

Best Mosquito Management - Santa Cruz County

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The Santa Cruz County Mosquito Abatement and Vector Control, County Service Area 53 (MVC) has provided mosquito control services to Santa Cruz County since 1994. Our formation came about as a result of prodigious public and local government support following years of complaints about biting mosquitoes. MVC was formed through the Government Code, though responsibilities are similar to those outlined within the California Health and Safety Code (Section 2000 *et seq.*). The language contained in these codes underscore the landowner's responsibility to manage their property, including wetlands, to prevent mosquito breeding.

A **vector** is any insect, rodent, or other arthropod or animal that can threaten health by transmitting disease agents or causing discomfort.

Our public health program employs sustainable **Integrated Mosquito Management (IMM)** methods and emphasizes the prevention of mosquito production by reducing breeding sources and the control of aquatic stages to interrupt the mosquito cycle. This includes the use of least-toxic biorational materials selected on the basis of maximum safety to the public, applicator and environment, and otherwise follows general guidelines of the California Department of Public Health (CDPH), the Mosquito and Vector Control Association of California (MVCAC), the American Mosquito Control Association (AMCA) and the University of California (UC).

California has had periodic outbreaks of mosquito-borne encephalitis and malaria, and currently is involved in an outbreak of West Nile virus. Although human cases have not yet been documented in this County, we have the mosquito vectors that can transmit these diseases and dead wild birds submitted for virus testing have been confirmed positive every year since 2004. As well as the dead bird submission program, the MVC currently maintains two sentinel chicken flocks as part of a statewide disease surveillance program. The birds are tested routinely and these and trapped adult mosquito samples are sent to a laboratory, allowing early warning of the presence of virus in local mosquitoes.

The sources of mosquitoes will always be with us, as a result of rainfall and myriad natural, residential and agricultural standing water sources. With a warming climate and vulnerable population, it is important that this MVC be increasingly diligent in providing reduction of mosquitoes, protection from mosquito-borne diseases and relief from annoyance and biting nuisance. We continue to strive for the support from property owners and residents, regulators and government officials that is necessary for us to be successful in this endeavor.

Seasonality and Variability

Wetlands have the potential to produce large outbreaks of mosquitoes at certain times of year. This MVC, through education and pro-active aerial, boat and ground-based treatments of larval stages, can reduce significantly but not eliminate the nuisance. The MVC makes frequent outreach appeals to the public to reduce standing water and artificial container breeding on their property, but the **proximity** of residents to large bodies of standing water increases their chance of exposure to mosquitoes and mosquito-borne diseases.

Restoration, enhancement and disturbance of wetlands for wildlife can produce mosquitoes as an unintended byproduct. Also, mosquitoes are often the pioneering species in newly flooded or disturbed sites. They can spike to high levels and disperse before a diverse aquatic regime and stasis through predation and competition is established. At least fifteen mosquito species can

exploit sites as diverse as tree holes, neglected septic systems and swimming pools, small containers, stormwater systems, freshwater sloughs and brackish marshes in the mild climate of our County.

Our Vector Control Specialists follow a route list of known breeding sources on a routine basis. The water sources are dip sampled to determine breeding using a standard twelve ounce dipper cup. Mosquito management decisions are made by first establishing a **threshold** level of breeding for each aquatic site on the basis of species type, number per dip, distance to residents, larval age, the presence of predators, presence of sensitive species and other factors. The attached Treatment Criteria table illustrates problem species type and uses human proximity, breeding density and source size as a general basis on which the threshold analysis is built.

Even when protected species are not present, marshes have great ecological and educational value as suburban wetlands, a rarely encountered environment whose biological diversity must be protected. Yet it is because of this proximity to human habitation and activity that management of mosquitoes is occasionally required to protect human health and the annoyance associated with the nuisance species. Selective reduction of mosquito populations in this protected environment is a marsh management element that increases the benefit values to the surrounding community and has benefits to avian life, particularly nestlings and species susceptible to West Nile virus. West Nile Virus has the potential to cause human, wildlife and equine mortality in this area.

Materials and Techniques

There have been remarkable advances in mosquito control materials and methodology, incorporating IMM approaches anchored around the use of public education, source reduction and biocontrols. When appropriate, larvicides are employed, preferably the selective and non-persistent mosquito growth regulator methoprene (synthesized juvenile hormone) and organic microbials (*Bacillus thuringiensis israelensis* and *B. sphaericus*). These are “least toxic” materials that provide maximum safety for the applicator, non-target organisms, residents and their pets and property, with minimum impact on the food web of the marsh. Dosage rates must be both sufficiently high to kill targeted species (and delay resistance) and sufficiently low to minimize non-target effects. Also, it is important that materials be rotated periodically to forestall resistance.

To achieve satisfactory, sustainable and consistent results with these materials, it is essential that they be alternated and used in conjunction with a comprehensive monitoring and source reduction program, and with other elements incorporating Best Management Practices. Knowledge of the biology of the target species and habitat, the timing of the application and other environmental factors related to operational success is crucial to obtaining cost-effective results of a reduction in the mosquito population. MVC staff is experienced in non-intrusive monitoring and treatments of mosquito larvae and adults, and in determining threshold levels for different species that would initiate treatments.

Treatments are conducted on foot using backpack sprayers for liquids or backpack blowers for granular materials, from a marsh boat or by contracted helicopter. Pesticides are applied when winds are less than 10 mph to avoid drift. Compromises are made in sensitive sites, such as a less intrusive management protocol arranged with the Fish and Wildlife Service for the federal refuge (see Wildlife Refuges section or Draft Monitoring and Treatment Plan). Temporary trails may be cut to less accessible areas, invasive plants reduced and poison oak kept at bay with spot treatments of herbicide, where appropriate. If brushing a trail is necessary, care is taken to reduce impact to green limbs or saplings.

The MVC is involved in the development review process in a pro-active role as part of our source reduction program. Due to the potential disruption and public health threat that infestations of mosquitoes could cause residents of this area, their existence is a **significant biological hazard** that requires mitigation, and should be included on initial study checklists.

Intense efforts are made to monitor mosquito breeding through trapping of adult mosquitoes, and inspecting breeding sites by sampling water for larvae and treating when necessary to reduce emergence. Alternative management strategies and biological control methods are first considered. Some sources require checking and treatment several times a year. Pre-treatment and post-treatment larval counts are taken to measure effectiveness and detect resistance. The contents of adult mosquito monitoring traps are also identified and counted to determine abundance and distribution of species.

Quality of life would seriously be impacted without mosquito reduction measures. With present or even increased levels of service, surviving mosquitoes in some years could still be numerous enough to result in complaints because breeding sites are frequently in close proximity to human activity and mosquitoes can disperse long distances. Priority is placed upon requests for service from residents, ahead of our other routine operations.

The MVC would rarely consider adulticiding measures (fogging), as it is more selective and effective to control larvae before they emerge and disperse as adults. Although fogging is perceived by some citizens as offering the best relief, it is unacceptable to others and is not a proactive strategy. It would be considered by the MVC as a last alternative and after approval by the West Nile Virus Technical Advisory Committee and the County Board of Supervisors, in cases of public health emergency.

Mosquitoes of Santa Cruz County / Establishing the Treatment Threshold

The floodwater mosquito, *Aedes washinoi*, is a day-biting species whose aquatic larvae appear in temporary pools in late winter, emerging to seek blood meals in the spring. This mosquito results in more complaints than any other does. *A. washinoi* is an aggressive daytime biting pest in the spring, and breeds densely in flooded willows and brackish marshes.

The County is home to a dozen mosquito species, but of particular concern is *Culex tarsalis*, the encephalitis mosquito, which breeds through summer and fall and has the potential of transmitting the disease to humans. They can be found among emergent vegetation such as smartweed and flooded grasses, algal mats and inundated blackberry thickets. The increase in warm-season human activity at dusk in areas surrounding the sloughs requires that we lower the threshold at which treatments are initiated in order to reduce mosquito breeding to acceptable levels. Criteria used to determine the threshold are larval density, species significance (nuisance or disease), flight range, dispersal patterns and other environmental and meteorological factors.

Treatment decisions are based on threshold levels of larvae determined for particular sites by evaluating species risk, proximity to residents, stage of development and abundance, ecological value, presence, number and type of predators and other aquatic life and other environmental factors. This number is dynamic, based on qualitative as well as quantitative observation and may change spatially and temporally. When threshold levels are exceeded, larviciding ensues using material appropriate for larval instars present: either the microbial formulations described below, or the insect growth regulator methoprene, or a duplex combination of the two. Pre-flood treatments are employed in some seasonal sources where historical breeding has existed and access is a problem post-flooding.

At present the program makes maximum effort to use *Bacillus thuringiensis israelensis* larviciding products for their efficacy and selectivity. A similar microbial larvicide, *Bacillus sphaericus* is used increasingly in highly organic sources such as sewage, dairy and apple processing ponds because the live bacteria recycles in the mosquito larvae it controls, thereby providing residual control. This is also OMRI accepted for use around organic crops. A new minimum risk biological larvicide, spinosad, is expected to be registered for mosquitoes in 2010.

The larvicide methoprene is used with water or granular carriers (for penetration of vegetation), or in pellet or briquet form for residual slow release and pre-flood. This insect juvenile growth hormone mimic prevents emergence of live adult mosquitoes and so requires post-treatment collection and emergence of pupae to determine efficacy. These products and the bacilli are applied to larger aquatic areas by contracted helicopter, by boat or on foot by backpack sprayer or motorized backpack blower.

Mature marshes with diverse species seldom contain many mosquitoes, as predators, parasites and competing invertebrates keep them in check. Open water areas with good circulation seldom breed because of wave action and access by predators. Permanent aquatic sources usually contain natural mosquito predators and do not require treatment, unless vegetation is so dense that it prevents natural predation.

Mosquitoes are often the pioneer species, which accounts for their peak density in disturbed aquatic environments. Frequently, the aquatic habitats targeted for larviciding are temporary or semi-permanent. Temporary sites such as marshes and flooded agricultural areas or woodland depressions produce prolific numbers of floodwater mosquitoes. These sites are generally very low in species diversity and mosquito predators, due to the time needed for most species to locate and colonize them.

While floodwater mosquitoes start development during the first week post-inundation, it may take two to three weeks for the first macro invertebrate predators to become established. Microbials and the growth hormone work effectively in the first week or so against larval mosquito stages. If ponds are treated late in the mosquito cycle for pupal mosquitoes by using less selective surface films, non-target aquatic invertebrates may also be killed. Although these are capable of eventually recovering from localized population declines via recruitment and re-colonization from proximal areas, surface films are usually used only in stagnant conditions where mosquitoes are the dominant organisms.

Mosquito management and source reduction decisions are enhanced by knowledge of the distribution and life stage of protected species. The most important aim is to reduce mosquitoes below the nuisance threshold without harm to the environment, with emphasis on targeting vector species. The MVC is signatory to CDPH compliance agreements (H&S Code section 116180) including the reporting of any adverse pesticide-related effects. We give high consideration to the direction and research provided by vector ecologists and IPM specialists within the UC system, and consultation with wetland managers and health authorities.

The MVC is committed to improving the quality of life and economic productivity of area residents and the habitability of the surrounding community. A wide variety of aquatic habitats, ranging from residential receptacles to larger agricultural and marshland areas, may be treated with larvicides. Fauna inhabiting the latter sites may include amphibians, fish, other vertebrates and invertebrates, particularly insects and crustaceans. The use, description, and safety of our selected larviciding materials are detailed in the attached appendix and in our environmental review documents online at <http://www.agdept.com/mvc.html>.

Other Biological Control

Although established in California in many permanent natural sources, mosquitofish are non-native and it is **not** the policy of the MVC to stock these opportunistic feeders in natural sources. The MVC warns against the re-release of *Gambusia affinis* before stocking these useful fish for the public in ornamental ponds, swimming pools and troughs.

Bats have proven their usefulness against arthropod pests when they can be lured to stay in properly built bat-houses. However, besides preferring larger (sometimes beneficial) flying prey

to maximize protein intake vs. energy expenditures, bats feed at dusk. The primary mosquito problem following winter rains are day-feeding *Aedes spp.* The CDPH warns that a small percentage of bats are rabid, and that bats often prefer attics to a bat-house.

Violet-green swallows are likewise often recognized as a supplement to mosquito management when they can be established, but are likewise not preferential in feeding or nest sites, and their mud homes, mites and excrement can, unfortunately, be a nuisance around structures.

Environmental Protection

Our area has myriad mosquito breeding sites near and within populated areas. Without ongoing and effective vector control, substantial mosquito activity would significantly and adversely effect the human environment. The MVC's mosquito control program, including chemical materials, is essential to maintain the vectors in the environment at a tolerable level. The MVC's program will never alleviate all mosquitoes. Rather, it is a resource maintenance program aimed at striking a balance to allow comfortable and healthful human existence within the natural environment, while protecting and maintaining the environment.

History has shown us that the control and abatement of vectors are necessary for our human environment to continue to be habitable. Malaria was largely responsible for the tragic and precipitous decline of native Californians in the 19th century, and with yellow fever, encephalitis and dengue continued to sicken thousands of Americans into the 20th century. Mosquito-borne diseases are currently a leading cause of human mortality worldwide. Modern recognition of the value of wetlands should not overshadow their potential for pestilence.

The Endangered Species Act has language that limits the regulations from placing undue burden upon essential local operations, including mosquito management, and places the burden of proof of harm on the regulators. Indirect effects causing harm must be close and actual, without remote causal links (effect on effect on effect). Ecologists recognize that the value to the food web of mosquitoes is not substantially missed when they are reduced in wetlands close to human activity, as that niche is adequately filled by other invertebrate scavengers.

In addition to the environmental protection measures and procedures inherent in the MVC's IMM program as discussed above, there are other practices unique to the MVC's chemical control program that protect the environment:

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.) The MVC applies aquatic larvicides under an NPDES permit required in waters of the U.S. and reports use to the Central Coast Regional Water Quality Control Board and has a Best Management Plan on file with that agency. The MVC and its staff consistently comply with these laws and regulations.

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

Consistent with the MVC's integrated mosquito management principles, when using pesticides, the MVC selects the least hazardous material that will meet its goals and the MVC avoids using restricted materials-type pesticides.

The MVC is an active member of the MVCAC, a statewide association representing the interests of vector control districts throughout the state. The Association, and its member districts, participates in the U.S. Environmental Protection Agency's Pesticide Environmental Stewardship Program, a program to encourage less pesticide use and greater environmental stewardship by vector control districts.

Pesticides are applied only by CDPH-certified and trained vector control technicians. The frequent training includes continuing education on appropriate practices to avoid environmental impacts and assure compliance with regulatory requirements.

The MVC regularly calibrates its pesticide application equipment to ensure that it distributes the proper quantities of material. It stores the material safely and under scrutiny of the Agricultural Commissioner and Environmental Health departments. Pesticide use is well documented and overseen by the Agricultural Commissioner. Application sites are measured and digitized into geographic information systems maps and related to trap monitoring and field records in the database, which is shared on a UC server with MVCAC and CDPH.

Wildlife Refuges

The MVC maintains good communication and cooperation with environmental regulatory agencies. Consultation is provided to these agencies for their wetlands restoration projects to ensure compatibility with mosquito management goals.

In our current Zones of Benefit, there are about 15 acres of wetland within the 300 acre Ellicott Slough Refuge/Reserve jointly managed by U.S. Fish and Wildlife Service (USFWS) and California Fish and Game (CDFG) that offers protection for the endangered Santa Cruz long-toed salamander and threatened red-legged frog and tiger salamander, and another 130 acres managed by CDFG as ecological reserve in the Watsonville Slough. The MVC also reports pesticide applications to the State Park system on its coastal freshwater marshes at Sunset State Beach, Twin Lakes and Natural Bridges.

In 2000 the MVC met with USFWS representatives and it was determined to defer using the larvicide methoprene in the Santa Cruz Long-toed Salamander ponds on the Ellicott Slough State Ecological Reserve and at the Calabassas unit of the Ellicott Slough National Wildlife Refuge (ESNWR) pending a USFWS Section 7 (inter-agency consultation) for the Refuge, which will result in a permit or Comprehensive Conservation Plan that will determine mosquito management alternatives. The MVC has since submitted Pesticide Use Proposals (PUP's) to the USFWS for larviciding materials to be used in the ESNWR. The PUP's outline application rates, target mosquito species, methods of application and the listed sensitive species and helps to ensure compatible use through the MVC conforming to Best Management Practices (BMP) in the Refuge area. A draft Monitoring and Treatment Plan has recently been submitted.

A protocol agreed upon with the USFWS for treatment of mosquito larvae in ponds and ditches of the Ellicott area amphibian refuge reduces the possibility of non-target impacts. Applications are made under the following conditions:

- Applications are made from shorelines of ponds. The MVC staff does not enter the water.
- The San Francisco Bay Area National Wildlife Refuge office is notified so that a USFWS biologist can be present when larvicides are applied.
- All staff sampling for mosquito larvae with a dipper are informed of the appropriate techniques for avoiding the capture of amphibian larvae or dislocating eggs and egg masses and for their release if they are inadvertently caught. Staff conducting monitoring activities does not enter the water.

Note: In addition, the MVC informs USFWS of surveillance activities at Ellicott and faxes a treatment map following applications. Similarly, amphibian protection measures are taken on a voluntary basis on other properties where amphibian recovery efforts are being conducted. Currently, the USFWS is reviewing mosquito management in its refuge. Other areas within the South County are being considered for acquisition within the federal refuge and State reserve systems.

Previously Submitted to the USFWS:

1. Appendix: The use, description, and safety of methoprene and microbial materials
2. Treatment Criteria table used as basis for building treatment threshold determination.
3. "Long-term effects of the mosquito control agents Bti and methoprene on non-target macro-invertebrates in wetlands in Wright County, MN (1997-98)", Lake Superior Research Institute.
4. USGS News Release: X-ray Studies Shed Light on Frog Deformities.
5. "Methoprene concentrations in freshwater microcosms treated with sustained-release Altosid formulations", Ross, Judy, Jacobsen and Howell, 1994.
6. "Nontarget effects of mosquito larvicides used on national wildlife refuges", Lawler, Jensen and Dritz, 1997.
7. "Laboratory and field evaluation of the efficacy of four insecticides for *Aedes vigilax* and toxicity to the non-target shrimp *Leander tenuicornis*." Brown, Thomas, Mason, Greenwood and Kay, 1999.
8. "Insect developmental inhibitors. 3. Effects on nontarget aquatic organisms." Miura and Takahashi, 1973.
9. "Field evaluation of the effects of slow-release wettable powder formulation of Altosid on nontarget organisms." Creekmur, Russell and Hazelrigg.
10. "Potential effects of Altosid briquet treatments on *Eubbranchipus bundyi*." Batzer and Sjogren.
11. "Environmental degradation of the insect growth regulator methoprene. II. Metabolism by aquatic microorganisms." Schooley, Bergot, Dunham and Siddall.
12. "Effects of methoprene on nontarget organisms when applied as a mosquito larvicide." Hester, Rathburn and Boike.
13. Report for the Ministry of Health (New Zealand): Environmental and health impacts of *Bacillus thuringiensis israelensis*. Glare and O'Callaghan, 1998.
14. Report for the Ministry of Health (New Zealand): Environmental and health impacts of the insect juvenile hormone analogue, s-methoprene. Glare and O'Callaghan, 1999.

APPENDIX

More about Methoprene

INTRODUCTION. s-Methoprene does not produce non-discriminatory, rapid toxic effects that are associated with nervous system toxins. s-Methoprene is a true analogue and synthetic 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 larva 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 becomes unbalanced. When this happens during the sensitive period, the unbalance 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. For these and perhaps other reasons, s-Methoprene is considered an insect growth regulator (IGR). An advantage that methoprene has over other larvicides is that the mosquito immatures remain alive as prey for aquatic predators.

There have been widely distributed reports regarding the effect methoprene may have on certain amphibians. Reports of frog abnormalities have been widely circulated, but these reports have not stood up to scientific scrutiny.

FORMULATIONS AND DOSAGES. Currently, seven s-methoprene formulations are sold under the trade name of Altosid. These include Altosid Liquid Larvicide (A.L.L.) and Altosid Liquid Larvicide Concentrate, Altosid Briquets, Altosid XR Briquets, Altosid SBG (single-brood granules), Altosid XR-G and Altosid Pellets. Altosid labels contain the signal word "CAUTION" and all products are Category 4.

ALTOSID LIQUID LARVICIDE (A.L.L.) & A.L.L. CONCENTRATE. These two flowable formulations have identical components except for the difference in the concentration of active ingredients. 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.

DOSAGES. Use rates are 3 to 4 ounces of A.L.L. 5% and $\frac{3}{4}$ to 1 ounce of A.L.L. Concentrate (both equivalent to 0.01008 to 0.01344 lb. AI) per acre, mixed in water as a carrier and dispensed by spraying with conventional ground and aerial equipment. Because the specific gravity of Altosid Liquid is about that of water, it tends to stay near the target surface. No rate adjustment is necessary for varying water depths when treating species that breathe air at the surface.

TARGET SPECIES. Liquid formulations are designed to control fresh and saline floodwater mosquitoes with synchronous development patterns. Cold, cloudy weather and cool water slow the release and degradation of the active ingredient as well as the development of the mosquito larvae. Accordingly, formulation activity automatically tracks developing broods.

ALTOSID BRIQUETS. The Altosid Briquet was the first solid methoprene product marketed for mosquito control beginning in 1978. It is made of plaster (calcium sulfate), 3.85 % (wt./wt.) r-methoprene, 3.85% s-methoprene (.000458 lb. AI/briquet) and charcoal (to retard ultra violet light degradation). Altosid Briquets release methoprene for about 30 days under normal weather conditions.

DOSAGES. Application should be made at the beginning of the mosquito season, and under normal weather conditions, repeat treatments should be carried out at 30-day intervals. The recommended application rate is 1 Briquet per 100 sq. ft. in non-flowing or low-flowing water up to 2 feet deep.

TARGET SPECIES. Flood water *Aedes* and permanent water *Anopheles*, *Culex*, and *Culiseta* larvae are usual targets. Typical treatment sites include storm drains, catch basins, roadside ditches, ornamental ponds and fountains, cesspools and septic tanks, waste treatment and settlement ponds, flooded crypts, transformer vaults, abandoned swimming pools, construction and other man-made depressions.

ALTOSID XR BRIQUETS. It is made of hard dental plaster (calcium sulfate), 1.8% (wt./wt.) s-methoprene (.00145 lb. AI/briquet) and charcoal (to retard ultra violet light degradation). 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 up to 150 days in normal weather.

DOSAGES. XR Briquets should be applied 1 to 2 per 200 sq. ft. in no-flow or low-flow water conditions, depending on the species.

TARGET SPECIES. Targets are the same as for the smaller briquets. Appropriate treatment sites for XR Briquets include storm drains, catch basins, roadside ditches, ornamental ponds and fountains, cesspools and septic tanks, waste treatment settlement ponds, flooded crypts, transformer vaults, abandoned swimming pools, construction and other man-made depressions, cattail swamps and marshes, water hyacinth beds, pastures, meadows, rice fields, 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% (wt./wt.) s-methoprene (0.04 lb. AI/lb.), dental plaster (calcium sulfate), and charcoal. Like the Briquets discussed above, Pellets are designed to slowly release s-methoprene as they erode. Under normal weather conditions, control can be achieved for up to 30 days.

DOSAGES. 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.

TARGET SPECIES. The species are the same as listed for the briquet 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, flooded crypts, transformer vaults, abandoned swimming pools, construction and other man-made depressions, tree holes, storm drains, catch basins, and waste water treatment settling ponds.

ALTOSID SBG is the newest formulation, at 1.5% (wt./wt.) s-methoprene it has a five to seven day residual for use with mosquitoes having synchronous development patterns (single-brood). It has a small particle size but high density and a broad target site list.

DOSAGES. Label application rates range from 5 lbs. to 20.0 lbs. per acre, depending on the target species and/or habitat.

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.

DOSAGES. Label application rates range from 5 lbs. to 20.0 lbs. per acre, depending on the target species and/or habitat.

TARGET SPECIES AND APPLICATION SITES. The species are the same as listed for the briquet formulations. Listed target sites include snow pools, 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.

More About Bacillus Products

Mosquito control makes use of two stomach 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 surface-active agents. 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 (butterflies and moths) 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 larvicide properties.

INTRODUCTION. Like a tiny chemical factory capable of only one production run, each **B.t.i.** organism may produce, if the environmental conditions around it 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 the d-endotoxin is ingested, these five proteins are released in the alkaline environment of an insect larvae's gut. The five proteins are converted into five different toxins if specific enzymes also are present in the gut. Once converted, these toxins work alone or in combination to destroy the gut wall. This leads to paralysis and death of the larvae.

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 microfiltration of the slurry. It can then be dried for processing and packaging as a solid formulation(s) or further processed as a liquid formulation(s). Since some fermentation medium (e.g. fish meal) is always present in liquid formulations, they generally smell somewhat like the medium.

FORMULATIONS AND DOSAGES. There are five basic B.t.i. formulations available for use: liquids, powders, 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. Powders, in contrast to liquids, may not always have a uniformly small particle size. Clumping, resulting in larger sizes and heavier weights, can cause particles to settle out of the feeding zone of some target mosquito larvae, preventing their ingestion as a food item. Powders must be tank mixed before application to an inert carrier or to the larval habitat, and it may be necessary to mix them thoroughly to achieve a uniformly small consistency. 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" and B.t.i. is Category 4.

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 1ST to 2ND, 2ND to 3RD, and 3RD to 4TH instars. If we apply B.t.i. at these points in their development, the toxic crystals may settle out before the larvae resume feeding, and with synchronous broods of mosquitoes, complete control failures may result. With asynchronous broods, efficacy may be reduced. Kills are usually observed within 24 hours of toxin ingestion.

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 (ITU's) and *Aedes 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.

BTI LIQUIDS. Currently, three commercial brands of B.t.i. liquids are available: Aquabac XT, Teknar HP-D, and Vectobac 12AS.

DOSAGES AND FORMULATIONS. 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 organic 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 "Duplexed" with the Altosid Liquid Larvicide discussed above. 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) allows a public health agency 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

BTI CORNCOB GRANULES. Granular formulations use a carrier that is dense enough to penetrate heavy vegetation. 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 highly organic water situations.

BTI PELLETS. Bactimos Pellets are the only extruded B.t.i. product on the market today. They are manufactured using a larval food as the B.t.i. carrier, and the manufacturer claims that this helps attract feeding larvae. The Pellets contain twice the amount of toxic units as Bactimos (corn cob) Granules, and the label correspondingly recommends using only half as much by weight in both clean water and organic water situations.

BTI BRIQUETS (donuts). B.t.i. donuts are a sole source product manufactured by Summit Chemical Company under a Bactimos B.t.i. subregistration. They are a mixture of B.t.i., additives, and cork. They are designed to float and slowly release B.t.i. particles for up to 30 days. They apparently are attractive to raccoons and possibly other wildlife because of their odor, and may sometimes be disturbed or carried off. Donuts may be staked in place to prevent wind from moving them from a site's littoral zone into open water. The use rate is one donut per 100 square feet in clean water and up to four donuts per 100 square feet in dirty water. Many districts

have not found these to be practical in most larval sites due to their expense and the possibility of them being moved by wind or animals. Homeowners, however, may find practical uses for these in ornamental ponds or other very small habitats.

TARGET SPECIES. B.t.i. adversely affects larval stages of insect species in the Order Diptera, Suborder Nematocera, Families Culicidae (Mosquitoes) and Simuliidae (Black Flies). B.t.i. has been shown to be effective for numerous mosquito species, including members of the mosquito genera *Aedes*, *Anopheles*, *Culex*, and *Culiseta*, commonly targeted in California.

Products containing B.t.i. are ideally suited for use in integrated pest management programs because the active ingredient does not interrupt activities of most beneficial insects and predators. Since B.t.i. has a highly specific mode of action, it is an insecticide of minimal environmental concern. B.t.i. 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. B.t.i. is fast acting and its efficacy can be evaluated almost immediately. It usually kills larvae within 1 hour after ingestion, and since each instar must eat in order for the larvae to grow that means B.t.i. usually kills mosquito larvae within 24 hours of application. It leaves no residues, and it is quickly biodegraded. Resistance is unlikely to develop simultaneously to the five different toxins derived from the B.t.i. delta-endotoxin since they have five different modes of action. This suggests that this mosquito larvicide will continue to be effective for many years.

B.t.i. 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. Toxicology profiles also suggest that the inert ingredients (not the B.t.i.) in liquid formulations, may cause minor eye irritations 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.

B.t.i. 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. However, non-target activity on larvae of insect species normally associated with mosquito larvae in aquatic habitats has been observed. There have reported impacts in larvae in the Order Diptera, Suborder Nematocera, Families Chironomidae (midges), Ceratopogonidae (biting midges) and Dixidae (dixid midges). 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. However, the concentration of B.t.i. required to cause these effects is 10 to 1,000 times higher than normal use rates.

Further, studies report these impacts are short-lived, with the population of these species rebounding quickly. **See study "Long-term effects of the mosquito control agents Bti and methoprene on non-target macroinvertebrates in wetlands in Wright County, Minnesota (1997-1998)".**

Concerning the operational use of B.t.i., timing of application is extremely important. Optimal benefits are obtained when treating 2nd or 3rd instar larvae. Treatments at other development stages may provide less than desired results. Therefore a disadvantage of using B.t.i. is the limited treatment window available.

INTRODUCTION. *Bacillus sphaericus* is a commonly occurring spore-forming bacterium found throughout the world in soil and aquatic environments. Some strains produce a protein endotoxin at the time of sporulation. It is grown in fermentation vats and formulated for end use

with processes similar to that of B.t.i. A standard bioassay similar to that used for B.t.i. has been developed to determine preparation potencies. The bioassay utilizes *Culex quinquefasciatus* 3rd-4th instar larvae. The endotoxin destroys the insect's gut in a way similar to B.t.i. and has been shown to have activity against larvae of many mosquito genera such as *Culex*, *Culiseta*, and *Anopheles*. The toxin is only active against the feeding larval stages and must be partially digested before it becomes activated. At present, the molecular action of *B. sphaericus* is unknown. 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.

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.

DOSAGES. VectoLex-CG is intended for use in mosquito breeding 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 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 1st to 3rd 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. VectoLex-G should be stored in a cool, dry place, in an intact product package. Once the VectoLex-G package is opened, moisture can be absorbed by the product leading to loss of activity over time. Refrigeration is not necessary.

TARGET SPECIES. *B. sphaericus* adversely affects larval stages of insect species in the Order Diptera, Suborder Nematocera, Family Culicidae (mosquitoes). *Culex* species are the most sensitive to *Bacillus sphaericus*, followed by *Anopheles* and some *Aedes* species. In California, *Culex* spp. and *Anopheles* spp. may be effectively controlled. Several species of *Aedes* have shown little or no susceptibility, and salt marsh *Aedes* species are not susceptible. *Bacillus sphaericus*, in contrast to B.t.i., is virtually non-toxic to Black Flies (Simuliidae).

B. sphaericus has demonstrated the unique property of being able to control mosquito larvae in highly organic aquatic environments, including sewage waste lagoons, animal waste ponds, and septic ditches. After a single application at labeled rates, field evaluations have shown VectoLex-CG to persist for 2-4 weeks. Field evaluations with VectoLex-CG have shown that *Bacillus sphaericus* may undergo limited recycling in mosquitoes in certain organically rich environments.

VectoLex-CG has been extensively tested and has had no adverse effects on mammals or non-target organisms. *B. sphaericus* technical material was not infective or pathogenic when administered as a single oral, intravenous or intratracheal installation in rats. 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 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% w/w 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 toxicologic effects including hypoactivity, tremors, ataxia and emaciation. The LD 50 value was greater than 1.5 mg/kg.

Acute fresh water fish toxicity tests were done on bluegill sunfish, rainbow trout and daphnids. The 96-hour LC 50 and NOEC 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 fish toxicity tests were done on sheep head minnows, shrimp and oysters. The 96 hour LC 50 value for both sheep head minnows and shrimp was 71 mg/liter, while the NOEC (no observable effect concentration) value was 22 mg/liter for sheep head 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.

Invertebrate toxicity tests were done on mayfly larvae and honeybees. The LC 50 and NOEC value for mayfly larvae was 15.5 mg/liter. Honeybees exposed to 10E4-10E8 spores/ml for up to 28 days demonstrated no significant decrease in survival when compared to controls. Acute toxicity of *B. sphaericus* to non-target plants was evaluated in green algae. The 120-hour EC 50 and NOEC values were greater than 212 mg/liter.

Bacillus sphaericus will not regenerate in salt water, rendering its use impractical for control of salt-water mosquitoes. Cycling is limited to permanent fresh water bodies, and if organics are very high, recycling may be minimal.

VectoMax™ is a biological larvicide that combines B.t.i. and *Bacillus sphaericus* in one homogenous formulation. These two bacteria are combined in one molecule which ensures that larvae feed on both at the same time. VectoMax offers the faster, broad spectrum control of VectoBac® (B.t.i.), with the residual control of VectoLex® (*B. sphaericus*).

B.t.i. and *B. sphaericus* are naturally occurring bacteria that have insecticidal activity on mosquitoes. This bacteria contain protein crystals that, when ingested by mosquito larvae, will rupture the gut wall or the larvae. This results in rapid death of the larvae. Can be used in wide range of sources and habitat. Urban and rural, agricultural sites, crop and non-crop, lakes, ponds, wetland and marshes, catch-basins and swimming pools, etc.

Spinosad is biologically derived from the fermentation of *Saccharopolyspora spinosa*, a naturally occurring soil organism. This Group 5, organic-rated larvicide overstimulates the nervous system of mosquito larvae. Available in CA by summer 2010.

* Criteria used to determine the action threshold are larval density, species significance (nuisance or disease), flight range, dispersal patterns and other environmental and meteorological factors combined with human and domestic animal activity, injury and proximity. Treatment decisions are based on threshold levels of larvae determined for particular sites by evaluating species, proximity to residents, stage of development and abundance, ecological value, presence, number and type of predators and other aquatic life and other environmental factors.