APPENDIX

SUPPLEMENTAL IVMP INFORMATION
CALIFORNIA
MOSQUITO-BORNE VIRUS
SURVEILLANCE
&
RESPONSE PLAN

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# CALIFORNIA MOSQUITO-BORNE VIRUS SURVEILLANCE AND RESPONSE PLAN

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Objectives

The California Mosquito-borne Virus Surveillance and Response Plan was developed to meet several objectives. Specifically, the Plan:

- Provides guidelines and information on the surveillance and control of mosquito-borne viruses in California, including West Nile, St. Louis encephalitis, and western equine encephalomyelitis viruses;
- Incorporates surveillance data into risk assessment models;
- Prompts surveillance and control activities associated with virus transmission risk level;
- Provides local and state agencies with a decision support system; and
- Outlines the roles and responsibilities of local and state agencies involved with mosquito-borne virus surveillance and response.

This document provides statewide guidelines, but can be modified to meet local or regional conditions.

Introduction

California has a comprehensive mosquito-borne disease surveillance program that has monitored mosquito abundance and mosquito-borne virus activity since 1969 (Reeves et al. 1990) and is an integral part of integrated mosquito management programs conducted by local mosquito and vector control agencies. Surveillance and interagency response guidelines have been published previously by the California Department of Public Health formerly known as the California Department of Health Services (Walsh 1987) and the Mosquito and Vector Control Association of California (Reisen 1995). The detection of West Nile virus (WNV) in New York, a virus not recognized in the Western Hemisphere prior to 1999, prompted the review and enhancement of existing guidelines to ensure that surveillance, prevention, and control activities were appropriate for WNV. From New York, WNV spread rapidly westward and by 2004 had been detected in all 48 of the continental United States. In addition to WNV, California is vulnerable to introduction of other highly virulent mosquito-borne viruses of public and veterinary health concern, such as Japanese encephalitis, dengue, yellow fever, Rift Valley fever, chikungunya and Venezuelan encephalitis viruses. If an existing or introduced virus is detected, it is critical that local and state agencies are prepared to respond in a concerted effort to protect people and animals from infection and disease. The current document describes an enhanced surveillance and response program for mosquito-borne viruses in the State of California. Its contents represent the collective effort of the California Department of Public Health (CDPH), the Mosquito and Vector Control Association of California (MVCAC), and the University of California at Davis (UCD).

Background

Mosquito-borne viruses belong to a group of viruses commonly referred to as arboviruses (for arthropod-borne). Although 12 mosquito-borne viruses are known to occur in California, only WNV, western equine encephalomyelitis virus (WEE) and St. Louis encephalitis virus (SLE) are significant causes of human disease. WNV continues to seriously impact the health of humans, horses, and wild birds throughout the state. Since 2004, there have been 3,622 WNV human cases with 129 deaths and 1,188 horse cases. Consequently, the California Arbovirus Surveillance Program emphasizes forecasting and monitoring the temporal and spatial activity of
WNV, WEE, and SLE. These viruses are maintained in wild bird-mosquito cycles that do not depend upon infections of humans or domestic animals to persist. Surveillance and control activities focus on this maintenance cycle, which involves primarily Culex mosquitoes, such as the western encephalitis mosquito, Culex tarsalis, and birds such as house finches and house sparrows.

Immature stages (called larvae and pupae) of Culex tarsalis can be found throughout California in a wide variety of aquatic sources, ranging from clean to highly polluted waters. Most such water is associated with irrigation of agricultural crops or urban wastewater. Other mosquito species, such as Culex pipiens, Culex quinquefasciatus, and Culex stigmatosoma, play an important role in WNV, and possibly SLE, transmission cycles in urban and suburban areas. Historically, Aedes melanimon, a floodwater mosquito, played a role in a secondary transmission cycle of WEE involving rabbits. Additional mosquitoes such as Aedes vexans and Culex erythrothorax also could be important bridge (i.e. bird to mammal) vectors in transmission.

Mosquito control is the only practical method of protecting the human population from infection. There are no known specific treatments or cures for diseases caused by these viruses and vaccines are not available for public use. Infection by WEE virus tends to be most serious in very young children, whereas infections caused by WN and SLE viruses affect the elderly most seriously. WNV also kills a wide variety of native and non-native birds. There are WEE and WNV vaccines available to protect horses since both viruses can cause severe disease in horses. Mosquito-borne disease prevention strategies must be based on a well-planned integrated pest management (IPM) program that uses real-time surveillance to detect problem areas, focus control, and evaluate operational efficacy. The primary components of an IPM program include education, surveillance, and mosquito control.

**Education**

Residents, farmers, and duck club owners can play an important role in reducing the number of adult mosquitoes by eliminating standing water that may support the development of immature mosquitoes. For instance, residents can help by properly disposing of discarded tires, cans, or buckets; emptying plastic or unused swimming pools; and unclogging blocked rain gutters around homes or businesses. Farmers and ranchers can be instructed to use irrigation practices that do not allow water to stand for extended periods, and duck club owners can work with mosquito control agencies to determine optimal flooding schedules. Educating the general public to curtail outdoor activities during peak mosquito biting times, use insect repellents, and wear long-sleeved clothing will help reduce exposure to mosquitoes. Clinical surveillance is enhanced through education of the medical and veterinary communities to recognize the symptoms of WEE, SLE, and WNV and to request appropriate laboratory tests. Public health officials need to be alerted if a mosquito-borne viral disease case is detected, especially if the public health risk is high.

**Surveillance**

Surveillance includes the monitoring, visualization, and analysis of data on climatic factors, immature and adult mosquito abundance, and virus activity measured by testing mosquitoes, sentinel chickens, wild birds (including dead birds for WNV), horses, and humans for evidence
of infection. Surveillance must focus not only on mosquito-borne viruses known to exist in California, but be sufficiently broad to also detect newly introduced viruses.

Climate Variation

The California Mediterranean climate provides ideal opportunities for forecasting mosquito abundance and arbovirus activity, because most precipitation falls during winter, as rain at lower elevations or as snow at higher elevations. Spring and summer temperatures then determine the rate of snow melt and runoff, mosquito population growth, the frequency of blood feeding, the rate of virus development in the mosquito, and therefore the frequency of virus transmission. In general, WEE virus outbreaks have occurred in the Central Valley when wet winters are followed by warm summers, whereas SLE and WN virus outbreaks seemed linked to warm dry conditions that lead to large populations of urban Culex. Although climate variation may forecast conditions conducive for virus amplification, a critical sequence of events is required for amplification to reach outbreak levels.

Mosquito Abundance

Mosquito abundance can be estimated through collection of immature or adult mosquitoes. The immature stages (larvae and pupae) can be collected from water sources where mosquitoes lay their eggs. A long-handled ladle (“dipper”) is used to collect water samples and the number of immature mosquitoes per "dip" estimated. In most local mosquito control agencies, technicians search for new sources and inspect known habitats for mosquitoes on a 7 to 14-day cycle. These data are used to direct control operations. Maintaining careful records of immature mosquito occurrence and abundance, developmental stages treated, source sizes, and control effectiveness can be useful for estimating the expected sizes of future adult populations.

Adult mosquito abundance is a key factor contributing to the risk of virus transmission. Monitoring the abundance of adult mosquito populations provides important information on the size of the vector population as it responds to changing climatic factors and to control efforts. Four adult mosquito sampling methods are currently used in California: New Jersey light traps, carbon dioxide-baited traps, gravid female traps, and resting adult mosquito collections. The advantages and disadvantages of these sampling methods, and guidelines for the design, operation, and processing of the traps have been discussed in Guidelines for Integrated Mosquito Surveillance (Meyer et al. 2003) and are summarized in Appendix A.

Mosquito Infections

Virus activity can be monitored by testing adult mosquitoes for virus infection. Because Culex tarsalis is the primary rural vector of WNV, SLE, and WEE, and Culex quinquefasciatus and Culex pipiens are important urban vectors of WNV and SLE, surveillance efforts emphasize the testing of these species. Another species that should be tested is Culex stigmatosoma, which is a highly competent but less widely distributed vector of WNV and SLE that feeds on birds and is probably important in enzootic transmission where it is found in high abundance. Female mosquitoes are trapped, usually using carbon dioxide-baited or gravid traps, identified to species, and counted into groups (pools) of 50 females each for testing at the Center for Vectorborne Diseases (CVEC) at UC Davis or by local agencies that pass annual proficiency testing. Procedures for submitting and processing mosquitoes for detecting virus infection are detailed in
Appendix B. The current surveillance system is designed to detect and measure levels of infection with WNV, SLE, and WEE. Although generally less sensitive than sentinel chickens, mosquito infections may be detected earlier in the season and over a finer scale than chicken seroconversions and therefore provide an early warning of virus activity. Testing adult mosquitoes for infection is one of the best methods to detect newly introduced or emerging mosquito-borne viruses. Testing mosquito species other than Culex may be necessary to detect the introduction of viruses that do not have a primary avian-Culex transmission cycle.

Avian Infections

Detection of arboviral transmission within bird populations can be accomplished by 1) using caged chickens as sentinels and bleeding them routinely to detect viral antibodies (seroconversions), 2) collecting and bleeding wild birds to detect viral antibodies (seroprevalence), and 3) testing dead birds reported by the public for WNV.

In California, flocks of 5-10 chickens are placed in locations where mosquito abundance is known to be high or where there is a history of virus activity. Each chicken is bled every two weeks by pricking the comb and collecting blood on a filter paper strip. The blood is tested at the CDPH Vector-Borne Disease Section for antibodies to SLE, WEE, and WNV. Some agencies conduct their own testing, but send positive samples to CDPH for confirmation and official reporting. Because SLE cross-reacts with WNV in antibody testing, SLE or WNV positive chickens are confirmed and the infecting virus is identified by western blot or cross-neutralization tests. Frequent testing of strategically placed flocks of sentinel chickens provides the most sensitive and cost-effective method to monitor encephalitis virus transmission in an area. Because chickens are continuously available to host-seeking mosquitoes, they are usually exposed to more mosquitoes than can be collected by trapping, especially when adult mosquito abundance or viral infection rates are low. Sentinel housing, bleeding instructions, and testing protocols are provided in Appendix C.

Virus activity in wild bird populations can be monitored by bleeding young (hatching year) birds to detect initial virus infection or by bleeding a cross-section of birds in an area and comparing seroprevalence among age strata to determine if the prevalence of the virus in the region has changed. Elevated seroprevalence levels (“herd immunity”) among key species during spring may limit virus transmission and dampen amplification. New infections also can be detected by bleeding banded birds in a capture-recapture scheme. In contrast to the convenience of using sentinel chickens, the repeated collection and bleeding of wild birds generally is too labor intensive, technically difficult, and expensive for most local mosquito control agencies to perform routinely. In addition, the actual place where a wild bird became infected is rarely known, because birds may travel over relatively long distances and usually are collected during daylight foraging flights and not at nighttime roosting sites where they are bitten by mosquitoes.

Unlike WEE and SLE, WNV frequently causes death in North American birds, especially those in the family Corvidae (e.g. crows, ravens, magpies, jays). Dead bird surveillance was initiated by CDPH in 2000 to provide early detection of WNV. Dead bird surveillance has been shown to be one of the earliest indicators of WNV activity in a new area. Birds that meet certain criteria are necropsied at the California Animal Health and Food Safety Laboratory and kidney snips tested for WNV RNA by RT-PCR at CVEC or oral swabs of American crows tested by rapid antigen tests by local agencies. Dead birds are reported to CDPH’s dead bird hotline (1-877-
WNV-BIRD) or via the website, http://westnile.ca.gov. Beginning in 2010, results from RT-PCR testing at CVEC distinguished between WNV recent and chronic positive birds based on qRT-PCR cycle threshold (Ct) values. In general, birds tested by RT-PCR with a Ct value of <30 and those positive by antigen tests are considered to be recently infected, whereas those with Ct values >30 are considered to have been chronically infected and the time since infection unknown. Chronic positive birds did not likely die from WNV infection and are of limited value for surveillance. The communication and testing algorithm for the dead bird surveillance program is detailed in Appendix D.

Tree Squirrel Infections

In 2004, tree squirrels were included as a WNV surveillance tool, based upon evidence that they were susceptible to WNV and could provide information on localized WNV transmission (Padgett et al. 2007). In conjunction with dead birds, tree squirrels were reported to the California WNV hotline, necropsied at the California Animal Health and Food Safety Laboratory and kidney tissue was tested by RT-PCR at CVEC. Tree squirrels will continue to be tested for WNV in 2013 and are included in the submission protocol in Appendix D.

Equine Infections

Currently, equine disease due to WEE and WNV is no longer a sensitive indicator of epizootic activity (unusually high incidence of infections in animals other than humans) in California because of the widespread vaccination or natural immunization of equids (horses, donkeys, and mules). Nevertheless, confirmed cases in horses can indicate that WEE or WNV has amplified to levels where tangential transmission has occurred and risk to humans is elevated in that region of the state. Each year, CDPH and the California Department of Food and Agriculture (CDFA) alert veterinarians to the risk of WNV in horses, advocate for vaccination, and provide information on diagnostic services that are available for suspected cases of WEE or WNV encephalitis. Numerous infectious and non-infectious causes, including other mosquito-borne viruses, can contribute to encephalitis and neurologic signs in horses. Testing of equine specimens for these possible etiologies is available through the California Animal Health and Food Safety Laboratory (see Appendix E).

Human Infections

Local mosquito control agencies rely on the rapid detection and reporting of confirmed human cases to plan and implement emergency control activities to prevent additional infections. However, human cases of arboviral infection are an insensitive surveillance indicator of virus activity because most persons who become infected develop no symptoms. For those individuals who do become ill, it may take up to two weeks for symptoms to appear, followed by additional time until the case is recognized and reported. No human cases of SLE or WEE have been reported in California in recent years. However, a total of 3,625 cases of WNV have been reported in California from 2003-2012.

To enhance human WNV testing and surveillance efforts throughout the state, a regional public health laboratory network was established in 2002. The laboratory network consists of the state Viral and Rickettsial Disease Laboratory (VRDL) as well as 20 county public health laboratories that are able to conduct WNV testing. Providers are encouraged to submit specimens for suspect
WNV cases to their local public health laboratories. Specimens for patients with encephalitis may also be submitted directly to Neurologic Surveillance and Testing, which is based in the VRDL and offers diagnostic testing for many agents known to cause encephalitis, including WNV and other arboviruses. In addition, VRDL collaborates with reference laboratories such as the regional laboratories of Kaiser Permanente to ascertain additional suspect WNV cases.

In accordance with Title 17 of the California Code of Regulations (Sections 2500 and 2505), physicians and laboratories are required to report cases of WNV infection or positive test results to their local health department. Positive WNV or other arbovirus test results are investigated by local health department officials to determine whether a patient meets the clinical and laboratory criteria for a WNV diagnosis. If so, the local health department collects demographic and clinical information on the patient using a standardized WNV infection case report, and forwards the report to the state health department. The local health department also determines whether the infection was acquired locally, imported from a region outside the patient’s residence, or acquired by a non-mosquito route of transmission such as blood transfusion or organ transplantation. Appendix F contains the protocol for submission of specimens to the regional public health laboratory network for WNV testing. Appendix G provides the national surveillance case definition for WNV infection.

**Mosquito Control**

Problems detected by surveillance are mitigated through larval and adult mosquito control. Mosquito control is the only practical method of protecting people from mosquito-borne diseases. Mosquito control in California is conducted by approximately 80 local agencies, including mosquito and vector control districts, county environmental and health departments, and county agriculture departments. Agencies applying pesticides directly to a water of the United States, or where deposition may enter a water of the United States, must obtain a National Pollutant Discharge Elimination System (NPDES) Permit for Biological and Residual Pesticide Discharges to Waters of the United States from Vector Control Applications (Vector Control Permit). Agencies must comply with provisions of the permit, including use of approved pesticides, pesticide use reporting, and visual, chemical, and toxicity monitoring requirements included in the permit. [http://www.swrcb.ca.gov/water_issues/programs/npdes/aquatic.shtml](http://www.swrcb.ca.gov/water_issues/programs/npdes/aquatic.shtml)

Compounds currently approved for larval and adult mosquito control in California are listed in Appendix H. Please refer to the Vector Control Permit, Attachments E and F, for a list of vector control pesticides that may be applied to waters of the United States, unless the receiving water has an existing impairment from a pesticide with the same active ingredient. Please review the California State Water Resources Control Board listing of impaired water bodies (303d list) prior to applying any pesticide. [http://www.swrcb.ca.gov/rwqcb4/water_issues/programs/303d_list.shtml](http://www.swrcb.ca.gov/rwqcb4/water_issues/programs/303d_list.shtml)

Additional considerations regarding adult mosquito control in urban areas are described in Appendix I.

**Larval Control**

Mosquito larval and pupal control methods are target-specific and prevent the emergence of adult female mosquitoes which are capable of transmitting pathogens, causing discomfort, and
ultimately producing another generation of mosquitoes. For these reasons, most mosquito control agencies in California target the immature stages rather than the adult stage of the mosquito. Larval mosquito control has three key components: environmental management, biological control, and chemical control.

Environmental management decreases habitat availability or suitability for immature mosquitoes, and may include water management, such as increasing the water disposal rate through evaporation, percolation, recirculation, or drainage. Laser-leveling of fields minimizes pooling at low spots, allows even distribution of irrigation water, and precludes standing water for long periods. Controlled irrigation or the careful timing of wetland flooding for waterfowl can reduce mosquito production or limit emergence to times of the year when virus activity is unlikely. Environmental management may include vegetation management because emergent vegetation provides food and refuge for mosquito larvae. Management strategies include the periodic removal or thinning of vegetation, restricting growth of vegetation, and controlling algae.

Biological control uses natural predators, parasites, or pathogens to reduce immature mosquito numbers. Mosquitofish, Gambusia affinis, are the most widely used biological control agent in California. These fish are released annually in a variety of habitats, such as rice fields, small ponds, and canals.

There are several mosquito control products that are highly specific and thus have minimal impact on non-target organisms. These include microbial control agents, such as Bacillus thuringiensis israelensis (Bti) and Bacillus sphaericus, and insect growth regulators, such as methoprene, that prevent immature mosquitoes from developing into adults. Surface films are very effective against both larvae and pupae, but also may suffocate other surface breathing aquatic insects. Organophosphate pesticides are used infrequently because of their impact on nontarget organisms and the environment.

**Adult Control**

When larval control is not possible or more immediate control measures are needed, adult mosquito control may be required to suppress populations of infected mosquitoes and interrupt epidemic virus transmission. Adult mosquito control products may be applied using ground-based equipment, fixed wing airplanes, or helicopters. Products applied in ultralow volume [ULV] formulations and dosages include organophosphates, such as malathion and naled, pyrethroids, such as resmethrin, sumithrin, and permethrin, and pyrethrins such as Pyrenone crop spray. Factors to consider when selecting an adulticide include: 1) efficacy against the target species or life cycle stage, 2) resistance status, 3) pesticide label requirements, 4) availability of pesticide and application equipment, 5) environmental conditions, 6) cost, and 7) toxicity to nontarget species, including humans.

For more information about mosquito control please see “Best Management Practices for Mosquito Control in California”. [http://www.westnile.ca.gov/resources.php](http://www.westnile.ca.gov/resources.php)
Response Levels

The California Mosquito-borne Virus Surveillance and Response Plan was developed to provide a semi-quantitative measure of virus transmission risk to humans that could be used by local mosquito control agencies to plan and modulate control activities. Independent models are presented for WEE, SLE and WNV to accommodate the different ecological dynamics of these viruses (Barker et al. 2003). SLE and WN viruses are closely related, require similar environmental conditions, and employ the same Culex vectors. Seven surveillance factors are measured and analyzed to determine the level of risk for human involvement and thereby gauge the appropriate response level:

1. Environmental or climatic conditions (snowpack, rainfall, temperature, season)
2. Adult Culex vector abundance
3. Virus infection rate in Culex mosquito vectors
4. Sentinel chicken seroconversions
5. Fatal infections in birds (WNV only)
6. Infections in humans
7. Proximity of detected virus activity to urban or suburban regions (WEE only)

Each factor is scored on an ordinal scale from 1 (lowest risk) to 5 (highest risk). The mean score calculated from these factors corresponds to a response level as follows: normal season (1.0 to 2.5), emergency planning (2.6 to 4.0), and epidemic (4.1 to 5.0). Table 1 provides a worksheet to assist in determining the appropriate rating for each of the risk factors for each of the three viruses. Appendix J shows sources of data useful in the calculation of risk in Table 1. Surveillance data can be managed and risk level calculated in time and space using the Surveillance Gateway.

For surveillance factor 2 (vector abundance), abundance is expressed as a percentage of normal by comparing the current level for an area to the average over the previous 5 years for the same two-week period. An “area” typically encompasses the boundaries of a local mosquito and vector control district but could represent a smaller unit if desired. Mosquitoes’ virus infection rate should be calculated using the most recent data (prior two week period) and expressed as the minimum infection rate (MIR) per 1,000 female mosquitoes tested. Calculations may also use maximum likelihood estimates (Centers for Disease Control and Prevention 2011), which account for varying numbers of specimens in pools and the possibility that more than one mosquito could be infected in each positive pool when infection rates are high. For WNV and SLE, risk may be estimated separately for Cx. tarsalis and the Cx. pipiens complex, respectively, because these species generally have different habitat requirements and therefore spatial distributions (e.g., rural vs. urban).

Each of the three viruses differs in its response to ecological conditions. WEE activity typically is greatest during El Niño conditions of wet winters, excessive run-off and flooding, cool springs, and increased Culex tarsalis abundance. Historically, WEE virus spillover into a secondary Aedes-rabbit cycle was common in the Central Valley, but has not been detected for more than 25 years. In contrast, SLE and perhaps WNV activity appears to be greatest during La Niña conditions of drought and hot summer temperatures and both SLE and WNV transmission risk increases when temperatures are above normal. Abundance and infection of the Culex pipiens complex are included in both SLE and WNV estimates of risk because these mosquito species are important vectors, particularly in suburban/urban environments. The occurrence of dead bird infections is included as a risk factor in the WNV calculations. For surveillance...
factors 4-6 (chickens, birds, humans), the specific region is defined as the area within the agency’s boundary and the broad region includes the area within 150 miles (~241 km) of the agency’s boundary.

Proximity of virus activity to human population centers is considered an important risk factor for all three viruses of public health concern. In the risk assessment model in Table 1 this was accommodated in two different ways. WEE virus transmitted by *Culex tarsalis* typically amplifies first in rural areas and may eventually spread into small and then larger communities. A risk score was included to account for where virus activity was detected. WNV and SLE virus may be amplified concurrently or sequentially in rural and urban cycles. The rural cycle is similar to WEE virus and is transmitted primarily by *Culex tarsalis*, whereas the urban cycle is transmitted primarily by members of the *Culex pipiens* complex. If the spatial distributions of key *Culex* species differ within an area (e.g., rural vs. urban), it may be advantageous to assess risk separately by species for abundance and infection rates in *Culex tarsalis* and the *Culex pipiens* complex. This would result in two estimates of overall risk for the areas dominated by each species.

Each of these surveillance factors can differ in impact and significance according to time of year and geographic region. Climate is used prospectively to forecast risk during the coming season. Climatic factors provide the earliest indication of the potential for increased mosquito abundance and virus transmission and constitute the only risk factor actually measured from the start of the calendar year through mid-spring when enzootic surveillance commences in most areas. Other factors that may inform control efforts as the season progresses are typically, in chronological order: mosquito abundance, infections in non-humans (e.g., dead birds for WNV, mosquitoes, sentinel chickens), and infections in humans. Enzootic indicators measure virus amplification within the *Culex*-bird cycle and provide nowcasts of risk, whereas human infections document tangential transmission and are the outcome measure of forecasts and nowcasts. Response to the calculated risk level should consider the time of year; e.g., epidemic conditions in October would warrant a less aggressive response compared to epidemic conditions in July because cooler weather in late fall will contribute to declining risk of arbovirus transmission.

The ratings listed in Table 1 are benchmarks only and may be modified as appropriate to the conditions in each specific region or biome of the state. Calculation and mapping of risk has been enabled by tools included in the CalSurv Gateway. Roles and responsibilities of key agencies involved in carrying out the surveillance and response plan are outlined in “Key Agency Responsibilities.”
Table 1. Mosquito-borne Virus Risk Assessment.

<table>
<thead>
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<th>WNV Surveillance Factor</th>
<th>Assessment Value</th>
<th>Benchmark</th>
<th>Assigned Value</th>
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<tbody>
<tr>
<td>1. Environmental Conditions</td>
<td>1</td>
<td>Avg daily temperature during prior 2 weeks ≤ 56 °F</td>
<td></td>
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<tr>
<td>High-risk environmental conditions include above-normal temperatures with or without above-normal rainfall, runoff, or snowpack.</td>
<td>2</td>
<td>Avg daily temperature during prior 2 weeks 57 – 65 °F</td>
<td></td>
</tr>
<tr>
<td>Weather data link: <a href="http://ipm.ucdavis.edu">http://ipm.ucdavis.edu</a></td>
<td>3</td>
<td>Avg daily temperature during prior 2 weeks 66 – 72 °F</td>
<td></td>
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<tr>
<td></td>
<td>4</td>
<td>Avg daily temperature during prior 2 weeks 73 – 79 °F</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>Avg daily temperature during prior 2 weeks &gt; 79 °F</td>
<td></td>
</tr>
<tr>
<td>2. Adult Culex tarsalis and Cx. pipiens complex relative abundance*</td>
<td>1</td>
<td>Vector abundance well below average (≤ 50%)</td>
<td></td>
</tr>
<tr>
<td>Determined by trapping adults, enumerating them by species, and comparing numbers to those previously documented for an area for the prior 2-week period.</td>
<td>2</td>
<td>Vector abundance below average (51 - 90%)</td>
<td></td>
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<tr>
<td></td>
<td>3</td>
<td>Vector abundance average (91 - 150%)</td>
<td></td>
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<tr>
<td></td>
<td>4</td>
<td>Vector abundance above average (151 - 300%)</td>
<td></td>
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<tr>
<td></td>
<td>5</td>
<td>Vector abundance well above average (&gt; 300%)</td>
<td></td>
</tr>
<tr>
<td>3. Virus infection rate in Culex tarsalis and Cx. pipiens complex mosquitoes*</td>
<td>1</td>
<td>MIR = 0</td>
<td></td>
</tr>
<tr>
<td>Tested in pools of 50. Test results expressed as minimum infection rate per 1,000 female mosquitoes tested (MIR) for the prior 2-week period.</td>
<td>2</td>
<td>MIR = 0.1 - 1.0</td>
<td></td>
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<tr>
<td></td>
<td>3</td>
<td>MIR = 1.1 - 2.0</td>
<td></td>
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<tr>
<td></td>
<td>4</td>
<td>MIR = 2.1 - 5.0</td>
<td></td>
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<tr>
<td></td>
<td>5</td>
<td>MIR &gt; 5.0</td>
<td></td>
</tr>
<tr>
<td>4. Sentinel chicken seroconversion</td>
<td>1</td>
<td>No seroconversions in broad region</td>
<td></td>
</tr>
<tr>
<td>Number of chickens in a flock that develop antibodies to WNV during the prior 2-week period. If more than one flock is present in a region, number of flocks with seropositive chickens is an additional consideration. Typically 10 chickens per flock.</td>
<td>2</td>
<td>One or more seroconversions in broad region</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>One or two seroconversions in a single flock in specific region</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>More than two seroconversions in a single flock or two flocks with one or two seroconversions in specific region</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>More than two seroconversions per flock in multiple flocks in specific region</td>
<td></td>
</tr>
<tr>
<td>5. Dead bird infection</td>
<td>1</td>
<td>No positive dead birds in broad region</td>
<td></td>
</tr>
<tr>
<td>Number of birds that have tested positive (recent infections only) for WNV during the prior 3-month period. This longer time period reduces the impact of zip code closures during periods of increased WNV transmission.</td>
<td>2</td>
<td>One or more positive dead birds in broad region</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>One positive dead bird in specific region</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>Two to five positive dead birds in specific region</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>More than five positive dead birds in specific region</td>
<td></td>
</tr>
<tr>
<td>6. Human cases</td>
<td>3</td>
<td>One or more human infections in broad region</td>
<td></td>
</tr>
<tr>
<td>Do not include this factor in calculations if no cases are detected in region.</td>
<td>4</td>
<td>One human infection in specific region</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>More than one human infection in specific region</td>
<td></td>
</tr>
<tr>
<td>Response Level / Average Rating:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal Season (1.0 to 2.5)</td>
<td></td>
<td></td>
<td>TOTAL</td>
</tr>
<tr>
<td>Emergency Planning (2.6 to 4.0)</td>
<td></td>
<td></td>
<td>AVERAGE</td>
</tr>
</tbody>
</table>

* Calculation of separate risk values for Cx. tarsalis and the Cx. pipiens complex may be useful if their spatial distributions (e.g., rural vs. urban) differ within the assessment area.
### SLE Surveillance Factor

**1. Environmental Conditions**
High-risk environmental conditions include above-normal temperatures with or without above-normal rainfall, runoff, or snowpack. Weather data link: [http://ipm.ucdavis.edu](http://ipm.ucdavis.edu)

<table>
<thead>
<tr>
<th>Assessment Value</th>
<th>Benchmark</th>
<th>Assigned Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Avg daily temperature during prior 2 weeks ≤ 56 °F</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Avg daily temperature during prior 2 weeks 57 – 65 °F</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Avg daily temperature during prior 2 weeks 66 – 72 °F</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Avg daily temperature during prior 2 weeks 73 – 79 °F</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Avg daily temperature during prior 2 weeks &gt; 79 °F</td>
<td></td>
</tr>
</tbody>
</table>

**2. Adult *Culex tarsalis* and *Cx. pipiens* complex relative abundance**
Determined by trapping adults, enumerating them by species, and comparing numbers to those previously documented for an area for the prior 2-week period.

<table>
<thead>
<tr>
<th>Assessment Value</th>
<th>Benchmark</th>
<th>Assigned Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Vector abundance well below average (≤ 50%)</td>
<td>Cx tars Cx pip</td>
</tr>
<tr>
<td>2</td>
<td>Vector abundance below average (51 - 90%)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Vector abundance average (91 - 150%)</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Vector abundance above average (151 - 300%)</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Vector abundance well above average (&gt; 300%)</td>
<td></td>
</tr>
</tbody>
</table>

**3. Virus infection rate in *Culex tarsalis* and *Cx. pipiens* complex mosquitoes**
Tested in pools of 50. Test results expressed as minimum infection rate per 1,000 female mosquitoes tested (MIR) for the prior 2-week collection period.

<table>
<thead>
<tr>
<th>Assessment Value</th>
<th>Benchmark</th>
<th>Assigned Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>MIR = 0</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>MIR = 0.1 - 1.0</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>MIR = 1.1 - 2.0</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>MIR = 2.1 - 5.0</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>MIR &gt; 5.0</td>
<td></td>
</tr>
</tbody>
</table>

**4. Sentinel chicken seroconversion**
Number of chickens in a flock that develop antibodies to SLEV during the prior 2-week period. If more than one flock is present in a region, number of flocks with seropositive chickens is an additional consideration. Typically 10 chickens per flock.

<table>
<thead>
<tr>
<th>Assessment Value</th>
<th>Benchmark</th>
<th>Assigned Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>No seroconversions in broad region</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>One or more seroconversions in broad region</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>One or two seroconversions in a single flock in specific region</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>More than two seroconversions in a single flock or two flocks with one or two seroconversions in specific region</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>More than two seroconversions per flock in multiple flocks in specific region</td>
<td></td>
</tr>
</tbody>
</table>

**5. Human cases**
Do not include this factor in calculations if no cases are detected in region.

<table>
<thead>
<tr>
<th>Assessment Value</th>
<th>Benchmark</th>
<th>Assigned Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>One or more human cases in broad region</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>One human case in specific region</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>More than one human case in specific region</td>
<td></td>
</tr>
</tbody>
</table>

**Response Level / Average Rating:**
- Normal Season (1.0 to 2.5)
- Emergency Planning (2.6 to 4.0)
- Epidemic (4.1 to 5.0)

<table>
<thead>
<tr>
<th>Cx tars Cx pip</th>
<th>TOTAL</th>
<th>AVERAGE</th>
</tr>
</thead>
</table>

* Calculation of separate risk values for *C. tarsalis* and the *Cx. pipiens* complex may be useful if their spatial distributions (e.g., rural vs. urban) differ within the assessment area.
### WEE Surveillance Factor

<table>
<thead>
<tr>
<th>Factor</th>
<th>Assessment Value</th>
<th>Benchmark</th>
<th>Assigned Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Environmental Conditions</td>
<td>1</td>
<td>Cumulative rainfall and runoff well below average</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>Cumulative rainfall and runoff below average</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>Cumulative rainfall and runoff average</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>Cumulative rainfall and runoff above average</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>Cumulative rainfall and runoff well above average</td>
<td></td>
</tr>
<tr>
<td>2. Adult <em>Culex tarsalis</em> abundance</td>
<td>1</td>
<td><em>Cx. tarsalis</em> abundance well below average (≤ 50%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td><em>Cx. tarsalis</em> abundance below average (51 - 90%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td><em>Cx. tarsalis</em> abundance average (91 - 150%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td><em>Cx. tarsalis</em> abundance above average (151 - 300%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5</td>
<td><em>Cx. tarsalis</em> abundance well above average (&gt; 300%)</td>
<td></td>
</tr>
<tr>
<td>3. Virus infection rate in <em>Cx. tarsalis</em> mosquitoes</td>
<td>1</td>
<td><em>Cx. tarsalis</em> MIR = 0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td><em>Cx. tarsalis</em> MIR = 0.1 - 1.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td><em>Cx. tarsalis</em> MIR = 1.1 - 2.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td><em>Cx. tarsalis</em> MIR = 2.1 - 5.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5</td>
<td><em>Cx. tarsalis</em> MIR &gt; 5.0</td>
<td></td>
</tr>
<tr>
<td>4. Sentinel chicken seroconversion</td>
<td>1</td>
<td>No seroconversions in broad region</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>One or more seroconversions in broad region</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>One or two seroconversions in a single flock in specific region</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>More than two seroconversions in a single flock or two flocks with one or two seroconversions in specific region</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>More than two seroconversions per flock in multiple flocks in specific region</td>
<td></td>
</tr>
<tr>
<td>5. Proximity to urban or suburban regions</td>
<td>1</td>
<td>Virus detected in rural area</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>Virus detected in small town or suburban area</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>Virus detected in urban area</td>
<td></td>
</tr>
<tr>
<td>6. Human cases</td>
<td>3</td>
<td>One or more human cases in broad region</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>One human case in specific region</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>More than one human case in specific region</td>
<td></td>
</tr>
</tbody>
</table>

**Response Level / Average Rating:**
- Normal Season (1.0 to 2.5)
- Emergency Planning (2.6 to 4.0)
- Epidemic (4.1 to 5.0)

<table>
<thead>
<tr>
<th>TOTAL</th>
</tr>
</thead>
</table>
General suggestions for applying the risk assessment model locally

- Use a consistent time period for environmental conditions, adult mosquito abundance, mosquito infection rates, and human cases. If you use a period that differs from the prior two-week period defined in the risk assessment -- such as the prior month -- use the same period for all other relevant measures. Note that sentinel seroconversions and dead bird infections may need special treatment to accommodate bleeding schedules and zip code closures, respectively. For sentinel seroconversions, use data from the most recent collection.
- If you have multiple trap types in your surveillance program, determine the vector abundance anomaly for each trap type and species and use the most sensitive trap type’s value in the risk assessment.
- When determining the vector abundance anomaly, there should be at least two and preferably five years of prior data to provide a comparative baseline for the particular trap type. Ideally, the prior years should use the same or very similar trap locations and be contiguous and immediately precede the time period being evaluated.

Risk assessment as implemented by the CalSurv Gateway (http://gateway.calsurv.org)

- Statewide maps at a resolution of 1 km\(^2\) will be generated and delivered to the primary contacts of each agency by email every Monday.
- Only those agencies with active Gateway accounts and defined primary contacts will receive the weekly maps.
- Mapped risk surfaces are generated for all areas of California that have one or more surveillance inputs within 8 km. The risk for each pixel in the map image is based on a spatially weighted summary of all available surveillance data within 8 km. Pixels > 8 km from the nearest surveillance do not have assigned risk values.
- Due to privacy concerns and delays in detection and reporting, human cases are not part of the Gateway’s risk assessment.
- All of the general suggestions from the prior section are used in the Gateway’s implementation.
- Risk estimates based on mosquito abundance and infection rates will be calculated separately for the key mosquito taxa, Cx. tarsalis and the Cx. pipiens complex.
- The risk assessment model is implemented also in an online calculator for use by local vector control agencies that allows user definition of locations and date ranges for calculation.
Characterization of Conditions and Responses

Level 1: Normal Season

Risk rating: 1.0 to 2.5

<table>
<thead>
<tr>
<th>CONDITIONS</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Average or below average snowpack and rainfall; below or average seasonal temperatures (&lt;65F)</td>
</tr>
<tr>
<td>• <em>Culex</em> mosquito abundance at or below five year average (key indicator = adults of vector species)</td>
</tr>
<tr>
<td>• No virus infection detected in mosquitoes</td>
</tr>
<tr>
<td>• No seroconversions in sentinel chickens</td>
</tr>
<tr>
<td>• No recently infected WNV-positive dead birds</td>
</tr>
<tr>
<td>• No human cases</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>RESPONSE</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Conduct routine public education (eliminate standing water around homes, use personal protection measures)</td>
</tr>
<tr>
<td>• Conduct routine mosquito and virus surveillance activities</td>
</tr>
<tr>
<td>• Comply with National Pollutant Discharge Eliminations System (NPDES) permit if applying pesticides to waters of the United States</td>
</tr>
<tr>
<td>• Conduct routine mosquito control, with emphasis on larval control</td>
</tr>
<tr>
<td>• Inventory pesticides and equipment</td>
</tr>
<tr>
<td>• Evaluate pesticide resistance in vector species</td>
</tr>
<tr>
<td>• Ensure adequate emergency funding</td>
</tr>
<tr>
<td>• Release routine press notices</td>
</tr>
<tr>
<td>• Send routine notifications to physicians and veterinarians</td>
</tr>
<tr>
<td>• Establish and maintain routine communication with local office of emergency services personnel; obtain Standardized Emergency Management System (SEMS) training</td>
</tr>
</tbody>
</table>

Level 2: Emergency Planning

Risk rating: 2.6 to 4.0

<table>
<thead>
<tr>
<th>CONDITIONS</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Snowpack and rainfall and/or temperature above average (66-79F)</td>
</tr>
<tr>
<td>• Adult <em>Culex</em> mosquito abundance greater than 5-year average (150% to 300% above normal)</td>
</tr>
<tr>
<td>• One or more virus infections detected in <em>Culex</em> mosquitoes (MIR / 1000 is &lt;5)</td>
</tr>
<tr>
<td>• One or more seroconversions in single flock or one to two seroconversions in multiple flocks in specific region</td>
</tr>
<tr>
<td>• One to five recently infected WNV-positive dead birds in specific region</td>
</tr>
<tr>
<td>• One human case in broad or specific region</td>
</tr>
<tr>
<td>• WEE virus detected in small towns or suburban area</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>RESPONSE</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Review epidemic response plan</td>
</tr>
<tr>
<td>• Enhance public education (include messages on the signs and symptoms of encephalitis; seek medical care if needed; inform public about pesticide applications if appropriate)</td>
</tr>
<tr>
<td>• Enhance information to public health providers</td>
</tr>
<tr>
<td>• Conduct epidemiological investigations of cases of equine or human disease</td>
</tr>
<tr>
<td>• Increase surveillance and control of mosquito larvae</td>
</tr>
<tr>
<td>• Increase adult mosquito surveillance</td>
</tr>
<tr>
<td>• Increase number of mosquito pools tested for virus</td>
</tr>
<tr>
<td>• Conduct or increase localized chemical control of adult mosquitoes as appropriate</td>
</tr>
<tr>
<td>• Contact commercial applicators in anticipation of large scale adulticiding</td>
</tr>
<tr>
<td>• Review candidate pesticides for availability and susceptibility of vector mosquito species</td>
</tr>
<tr>
<td>• Ensure notification of key agencies of presence of viral activity, including the local office of emergency services</td>
</tr>
</tbody>
</table>
**Level 3: Epidemic Conditions**

**Risk rating: 4.1 to 5.0**

<table>
<thead>
<tr>
<th>CONDITIONS</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Snowpack, rainfall, and water release rates from flood control dams and/or temperature well above average (&gt;79°F)</td>
</tr>
<tr>
<td>• Adult vector population extremely high (&gt;300%)</td>
</tr>
<tr>
<td>• Virus infections detected in multiple pools of <em>Culex tarsalis</em> or <em>Cx. pipiens</em> mosquitoes (MIR / 1000 &gt; 5.0)</td>
</tr>
<tr>
<td>• More than two seroconversions per flock in multiple flocks in specific region</td>
</tr>
<tr>
<td>• More than five recently infected WNV-positive dead birds and multiple reports of dead birds in specific region</td>
</tr>
<tr>
<td>• More than one human case in specific region</td>
</tr>
<tr>
<td>• WEE virus detection in urban or suburban areas</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>RESPONSE</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Conduct full scale media campaign</td>
</tr>
<tr>
<td>• Alert physicians and veterinarians to expect cases</td>
</tr>
<tr>
<td>• Conduct active human case detection</td>
</tr>
<tr>
<td>• Conduct epidemiological investigations of cases of equine or human disease</td>
</tr>
<tr>
<td>• Continue enhanced larval surveillance and control of immature mosquitoes</td>
</tr>
<tr>
<td>• Broaden geographic coverage of adult mosquito surveillance</td>
</tr>
<tr>
<td>• Accelerate adult mosquito control as appropriate by ground and/or air</td>
</tr>
<tr>
<td>• Coordinate the response with the local Office of Emergency Services or if activated, the Emergency Operation Center (EOC)</td>
</tr>
<tr>
<td>• Initiate mosquito surveillance and control in geographic regions without an organized vector control program</td>
</tr>
<tr>
<td>• Determine whether declaration of a local emergency should be considered by the County Board of Supervisors (or Local Health Officer)</td>
</tr>
<tr>
<td>• Determine whether declaration of a “State of Emergency” should be considered by the Governor at the request of designated county or city officials</td>
</tr>
<tr>
<td>• Ensure state funds and resources are available to assist local agencies at their request</td>
</tr>
<tr>
<td>• Determine whether to activate a Standardized Emergency Management System (SEMS) plan at the local or state level</td>
</tr>
<tr>
<td>• Continue mosquito education and control programs until mosquito abundance is substantially reduced and no additional human cases are detected</td>
</tr>
</tbody>
</table>

For more detailed information on responding to a mosquito-borne disease outbreak, please refer to:

Key Agency Responsibilities

**Local Mosquito and Vector Control Agencies**
- Gather, collate, and interpret regional climate and weather data.
- Monitor abundance of immature and adult mosquitoes.
- Collect and submit mosquito pools to CVEC for virus detection.
- Maintain sentinel chicken flocks, obtain blood samples, and send samples to VBDS.
- Pick-up and ship dead birds for necropsy and WNV testing, or test oral swabs from American crows locally via rapid antigen screening assays.
- Update CDPH weekly of all birds that are independently reported and/or tested by VecTOR Test, RAMP or RT-PCR.
- Update the Surveillance Gateway weekly with mosquito pool results that are independently tested by RAMP or RT-PCR.
- Conduct routine control of immature mosquitoes.
- Comply with NPDES permit if applying pesticides to waters of the United States.
- Conduct control of adult mosquitoes when needed.
- Educate public on mosquito avoidance and reduction of mosquito breeding sites.
- Coordinate with local Office of Emergency Services personnel.
- Communicate regularly with neighboring agencies.

**Mosquito and Vector Control Association of California**
- Coordinate purchase of sentinel chickens.
- Receive, track, and disburse payment for mosquito surveillance expenses.
- Coordinate surveillance and response activities among member agencies.
- Serve as spokesperson for member agencies.
- Establish liaisons with press and government officials.

**California Department of Public Health**
- Collate adult mosquito abundance data submitted by local agencies; provide summary of data to local agencies.
- Maintain a WNV information and dead bird reporting hotline, 1-877-WNV-BIRD, and a WNV website: [http://westnile.ca.gov](http://westnile.ca.gov).
- Coordinate submission of specimens for virus testing.
- Provide supplies for processing mosquito pool and sentinel chicken diagnostic specimens.
- Test sentinel chicken sera for viral antibodies.
- Test human specimens for virus.
- Distribute a weekly bulletin summarizing surveillance test results.
- Send weekly surveillance results to the UC Davis interactive website.
- Immediately notify local vector control agency and public health officials when evidence of viral activity is found.
- Conduct epidemiological investigations of cases of human disease.
- Coordinate and participate in a regional emergency response in conjunction with California Emergency Management Agency.
- Conduct surveillance for human cases.
• Provide oversight to local jurisdictions without defined vector-borne disease control program.
• Maintain inventory of antigens and antisera to detect exotic viruses.
• Provide confirmation of tests done by local agencies.

University of California at Davis
• Conduct research on arbovirus surveillance, transmission of mosquito-borne diseases, and mosquito ecology and control.
• Test mosquito pools and dead birds for endemic and introduced viruses.
• Provide a proficiency panel of tests for identification of viruses from human, equine, bird, or arthropod vectors to local agencies to ensure quality control.
• Maintain an interactive website (http://gateway.calsurv.org) for dissemination of mosquito-borne virus information and data.
• Maintain inventory of antigens, antisera, and viruses to detect the introduction of exotic viruses.
• Provide confirmation of tests done by local or state agencies.

California Department of Food and Agriculture
• Notify veterinarians and veterinary diagnostic laboratories about WEE and WNV and testing available at CAHFS
• Provide outreach to general public and livestock managers of the need to monitor and report equine and ratite encephalitides.
• Facilitate equine and ratite sample submission from veterinarians.
• Conduct investigations of equine confirmed WNV and WEE cases.

California Animal Health and Food Safety Laboratory
• Identify species of dead birds submitted for WNV testing.
• Conduct necropsies and testing on dead birds.
• Submit bird tissues to CVEC for testing.
• Test equine specimens for WNV.

Local Health Departments and Public Health Laboratories
• Test human specimens for WNV.
• Refer human specimens to CDPH for further testing.
• Notify local medical community, including hospitals and laboratories, if evidence of viral activity is present.
• Collect dead birds and ship carcasses to testing laboratories when needed.
• Test American crows via rapid assay or RT-PCR as resources allow.
• Participate in emergency response.
• Conduct epidemiological investigations of cases of human disease.
• Report WNV cases to CDPH.
• Conduct public education.
California Emergency Management Agency
- Coordinate the local, regional, or statewide emergency response under epidemic conditions in conjunction with CDPH via the Standardized Emergency Management System (SEMS).
- Serve as liaison with the Federal Emergency Management Agency (FEMA) in the event that a federal disaster has been declared.

Federal Centers for Disease Control and Prevention
- Provide consultation to state and local agencies in California if epidemic conditions exist.
- Provide national surveillance data to state health departments.

State Water Resources Control Board
- Review NPDES permit applications and respond in a timely manner.
- Review vector control pesticides registered by the California Department of Pesticide Regulation for inclusion on the Vector Control NPDES permit.
Appendix A: Guidelines for Adult Mosquito Surveillance

The objective of Appendix A is to standardize mosquito sampling and reporting procedures to provide comparable and interpretable abundance measures among collaborating mosquito control agencies in California. This section summarizes information from Integrated Mosquito Surveillance Program Guidelines for California that has been adopted by the Mosquito and Vector Control Association (MVCAC) (Meyer et al. 2003). The MVCAC guidelines recommend stratifying the use of different sampling methods in rural, small town, and urban environments for each of the major biomes of California and provide a listing of target vector and nuisance mosquito species. The stratified sampling approach monitors vector populations and virus activity in rural enzootic foci, agricultural or suburban amplification sites, and densely populated urban centers to provide estimates of early, eminent, and current epidemic risk.

The four sampling methods currently used by mosquito control agencies are: 1) New Jersey (American) light trap (Mulhern 1942), 2) CDC/ EVS style, or other CO₂-baited trap (Newhouse et al. 1966; Sudia and Chamberlain 1962), 3) gravid trap (Cummings 1992; Reiter 1983), and 4) adult resting collections (Loomis and Sherman 1959). Collection location sites should be geocoded and registered using the Surveillance Gateway [http://gateway.calsurv.org/]. Studies comparing trap design and efficiency for surveillance purposes have been published (Reisen et al. 2000; Reisen et al. 2002). These guidelines describe: 1) a comparison of the sampling methods, 2) equipment design, 3) operation, 4) specimen processing, 5) data recording and analysis, and 6) data usage.

Advantages and Disadvantages of Mosquito Sampling Methods:

<table>
<thead>
<tr>
<th>New Jersey Light Trap</th>
<th>CDC/EVS CO₂ Trap</th>
<th>Gravid Trap</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pros</strong></td>
<td><strong>Cons</strong></td>
<td><strong>Pros</strong></td>
</tr>
<tr>
<td>• All female metabolic states and males collected</td>
<td>• Selective for phototactic nocturnally active mosquitoes</td>
<td>• Collects females that have bloodfed and digested a blood meal; may have higher infection rate than CO₂ trap</td>
</tr>
<tr>
<td>• Minimal collection effort (can be run nightly without service)</td>
<td>• Ineffective in the presence of competing light sources</td>
<td>• Specimens are alive and suitable for virus detection</td>
</tr>
<tr>
<td>• Long history of use in California</td>
<td>• Sorting time excessive because of other insects in traps</td>
<td>• Without light, collects mostly mosquitoes thus reducing sorting time</td>
</tr>
</tbody>
</table>

Collection location sites should be geocoded and registered using the Surveillance Gateway [http://gateway.calsurv.org/]. Studies comparing trap design and efficiency for surveillance purposes have been published (Reisen et al. 2000; Reisen et al. 2002). These guidelines describe: 1) a comparison of the sampling methods, 2) equipment design, 3) operation, 4) specimen processing, 5) data recording and analysis, and 6) data usage.
### Appendix A

#### Resting Catches

<table>
<thead>
<tr>
<th><strong>Pros</strong></th>
<th><strong>Cons</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>- All metabolic states collected</td>
<td>- Standardization is difficult due to:</td>
</tr>
<tr>
<td>- Minimal equipment needed</td>
<td>1. Variable shelter size and type</td>
</tr>
<tr>
<td>- Specimens are alive and suitable for virus detection</td>
<td>2. Variable collector efficiency</td>
</tr>
<tr>
<td>- Blooded and gravid specimens can be tested to improve sensitivity of virus surveillance</td>
<td>- Labor intensive; difficult to concurrently sample a large number of sites</td>
</tr>
</tbody>
</table>

### New Jersey (American) Light Trap (NJLT)

#### Operation

At a minimum, one trap should be located in each principal municipality of a district or have a density of one trap/township (36 sq. mi.). Correct placement of the NJLT is a critical factor in its performance as an effective surveillance mechanism for measuring the relative abundance of phototaxic mosquitoes. Place the traps at six-foot height. This can be done by using a metal standard, or by hanging the traps from tree limbs or roof eaves. These distances should maximize attractancy over a 360 degree radius. The trap should be placed on the leeward side of a structure or tree line to decrease the influence of wind on trap catch.

Traps should be kept away from smoke or chemical odors that may be repellent to the mosquitoes. Traps should be away from buildings in which animals are housed and not be in the immediate vicinity of sentinel flocks to diminish attractancy competition. Traps should be placed away from street and security lights that may diminish attractancy of the trap bulb. A trap should be placed approximately 100-200 feet from each sentinel chicken flock when possible to link abundance with seroconversions.

Traps should be operated from week 14 to week 44 of the calendar year for districts north of the Tehachapi Mountains and all year long for districts south of the Tehachapi. Ideally, the traps should run for four to seven nights before the collection is retrieved (Loomis and Hanks 1959). The trap should be thoroughly cleaned with a brush to remove spider webs or any other debris that may hinder airflow through the trap. A regular cleaning schedule should be maintained during the trapping season to maintain trap efficiency.

#### Processing

Adult mosquitoes from the NJLT collection should be sorted from the other insects in an enamel pan before being identified and counted at 10x magnification under a dissecting microscope. Counting aliquots or subsamples of all specimen samples should be discouraged, because vector species may comprise only a small fraction of the total mosquito collection.

### CDC style CO₂-baited trap

#### Operation

Carbon dioxide-baited traps can be used for abundance monitoring or capturing mosquitoes for virus testing. Traps should be hung from a 6-foot tall standard pole (approximately 4 feet above ground level) to standardize trap placement for population and virus infection rate monitoring. Knowledge of the host-seeking patterns of the target species is essential in
Appendix A

determining CO$_2$-baited trap placement in the habitat to enhance catch size and therefore sampling sensitivity. *Culex tarsalis* primarily bloodfeed on birds and hunt along vegetative borders and tree canopies where birds roost and nest. *Culex erythrothorax* are best collected within wetland areas near dense stands of tules and cattails. In large, open breeding sources such as rice fields, CO$_2$-baited traps could be hung on standards on the up-wind side of the source for *Culex tarsalis* and *Anopheles freeborni* collections. *Aedes melanimon* and *Aedes nigromaculis* are mammal feeders and typically seek hosts over open fields.

When used to supplement sentinel chickens for arbovirus surveillance, traps should be operated at different locations to enhance geographical coverage and thus surveillance sensitivity. Labor and time constraints determine the extent of sampling. When used to monitor population abundance, traps should be operated weekly or biweekly at the same fixed stations. Temperature, wind speed, wind direction, and rainfall should be recorded because these factors affect catch size. The mini-light may be removed, because it attracts other phototactic insects that may hinder sorting and/or damage female mosquitoes in the collection container and may repel members of the *Culex pipiens* complex. The CO$_2$-baited trap should not be placed in immediate proximity to the sentinel chicken flock because it will compete with, and therefore lessen, exposure of the sentinel birds, but may be placed within a 100-200 foot radius of the sentinel flock site, but no closer than 100 feet from the flock.

**Processing**

Mosquitoes collected for arbovirus surveillance should be processed according to the procedures outlined in Appendix B. If possible, ten pools of a species (*Culex tarsalis, Culex pipiens, Culex quinquefasciatus, Culex stigmatosoma, Aedes melanimon,* and *Aedes dorsalis*) should be submitted for virus testing from a given geographical location at a given time. Only live mosquitoes should be pooled for virus testing. Dead, dried specimens should be counted and discarded. Only whole specimens should be submitted; avoid including detached body parts (which may be from other mosquito species) or other Diptera (i.e., *Culicoides*, etc.) in the pool to prevent sample contamination. Avoid freezing specimens before sorting and counting. Mosquitoes collected for population monitoring should be anesthetized in a well-ventilated area or under a chemical hood using triethylamine, identified to species under a dissecting microscope, counted, pooled and immediately frozen at -80°C or on dry ice for later virus testing.

**Reiter/Cummings gravid traps**

**Trap design and components**

The Reiter/Cummings gravid traps consist of a rectangular trap housing (plastic tool box) with an inlet tube on the bottom and an outlet tube on the side or top. The rectangular housing is provided with legs to stabilize the trap over the attractant basin containing the hay-infusion mixture. (Cummings 1992). The oviposition attractant consists of a fermented infusion made by mixing hay, Brewer’s yeast and water. The mixture should sit at ambient temperature for a minimum of three to four days prior to use to allow fermentation and increase attractancy. New solutions should be made at least biweekly to maintain consistent attractancy.

**Operation**

The Reiter/Cummings gravid trap is primarily used in suburban and urban residential settings for surveillance of gravid females in the *Culex pipiens* complex. The trap is placed on
the ground near dense vegetation that serves as resting sites for gravid females. Specimens may be retrieved on a one to three day basis.

**Processing**

*Culex pipiens* complex females collected with the gravid trap for arbovirus surveillance should be retrieved daily and the protocol for mosquito pool submission as outlined in Appendix B should be followed. For population monitoring of the *Culex pipiens* complex, collections may be retrieved every third day. The females are killed, identified and counted before being discarded. Autogenous females may also be attracted to the gravid trap.

**Adult resting collections**

**Trap design and operation**

A flashlight and mechanical aspirator can be used to collect adult mosquitoes resting in habitats such as shady alcoves, buildings, culverts, or spaces under bridges. Highest numbers usually are collected at humid sites protected from strong air currents. Adults resting in vegetation may be collected using a mechanical sweeper such as the AFS (Arbovirus Field Station) sweeper (Meyer et al. 1983). For quantification, time spent searching is recorded and abundance expressed as the number collected per person-hour.

Red boxes were developed to standardize collections spatially. Different researchers have used red boxes of varying dimensions. Largest catches are made in semi-permanent walk-in red boxes which measure 4’ x 4’ x 6’ (Meyer 1985). Smaller 1’ x 1’ x 1’ foot boxes typically collect fewer specimens, but are readily portable. The entrance of the walk-in red box should be left open, draped with canvas, or closed with a plywood door. The canvas or plywood door should have a 1 or 2 ft gap at the bottom to allow entry of mosquitoes, while affording some protection from the wind and decreasing the light intensity within the box. The box entrance should not face eastward into the morning sun or into the predominant wind direction.

**Processing**

Mosquitoes should be anesthetized with triethylamine, identified under a dissecting microscope, sorted by sex and female metabolic status (i.e., empty or unfed, blood fed or gravid), and counted. Females may be counted into ten pools of approximately 50 females per site per collection date for virus monitoring (see Appendix B). Only living females should be used for arbovirus surveillance. Data on metabolic status may indicate population reproductive age as well as diapause status.

**Data recording and analysis**

Counts from NJLTs, EVS, and gravid traps and information on pools submitted for testing or tested locally should be entered directly in electronic format through the California Vectorborne Disease Surveillance Gateway (http://gateway.calsurv.org). Import from local or proprietary data systems is available. For comparisons of abundance over time, space, or collection methods, refer to Bidlingmayer (1969).
Data usage

Mosquito collections from some or all of the four sampling methods collectively can be used to:

1. Assess control efforts.
2. Monitor arbovirus vector abundance and infection rates.
3. Compare mosquito abundance from collections with the number of service requests from the public to determine the tolerance of neighborhoods to mosquito abundance.
4. Determine proximity of breeding source(s) by the number of males present in collections from the NJLTs and red boxes.
5. Determine age structure of females collected by CO$_2$ traps and resting adult collections; such data are critical to evaluating the vector potential of the population.
Appendix B: Procedures for Processing Mosquitoes for Arbovirus Detection

1. Collect mosquitoes alive and return them immediately to the laboratory. Collections should be kept humid during transport with moist toweling to prevent desiccation. Females should be offered 5-10 percent sucrose if held overnight or longer before processing.

2. Anesthetize mosquitoes by cold, carbon dioxide, or triethylamine (TEA). TEA is recommended because specimens are permanently immobilized with minimal mortality and with no loss of virus titer (Kramer et al. 1990). TEA should be used either outdoors or under a chemical hood. Collections can be anesthetized outdoors using a few drops of TEA, the specimens transferred to Petri dishes, and then taken into the laboratory for processing. If refrigerated and kept humid, mosquitoes will remain alive in covered Petri dishes for one or two days without additional anesthesia. If mosquitoes are frozen before processing, sorting to species and enumeration must be done on a chill table to prevent virus loss.

3. Sort mosquito collections to species under a dissecting microscope at 10X to ensure correct identification and to make sure that extraneous mosquito parts (i.e., legs, wings) or other small insects such as chironomids or Culicoides are not inadvertently included in the pools. This is extremely important because diagnostics have transitioned from virus isolation to sensitive RT-PCR methods of viral detection. Count and discard dead and dried mosquitoes. 50 females of each vector species per pool from each collection site are then counted into individual polystyrene vials with snap caps containing two 5mm glass beads. Recommended sampling effort is ten pools of 50 females of each species from each site per week to detect minimum infection rates (MIRs) ranging from 0 to 20 per 1,000 females tested. Vials with pools should be labeled sequentially starting with #1 each year after the site code; e.g., KERN-1-13; where 13 refers to year 2013. Data on each pool can be entered directly in electronic format through the California Vectorborne Disease Surveillance Gateway (http://gateway.calsurv.org/). POOLS MUST BE ACCOMPANIED BY “MOSQUITO POOLS SUBMITTED FORM MBVS-3” AND CAN ONLY BE TESTED FROM REGISTERED SITES. Surveillance sites should be registered online at: http://gateway.calsurv.org/. Faxed registration forms (MBVS-1) will be accepted from agencies without adequate internet access.

List the site code for each pool that consists of a designated four-letter agency code followed by six digits identifying the site, i.e., KERN000001. Keep the pool numbers in sequence for the whole year regardless of the number of site codes: e.g., pool #1 may be from KERN000001, and pool #2 may be from KERN000004.

4. Freeze pools immediately at -70°C either on dry ice in an insulated container or in an ultra-low temperature freezer. Pools should be shipped frozen on dry ice to CVEC for testing by real time multiplex RT-PCR. Pools received by noon on Wednesday will be tested and reported by Friday or sooner. Agencies will receive an automated email notification that results have been entered into the CalSurv Gateway as well as a summary of positive pools; additionally, positive pools will be reported weekly in the California Arbovirus Surveillance Bulletin. Each pool is screened for WNV, SLE, and WEE viruses by a multiplex qRT-PCR assay, with positives with Ct scores >30 confirmed by a singleplex RT-PCR. Pools from
selected areas also are screened for additional viruses using Vero cell culture with isolates identified following sequencing. Care must be taken not to allow pools to defrost during storage or shipment, because each freeze-thaw cycle may result in a 10-fold decrease in viral titer, and all virus will be lost if the specimens sit at room temperature for extended periods. Address shipment to: Ying Fang, Center for Vectorborne Diseases, VetMed 3A room 3336, University of California at Davis, Davis CA 95616.

5. Local agencies that conduct their own testing by RT-PCR or RAMP® tests need to complete and pass a proficiency panel each year for the results to be reported by CDPH.
Appendix C: Procedures for Maintaining and Bleeding Sentinel Chickens

1. Procure hens in March or when they become available as notified by MVCAC when the chickens are 14-18 weeks of age to ensure minimal mortality during handling. Hens at this age have not yet begun to lay eggs, but they should have received all their vaccinations and been dewormed.

2. Recommended housing for chickens. Ten sentinel chickens can be housed in a 3Wx6Lx3H ft coop framed with 2x2 and 2x4 inch construction lumber and screened with no smaller than 1x1 inch welded wire. It is critical that the wire mesh be large enough to allow the mosquitoes to easily enter the coop and the coops be placed in locations with a history of arbovirus transmission and/or high mosquito abundance. The site of and band numbers located at each coop must be registered online at: http://gateway.calsurv.org/. Faxed registration forms (MBVS-1) will be accepted from agencies without adequate internet access. Coops should be at least two feet off the ground to reduce predator access, facilitate capture of the birds for bleeding, and allow the free passage of the feces through the wire floor to the ground. A single, hinged door should be placed in the middle of the coop, so that the entire coop is accessible during chicken capture. After construction, the lumber and roof should be protected with water seal. A self-filling watering device should be fitted to one end of the coop and a 25 lb. feeder suspended in the center for easy access. In exchange for the eggs, a local person (usually the home owner, farm manager, etc.) should check the birds (especially the watering device) and remove the eggs daily. If hung so the bottom is about four inches above the cage floor and adjusted properly, the feeder should only have to be refilled weekly (i.e., 100 lb. of feed per month per flock of ten birds). Therefore, if proper arrangements can be made and an empty 55-gallon drum provided to store extra feed, sentinel flocks need only be visited biweekly when blood samples are collected.

3. Band each bird in the web of the wing using metal hog ear tags and appropriate pliers. This band number, the date, and site registration number must accompany each blood sample sent to the laboratory for testing.

4. Bleed each hen from the distal portion of the comb using a standard lancet used for human finger "prick" blood samples. The bird can be immobilized by wedging the wings between the bleeder's forearm and thigh, thereby leaving the hand free to hold the head by grabbing the base of the comb with the thumb and forefinger. Use alcohol swabs on comb before bleeding. Blood samples are collected on half-inch wide filter paper strips, which should be labeled with the date bled and wing band number. The comb should be "pricked" with the lancet and blood allowed to flow from the "wound" to form a drop. Collect the blood by touching the opposite end of the pre-labeled filter paper strip to the wound. THE BLOOD MUST COMPLETELY SOAK THROUGH ON A ¾ INCH LONG PORTION OF THE STRIP. Place the labeled end of the strip into the slot of the holder (or "jaws" of the clothes pin) leaving the blood soaked end exposed to air dry.

5. Attach the completely dry filter paper strips to a 5x7 card in sequential order, from left to right by stapling the labeled end towards the top edge of the card, and leaving the blood soaked end free so that the laboratory staff can readily remove a standard punch sample. Write the County, Agency Code, Site, and Date Bled onto the card and place it into a zip lock plastic bag. Do not put more than one sample card per bag. It is important that bleded ends do not become dirty, wet, or touch each other. VERY IMPORTANT:
CHICKEN SERA MUST BE ACCOMPANIED BY SENTINEL CHICKEN BLOOD FORM (MBVS-2) OUTSIDE THE ZIP-LOCK BAG. Do not staple the form to the bag. Samples from each bleeding date then can be placed into a mailing envelope and sent to:

Department of Public Health, Richmond Campus
Specimen Receiving Unit Room B106 (ATTN: ARBO)
850 Marina Bay Parkway
Richmond, CA 94804

Specimens will be tested within 1-3 days upon receipt by the laboratory.

6. In the laboratory, a single punch is removed from the blooded end of the paper and tested for WEE, SLE, and WNV IgG antibodies using ELISA (Patiris et al. 2008; Taketa-Graham et al. 2010). Positive specimens are tested further with an indirect fluorescent antibody test and confirmed with a Western blot. Inconclusive SLE or WNV positives are confirmed and identified by cross-neutralization tests. Agencies will receive an automated email notification that results have been entered into the CalSurv Gateway; additionally, positive chickens will be reported in the weekly California Arbovirus Surveillance Bulletin.
California Procedure for Testing Sentinel Chickens for the Presence of Antibodies to Flaviviruses (SLE and WNV) and WEE

MVCD collects blood from comb of each chicken onto filter paper approx. every other week and enters data into Surveillance Gateway

Local labs that test their own flocks send positive samples to CDPH for confirmation

MVCD sends filter paper strips and submission report form to CDPH for arbovirus testing by EIA

Local labs that test their own flocks send negative results to CDPH

EIA positive samples tested by IFA and Western blot at CDPH

Negative results reported immediately to submitting agency via Surveillance Gateway

Inconclusive results may warrant CDPH request for whole blood sample

Final test results reported immediately to submitting agency via Surveillance Gateway and listed in weekly bulletin

Key:
EIA: Enzyme immunoassay test
IFA: Indirect fluorescent antibody test
MVCD: Local Mosquito and Vector Control District/Health Dept.
SLE: St. Louis encephalitis
CDPH: CDPH Vector-Borne Disease Section, Richmond
WEE: Western equine encephalitis
WNV: West Nile virus encephalitis
Surveillance for Mosquito-borne Viruses
Registration of Agencies and Sites

1. Participation of agencies

Agencies interested in participating in the statewide surveillance program for mosquito-borne viruses should place orders for mosquito pool testing by UC Davis Center for Vectorborne Diseases (CVEC) through the Mosquito and Vector Control Association (MVCAC). Sentinel chicken testing should be ordered through the California Department of Public Health (CDPH). Agencies will be billed in advance for the number of samples to be tested.

Agencies are responsible for registering and maintaining updated information for their sites online at: [http://gateway.calsurv.org/](http://gateway.calsurv.org/).

2. Registration of sentinel flock sites and wing band numbers

Agencies must use the unique band numbers assigned to their district by CDPH each year. Prior to submitting any sentinel chicken blood samples to CDPH, each agency must ensure that each flock site and accompanying band numbers are registered online at: [http://gateway.calsurv.org](http://gateway.calsurv.org). CDPH will only test samples if they are accompanied by the form “SENTINEL CHICKEN BLOOD – 2013” (MBVS-2) for each flock site, which includes the registered agency code, the registered site code (assigned by local agency), the wing band numbers assigned to that site, and date bled. **Also, the form should indicate any changes made and match the sample card exactly.**

3. Registration of mosquito sampling sites

Registration of new sites used for collection of mosquitoes for virus testing may be accomplished by accessing the California Vectorborne Disease Surveillance Gateway [http://gateway.calsurv.org/](http://gateway.calsurv.org/). Since 2010, the CalSurv Gateway has included enhanced spatial capabilities that allow users the option of directly entering geographic coordinates for sites or interactively selecting the location using a new Google Maps-based interface. The laboratory will test the pools provided that adequate information is provided on the “MOSQUITO POOL SUBMISSION” form (MBVS-3, revised 01/12/06), including your agency code, your site code for the site and geographic coordinates.

The geographic coordinates will be used to generate computer maps that show all registered sites and test results for each site. Also, as part of a collaborative effort, CVEC will host real-time maps in ArcGIS format at [http://maps.calsurv.org](http://maps.calsurv.org). In addition to these maps, agencies can access maps using Google Earth through the California Vectorborne Disease Surveillance Gateway ([http://gateway.calsurv.org](http://gateway.calsurv.org)) that provide enhanced functionality and detail.
Appendix D: Procedures for Testing Dead Birds and Squirrels

In 2000, CDHS initiated a dead bird surveillance program in collaboration with other public agencies. CDPH annually notifies about 600 agencies, organizations, and veterinarians involved with wildlife, including rehabilitation centers, about the program. The public is also notified about the program through the media and outreach materials. Dead birds and squirrels are reported to CDPH or data entered electronically through the Surveillance Gateway [http://gateway.calsurv.org/] and shipped to the California Animal Health & Food Safety (CAHFS) laboratory at UC Davis for screening and removal of kidney tissue (an oral swab is taken instead if the bird is an American Crow), which is then sent to the UC Davis Center for Vectorborne Diseases (CVEC) for WNV RNA detection via RT-PCR. Beginning in 2010, results from RT-PCR testing at CVEC distinguished between WNV recent and chronic positive birds based on cycle threshold (Ct) values. Chronic positive birds did not likely die from WNV infection and are of limited value for surveillance. Overviews of the dead bird reporting and testing algorithms are provided below.

**Sick / Dead Bird Reporting Protocol for Public and Local Agencies**

```
Dead

Wild Bird

CDPH Hotline / Web

B.I.R.D. System

MVCA or local pick-up (AC etc.)

WNV testing

Disposal

Sick

Domestic Poultry

CDFA

** AUTOMATED EMAIL REPORTS **

AI testing (CAHFS)

Local agency (AC, Rescue Group, CDFG, etc.)

Wild Bird

CDPH Hotline / Web

B.I.R.D. System

MVCA or local pick-up (AC etc.)

WNV testing

Disposal

```

* domestic poultry, designated spp.
** ≥ 5 birds, designated AI spp., water birds, shorebirds
AC Animal Control
AI Avian Influenza
BIRD Bird Information Reporting Database (CDPH SQL Server)
CAHFS CA Animal Health & Food Safety Laboratory
CDFA California Department of Food & Agriculture:
California Bird Flu Hotline: 1-866-922-BIRD
CDFG California Department of Fish & Game
http://www.dfg.ca.gov/regions/index.html
CDPH California Department of Public Health
West Nile virus & Dead Bird hotline: 1-877-968-BIRD
website: www.westnile.ca.gov
MVCA Mosquito & Vector Control Agency
Procedures for Testing Dead Birds: RT-PCR

For multiple bird die-offs, VBDS contacts CDFG.

Dead Bird Found:
Call CDPH Vector-Borne Disease Section (1-877-WNV-BIRD) or go to http://www.westnile.ca.gov for more information. Enter into Surveillance Gateway [http://gateway.calsurv.org/]

Dead > 24hrs (e.g. stiff, presence of maggots); not a species targeted for testing.

Found within 24 hours of death and meets testing criteria; zip code “open” for testing.

Local agency obtains dead bird and delivers or ships on blue ice to CAHFS.

Report will be recorded and noted in weekly bulletin, forwarded to agencies.

VBDS contacts local agency to pick up dead bird, or coordinates for public drop-off when appropriate. Information on dead bird is faxed/emailed to local agency and CAHFS. VBDS reports submission by county in weekly Arbovirus Bulletin.

CAHFS screens specimen to verify carcass is in a testable condition, then notifies VBDS of status. CAHFS removes kidney tissue/takes oral swab for RT-PCR testing by CVEC.

Laboratories enter test results into Surveillance Gateway

VBDS sends dead bird results to:

CAHFS, local CD, local MVCD, CDFG, and other public agencies.

Key:
CAHFS: CA Animal Health and Food Safety Laboratory
CD: Local Agency Communicable Disease Office
CDFA: CA Dept. of Food and Agriculture
CDFG: CA Dept. of Fish and Game
CVEC: UC Davis Center for Vectorborne Diseases
MVCD: Local Mosquito and Vector Control District
USFWS: US Fish and Wildlife Service
VBDS: CDHS Vector-Borne Disease Section, Richmond
VPHS: CDHS Veterinary Public Health Section, Sacramento
IHC: Immunohistochemistry

Negative Results: Submitting agency, CAHFS, local CD, local MVCD, CDFG, and other public agencies.

Positive Results: Submitting agency, CAHFS, VPHS, local CD, USFWS, CDFA, local MVCD, CDFG, and other public agencies.
Public reports dead bird to VBDS:
Is bird acceptable for West Nile virus (WNV) testing?

Yes

Bird assigned state number and picked up by local agency or dropped off by public

Non-corvid

VBDS assigns primary identification Corvid or Non-Corvid?

Corvid

Has local vector control agency passed proficiency panel for VecTest or RAMP?

Yes

Test oral swab by VecTest or RAMP

Negative Non-Crow

Send carcass to CAHFS

Negative Crow

STOP, submit results to VBDS by Friday by 4:00pm

Yes

Send carcass to CVEC; Tissue to CVEC; Results to CDPH

No

Dead bird reports available to agencies on request

STOP, submit results to VBDS by Friday by 4:00pm
Dead Bird and Tree Squirrel Reporting and Submission Instructions for Local Agencies
California West Nile Virus (WNV) Dead Bird & Tree Squirrel Surveillance Program
California Department of Public Health (CDPH)
Division of Communicable Disease Control

When your agency receives a call from the public about a dead bird (especially recently dead crows, ravens, magpies, jays, or raptors) or dead tree squirrel, or one of your staff finds any dead bird, please immediately refer them to the CDPH West Nile Virus and Dead Bird Hotline at 1-877-WNV-BIRD (968-2473).

The Dead Bird Hotline is monitored 8am - 5pm, Monday-Friday (7 days a week May-October). Reports can also be made after hours via voicemail prompts and on the WNV website: www.westnile.ca.gov. CDPH will assess the suitability of the dead bird or tree squirrel for testing and contact your agency if the carcass is approved for pickup. Any carcasses sent without prior notification will not be tested.

Agencies may call the agency phone number, 510-412-4601, to coordinate bird pick-ups with hotline operators. If your agency collects a dead bird for testing and it is in suitable condition, you can call this number to receive a dead bird number and submission form prior to shipping.

Only agencies listed under the permit issued to CDPH from the California Department of Fish & Wildlife are authorized to pick up dead birds and tree squirrels. The agencies covered include local mosquito abatement districts, environmental health departments, and other designated agencies.

Members of the public may salvage dead birds found on their property or place of residence if the local agency has indicated to CDPH they will accept public salvage. The public must first call the Dead Bird Hotline and obtain a Dead Bird Number; a corresponding public salvage submission form will then be faxed to the appropriate agency. The public will be instructed by the hotline staff to double-bag the carcasses and drop them off at the designated agency within 24 hours, between 9 am - 3 pm, Monday – Friday. Note: only dead birds may be brought in by the public to local agencies for shipping. We discourage public salvage of all squirrels because ground squirrels, which could be infected with plague, may be misidentified as tree squirrels.

web links: bird and tree squirrel ID chart (pdf) tree squirrel surveillance Q&A (pdf)

Once the submission is approved, your agency can ship the carcass to the California Animal Health & Food Safety laboratory at UC Davis (CAHFS Central). CAHFS Central removes specific tissues and forwards the samples to the UC Davis Center for Vectorborne Diseases (CVEC) for WNV testing. Shipping and testing expenses will be paid by CDPH. Carcasses are considered Category B, Biological Substances. This replaces the old designation, “Diagnostic Specimen”.

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To ensure the carcass arrives at CAHFS in a testable condition, to protect your safety, and to comply with shipping regulations, please follow these instructions:

- Only dead birds and tree squirrels can be picked up under our permit.

- Wear rubber or latex gloves when handling all carcasses. If gloves are not available, use a plastic bag -- turned inside out -- over your hand and invert the bag to surround the carcass. Do not touch a carcass with bare hands.

- Collect fresh carcasses. Badly decomposed or scavenged carcasses are of limited diagnostic value. Signs that a bird or squirrel has been dead for too long (over 24-48 hours) are the presence of maggots, an extremely lightweight carcass, missing eyes, skin discoloration, skin or feathers that rub off easily, strong odor, or a soft, mushy carcass.

- If upon pick-up the carcass is found to be unacceptable (e.g. a species your agency or CDPH is not accepting or a badly decomposed specimen), please collect the carcass, double-bag it, and dispose of it in a secure garbage can or dumpster. California Department of Fish & Game prefers that you burn or bury the carcass, but disposing of it in a dumpster is also acceptable. Please call CDPH immediately and notify us that the animal will no longer be submitted.

- Place each carcass into a sealed (zip-locked) plastic bag. This prevents cross-contamination and leakage. There should always be a bag separating the carcass from shipping documents.

- Enclose the shipping documents and the carcass (sealed in a zip-locked bag) into a second zip-locked bag. The primary shipping document is a copy of the dead bird submission form which contains the dead bird number and which is located on the Surveillance Gateway [http://gateway.calsurv.org/] or faxed by CDPH. CAHFS prefers that you put this inside the outer bag containing the dead bird or squirrel.

- Pack the carcass with blue ice packs. Please limit the number of ice packs to the number required to keep the carcass fresh, as the weight of extra ice packs add to the shipping charges. In accordance to shipping regulations, an absorbent material such as newspaper must be included in the box to prevent any leakage.

- Ship the carcass in a hard-sided plastic cooler or a Styrofoam cooler placed in a cardboard box. Unprotected Styrofoam containers cannot be shipped without an outer box or container, as they may break into pieces during shipment. Contact GSO directly to arrange for carrier pickup Monday through Thursday; this guarantees arrival at CAHFS before the weekend.

- Contact GSO to pick up carcasses at 1-800-322-5555. Our shipping account is 22971 and our zip code is 94804.
• Carcasses that need to be stored for an extended time period (over 2 days) should be put on dry ice or stored at -70°C. If it is not possible to store carcass at -70°C, a carcass may be stored at 0°C (regular freezer) for a short period of time. Refrigerating the carcass is recommended for overnight storage only (this slows virus deterioration, but does not stop it).

• CDPH will provide prepared shipping boxes with appropriate labels. Any empty boxes shipped to your agency from CDPH will have its caution labels covered by a sheet of paper with “EMPTY BOX” printed on it. Please discard this sheet of paper before using the box to ship out a dead bird. If you need additional boxes, please contact VBDS at (510) 412-6251 or email arbovirus@cdph.ca.gov.

• Once agencies pass the yearly proficiency panel, agencies may conduct in-house testing. Results can be entered directly into the Surveillance Gateway. Please enter results by 4:00pm Tuesday of each week to have results included in reports for that week’s State WNV updates. Note: any positive bird must be disposed of as biomedical waste (incineration).

Dead Bird Shipping List

Please verify that your agency has the following items:

- CAHFS Address (see below)
- GSO preprinted labels
- Agency-only number for hotline (510-412-4601)
- WNV hotline number, 1-877-WNV-BIRD (968-2473); staffed 8am - 5pm, 7 days a week during summer
- Crumpled newspapers or another absorbent material
- Rubber or Latex Gloves
- Packing tape
- Dead Bird Shipping Boxes
  - inner zip-lock bag
  - outer zip-lock bag
  - inner styrofoam box
  - outer cardboard box
  - blue ice packs

California Animal Health & Food Safety (CAHFS) laboratory:

CAHFS Central (530) 754-7372
ATTN: WNV
Jacquelyn Parker
University of California, Davis
West Health Science Drive
Davis, CA 95616
Appendix E: Procedures for Testing Equines and Ratites

The California Departments of Public Health (CDPH) and Food and Agriculture (CDFA) developed a cooperative passive surveillance program for equine and ratite encephalomyelitis. Primary responsibility for equine and ratite West Nile virus (WNV) surveillance rests with CDFA. Equine encephalomyelitides are legally reportable to CDFA by veterinarians and diagnostic laboratories pursuant to Section 9101 of the Food and Agricultural Code. Venezuelan equine encephalomyelitis is an emergency animal disease that must be reported to CDFA by telephone within 24 hours.

This appendix contains information sent to veterinarians, public health lab directors, local health officers, public health veterinarians, animal health branch personnel, and interested parties every spring to inform them about the California Equine and Ratite Arbovirus Surveillance Program. The mailing includes a case definition for equine encephalomyelitides and instructions for specimen collection and submission for both equine and ratite samples. The information is distributed to approximately 1,200 practitioners, equine organizations, and other interested parties. Specimen submission is coordinated through the California Animal Health and Food Safety Laboratory System (CAHFS) and other laboratories or individual veterinarians. Equine serum and cerebrospinal fluid are tested by CAHFS using the ELISA test to detect anti-WNV IgM. Equine neurologic tissue specimens are also sent to CAHFS for microscopic examination and, as indicated by clinical findings, forwarded to the USDA National Veterinary Services Laboratories (NVSL) for further arbovirus testing. All fatal cases of equine encephalitis are first evaluated for rabies at the local public health laboratory. An algorithm outlining the protocol for specimen submission and reporting is available for participants in the program and is included in this appendix.

Outreach is an important component of the program. CDPH and CDFA have developed and distributed educational materials about the diagnosis and reporting of arboviruses in equines and ratites.

Additional information on WNV for veterinarians, horse owners, and ratite owners, is available from CDFA, Animal Health Branch (916) 900-5002, and at the CDFA website: http://www.cdfa.ca.gov/AHFSS/Animal_Health/WNV_Info.html. Information on submission of laboratory samples is available from CAHFS (530) 752-8700 and at CAHFS website: http://cahfs.ucdavis.edu. A brochure containing facts about California WNV surveillance and general information about prevention and control is available from CDPH (916) 552-9730 and at CDPH’s website: http://www.westnile.ca.gov; a special section for veterinarians and horse owners is available at: http://www.westnile.ca.gov/resources.php.
Algorithm for Submission of Specimens from Domestic Animals with Neurologic Symptoms

**Species:**
- Horse
- Emu
- Ostrich
- Other

**Alive**
- Send acute and convalescent sera or CSF to CAHFS or other diagnostic lab for arbovirus serologic testing including the WNV IgM Capture ELISA test. Some arboviruses will be tested at NVSL or other diagnostic lab. If questions, call CAHFS at (530) 752-7578.

**Dead**
- Submit carcass to CAHFS for necropsy / histopath. Questions/Shipping Information: Call CAHFS at (530) 752-7578.
- If rabies negative and viral encephalitis still suspected, brain sent to CAHFS for microscopic examination and WNV testing. Some arboviruses will be tested at NVSL or other diagnostic lab. Questions/Shipping Information: Call CAHFS at (530) 752-7578.

CAHFS or other diagnostic lab reports results to submitter. Positive results reported by phone or email to CDFA. A copy of the report is sent to CDPH/VPHS.

CDFA conducts investigation of lab-positive case. CDPH/VPHS reports preliminary results to CDPH/VBDS for notification of local agencies.

**Key:**
- AHB: Animal Health Branch
- CAHFS: California Animal Health and Food Safety Laboratory
- NVSL: National Veterinary Services Laboratory
- VBDS: CDPH Vector-Borne Disease Section
- VPHS: CDPH Veterinary Public Health Section
- CDFA: California Department of Food and Agriculture
- CDPH: California Department of Public Health
SURVEILLANCE CASE DEFINITIONS FOR WEST NILE VIRUS DISEASE IN EQUINES

NOTE: A HORSE WITH SIGNS OF ENCEPHALITIS MAY HAVE RABIES – TAKE PROPER PRECAUTIONS

CONFIRMED CLINICAL CASE:

A horse with compatible clinical signs including ataxia (stumbling, staggering, wobbly gait, or in-coordination) or at least two of the following: fever, circling, hind limb weakness, inability to stand, multiple limb paralysis, muscle fasciculation, proprioceptive deficits, blindness, lip droop/paralysis, teeth grinding, acute death.

Plus one or more of the following:

- Isolation of West Nile (WNV) virus from tissues
- Detection of IgM antibody to WNV by IgM-capture ELISA in serum or CSF
- A four-fold or greater change in plaque-reduction neutralization test (PRNT) antibody titer to WNV in appropriately timed, paired sera
- Positive polymerase chain reaction (PCR) for WNV genomic sequences in tissues
- Positive IHC for WNV antigen in tissue (Note: this test has low sensitivity in equids)

SUSPECT CLINICAL CASE:

- Compatible clinical signs

EXPOSED EQUID:

- Detection of IgM antibody to WNV by IgM-capture ELISA in serum or CSF without any observable or noted clinical signs.

Assumptions on which case definition is based:

- Antibody in serum may be due to vaccination or a natural exposure; additional testing must be done to confirm WNV infection in a vaccinated horse.
- IgM antibody in equine serum is relatively short-lived; a positive IgM-capture ELISA means exposure to WNV or rarely a closely related flavivirus (SLE) has occurred, very likely within the last three months.

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1 Preferred diagnostic tissue are equine brain or spinal cord; although tissues may include blood or CSF, the only known reports of WNV isolation or positive PCR from equine blood or CSF have been from experimentally infected animals.
2 The first serum should be drawn as soon as possible after onset of clinical signs and the second drawn at least seven days after the first.
3 For horses it is recommended that RT-nested polymerase chain reaction assay be used to maximize sensitivity of the test (Emerg. Infect. Dis. 2001 Jul-Aug; 7(4):739-41)
4 An equine case classified as a suspect case should, if possible, undergo further diagnostic testing to confirm or rule out WNV as the cause of the clinical illness.
Protocol for Submission of Laboratory Specimens for Equine Neurological Disease Diagnosis and Surveillance

Complete information on specimen collection and submission is available on the CDFA website at: http://www.cdfa.ca.gov/ahfss/Animal_Health/WNV_Lab_Submission.html

1. Specimen collection and submission:
   A. Blood
      • Acute sample (5-10 ml) / no later than 7 days after onset
      • Convalescent sample (5-10 ml) / 14-21 days after onset
         Red top tubes of whole blood or serum (no preservatives or anticoagulants) should be submitted at ambient temperature to the California Animal Health and Food Safety (CAHFS) Laboratory* in your area. Do not freeze whole blood.
      • NOTE: For WNV, an acute sample only is required since the assay used detects IgM (and vaccine does not interfere). For the other encephalitis viruses, the acute sample should be submitted immediately, and a convalescent sample may be requested later to assist with the interpretation and differentiation of vaccine titers from active infection.
   
   B. Brain
      • The local health department and CDFA/Animal Health District Office should be contacted if rabies is suspected.
      • The animal or intact head should be submitted to a CAHFS laboratory in your area as quickly as possible. The intact head should be refrigerated, not frozen, immediately after removal using a leak-proof insulated transporting container with "cold packs" to keep the specimen at 4° C while in transit. When it is impossible for the CAHFS Laboratory to receive the carcass or chilled intact head within 48 hours, the submission protocol should be coordinated with the CAHFS Laboratory. Submission of the head intact is preferable to removal of the brain because: 1) the brain is better preserved (anatomically and virus titer) when left in the skull during transport, 2) specimens may be compromised if removal is not performed correctly, and 3) brain removal in field conditions may increase the risk of exposure to rabies.
      • All equine specimens submitted first to the CAHFS Laboratory will be forwarded to: 1) a Public Health Laboratory to initially confirm or rule out rabies, then to 2) The National Veterinary Services Laboratories (NVSL) for arboviral testing, in addition to a complete diagnostic work-up at the CAHFS.
      • All equine specimens submitted first to local public health laboratories for rabies testing and found to be negative should be forwarded to the local CAHFS Laboratory for arboviral testing.
   
   C. Other specimens for differential neurological diagnoses
      • Protocol for submission of these specimens may be coordinated through the CAHFS Laboratory, and may include sampling for equine herpes virus, EPM, or other agents associated with clinical neurological presentations.
2. **Submission forms**: Complete and include the transmittal forms supplied by the CAHFS Laboratory. See attached sample or download the form from their website: [http://cahfs.ucdavis.edu/](http://cahfs.ucdavis.edu/). The submission form for each specimen should be placed in a leak-proof plastic bag and attached to the corresponding container.

3. **Imperative information to include on the submission forms if West Nile virus is suspected**: Precision case reporting by the veterinary practitioner is critical to mosquito control efforts that prevent WNV infection in both horses and humans. It is very important that each laboratory submission form is completed in its entirety. More specifically, it is imperative to include and communicate to us:
   - The locations(s) of the horse during the two weeks prior to the onset of clinical disease. (Please ensure that this information is included in the addition to the owner’s address)
   - Detailed clinical signs
   - The present condition of the horse (include dead and euthanized)
   - An accurate vaccination history

4. **Shipment**: For information on sample shipping including regulations governing the transportation of infectious materials contact CAHFS at 530-752-7578.
Appendix F: Protocol for Submission of Laboratory Specimens for Human West Nile Virus Testing

West Nile virus (WNV) testing within the regional public health laboratory network (i.e., the California Department of Public Health Viral and Rickettsial Disease Laboratory and participating local public health laboratories) is recommended for individuals with the following symptoms, particularly during West Nile virus “season,” which typically occurs from July through October in California:

A. Encephalitis
B. Aseptic meningitis (Note: Consider enterovirus for individuals ≤ 18 years of age)
C. Acute flaccid paralysis; atypical Guillain-Barré Syndrome; transverse myelitis; or
D. Febrile illness*
   - Illness compatible with West Nile fever and lasting ≥ 7 days
   - Must be seen by a health care provider

* The West Nile fever syndrome can be variable and often includes headache and fever (T ≥ 38°C). Other symptoms include rash, swollen lymph nodes, eye pain, nausea, or vomiting. After initial symptoms, the patient may experience several days of fatigue and lethargy.

Required specimens:

- Acute serum: ≥ 2cc serum

If a lumbar puncture is performed and residual CSF is available:

- Cerebral spinal fluid (CSF): 1-2cc CSF

If West Nile virus is highly suspected and acute serum is negative or inconclusive, request:

- 2\textsuperscript{nd} serum: ≥ 2cc serum collected 3-5 days after acute serum

Contact your local health department for instructions on where to send specimens.
Appendix G: Surveillance Case Definition for West Nile Virus Infection in Humans

West Nile virus infection is reportable to local health departments under Title 17 of the California Code of Regulations. Blood donors that test positive for West Nile virus through blood bank screening should also be reported to CDPH, regardless of clinical presentation.

CASE DEFINITION: West Nile Virus

NOTE: This definition is for public health surveillance purposes only. It is not intended for use in clinical diagnosis.

Symptomatic Cases (adapted from 2011 CSTE case definition http://www.cdc.gov/osels/ph_surveillance/nndss/casedef/arboviral_current.htm)

Clinical criteria for diagnosis

Neuroinvasive disease
- Fever (≥100.4°F or 38°C) as reported by the patient or a health-care provider, AND
- Meningitis, encephalitis, acute flaccid paralysis, or other acute signs of central or peripheral neurologic dysfunction, as documented by a physician, AND
- Absence of a more likely clinical explanation.

Non-neuroinvasive disease
- Fever (≥100.4°F or 38°C) as reported by the patient or a health-care provider, AND
- Absence of neuroinvasive disease, AND
- Absence of a more likely clinical explanation.

Case classification

Confirmed = A case that meets the above clinical criteria and one or more of the following laboratory criteria for a confirmed case:
- Isolation of virus from, or demonstration of specific viral antigen or nucleic acid in, tissue, blood, CSF, or other body fluid, OR
- Four-fold or greater change in virus-specific quantitative antibody titers in paired sera, OR
- Virus-specific immunoglobulin M (IgM) antibodies in serum with confirmatory virus-specific neutralizing antibodies in the same or a later specimen, OR
- Virus-specific IgM antibodies in CSF and a negative result for other IgM antibodies in CSF for arboviruses endemic to the region where exposure occurred.

Probable = A case that meets the above clinical criteria and the following laboratory criteria:
- Virus-specific IgM antibodies in CSF or serum but with no other testing.*
  *CDPH recommends that virus-specific IgG antibody testing (e.g. EIA or IFA) also be performed. A specimen that is IgM-positive only (i.e. IgG-negative) may be a false
positive, while a specimen that is both WNV IgM- and IgG-positive is more likely a true infection.

**Presumptive Viremic Donors (Asymptomatic)**

Asymptomatic infection with WNV, which is generally identified in blood donors, is also reportable. Blood donors who test positive for WNV may not necessarily be ill, nor will they initially have positive IgM or IgG antibody test results. Local health departments should report blood donors who meet the following criteria for being a presumptively viremic donor to CDPH-CDER:

A presumptively viremic donor (PVD) is a person with a blood donation that meets at least one of the following criteria:

- a) One reactive nucleic acid-amplification (NAT) test with signal-to-cutoff (S/CO) ≥ 17
- b) Two reactive NATs

Additional serological testing is not required. Local health departments should follow up with the donor after two weeks of the date of donation to assess if the patient subsequently became ill. If the donor did become ill as a result of WNV infection, the disease incident should be reclassified as “West Nile virus – Non-neuroinvasive” or “West Nile virus – Neuroinvasive,” depending on the individual’s clinical symptoms.
Appendix H: Compounds Approved for Mosquito Control in California

Label rates and usage vary from year to year and geographically; consult your County Agricultural Commissioner and the California Department of Fish and Game before application. Examples of products containing specific active ingredients are provided below, but this is not an inclusive list nor constitutes product endorsement. For more information on pesticides and mosquito control, please refer to the Environmental Protection Agency (EPA) Web site: http://www.epa.gov/opp00001/factsheets/westnile.htm

**Larvicides:**

1. *Bacillus thuringiensis* subspecies *israelensis* (Bti: e.g. Aquabac 200G, VectoBac® 12AS, Teknar HP-D)
   - **Use:** Approved for most permanent and temporary bodies of water.
   - **Limitations:** Only works on actively feeding stages. Does not persist well in the water column.

2. *Bacillus sphaericus* (Bs: e.g. VectoLex® CG)
   - **Use:** Approved for most permanent and temporary bodies of water.
   - **Limitations:** Only works on actively feeding stages. Does not work well on all species. May persist and have residual activity in some sites.

3. Spinosad (e.g. Natular™ G30)
   - **Limitations:** Effective against all larval stages and moderately effective against pupal stage. Toxic via ingestion and contact. Some formulations approved for use in OMRI certified organic crops.

4. IGRs (Insect Growth Regulators)
   a. (S)-Methoprene (e.g. Altosid® Pellets)
      - **Use:** Approved for most permanent and temporary bodies of water.
      - **Limitations:** Works best on older instars. Some populations of mosquitoes may show some resistance.
   b. Diflubenzamide (e.g. Dimilin®25W)
      - **Use:** Impounded tail water, sewage effluent, urban drains and catch basins.
      - **Limitations:** Cannot be applied to wetlands, crops, or near estuaries.

5. Larviciding oils (e.g. Bonide)
   - **Use:** Ditches, dairy lagoons, floodwater. Effective against all stages, including pupae.
   - **Limitations:** Consult with the California Department of Fish and Game for local restrictions.

6. Monomolecular films (e.g. Agnique® MMF)
   - **Use:** Most standing water including certain crops.
   - **Limitations:** Does not work well in areas with unidirectional winds in excess of ten mph.

7. Temephos (e.g. Abate® 2-BG)
   - **Use:** Non-potable water; marshes; polluted water sites
**Limitations:** Cannot be applied to crops for food, forage, or pasture. This material is an organophosphate compound and may not be effective on some *Culex tarsalis* populations in the Central Valley. May require sampling and testing per General Vector Control NPDES permit requirements if applied to waters of the United States.

**Adulticides:**

1. **Organophosphate compounds**  
   **Note:** Many *Culex tarsalis* populations in the Central Valley are resistant at label OP application rates.  
   a. Malathion (e.g. Fyfanon® ULV)  
      **Use:** May be applied by air or ground equipment over urban areas, some crops including rice, wetlands.  
      **Limitations:** Paint damage to cars; toxic to fish, wildlife and bees; crop residue limitations restrict application before harvest.  
   b. Naled (e.g. Dibrom® Concentrate, Trumpet® EC)  
      **Use:** Air or ground application on fodder crops, swamps, floodwater, residential areas.  
      **Limitations:** Similar to malathion.

2. **Pyrethrins** (natural pyrethrin products: e.g. Pyrenone® Crop Spray, Pyrenone® 25-5, Evergreen)  
   **Use:** Wetlands, floodwater, residential areas, some crops.  
   **Limitations:** Do not apply to drinking water, milking areas; may be toxic to bees, fish, and some wildlife. Some formulations with synergists have greater limitations.

3. **Pyrethroids** (synthetic pyrethrin products containing deltamethrin, cyfluthrin, permethrin, resmethrin, sumithrin or etofenprox: e.g. Suspend® SC, Tempo Ultra SC, Aqua-Reslin®, Scourge® Insecticide, Anvil® 10+10 ULV, Zenivex E20, and Duet – which also contains the mosquito exciter prallethrin)  
   **Use:** All non-crop areas including wetlands and floodwater.  
   **Limitations:** May be toxic to bees, fish, and some wildlife; avoid treating food crops, drinking water or milk production.
### Pesticides Used for Mosquito Control in California

#### Larvicides

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<thead>
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<th>Active Ingredient</th>
<th>Trade name</th>
<th>EPA Reg. No.</th>
<th>Mfgr.</th>
<th>Formulation</th>
<th>Application</th>
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## PESTICIDES USED FOR MOSQUITO CONTROL IN CALIFORNIA

### Adulticides

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<th>Active Ingredient</th>
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<th>Mfgr.</th>
<th>Formulation</th>
<th>Stage</th>
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Appendix I: Adult Mosquito Control in Urban Areas

Adult mosquito control via ultra low volume (ULV) application is an integral part of an integrated mosquito management program. This response plan recommends the consideration of adult mosquito control to break local virus transmission cycles and reduce the risk of human infection. The following provides guidelines for local agencies considering ground or aerial ULV control of adult mosquitoes. Agencies should ensure they are complying with NPDES permit requirements.

Preparatory steps for aerial application contracts

- Send out request for proposals (RFP) to commercial applicators well in advance of any potential need for actual treatment. Specify required equipment and abilities in the RFP such as: 1) application equipment capable of producing desired droplet spectrum and application rate, 2) aircraft availability time frames (remember FAA requires 2-engine aircraft for applications over urban areas), and 3) the demonstrated ability to apply the chosen product to the target area in accordance with label requirements.
- Outline the desired capabilities and equipment within the RFP such as: 1) onboard real time weather systems, and 2) advanced onboard drift optimization and guidance software.
- Determine in advance whether the vector control agency or contractor will secure and provide pesticides. If the contractor will supply the pesticide, verify their knowledge of and ability to comply with regulations regarding the transport, use, and disposal of all pesticide and containers.
- Enter into a contingency contract with the commercial applicator.
- Consider acquiring non-owned, multiple engine aircraft insurance with urban application endorsement for added protection.
- Determine product and application rate to be used, along with a contingency plan. The product choice may be subject to change depending on product availability, the determination of resistance, labeling restrictions, environmental conditions, or other unforeseen factors.

Preparatory steps for ground-based applications

- Ensure that application equipment has been properly calibrated and tested for droplet size and flow rate. The vector control agency should have enough equipment, operators, and product available to finish the desired application(s) between sunset and midnight, or within 2-3 hours pre-sunrise (or when mosquitoes are demonstrated to be most active) to maximize efficacy.
- Ensure that vehicles are equipped with safety lighting and appropriate identifying signs; use sufficient personnel.
- Contact local law enforcement and provide them with locations to be treated and approximate time frames.
- Consider using lead and trailing vehicles particularly if the area has not been treated before and personnel are available.
Implementing an aerial application contract

- Contact commercial applicator and determine availability.
- Review long-term weather forecasts. Ideally applications should be scheduled during periods of mild winds to avoid last minute cancellations.

Contractor should:

- Contact Local Flight Standards District Office (FSDO) for low flying waiver.
- Arrange for suitable airport facilities.
- Contact local air traffic control.
- Locate potential hazards prior to any application and implement a strategy to avoid those hazards during the application – often in darkness.
- Provide equipment and personnel for mixing and loading of material (if previously agreed upon in contract).
- Register with applicable County Agricultural Commissioner’s office.

Vector control agency should:

- Delineate treatment block in a GIS format and send to contractor.
- Identify areas that must be avoided during an application and include detailed maps of those areas to contract applicators (e.g. open water, registered organic farms, any area excluded by product label).
- Send authorization letter to FSDO authorizing contractor to fly on the agency's behalf; contractor should provide contact information and assistance.
- Send map of application area and flight times / dates to local air traffic control; contractor should provide contact information and assistance.
- Consult with County Agricultural Commissioner’s office. Commissioner's office can provide guidance on contacting registered bee keepers and help identify any registered organic farms that may need to be excluded from application.
- If vector control agency is providing material, ensure adequate quantity to complete mission and that the agency has means to transport material.

Efficacy evaluation for aerial or ground based application

- Choose appropriate method(s) for evaluating efficacy of application
  - Determine changes in adult mosquito population via routine or enhanced surveillance.
  - Conduct three day pre and post-trapping in all treatment and control areas.
  - Set out bioassay cages with wild caught and laboratory reared (susceptible) mosquitoes during application.

- Ensure adequate planning so surveillance staff is available and trained, equipment is available, and trap / bioassay cage test locations are selected prior to application.
- Ensure efficacy evaluation activities are timed appropriately with applications.
- Enlist an outside agency such as CDPH and/or university personnel to help evaluate efficacy of application as appropriate.
Actions at time of application

- Confirm application rate with contractor.
- Confirm treatment block.
- Coordinate efficacy evaluations.

Public notification

Notification of the public prior to a mosquito control pesticide application by a vector control agency signatory to a Cooperative Agreement with CDPH, or under contract for such agency is not a legal requirement in California (California Code of Regulations – Title 3: Food and Agriculture: Division 6. Pesticides and Pest Control Operations: Section 6620a). However, public notification of pending adult mosquito control is recommended as early as possible prior to the treatment event.

Basic notification steps

- Provide notification of pending application as early as possible.
- Post clearly defined treatment block map online or through appropriate media outlet.
- Post product label and material safety data sheet (MSDS) online or through appropriate media outlet.
- Post and/or have available scientific publications regarding the efficacy of aerial or ground based applications (as appropriate), including effects on non-target organisms and risk-assessments.

Public relations considerations

- Ensure staffing is adequate to handle a significant increase in phone calls.
- Ensure website capability is adequate to handle a rapid increase in visitors.
- Train personnel answering phones to address calls from citizens concerned about personal and environmental pesticide exposure.
- Ensure adequate follow-through for calls related to sporting events, concerts, weddings, and other outdoor events that may be scheduled during the application and within the treatment block.
## Appendix J: Websites Related to Arbovirus Surveillance, Mosquito Control, Weather Conditions and Forecasts, and Crop Acreage and Production in California

<table>
<thead>
<tr>
<th>Website</th>
<th>URL</th>
<th>Available information</th>
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</thead>
<tbody>
<tr>
<td>California West Nile Virus Website</td>
<td><a href="http://westnile.ca.gov">http://westnile.ca.gov</a></td>
<td>Up to date information on the spread of West Nile virus throughout California, personal protection measures, online dead bird reporting, bird identification charts, mosquito control information and links, clinician information, local agency information, public education materials.</td>
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<tr>
<td>UC Davis Center for Vectorborne Diseases</td>
<td><a href="http://cvec.ucdavis.edu/">http://cvec.ucdavis.edu/</a></td>
<td>Frequently updated reports and interactive maps on arbovirus surveillance and mosquito occurrence in California.</td>
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<tr>
<td>Mosquito and Vector Control Association of California</td>
<td><a href="http://www.mv">http://www.mv</a> cac.org</td>
<td>News, membership information, event calendars, and other topics of interest to California’s mosquito control agencies.</td>
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<tr>
<td>California Vectorborne Disease Surveillance Gateway</td>
<td><a href="http://gateway.calsurv.org">http://gateway.calsurv.org</a></td>
<td>Data management system for California’s mosquito control agencies.</td>
</tr>
<tr>
<td>California Data Exchange Center</td>
<td><a href="http://cdec.water.ca.gov">http://cdec.water.ca.gov</a></td>
<td>Water-related data from the California Department of Water Resources, including historical and current stream flow, snow pack, and precipitation information.</td>
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<tr>
<td>UC IPM Online</td>
<td><a href="http://www.ipm.ucdavis.edu">http://www.ipm.ucdavis.edu</a></td>
<td>Precipitation and temperature data for stations throughout California; also allows calculation of degree-days based on user-defined data and parameters.</td>
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<tr>
<td>National Weather Service – Climate Prediction Center</td>
<td><a href="http://www.cpc.ncep.noaa.gov/products/predictions/">http://www.cpc.ncep.noaa.gov/products/predictions/</a></td>
<td>Short-range (daily) to long-range (seasonal) temperature and precipitation forecasts. Also provides El Niño-related forecasts.</td>
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<tr>
<td>California Agricultural Statistics Service</td>
<td><a href="http://www.nass.usda.gov/Statistics_by_State/California">http://www.nass.usda.gov/Statistics_by_State/California</a></td>
<td>Crop acreage, yield, and production estimates for past years and the current year’s projections. Reports for particular crops are published at specific times during the year – see the calendar on the website.</td>
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<tr>
<td>US Environmental Protection Agency – Mosquito Control</td>
<td><a href="http://www.epa.gov/pesticides/health/mosquitoes">http://www.epa.gov/pesticides/health/mosquitoes</a></td>
<td>Describes the role of mosquito control agencies and products used for mosquito control.</td>
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</table>
Appendix K

Appendix K: Reference List


Appendix K


Best Management Practices for Mosquito Control in California

Recommendations of the California Department of Public Health and the Mosquito and Vector Control Association of California

July 2012
An electronic version of this manual and the companion document “Best Management Practices for Mosquito Control on California State Properties” are available from the California West Nile virus website at http://www.westnile.ca.gov/resources.php. Please see Table 1, page 22, for a list of California mosquito control agencies or visit http://mvcac.org.

For more information, please contact:
Vector-Borne Disease Section
California Department of Public Health
vbds@cdph.ca.gov
(916) 552-9730
http://www.cdph.ca.gov
http://www.westnile.ca.gov
**Purpose of this Manual**
This manual provides landowners with Best Management Practices (BMPs) for mosquito control. The term BMP is used to describe actions landowners can take to reduce mosquito production from permanent water sources, reduce or eliminate mosquito production from temporary water sources, and reduce the potential for disease transmission to humans on their property.

**General Recommendations**
- **Implement universal BMPs**
  - Use personal protective measures
  - Eliminate unnecessary standing water

- **Identify and implement applicable mosquito control BMPs**
  - Reduce stagnation by providing water flow and manage vegetation in ponds or other water bodies.
  - Collaborate with local vector control agencies to develop and implement appropriate Integrated Pest Management (IPM) strategies that are most suitable for specific land-use type(s).

---

Use personal protective measures when potentially exposed to adult mosquitoes.

Eliminate unnecessary standing water, reduce stagnation by providing water flow, and manage vegetation in ponds or other water bodies.

Collaborate with local vector control agencies to coordinate activities on your property within a larger Integrated Pest Management mosquito control program.
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Executive Summary

The California Department of Public Health (CDPH) in collaboration with the Mosquito and Vector Control Association of California (MVCAC) developed this Best Management Practices (BMPs) plan to promote mosquito control on California properties, and enhance early detection of West Nile virus (WNV).

This plan describes mosquito control BMPs to be implemented by property owners and managers. These recommended practices, when properly implemented, can reduce mosquito populations through a variety of means including: 1) reducing or eliminating breeding sites, 2) increasing the efficacy of biological control, and 3) decrease the amount of pesticides applied while increasing the efficacy of chemical control measures. It is critical that property owners and managers communicate regularly with local vector control agencies regarding control practices on lands that are located within or near a local agency’s jurisdiction. Local vector control agencies may have more specific policies regarding the implementation of BMPs and other control operations, which may include use of enforcement powers authorized by the California Health and Safety Code.

There are many different BMPs included in this document and they are intended to provide overall guidance to reduce mosquito production on properties throughout California, though not all mosquito sources and land uses will be addressed in this document. If it is deemed necessary, site-specific BMP plans may be developed in collaboration with CDPH and the respective local mosquito and vector control agency.

Effective mosquito-borne disease surveillance and mosquito control to protect public health are dependent upon factors that may fluctuate temporally and regionally. Such factors include mosquito and pathogen biology, environmental factors, land-use patterns, resource availability; strategies that incorporate BMPs are the most effective means by which mosquito control can be conducted and individualized to specific situations. Best management practices included in this plan emphasize the fundamentals of integrated pest management (IPM) which include:

1. Knowledge of mosquito species composition and corresponding mosquito behavior and habitat, for both immature and adult stages.

2. Detecting and monitoring WNV activity by testing mosquitoes, birds, sentinel chickens, horses, and humans. Identifying the mosquito species present, locations, densities, and disease potential.

3. Managing mosquito populations by source reduction, habitat modification, and biological control (e.g., introduced predators and parasites). Pesticides are used to target immature and, when indicated, adult stages of the mosquito. Mosquito control products are selected and applied in a manner that minimizes risks to human health, beneficial and non-target organisms, and the environment.

4. Educating the general public about reducing mosquito production and minimizing their risk of exposure to WNV.
RECOMMENDATIONS FOR PROPERTY OWNERS AND MANAGERS

• Use this plan to identify and implement appropriate Best Management Practices to control mosquitoes.

• Eliminate unnecessary standing water, reduce stagnation by providing water flow, and manage vegetation in ponds or other water bodies.

• Collaborate with local vector control agencies to develop and implement appropriate integrated pest management strategies that are most suitable for specific land-use type(s).

• Ensure individuals use personal protective measures when potentially exposed to adult mosquitoes.
Introduction

Controlling mosquitoes is critical to maintaining both a high quality of life and protecting people from mosquito-transmitted (vectored) diseases such as West Nile virus (WNV). In many parts of California, residents have voted to form local mosquito control programs or agencies. As a result, approximately half the land area and 85% of the population of California are within the boundaries of a mosquito control program. Landowners and land managers have a responsibility to minimize mosquito production on their lands and play a key role in reducing mosquito populations throughout the State, regardless whether their property is inside or outside the jurisdiction of a mosquito control program. Information about mosquito surveillance, mosquito-borne diseases, and mosquito control is available in Appendices A and B.

Best Management Practices (BMPs) are defined as actions landowners can take to reduce or eliminate mosquito production from water sources on their property in an environmentally and fiscally responsible manner, and to reduce the potential for transmission of disease from mosquitoes to humans.

Each property is unique, and the BMPs listed in this manual will apply to some properties, but not others. Landowners should implement universally applicable BMPs and after evaluating their own property, also employ the mosquito control BMPs that are applicable to their situation.

Landowner Responsibility

According to the California Health and Safety Code, landowners in California are legally responsible to abate (eliminate the source of) a public nuisance arising from their property, including mosquitoes [H&S Code Sections 2001 - 4(d); 2002; 2060 (b)]. In areas that are within the jurisdictional boundaries of a mosquito control program, landowners should work with staff to address mosquito problems, particularly in areas where irrigation is used for agricultural purposes. Landowners that are not within the jurisdictional boundary of an established mosquito control program should seek advice from the nearest mosquito control agency or health department. Landowners may also contact the California Department of Public Health (CDPH) or consult the CDPH West Nile virus website for additional information about mosquitoes and mosquito control. http://www.westnile.ca.gov/resources.php.

Mosquito control programs have substantial authority to access private property, inspect known or suspected sources of mosquitoes, abate the source of a mosquito problem, and charge the landowner for work performed and/or charge fees if a landowner is unwilling or unable to address a mosquito problem arising from their property [H&S Code sections 2060-2067, 100170, and 100175]. Applicable sections of the California Health and Safety Code are summarized in Appendix C.
Mosquito Biology

The more than 50 species of mosquitoes in California share one common life history trait: the mosquito life cycle requires standing water. Management of standing water is the key to most of the mosquito control BMPs presented in this manual and is one of the oldest and most cost effective forms of mosquito control.

Mosquito species are broadly separated into two groups according to where they lay eggs, floodwater mosquitoes and standing water mosquitoes. Adult female floodwater mosquitoes lay eggs on mud or previously submerged vegetation. The eggs may remain dormant for days, months, or even years until they are flooded, at which time larvae hatch. Standing water mosquitoes lay eggs on the water surface. The eggs float on the surface for a few hours to a few days until the larvae hatch into the water.

Floodwater mosquito larval development (breeding) sites include irrigated pastures, rice fields, seasonally flooded duck clubs and other managed wetlands, tidal wetlands, riparian corridors, and snowmelt pools. These intermittent or seasonally flooded habitats can be among the most productive sources of mosquitoes because they are often free of natural predators.

Standing water mosquito breeding sites include artificial containers, treeholes, catch basins, open ditches, retention/detention ponds, natural or constructed ponds and wetlands, stormwater management devices, and along the edges of flowing streams. Sources are found everywhere from highly urban areas to natural wetlands and often produce multiple generations of mosquitoes each season. In southern California, urban sources can produce some species of mosquitoes year round.

Landowners or land managers can identify the presence of immature mosquitoes in water on their property. Mosquito larvae breathe air from above the water surface and most hang at an angle from or lay parallel with the surface of the water while consuming small bits of organic matter. When disturbed, larvae swim down into the water column in a serpentine motion. Mosquitoes may live as larvae from a couple of days to more than a month depending on the species, water temperature, and the amount of food available.

Mosquitoes then go through a non-feeding stage called a pupa. During this stage the mosquito changes into the winged adult form. The easily identified comma-shaped pupae hang from the water surface and move down through the water column in a rolling or tumbling motion when disturbed. This life stage typically lasts about a day, with the mosquito emerging from the back of the pupal case (above the water) as a flying adult. (See Figure 1: Mosquito Life Cycle).
Figure 1. The life cycle of all mosquito species consists of four stages: egg, larva, pupa, and adult.

All adult mosquitoes feed on plant nectar; however blood is essential for female mosquitoes to produce eggs. To take a blood meal, the female’s mouth parts pierce the skin, inject saliva, and suck blood out. It is through the injection of saliva that a mosquito causes the typical itchy bump and can infect a person or domestic animal with a disease causing organism. Depending on an individual’s immune response, even a single bite can be a significant nuisance.

For more information on mosquito biology and key mosquito species found in California, please see Appendix D.

For additional information on the larval habitats of California mosquitoes, please see Appendix E.
Best Management Practices (BMPs)

Mosquito Control Best Management Practices At-A-Glance

- Eliminate artificial mosquito sources.
- Ensure man-made temporary sources of surface water drain within four days (96 hours) to prevent development of adult mosquitoes.
- Control plant growth in ponds, ditches, and shallow wetlands.
- Design facilities and water conveyance and/or holding structures to minimize the potential for producing mosquitoes.
- Use appropriate bio-rational products to control mosquito larvae.
- Use personal protective measures to prevent mosquito bites.

Each property is unique. Landowners should implement universally applicable mosquito control BMPs, and after evaluating their own property, also employ the mosquito control BMPs that are applicable to their property and circumstances. Using appropriate BMPs is an efficient and effective way to help prevent a mosquito problem.

Universally Applicable Mosquito Control BMPs

Eliminate Artificial Mosquito Breeding Sites and Harborage

- Examine outdoor areas and drain temporary and unnecessary water that may stand longer than 96 hours.
- Dispose of unwanted or unused artificial containers.
- Properly dispose of old tires.
- If possible, drill drainage holes, cover, or invert any container or object that holds standing water that must remain outdoors. Be sure to check for containers or trash in places that may be hard to see, such as under bushes or buildings.
- Clean clogged rain gutters and storm drains. Keep outdoor drains flowing freely and clear of leaves, vegetation, and other debris.
- Aerate ornamental ponds to avoid letting water stagnate.
- Change water in birdbaths, fountains, and animal troughs at least once per week.
- Ensure rain and/or irrigation water does not stand in plant containers, trash cans, boats, or other containers on commercial or residential properties.
- Regularly chlorinate swimming pools and keep pumps and filters operating. Unused or unwanted pools should be kept empty and dry, or buried.
- Maintain irrigation systems to avoid excess water use and runoff into storm drains.
- Minimize sites mosquitoes can use for refuge (harborage) by thinning branches, trimming and pruning ornamental shrubs and bushes, and keeping grass mowed short.
Use Personal Protective Measures

- Apply an EPA-registered mosquito repellent when outdoors; especially around dusk and dawn when mosquitoes are most active (see Appendix F for additional information on insect repellents).
- Wearing loose-fitting protective clothing including long sleeves and pant legs.
- Install and properly maintain fine mesh screens on windows and doors to prevent mosquito entry into homes.

Provide Mosquito Management Related Information to Property Managers

- Off-site landowners should provide property managers with basic information about mosquitoes and appropriate measures to minimize mosquito habitats.

Contact Local Mosquito Control Program

- Contact the local mosquito control program to evaluate your property for mosquito breeding sites and work cooperatively to prevent a mosquito problem on your property. A contact list for mosquito control programs is provided in Table 1.

Where local mosquito control programs do not exist, landowners may contact CDPH for assistance or consult the California West Nile virus website for additional information about mosquito control: http://www.westnile.ca.gov/resources.php

Mosquito Control BMPs for Residential and Landscaped Properties

Many residential and commercial properties have potential mosquito sources around buildings and grounds associated with excess or poorly managed irrigation, poor drainage, and miscellaneous landscape features. Mosquitoes can develop in the standing water associated with over-irrigation, irrigation breaks and/or runoff, clogged gutters, stormwater management structures, ornamental ponds, swimming pools, trash cans and flower pots, low areas or holes in turf where water collects and stands and low areas underneath pier and beam homes or buildings.

Mosquito sources can be minimized by taking precautions such as regular inspection and proper maintenance of irrigation systems and other water features, and elimination of unwanted standing water.

- Avoid over-irrigating to prevent excess pooling and runoff.
- Routinely inspect, maintain, and repair irrigation system components.
• All underground drain pipes should be laid to grade to avoid low areas that may hold water for longer than 96 hours.
• Back-fill tire ruts or other low areas that hold water for more than 96 hours.
• Improve drainage channels and grading to minimize potential for standing water.
• Keep drainage ditches free of excessive vegetation and debris to provide rapid drainage.
• Check and repair leaky outdoor faucets.
• Report any evidence of standing water to responsible maintenance personnel.
• Use waterfalls, fountains, aerators and/or mosquitofish in ponds and ornamental water features. Land owners must consult with the local mosquito control agencies or California Fish and Game regarding proper use of mosquitofish.
• Prevent mosquito breeding in rain barrels by properly screening all openings, preventing mosquito access to the stored water.
• For ponds and ornamental water features where mosquitofish cannot be used, landowners should use one of several readily available larval mosquito control products to treat water when they see immature mosquitoes.

Landowners should also review the stormwater runoff section of this manual because building rooftops, parking lots, etc. may have associated stormwater management features that produce mosquitoes.

Mosquito Control BMPs for Rural Properties

Mosquito breeding on rural properties is highly variable due to differences in location, terrain, and land use. This list is intended to provide general guidance, not site-specific requirements. BMPs that are most applicable and relevant to a specific mosquito source may be selected from the list and incorporated into the overall property management plan. Ideally, activities should be coordinated with those of a local mosquito control program.

Flood irrigation is a common practice in rural areas throughout California and always poses the potential for creating mosquito breeding sites. Mosquitoes commonly develop within irrigation infrastructure including in ditches clogged with vegetation, irrigation tail water areas and return sumps, blocked ditches or culverts, vegetated ditches; and leaking irrigation pipes, head gates, pumps, stand pipes, etc. The fields, orchards, and pastures being irrigated may also produce mosquitoes, particularly where natural undulation or poor grading create low lying areas where water collects and stands.

Recommendations for rural properties are based on “Mosquito Control Best Management Practices” produced by the Sacramento-Yolo Mosquito and Vector Control District, and from Lawler and Lanzaro (2005).
Mosquito Control BMPs for Ditches and Drains

- Construct or improve large ditches to a slope of at least 2:1 (vertical: horizontal) and a minimum 4 foot wide bottom. Consider a 3:1 slope or greater to discourage burrowing animal damage, potential seepage problems, and prevent unwanted vegetation growth.
- Keep ditches clean and well-maintained. Periodically remove accumulated sediment and vegetation. Maintain ditch grade and prevent areas of standing water.
- Design irrigation systems to use water efficiently and drain completely to avoid standing water.
- Prevent wet areas associated with seepage by repairing leaks in dams, ditches, and drains.

Mosquito Control BMPs for Irrigated Pastures and Cropland

- Grade to eliminate standing water from pastures and fields. Use Natural Resource Conservation Service (NRCS) guidelines: Laser leveling and periodic maintenance may be needed to allow proper drainage, efficient water flow, and reduce low-lying areas where standing water may accumulate.
- Reuse wastewater through return flow systems to effectively minimize mosquito production and conserve water. Eliminate and reuse excess water that may typically stagnate and collect at lower levels of irrigated fields.
- Irrigate only as frequently as is needed to maintain proper soil moisture. Check soil moisture regularly.
- Drain water as quickly as possible following irrigation. Check slopes may be used to direct water movement and drainage. Drainage ditches may be used to remove water from the lower end of the field.
- Install surface drains to remove excess water that collects at lower levels of irrigated fields.
- Inspect fields for drainage and broken checks to see whether re-leveling or reconstruction of levees is needed. Broken checks create cross-leakage that may provide habitat for mosquitoes.
- If possible, use closed conduits instead of open canals for water conveyance.
- Do not over fertilize. Over-fertilization can leach into irrigation run-off making mosquito production more likely in ditches or further downstream.
- When possible, use sprinklers or drip systems rather than flood irrigation.
- Keep animals off the pasture while the soil is soft. Mosquito habitat is created in irrigated pastures when water collects in hoof prints.

Mosquito Control BMPs for Rice Fields

Flooded rice fields can always support the development of mosquitoes. As the rice stand develops and grows denser, the production of mosquitoes tends to increase while the ability for chemical control agents to penetrate the canopy decreases. The BMPs
presented in this section attempt to balance the needs of the grower with the need to control mosquitoes.

In California there is a long-standing cooperative effort among the Rice Commission, individual growers, and mosquito control agencies to manage mosquitoes on rice lands. Close cooperation between growers and vector control is particularly important with organic rice producers. With severe limits on chemical control options and greater expense for organic-compatible larvicides, organic rice growers should implement as many mosquito control BMPs as possible.

- Wherever feasible, maintain stable water levels during mosquito season by ensuring constant flow of water into ponds or rice fields to reduce water fluctuation due to evaporation, transpiration, outflow, and seepage.
- Inspect and repair levees to minimize seepage.
- Drain and fill in borrow pits and seepage areas external to the fields.
- Wherever feasible, maintain at least 4” – 6” (10-15 cm) of water in the rice field after rice seedlings have begun to stand upright. Any drainage should be coordinated with local vector control (where possible). Restocking of mosquitofish or use of alternative mosquito control measures should be instituted as soon as possible when fields are re-flooded.
- Whenever feasible, remove vegetation on the outer-most portions of field levees and checks, specifically where they interface with standing water.
- Control algae and weed growth as effectively as possible.
- Communicate frequently with your local mosquito control program regarding your crop management activities.
- Wherever feasible, maintain borrow pits (12” – 18” deep) (30-45 cm) on both sides of each check throughout rice fields to provide refuge for mosquitofish during low water periods.
- If a pyrethroid pesticide is to be applied to the fields stocked with mosquitofish, contact your local mosquito control program for advice on minimizing fish mortality.
- If a pesticide is applied, fields should be inspected for mosquitofish afterward and if needed, fish should be restocked as soon as feasible.

**Mosquito Control BMPs for Dairies and Animal Holding Operations**

Frequently infrastructure associated with dairies, feedlots, or other animal holding facilities can produce mosquitoes. Watering troughs and irrigated fields associated with the operation can create mosquito problems. Animal washing areas may also create mosquito problems, particularly drains and ditches, sumps, ponds, and wastewater lagoons.

The following activities can reduce mosquito production and simplify control activities around dairies and animal holding operations:
• All holding ponds should be surrounded by lanes of adequate width to allow safe passage of mosquito control equipment. This includes keeping the lanes clear of any materials or equipment (e.g. trees, calf pens, hay stacks, silage, tires, equipment, etc.).
• If fencing is used around the holding ponds, it should be placed on the outside of the lanes with gates provided for vehicle access.
• Large ponds should be divided into a series of smaller ponds that can be drained for removal of solid waste material.
• Ponds and lagoons should be narrow enough to allow solid waste removal after drying.
• All interior banks of the holding ponds should have a grade of at least 2:1.
• If possible, an effective solids separation system should be utilized such as a mechanical separator or two or more solids separator ponds. If ponds are used, they should not exceed 60’ (18m) in surface width.
• Drainage lines should never by-pass the separator ponds, except those that provide for normal corral run-off and do not contain solids.
• When possible, floating debris should be removed from ponds prior to crust formation.
• If a thick crust exists (grass growing on crust), it should be left intact until the pond can be drained and the solid material removed.
• Vegetation should be controlled regularly to prevent emergent vegetation and barriers to access. This includes access lanes, interior pond embankments, and any weed growth that might become established within the pond surface.
• Dairy wastewater discharge for irrigation purposes should be managed so it does not stand for more than 4 days.
• Tire sidewalls or other objects that will not hold water should be used to hold down tarps (e.g. on silage piles). Whole tires or other water-holding objects should be replaced.

Mosquito Control BMPs for Wetlands

Wetlands are an important source of mosquito production on public and privately owned lands. Under the California Wildlife Protection Act, the term “wetlands” is defined as any lands which may be covered periodically or permanently with shallow water, which
include freshwater and saltwater marshes, open or closed brackish water marshes, swamps, mudflats, fens, and vernal pools (Fish & Game Code Section 2785). Many wetlands are protected by federal and state laws.

By definition, “natural” wetlands are not intensely managed and options for implementing mosquito control BMPs in these areas are very limited. Even in managed wetlands, not all BMPs listed below may be suitable for use in all wetlands. It is the responsibility of the landowner to become informed on timing and extent of acceptable activities in a given wetland habitat. Intermittently or seasonally flooded wetlands can produce formidable numbers of mosquitoes, whereas well-managed semi-permanent and permanent wetlands usually produce fewer mosquitoes because of their limited acreage, stable water levels, and abundance of natural predators of mosquito larvae.

Information within this section has been partially adapted from Kwasny et al. (2004). Based on the site activities and potential for mosquito production, the existing BMPs may need to be modified or supplemented to address public health risk, goals and management strategy issues, and requirements of California Department of Fish and Game (DFG), the local mosquito and vector control program, and CDPH.

Due to the delicate and sometimes protected wetlands ecosystems, landowners, biologists, managers, and staff from mosquito control programs should collaborate to control mosquitoes. Source reduction and source maintenance can be combined with the judicious use of specific larvicides to minimize mosquito production from these wetlands.

General Mosquito Control BMPs for Wetlands

- Manage vegetation routinely; activities such as annual thinning of rushes and cattails and removing excess vegetative debris enables natural predators to hunt mosquito larvae more effectively in permanent wetlands. Vegetation in shallow, temporary wetlands can be mowed when dry.
- Time flooding of seasonal wetlands to reduce overlap with peak mosquito activity.
- Flood wetlands from permanent-water sources containing mosquito predators (e.g., mosquito-eating fish or invertebrate predators) to passively introduce mosquito predators. Permanent wetlands and brood ponds can be stocked with mosquitofish or native predatory species.
- Maintain permanent or semi-permanent water within the wetland to maintain populations of larval mosquito predators. Discourage the use of broad spectrum pesticides.
- Use fertilizers conservatively and manage irrigation drainage to prevent or minimize fertilizer and/or manure flowing into wetlands. Buffers between agriculture fields and wetlands should be established.
- Comply with all Federal and State Environmental Laws and the California Health and Safety Code to prevent environmental harm while reducing or eliminating mosquito production.
Mosquito Control BMPs for Design and Maintenance of Wetlands

- Provide reasonable access on existing roads and levees to allow for monitoring, abatement, and implementation of BMPs. Make shorelines of natural, agricultural, and constructed water bodies accessible for periodic maintenance, mosquito monitoring and abatement procedures, and removal of emergent vegetation.
- Construct, improve, or maintain ditches with 2:1 slopes and a minimum 4 foot (1.2 m) width at the bottom. Consider a 3:1 slope or greater to discourage burrowing animal damage, potential seepage problems, and prevent unwanted vegetation growth.
- Construct, improve, or maintain levees to quality standards that ensure stability and prevent unwanted seepage. Ideally build levees with >3:1 slopes and > 80% compaction; consider 5:1 slope or greater in areas prone to overland flooding and levee erosion.
- Provide adequate water control structures for complete draw-down and rapid flooding.
- When possible, include independent inlets and outlets in the design of each wetland unit.
- Construct or enhance swales so they are sloped from inlet to outlet and allow maximum draw-down.
- Excavate deep channels or basins to maintain permanent water areas (>2.5 feet deep) within a portion of seasonal managed wetlands. This provides year-round habitat for mosquito predators that can inoculate seasonal wetlands when they are irrigated or flooded.

Wetland Infrastructure Maintenance Mosquito Control BMPs

- Inspect levees at least annually and repair as needed.
- Periodically inspect, repair, and clean water control structures.
  - Remove all debris, including silt and vegetation, which can impede drainage and water flow.
  - Ensure water control structures are watertight to prevent unnecessary water flow or seepage.
- Regularly remove trash, silt and vegetation from water delivery ditches to allow efficient water delivery and drainage.
  - Remove problem vegetation that inhibits water flow using herbicides or periodic dredging.
  - If possible, use closed conduits instead of open canals for water conveyance.
- Periodically test and repair pumps used for wetland flooding to maximize pump output.
Water Management Mosquito Control BMPs for Seasonal Wetlands

- Timing of flooding
  o Delay or "phase" fall flooding of wetlands as long as possible in consultation with local vector control agencies. Fall flooding is known to produce large numbers of mosquitoes and/or those in close proximity to urban areas to minimize late season mosquito production.
  o Strategically locate wetlands identified for early flooding. Wetlands that are flooded in early fall should not be close to urban areas or historically produce great numbers of mosquitoes.
  o When possible, water in managed wetlands should be drawn-down in late March or early April.
  o Use a flood-drain-flood regime to control floodwater mosquitoes; flood to trigger hatching of dormant mosquito eggs, drain water and larvae into an area where they can be easily treated, drowned in moving water, or consumed by predators, and immediately re-flood wetland. This water management regime should be used only when it does not conflict with water quality regulations.
- Speed of flooding
  o Flood wetlands as quickly as possible to reduce the potential for large numbers of mosquitoes. Coordinate flooding with neighbors and/or the water district to maximize flood-up rate.
- Water source
  o Flood wetlands with water from permanent water sources containing mosquito predators (i.e., mosquito-eating fish or invertebrate predators) to passively introduce mosquito predators. Permanent wetlands and brood ponds used as flooding sources can be stocked with mosquito-eating fish or maintained to encourage natural predator populations.
  o Maintain a separate permanent water reservoir that conveys water to seasonal wetlands that provides year-round habitat for mosquito predators that can inoculate seasonal wetlands when they are irrigated or flooded.
- Frequency and duration of irrigation
  o When possible, reduce the number and duration of irrigations to minimize standing water. The need to irrigate should be evaluated based on spring habitat conditions and plant growth. If extended duration irrigation
(generally 14-21 days) is considered for weed control (e.g., cocklebur),

- additional measures to offset the potential for increased mosquito production may be needed.
- Irrigate managed wetlands before soil completely dries after spring draw-down to discourage floodwater mosquitoes from laying eggs in the dry, cracked substrate.
- Drain irrigation water into ditches or other water sources with mosquito predators instead of nearby dry fields.
- Maintain high ground water levels by keeping channels or deep swales permanently flooded for subsurface irrigation to reduce the amount of irrigation water needed during the mosquito season.

- Communicate with your local mosquito control agency (if there is one)
  - Advise your local mosquito control agency when you intend to flood so that they can make timely applications of larvicide if necessary.

- Emergency preparedness
  - Whenever feasible, have an emergency plan that provides for immediate drainage into acceptable areas if a mosquito-borne disease related public health emergency occurs.

### Vegetation Management Mosquito Control BMPs

- Control floating vegetation conducive to mosquito production (i.e., water hyacinth, water primrose, parrot feather, duckweed, and filamentous algae mats).
- Perform routine maintenance to reduce problematic emergent plant densities to facilitate the ability of mosquito-eating fish to move through vegetated areas and allow good penetration of chemical control agents.
- Manage vegetation based on local land management objectives and associated habitat uses to minimize mosquito production. Methods of vegetation control for managed wetlands include mowing, burning, disking, and grazing.
- Manage the spread and density of invasive, non-native emergent wetland vegetation to increase native plant diversity, increase the mobility of larval mosquito predators, and allow for more efficient penetration of chemical control agents.

### Additional Water Management BMPs for Permanent Wetlands

- Maintain stable water levels in wetlands that are flooded during summer and early spring to prevent intermittent flooding of shoreline areas favorable to mosquito production. Water level fluctuation can be minimized by continuing a constant flow of water into the wetland.
- Circulate water to avoid stagnation (e.g., provide a constant influx of water equal to the net loss or discharge of water).
- Maintain water depths as deep as possible (18” – 24" [45-60 cm] or more) during the initial flood-up to minimize shallow habitats preferred by mosquito larvae. Shallow water levels can be maintained outside of the mosquito breeding season.
Additional Mosquito Control BMPs for Saltwater Marsh

- Improving water flow through the wetland system minimizes stagnant water and facilitates movement of fish and other natural predators. For example, mosquitoes in coastal tidal wetlands can be managed by constructing and maintaining ditches that drain off the water when the tide falls.

Mosquito Control BMPs for Stormwater Management and Associated Infrastructure

Federal and state environmental regulations require mitigation of the harmful effects of runoff water from storms, irrigation or other sources prior to entering natural waterways from point and non-point sources. Mitigation may include water capture, slowing flow velocity, reducing volume, and removal of pollutants. The term “stormwater” is used as a generic term for runoff water, regardless of source.

Stormwater infrastructure typically includes conveyance systems (e.g. drain inlets, catch basins, pipes, and channels), storage and infiltration systems (e.g. flood control basins, percolation basins), and more recently, structural treatment devices designed and installed specifically to remove suspended and dissolved pollutants from runoff (e.g., vegetated swales, dry detention basins, ponds and constructed wetlands, media filtration devices, and trash capturing devices). The size and variability of stormwater infrastructure, inconsistent quantity and timing of water flows, and propensity to carry and accumulate sediment, trash, and debris, makes these systems highly conducive to holding areas of standing water ideal for production of mosquitoes. Identification of the potential mosquito sources (often belowground) found within stormwater infrastructure is often more difficult than the solutions needed to minimize mosquitoes. Some of the information within this section has been adapted from Metzger (2004).

General Stormwater Management Mosquito Control BMPs

- Manage sprinkler and irrigation systems to minimize runoff entering stormwater infrastructure.
- Avoid intentionally running water into stormwater systems by not washing sidewalks and driveways, washing cars on streets or driveways, etc.
- Inspect facilities weekly during warm weather for the presence of standing water or immature mosquitoes.
- Remove emergent vegetation and debris from gutters and channels that accumulate water.
- Consider mosquito production during the design, construction, and maintenance of stormwater infrastructure.
- Design and maintain systems to fully discharge captured water in 96 hours or less.
- Include access for maintenance in system design.
• Design systems with permanent water sources such as wetlands, ponds, sumps, and basins to minimize mosquito habitat and plan for routine larval mosquito inspection and control activities with the assistance of a local mosquito control program.

**Stormwater Conveyance**

• Provide proper grades along conveyance structures to ensure that water flows freely.
• Inspect on a routine basis to ensure the grade remains as designed and to remove accumulations of sediment, trash, and debris.
• Keep inlets free of accumulations of sediment, trash, and debris to prevent standing water from backing up on roadways and gutters.
• Design outfalls to prevent scour depressions that can hold standing water.

**Stormwater Storage and Infiltration Systems (Aboveground)**

• Design structures so that they do not hold standing water for more than 96 hours to prevent mosquito development. Features to prevent or reduce the possibility of clogged discharge orifices (e.g., debris screens) should be incorporated into the design. The use of weep holes is not recommended due to rapid clogging.
• Provide a uniform grade between the inlets and outlets to ensure that all water is discharged in 96 hours or less. Routine inspection and maintenance are crucial to ensuring the grade remains as designed.
• Avoid the use of electric pumps. They are subject to failure and often require permanent-water sumps. Structures that do not require pumping should be favored over those that have this requirement.
• Avoid the use of loose rock rip-rap that may hold standing water.
• Design distribution pumping and containment basins with adequate slopes to drain fully. The design slope should take into consideration buildup of sediment between maintenance periods.

**Stormwater Structures with Permanent-Water Sumps or Basins (Belowground)**

• Where possible, seal access holes (e.g., pickholes in manhole covers) to belowground structures designed to retain water in sumps or basins to minimize
entry of adult mosquitoes. If using covers or screens, maximum allowable gaps of 1/16 inch (2 mm) will exclude entry of adult mosquitoes. Inspect barriers frequently and replace when needed.

- If the sump or basin is completely sealed against mosquitoes, with the exception of the inlet and outlet, the inlet and outlet should be completely submerged to reduce the available surface area of water for mosquitoes to lay eggs (female mosquitoes can fly through pipes).
- Where possible, design belowground sumps with the equipment necessary to allow for easy dewatering of the unit.
- Contact the local mosquito control program for advice with problem systems.

### Stormwater Treatment Ponds and Constructed Treatment Wetlands

- Whenever possible, stock stormwater ponds and constructed wetlands with mosquito-eating fish available from local mosquito control programs.
- Design and maintain accessible shorelines to allow for periodic maintenance and/or control of emergent and shoreline vegetation, and routine monitoring and control of mosquitoes. Emergent plant density should be routinely managed so mosquito predators can move throughout the vegetated areas and are not excluded from pond edges.
- Whenever possible, design and maintain deep zones in excess of four feet (1.2 m) to limit the spread of invasive emergent vegetation such as cattails. The edges below the water surface should be as steep as practicable and uniform to discourage dense plant growth that may provide immature mosquitoes with refuge from predators and increased nutrient availability.
- Use concrete or liners in shallow areas to discourage plant growth where vegetation is not necessary.
- Whenever possible, provide a means for easy dewatering if needed.
- Manage the spread and density of floating and submerged vegetation that encourages mosquito production (i.e., water hyacinth, water primrose, parrot’s feather, duckweed, and filamentous algal mats).
- If possible, compartmentalize managed treatment wetlands so the maximum width of ponds does not exceed two times the effective distance (40 feet [12 m]) of land-based application technologies for mosquito control agents.

### General Access Requirements for Stormwater Treatment Structures

- All structures should be easily and safely accessible, without the need for special requirements (e.g., Occupational Safety and Health Administration - OSHA - requirements for “confined space”). This will allow for monitoring and, if necessary, abatement of mosquitoes.
- If utilizing covers, the design should include spring-loaded or lightweight access hatches that can be easily opened.
- Provide all-weather road access (with provisions for turning a full-size work vehicle) along at least one side of large aboveground structures that are less
than seven meters wide, or both sides if shore-to-shore distance is greater than seven meters.  *Note:* Mosquito larvicides are applied with hand held equipment at small sites and with backpack or truck mounted high-pressure sprayers at large sites.  The effective swath width of most backpack or truck-mounted larvicide sprayers is approximately 20-25 feet (6-7 meters) on a windless day.

- Build access roads as close to the shoreline as possible to allow for maintenance and vector control crews to periodically maintain, control and remove emergent vegetation and conduct routine mosquito monitoring and abatement. Remove vegetation and/or other obstacles between the access road and the structure that might obstruct the path of larvicides to the water.
- Control vegetation (by removal, thinning, or mowing) periodically to prevent barriers to access.

### Mosquito Control BMPs for Right of Ways and Easements

Right of ways and easements for a variety of infrastructure exist throughout California. Roadways, power lines, pipelines, canals, bike paths, utility access, railroads, etc. have lands associated with them that may produce mosquitoes. It is the responsibility of the company or individual associated with the infrastructure to prevent a public nuisance arising from the property, including a mosquito problem. The lands are as varied as the terrain in California, but the mosquito breeding sites found on these properties will be similar to those found in other sections of this manual.

#### Inspection of Property and Identification of Mosquito Sources

- Inspect property for standing water or evidence of standing water that may become mosquito sources.

#### Review and Implement Mosquito Control BMPs as Appropriate

Some rights of way and easements are very long and may have multiple types of mosquito breeding sites that fall within every category listed below, others will have none. After inspecting the property, implement mosquito control BMPs found in the sections below.

- If the property is in an urban area and is managed as commercial property, please refer to the following section:
  - *Residential and landscaped properties*, see page 5.

- If the property is associated with an irrigation canal or similar rural water conveyance, please refer to the following sections:
  - *Rural properties*, see page 6.
  - *Wetlands*, see page 9.

- If the property is associated with a variety of habitats like a railroad or pipeline
right of way, please refer to the following sections:
  - Rural properties, see page 6.
  - Wetlands, see page 9.

- If the property is associated with a roadway or other structure that would require management of runoff water, please refer to the following section:
  - Stormwater management (associated BMPs), see page 14.

In many instances, right of ways and easements will simply fall to the local mosquito and vector control program or go completely unmanaged because they are very large and it is not possible to determine the responsible party.

**Mosquito Control BMPs for Wastewater Treatment Facilities**

Wastewater treatment facilities are designed to collect, treat, and release nutrient rich highly organic water. These facilities implement practices appropriate to removing contaminants from wastewater, but which may be in direct conflict with BMPs intended to prevent development of mosquito larvae. Further, managers are under intense pressure to meet water quality standards in effluent water and are frequently concerned that mosquito control BMPs will jeopardize compliance with effluent standards.

Wastewater facilities often include features that can produce mosquitoes. Examples include 1) a series of treatment or evaporation ponds, 2) the use of tules or other emergent vegetation to remove contaminants, 3) aerated and non-aerated ponds with emergent vegetation around the edges or throughout, 4) cracks and openings in crusted waste matter on the surface of treatment ponds, and 5) abandoned or unused pond basins that frequently hold shallow water. Certain activities may also create or enhance mosquito habitat including 1) allowing evaporation of wastewater from treatment ponds for maintenance or as a standard treatment method, 2) release of wastewater into marshes or floodplains for evaporation or infiltration, and 3) distribution of sludge onto irrigated agricultural lands.

For mosquito control around buildings and grounds, consult the residential and landscape section of this document. Similarly, many BMPs included in the wetlands and dairy sections of this document are pertinent to wastewater management facilities, particularly those sections related to construction and management of treatment ponds and wetlands and the use and distribution of wastewater or sludge onto agricultural lands. For mosquito control related to wastewater collection, conveyance, and distribution consult the stormwater management section of this manual.

- Monitor all treatment ponds for mosquito larvae – particularly in areas of emergent vegetation.
- Remove emergent vegetation from edges of aerated ponds.
- Immediately incorporate sludge into soil through plowing or disking.
- Insure all water distributed onto evaporation ponds dries completely in less than 96 hours.
- Check abandoned ponds or tanks weekly to ensure they are completely dry.
- Use mechanical agitation to prevent the formation of any crust on treatment ponds or tanks.
- Work closely with a local vector control program. If there is no local vector control agency, consult the closest vector control program, the local public health officer, or CDPH to prevent or abate a mosquito problem from the facility.

**Mosquito Control BMPs for Wildlands – Undeveloped Areas**

California encompasses about 100 million acres (40 million hectares) of land. Approximately 75 million acres (30 million hectares) are classified as wildlands, which include all undeveloped and non-cultivated property in the state. In many cases the properties are remote and mosquito control is neither feasible nor warranted. However, if you own a property that is near a town or are aware of a mosquito problem at the property, you may wish to contact the closest vector control program or CDPH to determine what if anything can be done to alleviate the problem.

**Mosquito Control BMPs that May be Applicable to Wildlands**

- Conduct routine mosquito surveillance by looking for immature mosquitoes in the water. Apply EPA-registered products (typically containing Bti, Bs, or methoprene) to control mosquito larvae.
- Evaluate reports of mosquito annoyance from visitors or the public, and if possible work with a local mosquito control program to be notified if there is an adult mosquito problem on or near your property.
- After a rainfall, pay particular attention to temporary water sources and ponds that rise. Treat sources with mosquito control products if needed.
- Stock ornamental ponds and other water features with mosquitofish available from local mosquito control programs. However, their use is restricted in natural bodies of water or in water features that drain into natural bodies of water. Land managers must consult with the local mosquito control agencies regarding proper use of mosquitofish or other available biological control agents.
- Work closely with a local mosquito control program to accurately identify, map, and monitor areas that may produce mosquitoes; and tailor control measures for each site, contingent on the species of mosquitoes that are present.
- Implement personal protective measures
  - Provide visitors and guests with information regarding the risk of mosquito-borne disease transmission and personal protective measures.
  - Install and maintain tight-fitting window and door screens on buildings.
  - If possible, minimize outdoor activities at dawn and dusk when mosquitoes are the most active.
  - Wear protective clothing such as long-sleeved shirts and long pants when going into mosquito-infested areas.
  - Use mosquito repellent when necessary, carefully following the directions on the label.
Evaluation of the Efficacy of BMPs

Landowners can easily evaluate the efficacy of the mosquito control BMPs they have implemented. You can do a simple evaluation as follows:

- Immature mosquitoes: Look for immature mosquitoes in standing water on your property – if the number is decreasing noticeably or immature mosquitoes can not be found, the BMPs you have implemented are working.
- Adult mosquitoes: Simply be aware of the level of mosquito annoyance you experience and ask guests or employees about their experience with regard to mosquitoes. People become accustomed to a certain level of mosquito activity and commonly notice increases or decreases in that level. If the annoyance level is increasing, you have more work to do; if the number is decreasing or mosquitoes are not noticeable – good job! The BMPs you have implemented are working.

The best way to evaluate the effectiveness of BMPs is through a comprehensive surveillance program of larval dipping and adult mosquito trapping, including species identification. Some important strengths of local mosquito control programs are their ability to evaluate treatment options, estimate treatment costs, recommend and implement those BMPs most appropriate for a property. Local mosquito abatement programs also are familiar with indigenous mosquito species and therefore know the type of habitat those mosquitoes come from, often monitor adult populations, and can identify if there is a mosquito problem in a particular area. Landowners can make substantial progress in solving mosquito problems on their own, but if possible, they should work closely with a local mosquito control program to implement and evaluate mosquito control BMPs.
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<td><a href="http://www.mosquitoes.org">http://www.mosquitoes.org</a></td>
<td>(510) 783-7744</td>
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<td>BUTTE</td>
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<td><a href="http://www.contracostamosquito.com/">http://www.contracostamosquito.com/</a></td>
<td>(925) 771-6100</td>
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<td><a href="http://www.edgov.us/VectorControl/">http://www.edgov.us/VectorControl/</a></td>
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<td>INYO</td>
<td>INYO COUNTY DEPT OF AG OWENS VALLEY MAP</td>
<td><a href="http://www.inyomoagriculture.com/ovmap.html">http://www.inyomoagriculture.com/ovmap.html</a></td>
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<td>NEVADA COUNTY COMMUNITY DEVELOPMENT AGENCY</td>
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<td>TULARE</td>
<td>DELTA VCD</td>
<td><a href="http://www.deltavcd.com">http://www.deltavcd.com</a></td>
<td>(559) 732-8606</td>
</tr>
<tr>
<td>TULARE</td>
<td>TULARE MAD</td>
<td>6575 Dale Fry Road, Tulare, CA 93274</td>
<td>(559) 686-6628</td>
</tr>
<tr>
<td>VENTURA</td>
<td>MOORPARK CITY VCD</td>
<td><a href="http://ci.moorpark.ca.us/cgi-bin/htmlos.exe/03565.1.1476605945000001294">http://ci.moorpark.ca.us/cgi-bin/htmlos.exe/03565.1.1476605945000001294</a></td>
<td>(805) 517-6248</td>
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Appendix A
Mosquito Control and Arbovirus Surveillance

Mosquito Control Practices

Mosquito control agencies and private landowners in California work cooperatively to implement an integrated pest management (IPM) approach to mosquito control. Source reduction (eliminating the places where mosquito larvae hatch and develop) is the most effective way of preventing adult mosquitoes; however, it may be possible to eliminate mosquito production from a source through other modifications of habitat and/or water management. Biological control agents, including native or introduced predators, are often utilized in combination with water management practices. Pesticides are an important part of an IPM program and mosquito specific larval control pesticides are often used to supplement other source reduction activities. When source reduction and larval control have not adequately reduced the mosquito population, the application of pesticides to control adult mosquitoes may be necessary. Personnel working for vector control agencies who apply pesticides in California are certified by California Department of Public Health (CDPH) after demonstrating the knowledge necessary to control mosquitoes safely and effectively using IPM techniques.

Larval Control

Larval control is the foundation of most mosquito control programs in California. Whereas adult mosquitoes are widespread in the environment, larvae must have water to develop; control efforts therefore can be focused on aquatic habitats. Minimizing the number of adults that emerge is crucial to reducing the incidence and risk of disease. The three key components of larval control are environmental management, biological control, and chemical control.

Environmental Management

Manipulating or eliminating potential mosquito breeding sources can provide dramatic reductions in mosquito populations. There are three levels of environmental management.

1. Source elimination: This approach completely eliminates potential habitats for mosquitoes. This strategy is generally limited to artificial habitats created by urbanization. Examples of source elimination include emptying or turning over containers holding water, filling in holes containing water with sand or gravel, cleaning drainage ditches of debris, and covering or inverting structures and vessels that could hold water.

2. Source reduction: This strategy aims to alter and sometimes eliminate available habitat for larvae which substantially reduces mosquito breeding and the need for
repeatedly applying pesticides. Unlike source elimination, standing water may exist but the total amount of water, or the time the water is left standing, is greatly reduced. Source reduction may require some maintenance (see below) to prevent further mosquito breeding. Examples of source reduction include limiting the growth of emergent vegetation in wetlands and ponds, constructing drainage ditches to remove water from areas prone to flooding, and clearing stormwater channels of silt and debris. Routine larval monitoring can indicate whether these efforts are effective or need further action.

3. Source maintenance: When eliminating or significantly altering mosquito breeding sources is prohibited and/or inappropriate, reducing the number of sheltered, predator-free habitats while having minimal impact on the surrounding environment can make an area unsuitable for mosquitoes. Source maintenance can include water management, vegetation management, wetland infrastructure maintenance, and wetland restoration. Strategic, focused plans must be developed for each site.

Biological Control

Biological control uses predators, parasites, or pathogens to reduce populations of mosquito larvae and is often combined with environmental management to enhance results. The mosquitofish (*Gambusia affinis*) has been used to control mosquitoes in California since 1921 and is the most widely used biological control agent in the world. These small fish are effective against mosquito larvae because they grow and reproduce rapidly, feed at the water surface where mosquito larvae are found, and tolerate a wide range of temperature and water quality.

Other fish are occasionally used with mixed success. Fish are most effective in permanent ponds and wetlands, but are also used in rice fields and stormwater canals with permanent water. Many local mosquito control agencies propagate mosquito-eating fish.

Although many other animals have been tested for mosquito control, and in natural wetlands predation is an important factor in reducing mosquito production, biological control by the intentional addition of mosquito predators other than mosquitofish is largely experimental rather than operational.

Chemical Control

Pesticides that control mosquito larvae are called larvicides. Four types of larvicides (bio-rational, surface films, growth regulators, and chemical products) encompassing seven active ingredients are registered for use in California. Larvicides are applied by hand, from hand-held or vehicle-mounted engine-driven blowers, or by aircraft, depending on the product, the formulation, and the target habitat. Applicators of any of these products must be certified by the CDPH or an appropriate regulatory authority.
1. Bio-rational products

Bio-rational products exploit insecticidal toxins found in certain naturally occurring bacteria. These bacteria are cultured in mass and packaged in various formulations. The bacteria must be ingested by mosquito larvae so the toxin is released. Therefore bio-rational products are only effective against larvae since pupae do not feed. The bacteria used to control mosquito larvae have no significant effects on non-target organisms when applied for mosquito control in accordance with product labels.

Two products that are used against mosquito larvae singly or in combination are *Bacillus thuringiensis israelensis* (Bti) and *Bacillus sphaericus* (Bs). Manufactured Bti contains dead bacteria and remains effective in the water for 24 to 48 hours; some slow release formulations provide longer control. In contrast, Bs products contain spores that in favorable conditions remain effective for more than 30 days. Both products are safe enough to be used in water that is consumed by humans.

Another bio-rational product available for mosquito control is derived from the soil bacterium *Saccharopolyspora spinosa*, which produces natural metabolites called spinosyns during fermentation. These metabolites are lethal to mosquito larvae when ingested or by contact. The most active metabolites are formulated into a product called “spinosad”. The product affects the central nervous system of the mosquito causing uncontrolled nervous impulses, ultimately killing the larvae.

2. Surface agents

Mosquito larvae and pupae breathe through tubes called “siphons” that extend above the water surface. Surface agents such as highly refined mineral oils or monomolecular films (alcohol derivatives) can spread across the surface of the water to prevent mosquitoes from breathing. Depending on the product, the film may remain on the water’s surface from a few hours to a few days. Surface films are the only available products that are effective against very late stage larvae and pupae.

3. Insect growth regulators

Insect growth regulators (IGRs) disrupt the physiological development of larvae thus preventing adults from emerging. The two products currently used for controlling mosquito larvae are methoprene and diflubenzuron.

The effective life of these products varies with the formulation. Methoprene can be applied in granular, liquid, pellet, or briquette formulation. Methoprene has minimal non-target effects and no use restrictions. Diflubenzuron is rarely used in California because it may affect growth of non-target aquatic invertebrates. IGRs for mosquito control can be used in sources of water that are consumed by humans.
4. Chemical larvicides

Chemical pesticides are rarely used to control mosquito larvae. Organophosphate larvicides are used infrequently because of their potential non-target effects and label restrictions. The organophosphate pesticides temephos and malathion are registered for use as larvicides in California. However, malathion is currently used exclusively for adult mosquito control in the state. Temephos can be safely and effectively used to treat temporary water or highly polluted water where there are few non-target organisms and/or livestock are not allowed access. The efficacy of temephos may be up to 30 days depending on the formulation.

**Adult Control**

<table>
<thead>
<tr>
<th>Table: Action Levels for Adult Control</th>
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<td>IPM mosquito control programs initiate adult mosquito control when action levels or thresholds are reached or exceeded. Thresholds are based on local sampling of the adult mosquito population and/or when the risk of mosquito-borne disease increases above levels established by a local agency, often following guidelines established in the California Mosquito-borne Virus Surveillance and Response Plan. Thresholds are an integral component of mosquito control because they provide a range of predetermined actions based on quantified data. Thresholds also establish expectations and boundaries for responses that ensure appropriate mosquito control activities are implemented at the appropriate time. The threshold for adult mosquito control depends on several factors including:</td>
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<tr>
<td>• How local citizens tolerate nuisance mosquitoes by evaluating public service requests.</td>
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<tr>
<td>• Overall mosquito abundance.</td>
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<tr>
<td>• Presence of mosquito-borne disease in the region.</td>
</tr>
<tr>
<td>• Abundance of mosquito species that are vectors of disease.</td>
</tr>
<tr>
<td>• Local acceptance of adult mosquito control activities.</td>
</tr>
<tr>
<td>• Climate data.</td>
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</table>

Adult mosquitoes can only be controlled with adulticides. Many mosquito control programs in California include adulticiding as an integral component of their IPM program. Adulticiding falls into two categories – barrier applications and ultra-low volume (ULV) applications. Barrier applications target resting mosquitoes by applying pesticides to vegetation and structures. Barrier applications typically cover relatively small areas and are applied to alleviate specific problems rather than an area wide adult mosquito problem.

ULV applications are used to control adult mosquitoes over large areas. An “ultra-low volume” (typically less than 2 oz / acre [140 ml / ha] total volume) of tiny oil or water droplets carrying an insecticide are emitted from specialized equipment mounted to trucks or aircraft. The droplets kill adult mosquitoes on contact. ULV applications are made after sunset or before sunrise to coincide with the time that mosquitoes are most
active, when non-target insects are least active, and when temperature inversions are most likely to occur. These applications are employed when mosquito populations must be reduced immediately to halt disease transmission. Multiple applications in a particular area may be utilized when the objective is to kill a high enough proportion of older adult mosquitoes to break a disease transmission cycle.

Adverse effects from ULV applications are rare; however, people with health problems should be aware when and where the applications are being conducted. This information can be obtained by contacting the local vector control agency. Chemicals currently registered for ULV applications against mosquitoes in California (as of June, 2010) include organophosphates (e.g., malathion and naled), pyrethrins, (e.g., pyrethrum) and pyrethroids (e.g., resmethrin, sumithrin, permethrin, and etofenprox). With the exception of the active ingredient etofenprox, formulations of both pyrethrins and pyrethroids include the synergist piperonyl butoxide (PBO), which increases their activity against mosquitoes.

1. Organophosphates

Malathion and naled are neurotoxins that act by blocking the enzyme cholinesterase, inhibiting neurologic transmission. Malathion or naled may be used as rotational products with pyrethroid insecticides to help prevent development of pesticide resistance.

2. Pyrethrins

Pyrethrins and pyrethroids are neurotoxins that act by causing uncontrolled firing of neurons. Pyrethrum is a natural insecticide derived from chrysanthemum flowers. Adult mosquitoes are rapidly paralyzed and killed on contact. Pyrethrins are degraded rapidly by sunlight and chemical processes. Residual pyrethrins from ULV applications typically remain less than one day on plants, soil, and water.

3. Pyrethroids

Pyrethroids are manufactured pyrethrins. They have very low toxicity to birds and mammals but are toxic to fish if misapplied.
Compounds currently approved for larval and adult mosquito control in California are listed in Appendix B.

**Mosquito Surveillance**

**Mosquito and Mosquito-Borne Disease Monitoring**

Monitoring mosquito populations and mosquito-borne disease levels provides the necessary data to make informed management decisions.

The application of any pesticide to control mosquitoes in an IPM program is done after establishing the need to do so through mosquito population monitoring (surveillance).

Larval mosquito surveillance is the process of identifying and checking likely larval developmental sites for immature mosquitoes and treating the water to kill the mosquitoes prior to them emerging as flying, biting adults.

Adult mosquito surveillance is accomplished through a network of traps and through mosquito annoyance reports. Adult mosquito surveillance is a critical component of determining where mosquitoes are coming from, the potential for disease transmission in an area, and the need for adult mosquito control. Districts also use adult surveillance as a feedback or quality control mechanism to determine how effective the overall program is in reducing mosquito populations. Trapping adult mosquitoes and submitting those mosquitoes to test for diseases is often one component of a mosquito-vectored disease surveillance program. Collecting baseline data on mosquito populations and mosquito-borne disease also helps target educational efforts.

**Mosquito Surveillance Techniques**

1. Larval surveillance

   Larval surveillance is the routine sampling of aquatic habitats for developing mosquitoes. The primary tool is the “dip count” which indicates whether a habitat is producing mosquitoes and estimates larval density. A one-pint cup attached to a long handle is used to collect a standard volume of water (“dip sample”). The “dip count” may be expressed as the number of immature (larvae and pupae) mosquitoes per dip, per unit volume, or per unit surface area of the site.

2. Adult surveillance

   Several types of traps are used for adult surveillance, because mosquitoes are attracted to different traps depending on their species, sex, and physiological condition. The most common traps use light, carbon dioxide, water for egg laying, and a resting area. Trapped adults provide information about local distribution,
density, and identity. The size of an adult mosquito population can also be assessed by the number and distribution of service requests from the public. Data are used to help locate new sources of mosquitoes or known sources with a recurrent problem.

Annoyance Biting

Many species of mosquitoes are not important as vectors of disease, but can cause serious injury and discomfort to humans and animals. Each time a female mosquito pierces the skin to take blood, she contaminates the wound with her saliva, creating the potential for a mild allergic reaction. The common symptom of mosquito bites is irritated and swollen skin surrounding the bite with persistent itching for several days. Scratching these bites to alleviate the itching can result in secondary bacterial infections. In addition, when mosquito populations explode, the sheer number of mosquitoes attempting to bite can make life miserable.

Mosquitoes as Disease Vectors

Mosquitoes are the most important insect vectors of disease worldwide, causing millions of human deaths every year. Mosquito-borne pathogens are typically transmitted or “vectored” when a mosquito ingests a disease causing organism, the organism reproduces inside the mosquito, and is subsequently injected along with saliva into another animal or human host. The potential or “competence” to vector any particular disease causing organism varies greatly among mosquito species.

California has a long history of mosquito-borne disease. Mosquito control programs were first developed in the early 1900s to combat malaria and other diseases, and to reduce populations of nuisance mosquitoes. Currently, there are 12 mosquito-borne viruses recognized in California; however, only West Nile virus (WNV), western equine encephalomyelitis (WEE), and Saint Louis encephalitis (SLE) are significant threats to public health. Global trade and travel will continue to provide an avenue for introducing or re-introducing other mosquito-borne pathogens and their vectors into California and the United States. The diseases of greatest concern include Japanese encephalitis, dengue, yellow fever, Rift Valley fever, chikungunya, Venezuelan encephalitis, and malaria.

Virus Surveillance

In 2000, CDPH collaborated with the University of California, Davis, the California Department of Food and Agriculture, local mosquito and vector control agencies, and other state and local agencies to develop a comprehensive statewide surveillance program to detect and monitor WNV activity. More than 70 local mosquito and vector control districts and agencies, environmental health agencies, and county public health departments throughout California routinely contribute to the program. Surveillance includes testing for WNV infections in humans, horses, mosquitoes, wild birds, and “sentinel” chicken flocks located throughout California. The program also includes
testing dead birds reported by the public for infections with WNV. A special website (http://www.westnile.ca.gov/) and toll-free hotline (877-WNV-BIRD) were created and are maintained by CDPH to support this surveillance program. The information from the program allows CDPH and local agencies to identify conditions conducive to WNV transmission and areas with elevated risk. This information is used by local mosquito control agencies to reduce the threat of WNV transmission to humans.

Mosquito Transmitted Diseases

| Landowners throughout California, mosquito and vector control agencies, health departments, and CDPH work together to protect Californians from mosquito-borne diseases. Work to minimize the risk of disease transmission includes 1) comprehensive mosquito surveillance and control efforts on private and public lands, 2) agencies providing technical guidance and information to the medical and veterinary communities, and 3) educating the public about mosquitoes, the diseases they carry, and personal protective measures. |

Encephalitis

Several mosquito-borne viruses that occur in California can cause encephalitis. The majority of human infections with these viruses have no symptoms. Those with so-called mild symptoms can still have significant illness and face prolonged recovery, and severe cases can be fatal or cause permanent neurological damage. There are several species of mosquitoes in California that can transmit WNV, SLE, and WEE viruses to people and animals. The most important species belong to the genus Culex. Specifically Cx. tarsalis, Cx. pipiens, and Cx. quinquefasciatus are significant public health concerns because of their widespread distribution throughout the state, their proximity to humans, and their capacity as very efficient vectors.

West Nile Virus

West Nile virus has become an endemic disease in California and like other encephalitic viruses, can cause serious illness. Many people who are infected do not get sick or may have a variety of symptoms that can include fever, head and body aches, nausea, vomiting, swollen lymph glands, and skin rash. Only about one in 150 infected people will develop a serious illness that may require hospitalization. Elderly people are at highest risk of developing the severe form of WNV and are at an increased risk of long-lasting physical and mental disorders. The severe form of the disease can be fatal.

Malaria

Malaria is caused by four species of protozoa. The parasites destroy red blood cells causing severe fever and anemia. Left untreated, malaria can cause kidney failure, coma, and death. Malaria was once a common public health threat in California and
much of the southern United States, but it was eradicated by intensive mosquito control efforts and the discovery of anti-malarial drugs. However, the disease still occurs in many other countries worldwide, creating a perpetual risk of re-introduction, especially from infected travelers and immigrants. The *Anopheles* mosquitoes capable of transmitting malaria still occur in many areas of California.

**Canine Heartworm**

Canine heartworm occurs worldwide. It is caused by a filarial nematode transmitted by *Aedes* and some *Culex* mosquitoes that can infect domestic dogs, wild canines (e.g., foxes, coyotes, wolves), and cats. The tiny worms migrate through the body to the heart and cause thickening and inflammation of the heart, which can lead to difficulty in breathing, chronic cough, vomiting, and can sometimes be fatal.
Appendix B

Compounds Approved for Mosquito Control in California

Pesticides used for mosquito control have been evaluated for this purpose by the U.S. Environmental Protection Agency (EPA) and found to pose minimal risks to human health and the environment when used according to label directions. For updated information on specific products approved for use in California, please refer to the California Department of Pesticide Regulation website: http://www.cdpr.ca.gov/docs/label/labelque.htm.

Mosquito and vector control programs that apply pesticides to a water of the United States for the purpose of controlling any vector are required to obtain a National Pollution Discharge Elimination System (NPDES) Permit for Biological and Residual Pesticide Discharges to Waters of the United States. More information on the permit, issued by the State Water Resources Control Board, can be found at: http://www.waterboards.ca.gov/water_issues/programs/npdes/aquatic.shtml#davcp.

The components of this appendix have been adapted from the California Mosquito-Borne Virus Surveillance and Response Plan; please refer to the following website for more information: http://www.westnile.ca.gov.

The use of pesticides to control mosquitoes should be the last resort after BMPs outlined in this manual have been implemented. Individuals considering applying a pesticide must be adequately trained and always apply pesticides according to label directions. In California, local mosquito control agency employees must pass a testing and certification process through CDPH before they can apply pesticides to control mosquitoes. Similarly, commercial pesticide applicators must be appropriately certified by the California Department of Pesticide Regulation. Private landowners applying general use pesticides to control mosquitoes solely on their own property are not required to be certified; however, landowners have the same legal responsibility with regard to pesticide and environment related laws. Private citizens considering using pesticides should consult their County Agricultural Commissioner and the California Department of Fish and Game before application.

Examples of products containing specific active ingredients are provided below, but this is not an inclusive list nor constitutes product endorsement. For more information on pesticides and mosquito control, please refer to the U.S. EPA website: http://www.epa.gov/pesticides/health/mosquitoes/mosquito.htm.

Larvicides

1. *Bacillus thuringiensis*, subspecies *israelensis* (Bti: e.g., Aquabac 200G, VectoBac® 12AS, Teknar HP-D)  
   **Use:** Approved for most permanent and temporary bodies of water.
Limitations: Only works on actively feeding stages. Does not persist well in the water column.

2. *Bacillus sphaericus* (Bs: e.g., VectoLex® CG)
   **Use**: Approved for most permanent and temporary bodies of water.
   **Limitations**: Only works on actively feeding stages. Does not work well on all species. May persist and have residual activity in some sites.

3. Spinosad (bacteria derived natural insecticide: e.g., Natular G)
   **Use**: Approved for most permanent and temporary bodies of water.
   **Limitations**: Only works on mosquito larvae.

4. IGRs (Insect Growth Regulators)
   a. (S)-Methoprene (e.g., Altosid® Pellets)
      **Use**: Approved for most permanent and temporary bodies of water.
      **Limitations**: Works best on older instars. Some populations of mosquitoes may show some resistance.
   b. Diflurobenzuron (e.g., Dimilin®25W)
      **Use**: Impounded tail water, sewage effluent, urban drains and catch basins.
      **Limitations**: Cannot be applied to wetlands, crops, or near estuaries.

5. Larviciding oils (e.g., GB-1111, BVA 2 Mosquito Larvicide Oil)
   **Use**: Ditches, dairy lagoons, floodwater. Effective against all stages, including pupae.
   **Limitations**: Consult with the California Department of Fish and Game for local restrictions.

6. Monomolecular films (e.g., Agnique® MMF)
   **Use**: Most standing water including certain crops.
   **Limitations**: Does not work well in areas with unidirectional winds in excess of 10 mph.

7. Organophosphate compounds
   Temephos (e.g., Abate® 2-BG)
   **Use**: Non-potable water; marshes; polluted water sites
   **Limitations**: Cannot be applied to crops for food, forage, or pasture. This material may not be effective on some *Culex tarsalis* populations in the Central Valley.

**Adulticides**

1. Organophosphate compounds
   **Note**: Many *Culex tarsalis* populations in the Central Valley have shown resistance to OP pesticides at approved label rates.
   a. Malathion (e.g., Fyfanon® ULV)
      **Use**: May be applied by air or ground equipment over urban areas, some
crops including rice, wetlands.

Limitations: Paint damage to cars; toxic to fish, wildlife and bees; crop residue limitations restrict application before harvest.

b. Naled (e.g., Dibrom® Concentrate, Trumpet® EC)

Use: Air or ground application on fodder crops, swamps, floodwater, residential areas.

Limitations: Similar to malathion.

c. Chlorpyrifos (e.g., Mosquitomaster 412)

Use: Air or ground application in urban or recreational areas

Limitations: Not registered for use over agricultural commodities or grazing lands and may be toxic to bees, fish, and some wildlife.

2. Pyrethrins (natural pyrethrin products: e.g., Pyrenone® Crop Spray, Pyrenone® 25-5, Evergreen®)

Use: Wetlands, floodwater, residential areas, some crops.

Limitations: Do not apply to drinking water, milking areas; may be toxic to bees, fish, and some wildlife. Some formulations with synergists have greater limitations.

3. Pyrethroids (synthetic pyrethrin products containing deltamethrin, cyfluthrin, permethrin, resmethrin, sumithrin, or etofenprox: e.g., Suspend® SC, Tempo Ultra SC, Aqua-Reslin®, Scourge® Insecticide, Anvil® 10+10 ULV, and Duet, which also contains the mosquito exciter prallethrin)

Use: All non-crop areas including wetlands and floodwater.

Limitations: May be toxic to bees, fish, and some wildlife; avoid treating food crops, drinking water or milk production.
### PESTICIDES USED FOR LARVAL MOSQUITO CONTROL IN CALIFORNIA

#### LARVICIDES

For updated information on specific products approved for use in California, please refer to the California Department of Pesticide Regulation website: [http://www.cdpr.ca.gov/docs/label/labelque.htm](http://www.cdpr.ca.gov/docs/label/labelque.htm)

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<th>Trade name</th>
<th>EPA Reg. No.</th>
<th>MFG</th>
<th>Formulation</th>
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<td>Adapco</td>
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PESTICIDES USED FOR ADULT MOSQUITO CONTROL IN CALIFORNIA

ADULTICIDES

For updated information on specific products approved for use in California, please refer to the California Department of Pesticide Regulation website: [http://www.cdpr.ca.gov/docs/label/labelque.htm](http://www.cdpr.ca.gov/docs/label/labelque.htm)

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Appendix C
Health and Safety Codes Pertinent to Mosquito Control

In California, mosquito and vector control agencies are regulated by sections of the California Health and Safety (H&S) Code, Food and Agriculture Code, California Code of Regulations, and others. The following components of this appendix have been adapted from the Overview of Mosquito Control Practices in California, California Department of Public Health: http://www.westnile.ca.gov/resources.php

Governing laws and regulations

Many federal and state laws govern the activities of vector control agencies, including the Clean Water Act (CWA), the Endangered Species Act (ESA), and the Federal Insecticide Fungicide and Rodenticide Act (FIFRA). Pesticide application by vector control agencies in California is regulated under FIFRA. FIFRA is administered through the U.S. Environmental Protection Agency, and regulates the registration, labeling, and sales of pesticides in the United States.

The California H&S Code encourages the formation of local mosquito control programs to protect the public health, safety, and welfare (H&S Code Section 2001-b) Website link: http://leginfo.ca.gov/cgi-bin/displaycode?section=hsc&group=01001-02000&file=2000-2007. The legal responsibility of landowners in California to avoid causing a public nuisance, including mosquitoes is implied in the section. The potential consequences of failing to prevent a public nuisance are described in the Code sections listed below.

Under the H&S Code, local vector control agencies have the authority to conduct surveillance for vectors, prevent the occurrence of vectors, and legally abate production of vectors or public nuisance defined as “Any water that is a breeding place for vectors” and “Any activity that supports the development, attraction, or harborage of vectors, or that facilitates the introduction or spread of vectors.”(H&S Code Section 2002(j) and 2040). Vector control agencies also have authority to participate in review, comment, and make recommendations regarding local, state, or federal land use planning and environmental quality processes, documents, permits, licenses, and entitlements for projects and their potential effects with respect to vector production. (H&S Code Section 2041) Website link: http://caselaw.lp.findlaw.com/cacodes/hsc/2040-2055.html

Additionally, agencies have broad authority to influence landowners to reduce or “abate” the source of a vector problem. Actions may include imposing civil penalties of up to $1000 per day plus costs associated with controlling the vector. Agencies have authority to “abate” vector sources on private and publicly owned properties. (H&S Code Sections 2060-2065). Website link: http://caselaw.lp.findlaw.com/cacodes/hsc/2060-2067.html
Mosquito and vector control programs that enter into a cooperative agreement with the California Department of Public Health are exempted from some pesticide related laws under Title 3 of the California Code of Regulations Section 6620. Specifically, these agencies are exempted from “Consent to Apply” (Title 3, California Code of Regulations, Section 6616), “Notice” (Title 3, California Code of Regulations, Section 6618), and the “Protection of Persons, Animals, and Property” (Title 3, California Code of Regulations, Section 6614). Essentially, these provisions obviate the vector control agency from having to notify or get permission from landowners prior to applying a pesticide to their property in the interest of preserving the public health. Website link: http://www.cdpr.ca.gov/docs/legbills/calcode/030201.htm#a6620

A vector control technician working at a vector control agency must be a “certified technician” or work under the direct supervision of a “certified technician” to apply pesticides. Vector control technicians achieve certification through an examination process administered by the California Department of Public Health.

Vector control agencies cannot use any pesticide not registered for use in California, and are required to keep detailed records of each pesticide application, including date, location, and amount applied. All pesticides must be applied in accordance with the labeling of the product as registered with the U.S. EPA.
Appendix D
Mosquitoes of California

The biology and key characteristics of the four major mosquito genera in California are described below.

**Aedes**
There are about 80 species of *Aedes* mosquitoes in the continental United States; 24 species occur in California. Certain species are widespread, may occur in very large numbers, and are among the worst biting pests. *Aedes* mosquitoes do not lay their eggs directly on the surface of standing water. Instead, they lay single eggs on intermittently flooded surfaces such as the damp soil around irrigated pastures and fields, along the edges of coastal tidal marshes, and inside dry treeholes and containers. Eggs are extremely resistant to drying and will lie dormant on dry surfaces until flooding occurs (eggs of *Ae. vexans* have been documented to lie dormant for up to three years). This can lead to many generations of eggs in a given habitat if female mosquitoes lay successive batches of eggs before the area is flooded. When flooding occurs, large numbers of eggs hatch spontaneously and develop rapidly to adults. Although larval developmental sites vary greatly, the most productive include transient ground pools, flooded areas along overflowing streams, flood and stormwater control basins, intermittently flooded agricultural lands, and container habitats such as tree holes, wheel ruts, and discarded tires.

*Aedes* are primarily summer-breeding mosquitoes. Because of their rapid larval development in newly-flooded habitats, adults often emerge before predators can colonize the water source. Most *Aedes* complete two to several generations per year depending on the frequency of habitat flooding from natural and artificial events. Adults cannot survive in colder weather. Therefore the majority of *Aedes* overwinter as eggs.

Typically, *Aedes* mosquitoes found in California will not enter buildings and homes; however, they are strong fliers and are known to travel many miles from their aquatic developmental sites to search for hosts. *Aedes* mosquitoes are diurnal (i.e., active during the day) during mild weather, especially around shaded areas, but will also bite at dusk. Most *Aedes* females feed on large mammals like cattle and horses, but will readily feed on humans. *Aedes* mosquitoes are aggressive and persistent biters causing people and animals to avoid areas where their numbers are great. One example is the species *Ae. nigromaculis*, which are currently not known to vector disease, but are considered a serious pest because they will seek out human hosts and bite during the day when people are most likely to be outdoors and active.

**Anopheles**
Approximately 22 species of *Anopheles* are found in the continental United States and of these, 5 occur in California. When feeding, *Anopheles* adults rest with their abdomens positioned at a distinct angle to the surface of the skin, whereas other species orient their bodies parallel. Females lay single floating eggs directly on the
surface of permanent or semi-permanent standing water. A female can lay successive batches of up to 300 eggs during the breeding season. Eggs are not resistant to drying and typically hatch within two-three days, although hatching may take up to two-three weeks in colder climates. Larvae develop in 12 to 20 days, but can take longer in cooler weather. Preferred larval habitats include clear, fresh seepage water in sunlit or partly shaded pools, wetlands, roadside ditches, rice fields, and poorly maintained water troughs.

Adult females bite at dusk and dawn and prefer to feed on mammals. Many Anopheles mosquitoes prefer to feed on rabbits, but will also feed on large mammals such as livestock and humans. In California, Anopheles species may undergo two or more generations per year. Most species over-winter in protected areas as mated females, resuming activity the following spring. These are among the first mosquitoes to emerge and bite humans each year.

Historically, Anopheles freeborni, the western malaria mosquito, was a vector of malaria in California. Currently, with the disease eradicated from California and the United States, it is considered a nuisance mosquito. This species is widespread throughout California and females will lay their eggs in any standing fresh water, although it is abundant in rice fields or other wetlands during late summer. While most adult mosquitoes stay within a few miles of their breeding source, they will migrate further when seeking hibernation sites in fall. This can lead to a large influx of mosquitoes from uncontrolled areas to residential areas during September and October.

**Culex**

Culex, with 11 species found throughout the state is the second largest genus of mosquitoes in California, second only to Aedes. Females can lay up to seven rafts of eggs over a two-month life span; each raft contains from 100-300 eggs which are laid on the surface of standing water. Culex larvae occur in a broad range of aquatic sites ranging from containers such as discarded tires, water barrels, and flower pots to clogged gutters, catch basins, and water for irrigation and urban wastewater. During summer and periods of drought, areas without regularly flowing water, street drainage systems, and contaminated streams, ponds and pools become productive larval habitats. Culex larvae are known for thriving in polluted sources of water with a high organic content.

Culex mosquitoes prefer to take blood meals at dusk or after dark and can be painful and persistent biters. Culex preferably feed on birds but also feed on mammals including humans and horses. They readily enter houses and buildings in search of a suitable host. Two or more generations of Culex can occur per year. Females that emerge in late summer will mate and overwinter until the following spring or mid-summer.

Several species of Culex can transmit viruses that can cause encephalitis (i.e., inflammation of the brain), including WNV, SLE, and WEE. These mosquitoes are
efficient and effective vectors of these diseases among birds, humans, horses and many other wild and domestic animals.

**Culex tarsalis**
*Culex tarsalis*, the Western encephalitis mosquito, is one of California’s most important and efficient vectors of WNV, SLE, and WEE. This species is widespread in California. *Cx. tarsalis* prefer to lay their eggs on fresh or lightly polluted standing water such as rice fields, ditches, pastures, waste water ponds, and seasonal wetlands. Other more urban freshwater sources include ornamental ponds, storm drains, and flood control channels. Larvae usually develop into adults in approximately 8-14 days; warmer water can shorten the developmental period. *Cx. tarsalis* are active from spring through fall; however the population in the Central Valley peaks in June to July with a secondary, smaller peak in September coinciding with flooding of seasonal wetlands. *Cx. tarsalis* survive through the winter as adults in barns, culverts, caves, and similar dark, protected places.

Adult *Cx. tarsalis* can disperse a great distance up to 10-15 miles (16-24 km) in search of blood meals, generally traveling along riparian corridors, but most stay close to the site where they emerged. Adults rest by day in shaded areas such as animal burrows and treeholes. Females prefer feeding between dusk and dawn but may bite during the day in deep shade. Females obtain blood meals from birds or mammals and can transmit diseases between these groups.

**Culex pipiens and Culex quinquefasciatus**
*Culex pipiens* (the northern house mosquito) and *Culex quinquefasciatus* (the southern house mosquito) appear to be identical. *Cx. quinquefasciatus* occurs in Southern California, whereas *Cx. pipiens* is found along the coastal regions and in Northern California and is the most widely distributed mosquito species in the world. Both species can transmit encephalitis viruses. They are common in and around households and prefer to lay eggs in polluted water that is high in organic content such as dairy runoff, wastewater catchment basins, stormwater ponds, dirty flower pots, bird baths, or any drainage systems where standing water exists.

In California, *Cx. pipiens* and *Cx. quinquefasciatus* typically do not disperse from where they emerged. Females feed at dusk or after dark, readily enter homes and prefer avian hosts but will also feed on large mammals including humans. *Cx. pipiens* and *Cx. quinquefasciatus* are vectors of WNV and SLE virus, and have also been implicated in transmitting canine heartworm.

**Other Culex mosquitoes.**
*Culex stigmatosoma*, the foul water mosquito, *Cx restuans* and *Cx. erythrothorax* can also be infected with WNV, but their distributions are limited (e.g., *Cx. erythrothorax* is mainly found close to bodies of water with tules).
**Culiseta**

Only eight species of *Culiseta* mosquitoes occur in the continental United States, of which four are found in California. Females lay clusters of floating eggs (rafts) on the surface of standing water. *Culiseta* mosquitoes are moderately aggressive biters, attacking in the evening hours or in shade during the day. Peak populations occur during the cooler months. These mosquitoes prefer to feed on larger domestic animals, such as cattle and horses, but will also feed on humans. The distribution of *Cs. inornata*, an unusually large mosquito, is widespread and can be found at elevations of up to 10,000 feet. Larvae of *Cs. inornata* develop in permanent water habitats, including shallow marshes, peat bogs, roadside ditches, abandoned gravel pits, and in standing water in soil cavities left by fallen trees. The common name of this mosquito—the Large Winter mosquito—reflects that it is most active in cool weather habitats.
# Appendix E

## Typical Larval Habitats of California Mosquitoes*

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<tr>
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Appendix F

Insect Repellents

A number of products have been developed and registered by the Environmental Protection Agency for human use that repel adult mosquitoes and thus reduce the chances of mosquito bites. The most commonly used mosquito repellents contain the active ingredient DEET (N,N-diethyl-meta-toluamide), which has been formulated and sold under a variety of trade names. Repellents are available in a variety of concentrations and are formulated as aerosol sprays (most commonly at 15%), lotions, and solids (up to 100%). Spray repellents can be used on outer clothing as well as sparingly on the skin to ensure complete coverage. Repellents should not be used under clothing. The percentage of DEET in the repellent reflects the approximate length of time the product will repel mosquitoes (e.g., 23.8% DEET = about five hours of protection, 20% = about four hours, and 6.6% DEET = about two hours).

Topical repellents that contain picaridin, IR-3535, and oil of lemon eucalyptus are similar in efficacy to those with DEET, but often require more frequent application. Clothing and other materials impregnated with permethrin during manufacture are also available. It is important to always carefully read and understand the benefits and limitations of repellents listed on the product label before use. By law, all repellent products must be used according to their labels.
Appendix G
Additional Resources and Information

Mosquito Biology

Additional information on mosquitoes and mosquito-borne diseases is easily obtainable from a variety of reputable sources. More information on mosquito biology and ecology is available on the American Mosquito Control Association (AMCA) and the Mosquito and Vector Control Association of California (MVCAC) websites. Local mosquito and vector control agencies and their respective websites can provide detailed information about local mosquito species. Information on mosquito-borne diseases is available from the Centers for Disease Control and Prevention (CDC) and the CDPH websites. Contact information for local mosquito and vector control agencies in California can be found through the CDPH website by entering the zip code of the location of interest under “Locate Your Local Mosquito and Vector Control Agency” at http://www.westnile.ca.gov/; more information is available on the MVCAC website.

Monitoring Mosquitoes and Diseases

More information about reporting dead birds and WNV surveillance in California can be found at http://www.westnile.ca.gov/. Methods for sampling adult mosquitoes and guidelines for designing, operating, and processing of traps are discussed in Guidelines for Integrated Mosquito Surveillance (Meyer et al. 2003) and are summarized in Appendix B of the California Mosquito-Borne Virus Surveillance and Response Plan which can be found at: http://www.westnile.ca.gov/resources.php


Health Department Websites

California Department of Public Health West Nile virus (WNV) website: http://www.westnile.ca.gov

United States Center for Disease Control and Prevention website: http://cdc.gov

Disease Surveillance Websites

UC Davis Center for Vectorborne Diseases website:  http://cvec.ucdavis.edu

California Vectorborne Disease Surveillance Gateway website: http://www.calsurv.org/

Best Management Practices


• For additional information on personal protective measures and the use of chemical repellents, go to the Centers for Disease Control and Prevention (CDC) web site at: http://www.cdc.gov/ncidod/dvbid/westnile/RepellentUpdates.htm

• For more information on evaluating the efficacy of BMPs on state of California-managed Wildlife Areas, see Walton 2005.

Mosquito Control

American Mosquito Control Association website: http://www.mosquito.org

Mosquito and Vector Control Association of California website: http://www.mvcac.org

University of California at Davis Center for Vectorborne Diseases website: http://cvec.ucdavis.edu

University of California IPM Online website: http://www.ipm.ucdavis.edu/

State Water Resources Control Board NPDES General Permits: http://www.waterboards.ca.gov/water_issues/programs/npdes/aquatic.shtml#davcp

Additional Online Resources

Climate Information

National Weather Service – Climate Prediction Center website: http://www.cpc.ncep.noaa.gov/products/predictions

Water Related Information

California Data Exchange Center website: http://cdec.water.ca.gov
Pesticide and Insect Repellent Information


National Pesticide Information Center website: http://npic.orst.edu/

Agriculture and Crop Related Information


Additional Reference Publications


References

Cited in Text


## List of Acronyms

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