterministic risk assessment was performed to assess a reasonable worst case scenario for larvicide applications that included *Bacillus thuringiensis israelensis* (Bti), *Bacillus sphaericus* (Bs), methoprene, and monomolecular films. The risk assessment included an even settling model into a standard farm pond that contained the chosen receptors. Only *Daphnia magna* exposed to monomolecular films had a risk quotient that breached a USEPA regulatory level of concern. Further description of the nature of monomolecular films mitigated this concern. The potential for Bacillus sphaericus to cause deleterious effects on non-target organisms remains unclear because quantative toxicity data are not available, although anecdotal studies suggest Bs is relatively non-toxic when released into the environment.

Introduction

West Nile Virus (WNV) has been a concern for people across the U.S. since the disease entered the U.S. in the summer of 1999. Since then, WNV has caused the largest arboviral encephalitis epidemic in U.S. history (Huhn et al. 2003). Despite the fact that the disease has resulted in thousands of morbidity cases and hundreds of human deaths, many people are also concerned about the risks associated with managing the mosquitoes (Culicidae) that vector WNV using a variety of insecticides (Peterson et al. 2006). This concern is related to the perception that ecological and human exposure to the insecticides will lead to risks that are more severe than WNV itself.

West Nile Virus is vectored by several mosquito species mostly from the *Culex* genus (Turrel et al. 2005). Mosquito management plans have been implemented in the U.S. and globally to manage mosquito vectors of WNV and many other diseases. Common mosquito larvicides are insect growth regulators (IGRs) such as methoprene (Altosid®), as well as bacterial spore by-products such as *Bacillus thuringiensis israelensis* (Bti) (Vectobac®), and *Bacillus sphaericus* (Vectolex®), and mono-molecular films (Arosurf®, Agnique®).

Methoprene is a chemical that mimics the growth hormone of certain insects. The agent hinders regular maturation of early mosquito instars. The larvae that consume methoprene are unable to reach adulthood (USEPA 2006c). Application timing of methoprene for mosquito management is critical; it works best when the insects are at earlier stages (Gordon and Burford 1984). Late instars and adults are not affected by methoprene. Larvicide toxicity to terrestrial vertebrates is very low. Fish are susceptible

to methoprene exposure as high concentrations of these insecticides will cause fish mortality. Methoprene degrades quickly in soil, groundwater, exposed water, and vegetation. Methoprene concentrations in water degrade rapidly; 80% will degrade within 13 days after application (USEPA 1991).

Bacillus thuringiensis (Bt) is a soil bacterium. Its insecticidal property resides in a crystalline by-product (endotoxin) of sporulation by the bacterium that attacks an insect's microvillar lining when consumed (Mittal 2003). The insecticide most likely opens the path for secondary infection by other bacteria that are common in the insect's midgut (Broderick et al. 2006). The bacterium is a highly regarded insecticide because its many strains target receptors of specific insect species or groups. Bt endotoxins are practically non-toxic to mammals, fish, and birds (Mittal 2003). They break down quickly in the environment.

Bacillus thuringiensis israelensis (Bti) is the strain of Bt that is used for mosquito management and is not persistent through seasons (Hajaij et al. 2005). *Bacillus sphaericus* (Bs) is another soil bacterium that has a similar insecticidal action as Bti (Mittal 2003). In this case, the insecticidal agent is included in the spore cell wall and is a by-product of spore production (Mittal 2003). When the agent is consumed by the larvae, it attacks the lining of the mosquito midgut. The insecticide has been more effective against *Anopheles* and *Culex* species than *Aedes* species (Mittal 2003). For *Aedes aegypti* the target binding sites for the toxin do not appear to be widespread at the gastric cecum and posterior midgut where the toxin is typically active (Baumann et al. 1991).

Monomolecular films are insecticides that form a thin film, as thin as one molecule, over the water in which they are applied. Their main mode of action is to add a buffer between the water oxygen interface so that the surface tension of the water is decreased. Thus the immature mosquito cannot maintain proper orientation to the air-water interface (Levy et al. 1985). Late instars and pupae are more susceptible to mono-molecular films because they are not able to use dissolved oxygen as easily as earlier stages (Nayar and Ali 2003).

Risks from mosquito management plans for control of WNV vectors can be quantified using a risk assessment framework. Risk assessment is a formal discipline that provides an objective evaluation of risk. The discipline has become widely used to assist managers in making decisions about new or controversial technologies that may pose a risk to the public or the environment. Scientific data as well as societal considerations are used to describe the nature of the risk and communicate risk with decision makers. Within a risk assessment, assumptions and uncertainties are clearly presented.

Ecological risk can be quantified as a function of hazard and exposure (NRC 1983). The approach uses a tiered modeling system that moves from deterministic models based on conservative assumptions erring on the side of environmental safety, to refined probabilistic models using more realistic assumptions (SETAC 1994). Risk assessment follows a logical framework. The process proceeds in stepwise fashion including: (1) problem formulation, (2) hazard identification, (3) dose response relationships, (4) exposure assessment, and (5) risk characterization. These steps allow

for the comparison of an estimated environmental exposure with a reference dose derived from a toxic effect (NRC 1983).

The objective of this risk assessment is to conservatively quantify the potential acute and chronic risks of larvicide applications to non-target aquatic organisms following a regimen of sprays. Acute risks are associated with peak environmental chemical concentrations and corresponding short term toxicity studies. Chronic risk are associated with long term environmental concentrations which consider chemical persistence in the environment and corresponding toxic endpoints.

Materials and Methods

Problem Formulation

Mosquito management is an important tool in managing mosquito-borne diseases such as WNV. Larviciding is a preemptive management strategy against future potential vectors to manage mosquitoes in early life stages. Larvicides may have non-target impacts on aquatic species, and have the potential to disrupt community interaction webs. Aquatic receptors may be especially susceptible because the materials are applied directly to the water. A tier-I aquatic ecological risk assessment was conducted to quantify potential risks to aquatic receptors. Avian and mammalian risk assessments were not conducted as the exposure pathways to insecticides that are exposed directly to water would be limited to drinking water. Also it is accepted that, these chemicals have very low toxicity to terrestrial vertebrates. Therefore, the risk to these animals would be negligible.

Hazard Identification

The ecological risk assessment focused on four mosquito larvicides: methoprene, *Bacillus thuringiensis israelensis* (Bti), *Bacillus sphaericus* (Bs) and monomolecular films. Each larvicide is applied as a direct spray in which a calibrated pump applies the concentrated or diluted liquid formulation containing the active ingredient. Larvicides are also available in granular or tablet form and can act as slow-release insecticides over a season.

Methoprene has been shown to have adverse effects on freshwater amphipods (*Gammarus* sp.) (Breaud et al. 1977), a mayfly species, *Callibaetis pacificus*, non-biting midges (Chironomidae*)*, and a dytiscid beetle, *Laccophilus* sp. (Norland et al. 1975). In a long-term study in which each site was treated at three-week intervals six times over a season, Hershey et al. (1998) concluded that methoprene had a negative effect on aquatic insect predators at treated sites. These impacts were considered to be both direct and indirect through food and interaction webs, as the chemical acted to cause mortality to the predator populations, but also decreased the availability of prey.

Bacillus thuringiensis israelensis is the main strain of Bt that is used for mosquito management; however, it may have negative effects on non-target black flies and midges (Hershey et al. 1998). Although black fly control may be considered a beneficial side effect. Ali (1981) found that applications of Bti to 18 4x6-m experimental ponds significantly lowered non-target chironomid numbers. At the highest treatment rate of 4,000 g/ha, there was a 54-92% reduction in chironomid abundance. The same product used in golf course ponds did not significantly affect chironomid abundance; in the golf

course setting at a treatment of 3,000 g/ha there was only 30-67% chironomid reduction. Normal numbers returned 14 days after treatment. Charbonneau et al. (1994) found that although Bti caused high mortality of chironomids in a laboratory, a much reduced mortality was observed in the field. They concluded that Bti did not significantly reduce chironomid numbers in the field. Hershey et al. (1998) conducted the most rigorous study on larviciding. Throughout a full season of treatments, they found that Bti effectively killed target dipteran larvae, and the food chain was disrupted. Predator numbers dropped as prey became less prominent.

When applied as a larvicide to water, mono-molecular films have a short half-life, ranging from 5 to 15 days. It is unlikely that mono-molecular films will have negative non-target effects as they target mosquitoes without integrating completely into the water. The application timing of mono-molecular films is critical because they work best on late instars and pupae (Nayar et al. 2003).

Dose-Response Relationships

Toxic endpoints related to the dose of a chemical an organism receives are defined acutely by EC_{50}s and LC_{50}s which are concentrations in the aquatic environment that will cause 50% mortality in a population of organisms. NOECs are the highest concentrations tested in which the organism had no measurable physiological or behavioral response to a stimulus. Surrogate organisms, model organisms used in ecological risk assessment, were chosen based on the consistency of the data available.

Methoprene is classified as highly toxic to *D. magna* with an acute EC_{50} of 0.07 ppm and a chronic NOEC of 0.03 ppm. It is moderately toxic to rainbow trout and

bluegill sunfish with acute LC_{50}s of 1.52 ppm and 1.01 ppm, respectively (USEPA 1997a) (Table 13).

Bti is non-toxic to most aquatic organisms except for target dipterans, because it targets specific receptors located in mosquitoes and other closely related species. The LC_{50} for bluegill sunfish and rainbow trout is 0.66 ppm. It is moderately toxic to *D. magna* with an acute LC_{50} of 13 ppm (Table 14) (USEPA 1997a).

There are few toxic endpoints available for Bs as it is suspected to be almost non-toxic to non-target species. An estimated environmental concentration (EEC) for Vectolex WDG® applied at a rate of 898 ml/ ha was calculated to be $5x10^{-5}$ ppm. It is unlikely that at low concentrations as predicted by an even-settling scenario that exposures of Bs would have widespread effects on non-target species.

Arosurf® is relatively non-toxic to mammals having an oral LD_{50} of >11,300 mg/kg in rats (USNLM 2005). Monomolecular films are classified as moderately toxic to *D. magna* and eastern oyster *(Crassostrea virginica* Gmelin*)* with an acute EC_{50} of 1.9 ppm and 1.5 ppm, respectively. It is also slightly toxic to both cold water and warm water fish. However, subsurface exposure is not typical or persistent. Subsurface organisms will most likely only be exposed if the larvicide is mixed into the water column. (Table 15) (USEPA 1997a).

Exposure Assessment

My larvicide risk assessment included the following assumptions for all three insecticides: (1) the insecticide was applied at the maximum application rate according to the label (Table 16); (2) the insecticide distributed evenly throughout a standard farm

pond that was 1 ha in surface area and 2-m deep, for a total of 20 million liters of water; (3) each surrogate species was exposed to the insecticide including monomolecular films, which tend to stay on the water's surface; (4) for acute exposures the insecticide was not degraded by environmental factors; and (5) insecticides did not bind to organic sediment.

For chronic exposures, I assumed each insecticide to be degraded by photolysis in water for the duration of the toxicity study length for each organism. The exposure characterization was based on a 42-day period and five spray events occurring on days 1, 4, 14, 17, and 27. The average daily chronic concentration is given by the following equation:

$$C = \sum_{j=1}^{n} Pe^{rt} \div n \qquad [1]$$

where C is the concentration of insecticide in the water at a given time, j is the spray event, P is the peak concentration of the insecticide after the larvicide is applied, r is the rate of decay calculated from the photolysis half-life, t is the time in hours after the spray occurred, and n is the decay period (Table 17).

Ecological risks for the larvicides were assessed by dividing the EEC determined from my exposure scenario by the toxic endpoints for each organism to obtain a risk quotient (RQ). The RQs calculated in the results were compared to the USEPA's RQ levels of concern. Levels of concern (LOC) are regulatory tools used as aides in the risk management process. Managers compare the RQ and LOC to determine if regulatory action is needed. The acute regulatory LOC's in my assessment were adapted from USEPA and are 0.5 for aquatic species and 0.05 for endangered aquatic species. Chronic LOCs are 1.0 for all animals. If an RQ breaches a regulatory LOC, then the manager

decides to either restrict the products used or progress to higher tiers to a probabilistic or field-verified model (USEPA 2006h).

Results

Only *D. magna* exposed to monomolecular films had an RQ that breached a USEPA regulatory LOC (Table 18). All other RQs for surrogates exposed to the larvicides in the water were well under LOCs. Acute RQs for *D. magna* exposed to Bti and methoprene were $2x10^{-5}$ and $3x10^{-5}$ respectively. Chronic risk to *D. magna* from methoprene was $6x10^{-5}$. Rainbow trout had acute RQs that ranged from $2x10^{-6}$ to $2x10^{-5}$ for methoprene and Bti; and the acute RQs for bluegill sunfish were $1x10^{-6}$ for methoprene and Bti. Chronic risk to bluegill sunfish from methoprene was $9x10^{-7}$. Bluegill sunfish and rainbow trout had chronic RQs to monomolecular film of $9x10^{-7}$ and $3x10^{-6}$ respectively (Table 18).

The risk quotient for *D. magna* exposed to monomolecular film breached a regulatory LOC. However, *D. magna* would most likely not have significant exposure to monomolecular film because the films settle at the surface of the water, whereas *D. magna* live in the water column. Therefore, it is unlikely that *D. magna* would be exposed to monomolecular films at concentrations predicted by the conservative even-settling scenario utilized in our assessment.

Discussion

Published analyses of environmental risk are rare for larvicides used in mosquito management by vector control districts and government health services. A risk

assessment contained in the Suffolk County EIS found that risks posed by larvicides would be negligible in geographically specific water bodies (Suffolk-County 2006). NYCDOH (2005) did not conduct a quantitative risk assessment for larvicides and concluded that risks from larvicides would be negligible in ponds exposed to chemicals. Controlled field experiments have had conflicting results when characterizing impacts attributable to larvicides. Milam et al. (2000) found that treatments of Bti were much more damaging to *Anopheles quadramaculus* than non-arthropod sentinel species, including *Ceriodaphnia dubia*, *D. magna*, *Daphnia pulex* de Geer, and *Pimephales promelas.* In another study, methoprene and Bti were much less toxic to non-target shrimp than the alternative larvicides temephos and pirimiphos-methyl (Brown et al. 2000).

Hershey et al. (1998) conducted a large-scale study using 27 ponds in Wright County, Minnesota. The focus of this study was to determine the impact of aerially applied direct applications of granular methoprene and Bti on non-target invertebrates. Bti and methoprene significantly lowered numbers of chironomids, tipulids ceratopogonids, and brachycerans in treatment ponds. Food web and interaction web disruption was hypothesized to have occured in many of these reductions because predators seemed to decline with prey. Populations rebounded in the years after the treatments. This is consistent with Hajaij et al. (2005) who found very little residual Bti after applications into four mosquito habitat types. Norland and Mulla (1975) found effects on mayflies (*Callibaetis pacificus*) when methoprene was applied at 302.5 grams/ha to experimental ponds, while midges were variably affected, dytiscid beetle

predator numbers were reduced. Niemi (1999) found changes in ecosystem diversity in Bti treated ponds, and reduced total insect numbers in ponds treated with both methoprene and Bti.

Pinkney et al. (2000) observed that methoprene applied to experimental ponds had no significant impact on non-target arthropods compared to control treatments. Two formulations of Bti had no effect on non-target invertebrates, including the amphipod, *Hyalella azteca* Saussure, in test ponds that had a Bti concentration of 100 ppm (Gharib and Hilsenhoff 1988). Lawler et al. (1999) found that Bti and methoprene had no measurable impact on sentinel amphipods in ephemeral mangrove swamps on Sanibel Island, Florida when treated with Bti granules applied at 5.6 kg/ha and a methoprene liquid formulation applied at 10.65 ml AI/ha for the control of *Aedes taeniorhyncus*. Experiments using Bti in a flowing river found no negative effects on amphipods or other insect groups (Merritt et al. 1989). Black fly management resulted in no measurable impacts after one methoprene treatment on a small New York stream (Molloy and Jamnback 1981). Furthermore, Molloy (1992) found Bti used in black fly control had little significant effects on stream insects other than black flies and filter feeding chironomids.

Bti and methoprene pose limited risk to vertebrates. Niemi et al. (1999) examined bird populations in relationship to breeding and foraging near to ponds treated with Bti and methoprene. They observed no differences in the reproduction and activity of red-winged blackbirds residing around treatment ponds. In a field and laboratory evaluation of Bti (Vectobac-G®) for mosquito management, Charboneau et al. (1994) observed no

measurable impacts of the insecticide on non-target invertebrates and characterized how midge vulnerability to the insecticide varied with environmental conditions. Granular Bti caused lower mortality to midges in deeper water, possibly due to the denaturing of Bti as it moves deeper into the water column (Charbonneau et al. 1994).

Researchers have specifically examined monolayer films and their effect on nontarget organisms. Mulla (1983) found monomolecular films had no measurable impact on mayflies, diving beetles, and ostracods in field evaluations. Takahashi (1984) tested monomolecular films (ISA-20E) in experimental ponds to examine effects on non-target arthropod mortality. Corixids (*Corisella spp.*), notonectids (*Notonecta unifasciata* Guerin), clam shrimp, and *Tropisternus lateralis* Say all showed acute mortality from applications. However, populations rebounded shortly after treatment. Mayflies were not affected by monomolecular films. Hester et al. (1991) found very little effect on several non-target species including amphipods, killifish, snails, isopods, and crayfish and observed no mortality after monomolecular film applications.

There is variability and uncertainty associated with this environmental modeling approach. When conducting tier I risk assessments, scenarios are chosen that will most likely overestimate exposure and present a reasonable worst case scenario. Water cycling through the pond system or partitioning of methoprene and Bti to organic sediment is not accounted for in the even-settling scenario. Methoprene strongly adheres to sediment with a sorption coefficient (Koc) of 23,000; Bti also adheres readily to sediment (Ohana et al. 1987). Therefore, these chemicals may pose a risk to sediment dwelling arthropods such as chironomids. Conversely, because methoprene and Bti quickly dissipate and bind

to sediment it is likely that the even-settling scenario overestimates risks to organisms in the water column. Furthermore, if the pond is diluted by incoming water from springs or streams the chemicals will be less concentrated both in the pond and downstream.

An obvious uncertainty is the lack of quantitative toxicity values for Bs on non-target organisms. Brown et al. (2004) found no toxicity to non-target Australian fauna including the fish *Pseudomugil signifier* Kner and the shrimp *Leander tenuicornis* Say. Merritt et al. (2005) found similar results in a three year study in two separate habitats in which 138 invertebrate taxa were exposed to Bs. Results indicated few impacts on taxa categorized into functional groups.

The tier-I risk assessment found that risks to the evaluated aquatic organisms were negligible when organisms are exposed over both short and long terms. Long term studies have found that larvicide applications applied directly to water will pose little long term risk to populations or ecological interaction webs, but have the potential for non target impacts when applied multiple times over a season. Interaction web interruption has the potential to cause decreased abundance in populations of organisms at higher trophic levels. The conclusion of this study and the weight of evidence suggests that the majority of use patterns would not result in unacceptable risks over a season.

Table 13. Toxic endpoints for methoprene (ppm)

Species	Acute		Chronic	
Daphnia magna	48 Hr EC_{50}	0.07	42 Day NOEC	0.03
Bluegill Sunfish	96 Hr LC_{50}	1.52	42 Day NOEC	2
Rainbow Trout	96 Hr LC_{50}	1.01	NA	

(USEPA 1997a)

Table 14. Toxic endpoints for Bt (ppm)

Species	Study time	Acute		
Bluegill sunfish	96 hr	LC50	>	0.66
Daphnia magna	48 hr	EC50	>	13
Rainbow trout	96 hr	LC50	>	0.66

(USEPA 1997a)

Table 15. Toxic endpoints for monomolecular film (Arosurf®) (ppm)

Species	Study time	Acute		
Bluegill sunfish	96 hr	LC50		290
Sheepshead minnow	96 hr	LC50	>	300
Rainbow trout	96 hr	LC50		98
Eastern oyster	96 hr	EC50		1.5
Daphnia magna	48 hr	EC50		1.9

(USEPA 1997a)

Table 16. Estimated larvicide application rates in standard farm pond

Insecticide	Application Rate (ml/ha)	Acute EEC (ppm)
Methoprene[1]	35.05	1.75×10^{-6}
Bti[2]	271.29	1.36×10^{-5}
Arosurf®[3]	12349.41	2.50×10^{-4}

[1](Wellmark-International 2003)

[2](Valent-Biosciences 2004)

[3](Cognis-Corporation 2000)

Table 17. Estimated chronic environmental concentrations of larvicides in a standard farm pond

Chemical	Aqeous half life (hours)	42-day Chronic EEC (ppm)
Methoprene[1]	134	1.70×10^{-6}
Bt[2]	96	1.00×10^{-5}
Monomolecular film[3]	336	4.59×10^{-4}

[1](USNLM 2005)
[2](USEPA 1998a)
[3](Cognis-Corporation 2000)

Table 18. Estimated risk quotients of larvicides to aquatic organisms

	Methoprene		Bt	MMF
Species	*Acute RQ*	*Chronic RQ*	*Acute RQ*	*Acute RQ*
Daphnia magna	3E-5	6E-5	2E-5	2
Bluegill Sunfish	1E-6	9E-7	1E-6	9E-7
Rainbow Trout	2E-6	NA	2E-5	3E-6

CHAPTER 4

ECOLOGICAL EFFECTS OF SINGLE AND MULTIPLE APPLICATIONS OF MOSQUITO MANAGEMENT INSECTICIDES

Abstract

Mosquito management plans have been implemented in the U.S. and globally to manage mosquito vectors of WNV and many other diseases. Adulticides are applied via ultra-low-volume (ULV) space sprays that target adult mosquitoes as they are flying through the air. Adulticides are broad spectrum insecticides that are toxic to arthropods; the synergist piperonyl butoxide was applied with permethrin and d-phenothrin in this study. Larvicides are applied directly to water in liquid or delayed release formulations; this study focused on *Bacillus thuringiensis israelensis* and methoprene. Two studies were conducted during the late summers of 2004 through 2006 at Benton Lake National Wildlife Refuge near Great Falls, Montana. The first experiment was conducted in 2004 and 2005 to assess acute impacts of mosquito adulticides and larvicides on non-target aquatic and terrestrial arthropods after a single application. The second experiment was conducted in 2005 and 2006 to assess longer term impacts of a mosquito adulticide on non-target terrestrial arthropods after multiple applications. In general most of the responses evaluated during either study exhibited few deleterious effects from insecticide application. For aquatic samples in the acute study, 1 potentially deleterious effect was observed for amphipods on sample date 1 in 2004. However, no overall treatment effects were observed. During the same study, 1 of 54 responses had a significant overall

treatment effect for sticky card samples for either year. Many of the responses for sticky card samples suggested significant time effects and time by treatment effects. Three response variables were associated with fewer individuals present in the treatment plots. For the multiple spray study conducted in 2005 and 2006, six of the response variables collected via sticky card exhibited significant overall treatment effects, but none were associated with fewer individuals in the treatment plots. None of the responses collected using sweep net sampling suggested overall treatment effects. Time and time by treatment effects were prevalent in 2005 but no discernable pattern was evident. Power to detect significant treatment effects was low for many of the responses.

Introduction

West Nile Virus (WNV) has been a concern for people across the U.S. since the disease was initially observed in North America during the summer of 1999. Since that year, WNV has caused the largest arboviral encephalitis epidemic in U.S. history (Huhn et al. 2003). Despite the fact that the disease has resulted in thousands of morbidity cases and hundreds of deaths in humans, many people are concerned about the risks associated with managing mosquitoes that vector WNV using a variety of insecticides (Peterson et al. 2006). This concern is related to the perception that ecological and human exposure to the insecticides will lead to risks that are more severe than WNV itself.

West Nile Virus is vectored by several mosquito species, mostly from the *Culex* genus (Turell et al. 2005). Mosquito management plans have been implemented in the U.S. and globally to manage mosquito vectors of WNV and many other diseases.

Common mosquito adulticides are pyrethroids such as d-phenothrin, resmethrin, and permethrin, as well as organophosphates such as malathion and naled. Natural pyrethrins are also available as mosquito management agents. Permethrin and d-phenothrin are the adulticides evaluated in this study. Adulticides are applied via ultra-low-volume (ULV) space sprays that target adult mosquitoes as they are flying through the air. Adulticides are broad spectrum insecticides that are toxic to arthropods. All insects in the spray zone may be deleteriously affected by the application of adulticides. Adulticides used as space sprays become effective when they are released in the atmosphere as extremely small liquid droplets ranging from 8-30 microns in ULV formulations. Small droplets increase the surface area available to contact target mosquitoes. The insecticide is absorbed through an insect's cuticle and takes effect soon after contact. Mosquito management programs often aggressively apply adulticides in the midst of a disease outbreak. Most are sprayed in the evening or early morning when female mosquitoes are seeking a blood meal, and many other arthropods, particularly pollinators, are inactive.

Many terrestrial arthropods will be exposed in the same way as flying or resting mosquitoes. Spray events that occur while mosquitoes are active likely limit exposure of honey bees and other terrestrial arthropods in the spray zone; however, some non-target mortality is likely in the sprayed areas. Because of the nature of the applications and their frequency, it is unclear how arthropod communities will be affected in the field (Caron 1979).

Many of the studies conducted to measure impacts of mosquito management chemicals have focused on effects of adulticides on honey bees. Coldburn and Langford

(1970) found high bee mortality from applications of naled, malathion, and pyrethrum with the synergist piperonyl butoxide (PBO). These field tests did not use a ULV sprayer, but the study suggests that chemicals commonly used in mosquito management may lead to non-target arthropod mortality. Caron (1979) exposed caged honey bees and hives to ULV sprays of malathion, naled, and pyrethrum. This study showed decreasing mortality as distance increased from the insecticide application. Pankiw and Jay (1992) found that honey bees in cages experienced significant mortality from malathion spray drift. Hester et al. (2001) observed significant bee mortality in hives that were exposed to malathion spray events both in open fields and in a forested environment. Tietze et al. (1996) used sentinel crickets to measure spray deposition in a peri-domestic environment. Cricket mortality varied between 12.5% to 48.7% depending on where crickets were put in residential yards in relationship to the spray zone. Zhong et al. (2003) found that aerially applied naled had a negative effect on honey bees and reduced their honey production over a season.

Other researchers have focused on aquatic invertebrates. Siegfried (1993) found permethrin to be toxic at low concentrations (2.9-5.9 μg/L) to several aquatic insects, including black flies, caddisflies, mayflies, and damselflies. Milam et al. (2000) found that when permethrin was applied at 219 ml/ha in field test chambers the non-targets *Daphnia pulex, Ceriodaphnia dubia, and Pimephales promelas* had 90% survival in 8 of 10 experiments. Little experimental data are available for d-phenothrin.

Common larvicides include *Bacillus thuringiensis israelensis* and methoprene. Larvicides are applied directly to water in liquid or delayed release formulations. *Bacillus*

thuringiensis (Bt) is a soil bacterium. Its insecticidal property resides in a crystalline by-product of spore production (endotoxin) by the bacterium that attacks an insect's microvillar lining when consumed (Mittal 2003). The insecticide most likely opens the path for secondary infection by a bacterium that is common in the insect's midgut (Broderick et al. 2006). Bt is a highly regarded insecticide because its many strains target receptors of specific insect species or groups. Bt endotoxins are almost non-toxic to mammals, fish, and birds (Mittal 2003). Impact on aquatic invertebrates is the most likely non-target risk associated with larvicides. Milam (2000) found that toxicity of Bti was much greater to *Daphnia pulex* than to *Anopheles quadramaculatus* when applied as a liquid formulation.

Ecological effects have been monitored for Bti used for black fly and mosquito management. Merritt (1989) found few changes in indices used to measure treatment effects of Bti used for black fly management in a Michigan river. Drift samples taken at a control and treatment site did not differ for chironomids, baetids, gammarids, or hydropsychids, but did have some treatment effects on perlid stoneflies, and elmid beetles. Similar results were observed in 10 stream trials measuring stream insect density of selected taxa. Molloy (1992) observed that Bti applied for black fly control within a New York stream affected filter feeding chironomids, but not surface dwelling or tube-dwelling members of the same family. Caddisflies and mayflies also showed no response to Bti treatments.

Methoprene is a chemical that mimics the growth hormone of certain insects. The larvae that consume methoprene are unable to reach adulthood (USEPA 2006c).

Application timing of methoprene for mosquito management is critical; it works best when the insects are at earlier stages (Gordon et al. 1984). Late instars and adults are not affected by methoprene. Larvicidal toxicity to terrestrial vertebrates is very low. Fish are susceptible to methoprene and Bti exposure. Methoprene degrades quickly in soil, groundwater, exposed water, and on vegetation (USEPA 1991). Methoprene degrades rapidly in water; 80% will degrade within 13 days after application (USEPA 1991). Methoprene has been shown to have adverse effects on the scud (*Gammarus* sp.), a freshwater amphipod (Breaud et al. 1977), the mayfly species *Callibaetis pacificus*, chironomids and a dytiscid beetle, *Laccophilus* sp. (Norland et al. 1975).

This project attempts to assess some of the potential ecological effects associated with mosquito management agents, both adulticides and larvicides, at a field site in central Montana. Two adulticides, permethrin (Biomist®) and d-phenothrin (Anvil®), and 2 larvicides Bti (Vectobac®) and methoprene (Altosid®) were evaluated in an environmental setting that includes a permanent water body. A study was also conducted to monitor ecological indices of a worst case spray regimen of terrestrial arthropods.

Materials and Methods

Two studies (representing 2 replications of 2 separate experiments) were conducted during the late summers of 2004 through 2006 at Benton Lake National Wildlife Refuge near Great Falls, Montana. The first experiment was conducted in 2004 and 2005 to assess acute impacts of mosquito adulticides and larvicides on non-target aquatic and terrestrial arthropods after a single application. The second experiment was

conducted in 2005 and 2006 to assess longer term impacts of a mosquito adulticide on non-target terrestrial arthropods after multiple applications

Terrestrial and Aquatic Single Spray Study, 2004-2005

The site for the first experiment was near a road that parallels Pond 2 within the wildlife refuge (47°41'34.43"N, 111°20'47.69"W- 47°41'5.68"N, 111°20'54.01"W) (Figure 1). The experiment was arranged in a randomized complete block design (RCBD) with 5 treatments replicated 3 times. The blocking factor was location along the length of the experimental site. Experimental units were 30.48 m in length, 22.86 m in width, with a buffer zone of 15.24 m between each unit (Fig. 1). The total length of the site was approximately 686 m, and the area was approximately 15,678 m^2. Treatments consisted of 2 adulticides applied at their maximum labeled rates, d-phenothrin (4 g/ha) + PBO (39.2 g/ha) (Anvil 10+10®) and permethrin (7.8 g/ha) + PBO (39.2 g/ha) (Aquareslin®), 2 larvicides applied directly to water, *Bacillus thuringiensis israelensis* (302.6 g/ha) (Vectobac®) and methoprene (14 g/ha) (Altosid®), and an untreated control. Adulticides were applied via ULV sprayer downwind to the pond at dusk to represent a worst case acute scenario; larvicides were applied as liquids directly to water.

Samples were taken from each plot 1, 7, 14, and 28 days after the treatments were applied using a variety of techniques to capture both terrestrial and aquatic invertebrates. For terrestrial arthropods, 2 Olson (Olson Products, Medina, OH) yellow sticky cards (7.62 x 12.7 cm) were placed in each plot at 1-m high, 1 upwind and 1 downwind of the spray zone, to survey flying insects (in 2004 2 sticky cards were used on the same stake facing in perpendicular directions). Sticky card samples were not taken during the first

sample date (1 day after treatment). Sticky card samples were gathered 1, 2, and 4 weeks after treatments. Samples were taken to gain per week estimates of individuals captured. The sticky card counts for the last sample date were divided by 2 to represent average weekly counts.

For aquatic arthropods, a 500 micron D-net was used to capture sediment-dwelling aquatic invertebrates. Dipper samples were taken to collect free swimming aquatic invertebrates (e.g., *Daphnia magna*). Finally, bottom dredge samples were taken at random locations (2004 only) to capture benthic invertebrates that live in the pond sediment. Fauna captured in D-nets in 2004 was similar to that captured in the dredge samples, thus I took only D-net samples in the second year. At each sampling date, 2 D-net, dipper, and in 2004 dredge sub-samples were taken from each experimental unit approximately 3 m from the water's edge. Each sample from the aquatic environment was preserved in 95% ethyl alcohol, then sorted and counted.

Arthropod counts were recorded for each sampling type. Insects were classified into orders and families. Non-insects (such as amphipods and spiders) were classified to order. The sum of each of 2 samples from dipper and the D-net was used to estimate abundance within each plot. Family diversity, richness, and evenness diagnostics were calculated for each sticky card samples using the following equations:

$$\text{if } a_i=0 \text{ then } Di=0, \text{ if } a_i>0 \text{ then } Di=-(a/t)*\ln(a/t) \quad D=\sum_{i=1}^{n} D_i \qquad [1]$$

where D is the diversity index for the treatment, n is the number of families observed, a is the abundance of the family within the sample and t is the total of all the organisms within the sample (Magurran 1988),

$$\text{if } a_i=0 \text{ then } r_i=0, \text{ if } a_i>0 \text{ then } r_i=1 \; R=\sum_{i=1}^{n} r_i \qquad [2]$$

where a is the abundance of the family within the sample, r is the marker if the species is present, and R is the richness, counted as the overall number of families within the sample, and

$$E=D/\ln R \qquad [3]$$

where E is the evenness index from the sample, D is the diversity index from the sample and R is the richness of the sample. These indices capture relevant changes within the community structure. Significant treatment effects on these indices may indicate changes in community structure that may not be apparent from treatment effects on individual taxa (Magurran 1988).

Sticky card samples were classified into functional guilds and size classes. Functional guilds were arranged by the most probable feeding habits of the adults and included nectar/pollen foragers, predators, parasitoids, general scavengers, phloem feeders, blood feeders and leaf feeders. These are not exclusive groups; for example some non-plant feeders supplement their diet by obtaining plant carbohydrates and may exhibit some of feeding characteristics of nectar/pollen foragers. Insect sizes were categorized by medium-small insects (<5 mm), medium sized insects (5-10 mm), and large insects (>10), members of the same group were generally put into the same size class.

A linear model was fit to each of the response variables within the measurement types of the form:

$$Y=(Block+Treatment)*Time \qquad [4]$$

where Y is the predicted family, order, total count, or community index present in the sample, *Block* and *Treatment* are the experimental design elements of the model, and *Time* is the time series variable included for repeated measures over each of the time periods.

The Shapiro-Wilke test for normality was utilized to check the normality of the response data, for all of the samples and at each measurement date. A type III ANOVA was then performed on data that fit the assumptions of the linear model to estimate effects of the predictor variables on the response, and to check for interaction terms among the predictors.

Pairwise contrasts were calculated to detect significant differences (α=0.05) between the control plots and insecticide treatment plots on specific measurement days when significant treatment effects were detected. Retrospective power analyses were done to identify the probability of making a Type II error within a multivariate repeated measures test.

Terrestrial Study Multiple Spray Events, 2005-2006

The multiple-spray, terrestrial study was conducted adjacent to a 4.83-km portion of Bootlegger Trail Road (47°39'52.56"N, 111°16'55.17"W- 47°37'39.39"N, 111°16'55.68"W) (Figure 2) on the eastern border of the refuge. Two treatments were arranged in an RCBD with 3 replicates. The blocking factor was location along the length of the experimental site. Experimental units were 0.4-km long, with 0.4-km buffers between them. Treatments consisted of permethrin (7.8 g/ha) + PBO (39.2 g/ha) (Aquareslin®) at the maximum labeled rate and an untreated control. Spray applications

were made via truck-mounted ULV sprayer 4 separate times in 1-week intervals in August of 2005 and 2006.

For arthropod sampling, 3 Olson yellow sticky cards, at 7.62 m, 15.24 m, and 45.72 m from the road were placed in each plot on both the east (2006 only) and west side of the road. Also, sweep net samples (50 sweeps) were taken at the same distances within each plot. Richness, diversity, and evenness were calculated from each sample as shown above (Equations 1-3). Each group from each sample was put into functional guilds and size classes as mentioned above.

A model was fit to the data of the form:

$$y=(Block+Treatment+Treatment*Distance)*Time \quad [5]$$

where *y* is the predicted family, order, total count, or community index present in the sample, *Block* and *Treatment* are the experimental design elements of the model, *Distance* is the distance from the road, and *Time* is the time series variable included for repeated measures over each of the time periods. The *Treatment*Distance* interaction term was included in the model as it is likely an interaction could occur between those predictors along with a *Treatment*Distance*Time* interaction. The Shapiro-Wilke test for normality was utilized to check the normality of the response data, for all of the total counts, and at each measurement date. A type III ANOVA was performed on data that fit the assumptions of the linear model to estimate effects of the predictor variables on the observed responses, utilizing both multivariate and univariate tests, and to check for interaction terms among the predictors

Furthermore, the pairwise contrasts were calculated to detect significant differences (α=0.05) between the control plots, and insecticide treatment plots on specific measurement days when significant treatment effects were detected. Retrospective power analyses were done to identify the probability of making a type II error.

Results and Discussion

Terrestrial and Aquatic Single Spray Study, 2004-2005

D-net Samples, 2004-05 D-net samples collected from Pond 2 during the summers of 2004-2005 captured several aquatic invertebrates including scuds (*Gammarus* sp.), water boatmen (Hemiptera: Corixidae), beetles (Coleoptera: Dytiscidae), flies (Diptera: Chironomidae & Culicidae), and dragonflies and damselflies (Odonata). The model above was fitted to each group, along with a total count. No overall treatment effects were identified for any receptor (df=4,8; P=0.16-0.86) for 2004 or 2005 (Table 19). Other significant predictors for models fitted to both year's response variables included a block effect for both coleopterans and hemipterans (df=2,8; P=0.025,0.0014). Also, there was a time effect (df=3,24; P=0.013) (Table 19), as well as a block by time interaction effect (df=12,24; P=0.0151). Overall multivariate treatment effects were significant in 2004 for scuds on the first sampling date (df=4,8; P=0.014.). Pairwise contrasts detected significant differences between the control and Bti, methoprene, and d-phenothrin on date 1 (P=0.001,0.01,0.02). A significant time by treatment interaction was observed for coleopterans (df=12,24; P=0.05) (Table 19) related to a pairwise contrast which detected a significant difference between the control

plots and the permethrin treated plots on date 2. On average more beetles were observed in the permethrin plots (P=0.025). The same experiment conducted in 2005 reveled no overall treatment effects for amphipods on date 1 (df=4,8 P=0.37-0.74) (Figure 3). Total D-net counts in 2004 had overall treatment effects that mimicked treatment effects found in the analysis of the scuds because scuds contributed most of the individuals for each sample. Power to detect overall multivariate treatment effects among any taxa during either year was low (<0.532), except for dipterans on date 2 in 2004 (0.99).

Sediment Samples, 2004 Sediment samples were taken in pond 2 during 2004. Taxa captured in these samples were scuds, beetles (Coleoptera: Dytiscidae), and non-biting midge larvae (Diptera: Chironomidae). No overall treatment effects were detected for either of these 3 groups (df=4,8; P=0.42-0.88) (Table 19). Block was a significant predictor for scuds and diving beetles (df=2,8; P=0.03,0.04). Time was also a significant predictor for diving beetles (df=3,24; P=0.01) and more beetles were observed in the methoprene treatment plots on date 3 (P=0.03). Multivariate tests for treatment effects in this experiment had low power (<0.221).

Dipper Samples, 2004-05 Dipper samples were taken in the same locations during 2004 and 2005. Two taxa were collected in the dipper samples including *Daphnia* sp. and rotifers (phylum Rotifera). No overall treatment effects were observed during either year using the (df=4,8; P=0.28-0.94) (Table 19). Time was significant in the univariate analysis during 2004 (df=3,24; P=<0.001) as was block on date 3 in 2004 (df=2,8; P=0.01). Fewer waterfleas were collected in 2004 in the permethrin treated plots

(P=0.05). Power to detect treatment effects was reasonable on dates 1, 2, and 3 in 2004 (0.795-0.999) but was low in 2005 (<0.522).

Sticky Card Samples, 2004 Sticky card samples were taken in 2004 to survey flying insects in each treatment plot. Samples close to the pond (~1 m, West) were analyzed separately from those farther from the pond (~25 m, East). Each sample type came from different environments, one on the edge of the pond the other on dry prairie, and captured separate fauna. Approximately 50 families of insects were identified in 2004. Of the families identified in 2004, 30 had sufficient numbers to be analyzed in at least 1 of the environments (see Appendix A for a complete list of collected families). Individuals were categorized into 8 orders, 6 functional guilds, 3 size classes, and 3 ecological indices. Univariate tests did not suggest any overall treatment effects for any family group (df=4,8; P=0.08-0.96), order group (P=0.09-0.93), size class (P=0.21-0.9), or ecological index (P=0.22-0.55) for either dataset (Table 20). A significant overall treatment effect was observed for parasitoids for the eastern dataset (P=0.05) Pairwise contrasts did indicate a greater numbers of parasitoids in the control plots than the d-phenothrin plots on date 2. No significant overall treatment effect was observed for the 5 remaining functional guilds (P=0.08-0.82) (Table 20). Total arthropod counts did not have a significant overall treatment effect either distance from the pond (P=0.14,0.77).

Multivariate analysis suggested more Ceratopogonidae within methoprene and permethrin plots vs. the control on date 2, further from the pond (P=0.02) (Figure 4), and pairwise contrasts detected significantly fewer Calliphoridae for the same plots on date 1 for the west samples (df=1,8; P=0.04,0.02) (Figure 5). Relatively more predators were

observed in the Bti plots close to the pond on date 1 (P= 0.01) (Figure 6). Fewer scavengers were observed on dates 1 and 2 close to the pond for d-phenothrin as detected by pairwise contrasts (P=0.0438,0.0428) (Figure 7). A significant time effect was observed for almost all of the responses studied (df=2,16 P=<0.0001-0.23) (Table 20). Interactions between treatment and time were significant for Odonata. Samples had relatively lower counts of odonates for the larvicide treated plots on the first sampling date followed by a slight increase on date 2. Each of the adulticide treated plots and the control plots started with relatively more odonates on date 1 followed by a decrease on date 2 (Figure 8). Power to detect multivariate overall treatment effects was generally low for sticky card samples during 2004 (0.05-0.717) with some exceptions within certain dates including: Araneae, Coleoptera, and the large size class in the eastern plots, as well as, Bombyliidae, Ceratopogonidae, Chironomidae, Hymenoptera, Odonata, and Predators in the western plots (>0.85).

Sticky Card Samples 2005 The same experiment was conducted in 2005. Fewer individuals were observed in these samples. Only 7 families had sufficient individuals to be analyzed. Five orders, 3 ecological indices, 3 size classes, and 3 functional guilds were also analyzed (Appendix A). None of the response variables showed an overall treatment effect (df=4,8; P=0.11-0.9) (Table 20). Six of the analyzed groups were associated with a significant time effect (Table 20). Multivariate power was low to moderate (<0.85), with a few exceptions on certain dates including: Lestidae, Limnephillidae, Syrphidae, Araneae, Diptera, Odonata, Trichoptera, and the medium size class in the eastern plots, and Lesitidae and Odonata in the western plots (>0.85)

Terrestrial Study Multiple Spray Events, 2005-2006

Sticky Card Samples, 2005 Few arthropods were collected from each sample. The greatest number of identified arthropods was 17 on 1 card. Family richness was low. The greatest number of families sampled for 1 card was 7. Three families, (Pieridae and Bombylliidae, and Formicidae), 3 orders, (Hymenoptera, Lepidoptera, and Diptera), 3 ecological indices, 3 size classes, and 1 functional guild, (nectar/pollen foragers) were included (Appndix A). Two indicators suggested overall treatment effects; total lepidopterans and the "largest" size class were associated with more individuals in permethrin treated plots (df=1,12; P=0.03). The remaining groups did not have significant overall treatment effects (P=0.054-0.75). Distance by treatment interactions occurred for each of these groups along with Pieridae and the nectar pollen foraging guild (df=1,12; P=0.02-0.05) (Table 21). Multivariate power to detect treatment effects in the multivariate analysis was generally low with some exceptions on specific dates including: Lepidopterans and the large size category (>0.8) with most groups having power less than 0.8 on each date.

Sticky Card Samples, 2006 Samples were gathered the same way in 2006 as in 2005 (Appendix A). Overall total counts were low, as in 2005. One sample had 53 identified individuals; the rest had fewer than 21. Five families had enough individuals for analysis: Bombylliidae, Formicidae, Muscidae, Pieridae, and Syrphidae. Four orders were included (Araneae, Diptera, Hymenoptera, and Lepidoptera) as well as 3 size classes, 3 ecological indices, 4 functional guilds (nectar/pollen foragers, predators,

parasitoids, and scavengers), and total counts. No treatment effects were seen within the family groups (df=2,29; P=0.41-0.92), or order groups (P=0.11-0.57) (Table 21). The parasitoid guild showed a significant treatment effect (P=0.05) as did each of the ecological indices (P=0.003-0.05) which were associated with higher abundance of individuals within permethrin treated plots. Significant treatment effects were not observed for any of the other functional guilds (P=0.73-0.8) or the total arthropod count (P=0.86) (Table 21). Multivariate power for these analyses was generally low with some exceptions on specific dates including nectar-pollen foragers and the small size class. Most groups observed had power of less than 0.9 on each date.

Sweep Net Samples, 2005 Five families, 4 orders, 3 functional guilds, 3 ecological indices, and 3 size classes and total arthropod counts were analyzed from sweep net samples in 2005 (Appendix A). No overall treatment effect was noted for any of the response variables (df=1,12; P=0.06-0.99) (Table 21). Six groups had significant time effects including Acrididae, Formicidae, Membracidae, Diptera, the medium size class, and total counts; in general fewer individuals were collected later in the season (df=1,24; P=0.01-0.05). Five of the responses showed time by treatment effects: Cercopidae, Formicidae, Araneae, Hymenoptera, and predators (df=2,24; P=0.002-0.05) (Table 21). In general, average counts were low for these groups and a discernable pattern was not evident. The multivariate test revealed more Araneae in the permethrin treated plots on date 1 (df=1,12; P=0.01), low counts of Coleoptera revealed a similar result (df=1,12; P=0.01). Multivariate power for sweep-net samples was generally moderate to low with all of the samples having power lower than 0.9 ($\leq$0.752).

Sweep Net Samples, 2006 Sufficient individuals were collected in 8 families, 4 orders, 4 functional guilds, 3 size classes and total arthropod count from sweep net samples for 2006 for analysis (Appendix A). No response variables showed an overall treatment effect (df=1,23; P=0.24-0.97). Sixteen of the observed responses had observed significant time effects related to decreasing abundance later in the season (df=3,69 P=<0.0001-0.03) (Table 21). Power for these tests was generally low to moderate, with some groups having high power (>0.9) on particular dates including: Acrididae, Cicadellidae, and the large size class. Other groups had lower power on all dates of the multivariate tests (<0.9).

Summary of Results

The aquatic effects observed for the acute spray experiment conducted in 2004 and 2005 are consistent with those found by previous authors that examined similar single treatment scenarios. Most of the responses evaluated during my study found few deleterious effects from insecticide application.

Of the 5 response variables examined for D-net samples in either year of the single application study, none showed overall treatment effects. This was also true for the three analyzed responses for sediment samples in 2004, and the 2 responses analyzed in dipper samples in 2004 and 2005 (Table 19). Multivariate analysis considering pairwise contrasts coupled with repeated measures analysis revealed 2 overall treatment effects out of a total of 14 response variables between all 3 sample types and both years; these were associated with fewer individuals in the insecticide treated plots (Figure 3). These potential reductions in aquatic non-target populations did not suggest any trends or

persistent deleterious biological effects following a single adulticide or a single larvicide application (Table 19). Benthic invertebrates that are mostly static in the pond would be the best indicators to measure effects of adulticides drifting into the pond and liquid larvicides applied directly to water. Data from D-net samples showed possible impact on amphipods that may have represented relatively stable populations within the treatment plots (Figure 3). Significant differences for the pond study were found on the dates closest to the spray event when treatment effects were most likely observable. Detection of significant differences between treatments and control plots were minimal and may have been observed based only on the α-level and the relatively large number of recorded response variables. Additionally no significant treatment effects were observed in 2005 (Table 19).

Terrestrial sampling via Olson yellow sticky cards for the same experiment revealed similar results for terrestrial organisms in both 2004 and 2005. For both years, only 1 out of 54 observed response variables exhibited a significant overall treatment effect (Table 20). A time effect was observed for more than 30 of the responses and a time by treatment effect was observed for 6 of the responses (Table 20). Three of the responses were associated with a mean reduction in specific non-target responses on particular dates (Figures 2,3, & 5) . Time by treatment interactions did not exhibit any identifiable pattern. Like the aquatic samples, potential reductions in terrestrial non-target populations did not exhibit trends across dependent groups and persistent biological effects were not observed.

Sticky card results for the terrestrial multiple spray experiment conducted in 2005-2006 did not seem to indicate any potential deleterious effects. Six of the 20 analyzed responses exhibited a significant overall treatment effect (Table 21). None of the significant treatment effects in either year were associated with a reduction of the given response in the permethrin treated plots. Treatment by distance effects were present in responses where an overall treatment effect occurred but did not result in fewer numbers closer to where the spray truck applied the insecticide.

None of the 23 groups collected via sweep net showed overall treatment effects. Six of 19 groups collected in 2005 and 16 of 22 groups collected in 2006 exhibited significant time effects. There was a general decrease in individuals collected particularly later in the season for most of the groups observed. Five of 19 groups in 2005 also exhibited time by treatment effects, although no discernable patterns were associated with these effects (Table 21). This observation was not repeated in 2006. However, multivariate test results did not signify deleterious effects on any particular date. In general, the terrestrial study did not measure deleterious effects on non-target arthropods. No persistent or biological patterns were noticeable in this study.

Type I error rates were set at 5% for each response variable in each year and measurement. With more than two hundred responses examined between both studies, type I errors would not be uncommon. Conversely, power to detect departures from the null hypothesis of "no treatment effects" was low in most cases and had the potential to mask potential deleterious trends within the datasets. For most sample types except for dipper samples in 2004 the average power was less than 0.2. This is not surprising due to

the small sample sizes, but could represent confounding variables between treatment plots, especially in the first study, where flying insects may have moved throughout treatment plots.

Discussion

My results are similar to those of, Lawler et al. (1999) who did not find a decrease in amphipods when Bti and methoprene were applied to seasonal pools in Florida mangrove swamps. While my study was only 2 years it yielded similar results when compared to a long-term study conducted by Niemi et al. (1999) in Minnesota wetlands found no significant decrease in zooplankton when methoprene or Bti was applied in granular formulstions to nine permanent ponds over a three-year period. Charbonneau et al. (1994) found that although Bti caused high mortality of chironomids in a lab environment, a much smaller mortality was observed in the field. Because I had no laboratory studies of mortality this is difficult to assess in the field.

Hershey et al. (1998) conducted the most rigorous non-target organism study on larviciding. Throughout a full season of numerous treatments they found that Bti was effective at killing target dipterans and the food chain was disrupted. Predator numbers dropped as prey became less prominent. Ali (1981) found that treatments of Bti in 4x6-m experimental ponds significantly lowered non-target chironomid numbers. At the highest treatment rate of 4 kg ai/ha there was a 54-92% reduction in chironomid numbers. But the same product used in golf course ponds had no significant effect on chironomid numbers; in this setting at a treatment of 3 kg/ha there was only a 30-67% chironomid reduction. Normal numbers returned 14 days after treatment. As mentioned in Chapter 3

it is unlikely that risks from the larvicides applied directly to water will cause significant mortality to sentinel non-target invertebrates. Exposure of methoprene to lenthic invertebrates may be mitigated by its tendency to bind to organic compounds. However, it is unclear if methoprene remains bioavailable when bound to sediment.

In a study similar to mine, effects from adulticides were minimal. Jensen et al. found no treatment effects on non-target aquatic invertebrates downwind from ULV sprays of pyrethrins, malathion, and permethrin. Further, they found no impacts on species diversity within the seasonally impounded ponds. Studies for terrestrial nontarget receptors other than honey bees are lacking.

Although it is likely that collateral mortality occurs especially to small insects that are active at the same time as mosquitoes, evidence gathered in my study suggests that measurable and persistent biological effects on non-target arthropods exposed to both larvicides and adulticides applied via ULV sprayer are small.

Figure 1. Experiment site, Pond 2, Benton Lake National Wildlife Refuge

Figure 2. Experiment site, Bootlegger Trail Road, Benton Lake National Wildlife Refuge

Table 19. P-values for overall treatment effects, aquatic counts 2004-2005 for single spray study

D-net			P-Values				
	Predictor	df	Amphipod	Coleoptera	Hemiptera	Odonata	Total including all taxa
2004	Treatment	(4,8)	0.23	0.86	0.16	-	0.35
	Time	(3,24)	0.55	0.52	0.01	-	0.03
	Time*Treatment	(12,24)	0.68	0.05	0.6	-	0.24
2005	Treatment	(4,8)	0.86	-	0.5	0.28	0.86
	Time	(3,24)	0.19	-	0.11	0.006	0.16
	Time*Treatment	(12,24)	0.54	-	0.73	0.28	0.56

Sediment						
	Predictor	df	Amphipod	Coleoptera	Diptera	Total
2004	Treatment	(4,8)	0.42	0.5	0.88	0.67
	Time	(3,24)	0.66	0.01	0.39	0.74
	Time*Treatment	(12,24)	0.27	0.84	0.55	0.23

Dipper				
	Predictor	df	*Daphnia* sp.	Rotifer
2004	Treatment	(4,8)	0.28	-
	Time	(3,24)	<0.0001	-
	Time*Treatment	(12,24)	0.66	-
	Predictor	df		
2005	Treatment	(4,8)	0.93	0.94
	Time	(3,24)	0.85	0.69
	Time*Treatment	(12,24)	0.64	0.27

Table 20. P-values for overall treatment effects, sticky card counts 2004-2005 for single spray study both east and west plots

2004			Families	Orders	Functional Guilds	Size Classes	Indices	
	Total number analyzed		30	8	6	3	3	
	Predictors	df	Families	Orders	Functional Guilds	Size Classes	Indices	Total including all taxa
	Treatment	(4,8)	0.08-0.96	0.09-0.93	0.05-0.82	0.21-0.90	0.22-0.55	0.14
	Time	(2,16	<0.0001-0.23	<0.001-0.1	<0.0001-0.11	<0.0001-0.001	<0.0001-0.01	0.003-0.007
	Time*Treatment	(8,16)	0.03-0.99	0.004-0.96	0.005-0.98	0.31-0.69	0.003-0.51	0.24-0.51
2005			Families	Orders	Functional Guilds	Size Classes	Indices	
	Total number analyzed		7	5	3	3	3	
	Predictors	df	Families	Orders	Functional Guilds	Size Classes	Indices	Total including all taxa
	Treatment	(4,8)	0.03-0.84	0.03-0.84	0.48-0.67	0.17-0.86	0.21-0.90	0.77
	Time	(2,16)	0.0001-0.7	0.002-0.98	0.002-0.34	0.0002-0.61	0.23-0.86	0.003-0.17
	Time*Treatment	(8,16)	0.17-0.92	0.09-0.92	0.21-0.56	0.18-0.91	0.14-0.98	0.24-0.51

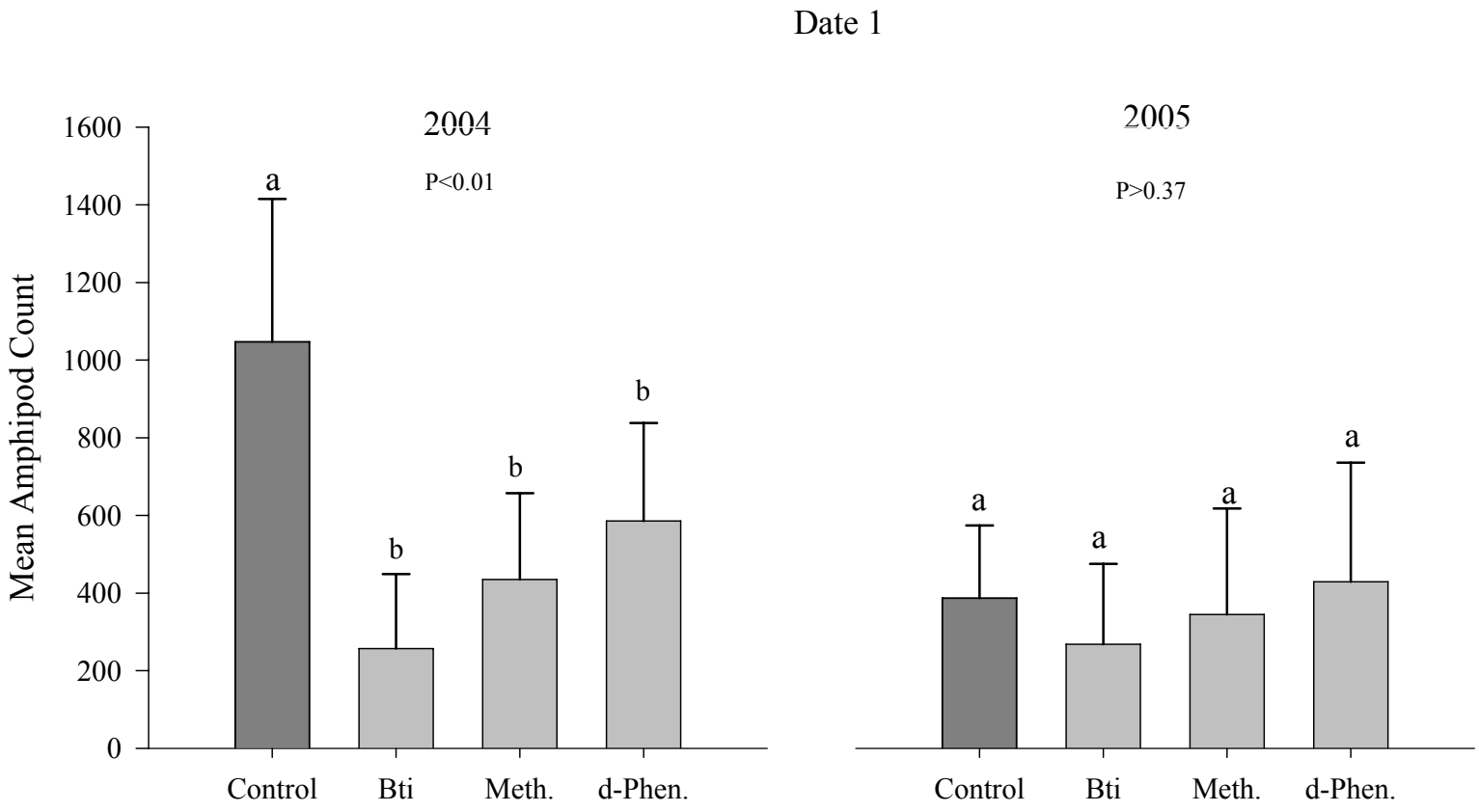

Figure 3. Amphipods per D-net sample, Date 1, 2004-2005, single spray study

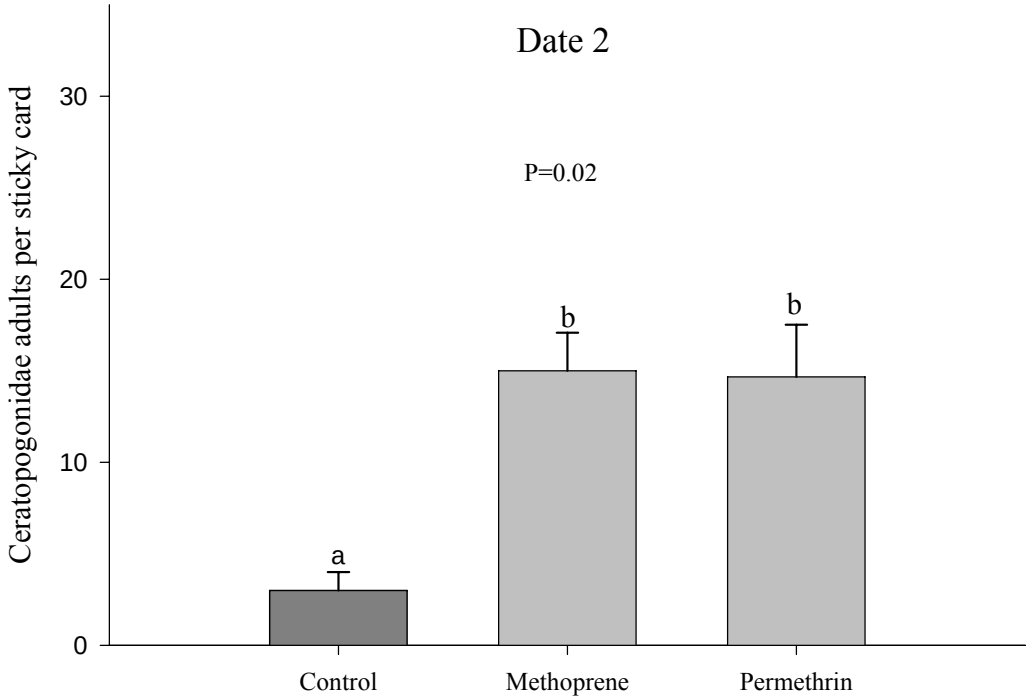

Figure 4. Ceratopogonidae adults per sticky card, East, Date 2, 2004, single spray study

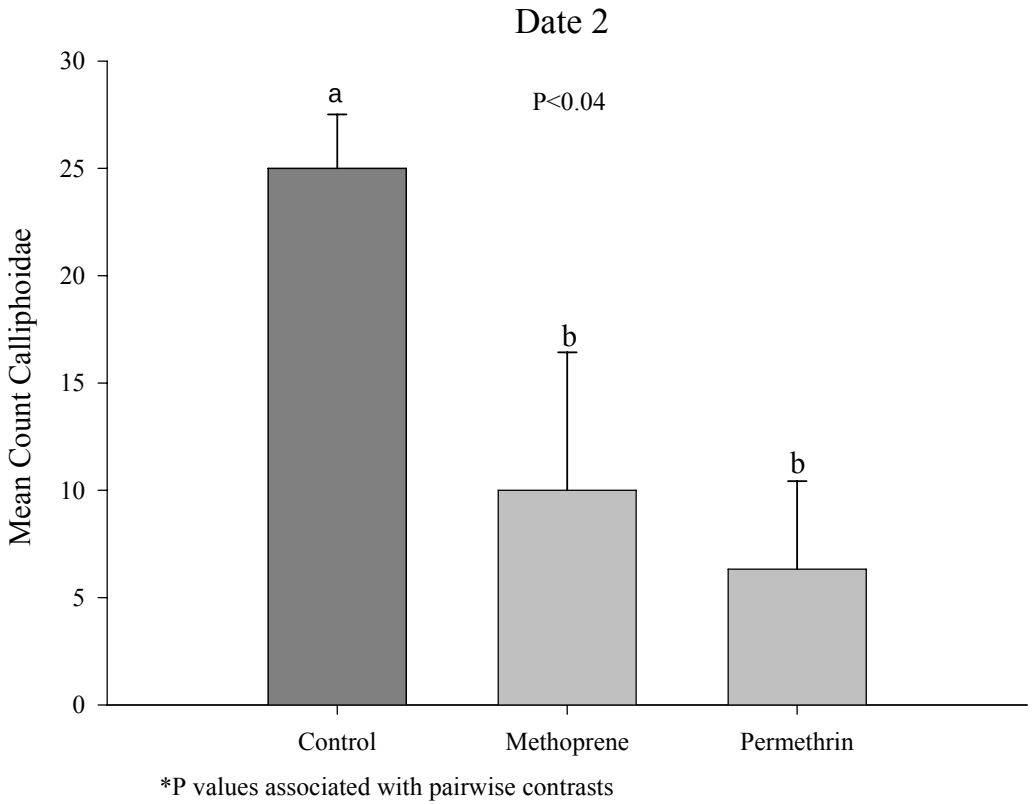

Figure 5. Calliphoridae per sticky card, West, Date 2, 2004, single spray study

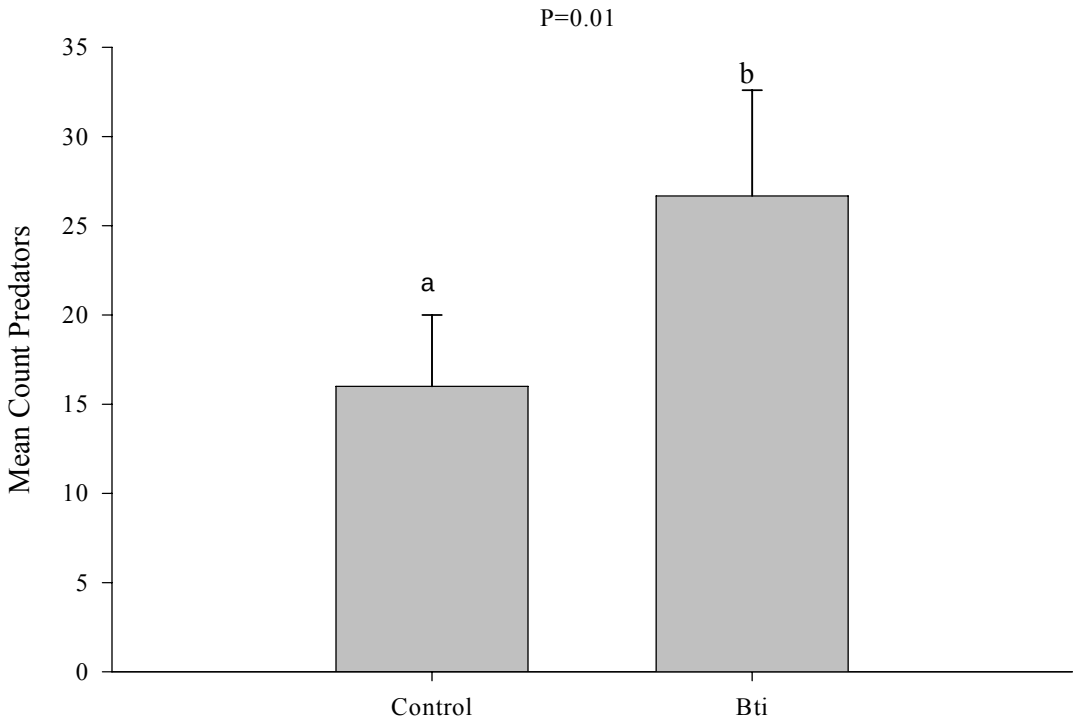

Figure 6. Predators per sticky card, West, Date 1, 2004, single spray study

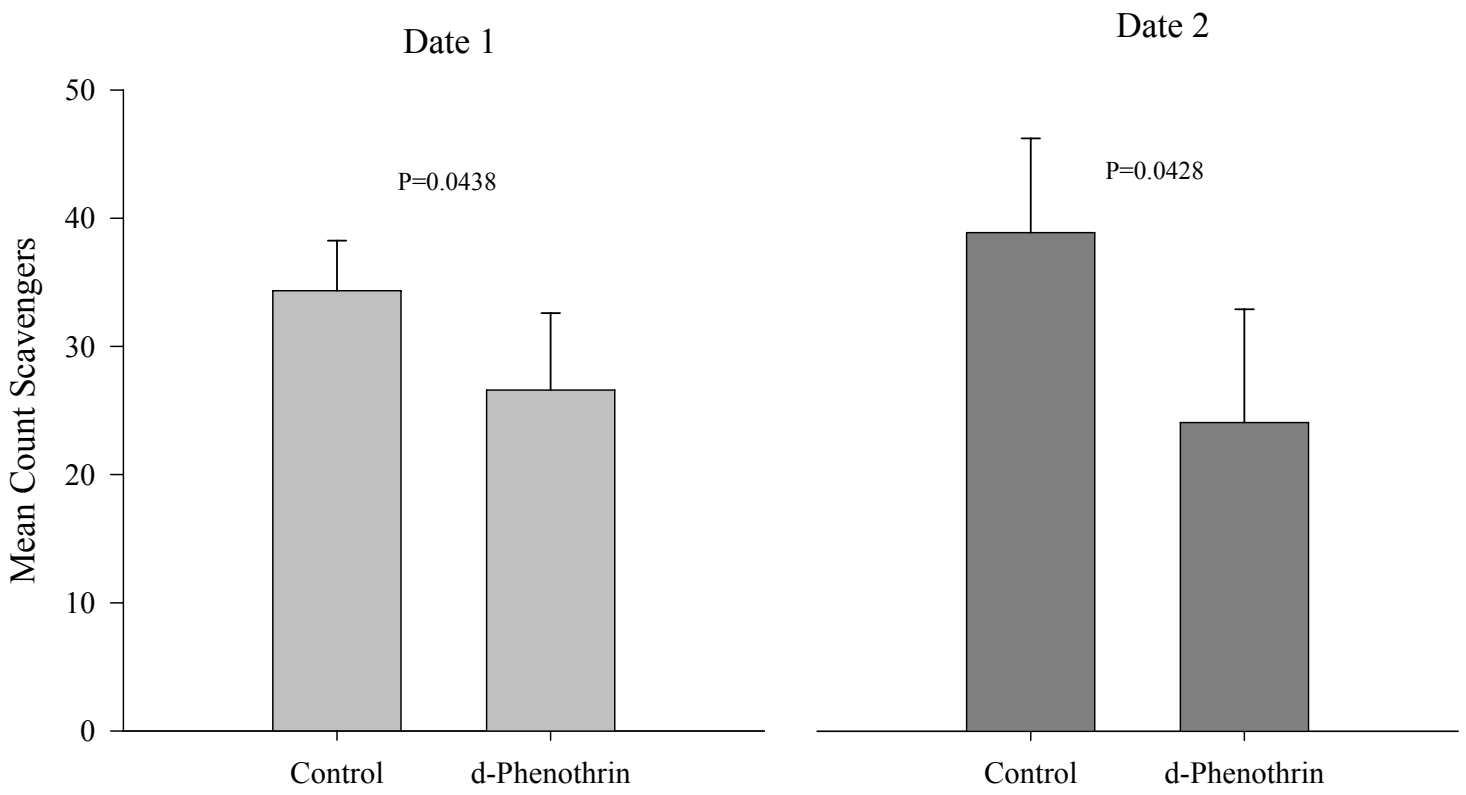

Figure 7. Scavengers per sticky card, West, Dates 1 & 2,2004, single spray study

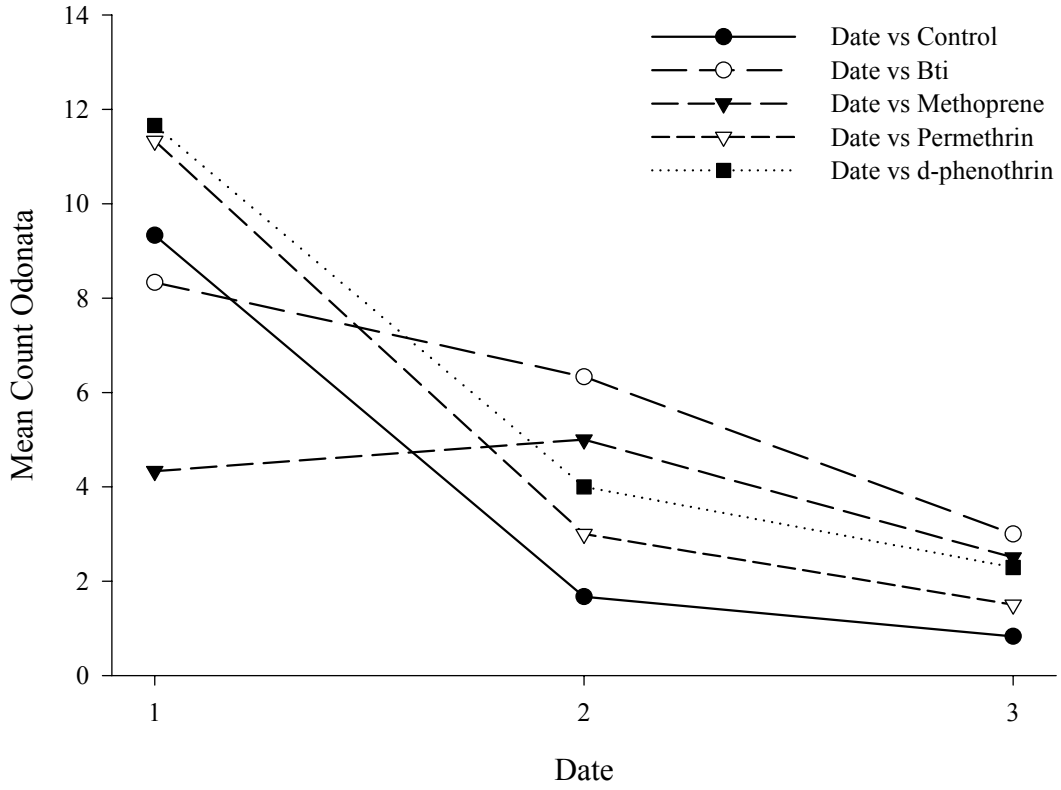

Figure 8. Odonata per sticky card interaction between Date and Treatment, West, 2004, single spray study

Table 21. P-values for overall treatment effects for sticky cards and sweep samples for 2005-2006, multiple spray study

Sticky Card Samples

2005		Families	Orders	Functional Guild	Size Classes	Indices	
Total Number Analyzed		3	3	1	3	3	
Predictors	**df**	**Families**	**Orders**	**Functional Guilds**	**Size Classes**	**Indices**	**Total**
Treatment	(1,12)	0.07-0.75	0.03-0.35	0.054	0.03-0.58	0.12-0.23	0.23
Time	(2,24)	0.1-0.17	0.04-0.17	0.01	0.03-0.9	0.43-0.93	0.05
Time*Treatment	(2,24)	0.05-0.7	0.07-0.52	0.33	0.08-0.69	0.04-0.13	0.08
Distance*Treatment	(1,12)	0.05-0.51	0.03-0.51	0.02	0.03-0.98	0.09-0.13	0.09
2006		Families	Orders	Functional Guilds	Size Classes	Indices	
Total Number Analyzed		5	4	4	3	3	
Predictors	**df**	**Families**	**Orders**	**Functional Guilds**	**Size Classes**	**Indices**	**Total**
Treatment	(1,28)	0.41-0.92	0.11-0.57	0.05-0.8	0.29-0.7	0.003-0.005	0.86
Time	(2,56)	0.004-0.64	0.0002-0.33	0.07-0.27	<0.0001-0.3	0.0006-0.09	0.16
Time*Treatment	(2,56)	0.03-0.65	0.12-0.47	0.03-0.68	0.16-0.68	0.07-0.36	0.31
Distance*Treatment	(1,28)	0.4-0.99	0.23-0.77	0.11-0.9	0.41-0.61	0.02-0.4	096

Sweep Net Samples

2005		Families	Orders	Functional Guilds	Size Classes	Indices	
Total Number Analyzed		5	4	3	3	3	
Predictors	**df**	**Families**	**Orders**	**Functional Guilds**	**Size Classes**	**Indices**	**Total**
Treatment	(1,12)	0.06-0.64	0.16-0.9	0.29-0.85	0.15-0.63	0.32-0.99	0.83
Time	(2,24)	0.02-0.3	0.01-0.22	0.09-0.28	0.01-0.4	0.14-0.98	0.02
Time*Treatment	(2,24)	0.002-0.84	0.009-0.9	0.007-0.92	0.17-0.73	0.79-0.97	0.74
Distance*Treatment	(1,12)	0.06-0.99	0.31-0.6	0.15-0.89	0.23-0.69	0.25-0.63	0.95
2006		Families	Orders	Functional Guilds	Size Classes	Indices	
Total Number Analyzed		8	4	4	3	3	
Predictors	**df**	**Families**	**Orders**	**Functional Guilds**	**Size Classes**	**Indices**	**Total**
Treatment	(1,28)	0.31-.0.97	0.24-0.83	0.38-0.73	0.56-0.87	0.85-0.9	0.8
Time	(2,56)	<0.0001-0.33	<0.0001-0.03	<0.0001-0.002	<0.0001-0.25	<0.0001	0.02
Time*Treatment	(2,56)	0.08-0.87	0.31-0.94	0.26-0.66	0.06-0.63	0.39-0.69	0.74
Distance*Treatment	(1,28)	0.29-0.93	0.23-0.97	0.32-0.91	0.26-0.97	0.51-0.76	0.95

CHAPTER 5

CONCLUSION

Results from my deterministic and probabilistic ecological risk assessments, field experiments, and the weight of scientific evidence suggest that risks to ecological receptors most likely are small from mosquito insecticides applied within both single and multiple applications throughout a season.

Because I used several conservative exposure assumptions in the risk assessments, more realistic assessments most likely would result in RQ values lower than reported here. My tier-I risk assessments found that risks to the evaluated organisms were negligible when exposed over both short and long time periods. Long-term studies by other researchers have found that larvicide applications applied directly to water will pose little risk to populations or ecological interaction webs, but have the potential for non-target impacts when applied multiple times over a season.

Environmental exposures from adulticide and larvicide applications are not likely to add appreciably to background levels of the same active ingredients used in agricultural and urban settings. ULV application increases the efficiency of dissipation so that persistence in the environment is limited.

The probabilistic assessment outlined several uncertainties. These uncertainties verified the reasonable worst case scenario in the deterministic assessment. They also identified the need for data on the environmental fate of adulticides applied via ULV sprayer.

The ecological observations for the acute spray experiment conducted in 2004 and 2005 and the multiple spray study conducted in 2005 and 2006 are consistent with those done by previous researchers who examined similar treatment scenarios. In general most of the responses evaluated during my study exhibited few potentially deleterious effects from insecticide applications. It is likely that observed treatment effects could be attributed to type I errors from the analysis (α=0.05). Power was generally low for most of the response variables. No long term or persistent effects were observed in either study. Significant treatment effects related to specific dates were not repeated in both seasons. Observed response variables often changed throughout the season with overall individual abundance decreasing later in the season.

Limited toxicity data are available for non-target arthropods. Although, it is likely that collateral mortality occurs especially to small insects that are active at the same time as mosquitoes, evidence gathered in my field studies suggest that measurable and persistent biological effects on non-target arthropods exposed to both larvicides and adulticides are small.

Although the same compounds used at much higher rates have been noted to cause some ecological disruption, persistent ecological effects for those applied at low rates, beyond several cases of mosquito resistance, are not noted in any of the literature. The combination of results Chapters 2-4 suggest large scale interaction web disruption from mosquito management plans is unlikely. Direct risk to terrestrial and aquatic vertebrates and aquatic invertebrates is probably low. Direct effects on arthropod communities were not significant within the field experiments. Mosquito management insecticides applied at low rates are unlikely to have deleterious ecological effects.

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APPENDICES

APPENDIX A

LIST OF ORDERS AND FAMILIES IDENTIFIED FROM BENTON LAKE NATIONAL WILDLIFE REFUGE

Table 22. Summary of taxa collected in terrestrial samples 2004-2006

		Single Spray Study		Multiple Spray Study			
Order	Family	Sticky 2004	Sticky 2005	Sticky 2005	Sticky 2006	Sweep 2005	Sweep 2006
Araneae		X	X		X	X	X
Coleoptera	Anthicidae	X					
	Cantharidae	X					
	Carabidae	X				X	X
	Chrysomelidae	X	X			X	
	Cicindelidae (subfamily)					X	X
	Clambidae	X					
	Coccinellidae	X	X	X	X	X	X
	Curculionidae	X				X	X
	Dermestidae						X
	Hydrophilidae	X					
	Lycidae	X					
	Meloidae					X	X
	Melyridae	X					
	Nitidulidae		X				
	Phalacridae						X
	Ptiliidae	X					
	Staphylinidae	X	X	X		X	X
	Tenebrionidae					X	
Dermaptera	Chelisochidae	X	X				

Table 22. Continued

		Single Spray Study		Multiple Spray Study			
Order	Family	Sticky 2004	Sticky 2005	Sticky 2005	Sticky 2006	Sweep 2005	Sweep 2006
Diptera	Acroceridae	X	X				
	Anthomyiidae		X	X		X	X
	Anthomyzidae	X					
	Asilidae		X		X		X
	Bombyliidae	X		X	X		
	Calliphoridae	X		X	X		X
	Ceratopogonidae	X					
	Chironomidae	X	X	X	X		X
	Chloropidae		X				
	Culicidae	X			X		X
	Dolichopodidae	X	X		X		
	Ephydridae	X					
	Lauxaniidae		X				
	Muscidae	X	X	X	X	X	X
	Mydidae		X	X			X
	Phoridae		X		X		X
	Sarcophagidae		X	X			
	Scatopsidae			X			
	Sciomyzidae	X					X
	Simuliidae	X				X	
	Stratiomyidae	X					
	Syrphidae	X	X	X	X		X

Table 22. Continued

		Single Spray Study		Multiple Spray Study			
Order	Family	Sticky 2004	Sticky 2005	Sticky 2005	Sticky 2006	Sweep 2005	Sweep 2006
Diptera	Tabanidae	X			X		
	Tachinidae	X		X	X	X	X
	Therevidae	X		X			X
	Tipulidae	X					
Hemiptera	Aphidoidea (superfamily)	X					X
	Aradidae					X	X
	Berytidae	X					
	Cercopidae					X	
	Cicadellidae	X	X	X	X	X	X
	Cicadoidea					X	
	Fulgoroidea (superfamily)	X				X	X
	Issidae						X
	Lygaeoidea (superfamily)	X				X	X
	Membracidae					X	
	Miridae	X			X	X	X
	Nabidae					X	
	Pentatomidae		X			X	X
	Reduviidae					X	X
	Saldidae	X					X
	Scutelleridae	X				X	X
Hymenoptera	Apidae	X					X
	Braconidae	X			X		X

Table 22. Continued

		Single Spray Study		Multiple Spray Study			
Order	Family	Sticky 2004	Sticky 2005	Sticky 2005	Sticky 2006	Sweep 2005	Sweep 2006
Hymenoptera	Chalcidoidea (superfamily)	X			X		
	Evanoidea (superfamily)		X			X	
	Formicidae	X	X	X	X	X	X
	Ichneumonoidea (superfamily)	X			X		X
	Proctotrupoidea (superfamily)		X		X	X	X
	Sphecidae	X					
	Vespidoidea (superfamily)	X			X		X
Lepidoptera	Arctiidae	X					
	Lycaenidae	X					
	Noctuidae	X	X	X	X		X
	Pieridae	X	X	X	X		
	Tortricidae	X					
Odonata	Coenagrionidae	X					
	Gomphidae				X		X
	Lestidae		X				
	Libellulidae	X			X		X
Orthoptera	Acrididae	X		X			X
Trichoptera	Hydroptilidae	X					
	Limnephilidae		X				