

*Suffolk County
Vector Control
& Wetlands
Management
Long Term Plan
& Environmental
Impact Statement*



**TASK 3 LITERATURE REVIEW
BOOK 8: MOSQUITO CONTROL
AND THE FOOD CHAIN**

Prepared for:

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**SUFFOLK COUNTY VECTOR CONTROL AND WETLANDS MANAGEMENT
LONG - TERM PLAN AND ENVIRONMENTAL IMPACT STATEMENT**

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LIST OF ABBREVIATIONS AND ACRONYMS

<i>Bti</i>	<i>Bacillus thuringiensis</i> var. <i>israelensis</i>
CA	Cashin Associates
CDC	Centers for Disease Control and Prevention
CE	Cameron Engineering
CTDEP	Connecticut Department of Environmental Protection
EEE	Eastern Equine Encephalitis
EPA	United States Environmental Protection Agency
HNA	Hazard Normalized Application
MSF	Monomolecular Surface Films
NOAA	National Oceanographic and Atmospheric Administration
OMWM	Open Marsh Water Management
PBO	Piperonyl Butoxide
PAN	Pesticide Action Network
RED	Re-registration Eligibility Decision
USFWS	The United States Fish and Wildlife Service
WNV	West Nile Virus

EXECUTIVE SUMMARY

Mosquito control can affect estuarine and freshwater organisms directly by unintended, non-target effects, or indirectly by impacting prey, competitor, or predator species. Non-target effects are not well studied, although pesticides are tested for their toxicity to organisms other than mosquitoes. However, indirect effects of mosquito control are even less studied.

Mosquitoes are prey for a wide variety of organisms. Frogs, birds, bats, and dragonflies eat adult mosquitoes, while organisms such as tadpoles, dragonfly naiads, fish, water beetles, and various aquatic invertebrates consume mosquito larvae. Birds, bats, dragonflies, and frogs are said to consume large quantities of adult mosquitoes following brood emergence. Studies have shown that the consumption rates may have been overstated in some cases, and that larger, more easily caught insects are generally preferred over mosquitoes. Fish, ducks, and predacious aquatic invertebrates can consume larvae almost as quickly as they are produced. Access to breeding areas can reduce the effectiveness of these predators, however. There does not seem to be any mosquito predator that specializes in mosquitoes, however. The predators discussed here are all opportunistic generalists that feed on whatever prey is available, and consequently switch to other prey items when mosquitoes are exhausted or difficult to catch.

Non-target impacts from insecticides can directly impact particular species. The toxicity studies that have been undertaken were discussed in Book 7 of the Literature Search, and in the other three parts of this Book 8. Ecosystem impacts from any non-target impacts are more difficult to determine. Keystone species are rare in coastal systems, as most predators tend to consume a wide variety of prey items, and food chains are often complex. Specialization may be rare because environmental conditions such as temperature, salinity, dissolved oxygen concentrations, and nutrient inputs all tend to not be constant. Such habitats reward species which can adjust to environmental change, including resultant changes in abundances of potential prey or predators.

A few studies have looked for ecosystem-level changes resulting from mosquito management. The installation of grid ditches was found, by some, to have had major impacts on the kinds of life found in salt marshes. Others did not find these impacts. It seems likely that the impacts varied, depending on the particular ecological setting. Similarly, more progressive means of

marsh management used for mosquito control are generally thought to either have many fewer impacts than ditching, or to actually serve as a means of restoring degraded marshes to better ecological conditions. The scientific literature on these potential impacts was extensively discussed in Book 9, Part 3, *Natural and Managed Salt Marshes*.

Pesticide use has also been found to cause ecological change, and not to cause any changes. This is at least partially because of the difficulty in constructing a well-designed experiment to look for ecosystem impacts. The application of mosquito control pesticides is usually for some practical purpose, and therefore the identification of control areas that are in similar to treatment sites, and are close enough to share other environmental conditions, can be difficult. For observational studies, determining ecological differences between treatment and control sites may actually measure inherent differences between the sites, rather than the effects of insecticides. Thus, most of the few studies that have been conducted reach conclusions by inference, rather than through clear demonstrations of cause and effect.

The largest study of ecological impacts from mosquito control pesticides was conducted in Minnesota. Early results indicated that larvicides reduced important invertebrate populations. Sampling several years later found the impacts had disappeared. Analysis of the later data sets suggested that either climate was much more important than insecticide applications, as droughty conditions that accompanied the determination of impacts yielded to wetter conditions that resulted in no impacts, or that reductions to the proper application rates for the larvicides eliminated the negative impacts, as application rates in the earliest years may have exceeded label restrictions.

Most other studies of larvicides either found no measurable impacts, or found the impacts to be ephemeral, or to result from application rates far in excess of those typically used for mosquito control. Although adulticides clearly have the potential to impact non-target species at low concentrations, similar reports were produced by the few studies that tried to find larger, ecosystem-wide changes from their application.

Therefore, studies on the ecological impacts of mosquito control tend to reinforce the concept that it is very difficult to conduct ecological systems research, especially in environmental settings. Impacts are difficult to detect, and confounding factors tend to multiply. Ecosystem

impacts from mosquito control have certainly not been well-documented, other than those from mosquito ditching in certain, particular marshes. The determination of whether the use of modern mosquito control pesticides is likely to cause ecological impacts is likely to be clearest when accomplished through modeling exercises, such as will be carried out as part of the Long-Term Plan impact assessment, than through direct measurements of treated versus untreated areas.

1 Introduction

1.1 Mosquito Control and the Food Chain

Mosquito control pesticides and marsh management can impact estuarine and freshwater ecosystems. Other parts of this literature search have reviewed direct impacts from pesticides on a variety of non-target organisms. However, it may be possible that the reduction of mosquitoes in the environment may have impacts on the food web. In addition, the previously discussed direct impacts to non-target organisms by pesticides may also have overall impacts on the greater food web.

To address these issues, a review of the literature on mosquito food chains was undertaken. In addition, reports discussing the direct and indirect impacts of pesticides and marsh management on species diversity and species inter-relationships were reviewed. Cumulative and long-term impacts such as bioaccumulation, bioconcentration, and biomagnification were also assessed.

Several of the other parts of the literature review for the Suffolk County Vector Control and Wetlands Management Long-Term Plan were used in the preparation of this report:

- Book Seven – Ecotoxicity Review of Primary List Mosquito Control Agents
- Book Eight, Part One – Aquatic Invertebrates and Vector Control Pesticide Impacts
- Book Eight, Part Two – Fish and Vector Control Pesticide Impacts
- Book Nine, Part Three – Natural and Managed Salt Marshes
- Book Ten – Freshwater Wetlands
- Book One – Long Island Mosquitoes

1.2 Vector Control and Wetlands Management

Mosquito management can impact marsh food chains through applications of larvicides and adulticides, and also through wetlands management. Wetlands management methods include construction of new ditches in salt marshes and maintenance of previously constructed ditches, and the various techniques of Open Marsh Water Management (OMWM). The particular mosquito larvicides and adulticides examined for this study are presented in Table 1-1 and Table 1-2.

Table 1-1 Mosquito Control Larvicides

Name	Type	Trade Name
Methoprene	Insect growth regulator	Altosid
<i>Bacillus thuringiensis</i> var. <i>israelensis</i> (Bti)	Bacterial	Vectobac, Teknar

Table 1-2. Mosquito Control Adulticides

Name	Type	Trade Name
Malathion	Organophosphate	Fyfanon, Atrapa
Permethrin	Pyrethroid	Permanone, Biomist
Resmethrin	Pyrethroid	Scourge, Purge
Sumethrin	Pyrethroid	Anvil
Piperonyl Butoxide	Microsomal enzyme inhibitor	Butacide, Nusyn

1.3 Mosquito-Borne Diseases

With the emergence of West Nile Virus (WNV) in New York City in 1999, vector control and wetlands management activities have increased to combat the arbovirus (Marra *et al.*, 2004). In addition to its effects on humans, the virus has a significant impact on several animal species, most notably avian and equine species. Raptors, songbirds, geese, sage-grouses, and domesticated chickens and turkeys have been among the avian species infected to date. As of 2004, 29 mammalian species have been infected including horses, chipmunks, skunks, squirrels, bats, and domestic cats and dogs.

Mosquitoes are the vector for WNV. At least 40 species of mosquitoes have tested positive for WNV virus in North America. However, most authorities identify *Culex spp.* (mostly *Cx. pipiens*, *Cx. restuans*, *Cx. quinquefasciatus*, and *Cx. tarsalis*) as the primary vectors (Cornell, 2004; CDC, 2003). Therefore, it can be assumed that mosquito control, especially of *Culex spp.*, will have beneficial aspects for certain portions of the ecosystem, by diminishing non-human impacts from WNV.

A fuller discussion of the ecological impacts of WNV is contained in Book 2, Part 3, *Susceptibility of Other Organisms to West Nile Virus*.

2 Mosquito Ecological Value

2.1 Mosquito Ecology

Mosquitoes are present in many of the estuarine and freshwater environments of Suffolk County. Forty-one native mosquito species have been observed in the region, with twelve of these considered to be of concern because they are involved in disease transmission cycles and/or bite humans (CA-CE, 2004a). The species of concern are shown in Table 2-1.

Table 2-1 - Mosquito species of concern

Scientific Names
<i>Aedes vexans</i>
<i>Coquillettidia perturbans</i>
<i>Culex pipiens</i>
<i>Culex restuans</i>
<i>Culiseta melanura</i>
<i>Ochlerotatus cantator</i>
<i>Ochlerotatus trivittatus</i>
<i>Ochlerotatus triseriatus</i>
<i>Ochlerotatus canadaensis</i>
<i>Ochlerotatus japonicus</i>
<i>Ochlerotatus sollicitans</i>
<i>Ochlerotatus taeniorhynchus</i>

The mosquito life cycle includes four stages of development:

- egg
- larva
- pupa
- adult.

A major means of differentiating between mosquito species is whether or not the species' eggs are deposited directly into water, or if the eggs require a period of desiccation. Both male and female adult mosquitoes feed on plant sugars, but females require a blood meal in order to produce fertile eggs (CA-CE, 2004a).

The number of broods produced per year is also used to classify mosquitoes. Univoltine species have a life cycle that results in a single generation of mosquitoes per year, while multivoltine species produce multiple generations per year.

Table 2-2 - Primary Ecological Groupings of Mosquitoes found in the Northeast

	FLOODWATER MOSQUITOES		PERMANENT WATER MOSQUITOES
	Univoltine Aedine Life Cycle	Multivoltine Aedine Life Cycle	Culex/Anopheles Life Cycle
Representative Habitats	Woodland Pools Freshwater Swamps Roadside Ditches	Fresh Floodwater Saltmarsh Floodwater Containers	Freshwater Swamps Brackish Water Swamps Standing Polluted Water Containers
Number of Generations	1 per Year	Rain/Tide Dependent	Continuous
Overwintering Mechanism	Egg Stage	Egg Stage	Mated Female
Seasonal Distribution	April - May	June - October	May - October

Larval mosquitoes feed on microorganisms and organic particulates in the water column (Merritt et al., 1992). Adult mosquitoes feed on nectar for energy requirements (Foster, 1995); females of almost all species require blood meals to allow for egg production (CA-CE, 2004a).

It is sometimes assumed that mosquitoes are important ecologically (Hammerschmidt and Fitzgerald, 2005), usually because they consume and in turn are consumed. Mosquitoes are identified as being among the dominant invertebrates in particular wetland settings (Batzer and Wissinger, 1996); nonetheless, they were not described as ecologically important. Partially this is because benthic invertebrates and, particularly, bacteria have been shown to be much more important consumers of detritus than water column insect detritivores. Partly, it is because mosquitoes have not been shown to be important prey species for any wetland predators.

2.2 Mosquito Predators

Human interest in mosquitoes stems from their impacts through nuisance and potential health threats. However, they also serve as prey for a wide variety of organisms. Mosquito predators include:

- Mature terrestrial organisms, such as frogs, birds, bats, and dragonflies, which tend to prey on flying adult mosquitoes
- Immature (developing) terrestrial organisms, such as tadpoles and dragonfly naiads, which when developing in aquatic settings prey on mosquito larvae or pupae

- Mature aquatic organisms, such as fish and water beetles, which consume mosquito larvae
- Immature aquatic organisms and aquatic invertebrates, which compete with and potentially may consume mosquito larvae

(Sutter-Yuba Mosquito and Vector Control District, 2005; Cashin Associates, 2004a)

Table 2-3 and Table 2-4 list some particular species that have been identified as mosquito predators.

Table 2-3. Adult mosquito predators

Grouping	Common Name	Scientific Name
Bats	Little Brown Bat	<i>Myotis lucifragus</i>
	Big Brown Bat	<i>Eptesicus fuscus</i>
Birds	Red Wing Black Bird	<i>Agelaius phoeniceus</i>
	Tree Swallows	<i>Tachycineta bicolor</i>
	Marsh Wrens	<i>Cistothorus palustris</i>
	Purple Martins	<i>Progne s. subis</i>
Toads and Frogs	Eastern Spadefoot Toad	<i>Scaphiopus holbrookii</i>
	Fowler's Toad	<i>Bufo fowleri</i>
	Spring Peepers	<i>Hyla crucifer</i>
	Wood Frogs	<i>Rana sylvatica</i>
	American Green Frogs	<i>Hyla cinerea</i>
Salamanders	Red Backed Salamander	<i>Plethodon cinereus</i>
	Tiger Salamander	<i>Ambystoma tigrinum</i>
	Spotted Salamander	<i>A. maculatum</i>
	Common Red-Backed Salamander	<i>Plethodon cinereus</i>
Dragonflies	Salt Marsh Dragonfly	<i>Erythrodiplaz bernice</i>

Table 2-4. Larval mosquito predators

Grouping	Common Name	Scientific Name
Fish	Mosquito Fish	<i>Gambusia affinis</i>
	Mummichogs	<i>Fundulus heteroclitus</i>
	Sheepshead Minnow	<i>Cyprindoon variegatus</i>
Frogs	Tadpoles	<i>Rana spp.</i>
Insects	Dragonfly Naiads	Order: <i>Odontata</i>
	Horsefly Larvae	Genus: <i>Chrysops</i>
	Backswimmer	<i>Notonecta undulata</i>
	Diving Beetle	<i>Dystiscus dauricus</i>
	Aquatic Spiders	Family: <i>Pisauridae</i>
Snakes	Northern Water Snake	<i>Nerodia s. sipedon</i>
	Common Garter Snake	<i>Thamnophis sirtalis</i>
	Eastern Milk Snake	<i>Lampropeltis t. triangulum</i>
Ducks	Mallard Ducks	<i>Anas platyrhynchos</i>

Vector control and wetland management specialists are concerned with the impact pesticides may have on mosquito predators because they can reduce mosquito populations. The popular press has identified birds, bats, dragonflies, and frogs as major controls on adult mosquitoes. Certain aquatic species are also identified as voracious consumers of larvae, particularly killifish (salt water environments, mosquitofish (fresh water environments), and ducks. Kale (1968) and others (Corrigan, 1997; Purple Martin Conservation Association, 2002) have shown that, with the exception of fish, these predators do not consume mosquitoes to the degree shown in some other work (e.g., Campbell, 1907, and Wade, 1966). Most of the predators prefer larger prey items that provide more energy per unit of foraging time. For example, bats prefer moths and beetles, dragonflies prefer butterflies, bees and other dragonflies, and purple martins prefer larger insects such as grasshoppers, along with bees and butterflies. Tree swallows and red wing black birds will switch to seeds when environmental conditions, such as cold weather or drought, reduce insect abundance (Cashin Associates, 2004a; CA-CE, 2004b; Sutter-Yuba Mosquito and Vector Control District, 2005). In addition, because most mosquitoes have adopted the species survival mechanism of producing large emerging broods from synchronous larval hatches, as many other insects also do, predators must find other prey items when the brood disperses or the larvae pool is consumed. Nearly all predatory insects in ephemeral pools are generalists, for example. Fish and ducks that prey on mosquitoes also consume other organisms or aquatic vegetation. Therefore, although some wetland insects (such as chironomids) are found to be key prey for some other biota (such as ducks), mosquitoes are not identified as such (Batzner and Wissinger, 1996).

2.3 Habitat Management

Changing the hydrology of a marsh by installing mosquito control ditches has been asserted to have had a major impact on salt marsh ecologies (see for example, Bourn and Cottam, 1950). Others, examining the same environments, disagree passionately (Provost, 1977). Some of these disagreements are the result of different perspectives on the nature of salt marshes (Nixon, 1982). Other differences arise because most salt marsh studies were not generalized, but rather were restricted to specific marshes. The conditions at one marsh may be different enough from those at another that generalizing about marshes in general on the basis of geographically-limited studies may have provided the basis for the conflicts found in the literature (Dale and Hulsman,

1990; Pomeroy and Imberger, 1981). Certainly, the Literature Search found it was commonly asserted that ditches promote drainage of tidal flooding, decrease the water table in the marsh, and increase soil salinities. Most observers found an expansion of *Spartina patens* acreage, usually at the expense of low marsh *S. alterniflora* (Cashin Associates, 2004a). If these effects were generally the result of the installation of mosquito ditching, there is no doubt that ditching would be described as having had major ecological impacts on salt marshes. But the impacts are not universally found, and so it is clear that ditching and maintenance of a ditch system does not always lead to ecological degradation of a salt marsh.

Other means of water management for mosquito control have been developed. These are generally described as being more progressive because they are more selective in determining how the hydrology of the marsh will be manipulated and managed than universal grid ditching was. These more careful approaches are grouped under the loose term Open Marsh Water Management (OMWM) (Wolfe, 1996). Generally, OMWM intends to improve mosquito control by enhancing fish predation on larvae. Since it achieves this end by improving fish habitat, most observers find OMWM, at worst, neutral ecologically, and many describe it as a restoration of marsh ecology (Cashin Associates, 2004a).

Changing marsh habitats seems likely to result in changes of, at a minimum, parts of the ecology of the marsh. However, some studies have found overall marsh systems to be very resilient (McLetchie and Goodbred, 2005), and so it is not clear that even large manipulations of the marsh necessarily result in ecological impacts. These issues are discussed in much greater depth in Book 9, Part 3, *Natural and Managed Salt Marshes*.

2.4 Rare and Endangered Species and Species of Special Concern

The USEPA Office of Pesticide Programs includes endangered species considerations in its risk assessments of pesticides. Limitations on pesticide use based on endangered species are not law, but are voluntary guidelines. Under the Endangered Species Act, the USEPA must ensure that the use of pesticides that it registers has no deleterious effect on species listed as endangered and threatened by the US Fish and Wildlife Service or to habitat that is critical to those species.

A number of rare and endangered species of aquatic organisms are found in Suffolk County wetlands. The Peconic River corridor (Table 2-5) and some of Suffolk County’s coastal plain ponds (Table 2-6) are examples of areas where endangered species are found.

Table 2-5. Rare wildlife species associated with Peconic River ponds and wetlands

Common Name	Scientific Name
Spotted Salamander	<i>Ambystoma maculatum</i>
Common Red-Backed Salamander	<i>Plethodon cinereus</i>
Tiger Salamander	<i>A. tigrinum</i>
Wood Frog	<i>Rana sylvatica</i>
Spring Peeper	<i>Pseudacris c. crucifer</i>
Eastern Spadefoot Toad	<i>Scaphiopus h. holbrooki</i>
Spotted Turtle	<i>Clemmys guttata</i>
Eastern Hognose Snake	<i>Heterodon platyrhinos</i>
Banded Sunfish	<i>Enneacanthus obesus</i>
River Otter	<i>Lutra Canadensis</i>

Source: NYS DEC, 1999

Table 2-6. Rare insect species associated with coastal plain ponds

Common Name	Scientific Name
Barrens Bluet Damselflies	<i>Enallagma curvatum</i>
Round Necked Damselfly	<i>Nehalennia intergricollis</i>
Violet Dart	<i>Euxoa violaris</i>
Pink Sallow	<i>Psectaglaea carnososa</i>

Source: NYS DEC, 1999

Impacts to these species would generally be considered to constitute an ecosystem impact. The most likely effect would be as a non-target impact from the use of mosquito control pesticides, as water management is generally prohibited under State law in fresh water environments.

3 Environmental Fate of Larvicides and Adulticides

3.1 Bioaccumulation, Bioconcentration, and Biomagnification

Bioaccumulation is the process by which organisms absorb chemicals directly from the environment. Bioaccumulation occurs when a chemical's concentration increases in the organism as absorption exceeds metabolism. Bioconcentration is the accumulation of a chemical in tissues of an organism to levels greater than in the environment in which the organism lives. Biomagnification is the process by which the concentration of toxic substances increases in each successive link in the food chain.

In general, bioaccumulation is greater in larger, longer-lived individuals or species and those with a greater fat content, than smaller, leaner, thinner, more short-lived individuals or species. Bioaccumulation is also a function of metabolic rates and intake and excretion frequencies.

Bioconcentration rates are cited in the following sections for each of the pesticides from research cited in the Hazardous Substances Database (HSDB) of the National Institutes of Health's National Library of Medicine.

3.2 Environmental Persistence

Wurster (1971) found that most insecticides break down rapidly in the environment and do not persist due to their chemical instability. Wurster asserted that the major effects of most pesticides were restricted to treated areas. He found that residues do not accumulate extensively in ecological systems. Those pesticides for which this is not true have been identified as major environmental problems. Malathion residues, for example, have been found to be high enough to kill the larvae of the mud crab, *Rhithropanopeus harrisi* and the commercial blue crab, *Callinectes sapidus* (Bookhout and Monroe, 1977).

Toxicity tests have shown that organisms exposed over a longer duration may experience greater mortality. Relyea (2004) ran malathion toxicity experiments with tadpoles over 16 days rather than the more usual one to four day test. He found that had he ended the experiment on day 3, that 5 mg/L of malathion caused only 5 percent mortality. However, at the end of the 16-day test, 100 percent of the tadpoles were dead.

The impact of pesticides on food chains is governed in part by their persistence in air, soils, and water. Particular pathways can be formed, or not formed, depending on how long-lived the pesticides are in the various media. Generally, there will be less opportunity for impacts to food chains by pesticides that are relatively short-lived in the environment. Important issues to consider in these determinations include:

- Pesticides that degrade rapidly in the atmosphere tend not to be deposited in significant concentrations in water or soils.
- Pesticides that degrade through exposure to sunlight may not persist on surfaces long enough to significantly impact food chains.
- Pesticides can differentially accumulate in parts of plants, either because of direct deposition, or through uptake from soils, and so specific grazing practices by herbivores may be important in terms of differential exposures
- Organic compounds, such as pesticides, tend not to be very soluble, and so pathways tracing dissolved compounds may not be as important as some other pathways.
- Organic compounds may be enriched in surface films.
- Pesticides in aqueous environments are likely to be scavenged by particulate matter. Therefore, organisms consuming particles in the water column, or those that feed on recently deposited material, may have greater exposures. Food chains associated with filter feeders or benthic deposit-feeding organisms may contain greater burdens.

Although many mosquito control pesticides have been found to degrade rapidly, their photoproducts and metabolites may be toxic to certain aquatic organisms. La Clair *et al.* (1998) subjected African clawed frog (*Xenopus laevis*) embryos to 1 µg/L of several of the degradation products of S-methoprene. The juvenile frogs that developed from the exposed embryos were deformed, suggesting that methoprene is degraded to a material that may be more detrimental to aquatic organisms than the parent compound. An endnote was added to this paper detailing follow-up work that included measuring the degradation products of S-methoprene in the water and sediments from three field sites. Traces of two of the degradation products used in the original study were found. Although the detected concentrations were lower than that required

to cause deformations in *Xenopus*, the presence of the compounds several months after methoprene application was noted with concern. As a check on these concerns, the concentration of a methoprene degradate normally found in the commercially applied product when applied to a pond under recommended dosage regime was calculated. It was found to be 0.0044 to 0.0060 µg/L, considerably lower than the 1 µg/L found to cause amphibian deformities. However, the work suggests that methoprene metabolites may nonetheless be a problem due to the cumulative affect of multiple applications and potential bioaccumulation. In addition, S-methoprene is added to sprays against fleas that can be purchased at many pharmacies and grocery and pet stores, and the level of methoprene present in these sprays is orders of magnitude higher than the level found in mosquito control products. One of the degradation products is also added to certain agricultural products. Therefore, measurement of methoprene degradation products in the environment may be affected by inputs from non-mosquito control applications.

In a follow-up study of amphibian deformities and methoprene degradation products, Hendrick *et al.* (2002) concluded that factors other than Altosid (methoprene) and its degradation products contributed to the outbreak of frog deformities. Data were collected from 60 counties in Minnesota that were part of a network reporting anuran deformities in 1997 and 1998. Seven of the 60 counties reported use of Altosid, and 53 did not. Five of the seven counties where Altosid was used reported anuran deformities, but two reported no deformities. There were no statistically significant differences in frog deformity rates between the counties where methoprene was used, and those where it was not used. Analyses of pond water after methoprene treatment demonstrated rapid degradation of the pesticide. Even at 100 times the maximum field application rate, concentrations were measured in the single digit parts per billion (ppb), or at non-detect levels. Thus, methoprene degradation products have minimal potential for affecting anurans, even under “considerable overdose situations.” Degitz *et al.* (2001) support these results, finding that methoprene degradation products, for *Xenopus* development, had a no-observable-effect-level of greater than 10,000 ppb for the degradation product 7-methoxycitronellic acid, and greater than 1,250 ppb for s-methoprene acid.

3.3 Particular Vector Control Chemicals

The following section discusses factors affecting environmental persistence, bioaccumulation, bioconcentration, and biomagnification for Primary List pesticides.

3.3.1 Methoprene

The half-life of methoprene varies from 30 hours to 14 days, depending on environmental conditions such as temperature and salinity (Glare and O'Callaghan, 1999; Madder 1980; Schaefer and Dupras, 1973).

Degradation in Water – Methoprene is only slightly soluble in water (Kidd and James, 1991). Studies found half-lives in pond water of 30 and 40 hours at initial concentrations of one and ten µg/L, respectively (Menzie, 1980). If released into water, methoprene adsorbs to suspended solids and sediments (HSDB, 2005). It volatilizes from water surfaces with an estimated volatilization half-life for rivers and lakes of 6.3 and 75 days, respectively. Volatilization from water surfaces is attenuated by adsorption to suspended solids and sediments in the water column. It is rapidly degraded in both sterile and nonsterile pond water when exposed to sunlight (more than 80 percent within 13 days). Degradation is reported to be less rapid under sterile conditions than under nonsterile conditions, which suggests that microbial processes are contributory. Menzie (1980) found that aquatic microorganisms and sunlight rapidly degrade methoprene.

It should be understood that many formulations of methoprene are microencapsulated to ensure slow release over a pre-determined time period. Most formulations used in marshes are intended to dissolve within one week of application. Certain long-lasting briquets have longer lifespans in water. Intended for use in catch basins and similar environments, formulations have been prepared to last 60 days up to 120 days before they are completely dissolved. The lifespan of the product in no way influences the fate of the chemical, once it is released to the water column.

Atmospheric Degradation - Methoprene degrades rapidly in water and on inert surfaces exposed to sunlight (USEPA, 1991). Photolysis and microbial metabolism degrade 90 percent of the product, while photolysis alone degrades 80 percent of the product within 13 days (USEPA, 2001). According to research cited in the National Library of Medicine Hazardous Substance

Database (HSDB) (HSDB, 2005), if methoprene is released to the air, it will exist in both vapor and particulate phases. The vapor-phase product is degraded by reaction with photochemically produced hydroxyl radicals and ozone with half-lives estimated at 1.5 and 48 minutes, respectively.

Degradation in Soils - Methoprene demonstrates low persistence in soils with a reported half-life of up to ten days. In sandy loam, its half-life was calculated to be about 10 days. When applied at an extremely high application rate of one pound per acre, its half-life was less than ten days (USEPA, 1982). Although methoprene volatilization from moist soil surfaces may be important, adsorption to soil tends to attenuate volatilization. Volatilization from dry soil surfaces is reported to be minimal. In soil, microbial degradation is rapid and appears to be the major degradation pathway (USEPA, 1982; USEPA, 1991). Methoprene is rapidly and tightly absorbed to most soils (EPA, 1982) and in field leaching studies was observed only in the top few inches of the soil even after repeated washings with water (USEPA, 1982). Based on these properties and its low environmental persistence it is unlikely to be significantly mobile.

Bioconcentration - An estimated bioconcentration factor of 3,400 suggests a potential for bioconcentration in aquatic organisms exists (HSDB, 2005).

3.3.2 *Bacillus Thuringiensis* var. *Israelensis*

Bti spores and endotoxins are inactivated when exposed to ultraviolet light wavelengths of 300-400 nanometers, which falls within the spectrum of sunlight. The half-life of *Bti* on plant surfaces ranges from one to four days and several months on soil surfaces. In water, the agent precipitates out of the water column as it binds to particulate matter. This process renders *Bti* unavailable to larvae, reducing its efficacy in aquatic systems where large amounts of particulate matter are commonly present (CA-CE, 2005b).

3.3.3 *Bacillus Sphaericus*

Bacillus sphaericus (Bs) spores are not persistent when used as a larvicide and applied directly to the water (Paul and Sinnott, 2000). It is especially effective in highly organic waterbodies. According to Paul; and Sinnott, Bs does not affect most other species of aquatic insects, its

potential fish toxicity is “so small as to be considered negligible ... and it does not accumulate in fish and wildlife.”

3.3.4 Malathion

Malathion may pose the greatest aquatic risk to aquatic organisms of the three commonly used organophosphates, malathion, fenthion, and naled (Rand, 1995). The ranking is based on acute toxicity, degradation, and usage. Malathion is more bioavailable than resmethrin due to its solubility, density and application rate. Malathion is more water-soluble, reaching the ppm range compared to the ppb range for resmethrin, and has a greater density than resmethrin, 1.23 to 1.96 g/cm³ vs. 0.84 to 1.02 g/cm³, respectively. The maximum application rate for resmethrin is 1.3 g active ingredient (a.i.)/hectare compared to 18.4 to 36.8 g a.i./hectare. Organophosphates form a thicker layer after application than resmethrin, 17.1 nm compared to 0.9 nm in a 0.4 hectare pond at recommended application rates. Because of these characteristics, aquatic organisms experience greater exposure to organophosphates such as malathion than resmethrin (Rand, 1995).

Malathion was one of the compounds included in an extensive study of agricultural pesticides in coastal areas conducted by the National Oceanographic and Atmospheric Administration (NOAA) (Pait, *et al.*, 1992). Malathion had only been found in two samples of the water column and sediments by 1990; nonetheless, due to its measured toxicity to estuarine life, it was said to be “quite toxic.” A preliminary study for this overall assessment of agricultural pesticide impacts on aquatic life looked at fish kills in coastal waters (Lowe *et al.*, 1991). Pesticides were suspected to be the cause of 150 of 3,600 documented fish kills between 1980 and 1989 – approximately four percent. Particular pesticides were determined to be the cause of 44 of the fish kills, with endosulfan and malathion being tabbed in 28 of the events. Over half of the malathion-linked fish kills were linked to non-agricultural applications of malathion, with mosquito control identified as the exemplary non-agricultural use of malathion. However, the report does not specify exactly how many of the fish kills are conclusively linked to mosquito control applications of malathion (the implication is it is something between five and 10 events).

Atmospheric Degradation – The HSDB cites research that shows that malathion exists solely as a vapor in the atmosphere. It degrades in the atmosphere by reacting with photochemically

produced hydroxyl radicals and has a half-life in air of five hours, with 80 to 90 percent degradation over ten days (HSDB, 2005).

Degradation in Water – Malathion is not expected to adsorb to suspended solids and sediments in water. Biodegradation in raw river water reached 90 percent in two weeks (HSDB, 2005). The half-life of malathion via hydrolysis in seawater and freshwater ranges from two to 11 days. Direct volatilization from water surfaces was not expected to be an important fate process. EXTTOXNET (1993) reported on research that showed biological and physical processes were able to completely break down malathion in water after 25 days, with only one percent remaining after 18 days. The process was accelerated by increased salinity. Wang (1991) conducted a persistence study in the Indian River estuary in Florida after aerial and truck mounted sprays of malathion. Maximum deposits of malathion (492 ng/cm^3) on the water surface occurred 36 minutes after aerial spraying, representing only 20 percent of the amount applied. Peak water concentration of five $\mu\text{g/L}$ was observed 84 minutes after aerial spraying. The concentration decreased to 0.8, 0.22, and less than 0.05 $\mu\text{g/L}$ at 12.4, 24.4, and 48.4 hours after spraying. Wang reported that no significant mortalities of fish or copepods occurred. Similar results were observed in a study of aerial applications of malathion to control the Mediterranean fruit fly (Medfly). Hundreds of thousands of acres in several California counties were treated with between one to 12 applications of malathion at approximately 3.6 gm a.i. per hectare (Rand, 1995). One to three weeks elapsed between treatments. Residues were detected in freshwater ponds and swimming pools of zero to 90 ppb. Samples collected in fishing areas just after applications averaged 6.22 and 2.26 ppb for malathion and malaoxon (the oxidation product).

Degradation in Soils - Soil degradation is generally completed after three days and is directly related to the degree of binding between the pesticide and soil particles (EXTTOXNET, 1993). Malathion is expected to have very high mobility in soil. It is not expected to volatilize from moist or dry soil surfaces. Biodegradation in soil is rapid, with 80 to 95 percent degraded in ten days, and increases with the organic content of the soil. This results in half-lives ranging from one to six days (HSDB, 2005).

Bioconcentration - An estimated bioconcentration factor of 13 in aquatic organisms means there is a low potential for amplification up the food chain (HSDB, 2005).

3.3.5 Permethrin

Atmospheric Degradation - Permethrin exists in both a vapor and particulate phase in the atmosphere. Atmospheric permethrin in the vapor phase is degraded by reaction with photochemically produced hydroxyl radicals and ozone, with half-lives estimated to be 9.8 hours and 49 days, respectively (HSDB, 2005). When exposed to sunlight, the half-life of permethrin is 4.6 days (WHO, 1990). Permethrin in water was found to be nontoxic (at 0.05 mg/L) after gradually loses its toxicity over 48 hours in sunlight (Wagenet, 1985).

Degradation in Water – When released into water, permethrin adsorbs to suspended solids and sediments. Permethrin was found to biodegrade in a sediment-seawater solution, with a half-life of less than 2.5 days. Volatilization from water surfaces is estimated to result in a half-life in rivers and lakes of 26 and 290 days, respectively. However, water surface volatilization may be attenuated when permethrin is adsorbed to water column suspended solids and sediments. The half-life of permethrin in water from photolysis is 33 days (HSDB, 2005).

Degradation in Soils - Permethrin is expected to have no mobility if released to the soil. Volatilization from moist soil surfaces is expected to be an important fate process, although soil adsorption can attenuate volatilization. Permethrin has low to moderate soil persistence, with reported half-lives of 30 to 38 days (Kidd and James, 1991, Wauchope *et al.*, 1992). According to research cited in EXTNET (1994), permethrin is readily decomposed in most soils by microbes. The addition of nutrients to soil may increase the degradation of permethrin, probably by increasing microbial activity. Wagenet (1985) reported that there is very little leaching of permethrin. Permethrin is not very mobile in a wide range of soil types (Penick Corp., 1979). Because permethrin binds very strongly to soil particles and is nearly insoluble in water, it is not expected to leach or to contaminate groundwater.

Bioconcentration - Bioconcentration values for rainbow trout and sheepshead minnow are 560 and 480, respectively (HSDB, 2005), suggesting a potential for aquatic bioconcentration.

3.3.6 Resmethrin

Hydrolysis, photodegradation, and biodegradation all rapidly break down resmethrin. It has a half-life ranging from 15 minutes to 36 days, depending upon environmental conditions.

Reported degradation end products include chrysanthemic acid, benzaldehyde, benzyl alcohol, benzoic acid, phenylacetic acid, and various esters (Penick Corp., 1976).

Rand (1995) reports that resmethrin “floats” on the air water interface (the surface microlayer) that represents the hydrophobic, organic carbon rich layer of most waterbodies. Rand estimated that a 0.91 nm film of resmethrin would form on a one-acre pond if applied directly and evenly. He suggests that even though resmethrin has a low vapor pressure it would more likely be transferred to the air than the water because of its extremely low water solubility and low density. He adds that due to its solubility, density and the application solvents typically used, resmethrin will remain in contact with the air and light for a longer time than organophosphate pesticides such as malathion (see section 3.3.3). Because resmethrin is not well distributed in the water column after application it will be minimally bioavailable to aquatic organisms. Rand concludes, “Although it appears that resmethrin is more acutely toxic than organophosphates [such as malathion] in the laboratory, under realistic field exposure it has less potential risk because exposure is minimal.”

Atmospheric Degradation - Resmethrin exists only in the particulate phase in the atmosphere. It undergoes direct photolysis with a half-life on glass plates ranging from 20 to 90 minutes when exposed to sunlight (HSDB, 2005).

Degradation in Soils - Resmethrin is of low to moderate persistence in soils. Resmethrin binds tightly to soil and may adsorb to sediments, suspended particles and plants. It is not expected to be mobile and contaminate groundwater, particularly because of its extremely low solubility in water (Augustijn-Beckers *et al.*, 1974). Its half-life in soil is 30 days and 36.5 days in sediments (EXTOXNET, 1996). Volatilization from moist or dry soils is not expected to be an important fate process. Resmethrin is expected to biodegrade as readily as other pyrethroids through the action of microorganisms.

Degradation in Water – If released into water, resmethrin is expected to adsorb to suspended particles and sediments. When added to soil four hours before flooding or mixed with sediment, the acute toxicity was 100-500 times lower than when in water alone (Rand, 1995). Photolysis in surface waters is also likely to be important with a predicted near-surface half-life of 0.2 hours (HSDB, 2005). EXTOXNET reports work that shows that pyrethroid concentrations decrease

rapidly in pond waters and in laboratory degradation studies due to sorption to sediment, suspended particles and plants. Microbial and photodegradation also occur (Muir *et al.*, 1985). EXTOWNET (1994) reported a half-life in water of 36.5 days.

Bioconcentration - HSDB (2005) cited a moderate bioconcentration factor of 68.

3.3.7 Sumithrin

Atmospheric Degradation - Sumithrin (phenothrin) exists in both vapor and particulate phases in the atmosphere. Sumithrin is degraded in the atmosphere by photochemically produced hydroxyl radicals and ozone with half-lives estimated at four hours and 38 minutes, respectively (HSDB, 2005).

Degradation in Soils - Sumithrin is expected to have no soil mobility, will easily volatilize from moist soil surfaces, though adsorption to soil can attenuate volatilization. Sumithrin rapidly photodecomposes and biomineralizes in soils and aqueous systems (HSDB, 2005). Degradation in soil has a half-life of one to two days under dry and high light conditions and two to four weeks under flood conditions. In the absence of light, the half life is extended and sumithrin has been known to remain intact for up to one year in dark storage conditions, such as in grain silos (Cashin Associates and Integral Consulting, 2005). Residues of trans-phenothrin fell to less than ten parts per billion (ppb) within 45 days in an aerobic soil (HSDB, 2005).

Degradation in Water – In water, sumithrin adsorbs to suspended solids and sediments, though volatilization from water surfaces is important. Volatilization half-lives in rivers and lakes were found to be seven and 81 days, respectively. Volatilization from water surfaces is attenuated by adsorption to water column suspended solids and sediments. Reported hydrolysis half-lives for d-trans-phenothrin were 301, 495-578, and 91-120 days at pH values of five, seven, and nine, respectively (HSDB, 2005).

Bioconcentration - A bioconcentration factor estimated at 266 suggests a potential for bioconcentration in aquatic organisms (HSDB, 2005). However, bioconcentration studies on similar compounds suggest that the impact might be less, as many aquatic organisms are able to readily metabolize these compounds.

3.3.8 Piperonyl Butoxide

Piperonyl butoxide (PBO) exists in vapor and particulate phases in the atmosphere. Photochemically produced hydroxyl radicals in the atmosphere degrade piperonyl butoxide with a half-life estimated at four hours (HSDB, 2005).

Piperonyl butoxide is rapidly degraded in soil with a half-life of 14 days (EXTOXNET, 1994). It has moderate to low mobility in soils. Little volatilization from moist or dry soil surfaces is expected.

Piperonyl butoxide adsorbs to suspended solids and sediment in water and volatilization from water surfaces is not significant. Little hydrolysis occurs at a pH of five, seven, and nine under sterile, dark conditions. When illuminated by sunlight, however, PBO in water is rapidly degraded with a half-life of 8.4 hours (HSDB, 2005). PBO has a moderate bioconcentration factor of 90 (HSDB, 2005).

4 Ecosystem Impacts

4.1 Long Term Exposure Studies

4.1.1 Artificial Environments

Most assessments of the toxicity of mosquito-control pesticides to aquatic organisms have been based on information about the estimated concentration of the pesticides in water and on concentration-effect relationships based on single-species toxicity tests. Model ecosystems have also been used to assess the hazard of pesticides. Model ecosystems, or micro- or mesocosms, typically contain water, sediment, and communities of plants and invertebrates from natural ponds that are established in laboratory aquaria. These microcosms can be used to measure the higher-level ecological effects of pesticides. Indoor microcosm studies measure the effects of pesticides on populations and communities under simulated natural conditions. Extended toxicity studies can be conducted with algae, invertebrates or fish. Microcosm studies usually include sediment to allow the pesticide to partition between water and sediment, as it would do naturally. Such systems allow for variation in the chemical concentration over time and can simulate exposure patterns typically observed in the field.

Leeuwangh *et al.*, (1994) studied the success of four types of freshwater model ecosystems in predicting the toxicity of pesticides. The systems in order of complexity include: single species toxicity tests; laboratory water-sediment columns; indoor trophic-level systems (algae, daphnia, bacteria in three separate containers); and indoor microcosms that have multiple species in a single container. Leeuwangh concluded that the major problem in assessing the toxicity of pesticides is determining the actual exposure. He also suggested that for the organophosphate pesticide studied, chlorpyrifos, and others (based on his literature review), pesticide effects could be accurately predicted on the basis of single-species toxicity data. Leeuwangh's model ecosystem studies demonstrated that the recovery of populations exposed to pesticides depends on factors other than the chemical's aquatic concentration. Population recovery is also affected by such factors as the presence of resistant life stages, species life stage at the time of exposure, and immigration of species from non-polluted sources. Leeuwangh found that mesocosm experiments demonstrated insect population recovery from the affects of chlorpyrifos applications.

Yasuno and Satake (1990) examined the effects of methoprene on the emergence of insects and their density in an outdoor experimental stream. They constructed an 80-meter long artificial stream with a pebble substrate that was naturally populated by several species of macrobenthos. The primary species included a caddisfly, mayfly, and four chironomids. The researchers found no decrease in macrobenthos, but chironomids and caddisflies disappeared. The concentrations used in the experiment were considerably higher than those that would be experienced using recommended rates for field conditions. Organisms were exposed to 1 and 10 milligrams per liter (1 and 10 ppm) of methoprene for 30 minutes, whereas typical vector control application concentrations are in the parts per billion range.

4.1.2 Ecosystem Studies

Ecosystem impact studies from vector control chemicals appear to have been limited to the larvicides, methoprene and *Bti*. Researchers acknowledge that even degradable pesticides can have an ecological impact that depends on the proximity of the pesticide application to the wetland, factors that affect degradation and sorption rates, and local wetland hydrology (Clark *et al.*, 1993).

The methods used for the analysis of ecosystem data is critical to separating out significant community effects of pesticide applications. Kreutzweiser and Faber (1999) examined the affects on community structure of pesticide applications to enclosures in a forest ecosystem and found that the statistical approaches traditionally used to analyze data are inadequate for such complex data sets. The common use of multiple univariate tests for large community data sets can result in a number of “significant hits,” some by chance alone. It is thus difficult or impossible to determine how many significant hits constitute a community-level perturbation. Multivariate analyses that can be used successfully to interpret complex data sets, as was the case here where they identified divergences in the community structure among the treatments. For example, after 64 days the density of one species of copepod in the pesticide treated enclosure was significantly reduced. However, that reduction had apparently triggered a significant secondary increase in the population of a cladoceran species. As both species are herbivorous, the increase in the cladocerans was presumed to be the result of release from competition with the copepods. This suggested there is a need for both aquatic mesocosm studies and the

appropriate multivariate statistical analyses to better understand the impact of pesticides on ecological communities.

4.1.3 Methoprene

A review of the impacts of larvicides, including methoprene, was undertaken by USFWS in 1998. This study found that some reports seem to indicate that there is a potential for ecological impacts from larvicide use, but did not clearly determine that impacts would be expected (Brown, 1998). A related study looked for invertebrate community differences between larvicide-treated and untreated areas of four East Coast National Wildlife Refuges. Differences in community structure were found between treated and untreated sites, as a rule. However, because of environmental differences between the treated and untreated sites, the study could only infer (using Brown, 1998, as a reference) that the larvicides were responsible for the qualitative variances. In addition, because most of the sites were treated with more than one larvicide over the course of each season, it was difficult to determine if methoprene or Bti played a role in the observed differences (USFWS, 2000).

A calculation of the Shannon diversity index for the Long Island results indicated that the marsh surface samples were more diverse for the untreated sample as compared to the two treated sites, but that the water column samples from the treated areas were more diverse at the treated sites than at the untreated sites. The analyst, Michael Higgins of the USFWS, warned that one set of samples is too few to draw firm conclusions from (M. Maghini, LI Complex, USFWS, personal communication, 2003).

A comprehensive review of the environmental and health impacts of S-methoprene was conducted for the New Zealand Ministry of Health to determine whether to permit the use of the pesticide (Glare and O'Callaghan, 1999). The lethal dose for mosquitoes was found to be in the parts per billion concentrations, compared to lethal doses for other insects that are 100 times higher. The study concluded that the concentrations associated with field applications for mosquito control would be unlikely to be lethal to many other insects. This study also cited 30 years of previous research that showed no impact from acute methoprene exposure on benthic aquatic invertebrates, mollusks, crustaceans, marine worms, and other organisms. However, continuous methoprene exposure for populations of non-target invertebrates has been found to

have impacts. For example, in California, repeated applications of 0.01 ppm of methoprene to experimental ponds eliminated larvae of the mosquito predator beetle, *Laccophilus* spp. Approximately 84 percent of the predator biomass was eliminated in one test period (Miura *et al.*, 1978). Because methoprene is not persistent in water or soil, affected aquatic populations recover once methoprene applications cease. This was cited as support for a study conclusion that any ecosystem effects from methoprene use are not permanent.

Other studies such as that of Bircher and Ruber (1988) documented methoprene impacts, but found recoveries in the population of affected species following cessation of the methoprene treatment. Bircher and Ruber found that although early stages of the estuarine copepod *Apocyclops spartinus* were sensitive to methoprene, transient decreases in copepod populations did not necessarily lead to decreases in their standing population.

Hershey *et al.* (1998) reported on exposure tests on non-target macroinvertebrates conducted in Minnesota wetlands over the period 1989 to 1993. Statistically significant differences between non-target invertebrate populations exposed to methoprene and *Bti* were found in comparison to control areas. There were no initial impacts from the larvicide applications measured in 1989. However, the area was suffering from a drought, and drought is the most limiting condition for freshwater wetland invertebrates. After several years of non-drought conditions, control area invertebrate populations recovered, but treatment populations did not, to the same degree, causing significant differences.

One treatment site impact was a reduction in certain invertebrate insect predator populations. These predaceous invertebrates are duck diet mainstays, which raised a concern that the impacts would propagate throughout the food chain. Hershey *et al.* speculated that some predators would have been killed directly by the pesticide, while others could have been affected by a reduction in the number of food species, particularly midges (chironomids). Chironomids are, in general, prey for many wetland insect predators; the measured population reduction from methoprene applications thus might result in the overall reduction in wetland species richness due to indirect impacts from repeated use of methoprene. Such indirect impacts were assumed to be the cause of the overall impacts found for *Bti* use, since *Bti* is toxic only to nematoceran Diptera (mosquitoes, fungus gnats, crane flies, gnats, and sand flies) (see below for a fuller discussion of *Bti* reports).

The results reported on by Hershey *et al.* were part of a larger study reported on in a review by the Scientific Peer Review Panel of the St. Paul Metropolitan Mosquito Control District (1996). This report concluded that methoprene did not affect zooplankton species diversity, in that there were no consistent or persistent changes in zooplankton density, size, or reproduction. Its statistical analysis was not as sophisticated as that in Hershey *et al.*, but did find an overall reduction in benthic invertebrates, largely due to a decrease in larval chironomids, which made up about 60% of all sampled insects. Populations of crane flies (Tipulidae), biting midges (Ceratopogonidae), and soldier flies (Stratiomyidae) were also reduced in the treatment areas.

These results were brought into question by a follow-up study that revisited many of the wetlands discussed in these two reports. Balcer *et al.* (1999) sampled under different climatic conditions from 1997-1998. Insect populations in the duplicated wetlands showed no statistical differences. The apparent confounding results were explained as follows:

- 1) The 1989-1993 data were collected following several years of drought, which may have resulted in pre-stressed populations that were extra-susceptible to the pesticides; and
- 2) Climatic conditions in 1997-1998 resulted in thick vegetative growth in the wetlands, which may have limited exposure to the pesticides for the targeted invertebrates and provided optimal environmental conditions for the invertebrates.

Long-term impacts from pesticide use were not found in the later study, suggesting that other environmental factors may be more important than mosquito larvicide use for freshwater invertebrate population trends. Further review of these data was completed and reviewed by the original Scientific Review Panel that had oversight of the 1988-1993 and the 1997-1998 work (Read, 2001). The researchers concluded that there was no effect of the pesticide use on zooplankton species richness, density, size, or reproduction. There was also no effect on breeding bird populations and no effect on red-winged blackbird reproduction or foraging. Ducklings feeding on treated and untreated sites showed no differences in foraging behavior or final weight. Amphibian growth and survival was not affected by methoprene even at high doses for 100 days. Frogs, crayfish, and amphibians fed *Bti*-killed mosquitoes showed no effects. A two-year field study was conducted of frogs exposed to methoprene and those in untreated areas. Of the 1,356 frogs examined during the study, no significant differences were observed in frog numbers or malformation rates, though high variability was reported between sites.

Either the earlier dates of treatment and or the higher than planned doses used in 1992 and 1993 (15 to 19 kg/h of *Bti* were used, instead of the planned nine kg/h that was used in 1991 and 1995-1998) may have contributed to the differences seen during those years. No differences between later treatments and controls suggested to the reviewers that there was no long-term cumulative impact of methoprene.

Other studies also tend not to find large impacts. Lawler *et al.* (2000), in a study conducted in California salt marshes, found no impacts on either caged or free-swimming invertebrate populations from sustained release methoprene and a combination formula of methoprene and *Bti*, although the dosages were effective for mosquito control. This included Diptera, which had been found to be the order most affected by the pesticides in Minnesota.

A review of methoprene by Antunes-Kenyon and Kennedy (2001) found that it is generally toxic to insects in Diptera, especially midges and mosquitoes. They found no impacts to amphibians, believed the weight of evidence was unclear regarding impacts to crustaceans, and suggested that due to rapid degradation, liquid formulation methoprene was unlikely to have any adverse impacts. Sustained-release briquettes, especially 150-day formulations, were thought to have the potential for some impacts, especially in poorly flushed waters. However, use of the sustained release formulations was shown long ago to be very important to the successful control of *Coquillettidia perturbans*, a vector of EEE (Sjogren *et al.*, 1986). Compared to temephos and *Bti*, only the sustained release methoprene was effective. Overall, Antunes-Kenyon and Kennedy determined there was no permanent ecosystem disruption from methoprene.

A study by Niemi *et al.* (1999), though it reported no negative effects of *Bti* or methoprene over a three-year period on zooplankton and breeding birds, found significant reductions in benthic insects in two of the three sample years. Total benthic insect density, biomass, and species richness were lower in *Bti* and methoprene treated sites for two of the three sample years. No trophic relationships were uncovered between insects and birds, and insects and zooplankton, for treated sites. The complexities of the food chain serve as an explanation for the lack of an observable effect of a decline in benthic insects on zooplankton and breeding bird abundance. It may be that the breeding birds foraged on adjacent untreated wetlands. Zooplankton abundance may be more influenced by other environmental factors rather than predation.

Hershey *et al.* specified that the impacts propagated up the food chain. Several studies have sought such impacts. Hanonowski *et al.* (1997) thought that, although their data showed no impacts from methoprene or *Bti* on marsh-breeding bird populations, under some conditions it was possible that these larvicides could have negative impacts – but impacts that would be less than those caused by weather or predation. A peer review of the original work in Minnesota (Anderson *et al.*, 1996) thought that the data presented were not conclusive regarding ecological impacts to ducks and other wetland birds, especially as it is possible that the ducks may vary their diet depending on available prey. This review did suggest that further research was needed.

It is possible that complex feedback mechanisms produced the differences between the Hershey and Balcer studies. If food chain impacts do propagate, then controls on the food chain base will be reduced over time in the treatment areas. This may allow the treatment area to rebound from its slower recovery from drought impacts. Another possibility is that a hidden confounding factor was responsible for the original difference, as perhaps whatever made these wetlands good for mosquito breeding also tended to inhibit other invertebrate population recoveries following a drought.

Pinckney *et al.* (2000) studied the effect of 0.011 kg a.i./ha methoprene on insect populations in experimental ponds, and found no significant differences between Altosid treated ponds and control ponds in terms of mean numbers of species or families. The results were analyzed in comparison to other work that applied Altosid to experimental ponds at 0.30 kg a.i./ha and found a substantial decrease in emergence rates of caged mayfly nymphs (*Callibaetis pacificus*) (Norland and Mulla, 1975). Pinckney *et al.* point out that Norland and Mulla's application rates were nearly 30 times higher, where the rate used by Pinckney *et al.*, corresponds to a concentration of 1.1 µg/L in the ponds. That concentration is similar to the median concentration (0.5 µg/L) used by Hershey *et al.* (1998) in Minnesota ponds. Pinckney *et al.* note the long-term Minnesota study used granular methoprene, which is more persistent than this study's liquid formulation. Pinckney *et al.* suggested that the more stable granular formulation used in Minnesota would have greater impacts than the shorter-lived liquid formulation. Pinckney also acknowledges that the multiyear nature of the Minnesota study exposed researchers to the variability of insect populations due to factors other than exposure to pesticides.

A number of studies have been conducted on the potential impacts of methoprene on non-target salt marsh species. Lawler *et al.*, (2000) tested a combination of methoprene and *Bti* on insects in salt marsh enclosures. They tested both the liquid formulation and the sustained release methoprene pellets. Immature *Aedes dorsalis* mosquitoes and water boatmen (*Trichocorixa reticulata*) reared in salt marsh pond predator exclusion cages were compared to those reared in untreated ponds. They also collected uncaged insect populations from the sites with sweep nets. They found that the pesticides killed caged mosquitoes and that the sustained release formula was exhibited was 80 percent effective for 99 days. No detectable effects of either pesticide were observed on the survival or maturation of the water boatmen or on the abundance of the uncaged invertebrates. Concerns were expressed about the potential for the development of resistance in mosquito populations exposed to the sustained release formulation, citing Dame *et al.* (1998), which documented resistance in *Aedes taeniorhynchus* exposed to 150-day sustained release methoprene briquettes for five years. Lawler *et al.*'s "conservative conclusion" was that the use of sustained release methoprene formulations was advantageous in environments where there are a limited number of nontarget species whose tolerance to methoprene is known and to protect public health from outbreaks of mosquito-borne disease. Otherwise, the study recommended the use of liquid formula methoprene, which degrades within a day or two and therefore carries less risk. It is unlikely that a variety of nontarget species would be reaching metamorphosis at the same time as a cohort of mosquitoes is being treated. Therefore, the periodic use of liquid methoprene to control mosquitoes would give the nontarget community time to recover in the event that there are effects. This approach could slow the development of resistance by reducing the amount of time that methoprene exerts selective pressure on the insects (Lawler *et al.*, 2000).

Horst and Walker (1999) examined the effect of methoprene on the morphogenesis and shell formation in the blue crab *Callinectes sapidus*, and found deleterious impacts of the pesticide on the crab larvae. However, the concentrations used in the research were orders of magnitude higher than typical field concentrations of the pesticide used according to the manufacturer's directions. For example, 50 percent of crab megalopae died at one ppm exposure to methoprene. That concentration is 250 times greater than the rate of release data cited by the USEPA Reregistration Eligibility Document (RED) Fact Sheet of less than or equal to four ppb (USEPA,

1991). This overapplication raises doubts about conclusions that methoprene is “capable of producing deleterious effects on the blue crab *at environmental concentrations* [emphasis added]” and “adverse effects are produced *at concentrations equal to or less than those considered necessary for mosquito control* [emphasis added]” (Horst and Walker, 1999). In fact, an often-cited early study of the effect of methoprene on the mud crab, *Rhithropanopeus harrisi*, tested three different concentrations of methoprene (1.0, 0.01, and 0.0001 ppm) on crab development and survival. Although 1.0 ppm of methoprene was lethal, concentrations below 0.1 ppm had no effect on crab survival or developmental processes including molting (Costlow, 1977). Another study examined the effect of methoprene on nitrogen fixation and growth by blue-green algae, using both field concentrations and much higher levels (Wurlbaugh and Apperson, 1978). Although high concentrations (500 ppb) had an impact, they found no significant affect at field concentrations (20 ppb).

Bircher and Ruber (1988) measured the toxicity of methoprene to all stages of the salt marsh copepod *Apocyclops spartinus*. Concentrations used were 0.1 to 10.0 ppm. The study found that when concentrations exceeded 0.1 ppm, transient effects on early copepod stages could be observed. It was noted that this concentration was much higher than those used for mosquito control. Methoprene had no impact on cyclopoid copepods.

4.1.4 *Bacillus thuringiensis israelensis*

In unpublished and officially unreleased work (which is nonetheless available on the Internet), Bobinchock and Popovich (1981) conducted a three-month study at the Fire Island National Seashore on the impact of *Bti* on various salt marsh species. They evaluated the impacts of *Bti* applications on the following non-target species: grass shrimp (*Hypolyte zostericola*), fiddler crabs (*Uca pugnax*), hermit crabs (*Pagurus longicarpus*), killifish (*Fundulus* sp.), water boatman (*Trichocorixa reticulata*), and backskimmers (*Notonecta undulate*). All species were adults, with the exception of grass shrimp, where juveniles were also assessed. The authors reported no mortality associated with *Bti* treatment for the killifish, fiddler crabs, water boatman, and backskimmers. Results for the grass shrimp and hermit crabs were inconclusive as significant mortality also occurred in the controls.

Back *et al.*, (1985) treated a Quebec stream with high doses of *Bti* in an effort to assess its efficacy against black fly larvae and its impact on non-target insects. *Bti* was highly efficacious when used against black fly larvae, but uncovered no evidence of impacts on non-target insects. They concluded, “considering the high dosage used in this experiment and the fact that the main targets of *Bti* formulations are two Nematocera families (Simuliidae and Culicidae), one may conclude that Teknar [*Bti*] is a highly selective larvicide.”

Although Charbonneau (1991) did not find a direct adverse impact of *Bti* on chironomids (midges), research by others was cited that did demonstrate significant mortality of chironomids subjected in the laboratory and in the field to *Bti*. Chironomid mortality is significant as it is an important food source for mallard ducks, suggesting a decrease in chironomids could impact waterfowl recruitment from a wetland. The Scientific Peer Review Panel of the Metropolitan Mosquito Control District (1996) found *Bti* applications caused impacts to invertebrate species richness. Reference site species richness doubled from six to 12 over the five years, due to increased Diptera (aquatic fly) taxa, mainly chironomids. However, richness stayed at about six taxa per sample in *Bti*-treated wetlands. No impact on benthic invertebrates was found in the first year of *Bti* wetland application, but significant impacts, particularly to benthic midges and other primitive flies in the second and third years of treatment. This may have been related to weather patterns (see Section 4.1.3).

A simpler analysis of the potential for *Bti* impacts to chironomids at Prince Edward Island streams (the treatments were for black fly control) found no decrease in numbers overall, although applications reduced the non-target insects at one site (they increased at the other site) (McCracken and Matthews, 1997). Similarly, a Delaware study of several mosquito control options (temephos, *Bti*, and xanthan gum) found that *Bti* use did not reduce chironomid larvae compared to control locations (Laskowski *et al.*, 1999).

As discussed above, USFWS conducted a general review of the impacts of larvicides, including *Bti*, in 1998, and found that there is a potential for ecological impacts from larvicide use, but did not clearly determine that impacts would be expected (Brown, 1998). Related to the literature search was field work conducted at four East Coast refuges. As reported above, differences in community structure were found between treated and untreated sites, but the effects could not be definitively traced to larvicide use, or in particular, to *Bti* applications (USFWS, 2000).

Although, as reported above, the diversity index for Long Island sites varied between treated and untreated sites, no firm conclusions could be drawn based on the limited sample sizes (M. Maghini, LI Complex, USFWS, personal communication, 2003).

USEPA (1998) reported on the potential impacts to non-target terrestrial and aquatic invertebrates and cited a reduction in the number of adult and larval lepidoptera the year of spray and some reduction extending into the following year due to reduction of larvae the previous year for undifferentiated *Bacillus thuringiensis*. *Bti*, however, in particular was found not to affect overall arthropod abundance, including beetles, sucking insects such as aphids, leafhoppers, or cicadas and spiders. The re-registration eligibility decision (RED) also reported that *Bti* has no appreciable effect on aquatic invertebrates. Field studies found no adverse affect on the abundance or composition of benthic organisms, or on the immature or adult stages of mayflies, caddisflies, dragonflies, damselflies, beetles, midges, and dobsonflies. The report did suggest that *Bti* might impact dipterans other than mosquitoes.

A study by Su and Mulla (1999) found that *Bti* and Bs suppressed the growth of two species of unialgae, *Closterium* and *Chlorella*. Reduced growth of these algae led to lower photosynthesis and hence lower dissolved oxygen as well as decreased turbidity. No explanation was suggested for the observed effects.

4.1.5 Malathion

The National Oceanographic and Atmospheric Administration (NOAA) conducted an inventory of national agricultural pesticide use in coastal areas. Malathion was identified (in 1992) as the most widely used adult mosquito control agent in the nation (Pait *et al.*, 1992). In a related report, Lowe *et al.* (1991) found that particular pesticides might be linked to certain fish kill events – approximately one percent of all fish kills. Malathion and endosulfan were said to caused approximately 64 percent of these (28 of total of over 3600 fish kills). Although the exact number was not specified, most of the malathion-linked fish kills were said to have been from non-agricultural uses of the pesticide, with mosquito control being cited as the exemplar of non-agricultural applications.

4.2 Pesticides and Stress

Research has shown that there are synergistic effects of pesticides and predatory stress on certain species, such as frogs. Relyea (2003) studied the effects of malathion on the survival of six tadpole species in the presence of predatory stress. He found that tadpoles exposed to malathion in the presence of a predator exhibited significantly greater mortality than controls without a predator.

Other factors can add further stress to the organisms. Two examples of indirect pesticide impacts to organisms such as frogs include the immigration of competitors from non-pesticide treated areas, or the presence of competitors that are resistant or otherwise unaffected by the pesticide (Leeuwangh, 1994).

An early study of the effect of methoprene on the mud crab, *Rhithropanopeus harrisi*, Costlow (1977) reported that 0.01 and 0.0001 ppm of methoprene affected crab development and survival, but only when combined with salinity stress. Crab larvae reared at five parts per thousand (ppt) salinity experienced reduced survival over those reared at 20 ppt and 35 ppt. Some abnormal development was observed at 35 ppt but not at the optimal salinity of 20 ppt. Costlow attributed the different responses to the combination of methoprene exposure and salinity induced stress. In a follow-up study by Costlow and colleagues (Christiansen *et al.* 1977), the percentage of abnormal megalopa was higher under salinity and temperature stress than under optimal conditions in the presence of methoprene. Survival was unaffected. The duration of zoeal development was lengthened with an increase in methoprene concentration under stress conditions.

4.3 Keystone Species and Generalists

Keystone species are those on which the persistence of a large number of other species in the ecosystem depends and whose impacts on an ecosystem or community are greater than would be expected from its relative abundance (Paine, 1974). Keystone species are usually identified when their removal from an ecosystem results in changes to the rest of community. Protecting keystone species is therefore a priority for conservationists and resource managers. Keystone species have been documented in a wide range of terrestrial ecosystems and are represented by a wide range of organisms, from plants to carnivores and detritivores (Bagheera and ESNB, 2000).

However, Jennings and Kaiser (1998) concluded that keystone species are rare in coastal systems, as most predators tend to consume a wide variety of prey items. This appears to be the case for most major mosquito predators, such as fish and dragonflies, which are generalists consuming other species when mosquitoes are not abundant. General ecological theory thus suggests that the removal of mosquitoes from an ecosystem is unlikely to impact a keystone species.

4.4 Indicator Species

The use of one or two species each from salt marsh and freshwater ecosystems as indicators of ecosystem response to pesticides may be useful for routine pesticide monitoring. In a study by Brown *et al.* (2000), freshwater shrimp were identified as an appropriate indicator species for the evaluation of the toxic effects of insecticides applied to freshwater habitats for mosquito control. The shrimp was selected because it occurs throughout Australia in a variety of vegetated aquatic habitats and because it is an important food source for fish. Some US researchers are also looking toward shrimp as an indicator species. The Marine Ecotoxicology Branch of the Center for Coastal Environmental Health and Biomolecular Research examines the toxicological and ecological impacts of contaminants on marine and estuarine ecosystems. They have utilized shrimp (*Palaemonetes pugio*), hard clams (*Mercenaria mercenaria*), oysters (*Crassostrea virginica*), and fiddler crabs (*Uca pugilator*) for estuarine contaminant studies. Shrimp are important prey species for estuarine fish and bivalves for some coastal bird species.

The NOAA Status and Trends program (Mussel Watch) since 1986 has monitored chemical contaminants in bivalves such as oysters and mussels. They are sampled biennially at over 280 coastal and estuarine sites; in addition, sediments are sampled once every decade. In the northeast and middle Atlantic, tissue contaminant concentrations are measured in Eastern oysters (*Crassostrea virginica*) and blue mussels (*Mytilus edulis*). Mussel Watch tests for persistent environmental contaminants. Bivalves are the organisms of choice because they are sessile filter feeders, and it has been shown that many contaminants accumulate in bivalve tissue. Because modern mosquito control pesticides are not classified as environmentally persistent, they are not sampled for in this program.

It should be noted that modern mosquito control pesticides, malathion degradates, and piperonyl butoxide (PBO, a synergist added to pyrethroids) are included in organic compound testing conducted by Suffolk County Department of Health Services, for groundwater and estuarine studies. The County has yet to detect these compounds in an aqueous sample. USGS and Stony Brook University researchers have measured these compounds, usually at extremely low concentrations (as low as parts per quadrillion), but only in the immediate wake of a spray event (although PBO has proven to be detectable in the water column up to a week after a pyrethroid application) (Brownawell et al., 2005).

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