SANTA CRUZ COUNTY MOSQUITO AND VECTOR CONTROL CSA 53

DETAILED TECHNICAL REVIEW OF THE INTEGRATED PEST MANAGEMENT PROGRAM

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SANTA CRUZ COUNTY MOSQUITO AND VECTOR CONTROL
CALIFORNIA ENVIRONMENTAL QUALITY ACT

TECHNICAL REVIEW

SECTION 1. INTRODUCTION

A. FOCUS OF THE EXISTING INTEGRATED VECTOR PEST MANAGEMENT PROGRAM (IVMP)

The principal purpose of the Santa Cruz County Mosquito and Vector Control (**MVC**) activities is to provide an Integrated Pest Management Program (**IVMP**) to meet human, domestic animal and wildlife needs for protection from the nuisance and disease that may be spread by vector insects and animals. The focus is on preventive methods because integrated, preventive activities can avoid the more reactive emergency measures that can be necessary if vector-borne disease and infestations go unchecked in early stages.

Manipulation and control of vectors must be based on careful surveillance of their abundance, habitat (potential abundance), pathogen load, and/or potential contact with people; the establishment of treatment criteria (thresholds), (Section 6.G.; Attachment 4); and appropriate selection from a wide range of control methods. This dynamic combination of surveillance, treatment criteria, and use of multiple control activities in a coordinated program is what is generally known as Integrated Pest Management (IPM) (Glass 1975, Davis et al 1979, Borror et al 1981, Durso 1996, Robinson 1996).

At the MVC, emphasis is squarely on prevention and intervention at the lowest level necessary to provide this service. Responsible integrated programs use the less targeted controls, such as adulticide spraying, only when more targeted controls, such as source reduction and larviciding have failed to adequately reduce vector populations. Likewise, in an IVMP, narrow spectrum larvicides (e.g., biological toxins, insect growth regulators) are preferred over broader-spectrum larvicides.

MVC has been recognized by the Environmental Protection Agency in its Pesticide Environmental Stewardship Program for using scientifically collected information to narrow the target of vector control. For example: not all mosquitoes are pestiferous. Targeting is achieved by identifying and controlling only the mosquitoes that are nuisance and disease vectors, and only when the population exceeds certain levels of abundance. The goal of the MVC is to choose the control tactic for the situation that is most effective while having the least environmental impact.

Vector control activities that may affect wildlife include the application of mosquito larvicides and adulticides, the manipulation of water sources to reduce their potential for producing mosquitoes, for example by reducing vegetation that provides cover and the introduction of non-native mosquito larvae eating fish (*Gambusia affinis*). If these activities were to cause mortality, reduce food supplies, or affect habitat availability or suitability for wildlife they could negatively impact some wildlife populations. Conversely, vector control activities benefit some wildlife species and populations by reducing vector-borne disease transmission within and among wildlife populations. For example: areas covered and targeted by the MVC are the same areas where wildlife is at risk from West Nile virus.

For each activity in the IVMP there is existing information, both in the experience of MVC staff and in published literature, on the specific implementation, mode of action, efficacy, cost-effectiveness, safety, and potential environmental impacts. The reader is directed to the attached bibliography, and especially to the publications of the MVC, the American Mosquito Control Association (AMCA), and the Mosquito & Vector Control Association of

California (MVCAC), for general discussions of mosquito control or for detailed information on the rationale, modes of action, and specific application of each vector surveillance and control technique. This Technical Review, only briefly summarizes general information on mosquito and vector control methods and focuses on the potential environmental impacts of those mosquito surveillance and control activities currently or potentially to be used by the MVC in the County wide area.

Note: In the interest of precision and clarity, both the common and scientific names are initially presented for organisms discussed in this report. In subsequent references the more well-known names will be used consistently. Tables of common and scientific names are compiled for mosquitoes and Special Status Species in Appendices B and D.

B. NEED FOR EXPANSION OF THE VECTOR CONTROL PROGRAM

The project is placement of a measure on the ballot that proposes to expand the area in which County Service Area #53 (also known as Mosquito and Vector Control, MVC) conducts vector control activities. If approved, the service area will expand from the current 70 square miles located in the south portion of the unincorporated area of the County including the City of Watsonville to the entire County of Santa Cruz, a 446 square mile area. The expanded area will include the three incorporated cities not currently being served (Capitola, Santa Cruz and Scotts Valley), which currently do not receive any protection from vectors or vector-borne disease.

The MVC was established by Board resolution in 1993 through CA Government Code (25210) in response to many years of public demand for relief from pestiferous mosquitoes. Currently, the program's mosquito control operations are geographically limited to activities in two "zones of benefit" which lie within the Aptos and Pajaro Supervisorial Districts (population 85,000). Because demand for services has since become countywide, and the threat of vector-borne disease has increased, the County is proposing the expansion of mosquito and other vector control services. Over the last few years, about 60% of calls for service have been from residents outside of our current service area.

A public opinion survey was conducted to determine public interest, willingness of property owners to pay for services, and predict the success of a ballot measure required by law. The poll was supportive of expanding services and boundaries. With the approval of the Santa Cruz County Board of Supervisors, the MVC is pursuing possible annexation of cities and services to unserved areas.

In response to inquiries made of the unserved cities as to interest in services, the Cities of Capitola, Scotts Valley and Santa Cruz have approved Resolutions of Inclusion for annexation, contingent on the County adopting a Resolution of Intention to expand the service area and initiate formation procedures for unserved areas, which would require property owner approval.

C. VECTORS ADDRESSED BY THE PROGRAM

The California Health and Safety Code (Division 3, Chapter 1) defines a vector as "any animal capable of transmitting the causative agent of human disease or capable of producing human discomfort or injury, including, but not limited to, mosquitoes, flies, mites, ticks, other arthropods, and rodents and other vertebrates" (Section 2002). The MVC undertakes activities through its Integrated Vector Management Program to control mosquitoes, biting flies, and other insects as vectors of disease and/or discomfort in the Service Area.

1. Mosquitoes

Sixteen species of mosquitoes frequently occur within Santa Cruz County and their control is the primary focus of the MVC activities. Attachment 4 lists some of the more important species known to occur within the MVC Service Area. The reader is referred to the publications by Bohart and Washino (1978), and Meyer and Durso (1993) for detailed information concerning the biology, ecology, and diseases vectored by these mosquitoes. In addition to these species, the MVC maintains surveillance for imported mosquito species that may have the potential to establish themselves in the Monterey Bay Area.

Certain species of mosquitoes found in Santa Cruz County can transmit malaria, St. Louis encephalitis, western equine encephalitis, West Nile virus (WNV), and potentially other viruses to humans. West Nile virus is also a threat to wildlife, primarily birds, and contributed to tens of thousands of wild bird deaths in California in 2004, including special status species. A few species of mosquitoes are also capable of transmitting dog heartworm and mixamatosis to both domestic and wild animals. Although some species of mosquitoes have not been shown to transmit disease, most species can cause human discomfort when the female mosquito bites to obtain blood. Reactions range from irritation in the area of the bite to severe allergic reactions or secondary infections resulting from scratching the irritated area. Additionally, an abundance of mosquitoes can cause economic losses, and loss of use or enjoyment of recreational, agricultural, or industrial areas.

2. Yellowjackets

Three species of pestiferous yellowjackets (*Vespula pensylvanica*, *V. vulgaris* and *Dolichovespula arenaria*) occur within Santa Cruz County. Yellowjackets are controlled at the request of public agencies when they are an immediate hazard on public property. Control is generally accomplished through application of non-persistent pesticides directly to the nest.

Currently, yellowjackets are not known to be vectors of any transmittable diseases, however, yellowjackets are still economically and medically significant. Yellowjackets have a painful sting, can fly moderate distances, and are found throughout the Project Area. Economic impact may include loss of use of public recreational areas, or as an occupational hazard to certain types of urban, suburban and rural employees. More significantly, yellowjacket stings can result in anaphylactic shock and rapid death for the approximately 0.5% of the public with severe allergies. Some individuals are allergic to all bee and wasp stings. Normal reaction to a bee or wasp sting includes redness, itching, swelling, and pain at the site of the sting. Allergic reactions may include swelling of an entire extremity, abdominal cramps, vomiting, diarrhea, upper respiratory distress, and constriction of the throat and chest.

3. Africanized Honey Bees

Africanized honeybees (AHB) were first detected in California on October 24, 1994, and have migrated up through the State and are now established as far north as Kern and San Luis Obispo Counties. AHB are not currently present in Santa Cruz County but may become established in the future. The County Agriculture Commissioner has determined that the Santa Cruz County Mosquito and Vector Control shall serve as the designated swarm removal agency if and when these bees arrive in this area. AHB are not known to be disease vectors and are no more venomous than European honeybees (EHB). However, AHB respond to threats more rapidly than EHB and will defend their hive with greater numbers of bees, resulting in a massive number of stings to an individual. Although individuals have died as a result of 100 - 300 stings, it is estimated that the average lethal dose of venom for an adult human is 1,100 bee stings; for a child or pet it can be substantially less. Bee stings, like yellow jacket stings, can result in anaphylactic shock and death within 15 to 30 minutes for the approximately 0.5% of the public with severe allergies.

4. Other Animals Of Importance

Although certain animal species such as rodents, flies, fleas and ticks will not be regularly controlled, these animals play important roles in the transmission of plague, cholera, amebic dysentery, typhoid fever, salmonellosis, anthrax, infectious hepatitis, murine typhus, hantavirus, Lyme disease, babesiosis, anaplasmosis or ehrlichiosis, and may be surveyed for diseases. The MVC routinely provides education and consulting services to the public about disease risk associated with these vectors and appropriate measures to protect human health.

Most of the vectors mentioned above are extremely mobile and cause the greatest hazard or discomfort away from their breeding site. Each of these potential vectors has a unique life cycle and occupies a different habitat. In order to effectively control these vectors, an integrated vector management program must be employed. MVC policy is to identify those species that are potential vectors, to recommend techniques for their prevention and control, and to anticipate and minimize any new interactions between vectors and humans.

D. PROGRAM ACTIVITIES

The MVC's Integrated Vector Management Program consists of six general types of coordinated activities:

- **Surveillance** for mosquito populations, mosquito habitats, disease pathogens, and public distress associated with mosquitoes. Mosquito surveillance activities include field counting, trapping, and laboratory analysis of mosquitoes, alternative hosts, and pathogens to evaluate populations and disease threats; field inspection of known or suspected mosquito habitats; maintenance of paths and the potential use of all-terrain vehicles to access mosquito habitat; analysis of public service requests and surveys; and other data collection methods.

- **Public Education** to encourage and assist reduction and prevention of mosquito and other vector habitats on private and public property.

- **Vegetation Management.** The MVC uses hand tools as a means of pruning vegetation along existing trails to allow access to mosquito breeding sites for surveillance and control. The MVC also uses herbicide (Roundup® and Aquamaster®) to keep trails free of poison oak and to reduce noxious, invasive or exotic plants in ponds.

- **Biological Control.** Application of the "mosquito fish" *Gambusia affinis*, the bacteria *Bacillus sphaericus* (Bs), and *Bacillus thuringiensis israelensis (Bti)*, predators and pathogens of mosquitoes, is known as "Biological Control." *Gambusia affinis*, is used only in ornamental ponds and other artificial settings. *Bacillus sphaericus* reproduce within and upon mosquitoes, for at least some time, after release. *Bacillus thuringiensis israelensis* materials applied by the MVC contain only spores made up of specific protein molecules produced by the *Bti* organism and not live bacteria. Because the potential environmental impacts of *Bs* and *Bti* application are generally similar to those of chemical pesticide applications, these materials are evaluated below under Chemical Control.

- **Chemical Control.** In the context of the MVC's IVMP "Chemical Control" is the application of insecticides to directly reduce populations of larval and adult mosquitoes and other insect vectors. (Note: Adulticides, pesticides that target adult mosquitoes, have been used only on one occasion in the ten year history of the MVC, to provide relief at a single site during the first year of the existence of the MVC. Further discussion of adulticides appears in sections 1.F. and 6.D.

While these activities are all elements of the IVMP, it is important to note that the specific activities performed by MVC staff vary considerably. Daily and regional activities fluctuate in response to mosquito species, activity, population size, densities, age, structure, species and spatial distributions, season, climate, vector potential, proximity to human populations, access by MVC staff to mosquito habitat, abundance of natural predators, availability and cost of control methods, effectiveness of previous control efforts at the site, potential resistance developments in mosquito populations, land-owner policies or concerns, proximity to special status species, existence of Endangered Species Recovery Plans, Habitat Conservation Plans, and local community concerns. Therefore, the specific actions taken in response to current or potential mosquito activity in any specific place and time depends on factors of mosquito and pathogen biology, biotic and abiotic environmental factors, human settlement patterns, local standards, available control methods, and institutional and legal constraints.

The IVMP of the MVC is regularly reviewed, in part or entirety by the MVC, the Agricultural Commissioner, the Department of Health Services, the Mosquito and Vector Control Association of California, the County's IPM Departmental Advisory Group and the County Grand Jury to ensure that its practices are effective, economical, safe, environmentally sensitive, and responsive to the needs of the public. The MVC periodically incorporates new materials, methods and treatment criteria. Over the 11-year history of the MVC these changes have generally reduced the potential environmental impacts of our activities through the introduction of more selective materials or more subtle or sophisticated interactions with existing ecological and hydraulic processes. Although the MVC does not foresee adoption of any new general project elements beyond those listed above, it is likely that specific activities and policies discussed below will evolve, and it is certain that their intensity of application will continue to vary from site to site and from year to year. However, because the modes of action and the intensity of proposed activities are similar to those in current use, the potential environmental impacts of the program as a whole should not increase as a result of these changes. Where substantially new materials or methods are proposed for routine operational use, the MVC will update the environmental review.

E. GENERAL VECTOR MANAGEMENT STRATEGY

Mosquito control activities implemented by the MVC primarily involve the application of larvicides on bodies of standing water and management of water sources in an effort to reduce their ability to produce large numbers of mosquitoes. Aerial or ground applications of adulticides, which have a greater potential for impacting humans and wildlife than larvicide treatments do, are restricted to circumstances where a health emergency exists. Adulticides, pesticides that target adult mosquitoes, were used during only one period in the ten year history of the MVC, during the first year of the existence of the District. Further discussion of adulticides appears in sections 1.F. and 6.D.

The MVC's IVMP, like any other IPM program, by definition involves procedures for minimizing potential environmental impacts. The MVC employs IPM principles by first determining the species and abundance of vectors through evaluation of public service requests and field surveys of immature and adult pest populations; and then, if the populations exceed predetermined criteria, using the most efficient, effective, and environmentally sensitive means of control. For all vector species, public education is an important control strategy, and for some vectors (rodents, ticks, fleas) it is the MVC's only control method. In some situations, the MVC also uses biological control such as the planting of mosquitofish in ornamental ponds. When these approaches are not effective or are otherwise inappropriate, pesticides are used to treat specific vector-producing or vector-harboring areas or vector populations.

1. <u>Mosquitoes</u>

Control of mosquitoes is the primary focus of the MVC's activities. In order to maximize familiarity by the operational staff with specific mosquito sources in the Project area, the MVC is divided into zones. Zones range from 30 to 50 square miles in area and each is assigned a full-time Mosquito Control Specialist. Each technician is responsible for inspection and treatment of known mosquito sources, finding and controlling new sources, and responding to service requests from the public within that zone. The zone concept will be extended if the MVC expands to cover the entire County.

Mosquito control activities are conducted at a wide variety of sites. Examples of natural sites in the Project area include marshes, lakes and ponds, streams, sloughs, seasonal wetlands. Examples of artificially created sites include stormwater detention basins, flood control channels, spreading grounds, street drains and gutters, wash drains, roadside ditches, animal troughs, tire piles, fountains, ornamental fish ponds, swimming pools and liquid waste detention ponds.

2. Other Vectors

As described in the Preliminary Review, the MVC's activities also address the management of yellowjackets, Africanized Honeybees, and other vector species. The MVC's IVMP principles for mosquitoes apply similarly to other vectors, including assessing threat to surrounding organisms, pesticide use in strict accordance with label requirements, eradication of breeding sources and educating the public.

F. EMERGENCY ACTIVITIES

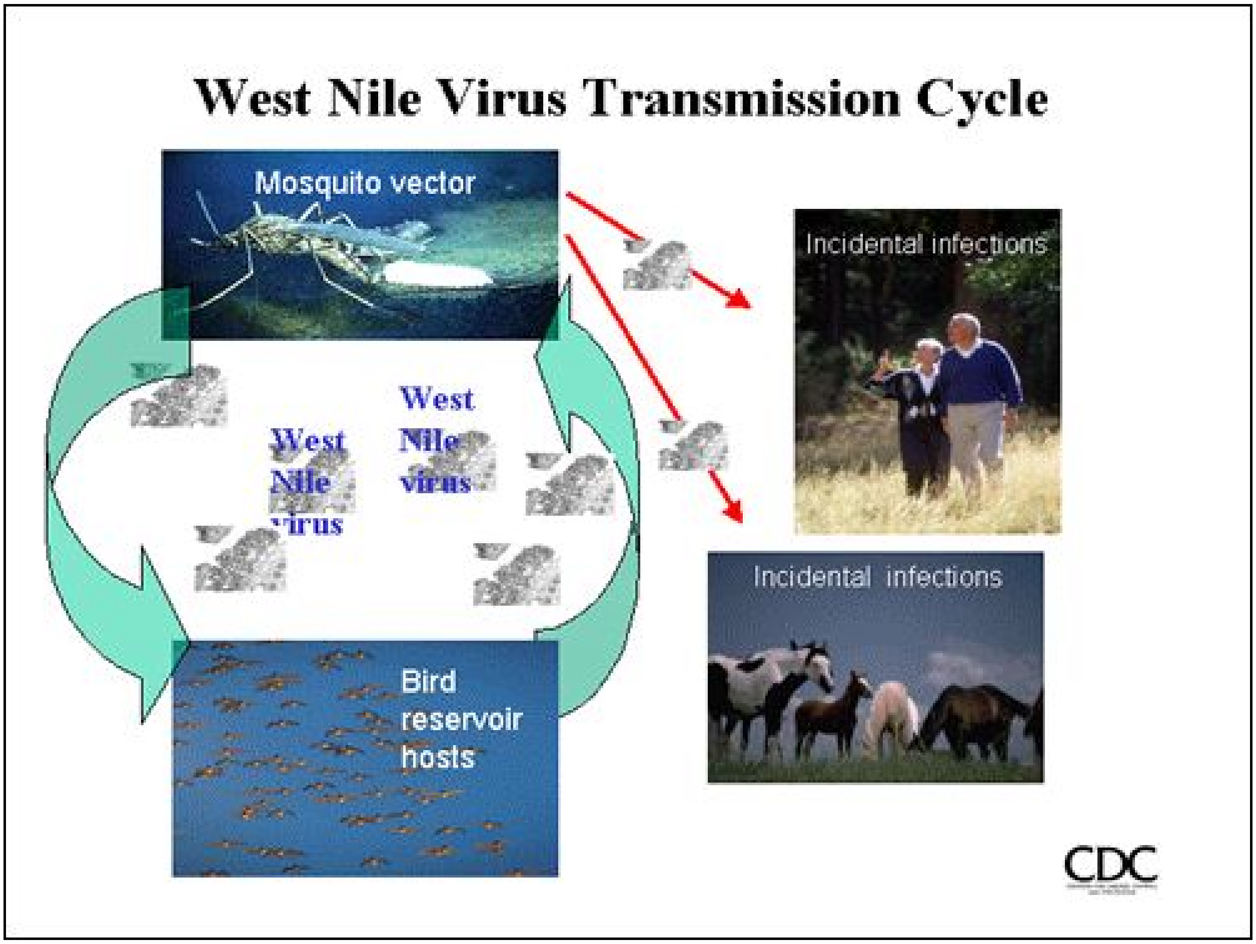
In the event of emergency conditions (actual or imminent disease outbreak), MVC actions temporarily vary from the routine operational actions through increases in scope or intensity and potentially through use of legal pesticides that target adult mosquitoes, in strict conformance with label requirements. There is a statewide structure in place to respond to outbreaks of West Nile Virus and other vector borne diseases which will in part dictate response at the local level. The MVC will seek County Board of Supervisors' approval before application should the necessity arise to conduct aerosol spraying of adulticides.

The Santa Cruz County West Nile Virus Technical Advisory Committee (WNV-TAC) has developed a 3-tiered Arbovirus Surveillance and Response Plan (Attachment 7) approved by the County Board of Supervisors, that calls for increasing levels of vector control as the threat of WNV transmission to humans increases. It is based on documents created by the California Department of Health Services (DHS) ("California Mosquito-Borne Virus Surveillance and Response Plan", Kramer, 2004). During a normal mosquito season when there is no evidence of active virus transmission (Level 1), larvicidal compounds are typically applied to standing bodies of water when surveillance indicates that mosquito larvae and pupae have reached some predetermined threshold. (section 6.G.)

Larvicides are used most effectively early in the mosquito season before large numbers of adult mosquitoes are present. In contrast, adulticide use will increase statewide if WNV transmission to people is actively occurring and when there is an immediate need to reduce mosquito numbers, particularly in areas where there is no larval control programs in place. This situation already occurred in California in summer 2004. As mentioned, appropriate and timely use of larvicides and/or other source reduction or water management measures reduces the adult mosquito population, the number of infected adult mosquitoes and infected birds, the possibility of human and horse infections, and therefore the need for reactive measures such as adulticiding. Because the MVC undertakes a regular program of larvicide the potential that adulticides will be necessary is reduced.

> It is important to note that adulticides are currently used for vector control elsewhere in California on a limited basis, and the use of adulticides will increase statewide wherever the WNV response is elevated by DHS to Level 2 (Emergency Planning Conditions) or Level 3 (Epidemic Conditions). Adulticides pose a comparatively greater threat to wildlife than larvacides since they are more toxic to both target and non-target species (Their, 2001; Logomasini, 2004). Adulticides may be applied in urban or suburban areas to target mosquitoes near people, or they may be applied in dispersal corridors or near wetlands to reduce adult mosquito populations near their source. Adulticides will only be used by the MVC if there is a public health emergency caused by actual cases of human vector borne disease such as West Nile Virus (WNV), the California Department of Health Services or the County Health Officer declares a Level 3 disease outbreak (epidemic conditions), and there is specific, prior approval by the County Board of Supervisors. Adulticides that might be used by the MVC are Pyrethrins (Pyrenone 25-5) and the synthetic pyrethroids Resmethrin and Permethrin.

Detailed information on adulticides and larvicides is available at http://npic.orst.edu/wnv/pesticideinfo.htm#mosqmanage, and pesticide use information for California is available at www.cdpr.ca.gov/docs/westnile/.



SECTION 2. PROGRAM/PROJECT SETTING

A. INTRODUCTION

The MVC's activities are now conducted within a 70 square mile jurisdiction within Santa Cruz County, California. Following the proposed annexation of territory the MVC's activities will be conducted within the entire County of Santa Cruz, an area of 446 square miles, including the incorporated cities of Watsonville, Santa Cruz, Capitola and Scotts Valley.

In addition, the MVC periodically cooperates with adjoining Mosquito & Vector Control District's and/or County and State Health Departments on activities that cross normal MVC boundaries; in these situations, the District or Department with jurisdiction over the locations where specific activities are performed has primary responsibility for these activities.

Mosquito control activities are associated with wet areas of all types and sizes. Because of the diversity of mosquito habitat, mosquito control activities are conducted in a wide variety of ecosystems and habitat types. These include marshes, ponds, lakes, creeks, sloughs and seasonal wetlands, wastewater ponds, storm-water detention basins, ditches, ornamental fishponds, impound areas, etc., as well as individual homes or commercial buildings. Other vectors, such as yellowjackets, inhabit an even wider range of natural and artificial habitats.

Mosquito control sites can be roughly divided into those where activities may have an effect on the natural environment either directly or indirectly through drainage to off-site areas ("Environmental Sites"), and sites where the potential environmental impacts are negligible ("Non-Environmental Sites"). Examples of "Environmental Sites" in the Project area include marshes, lakes and ponds, streams, sloughs, seasonal wetlands, stormwater detention basins, flood control channels, spreading grounds, street drains and gutters, wash drains, and roadside ditches. Examples of "Non-Environmental Sites" include animal troughs, artificial containers, tire piles, fountains, ornamental fishponds, swimming pools and liquid waste detention ponds.

B. URBAN AND DEVELOPED AREAS

The MVC services developed areas with artificial sources, including fishponds, stormwater detention basins, street gutters and drains, and swimming pools. The MVC's activities in these areas have little or no impact on sensitive wildlife or vegetation as long as mosquito fish planted in these sources cannot escape into natural areas and residual levels of biologic and chemical agents in drainage that reaches lagoons and the ocean are low.

C. WETLANDS AND OTHER SENSITIVE HABITATS

A number of specific habitat types that are considered "sensitive" are found within the existing and proposed MVC Service Area. This includes seasonal wetlands like the Ellicott Slough National Wildlife Refuge and Ellicott Slough State Ecological Reserve, Pajaro Lagoon, and others, and permanent wetlands such as Scott Creek and Waddell Creek marshes. In addition, riparian corridors are considered "sensitive habitats" by the County and state and federal resource agencies. Specific vector surveillance and control methods that are used in these areas are consistent with published management plans and MVC policies, defined throughout this report, to ensure their protection.

The MVC maintains detailed maps and databases of all areas where mosquito production takes place in the Service Areas (the "Source List"), and the location ("Source Number") is recorded for all surveillance and control activities. Therefore, the MVC has a detailed long-term database that allows evaluation of the intensity of control efforts and their relationship to specific wetland or riparian sites. This database and spatial data will be extended to cover the new service area if the MVC service area is expanded.

D. ENDANGERED AND OTHER SPECIAL STATUS SPECIES

The California Department of Fish and Game's (DFG) Natural Diversity Database (NDDB) lists 112 special status species[1] in Santa Cruz County (Attachment 6). DFG list of Fully Protected Animals, California Species of Special Concern, and other lists were also consulted. Many of the special status species are associated with specific locations where the MVC does not perform routine (non-emergency) operations.

Of the twenty-eight animals listed as "Endangered" under either the Federal or State Endangered Species Acts (ESA), five occur in habitats where the MVC has routine operations. These include the Santa Cruz Long-Toed Salamander (*Ambystoma macrodactylum croceum*), which is also a DFG Fully Protected Animal, the Tidewater Goby (*Eucyclogocius mykiss irideus),* the Coho Salmon (*Oncorhynchus kisutch*), the Least Bell's vireo (*Vireo belli pusillus*), the California Clapper Rail (*Rallus longirostris obsoletus*), which is also a DFG Fully Protected Animal. Habitat descriptions and current CNDDB maps of distribution and potential habitat of all endangered species in the Service Area are maintained by the MVC and incorporated into the operational guidelines of field personnel.

In addition to endangered species the NDDB shows additional taxa that are listed as "Threatened", "Rare" or are Species of Special Concern. This listing indicates that the species or subspecies is vulnerable to decline to endangered levels, and habitat loss is listed as the primary threat for each of these species. Of these, the South/Central Coast Steelhead trout (*Oncorhynchus mykiss irideus*), the California red-legged frog (*Rana aurora draytonii*), the California tiger salamander (*Ambystoma californiense*), the Southwestern pond turtle (*Clemmys marmorata*), the Western snowy *plover (Charadrius alexandrinus nivosus*), the bank swallow (*Riparia riparia*), marsh hawk *(Circus cyaneus*), and the California black rail (*Laterallus jamaicensis coturniculus*), which is also a DFG Fully Protected Animal, have habitat that might overlap in areas of routine MVC activity. Habitat descriptions and current CNDDB maps of distribution of all threatened species in the Service Area are maintained by the MVC and incorporated into the operational guidelines of field personnel.

The listed species that are most likely to intersect with MVC activities are those in one of the following habitat types: tidal marshlands, seasonal and permanent wetlands, and riparian zones.

1. Tidal Marshlands

Special Status Species in the MVC's tidal marshlands are Tidewater goby, California clapper rail, Steelhead trout, Coho salmon, California black rail and the Western snowy plover; as well as the marsh hawk.

2. Riparian Zones

Special Status Species in riparian zones in the MVC Service Area are Tidewater goby, Least Bell's vireo, Coho salmon; Steelhead trout, California red legged frog; Southwestern pond turtle, and foothill yellow legged frog.

3. Seasonal Wetlands

Special Status Species in seasonal wetland zones in the MVC Service Area are Santa Cruz long toed salamander, California tiger salamander, red legged frog, and the marsh hawk.

4. Permanent wetlands

Special Status Species in permanent wetland zones in the MVC Service Area are Santa Cruz long toed salamander, red legged frog, and the marsh hawk.

[1]Under the Federal and California Endangered Species Acts, the word "species" may also mean subspecies or other taxonomic groupings.

E. EFFECT OF MOSQUITO-BORNE VIRUS ON WILDLIFE

The MVC program creates some beneficial effects for wildlife by controlling WNV. In August through October of 2004 thirty-six dead wild birds collected were confirmed positive for WNV by a State laboratory. The birds were collected by the public throughout the County as illustrated in the map in Attachment 9.

A report prepared by the UC Davis Wildlife Health Center (June 2004) explored and mapped West Nile virus Transmission and Wildlife Exposure Risk in California. Santa Cruz County is given a medium to very high risk. WNV is transmitted and maintained in the environment primarily in a mosquito-avian cycle, and summer *Culex* species are considered to be the most important vectors of WNV for California wildlife species (Goddard et al., 2002).

The propensity of *Cx. tarsalis* to feed on both birds and mammals indicates that it will transmit the virus among avian hosts, as well as from birds to mammals. *Culex pipiens* transmit WNV less efficiently than *Cx. tarsalis*, but they will play an important role in transmitting WNV in wildlife near urbanized areas since they are common, widespread, and feed on both birds and mammals. Other important species common in Santa Cruz County include *Cx. stigmatosoma* that feeds preferentially on birds, and *Cx. erythrothorax* that is abundant in wetland habitats and feeds on both birds and mammals. The extent to which *Culex* species feed on reptiles and amphibians in California is poorly understood, but WNV transmission from birds to amphibians and reptiles is a possibility.

The population-level impacts of WNV on amphibians, reptiles, and mammals (non-domestic) are largely unknown. WNV exposure among these taxa will occur when they are fed upon by mosquitoes that have acquired WNV by feeding on birds. For reptiles and amphibians, this scenario seems unlikely because the mosquito species that feed on reptiles and amphibians are relatively low in abundance and rarely feed on non-reptile or non-amphibian hosts (Meyer and Durso, 1998). However, it has been speculated that incidental exposure may occur upon predation and consumption of WNV infected prey items.

The U. S. Geological Survey has stated that surveillance activities, public observations, and preliminary analysis of population survey data suggest that mosquito-borne WNV has caused extensive mortality in many avian species, particularly corvids (crows) and raptors (hawks). The virus could devastate flocks of threatened and endangered species, migratory birds, and other wildlife throughout the country.

To date in North America, WNV infection is known to cause death in over 200 species of native and exotic birds and 20 species of mammals (Komar, 2003). Experimental WNV infection caused death mainly in passerines (corvids, house sparrows, common grackles) and ring-billed gulls, but morbidity and mortality were also seen in species from the orders Anseriformes, Falconiformes, Galliformes, Gruiformes and Columbiformes, Psittaciformes, Strigiformes, and Piciformes.

SECTION 3. VECTOR AND DISEASE SURVEILLANCE ACTIVITIES

A. INTRODUCTION

The MVC's responsibility to protect public health and welfare involves monitoring the abundance of vectors, vector habitat, vector-borne pathogens, and interactions between vectors and people over time and space. Collectively, these monitoring activities are termed Vector Surveillance. Vector surveillance provides the MVC with valuable information on what vector species are present or likely to occur, when they occur, where they occur, how many there are, and if they are carrying disease or otherwise affecting humans. Vector surveillance is critical to an Integrated Vector Management Program because the information it provides is evaluated against treatment criteria to decide when and where to institute vector control measures. Equally important is the use of vector surveillance in evaluating the efficacy, cost effectiveness, and environmental impacts of specific vector control actions.

B. MOSQUITO SURVEILLANCE

Detailed descriptions of surveillance activities like those performed by MVC can be found in publications by Service (1993) and Durso (1996).

1. Immature Mosquitoes

Immature mosquito stages include eggs, four larval stages, and pupae. Mosquito control agencies routinely target the larval and pupal stages to preclude an emergence of adults. The abundance of the immature mosquitoes in any "breeding" source is measured through direct sampling with a 250ml dipper, which provides relative local abundance as number of immatures per unit volume or area of the source. This method requires access by field personnel to within about three feet of larval sites at least every two weeks. The spatial patchiness of larvae requires access to multiple locations within each source, rather than to single "bell-weather" stations.

2. Adult Mosquitoes

Mosquito adults, primarily females, are sampled to determine the direct threat posed by their distribution, abundance, species mix, and pathogen status. Direct surveillance is typically accomplished using a variety of traps that are configured to attract mosquitoes to the trap where they are captured by suction and sequestered in an escape-proof net or glass enclosure. Other direct surveillance strategies, less commonly used by the MVC, include landing counts, and artificial resting units (Service 1993, Durso 1996).

Another important measure of adult mosquito abundance is the number and distribution of service requests from the public. In combination with identification of the species causing the disturbance, this information is a powerful technique in identifying previously unknown mosquito sources, or known sources with resurgent mosquito production.

Host-seeking traps

Traps for host-seeking female mosquitoes include standard and modified (e.g. Fay; EVS = "Encephalitis Virus Surveillance") CDC-type portable light traps, which release carbon dioxide (dry ice and/or compressed gas) at a low rate (typically two pounds/night/trap) to attract female mosquitoes seeking blood meals. The number of females collected during each night of trap operation is expressed numerically as the number of females per trap night. Use of these traps requires direct access to the trap site by field personnel on two consecutive days, typically every two weeks. The MVC currently uses 20 of these traps every 2 weeks.

Light traps

Light traps use a source of photo-attraction (typically a 25 watt incandescent bulb) to lure mosquitoes to the trap where they are pulled in by the suction provided by an electric motor/fan combination. Mosquitoes picked up by the suction are directed through a cone to a collection jar where they are killed by a household insecticide[2]. The standard trap of this type used by most mosquito control agencies is the New Jersey Light Trap. This trap is considerably larger and less portable than the host-seeking trap and requires a source of 110v AC to operate. Like the host-seeking trap, the number of females collected during each night of trap operation is expressed as the number of females per trap night. This surveillance method requires one field visit per trap per week, and the MVC typically maintains about eight traps.

C. SURVEILLANCE FOR OTHER VECTORS

[2]A 1 inch piece of Shell No-Pest Strip© (Vapona) per trap, which is properly disposed of when no longer effective.

The MVC conducts surveillance for non-mosquito vectors, primarily indirectly, through analysis of public service requests. Direct surveillance is primarily a research tool for most non-mosquito vectors at this time, and MVC staff has assisted the California Department of Health Services (DHS) in conducting some tick and rodent collections.

D. SURVEILLANCE FOR VECTOR-BORNE DISEASES

1. <u>Arboviral Diseases</u>

The primary mosquito-borne human diseases for which routine surveillance occurs in the service area are known as "arboviruses" (ARthropod-BOrne viruses). The primary reservoir for the pathogens that cause these diseases are wild birds, and humans only become exposed as a consequence of an accidental exposure to the bite of infective mosquitoes. The arboviruses of greatest public health concern in California are western equine encephalitis virus (WEE), St. Louis encephalitis virus (SLE), and west Nile virus (WNV). Clinical illness caused by WEE is predominately seen in young children while SLE and WNV tend to affect the elderly.

Detecting the presence of these mosquito-borne viruses in nature requires the application of a number of sophisticated methodologies, which are discussed in detail in the recent technical report by Reisen, et al. (1995). Two methods of encephalitis virus surveillance involve 1) capturing and testing female mosquito mosquitoes for the presence of mosquito-borne WNV or encephalitis viruses and 2) periodically testing for the presence of WNV or encephalitis virus specific antibodies in the blood serum of either sentinel chickens or wild birds that are potentially exposed to infective mosquito bites.

2. <u>Malaria And Dog Heartworm</u>

The County Health Services Agency (HSA) has in place a program to track both endemic (contracted from local mosquitoes) and imported malaria cases. Information received from County and State health departments alert the MVC of people diagnosed with malaria. The HSA and MVC then follow up with the patients to identify the source of infection (local or imported) and to ensure that treatment regimes are followed correctly and completely. Known breeding sites of *Anopheles punctipennis* and *Anopheles hermsii*, the two malaria-carrying species, which occur in Santa Cruz County, are resurveyed within two miles of the patient's residence and, if needed, treated. This malaria surveillance program was established to prevent the malaria pathogen *Plasmodium vivax* from becoming established in Santa Cruz County *Anopheles* populations

In addition, dog heartworm is monitored through case reports submitted by veterinarians and by occasional testing of adult Western Treehole Mosquitoes (*Ochlerotatus sierrensis*) for the presence of the filarial worm *Dirofilaria immitis*.

3. <u>Other Diseases</u>

In collaboration with the County Environmental Health Services (EHS) and DHS, testing for the presence of plague, hantavirus, Lyme disease and murine typhus is occasionally conducted by collecting wild rodents, fleas, and ticks. Small animals may be trapped using live traps baited with food. The traps are set in late afternoon and are collected within 24 hours. The animals are anesthetized and blood, tissue, and flea samples are obtained. Special status species that may become trapped will be released immediately and will not be used in these tests.

E. POTENTIAL ENVIRONMENTAL IMPACTS OF SURVEILLANCE

The MVC's surveillance activities require access to mosquito habitat sites; the placement of mosquito traps, and sentinel birds in the field; the collection of mosquitoes in the field; and the direct or instrumental collection of physical data. Each of these activities poses a small potential for disturbance to natural or artificial environments.

<u>Non-invasive Sampling:</u> Non-invasive sampling does not impact the environment directly. Low impact methods include the placement of host-seeking mosquito traps, light traps, artificial resting units (ARUs), and sentinel

chicken flocks. In this situation, existing roads, trails, and clearings are usually utilized to accommodate this type of surveillance activity.

Invasive Sampling: Obtaining samples of immature mosquitoes involves removal of negligible quantities of water. This water may also include non-target organisms associated with the mosquito immatures. Technicians will either make a count of the immatures present or remove a small number for identification at the agency office laboratory, returning the contents of the dipper back into the source once the quantification and identification process is completed. Taking dipper samples also requires the technician to wade into the source and repetitively sample/dip along transects to assess the extent and magnitude of immature mosquito populations. Trampling of some vegetation can occur, but most sampling actions involve either walking the shore line or wading through open water gaps that border emergent vegetation (grasses, tulles, cattails, etc.) where mosquito immatures are most likely to be sampled. Technicians are advised not to penetrate dense vegetation for reasons of safety and unnecessary environmental impact.

At sites that have been identified by the United States Fish and Wildlife Service (USFWS) and/or DFG as sensitive because of listed amphibians, a surveillance protocol has been arranged with the USFWS to minimize impacts (Noda 1996, Barr 2000, Attachment 8). This protocol will be extended to include additional potential RLF and SCLTS habitats if the service area expands. MVC staff avoids entering the water and returns dipper contents to the same place after counting, except for one small mosquito larvae sample for laboratory identification. Staff also disinfects boots and dipper prior to going to these sites. The disinfection protocol conforms to recommendations of the declining amphibian population Task Force (DAPTF-Fieldwork Code of Practice). If the service area is expanded there will be more dip sampling sites that may be breeding locations for special status amphibians. Where these locations have been identified by USFWS, Department of Fish and Game (DFG) staff, or other qualified biologists the surveillance protocol will be followed so that eggs, egg sacs and individuals are unlikely to be disturbed by sampling. In addition, MVC staff meets with DFG biologists to receive training in egg recognition.

Special Use of Birds to Support Encephalitis Virus Surveillance (EVS) Activities: Placement of sentinel chickens is a necessary component of EVS. Therefore, their physical presence is required at sites where virus activity is to be monitored on a routine basis. Sentinel chickens in flocks of approximately ten are sequestered in a coop structure (usually 4'x4'x8' or larger) covered with 1" welded wire to exclude access by resident wildlife with perhaps the exception of mice and other small rodents. The existing coop is surrounded by a chain link run so that the chickens can free range. Feed (various commercially available chicken feeds) and water is housed within the coop enclosure. Manure is removed as needed to reduce fly production.

Access Policy: Technical staff use pre-existing accesses such as walkways, trails, roads, surfaced areas and compacted dirt and agricultural roads to reach sampling locations whenever possible. Occasionally, pruning trees and brush is necessary. This does not occur on a routine basis, and mature trees are not cut. Impacts of pruning and clearing are temporary and localized. When off-road travel is necessary, which occurs approximately 10 times per year in the current service area, this is usually over a fallow agricultural field. In the foreseeable future, but rarely, MVC staff may brush a trail up to 4 feet wide to allow passage of an ATV.

All Terrain Vehicles (ATVs): The MVC does not currently use ATV's but can foresee need for the use of all terrain vehicles to facilitate access into areas that are not accessible by conventional transportation means or by foot. MVC staff would not use ATV's where environmental conditions (e.g., impenetrable vegetation/terrain) can result in causing an accident, personal injury or environmental damage. The MVC would purchase weight-spreading tires for low ground pressure.

SECTION 4. VEGETATION MANAGEMENT

A. PHYSICAL CONTROL OF VEGETATION

The MVC occasionally clears plant matter that prevents access to mosquito breeding sites or that is preventing management practices that would minimize mosquito populations. "Brushing" is usually limited to cutting back weeds, dead limbs and brush. The MVC staff never removes live, mature trees and does not remove vegetation down to bare soil. Trimmed vegetation is broadcast in such a way as to minimize visual degradation of the habitat. Trimming is also kept to a minimum to reduce the possibility of the invasion of exotic species of plants and animals. Follow up surveys are also conducted to verify that the work undertaken was effective and that the physical manipulation of the vegetation did not result in any unintended overall habitat degradation.

B. HERBICIDES

For chemical control of vegetation the MVC uses the herbicides Round Up and Aquamaster, formerly labeled as Rodeo, which are both based on the active ingredient Glyphosate. These are selective materials with very low animal toxicity, and they are applied in strict conformance with label requirements. Roundup is occasionally used along trails, primarily to control growth of poison oak and Himalayan black berry vines that would otherwise prevent access to mosquito breeding habitats. Aquamaster/Rodeo is used only where dense infestations of exotic, invasive aquatic floating or emergent vegetation create harborage for larval mosquitoes of vector significance above the treatment threshold.

The MVC did not apply Round Up, which is labeled for terrestrial application, in the five years from 2000-2004 in Santa Cruz County. The MVC applied 13.5 gallons of Aquamaster or Rodeo over 2000-2004. These are chemically similar to Round Up, but are labeled for aquatic application. Glyphosate is a material that DFG approves for limited use in aquatic environments. The total applications by weights or volumes of pesticides applied by the MVC are shown in the Pesticide Use Table, along with the application rates and the number of acres treated in 2004. Proportionately similar amounts of these substances are expected to be used if the service area is expanded (see Table 1, Attachment 5).

The use of these herbicides is principally at disturbed sites. Furthermore, care is taken to make sure that potential drift is eliminated by using these chemicals only during periods when there is no wind. Application is also timed to maximize effectiveness and reduce the potential for additional applications.

1. Identification And Use

Glyphosate, N-(phosphonomethyl)glycine, is a nonselective, non residual, post emergence herbicide used for the control of pest plants and brush (Shipp et al., 1986; USDA, 1984). Glyphosate is effective against deep-rooted perennial species and against annual and biennial species of grasses, sedges and broadleaf weeds (EPA, 1986). It acts in plants by inhibiting amino acid synthesis. Its physical form is colorless crystals. The two most widely used formulations are Roundup (41% of the isopropylamine salt of glyphosate with surfactants) and Aquamaster, formerly labeled as Rodeo (53% of the isopropylamine salt of glyphosate without surfactants).

The pest plants MVC primarily targets are listed as such in the California Invasive Plant Council list (Cal-IPC List; formerly Cal-EPPC List) more formally known as "Exotic Pest Plants of Greatest Ecological Concern in California". The primary listed plant whose area has been reduced by the MVC is parrot's feather (*Myriophylum aquaticum*), on List B of the Cal-IPC List, described as "Wildland Pest Plants of Lesser Invasiveness; invasive pest plants that spread less rapidly and cause a lesser degree of habitat disruption; may be widespread or regional".

2. Fate And Transport In The Environment

The persistence of glyphosate in the environment is moderate and in soil is dependent on adsorption to soil particles, runoff, and microbial transformation. Complete microbial transformation occurs in soil and water (hydrolysis half-life 35 days). Depending on soil type, a half-life of 3-130 days has been calculated. The lower number is for silty, clay loam and the upper for a sandy loam with little organic matter (USDA, 1984). Glyphosate in soil is resistant to chemical degradation and phototransformation (Shipp et al., 1986). The vapor pressure of glyphosate is negligible; indicating that glyphosate would not volatilize (Hartley and Kidd, 1987).

In aquatic systems, glyphosate adsorbs strongly to organic and mineral matter where it is degraded primarily by microorganisms (Shipp et al., 1986). Its estimated half-life in natural waters is 7-10 weeks (Ghassemi et al, 1981). Glyphosate is relatively non-mobile in the environment due to its rapid and strong absorption to soil particles. The extent of absorption onto soil appears to be related to the clay content of soil and the action-exchange capacity of the soil (Glass, 1987). Absorption is greater in soils saturated with aluminum and iron compared with soils saturated with sodium and calcium. At low application rates, pH does not affect glyphosate binding to soil, while at high application rates glyphosate binding decreases with increasing soil pH (Shipp et al., 1986). The K_{oc} for glyphosate is calculated to be 25.4 ml/gm (Lyman et al, 1982), indicating that glyphosate would be expected to migrate with infiltrating groundwater. However, studies of glyphosate in the environment indicate that is not the case. Glyphosate was not detected in groundwater in California (CDFA, 1989). Data indicate that it is relatively non leachable and has a low tendency for transport in runoff (Shipp et al., 1986).

Glyphosate has virtually no tendency to bioaccumulate in animals (Ghassemi et al., 1981). The United States Department of Agriculture (1984) reports a default BCF of 1 as a conservative indicator of bioconcentration and suggests glyphosate has a low tendency for bioaccumulation.
See also http://www.cdpr.ca.gov/docs/empm/pubs/fatememo/glyphos.pdf

3. Potential For Human And Other Mammalian Toxicity

Ingestion of Roundup by humans has been reported to result in irritation of the mouth, nausea, intestinal discomfort, vomiting and diarrhea. Ingestion of large quantities has been reported to result in hypotension and pulmonary edema (Monsanto, 1989). Dose levels at which these effects were observed were not reported nor is it clear to what extent the surfactant component contributed to these effects. Dermal exposure to a 6.4% aqueous solution of Roundup by volume on human skin did not result in primary irritation or in dermal sensitization.

The acute oral, inhalation and dermal toxicity of glyphosate are low. The acute oral LD_{50} of glyphosate and Roundup in rats ranged from 4,300 - 5,600 mg/kg and in rabbits has been reported at 3,800 mg/kg. A four-hour LC_{50} of 25 mg Roundup/l of air has been reported in rats. In another inhalation study, no evidence of toxicity was observed in rats exposed to 4.89 mg Roundup/l of air. Percutaneous absorption studies indicate that glyphosate is not readily absorbed through the skin. No signs of toxicity have been observed in rabbits following dermal exposure to 5,000 mg/kg of glyphosate or Roundup. Therefore, the acute dermal LD_{50} in rabbits is greater than 5,000 mg/kg for both chemicals. The LD_{50} for dermal exposure to Roundup in rats exceeds 17,600 mg/kg. Glyphosate is nonirritating to the skin, but moderate skin irritation, attributed to the presence of surfactants, has been reported in laboratory animals after exposure to Roundup. The transient ocular irritation has been reported in rabbits exposed to glyphosate and Roundup in standard eye irritation tests (Shipp et al., 1986). No signs of allergic contact dermatitis or dermal sensitization have been reported in guinea pigs exposed to glyphosate.

No treatment-related alterations in clinical chemistry parameters or pathological changes in organs have been observed in rats or mice exposed orally to glyphosate either sub chronically or chronically. Adverse effects have been limited to depressed body weight or altered organ weights. In male rats, no toxicity occurred at 135 mg glyphosate/kg/day, but an increase in the absolute and relative lung weight was reported at 340 mg glyphosate/kg/day. In mice, there were no signs of toxicity reported at 2,305 mg glyphosate/kg/day, but a reduction in body weight gain did occur at 12,225 mg glyphosate/kg/day. Minor nasal irritation has been the only effect observed following sub chronic inhalation exposure of rats to 0.36 mg of an aqueous solution of Roundup (41% glyphosate/l of air) (Shipp et al., 1986).

No treatment-related toxicity has been observed following chronic exposure to glyphosate at doses up to 5,874 mg/kg/day in mice or 31 mg/kg.day in rats (Shipp et al., 1986). An apparent decrease in the absolute and relative pituitary weight has been observed in dogs administered 100 mg glyphosate/kg/day for one year (EPA, 1986).

No adverse effects on reproductive capability have been observed in rats fed 30 mg glyphosate/kg/day in a three-generation reproduction study. Focal renal tubular dilation was reported in third generation male rats exposed to 30 mg glyphosate/kg/day in this study with no effects observed in 10 mg glyphosate/kg/day. There was no evidence of a teratogenic effect in rats or rabbits exposed to 3,500 or 350 mg glyphosate/kg/day, respectively (Shipp et al., 1986).

Glyphosate has been evaluated for genotoxic activity in a variety of in vivo and in vitro systems. No evidence of genotoxicity has been reported (Shipp et al., 1986).

No significant differences in the total number of tumor-bearing animals or the total number of animals with malignant tumors were found in a 26-month feeding study in rats at doses up to 31 mg glyphosate/kg/day. In a 24-month study in mice at a dose of 3,900 mg glyphosate/kg/day, an increase in the incidence of renal tubular adenomas compared to historical controls was reported in male mice; however, they were not considered to be treatment-related (Shipp et al., 1986). An independent panel reviewed the pathology and concurred that the tumors were not treatment-related and EPA revised the cancer classification from C to D.

SECTION 5. BIOLOGICAL CONTROL OF VECTORS

A. INTRODUCTION

Biological control of mosquitoes and other pests is the intentional introduction or redistribution of pathogens, parasites or predators to reduce the size of target mosquito populations. Biological control of mosquito larvae is one of the principal components of the MVC's Integrated Vector Management Program. The MVC does not use biological control against adult mosquitoes at this time. Currently, the biological control agents in use or under consideration by the MVC as part of this project are *Bacillus thuringiensis israelensis*, *Bacillus sphaericus,* and the mosquitofish *Gambusia affinis*.

Intentional biological control of mosquitoes is a relatively recent development and can largely be traced to observations and ecological studies of fish predation on mosquito larvae beginning early in this century (Smith 1904). Early investigations studied the potential effects of indigenous, and later introduced, fish on mosquito larvae. Results of such studies have been adopted in developing strategies to use mosquito predators in providing economical and sustained levels of control. As resistance to pesticides and environmental concerns associated with their use became more prevalent after the mid-1960's, biological control of larval mosquitoes became used more often as a method of protecting the public from mosquitoes and the diseases they transmit. However, reliable biological control of adult mosquitoes has not been demonstrated, and is not currently pursued by the MVC. It should also be noted that biological control methods also have potential environmental impacts, and their proper use is as one component of an integrated management program based on surveillance, treatment criteria, and selection of the most appropriate control method at the time and place that mosquito control is required.

Predation of mosquito larvae by the mosquitofish *Gambusia affinis* is significant in ornamental fishponds within the MVC Service Area. In 2003, MVC staff stocked or distributed about 6 pounds of mosquitofish (over 3,000 fish) to the public for ornamental fishpond use.

Protein spores from the bacteria *Bacillus thuringiensis israelensis* (Bti) are used by the MVC as a “biological insecticide,” but because no live organisms are used, the MVC does not consider this Biological Control. Because the potential environmental impacts of applying either type of *Bacillus* are associated with the potential disturbance associated with the mode of application and the potential for non-target toxicity, these materials will be discussed in the Chemical Control section of this document.

B. BIOLOGICAL CONTROL AGENTS

Biological control agents of mosquitoes include a wide variety of pathogens, parasites and predators. As a rule, mosquito pathogens and parasites are usually highly specific to their mosquito host, whereas predators are more general in their feeding habits and opportunistically feed on mosquitoes.

1. <u>Mosquito Pathogens</u>

Mosquito pathogens include an assortment of viruses and bacteria. They are highly host-specific and usually infect mosquito larvae when they are ingested. Upon entering the host, these pathogens multiply rapidly, destroying internal organs and consuming nutrients. The pathogen can be spread to other mosquito larvae in some cases when larval tissue disintegrates and the pathogens are released into the water to be ingested by uninfected larvae.

Examples of bacteria pathogenic to mosquitoes are *Bacillus sphaericus* and several strains of *Bacillus thuringiensis israelensis*. These two bacteria produce proteins that are toxic to mosquito larvae. Both are produced commercially as mosquito larvicides. These are discussed in section 6.C.4.

2. <u>Mosquito Parasites</u>

The life cycles of mosquito parasites are biologically more complex than those of mosquito pathogens and involve intermediate hosts or organisms other than mosquitoes. Mosquito parasites are ingested by the feeding larva or actively penetrate the larval cuticle to gain access to the host interior. Once inside the host, parasites consume the internal organs and food reserves until the parasite's developmental process is complete. The host is killed when the parasite reaches maturity and leaves the host (*Romanomermis culicivorax)* or reproduces (*Lagenidium giganteum).* Once free of the host, the parasite can remain dormant in the environment until it can begin its developmental cycle in another suitable host.

Examples of mosquito parasites are the fungi *Coelomomyces spp., Lagenidium giganteum, Culicinomyces clavosporus* and *Metarhizium anisopliae*; the protozoa *Nosema algerae, Hazardia milleri, Vavraia culicis, Helicosporidium spp., Amblyospora californica, Lambornella clarki* and *Tetrahymena spp.*; and the nematode *Romanomermis culicivorax*. None of these are under consideration for use by the MVC as they are not currently commercially available.

3. <u>Mosquito Predators</u>

Mosquito predators are represented by highly complex organisms, such as insects, fish, birds, and bats, which consume larval or adult mosquitoes as prey. Predators are opportunistic in their feeding habits and typically forage on a variety of prey types. This allows the predators to build and maintain populations at levels sufficient to control mosquitoes, even when mosquitoes are scarce.

Examples of mosquito predators include representatives from a wide variety of taxa: coelenterates (*Hydra* spp.); platyhelminths (*Dugesia dorotocephala, Mesostoma lingua*, and *Planaria* spp.); insects (Anisoptera, Zygoptera, Belostomidae, Geridae, Notonectidae, Veliidae, Dytiscidae and Hydrophilidae); arachnids (*Pardosa* spp.); fish (*Gambusia affinis, Gasterosteus aculeatus, Poecillia reticula*); bats; and birds (anseriformes, apodiformes, charadriiformes and passeriformes). Because of their abundance and easy identification, MVC staff routinely monitor Notonectids and Dytiscids. Where these invertebrate predators of mosquito larvae are abundant, chemical control is rarely used (see Treatment Criteria in 6.G. and Attachment 4).

4. Environmental Relationships In Biological Control

The effectiveness of a mosquito biological control agent lies in its ability to reduce mosquito numbers as quickly as possible. An ideal biological agent 1) feeds preferentially on mosquitoes, 2) exhibits an extremely efficient hunting or parasitizing strategy, and 3) reproduces quickly. These traits determine suitability for practical application. New mosquito sources initially have few predators and other competing aquatic organisms. Mosquito control personnel use this knowledge to develop a control strategy that involves integrated pest management techniques.

Since mosquitoes are "pioneers", capable of colonizing sources within days of flooding, initial control efforts attempt to suppress the first generations of mosquitoes until natural predators or competitors can control them. Initial treatment includes the selective use of pesticides. Once biological control is established in a "managed" source, periodic inspections at timely intervals are adequate to monitor changes in larval abundance. Periodically, the source may require treatments with pesticides when 1) predators are not effective, 2) aquatic and shoreline vegetation provide too much shelter, 3) the water level changes, or 4) water quality does not support predators.

5. Conservation And Application Of Predators

Predation on mosquitoes is a natural process that will occur without human intervention. The ability of predators to control mosquitoes is related to four factors: 1) whether mosquitoes are preferred prey; 2) whether the hunting strategy of the predator maximizes contact with mosquitoes; 3) whether the predator consumes large numbers of mosquitoes; and 4) whether the predator is present in sufficient numbers to control mosquitoes. Predator effectiveness is enhanced when proper conditions are present.

Within a typical aquatic environment that produces mosquitoes, predators are distributed among different substrates. For example pond surfaces support water striders, planaria and spiders. Below the water surface, backswimmers, predaceous diving beetles and water scavenger beetles live and feed. If the pond contains vegetation, then the plant surfaces (periphyton) will support *Hydra*, damselfly and dragonfly nymphs, and giant water bug nymphs and adults. The benthos supports dragonfly and damselfly nymphs that feed on organisms associated with silts and organic detritus. Together, the different predators form a spatial network that accounts for predation throughout the pond. Ideally an adequate variety of vegetation should be present to maintain sufficient levels of predator diversity. Greater potential for an acceptable level of mosquito control exists when more predators are present. Care should be taken so that mosquitoes do not have an advantage when too much or too little vegetation is removed. The overall objective of using predators is to reduce the frequency of pesticide applications. This minimizes environmental impact and delays the development of mosquito resistance to pesticides.

C. PRACTICAL APPLICATIONS OF BIOLOGICAL CONTROL AGENTS

A wide range of organisms has been evaluated for their effectiveness as biological control agents against mosquito larvae, but only a relatively small number are currently in use in California. There have been a number of reasons for this, including 1) difficulties in mass production, 2) failure to produce a consistent level of larval control, 3) expense, and 4) restricted application because of environmental concerns. Most agents, particularly predators and parasites, have only demonstrated acceptable control when used in conjunction with mosquitofish and larvicides.

1. Microbial Agents And Mosquito Control

Commercial formulations of *Bacillus sphaericus* and *Bacillus thuringiensis israelensis* are extensively used as mosquito larvicides. Both are relatively selective for mosquitoes and are innocuous to associated non-target organisms and predators. *Bacillus thuringiensis israelensis* is also toxic to black flies, a pest and disease vector found in moving streams, and has varying toxicity to some midge species.

Bacillus thuringiensis israelensis and *Bacillus sphaericus* are often considered chemical control measures because they are available in commercial formulations that consist of granular, powdered or liquid concentrates. The use of these two microbials is discussed further under Chemical Control (Section 6.C.).

D. MOSQUITOFISH AND MOSQUITO CONTROL

Gambusia affinis, or mosquito fish, is the most commonly used biological control agent for mosquitoes in the world. Careful use of this fish can provide safe, effective, and persistent suppression of a variety of mosquito species in many types of mosquito sources. As with all control agents, the use of mosquitofish requires a good knowledge of operational techniques and ecological implications, careful evaluation of stocking sites, use of appropriate stocking methods. *G. affinis* have naturalized in some areas of Santa Cruz County from historical stocking of creeks and other naturally occurring habitat, prior to the formation of MVC. However, at this time waterways in Santa Cruz County are remarkably free of non-native fish infestations and this is an important positive environmental condition to preserve (personal communication, Kristen Schroeder, County of Santa Cruz).

The MVC has never stocked naturally occurring ponds and creeks and introduces mosquitofish only in ornamental ponds and other discrete artificial sources. This is to avoid escape of fish into natural environments. Fish are currently also distributed through nurseries, however this practice is proposed to cease if the MVC service area is expanded and MVC staff can personally control the release of *Gambusia* elsewhere in the County. For general information on the biology of mosquitofish and their application in mosquito control programs, the reader is referred to Downs (1991) and Swanson et al (1996).

Mosquitofish are also made available to the public for backyard water gardens, unmaintained spas and swimming pools, stock watering troughs and ornamental fishponds. Fish can be picked up Monday through Friday during normal office hours, but the MVC prefers to stock them in these sites if the residents live within the service area. All citizens that pick up fish are informed of the restrictions for use of mosquitofish and of the California Fish and Game regulations (15200-15202, 15005b) that may prohibit the placement of non-native fishes into certain creeks, lakes or other natural water bodies of the state. The public is also informed on how to properly care for and maintain the mosquitofish that they are given.

E. ENVIRONMENTAL CONSIDERATIONS OF MOSQUITOFISH USE

Though mosquitofish are not native to California, they are now ubiquitous throughout most of the state’s waterways and tributaries, in large part due to historical stocking by State and local agencies.

1. Advantages Of Mosquitofish For Biological Control

Mosquitofish possess characteristics that make them efficient predators of mosquito larvae. They thrive in shallow, calm, vegetated waters, which is the same environment where many mosquitoes prefer to lay eggs, and can tolerate wide ranges of water temperature and quality. Mosquitofish are surface-oriented predators where mosquito larvae are an accessible prey. The small size of the fish enables them to penetrate moderately vegetated and shallow areas within the mosquito source. Mosquitofish are livebearers that grow rapidly, mature at a young age, and reproduce quickly. This allows the fish to establish a high population in the source shortly after stocking. In many sources, seasonal peaks in mosquitofish activity and population growth coincide with mosquito reproduction times. Because of their omnivorous feeding habits, mosquitofish can thrive in habitats where mosquitoes occur intermittently.

Mosquitofish are hardy and easy to handle, transport, and stock. As a result of extensive research and practical experimentation in California, mosquitofish can be reliably cultured in large numbers. Problems still exist in some areas with winter survival rates and inadequate supplies of fish in the spring. Because the fish reproduce where they are stocked, long-term control can be achieved by stocking relatively few fish, often in a single application. Compared to pesticides, which require repeated applications, mosquitofish can provide inexpensive and safe long-term control, sometimes within days after application. Although not all introductions are successful, mosquitofish are an effective biological control component of an Integrated Vector Management Program.

2. Limitations To Use Of Mosquitofish For Biological Control

Not all types of mosquito sources are suitable for stocking with mosquitofish and mosquitofish are not effective in all situations. Since mosquitofish usually are not stocked in numbers sufficient to cause an immediate effect, they do not control mosquitoes as quickly as pesticides do.

3. Mosquitofish And Non-Mosquito Prey

Mosquitofish, despite their name, cannot survive solely on a diet of mosquito larvae (Reddy & Pandian 1972). Laboratory and field research have shown that mosquitofish also will eat a wide variety of food, including zooplankton, copepods, cladocerans, and immature stages of many insects, including midges, water beetles, damselflies, and mayflies (Washino & Hokama 1967, Ahmed et al 1970, Reed & Hoy 1970, Miura et al 1979, Farley 1980, Walters & Legner 1980, Bence 1988, Walton & Mulla 1991, Lawler et al 1999). Hess & Tarzwell (1942) concluded that mosquitofish were true opportunistic feeders, so that the simple availability of prey was the key criteria in prey selection by mosquitofish. As such, the selection of food items by mosquitofish apparently shifts away from specific prey as its abundance drops. Within their generally wide diet, mosquitofish do have some clear feeding preferences, including food at the water surface, prey size ranging from large zooplankton to very small fish or invertebrates, and prey that is not highly mobile (Swanson et al 1996).

4. Mosquitofish And Protected Amphibians

Mosquito fish can modify food chains in small experimental pools and can have significant impacts on endemic fish in these settings (Swanson et al 1996, USFWS 1996). Concerns have been raised in the literature about use of mosquitofish because the omnivorous feeding habit of mosquitofish may pose a threat to the juvenile forms of the threatened California Red-legged Frog (RLF) and endangered Santa Cruz Long Toed salamander (SCLT). Studies have shown that RLF tadpoles in ponds with mosquitofish suffer greater injuries and weigh 34% less at metamorphosis than those in ponds without mosquitofish (Lawler, S.P. et al, 1999), and some biologists managing RLF breeding habitat work actively to eliminate mosquitofish from the ponds. Further, the Draft recovery plan for the SCLT ("Draft Revised Recovery Plan for the Santa Cruz Long Toed Salamander", USFWS, December, 2004) identifies mosquitofish as a threat to SCLT because they prey on eggs and larvae. Given this information, it is prudent to use mosquitofish for mosquito control in a manner that does not bring them into contact with any special status species. Even though there is a small baseline population of mosquito fish that exists in local waterways from stocking decades ago, it is also prudent to take steps to prevent additional fish from reaching natural waterways and beginning uncontrollable infestations.

5. Policies That Minimize Environmental Impacts Of Mosquitofish

MVC uses the following practices to avoid significant environmental impact:

- Stocking of mosquitofish by the MVC is limited to artificial, isolated sources such as ornamental ponds, pools, troughs, etc.
- In publicizing this biocontrol method the MVC will always inform the public that mosquitofish are non-native and opportunistic feeders that could impact native species.

F. CONCLUSIONS

The increasingly limited availability of registered pesticides and increasing insect resistance to pesticides increases the need for alternatives including biological control. As agents for biological control of mosquitoes, mosquitofish deserve consideration for implementation in a biological control program for artificial sources of water as long as they can be kept separated from populations of sensitive species.

SECTION 6. CHEMICAL CONTROL

A. INTRODUCTION

Control of vectors with pesticides ("Chemical Control") is an essential portion of the MVC's Integrated Vector Management Program. When mosquito abundance exceeds MVC thresholds (see Section 6.G.), and physical or biological control would be ineffective, inefficient, or otherwise inappropriate, pesticides to control larval mosquitoes can be used. While larvicides are a part of MVC's everyday operations, the MVC does not currently use adulticides but may potentially use adulticides if certain conditions exist (see Emergency Activities 1.F). Where interactions between ground-nesting yellowjackets or potentially Africanized Honey Bees (AHBs) and people present a stinging risk or otherwise exceed local tolerance, insecticides are sometimes a part of the MVC response.

Pesticide use by the MVC varies spatially and temporally in response to a large number of variables. Characteristics of the vector that are evaluated include: composition, density, extent, and age (larval instar) structure; proximity to human settlements, including sensitive receptors; weather (water temperature, wind, evaporation rate, air temperature inversion); abundance of natural predators; regional or local pathogen (disease organism) activity; vegetation; previous control efficacy history at the specific site; and/or potential for development of resistance (see MVC Guidelines in 6.G. of this Section; also Durso 1996, Lawler 1997, etc.). Although application will vary from the current use if the service area is increased, the number of applications and quantities of various pesticides applied by the MVC from 2000 through 2004 are shown in Attachment 5.

In addition to the pesticides used routinely by the MVC, which are all discussed in detail in this Section, there are a number of other materials, especially adulticides and organophosphate (OP) larvicides (see below), which are labeled and registered for use against mosquitoes in California. The MVC does not use, nor does it plan to use, organophosphate pesticides. Therefore, although these materials are available for use, they will not be discussed in this report. Further information on any California registered pesticide is available from the California Department of Pesticide Registration (DPR 2004 http://www.cdpr.ca.gov/docs/registration/regmenu.htm#registration).

B. GENERAL DISCUSSION: POTENTIAL ENVIRONMENTAL IMPACTS OF CHEMICAL CONTROL AGENTS (LARVICIDE AND ADULTICIDE PESTICIDES).

Chemical control of mosquitoes or other pests presents a number of potential environmental impacts. These potential environmental impacts can be divided into those associated with the pesticide itself, including its inert ingredients and breakdown products, and those associated with its mode of application (noise and other disturbance effects). In addition, potential pesticide impacts are often divided into those that might affect people directly (safety, residue, chronic toxicity), and those affecting other non-target organisms (especially Special Status Species). This sub-section presents an overview of general issues and information on the potential environmental impacts associated with the mosquito control pesticides and pesticide application methods used or under consideration by the MVC, and the general policies and practices of the MVC, which limit negative impacts. Further information, specific to each material or application method, follows in sections 6.C. and 6.D.

The pesticides routinely used by the MVC are relatively selective and relatively non-persistent in the environment. The MVC uses only pesticides registered by the United States Environmental Protection Agency and California Environmental Protection Agency. Pesticide application is always done in strict accordance with the pesticide label instructions (labels and Material Safety Data Sheets (MSDS) for all pesticides used by the MVC are available from the MVC or from the California Department of Pesticide Registration).

Regarding the possible use of adulticides under emergency conditions, the potential effects of such use is extremely dependant on a multitude of factors such as where the chemicals are used, the conditions under which they are used, the frequency, dosage, weather during application, and type of infestation that is being controlled. As such, it is not possible to analyze in detail the potential impacts from adulticiding activities that cannot be clearly described at this time. Therefore general remarks about adulticiding follow, but conclusions about specific environmental impacts would be speculative at this time and therefore are not addressed.

1. <u>Human Safety</u>

Pesticides, by their nature, are toxic to some organisms. Toxicity varies considerably between different species, and to a lesser extent between individuals of the same species, when exposed to identical dosages. The safety of pesticides to humans is primarily assessed through measurements of acute (single-dose) toxicity in other animals, which is summarized using "signal words" on the pesticide label or the "LD-50" values (the level of exposure at which 50 percent of the laboratory study population dies) on the MSDS (high values indicate low toxicity; see Durso 1996). The following is an explanation of these signal words. Please note that the MVC does not propose to use pesticides with a "Warning" or "Danger" label, and adulticide aerosols will only be used under emergency conditions and with specific prior approval of the Board of Supervisors.

CAUTION. This word signals that the product is slightly toxic ("Category 3 or 4"). An ounce to more than a pint taken by mouth could kill the average adult. Any product, which is slightly toxic orally, dermally, or through inhalation or causes slight eye and skin irritation, will be labeled "CAUTION".

WARNING. This word signals that the product is moderately toxic ("Category 2"). As little as a teaspoonful to a tablespoonful by mouth could kill the average sized adult. Any product, which is moderately toxic orally, dermally, or through inhalation or causes moderate eye and skin irritation, will be labeled "warning".

DANGER. This word signals that the pesticide is highly toxic ("Category 1"). A taste to a teaspoonful taken by mouth could kill an average sized adult. Any product that is highly toxic orally, dermally, or through inhalation or causes severe eye and skin burning will be labeled "DANGER".

Regarding adulticides, Piperonyl Butoxicide, a synergistic ingredient used with some adulticides, was not carcinogenic in tested laboratory animals (U.S. Dep't of Health and Human Services, 1979). At least some pyrethrins caused benign tumors at moderate to high doses in rats, also associations with asthma and dermatitis allergens. Researchers consider piperonyl butoxide to be moderately acutely toxic to fish, highly acutely toxic to aquatic invertebrate species, and has a low bioconcentration potential. (NPTN-EPA, 1998). Some studies have suggested that at least some pyrethroids may be carcinogenic and many studies have identified pyrethroids as having hormonal effects. In addition, it is established that pyrethroids can be significantly allergenic, including a possible cause of asthma. In the amounts under consideration to potentially be used by the MVC, no human health risk can be expected (letter of Will Forrest, Epidemiologist, County of Santa Cruz Health Services Agency, December 6, 2004).

Pesticide safety is also evaluated in terms of Chronic Toxicity, or the response of organisms to repeated exposures to the material being tested. The California Department of Pesticide Registration (DPR) requires extensive testing for toxicity as a condition for allowing registration of pesticides in California, and as of 2003 did not report any studies showing evidence of chronic toxicity associated with any of the pesticides used by the MVC when used at or near label rates (DPR 2003). DPR has reported "possible adverse" effects associated with repeated exposures at extremely high dosages (exceeding legally allowed label rates by a factor of 100 or more) of the adulticides Permethrin, Pyrethrins, Resmethrin and Piperonyl Butoxide, (DPR 1999). MVC policies and practices, which are discussed in more detail below, ensure that the conditions encountered during these tests would not occur during potential MVC use of adulticides. Finally, standard toxicology measurements show average risk to the population as a whole. Therefore, a margin of safety is incorporated in label instructions and MVC application policies and practices to protect children, persons with compromised health, and other "sensitive receptors."

Applicator registration and testing by the California Department of Health Services, ongoing training provided by the MVC and the Mosquito & Vector Control Association of California, routine equipment calibration, and regular oversight by the Santa Cruz County Agricultural Commissioner all contribute to protection from misuse.

Summary information regarding human exposure to pesticides used in mosquito control is available from the Center for Disease Control (www.cdc.gov/mmwr/preview/mmw rhtml/mm5227a1.htm).

2. <u>Potential Effects On Non Human Non-Target Organisms</u>

In addition to potential toxicity to humans, pesticides are evaluated for their potential effects on other non-target organisms. Some labeled mosquito control pesticides can have effects such as the short-term reduction of other non-target invertebrates, or to the food source or direct impacts to fish, amphibians, and other aquatic organisms (see 6.C. and 6.D.).

The Environmental Hazards section on labels of pesticides used for mosquito control instructs applicators about how to avoid and minimize these non-target impacts, and the MVC rigorously follows these instructions. For some adulticides, the labels instruct the applicator to avoid direct application over water or drift into sensitive areas (i.e., wetlands) due to a potential toxicity of these compounds to fish and invertebrates. Although there is some variation in the habitats to be avoided, they usually include lakes, streams and marshes. If the MVC were to use adulticides, staff would strictly follow label instructions and carefully monitor environmental and meteorological conditions to maximize effectiveness while avoiding and minimizing non-target exposure, application over water when restricted by the label, and other environmental effects.

One type of non-target effect that has been reported is the disruption of food chains through the loss of wide ranges of insects or other prey organisms. Due to the relatively selective toxicity and lack of bioaccumulation of the pesticides currently used by the MVC, they generally do not have a significant effect on food chains. However, questions have been raised about the impacts of these materials on midges, which are physiologically similar to mosquitoes and are important in the food supply for some fish, waterfowl and wading birds. The MVC and other mosquito control districts have reviewed the scientific literature of the materials they use and have concluded that there is no substantial evidence to support this concern. Specifically, 1) there is no evidence of a spatial or temporal relationship between larvicide use and successful reproduction of waterfowl or wading birds (Hanowski, 1997; Scientific Peer Review Panel, Minnesota, 1996) (Attachment 10); 2) thin-surface film napthenic oil Golden Bear 1111 has no effect on benthic stages of midges, which do not breathe at the water surface (Mulla, 1981); 3) *Bti* and *Bs* have no detectible effect on midge larvae when applied at the maximum allowable label rates for mosquito control (Balcer, 1999); 4) Methoprene, at label rates for mosquito control, can reduce adult emergence of some species of midges but does not directly kill mosquito or midge larvae and therefore does not remove them from the food chain; also adults are not targeted allowing for recovery (Balcer, 1999; Scientific Peer Review Panel, 1996)(Attachment 10); 5) no bioaccumulation (food chain magnification) of larvicides in larva-eating animals has been demonstrated for larvicides used by the MVC (Glare 1998, 1999); and 6) the MVC does not use, and does not plan to use, other larvicides in areas where midges might be a significant portion of the food chain.

3. <u>Inert Ingredients, Synergism, And Environmental Fate</u>

Ideally, the safety and environmental effects of pesticides are evaluated not only for the active ingredient(s) of the pesticide, but also for inert ingredients, chemicals that are produced as the pesticide is broken down in the environment (environmental fate), and all possible combinations of interactions with other environmental compounds (synergisms). In the past, some persistent mosquito control pesticides (eg. DDT) both accumulated in animal tissues and were concentrated up food chains (bioaccumulation), and also created breakdown products that themselves posed environmental risks (e.g. DDE from DDT). As a practical matter, it has been impossible for DPR or any other institution to test every possible chemical interaction and breakdown product. However, pesticides are tested as mixtures, together with their inert compounds, prior to their registration by USEPA or DPR, and extensive information has been collected on their total toxicity, bioaccumulation, and environmental fate. In addition, these materials do not bioaccumulate (see specific material descriptions later in this Section).

4. <u>Resistance And Loss Of Effectiveness</u>

A number of examples of pesticide resistance have been published over the years, and one of the concerns with the development of resistance is the observed tendency of users to increase application frequency and/or intensity as pesticide effectiveness drops. The MVC has not experienced control failures due to resistance while using the current array of pesticides. However, to help guard against the development of resistance, and the monetary or environmental costs that can result, the MVC makes use of a number of pesticides with different modes of action, closely monitors research on resistance, and is committed to revising application practices if needed to avoid resistance. Reduced need for increasing levels of chemical use because of resistance is one of the benefits of the focus on prevention of an integrated pest management program.

5. <u>MVC Policies And Practices To Protect The Environment</u>

In addition to the environmental protection measures and procedures inherent in the MVC's IVMP as discussed earlier (especially application thresholds and other criteria that limit frequency of use of chemical control agents), there are other practices in the MVC's chemical control program that protect the environment:

a. There are numerous federal and state laws and regulations that strictly control and regulate the storage, transport, handling, use and disposal of the pesticides in order to protect against surface and groundwater contamination and other impacts to the environment and public health. (E.g., Federal Insecticide, Fungicide and Rodenticide Act; Cal. Food & Agric. Code divisions 6 & 7; Cal. Code of Regs., title 3, division 6.) MVC staff consistently complies with these laws and regulations and are routinely inspected by the County Agricultural Commissioner for compliance.

b. The MVC only uses pesticides registered by the U.S. Environmental Protection Agency and California Department of Pesticide Regulation. The MVC strictly complies with the pesticide label restrictions and requirements concerning the storage, transport, handling, use and disposal of the pesticides.

c. Consistent with the MVC's integrated mosquito management principles, when using pesticides, the MVC selects the least hazardous material that will meet its goals. The MVC does not use Category 1 pesticides, and would only use Category 2 pesticides in emergency conditions.

d. The MVC regularly calibrates the output of all of its pesticide application equipment.

e. The State Department of Health Services (DHS) regulates the MVC. Mosquito control activities are coordinated with DHS pursuant to an annual Cooperative Agreement, under which the MVC commits to comply with certain standards concerning mosquito control and pesticide use. State law and the Cooperative Agreement require MVC mosquito control employees to be certified by DHS as mosquito control technicians. This certification helps to ensure that the employees are adequately trained regarding safe and proper mosquito control techniques, including the handling and use of pesticides and compliance with laws and regulations relating to mosquito control and environmental protection.

f. MVC also works in close coordination with the County Agricultural Commissioner, including monthly reporting of its activities.

g. The MVC performs management within Ellicott Slough National Wildlife Refuge in conformance with site specific pesticide use agreements. These are arrangements between MVC and USFWS that outline application rates, target mosquito species, methods of application and the listed sensitive species. These provide the MVC with consultation with the resource managers and help to ensure that the MVC activities are compatible with wildlife. Similar agreements will be made between MVC and resource agencies for other areas should the MVC service area be expanded and anywhere they are requested by USFWS or DFG.

C. LARVICIDES

1. <u>Introduction</u>

Larviciding is a general term for the application of non-living natural materials or synthetic chemical products to aquatic habitats to kill mosquito larvae or pupae or to otherwise prevent emergence of adult mosquitoes. Larvicides can be applied in a wide variety of formulations using a broad range of application technologies. Larviciding was developed early this century for the control of malaria and yellow fever mosquitoes` and still represents the most extensive set of MVC chemical control activities.

MVC uses larvicides to treat a wide variety of aquatic habitats ranging from small domestic containers to large marshland areas. Frequently, the aquatic habitats targeted for larviciding are temporary or semi-permanent, since permanent aquatic sources usually contain natural mosquito predators such as fish and do not require further treatment, unless vegetation is so dense that it prevents natural predation. Tidal marshes and the margins of creeks produce prodigious numbers of floodwater mosquitoes. While floodwater mosquitoes develop during the first weeks after flooding, it often takes at least two to three weeks for the first macro invertebrate predators of mosquitoes to become established, and therefore biological or chemical control will be needed.

The major advantage of larviciding is the very small target area compared to the area that must be treated if adults are the target. Depending on water temperature, organic content, mosquito larval density, and other variables, pesticide applications may be repeated at any site at frequencies ranging from annually to weekly. Larvicides routinely used by the MVC are Golden Bear Oil 1111, Agnique (Mono-Molecular Film), Methoprene (Altosid), Bs (*Bacillus sphaericus*) and Bti (*Bacillus thuringiensis israelensis*). Typically, the MVC applies larvicides to less than one percent of the total MVC Service Area in any year.

There are times when larviciding is inappropriate (Durso 1996). Effective larviciding results are not always easy to achieve, and is critically dependent on timing when using non-persistent pesticides. The size, location, or topography of the mosquito source area may make timely larviciding impossible. Spatial accuracy of the larvicide application is also extremely important. Congregated larvae may be easy targets, but missing a relatively small area containing them is also easy and leads to the emergence of many adults. Finally, larvicide labels allow a range of legal application dosage rates; the selected rate must be sufficiently high to control the targeted mosquito species and sufficiently low to avoid or minimize non-target effects, especially where Special Status Species are present.

Fauna that may inhabit larvicide application sites include amphibians, fish, turtles, waterfowl, other vertebrates and invertebrates, particularly insects and crustaceans. The larvicides most used and as used by the MVC cause little impact to these species. (Glare & O'Callaghan 1998, 1999; Lawler 2000). Disturbed or temporarily flooded sites that meet MVC criteria to larvicide are generally very low in diversity of non-mosquito animal species at those times, due to the time needed for most non-mosquito species to locate and colonize these sites after flooding (Collins & Resh 1989). Also, because the MVC applies larvicides in limited areas at any time, and the short-acting formulation are preferred over slow-release formulations, most of the non-target species that do exploit temporary aquatic habitats are capable of quickly recovering from localized population declines via re-colonization from untreated proximal areas (Lawler 1997; Balcer 1999).

Impacts of larviciding on flora are insignificant because the materials have no toxicity to plants and the application methods involve very little disturbance to plants or soil.

The larvicides used by the MVC are each discussed below: Insect Growth Regulator S-Methoprene, Water Surface Films (GB1111 and Agnique), and Ingested Bacterial Larvicides (Bti and *Bacillus sphaericus*) followed by a discussion of application methods, which are similar for all these materials.
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2. <u>Insect Growth Regulators (IGR's), Methoprene</u>

<u>S-METHOPRENE.</u> S-Methoprene (known simply as Methoprene or as its trade name, Altosid) is a synthetic analogue (mimic) of a naturally occurring insect hormone called Juvenile Hormone (JH). JH is found during aquatic life stages of the mosquito and in other insects, but is most prevalent during the early instars. As mosquito larvae mature, the level of JH steadily declines until the 4th instar molt, when levels are very low. This is considered to be a sensitive period when all the physical features of the adult begin to develop. s-Methoprene in the aquatic habitat can be absorbed on contact and the insect's hormone system then becomes unbalanced. When this happens during the sensitive period, the imbalance interferes with 4th instar larval development. One effect is to prevent adults from emerging. Since pupae do not eat, they eventually deplete body stores of essential nutrients and then starve to death. Based on its mode of action, s-Methoprene is considered an insect growth regulator (IGR). This material has no effect on mosquito pupae and must be contacted by larvae to be effective.

Methoprene is applied either in response to observed high populations of mosquito larvae at a site, or as a sustained-release product that can be present for four months or longer. Application can be by hand, boat, or aircraft. For example, the MVC applied about 9.4 pounds of Altosid Pellets, 22.3 pounds of Altosid briquettes, 983 pounds of Altosid Single-Brood Granules and 0.1 gallons of Altosid Liquid Larvicide in the entire Service Area during 2004. This corresponds to 73 acres treated with methoprene in 2004. (see Pesticide Use Chart, Table 1, Attachment 5) for application rates and compare usage trends)

<u>FORMULATIONS AND DOSAGES</u>. s-Methoprene has a half-life of about two days in water, two days in plants, and ten days in soil (Wright 1976 in Glare & O'Callaghan 1999, La Clair et al 1998). The manufacturer has developed a number of formulations to maintain an effective level of the active material in the mosquito habitat (0.5-3.0 parts per billion = ppb[3]; (Ross et al., 1994) for a practical duration, thus minimizing the cost and potential impacts associated with high-frequency repeat applications. Altosid labels contain the signal word "CAUTION".

ALTOSID LIQUID LARVICIDE (A.L.L.) & A.L.L. CONCENTRATE. These two microencapsulated liquid formulations have identical components and only differ in their concentrations of active ingredients (AI). A.L.L. contains 5% (wt./wt.) s-Methoprene while A.L.L. Concentrate contains 20% (wt./wt.) s-Methoprene. The balance consists of inert ingredients that encapsulate the s-Methoprene, causing its slow release and retarding its ultraviolet light degradation. Maximum labeled use rates are 4 ounces of A.L.L. and 1 ounce of A.L.L. Concentrate (both equivalent to 0.0125 lb. AI) per acre, mixed in water as a carrier and dispensed by spraying with conventional ground and aerial equipment. In sites which average a foot deep, these application rates are equivalent to a maximum active ingredient concentrations of 4.8 ppb, although the actual concentration is substantially lower because the encapsulation does not allow instantaneous dissolution of all of the active ingredient into the water.

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[3]Note that this concentration is measured in parts per **billion**, and is equivalent to 0.0005 to 0.003 ppm (parts per **million**) when comparing application rates and toxicity studies.

Because the specific gravity of Altosid Liquid is about equivalent to that of water, it tends to stay near the water surface. Therefore, no adjustment to the application rate is necessary in varying water depths when treating species that breathe air at the surface. Cold, cloudy weather and cool water slow the release and degradation of the active ingredient as well as the development of the mosquito larvae.

ALTOSID BRIQUETS. Altosid Briquettes were the first solid methoprene product marketed for mosquito control, beginning in 1978. Briquettes consist of 4.125% s-methoprene (.000458 lb. AI/briquette), 4.125% (wt./wt.) r-methoprene (an inactive isomer), and plaster (calcium sulfate) and charcoal to retard ultra violet light degradation. Altosid Briquettes release methoprene for about 30 days under normal weather conditions and, as noted earlier, this means that the concentration of AI in the environment at any time is much lower than the value calculated from the weight of material applied.

Applications are usually made at the beginning of the mosquito season, and under normal weather conditions, repeat treatments occur at approximately 30-day intervals. The recommended application rate is 1 Briquette per 100 sq. ft. in non-flowing or low-flowing water up to 2 feet deep. Small sites with any mosquito genera may be treated with this formulation. Typical treatment sites include storm drains, catch basins, roadside ditches, ornamental ponds and fountains, cesspools and septic tanks, waste treatment and settlement ponds, transformer vaults, abandoned swimming pools, and construction and other man-made depressions.

Altosid Briquettes prevent the emergence of adult mosquitoes including *Anopheles, Culex, Culiseta, Coquillettidia, and Mansonia* spp., as well as those of the floodwater mosquito complex (*Aedes, Ochlerotatus* and *Psorophora* spp.) from treated water. Treated larvae continue to develop normally to the pupal stage where they die.

ALTOSID XR BRIQUETS. This formulation consists of 2.1% (wt./wt.) s-methoprene (.00145 lb. AI/briquet) embedded in hard dental plaster (calcium sulfate) and charcoal. Despite containing only 3 times the AI as the "30-day briquet", the comparatively harder plaster and larger size of the XR Briquet change the erosion rate allowing sustained s-methoprene release for up to 150 days in normal weather. The recommended application rate is 1 to 2 briquets per 200 sq. ft. in no-flow or low-flow water conditions, depending on the target species. Many applications are similar to those with the smaller briquets, although the longer duration of material release can also make this formulation economical in small cattail swamps and marshes, water hyacinth beds, meadows, freshwater swamps and marshes, woodland pools, flood plains and dredge spoil sites.

ALTOSID PELLETS. Altosid Pellets were approved for use in April 1990. They contain 4.25% (wt./wt.) s-methoprene (0.04 lb. AI/lb.), dental plaster (calcium sulfate), and charcoal in a small, hard pellet. Like the Briquets discussed above, Altosid Pellets are designed to slowly release s-methoprene as they erode. Under normal weather conditions, control can be achieved for up to 30 days of constant submersion or much longer in episodically flooded sites (Kramer 1993). Label application rates range from 2.5 lbs. to 10.0 lbs. per acre (0.1 to 0.4 lb. AI/acre), depending on the target species and/or habitat. At maximum label application rates, as with the Briquets, the slow release of material means that the actual concentration of active ingredient in the water never exceeds a few parts per billion.

The target species are the same as those for the briquet and liquid formulations. Listed target sites include pastures, meadows, rice fields, freshwater swamps and marshes, salt and tidal marshes, woodland pools, flood plains, tires and other artificial water holding containers, dredge spoil sites, waste treatment ponds, ditches, and other man-made depressions, ornamental pond and fountains, transformer vaults, construction and other man-made depressions, tree holes, storm drains, catch basins, and waste water treatment settling ponds.

ALTOSID XR-G. Altosid XR-G was approved for use in 1997. This product contains 1.5% (wt./wt.) s-methoprene. Granules are designed to slowly release s-methoprene as they erode. Under normal weather conditions, control can be achieved for up to 21 days. Label application rates range from 5 lbs. to 20.0 lbs. per acre, depending on the target species and/or habitat. The species are the same as listed for the briquet formulations. Listed target sites include meadows, rice fields, freshwater swamps and marshes, salt and tidal marshes, woodland pools, tires and other artificial water holding containers, dredge spoil sites, waste treatment ponds, ditches, and other natural and man-made depressions.

ALTOSID SBG. Altosid Single Brood Granules was approved for use in 2000, contains 0.2% (wt./wt.) s-methoprene and is effective for 5-10 days after application, so is designed for short-term control or for single broods of mosquitoes. Application rates range from 5-20 lbs. per acre and listed sites are uncultivated agricultural and non-agricultural non-food areas, salt and tidal marshes, freshwater swamps and marshes, woodland pools and meadows, dredging spoil sites, drainage areas, ditches, waste water treatment facilities, retention ponds, harvested timber stacks, swales, storm water drainage areas, sewers, catch basins, tree holes, water-holding receptacles (e.g., tires, urns, flower pots, cans and other containers) and other natural and manmade depressions. The target species are the same as listed for briquets.

ENVIRONMENTAL IMPACTS: Reviews of the published literature on this material found minimal environmental impact at the dosages that are and will be used. There is evidence of some sensitivity of crustaceans (Mian & Mulla 1982, Scientific Peer Review Panel, Minnesota, 1996 (Attachment 10) Glare & O'Callaghan 1999, Office of the Minnesota Legislative Auditor 1999) In Attachment 11 is a list of organisms impacted by S-Methoprene in studies reviewed by Glare & O'Callaghan (1999). For further information, see http://extoxnet.orst.edu/pips/methopre.htm.
For environmental fate see http://www.cdpr.ca.gov/docs/empm/pubs/pyrethfate.pdf
or http://www.cdpr.ca.gov/docs/empm/pubs/methofate.pdf

Wright (1976) reviewed the toxicology data collected for Methoprene registration and found no clinical signs of toxicosis in swine, sheep, hamsters, rats, dogs, rabbits, guinea pigs and cattle. Additionally, teratological (birth defect) studies in swine, sheep, hamsters, rats and rabbits showed no observable effects. Hester et al. (1980) found non-target organisms did not exhibit any adverse effects when exposed to treatments of sand granule and liquid formulations of methoprene up to a maximum of three and seven weeks, respectively. The acute, short-term toxicity of ZR-515 (methoprene) was also tested on 35 aquatic organisms including Protozoa, Platyhelminths, Rotatoria, Annelida, Arthropoda, Mollusca, Chordata and Thallophyta, and LC50 values of 0.9 to 5.0 ppm were calculated (250 to 1000 times label rates) (Miura and Takahashi 1973). Dosage rates used for larval mosquito control produced no adverse effect on the organisms tested, except for some sensitivity in larvae of some aquatic Diptera (Chironomidae, Ephydridae, and Psychodidae).

Bircher and Ruber (1988) assessed the toxicity of methoprene to all life cycle stages of the salt marsh copepod (*Apocyclops spartinus*) at concentrations ranging from 0.1 to 10.0 ppm. In general the copepods were resistant to concentrations of methoprene used to control mosquitoes, but early nauplii did show some mortalities to methoprene concentrations near the lower margins of mosquito susceptibility. Christiansen et al. (1977) showed a reduction in survival of larvae of the mud-crab *Rhithropanopeus harrisii* (Gould) under a range of salinity and temperature conditions, when exposed to 0.01, 0.1 and 1.0 ppm methoprene. MVC application rates are 0.5 - 3 ppb, order of magnitude lower than these concentrations. McKenney and Mathews (1988) reported that larval survival, growth and energy metabolism of an estuarine shrimp *Palaemonetes pugio* were altered by exposure to low ug/l concentrations of an insect growth regulator (the juvenile hormone analogue, methoprene).

An extensive early study of technical (powdered) methoprene on a Louisiana coastal marsh (Breaud et al 1977) showed reductions in some animal populations and increases in others. Specifically, populations of adult and young scud (*Hyalella azteca*), adult and young opossum shrimp (*Taphromysis louisianae*), adult and young freshwater prawns (*Palaemonetes paludosus*), immature mayflies (*Callibaetis* sp.), larval dance flies (*Notophila* sp.), larval midges *(Chironomidae*), adult and young fresh water snails (*Physa* sp.), immature damselflies and dragonflies (*Enallagma, Anax,* and *Belonia* spp.), adult burrowing water beetles (*Suphisellus* sp. and *Hydrocanthus* sp.), adult water scavenger beetles (*Berosus infuscatus*), and immature water scavenger beetles (*Berosus* spp.) decreased, immature water boatmen (*Trichocorixa louisianae*), larval moth flies (*Psychoda* sp.), adult and young crawfish (*Procambarus clarki* and *Cambarellus* sp.), and adult predaceous diving beetles (*Liodessus affinis*) increased, and no statistically significant ($P>0.05$) difference was seen between the test and control populations of 28 other aquatic organisms. Interpretation of this study is difficult in part because of the mixed nature of the results, however, given the fact that the application rate (28gm AI/ha technical powder) was at least twice the highest label rate allowed today, and was effectively much higher than that when the encapsulation and other coatings on modern formulations are considered.

Finally, Lawler, Dritz and Jensen (1999) concluded that occasional use of sustained-release methoprene does not decrease abundance of non-target salt marsh insects. Hershey *et al.* (1995) determined that many aquatic insects are insensitive to methoprene in amounts used for mosquito control, however, when the same material was continued for 3 years at maximum label rates, there were strong reductions in midges. However, Balcer, Schmude and Snitgen (1999) continued to assess the long-term effects of standard applications of methoprene and Bti on non-target macro invertebrates in the same wetlands in Minnesota, and found no significant reductions in the abundance of chironomids or any other taxa of macro invertebrates. Balcer et al concluded that the Hershey study was conducted during unusual drought conditions when these midge populations were stressed.

A study by Niemi *et al.* (1999) at the same time found no detectable effects on red-winged blackbirds and other breeding birds in freshwater riparian areas of Minnesota. Ankley *et al.* (1998) showed no effect of methoprene on Northern leopard frog (*Rana pipiens)* tadpoles at twice the maximum mosquito label rate.

After examining these and other studies, the MVC has concluded that 1) applications of methoprene (especially technical powder) at rates significantly higher than allowed by the label can adversely impact a number of aquatic animals 2) emergence of adults of some fly species (specifically, some types of midges) can be temporarily reduced at application rates similar to MVC practices; 3) larval flies affected by methoprene are not killed at label application rates, but are prevented from becoming adults; 4) for species that are affected by methoprene, recolonization and reestablishment of populations from neighboring sites is fast once intense control was relaxed, 5) the patchy distribution of mosquito larvae leads to maintenance of untreated refugia for non-targets, speeding recolonization (7) no bioaccumulation of methoprene has been seen in animals that have eaten mosquito or midge larvae treated with methoprene, and 8) any problems associated with repeated applications of methoprene can be diminished through rotation with other larvicides.

Finally, it has been suggested that breakdown products of methoprene under certain circumstances may be associated with deformities in frogs that have been observed in Minnesota and other states (Fort 1998, La Clair 1998). The MVC finds no substantial evidence to support this suggestion, and reviews of this literature by independent analysts in Minnesota and New Zealand also find no evidence to support this claim (Glare & O'Callaghan 1999; Minnesota State Auditor's Office 1999, Degitz 2004). First, there is no evidence of a spatial or temporal relationship between Altosid use and amphibian deformities noted in any literature and no significant evidence of frog deformities anywhere in California where methoprene use occurs (Fenn 1999). Second, there are well-documented alternative explanations for frog deformities where they do occur including infections with trematodes, and these explanations are more consistent with the epidemiological patterns observed than pesticide exposure (Ankley 1998, Sessions 1999, Henrick 2002, Degitz 2001, 2003, 2004). Third, the observations discussed to support the assertion have not been duplicated by other researchers (Ankley et al 1998, Glare & O'Callaghan 1999). Fourth, consultations with herpetologist Dr. Mark Jennings found no professional agreement with the claims of methoprene and frog deformities (communication with Dr. Karl Malamud-Roam). Finally, deficiencies in methodology and/or interpretation exist in the few reports that make this assertion, including failure to compensate for natural degradation of methoprene in the environment (La Clair 1998) and failure to evaluate parasitism (Sparling 1998).

2. Water Surface Films

INTRODUCTION. Water Surface Film larvicides spread across water surfaces and disrupt larval respiration, killing mosquitoes and some other classes of air-breathing aquatic insects. Water surface film larvicides used by the MVC include specially refined petroleum distillates (Golden Bear 1111) and ethoxylated Isostearyl Alcohols (Agnique). In addition to being safe agents for effective for the control of younger larval instars, these are the only currently registered larvicides used by the MVC that are effective against mosquito pupae. Therefore, when timely larval control is not possible or not successful, pupal control can usually be achieved using these products.

MOSQUITO LARVICIDE GB-1111 (GOLDEN BEAR 1111). This product, generally referred to as Golden Bear 1111 or simply GB-1111, is a highly-refined petroleum based "napthenic oil" with very low phytotoxicity and no detectible residual products within days after application. Volatility is very low ("non-volatile" according to the MSDS), and environmental breakdown presumably results primarily from natural microbial degradation into simple organic compounds. The label for GB-1111 contains the signal word "CAUTION". GB-1111 contains 99% (wt./wt.) oil and 1% (wt./wt.) inert ingredients including an emulsifier. The nominal dosage rate is 3 gallons per acre or less. Under special circumstances, such as when treating areas with high organic content, up to 5 gallons per acre may be used.

GB-1111 provides effective control on a wide range of mosquito species. Applied to breeding areas, GB-1111 is an effective material against any mosquito larvae and pupae obtaining atmospheric oxygen at the water surface. It can even be effective in treating adult mosquitoes as they emerge. Where pupal density is high, or where warm water indicates that this will occur soon, GB-1111 is used unless other materials are required by site-specific protocols or other application criteria. Low dosages (1 gallon per acre) of oil work slowly, especially in cold water, and can take 4 to 7 days to give a complete kill. Higher dosage rates are sometimes used (up to 5 gallons per acre) to lower the kill time. It is typically applied by hand, boat, or truck. Aerial application is possible for large areas, but is not routine and has only been conducted on one occasion in the first year of the MVC.

POTENTIAL ENVIRONMENTAL IMPACTS OF GB-1111. Little information has been published on the potential environmental impacts of this pesticide. GB-1111 was re-registered as a mosquito larvicide by the California Department of Pesticide Registration on April 20, 1999 (DPR 1999), and subsequent consultations with the Registration Specialist for this material at DPR indicate that the Department did not find evidence that GB-1111 has any potential significant environmental impacts when applied under label requirements and MVC application protocols (Duane Schnabel, DPR, pers. comm. May, 1999).

Four studies by Tietze et al (1991, 1992, 1993, 1994) tested three species of fish (Inland Silversides, Mosquitofish, and Sheepshead Minnows) and a range of microorganisms and concluded that this larvicide is not toxic to the tested organisms at label application rates. Mulla and Darwazeh (1981) experimented with GB-1111 in small experimental ponds and found that benthic invertebrates were unaffected while populations of surface breathing insects were temporarily reduced following application of this larvicide. Miles, Lawler and Dritz (UC Davis, 2002) completed a significant independent study of non-target effects of GB-1111, with financial assistance from USFWS, on the tidal marshes of Newark, CA, and observed the following effects: 1) surface breathing insect populations were reduced at the time of treatment; 2) this effect did not persist beyond a few days 3) those potentially affected animals with high mobility left the site, while some of those that could not leave died (especially water boatmen, Corixidae); 4) overall populations of invertebrate species were not affected, apparently because of recolonization from neighboring untreated sites.

The MVC reduces non-target impacts of GB-1111 by using it mainly for spot treatment in areas where more selective microbials (Bti, Bs) are ineffective, such as where mosquito pupae predominate and in highly polluted sources, while avoiding treating large areas of aquatic sites when non-targets are present, allowing for their recovery. Whereas there is concern regarding the oil sheen temporarily reducing the insulating properties of down on ducklings in freezing conditions (P. Binding correspondence with Lawler), the MVC will not use GB-1111 when air or water temperatures are below 50 degrees F.

AGNIQUE: Agnique is the trade name for a recently reissued surface film larvicide, comprised of ethoxylated alcohol. Agnique has very low vertebrate toxicity; an average persistence in the environment of 5-14 days at label application rates; and no toxic breakdown products, skin irritation, carcinogenicity, mutagenicity, or teratogenicity has been reported. Because of its similar mode of action and effectiveness against pupae, Agnique can be used as an alternative to Golden Bear 1111, especially in sites where the temporary oil sheen associated with GB-1111 might be objectionable. Because the application rate of Agnique (0.2-0.5 gallons/acre) is much lower than that of Golden Bear (3-5 gallons/acre), this potential shift would not include an increase in volume of materials applied.

<u>POTENTIAL ENVIRONMENTAL IMPACTS OF AGNIQUE</u>. A number of efficacy and non-target studies had been conducted on this material when it was registered under the name Aerosurf. The pesticide was reregistered in California in July 1999 and consultations with DPR indicate that the Department did not find evidence that Agnique has any potential significant environmental impacts when applied under label requirements and MVC application protocols (Duane Schnabel, DPR, pers. comm. May, 1999). Minor proprietary changes in preparation did not apparently change any of the material's potential environmental impacts, and therefore the earlier literature is referenced.

Most published studies conducted with this larvicide tested application rates of 3 to 100 times the maximum label rate. At these rates, no observable effect on mortality or development was noted in tests on green tree frogs, seven species of fresh and salt water fish, two species of shrimp, five species of water beetle, or one species each of fairy shrimp, crayfish, snail, polychaete worm, mayfly naiad, copepod, ostracod, or midge. In addition, no effect was seen on five species of plants. As with GB-1111, air (surface) breathing insects were temporarily adversely impacted. Waterboatmen, backswimmers, and one species of water beetle exhibited increased mortality at application rates above label limits. In addition, a clam shrimp, a crab, an amphipod, and one species of isopod exhibited minor to significant increases in mortality at levels several times the highest application rate allowed by the label. For more information, please see the table in Attachment 12. It should be noted that the greater persistence of this material (up to two weeks) relative to GB-1111 can reduce the need for repeated applications, but might also increase the duration of suppression of other air-breathing insects. Because MVC larvicide protocols require application of larvicides only in areas with immature stages of mosquitoes, and because larval distribution is highly patchy (Service 1993), recolonization of impacted non-targets from unsprayed areas can still occur.

3. <u>Bacterial (Ingestion) Larvicides</u>

<u>INTRODUCTION</u>. The MVC uses two types of ingested toxins whose active ingredients are manufactured by bacteria. These control agents are often designated as Bacterial Larvicides. Their mode of action requires that they be ingested to be effective, which can make them more difficult to use than the contact toxins and water surface films. Bacteria are single-celled parasitic or saprophytic microorganisms that exhibit both plant and animal properties, and range from harmless and beneficial to intensely virulent and lethal. A beneficial form, *Bacillus thuringiensis* (Bt), is the most widely used (especially in agriculture) microbial pesticide in the world. It was originally isolated from natural Lepidopteran (butterfly and moth) die-offs in Germany and Japan. Various Bt products have been available since the 1950's, and in 1976, Dr. Joel Margalit and Mr. Leonard Goldberg isolated from a stagnant riverbed pool in Israel, a subspecies of *B. thuringiensis* that had excellent mosquito larvicide activities. It was named *B.t.* variety *israelensis* (B.t.i.) and later designated *Bacillus thuringiensis* Serotype H-14.

Either of these two designations may be found on the labels of many bacterial mosquito larvicide formulations used today. Another species of bacteria, *B. sphaericus*, also exhibits mosquito larvicidal properties.

BTI (*Bacillus thuringiensis* var. *israelensis*). B.t.i. organisms produce, when environmental conditions are favorable, five different microscopic protein pro-toxins packaged inside one larger protein container or crystal. The crystal is commonly referred to as delta (d-) endotoxin. If a mosquito larva ingests the d-endotoxin, these five proteins are released in the alkaline environment of the insect larval gut. The five proteins are converted into five different toxins if specific enzymes also are present in the gut. Once converted, these toxins destroy the gut wall, which leads to paralysis and death of the larvae. B.t.i. is toxic to larval stages of all genera of mosquitoes and to black flies (Simuliidae).

B.t.i. is grown commercially in large fermentation vats using sophisticated techniques to control environmental variables such as temperature, moisture, oxygen, pH and nutrients. The process is similar to the production of beer, except that B.t.i. bacteria are grown on high protein substrates such as fishmeal or soy flour and the spore and delta endotoxin are the end products. At the end of the fermentation process, B.t.i. bacteria exhaust the nutrients in the fermentation machine, producing spores before they lyse and break apart. Coincidental with sporulation, the delta endotoxin is produced. The spores and delta endotoxins are then concentrated via centrifugation and micro filtration of the slurry. It can then be dried for processing and packaging as a solid formulation or further processed as a liquid formulation. Since some fermentation medium (e.g. fish meal) is always present in liquid formulations, they generally smell somewhat like the medium.

The MVC uses four B.t.i. formulations: liquids, granules, pellets, and briquets. Liquids, produced directly from a concentrated fermentation slurry, tend to have uniformly small (2-10 micron) particle sizes, which are suitable for ingestion by mosquito larvae. B.t.i. granules, pellets, and briquets are formulated from B.t.i. primary powders and an inert carrier. B.t.i. labels contain the signal word “CAUTION”.

The amount of toxins contained within B.t.i. products are reported indirectly as the result of at least two different bioassays and are difficult to equate to one another. Prepared volumes of toxins are applied to living mosquito larvae and the resulting mortality produces through formulae numerical measures known as International Toxic Units (ITUs) and *Ae. aegypti* International Toxic Units (AA-ITU’s). These measures are only roughly related to observed efficacy in the field, and are therefore inappropriate to consolidate and report on like other toxicants (active ingredients).

Bti is applied by the MVC as a liquid or sometimes bonded to an inert substrate (i.e.: corn cob granules) to assist penetration of vegetation. Application can be by hand, boat, or aircraft. Persistence in the environment is three to five days due to sensitivity to UV light. Kills are usually observed within 48 hours of toxin ingestion. As a practical matter, apparent failures are usually followed with surface film treatments.

Timing of application is extremely important in operational use of bacterial toxins. Optimal benefits are obtained when treating 2^{nd} or 3^{rd} instar larvae. Treatments at other development stages may provide poor control. Since fourth instar mosquito larvae quit feeding prior to becoming pupae, it is necessary to apply B.t.i. prior to this point in their development. Although the details are poorly understood, evidence suggests that larvae also undergo a period of reduced feeding or inactivity prior to molting from 1^{ST} to 2^{ND}, 2^{ND} to 3^{RD}, and 3^{RD} to 4^{TH} instars. If we apply B.t.i at these points in their development, the toxic crystals may settle out of the water column before the larvae resume feeding, and with synchronous broods of mosquitoes, complete control failures may result. With asynchronous broods, efficacy may also be reduced. Therefore a disadvantage of using B.t.i. is the limited application window available.

BTI LIQUIDS. Currently, three commercial brands of B.t.i. liquids are available: Aquabac XT, Teknar HP-D, and Vectobac 12AS. Labels for all three products recommend using 4 to 16 liquid oz/acre in unpolluted, low organic water with low populations of early instar larvae (collectively referred to below as clean water situations). The Aquabac XT and Vectobac 12 AS (but not Teknar HP-D) labels also recommend increasing the range from 16 to 32 liquid oz/acre when late 3rd or early 4th instar larvae predominate, larval populations are high, water is heavily polluted, and/or algae are abundant. The recommendation to increase dosages in these instances (collectively referred to below as dirty water situations) also is seen in various combinations on the labels for all other B.t.i. formulations discussed below.

B.t.i. liquid may also be combined with the Altosid Liquid Larvicide discussed earlier. This mixture is known as Duplex. Because B.t.i. is a stomach toxin and lethal dosages are somewhat proportional to a mosquito larvae's body size, earlier instars need to eat fewer toxic crystals to be adversely affected. Combining B.t.i. with methoprene (which is most effective when larvae are the oldest and largest or when you have various, asynchronous stages of one or more species) allows a MVC to use less of each product than they normally would if they would use one or the other. Financially, most savings are realized for treatments of mosquitoes with long larval development periods, asynchronous broods or areas with multiple species of mosquitoes.

BTI CORNCOB GRANULES. There are currently two popular corncob granule sizes used in commercial formulations. Aquabac 200G, Bactimos G, and Vectobac G are made with 5/8 grit crushed cob, while Aquabac 200 CG (Custom Granules) and Vectobac CG are made with 10/14 grit cob. Aquabac 200 CG is available by special request. The 5/8 grit is much larger and contains fewer granules per pound. The current labels of all B.t.i. granules recommend using 2.5 to 10 lb./acre in clean water and 10 to 20 lb./acre in dirty water situations.

ENVIRONMENTAL IMPACTS OF BTI. Products containing Bti are ideally suited for use in Integrated Vector Management Programs because the active ingredient has a highly specific mode of action and is therefore comparatively selective. Bti does not interrupt activities of most beneficial insects and predators. Bti controls all larval instars provided they have not quit feeding, and can be used in almost any aquatic habitat with no restrictions. It may be applied to irrigation water and any other water sites except treated finished drinking water. Bti is fast acting and its efficacy can be evaluated almost immediately. It can kill larvae within 1 hour after ingestion, and since each instar must eat in order for the larvae to grow, the Bti usually kills mosquito larvae within 48 hours of application. Bti leaves no residues, and persists in the environment three to five days. Resistance is unlikely to develop simultaneously to the five different toxins derived from the Bti delta-endotoxin since they have five different modes of action. This suggests that this mosquito larvicide will continue to be effective for many years.

Bti labels carry the CAUTION signal word, suggesting the material may be harmful if inhaled or absorbed through the skin. However, the 4-hr Inhalation LC 50 in rats is calculated to be greater than 2.1 mg/liter (actual) of air, the maximum attainable concentration. The acute Dermal LD 50 in rabbits is greater than 2,000 mg/kg body weight and is considered to be non-irritating to the eye or skin. That is equivalent to a 220 lb. man spilling more than a half-gallon of Bti liquid directly onto his skin or eyes and not washing it off. Toxicology profiles also suggest that the inert ingredients (not the Bti) in liquid formulations may cause minor eye irritation in humans. The acute Oral LD 50 in rats is greater than 5,000 mg/kg body weight (similar to an individual drinking over 5 quarts) suggesting the material is practically non-toxic in single doses. Common table salt has an LD 50 of 4,000 mg/kg of body weight.

Bti applied at label rates has virtually no adverse effects on applicators, livestock, or wildlife including beneficial insects, annelid worms, flatworms, crustaceans, mollusks, fish, amphibians, reptiles, birds or mammals (deBarjac et al 1980, Garcia et al 1981, Gharib and Hilsenhoff 1988, Holck and Meek 1987, Knepper and Walker 1989, Leclair et al 1988, Marten et al 1993, Merritt et al 1989, Molloy et al 1992, Miura et al 1980, Mulla et al 1983, Mulla et al 1982, Purcell 1981, Reish et al 1985, Shadduck 1980, Siegel et al 1987, Tietze et al 1993,1992,1991, Tozer and Garcia 1990). However, non-target activity on larvae of insect species normally associated with mosquito larvae in aquatic habitats has been observed at concentrations of Bti 10 to 1,000 times higher than maximum allowed label rates. There have been reported impacts in larvae belonging to the midge families Chironomidae, Ceratopogonidae, and Dixidae (Anderson et al 1996, Molloy 1992, Mulla et al 1990, Rodcharoen et al 1991, Tozer and Garcia 1990)

These non-target insect species, taxonomically closely related to mosquitoes and black flies, apparently contain the necessary gut pH and enzymes to activate delta-endotoxins.

Bacterial spores of Bti are uniquely toxic to nematoceran Diptera (mosquitoes, midges, black flies, psychodids and ceratopogonids) (Lacey and Mulla 1990). That result was reported after reviewing Bti studies conducted using a variety of Bti formulations and under a variety of test conditions. Lacey and Mulla (1990) concluded that Bti was a highly selective larvicide that produced minimal adverse impact on the environment. Garcia et al. (1981) tested a total of 23 species of aquatic organisms other than mosquito larvae using various formulations of Bti in his laboratory. No mortality was observed for these species with the exception of *Chironomus maturus*, which showed a degree of susceptibility similar to that of mosquito larvae. Miura et al. (1980) found Bti at rates used for mosquito control to be very safe to organisms associated with mosquito breeding habitats. A total of 28 species or species groups were treated with the bacterium under simulated or field conditions, with no adverse effects observed, except for chironomid larvae, which were slightly affected. However, the effect was so light that the population in the field continuously increased after the treatment. Miura et al. (1981) found Bti and *Bacillus sphaericus,* when applied at rates used for mosquito control, very safe to organisms associated with mosquito breeding habitats, including natural enemies of mosquito larvae. When various aquatic organisms were exposed to the bacteria under laboratory, simulated or field conditions, no adverse effect was noted on the organisms with the exceptions of chironomid and psychodid larvae. Chironomid larvae were slightly affected by Bti treatment at a rate used for mosquito control but pyschodid larvae were only affected at the higher concentration (50ppm)(Miura et al., 1981).

After testing mice, rats and rabbits, Siegal et al. (1987) concluded that Bti was not a virulent mammalian pathogen and that it could be used safely in environments where human exposure was likely to occur. Key and Scott (1992) conducted laboratory studies with Bti and *Bacillus sphaericus* against the grass shrimp *Palaemonetes pugio* and the mummichog *Fundulus heteroclitus*. Their study indicated that both Bti and *B. sphaericus* larvicides have large margins of safety. In a study by Aly and Mulla (1987), aquatic mosquito predators were fed with *Cx. quinquefasciatus* fourth-instar larvae intoxicated with either Bti or *Bacillus sphaericus* preparations. Although the mosquito larvae contained large amounts of the bacterial preparations in their gut, no effect upon longevity or ability to molt was observed in the backswimmer *Notonecta undulata*, in naiads of the dragonfly *Tarnetrum corruptum*, or in naiads of the damselfly *Enallagma civile*. Equally, the reproduction of *N. undulata* and the predation rate and ability to emerge normally in *T. corruptu*m and *E. civile* were not affected by ingestion of large amounts of bacterial toxins.

At extremely high doses, negative effects of Bti were obtained when solubilized parasporal crystalline proteins were injected into the intra-abdominal space of Japanese quail (Kallapur et al., 1992). Exposure of brook trout *Salvelinus fontinalis* fry to 4500 and 6000 mg/liter Teknar for 45 min resulted in 20 and 86.4% mortality, respectively (Fortin et al., 1986). Again, it should be noted that the rates tested were more than 50X the allowed label rate for mosquito control.

BACILLUS SPHAERICUS. *Bacillus sphaericus* is a commonly occurring spore-forming bacterium found throughout the world in soil and aquatic environments. This bacterium is also grown in fermentation vats and formulated for application using processes similar to that of Bti. Some strains produce a protein endotoxin at the time of sporulation. This endotoxin destroys the insect's gut in a way similar to Bti and the toxin is only active against the feeding larval stages and must be partially digested before it becomes activated. Bs persists in the environment for two to four weeks and has some ability to recycle (grow and reproduce) within mosquito larvae.

At present, the molecular action of *B. sphaericus* is now well understood. Isolation and identification of the primary toxin responsible for larval activity has demonstrated that it is a protein with a molecular weight of 43 to 55 kD. A standard bioassay similar to that used for Bti has been developed to determine preparation potencies. The bioassay utilizes *Culex quinquefasciatus* 3rd to 4th instar larvae.

B. sphaericus adversely affects larval mosquitoes but, in contrast to Bti, is virtually non-toxic to Black Flies (Simulidae). *Culex* species are the most sensitive to *Bacillus sphaericus*, followed by *Anopheles* and some *Aedes* and *Ochlerotatus* species. In California, *Culex* spp. and *Anopheles* spp. may be effectively controlled. Several species of *Aedes/Ochlerotatus* have shown little or no susceptibility, and salt marsh *Aedes* species are not susceptible. *B. sphaericus* differs from Bti in being able to control mosquito larvae in highly organic aquatic environments, including sewage waste lagoons, animal waste ponds, and septic ditches. Also in contrast to Bti, field evaluations of VectoLex-CG (a commercial formulation of *B. sphaericus*) have shown greater environmental persistence (2-4 weeks), and the ability for limited recycling within larval mosquitoes in some organically rich environments (Rodcharoen, 1991). Persistence varies with a number of environmental parameters, and is low in saline or highly organic environments.

VECTOLEX CG. VectoLex-CG is the trade name for Abbott Laboratories' granular formulation of *B. sphaericus* (strain 2362). The product has a potency of 50 BSITU/mg (*Bacillus sphaericus* International Units/mg) and is formulated on a 10/14 mesh ground corn cob carrier. The VectoLex-CG label carries the "CAUTION" hazard classification. VectoLex-CG is intended for use in mosquito breading sites that are polluted or highly organic in nature, such as dairy waste lagoons, sewage lagoons, septic ditches, tires, and storm sewer catch basins. VectoLex-CG is designed to be applied by ground (by hand, boat or truck-mounted blower) or aerially at rates of 5-10 lb./acre. Best results are obtained when applications are made to larvae in the 1^{st} to 3^{rd} instars. Use of the highest rate is recommended for dense larval populations. Larval mortality may be observed as soon as a few hours after ingestion but typically takes as long as 2-3 days, depending upon dosage and ambient temperature. *B sphaericus* is more expensive than similar Bti products and generally less effective against floodwater and freshwater marsh mosquitoes, and with fewer protein toxins is more susceptible to eventual resistance.

ENVIRONMENTAL IMPACTS OF *BACILLUS SPHAERICUS*. *B. sphaericus* has been extensively tested and has had no adverse effects on mammals or other non-target organisms. *B. sphaericus* technical material was not infective or pathogenic when administered as a single oral, intravenous or intratracheal dose to rats (Shadduck et al, 1980; Siegel and Shadduck, 1990). No mortalities or treatment-related evidence of toxicological effects were observed. The acute oral and dermal LD 50 values are greater than 5000 mg/kg and greater than 2000 mg/kg, respectively. The technical material is only moderately irritating to the skin and eye. Oral exposure of *B. sphaericus* is practically nontoxic to mallard ducks. No mortalities or signs of toxicity occurred following a 9000 mg/kg oral treatment. Birds fed diets containing 20% wt./wt. of the technical material experienced no apparent pathogenic or toxic effects during a 30-day treatment period. Mallards given an intraperitoneal injection of *B. sphaericus* demonstrated toxicological effects including hyperactivity, tremors, ataxia and emaciation. The LD 50 value was greater than 1.5 mg/kg. Acute aquatic fresh water organism toxicity tests were conducted on bluegill sunfish, rainbow trout and daphnids. The 96 hour LC 50 and NOEC (No Observable Effect Concentration) value for bluegill sunfish and rainbow trout was greater than 15.5 mg/liter; the 48 hour EC 50 and NOEC value for daphnids was greater than 15.5 mg/liter. Acute aquatic saltwater organism toxicity tests were conducted on sheepshead minnows, shrimp and oysters. The 96-hour LC 50 value for both sheepshead minnows and shrimp was 71 mg/liter, while the NOEC value was 22 mg/liter for sheepshead minnows and 50 mg/liter for shrimp. The 96-hour EC 50 value for oysters was 42 mg/liter with a NOEC of 15 mg/liter. The LC 50 and NOEC value for immature mayflies was 15.5 mg/liter. Honeybees exposed to l0E4-10E8 spores/ml for up to 28 days demonstrated no significant decrease in survival when compared to controls. Additional studies on various microorganisms and invertebrates, specifically cladocerans, copepods, ostracods, mayflies, chironomid midges, water beetles, backswimmers, water boatmen, giant water bugs, and crawfish, have shown no adverse effects or negative impacts (Holck and Meek 1987, Miura et al 1981, Mulla et al 1984, Rodcharoen et al 1991, Walton and Mulla 1991, Key and Scott 1992, Tietze et al 1993). Furthermore, Ali (1991) states that although *B. sphaericus* is known to be highly toxic to mosquito larvae, *B. sphaericus* does not offer any potential for midge control. Acute toxicity of *B. sphaericus* to non-target plants was also evaluated in green algae. The 120-hour EC 50 and NOEC values exceed 212 mg/liter.

5. Larviciding Techniques And Equipment

Because of the wide range of mosquito sources in the Service Area and the variety of pesticide formulations described above the MVC uses a variety of techniques and equipment to apply larvicides, including hand held sprayers and spreaders, truck- or boat-mounted spray rigs, and helicopters or other aircraft. For a brief description of these application methods, see Durso (1996).

Ground Application Equipment. The MVC uses conventional pick-up trucks as larvicide vehicles. A chemical container tank, high pressure, low volume electric or gas pump, and spray nozzle are mounted in the back of the truck bed, with a switch allowing the driver to operate the equipment and apply the larvicide from the truck's cab. If the MVC were to purchase an ATV, it would have a chemical container mounted on the vehicle, a 12 volt electric pump supplying high pressure low volume flow, and booms and/or hoses and spray tips allowing for application while steering the vehicle. ATV's are ideal for treating areas such as agricultural fields, pastures, and other off-road sites. Additional training in ATV safety and handling would be provided to employees before operating these machines.

Additional equipment used in ground applications includes hand-held sprayers and backpack blowers. Hand held sprayers (hand cans) are standard one or two gallon garden style pump-up sprayers used to treat small isolated areas. Backpack sprayers are gas-powered blowers with a chemical tank and calibrated proportioning slot. Generally a pellet or small granular material is applied with a backpack sprayer or "belly grinder" machine designed to distribute pellets or granules. Larvicides are applied when wind speeds are less than 10 mph.

There are several advantages of using ground application equipment, both when on foot and when conveyed by boat or vehicles. Ground larviciding allows applications while in proximity to the actual treatment area, and consequently treatments to only those microhabitats where larvae are actually present. This also reduces both the unnecessary pesticide load on the environment and the financial cost of the amount of material used and its application. Both the initial and the maintenance costs of ground equipment are generally less than those for aerial equipment. Ground larviciding applications are less affected by weather conditions than are aerial applications.

However, ground larviciding is impractical for large, deep or densely vegetated areas. There is also a greater risk of chemical exposure to applicators than there is during aerial larviciding operations. Damage may occur from the use of a ground vehicle in some areas. Ruts and vegetation damage may occur, although both these conditions are reversible and generally short-lived. Technicians are trained to recognize sensitive areas and to use good judgment to avoid significant impacts.

Aerial Application Equipment. When large areas are simultaneously producing mosquito larvae at densities exceeding MVC treatment thresholds, then the MVC may use helicopters or other aircraft to apply any of the larvicides discussed above. The MVC contracts with independent flying services to perform aerial applications, with guidance to the target site provided by MVC staff and GIS-generated aerial maps with the surveyed target site highlighted. Aerial application of larvicides is a relatively infrequent activity for the MVC, typically occurring only 2-3 times each year, with each application covering from about 30 to a few hundred acres. However, larval production can vary substantially and the MVC is capable of undertaking more frequent or extensive operations.

There are three advantages to using fixed or rotary wing (helicopter) aerial larvicide application equipment compared to ground application. First, it can be more economical for large target areas with extensive mosquito production. Second, by covering large areas quickly, it can free MVC staff to conduct other needed surveillance or control. Third, it can be more practical for remote or inaccessible areas, such as islands and large marshes, than ground larviciding. However, there is a greater risk of drift with aerial applications, especially with liquid aerial larviciding, and consequently there is more potential risk of non-target exposure. In addition, accuracy in hitting the target area temporarily requires additional manpower for on-target direction and electronic guidance systems such as GPS, which can increase costs. Finally, in addition to the timing constraints inherent in most larvicide use, the potential application window can be very narrow for aerial activities due to weather conditions. The MVC minimizes drift by conducting practically all aerial treatments using granular larvicides applied to target aquatic sites with low-flying helicopters equipped with GPS and with ambient wind speed at less than 10mph.

Ahead of the flights, the MVC notifies landowners, officials and media with a news release so that the public is informed. MVC staff warn off people found at the sites ahead of treatment, direct the pilot by radio, and inform the pilot to avoid known bird roosting sites. The helicopter only spends a few minutes at any one site.

6. Managing Larvicide Resistance

Selecting the proper class of larvicide and the formulation are both important in pesticide resistance management, as is rotation of larvicides. For example, use of sub-lethal dosages (below the lower end of the label recommended application rates) may encourage resistance. Insects with inherent tolerances for weakly applied pesticides may survive to produce tolerant offspring. Also, use of extended-release formulations beyond their recommended use period may encourage resistance by exposing mosquitoes to sub-lethal concentrations of active ingredients.

7. Larvicides And Other Control Options

Currently used mosquito larvicides, when applied properly, are efficacious and environmentally safe. These agents have been successfully integrated into the MVC's programs. Historically, Mosquito and Vector Control districts have viewed larviciding as less effective or less economical than physical control, water management, or biological control and as more effective than adulticiding. However, this view developed long ago when the values of wetlands were not as widely recognized as they are today and when relative control costs were different. To some extent, this philosophy has been evolving in recent decades as more selective larvicides have become available, and as physical and biological control have become more constrained by regulatory requirements. While it can be hard to compare the relative environmental impacts of different control strategies, it is now increasingly common to primarily use selective larvicides in relatively undisturbed sites and to emphasize physical control and biological control primarily in man-made or disturbed areas.

Compared to adulticides, larvicides are generally more selective and pose less risk for drift. Larvicides are usually applied directly into natural and man-made aquatic habitats as liquid or solid formulations, and aerial drift is negligible. Drift in water can result from flushing or rainwater runoff, but under those conditions rapid environmental breakdown and dilution reduce pesticide concentration and consequently minimize exposure to non-target organisms.

D. CHEMICAL CONTROL OF ADULT MOSQUITOES

1. Introduction

When physical, biological, and chemical control of larval mosquitoes fails or is otherwise insufficient to reduce disease transmission risk, the MVC may seek Board of Supervisor approval to use insecticides to directly reduce populations of adult mosquitoes (adulticiding) (See Emergency Activities Section 1.F.). Adulticides have been used by the MVC only at one site on one occurrence, early in the ten-year history of the MVC. If adult mosquito populations exceed MVC thresholds (Attachment 4), a public health emergency is declared based on human cases of vector borne disease such as WNV, and Board of Supervisor's approval is obtained MVC staff may use ULV (Ultra Low Volume) sprayers to generate aerosol mists of very small insecticide droplets, which are allowed to intentionally drift into and across areas harboring the target species. Insecticides for control of adult mosquitoes are known as adulticides, and the MVC could select from a variety of materials registered for this purpose. MVC staff could also choose to purchase a variety of adulticide application equipment, ranging from hand-held to vehicle-mounted, and could contract for aerial applications. Please note the distinction between "aerosol" pesticide applications, which describe all MVC adulticiding activity, and "aerial" pesticide applications, which refer to any application of pesticides from aircraft.

The effectiveness and efficiency of adulticiding depends on a number of related factors. First, the mosquito species to be treated must be susceptible to the insecticide applied. Some California mosquitoes are resistant or more tolerant to some adulticides thus affecting the selection of chemical. Second, insecticide applications must be made during periods of adult mosquito activity, which varies between species. Some species of mosquitoes are diurnal (biting in the daytime), others are crepuscular (biting at dawn or dusk), and still others are nocturnal (biting at night). Aerosol applications should be made when the target mosquitoes are flying and are maximally exposed to the aerosol mist. MVC criteria emphasize emergency adulticiding as a potential technique to reduce populations of *Cx. tarsalis* or *Cx. pipiens* (the primary vectors of encephalitis and WNV), or possibly *Och. sierrensis* (dog heartworm) which are primarily crepuscular species. Therefore, MVC adulticiding activity would potentially take place at dawn and dusk.

In addition, technical considerations can influence adulticide effectiveness. First, the application must generate a pesticide concentration in the air that is lethal to the target insect. Second, since the aerosol mist must move from the sprayer to the target mosquitoes; the size of the pesticide droplets is critical to ensure proper movement without rapid evaporation, settling to the ground, or drift away from the target site. Studies have shown that droplets within the 8-15 micron diameter range are most effective in controlling adult mosquitoes (Mount 1998). Third, whether the treatment is ground or aerially applied, sufficient insecticide must be distributed to cover the target site with an effective dose. Densely vegetated habitats may require a higher application rate than open areas to allow the wind to sufficiently carry droplets through the foliage.

Finally, environmental conditions may also affect the results of adulticiding. Wind determines how the ULV droplets will be moved from the sprayer to and within the treatment area. Conditions of no wind will result in the material not moving from the application point. High wind can inhibit mosquito activity and will quickly disperse the insecticide too widely to be effective. Light wind conditions (1-10 mph) are the most desirable both because mosquitoes are most likely to be active and because the aerosol is most likely to maintain the proper concentration as it moves through the target area. Also, ULV applications are generally avoided during hot daylight hours because thermal conditions will cause small droplets to rise, moving them away from mosquito habitats and flight zones. Preferred conditions include the presence of a thermal inversion near the ground, which can trap the aerosol in a mist in the lower ten or twenty feet of the atmosphere, maintaining the proper control dose with minimal material use. Ideal conditions of wind and temperature are generally found around sunrise or sunset, and adulticiding is usually conducted during these times. This practice minimizes exposure of non-target diurnal species such as bees or butterflies. Control of adults of some mosquito species may require modifications of this schedule to accommodate the species flight activity pattern.

MVC criteria on the use of ULV treatments are discussed in 1.F and Attachment 7, and address arboviral emergencies, mosquito species composition and abundance, pathogen (disease organism) presence, proximity of mosquitoes to human populations, presence of an open (no people) target area, and weather conditions. As with larvicides, adulticides would be applied in strict conformance with label requirements.

The adulticide that would potentially be used by the MVC is Pyrethrum (= Pyrethrins; Pyrenone 25-5®). The synthetic pyrethroids Resmethrin and Permethrin have not been used by the MVC, but might be used under some circumstances. For limited adult mosquito populations, an alternative to aerosol fogging for adult mosquitoes is the localized application of synthetic pyrethroid residual barrier sprays to resting surfaces.

2. Pyrethrins And Pyrethroids

INTRODUCTION Pyrethrin (pyrethrum) is a natural insecticide extracted from certain varieties of the flower *Chrysanthemum cinerariaefolium* and consists of six active ingredients collectively known as pyrethrins (Worthing & Hance 1991). This material provides effective control of adult mosquitoes and other insect pests at very low dosage and has little residual activity (persistence) due to its sensitivity to sunlight. The flowers are grown commercially in parts of Africa and Asia. Synthetic analogues of the natural pyrethrins reached commercial success in the 1950's. Like the natural pyrethrins, 'first generation' synthetic pyrethroids such as phenothrin and tetramethrin, are relatively unstable when exposed to light. During the 1960's-1970's, great progress was made in synthetic light-stable pyrethroids. These photostable pyrethroids represent the 'second generation' of these compounds. However, the low persistence of natural pyrethrum means that it is often required in agricultural areas, despite its significantly higher cost (see http://extoxnet.orst.edu/pips/pyrethri.htm).

Pyrethrins and pyrethroids exhibit rapid knockdown and kill of adult mosquitoes, characteristics that are considered a major benefit of their use. The mode of action of these compounds relates to their ability to affect sodium channel function in the insects' neural membranes. Their toxicity in insects is markedly increased by the addition of synergists (primarily piperonyl butoxide) that inhibit detoxification of the pyrethrins in insects. For a discussion of potential health effects of synergists see Section 6.B.1.

Pyrethrins and synthetic pyrethroids are not cholinesterase inhibitors, are non-corrosive and will not damage painted surfaces. They are less irritating than other mosquito adulticides and have a less offensive odor. In comparison to other adulticides, pyrethroids may be effectively applied at much lower rates of active ingredient per acre.

<u>NATURAL PYRETHRIN:</u> Pyrenone 25-5 is a California-registered natural pyrethrin formulation, with a label containing a CAUTION statement. Pyrenone 25-5 contains 5% pyrethrin and 25% piperonyl butoxide. Pyrenone 25-5 is applied as a ULV spray with a dosage per acre of typically 0.87 oz/acre (equivalent to 0.0027 lbs of pyrethrins and 0.0135 pounds of piperonyl butoxide per acre).
For DPR information on environmental fate see http://www.cdpr.ca.gov/docs/empm/pubs/pyrethfate.pdf

RESMETHRIN. Resmethrin is a 1^{st} generation synthetic pyrethroid and is the active ingredient in Scourge. Resmethrin provides rapid knockdown and quick kill of all species of adult mosquitoes, and is also effective against many other flying or crawling insects, although it is slower acting than natural pyrethrins. Resmethrin exhibits very low mammalian toxicity, degrades very rapidly in sunlight and provides little or no residual activity (Muir, 1985). Resmethrin products are available in several concentrations that range from 1.5% to 40% and may or may not contain piperonyl butoxide. Scourge contains 4.14% Resmethrin, 12.54% piperonyl butoxide, 5% aromatic petroleum solvent (a mixture of hydrocarbons) and other inert ingredients. Scourge is labeled with the signal word "Caution", and has a maximum rate of application of 0.007 lbs per acre of the active ingredient. (see http://extoxnet.orst.edu/pips/resmethr.htm.)

PERMETHRIN. Permethrin is a second-generation synthetic pyrethroid with a broad spectrum of activity against all mosquito species. It exhibits fast action, low volatility, good photo stability, low solubility in water, no odor, and low mammalian toxicity. Its photo stability (half life in sunlight 4.6 days) means that permethrin provides some residual activity when applied directly to surfaces. It is formulated as the active ingredient in products such as Permanone and Biomist. Permethrin is a general use pesticide with labels that may contain either the signal word WARNING (Category 2) or CAUTION (Category 3) depending on the particular product. The MVC does not use Category 2 pesticides except in emergency circumstances. Permethrin products are available in various concentrations, from 1.5% to 57% and may or may not be synergized with piperonyl butoxide. Synergized permethrin products may contain piperonyl butoxide in various ratios by weight but the maximum rate of application is 0.007 lbs. per acre of the active ingredient (see http://extoxnet.orst.edu/pips/permethr.htm and http://www.cdpr.ca.gov/docs/empm/pubs/fatememo/permethrin.pdf).

3. Adulticiding Techniques And Equipment

The MVC would apply adulticides, if needed, and following Board of Supervisors' approval, from truck mounted ULV aerosol equipment or from hand-held or ATV-mounted ULV equipment. It is less likely that application from the air would be used.

4. Potential Environmental Impacts Of Adulticiding

Adulticiding poses a number of potential environmental impacts associated with non-target toxicity, pesticide drift, and with disturbance associated with the applications. The mode of action of pyrethrins and pyrethroids means that these pesticides have a wider spectrum of potential non-target toxicity than the larvicides discussed before (Worthing & Hance 1991). In addition, the need for the aerosol mist to drift through the mosquito harborage (target area) generates some risk that materials will spread beyond the intended target area. Judicious use of these materials, based on the MVC's rigorous criteria for selecting and applying these materials and strict adherence to label requirements limits the potential environmental impacts to some extent.

Pyrethrins and pyrethroids are highly toxic to most insects, moderately toxic to many fish and some birds, and much less toxic to other organisms (Mallis 1990, Worthing & Hance 1991). Worthing & Hance (1991) report acute oral LD50 values of pyrethrin of about 150 ug/bee in honey bees, 584-9000 mg/kg for rats, and >10,000 mg/kg for mallard ducks[4]. Percutaneous LD50 values for pyrethrin include >1,500 mg/kg for rats and >5,000 mg/kg for rabbits. Toxicity values for Resmethrin in rats include oral LD50 >2,500 mg/kg and percutaneous LD50 >3,000 mg/kg. For Permethrin, typical oral LD50 values are 430-4,000 mg/kg in rats, 40-2690 mg/kg in mice, >3,000 mg/kg in chickens, and >13,500 mg/kg in Japanese quail. In addition, DPR has reported "possible adverse" chronic toxicity effects associated with repeated exposures at extremely high dosages (exceeding legally allowed label rates by a factor of 100 or more) of Permethrin, Pyrethrins, Resmethrin, and Piperonyl Butoxide (DPR 1999).

Translating these values to a risk assessment of field applications can be difficult because of the complex distributions of both target and non-target species in natural settings. While it is clear that these materials can cause significant immediate mortality in some desirable, non-target insects, recent studies by UC Davis and USFWS researchers have demonstrated rapid (24 - 48 hour) rebound of impacted insect populations following ULV activities with pyrethroids and malathion in the Central Valley (Lawler et al, 1997). In general, mosquito distribution is patchy, so adulticide application is discontinuous; this may allow non-target organisms to recolonize the treated area from nearby untreated areas.

Toxicity in fish is measured as LC50, or the concentration of toxicant in the water that is fatal to 50% of the sample by the end of a fixed time period (often 96 hours). Worthing & Hance (1991) report 96-hour LC50 values for Coho salmon and channel catfish exposed to pyrethrum of 39 mg/L and 114 mg/L respectively. It is not easy to relate these values to the volumes of active ingredient that might drift from a treatment area and settle on water, but the risk has been judged high enough that the pesticide labels for these materials warn not to apply them to lakes, streams, or ponds, or when drift from the application might settle on these areas. However, direct deleterious effects have not been documented for non-targets in aquatic habitats as a result of deposition of currently employed adulticides, probably due to a small mass depositing per unit area and dilution factors such as tidal flushing and water depth (Lawler et al, 1997).

In addition to label restrictions over drift onto sensitive environmental sites, there are operational limitations on these materials based on the need to minimize potential impacts on some classes of sensitive agricultural resources -- in particular, organic farms and honey bees and bee hives. Excessive drift onto organic farms can disrupt agricultural activities and/or lead to loss of organic registration for the farmer. Drift onto honey bees or hives with active bees can kill the bees and destroy the hive. For all these reasons, these types of impacts are not acceptable to the MVC. Target selection and trap surveillance to determine mosquito dispersal patterns is critical to narrowing treated area and reducing environmental risks.

5. Measures To Minimize Environmental Impacts Of Adulticiding

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[4]When an LD50 is reported as greater than a large number, it means that fewer than half of the test animals died at the highest concentration tested.

The most important measure to minimize the potential environmental impacts of adulticiding is spatial and temporal separation of aerosol applications from sensitive species or habitats. In general, this is accomplished by strict compliance with label requirements and MVC criteria. For example, the Environmental Hazards section on labels of pesticides used as mosquito adulticides instruct applicators to avoid direct application over water or drift into sensitive areas due to a potentially high toxicity of these compounds to fish and invertebrates. Although there is some variation in the habitats to be avoided, they usually include lakes, streams and marshes. Also, MVC staff would evaluate the wind speed and direction and the potential for a thermal inversion prior to initiating aerosol activities. Adulticides would not be applied, and in particular not to environmentally sensitive habitats except in a declared public health emergency and with Board of Supervisors approval as described in section 1. F and Attachment 7.

Specific measures include the MVC's emphasis on *Cx. tarsalis, Cx. pipiens and Och. sierrensis* in its emergency adulticide criteria, due to their high potentials as vector organisms. The MVC would apply aerosol adulticides at dawn or dusk, when these target species are active. Fortuitously, this is also when temperature inversions and light wind generally make drift easiest to predict and manage, and also when many non-target insects (bees, butterflies) are not active. The MVC would strive to identify and communicate with beekeepers, to avoid exposing their colonies to adulticiding activities undertaken by MVC staff.

In addition, the MVC maintains a list of sensitive receptors, and has established a policy to notify these receptors prior to aerosol applications and/or to avoid aerosol applications that may drift onto them, except under emergency conditions. Finally, conducting ULV operations in early mornings and late evenings would increase the efficacy of the control operation and minimizes exposure to people and their pets.

As described for larviciding, spray equipment would be calibrated regularly, and at least once a year. Measurements for output and droplet sizes of the pesticides being used would be confirmed to maximize efficiency and minimize potential adverse impacts.

All personnel who apply pesticides receive retraining at least once a year. This training consists of an annual review concerning all aspects of the pesticides the applicator will be handling that year. All applicators are certified by the Department of Health Services on the safe and proper use of pesticides. Applicators must also undergo a minimum of 20 hours of formal continuing education every two years to maintain their state certification.

E. CHEMICAL CONTROL OF OTHER INVERTEBRATES

Wasps in public areas that pose an imminent threat to humans or pets are controlled by the MVC. The MVC will also control Africanized Honey Bees, should they arrive in Santa Cruz County in the future. The MVC does not currently control European honeybees and does not control any wasps that are located on private property or on or inside a structure. Currently, if the Vector Control Specialist finds that the insect in question is a honey bee, the resident will be given a copy of a referral list which contains the names of companies in Santa Cruz County that are certified for control of bees, or beekeepers. In addition, if the insect is a wasp, but the nest is located on private property or on or inside a structure, the resident will be given a copy of a list of companies certified for structural pest control. If a MVC Vector Control Specialist elects to treat stinging insects, he or she will apply an insecticide directly to the insect or insect nest, in accordance with MVC policies to avoid any drift and harm to other organisms, or place tamper-resistant traps or bait stations, selective for the target insect, in the vicinity of the problem animals.

Spectracide®, Vikor®, Wasp Freeze® and PT 515 are insecticides used by the MVC against ground-nesting yellowjackets. Their active ingredients include Pyrethrins, Permethrin, and/or Tetramethrin, synergized by Piperonyl Butoxide (see 6.B.1). The potential environmental impact of this material is very small because the mode of application, deep into underground nests, limits the potential for environmental exposure from these materials. In 2000-2004, the MVC applied just over 1 gallon total of yellowjacket pesticides.

F. CONCLUSIONS: APPROPRIATE USE OF CHEMICAL CONTROL

Santa Cruz County contains many source areas that produce significant vector populations near populated areas. Without ongoing and effective vector control, the resulting vector activity would significantly and adversely effect the human environment. The use of larvicides limits the proliferation of mosquito larvae in aquatic sources, while adulticiding could be used to reduce harmful levels of adult mosquitoes in an emergency situation. Other registered pesticides help control other invertebrate threats to the public health and welfare. In concert with public education and biological control, this combination of control methods helps to provide protection from vector borne disease and nuisance.

G. TREATMENT DECISION MAKING

The Santa Cruz County Mosquito and Vector Control uses a phased approach to vector control. Pesticide treatments are used only after determining whether landowner education and source reduction is more applicable. In the choice of pesticide material MVC personnel choose the material with the least impact to control larvae and, in a public health emergency, localized adulticiding may be chosen. In general this progression of choices would be:

Bti or B. sphaericus → Duplex (Bti + methoprene) → Methoprene → Oil or Agnique → Pyrethroids

Decisions on where and when to treat are based on thresholds (see Larval Treatment Criteria chart, Attachment 4). These thresholds are meant to be guidelines since each site is different and other factors play a role in the levels of mosquitoes that can be tolerated. Some of these factors are as listed:

- The proximity of homes or heavy human use areas to the source
- The age and distribution of the immature mosquitoes in a source
- The number of mosquito service calls attributed to the source from previous seasons
- The expected weather conditions and the season of the year
- The accessibility to the source (including special restrictions)
- The pest or disease significance of the mosquito to be controlled in the source
- The size of the source (staff and equipment needs increase with size)
- The sampling method used to check the source
- The number of active sources and available personnel and equipment
- Abundance of predators
- Presence of sensitive species

The MVC does not treat all sites where mosquito breeding is found. That would be an inefficient use of time, materials and resources and could hasten resistance and cause unnecessary impact. Comprehensive and scientific criteria are used to determine the action threshold for appropriate intervention by means of source reduction (water or vegetation management), landowner education (or abatement enforcement), biological control or chemical larviciding treatment. These criteria are:

- Larval density and abundance
- Mosquito species significance (nuisance or disease)
- Stage of mosquito development
- Flight range
- Dispersal patterns
- Ecological sensitivity of the aquatic site
- The presence, number and type of predators and competitors
- Other environmental, meteorological or epidemiological factors when compared with human and domestic animal activity, injury and proximity.

Treatment decisions are based on adult mosquito surveillance findings (trap counts), complaints and larval dip sampling, and when possible all three. Typically, a suspected breeding site is sampled with a pint, long-handled dipper cup at various edge locations at intervals and an average number per ten dips established. The larvae are then identified or reared in our laboratory. Criteria for the site are then analyzed and often discussed before an action threshold level of larvae is determined for a particular site, beyond which intervention should take place. This action threshold number is then compared with the average number of larvae of that mosquito species per dip sample. The action threshold level is seasonally dynamic, partially intuitive and may change spatially and temporally or when more than one mosquito species are cohabiting.

H. GEOGRAPHIC INFORMATION SYSTEMS

Current GIS Use: The MVC uses GIS to map all mosquito sources. A supplemental database is linked to the location of mosquito sources as well as to the location of monitoring traps. Work activity is tracked by GIS, such as location and amounts of pesticide applications. School sites, California Department of Fish and Game parcels, organic farms, and urbanized areas are mapped and tracked to insure proper application of pesticides and to establish treatment thresholds and buffers. Service requests are also mapped to identify breeding source problems. Maps of wetland areas with mosquito breeding that exceeds threshold levels are provided to a helicopter pilot/Pest Control Adviser who is contracted to conduct aerial larvicide treatments highly targeted with GPS over target areas.

GIS was also used to map existing MVC boundaries and to establish proposed boundaries for expanding the MVC into other parts of the county, by analyzing the number of vector complaint calls per Assessor's parcel book.

Current Data Use: The MVC tracks data in several Access databases that include relevant information required to monitor work requests, pesticide application, and number of site visits. The database also includes trap numbers and common names of trap sites. In addition to the Access database the following GIS data is used; Ortho-Photos (aerials), Parcel Boundaries, School Properties, Streets, Streams, Lakes, Barclay Map Grid, Well Location data, and Assessors Map Pages.

Data Shared: The MVC supplies trap location data to the California Center for Disease Control. They produce hardcopy maps for consultation with Fish and Game that show mosquito breeding and proposed treatments, and for the Santa Cruz County Board of Supervisors that show the number and location of service requests.

Data to contribute to County's GIS Program: The Mosquito and Vector Control MVC has updated wetlands data and is actively mapping the locations of organic farms and sensitive species. This information is critical to help establish thresholds for treatment. The MVC has extensive records on pesticide application that could benefit other departments.

Work Order Tracking: The MVC is actively tracking wetland data that includes lakes, rivers and point sources. Tables are joined to the wetlands data to track the number of site visits, what type of work was conducted at the site, the hours spent, water temperature and other relevant information.