General Pesticide Permit Toxicity Study:
Monitoring Aquatic Toxicity of Spray Pesticides to Freshwater Organisms

Draft Final Report

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Executive Summary

Pesticides are applied to waterways in California to control invasive aquatic plants and animals, and insect vectors such as mosquitoes. In response to a 2009 court decision[1], the State Water Resources Control Board (State Water Board) adopted Order 2011-0002-DWQ in March 2011 (Vector Control Permit), a National Pollution Discharge Elimination System (NPDES) general permit, to cover the discharge of pesticides to waters of the United States resulting from adult and larval mosquito control. In April 2012, the State Water Board adopted Order 2012-0003-DWQ to add clarifying language to Order 2011-0002-DWQ.

Pesticides used in spray activities have the potential to cause toxicity to non-target organisms in receiving waters. As specified in the Vector Control Permit, the State Water Board funded this study to determine if toxicity testing is an appropriate tool to use in monitoring spray pesticide applications. The goal of this study was to determine if toxicity testing increased information regarding the potential risk these pesticides pose to receiving waters beyond information provided by the standard chemical analysis for residual active ingredient in the receiving water sample.

Monitoring included a combination of aquatic toxicity tests and chemical analyses. Three different environmental settings were monitored: agricultural, urban and wetland habitats. Combinations of water and sediment toxicity test protocols were used because of differences in the physico-chemical properties and short-term environmental fates of the different pesticides used in the Vector Control Permit. In all cases, samples were collected before and after spray activities. Monitoring emphasized high priority active ingredients of insecticides used in vector control throughout the state. This study was comprised solely of application events conducted by Mosquito and Vector Control Association of California (MVCAC) districts. The active ingredients monitored in this study were the organophosphate pesticides malathion and naled, the pyrethroid pesticides etofenprox, permethrin and sumithrin, and pyrethrins. The latter pesticide classes often were sprayed in combination with the synergist piperonyl butoxide (PBO), which was also monitored.

Approximately 16% of the post-application water samples were significantly toxic, however not all toxicity could be attributed to the mosquito control applications. Four of the 16 toxic water samples had toxicity that could not be attributed to the measured chemicals. The toxicity of nine samples was attributed to the naled breakdown product dichlorvos. One of two toxic samples that were observed after permethrin applications could be explained by the presence of bifenthrin, which was not applied as part of vector spray pesticide activities. Two toxic samples observed after the application of pyrethrin could have been caused by a synergizing effect from PBO with ambient concentrations of pyrethroids in the urban setting, as the concentrations of pyrethrins were well below toxic levels.

Four of the 43 post-application sediment samples were significantly more toxic than their corresponding pre-application sample. The toxicity of these samples was not influenced by the spray application.

[1] *National Cotton Council of America v. EPA (6th Cir., 2009) 553 F.3d 927*

Concentrations of detected active ingredients were evaluated relative to several different water quality thresholds. The U.S. EPA Office of water uses aquatic toxicity data to develop ambient water quality criteria that can be adopted by states and tribes to establish water quality standards under the Clean water Act. Since there are few water quality criteria available for pesticides, another source of numbers for comparison of thresholds is the U.S. Environmental Protection Agency's Office of Pesticide Program (OPP) aquatic life benchmarks. OPP uses aquatic toxicity data in ecological risk assessments for pesticide registration decisions under the Federal Insecticide, Fungicide and Rodenticide Act. OPP's procedure for effects assessment is based on the most sensitive, scientifically acceptable toxicity endpoint available to EPA for a given taxon.

Pyrethrin concentrations did not exceed any of the evaluation thresholds. There was a single detection of etofenprox and sumithrin that exceeded the Vector Control Permit trigger values. Of the six post-application permethrin detections, five exceeded the OPP acute benchmark value and all concentrations exceeded the chronic benchmark. Three detections also exceeded the median lethal concentration (LC50) for *Hyalella azteca*, but none of these samples exhibited significant toxicity. Two of the three detected concentrations of malathion exceeded the U.S. EPA ambient water quality criterion of 100 ng/L. All three concentrations exceeded the U.S. EPA OPP chronic benchmark, but not the corresponding acute benchmark or the LC50.

There is no acute benchmark for naled, but in both circumstances where this compound was detected the concentrations exceeded the Vector Control Permit's receiving water monitoring trigger value and the chronic benchmark value, but not the LC50. The naled breakdown product dichlorvos was detected in 13 of 18 post-application samples. All of these detections were greater than the chronic benchmark and nine of these detections were greater than the LC50 for the test organism (*Ceriodaphnia dubia*). Dichlorvos was considered as a receiving water problem associated with the naled spray events because the toxicity test results showed a high magnitude of toxicity in the absence of any other obvious cause. This is an example of where the implementation of receiving water toxicity testing provided additional useful information to the spray event monitoring beyond that provided through chemical analysis of the active ingredient.

This study was designed to determine if toxicity testing provided additional useful information regarding the potential for impacts in the receiving water system beyond that provided by the analysis of the active ingredient alone. In the case of naled, analysis of the active ingredient would have underestimated potential impacts to the receiving system. Toxicity testing provided information that led to the inclusion of dichlorvos to the analyte list. Because of the strong relationship between the concentration of dichlorvos and toxicity, it is likely that monitoring for dichlorvos under the permit would provide similar information regarding the potential for aquatic impacts as toxicity testing.

Permethrin concentrations in three post-application samples were greater than the toxicity threshold of the test organism (*H. azteca*), but none of the samples were toxic. Because the laboratory reporting limit for permethrin in these samples was approximately the same concentration as the toxicity threshold, it is possible that small influences of physical parameters in the receiving water, such as dissolved carbon, could have affected bioavailability of the pesticide and thus affected the toxicity.

Two toxic samples were observed after the application of pyrethrin. The elevated concentrations of PBO measured in these samples suggested the possibility of a synergistic effect with pyrethroids already present in these urban receiving waters. Pyrethroids were not measured in the water samples, but if this situation were to occur, then toxicity testing or additional chemical analysis would be necessary to demonstrate the ancillary effects.

Sediment toxicity results did not provide significant additional information during the first year of the study, but sediment chemical analysis demonstrated that sumithrin concentrations increased over multiple applications at individual sites. However, sumithrin concentrations remained below toxic levels. During the second year of the study, four samples had significant post-application toxicity. The toxicity of these samples was not related to the application of the active ingredient.

Introduction

Pesticides are applied to state and local waterways in California to control invasive aquatic plants and animals, and insect vectors such as mosquitoes. Because pesticides used in these activities have the potential to cause toxicity to non-target organisms in receiving waters, the California State Water Resources Control Board (State Water Board) adopted Order 2011-0002-DWQ in March 2011. This NPDES general permit covers the discharge of pesticides to waters of the United States resulting from adult and larval mosquito control. In lieu of requiring permittees under each permit to conduct toxicity testing, the State Water Board has funded the current study to determine if toxicity testing is an appropriate tool to use in monitoring pesticide applications. The goal of the study was to determine if toxicity testing increased information regarding the potential risk these pesticides pose to receiving waters beyond information provided by the standard analysis of the active ingredient in the receiving water sample.

Monitoring included a combination of aquatic toxicity tests and chemical analyses to determine whether pesticide applications had the potential to cause toxicity in receiving waters. Toxicity test protocols were selected based on existing dose-response data, so that the most sensitive test species was used for monitoring specific pesticide classes. A combination of water and sediment toxicity test protocols was used because of differences in the physico-chemical properties and short-term environmental fates of the different pesticides used in the Vector Control Permit. In all cases, samples were collected before and after spray activities. Pre- and post-event sampling was conducted as close to the application events as possible because of the potential for other chemicals in receiving waters to confound interpretation of toxicity test results.

Monitoring emphasized high priority active ingredients of insecticides used in mosquito vector control throughout the state, and for the control of specific insect species such as the glassy-winged sharpshooter and the beet leaf hopper. Mosquito control in the state is conducted by individual Districts that typically belong to the Mosquito and Vector Control Association of California (MVCAC). Control of pests related to agriculture is overseen by the California Department of Food and Agriculture (CDFA). As the study progressed, application events conducted by the MVCAC members far outnumbered those conducted by the CDFA in both magnitude and likelihood of impacts to aquatic life. CDFA malathion aerial events to control beet leaf hoppers in western Fresno County were cancelled due to minimal pest pressure under localized drought conditions. Other CDFA events were not monitored because after meetings with stakeholders it was determined that applications were tightly controlled and not proximate to aquatic habitat so that risk to aquatic life was determined to be minimal. Therefore, this study is comprised solely of application events conducted by MVCAC members and all further discussion concerns mosquito abatement spray activities.

Methods

Project Design and Monitoring Coordination

Mosquito and vector control spray applications are conducted by individual vector control districts throughout the state. For this study, MVCAC coordinated the monitoring of spray applications conducted by member districts. Most of the sampling events were coordinated by MVCAC staff. UC Davis researchers met with MVCAC staff and their consultants to develop sampling strategies, visit potential sampling areas, and select appropriate sampling sites prior to sampling events. Staff from Michael Johnson and Associates and URS Corporation also participated in field sampling of selected sites on behalf of MVCAC. Once sampling areas were designated for all chemicals and sample area types (agriculture, urban and wetland), UC Davis staff helped coordinate individual sampling events as applications progressed. Applications were monitored from July to October 2011 and from May to September 2012.

Spray pesticide active ingredients were prioritized for the toxicity study using U.S. EPA Office of Pesticide Programs (OPP) aquatic life benchmarks (Table 1). The aquatic life benchmarks (for freshwater species) are based on toxicity values reviewed by EPA and used in the Agency's most recent risk assessments developed as part of the decision-making process for pesticide registration. The OPP relies on studies required under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), as specified at 40 Code of Federal Regulations part 158, as well as a wide range of environmental laboratory and field studies available in the public scientific literature to assess environmental risk. Each aquatic life benchmark is based on sensitive, scientifically acceptable toxicity endpoints available to U.S. EPA for a given taxon (for example, freshwater fish) of all scientifically acceptable toxicity data available to U.S. EPA. The acreage treated and the use patterns of the active ingredients were also considered in pesticide prioritizations. Although CDFA application events were not monitored as part of this study, several chemicals used by the MVCAC are also used by the CDFA. Pesticides were categorized as high, moderate, or low priority based on the potential for receiving water toxicity to invertebrates and fish, the frequency of use, and the acreage sprayed. Active ingredients that were rated moderate or low priority were not monitored in this study. A complete list of all active ingredients is included in Appendix A.

Table 1. Final list of high priority active ingredients that were targeted for the current study. All active ingredients are insecticides.

Active Ingredients	MVCAC	CDFA*	Chemical Class	Target Organisms
Malathion	√	√	Organophosphate	Mosquito Adult and Beet Leaf Hopper
Naled	√	√	Organophosphate	Mosquito Adult
Temephos	√		Organophosphate	Mosquito Larvae
Cyfluthrin		√	Pyrethroid	Mosquito Adult
Etofenprox	√		Pyrethroid	Mosquito Adult
Permethrin	√		Pyrethroid	Mosquito Adult
Prallethrin	√		Pyrethroid	Mosquito Adult
Resmethrin	√		Pyrethroid	Mosquito Adult
Sumithrin/Piperonyl Butoxide	√		Pyrethroid and Synergist	Mosquito Adult
Pyrethrins/Piperonyl Butoxide	√	√	Pyrethrin and Synergist	Mosquito Adult

*Note: CDFA did not spray malathion for the beet leaf hopper due to low populations of this pest in 2012. CDFA did not monitor cyfluthrin spray events because targeted spraying of this pesticide for the Asian Citrus Psyllid posed minor risk to receiving waters due to lack of proximity to aquatic habitat.

Sample Collection, Toxicity Testing and Analytical Chemistry

Water and sediment samples were collected before and after spray events in three environmental settings: agricultural, urban and wetland. Up to six spray events were monitored for each active ingredient on the high priority list (Table 1). These active ingredients include the organophosphate pesticides malathion, naled and temephos, pyrethrins applied with piperonyl butoxide (PBO), and the pyrethroids cyfluthrin, etofenprox, permethrin, prallethrin, resmethrin, and sumithrin. Pyrethroids were also generally applied with PBO. Because spray events took place in the evenings, the target collection period for pre-application water and sediment samples was from 4:00 to 8:00 p.m. (designated as the Time 0h sample, or T0). The target sampling period for the first post-application water samples was from 6:00 to 10:00 a.m. the following morning (designated as the Time 12h sample, or T12), and the target period for the final post-application water samples was from 4:00 to 8:00 p.m. (designated as the Time 24h sample, or T24). Post-application sediment samples were collected four to seven days after the application (designated as the Time 7d sample, or T4-7). Water and sediment samples were collected according to the Standard Operating Procedure (SOP) for Conducting Field Measurements and Field Collections of Water and Bed Sediment Samples in the Surface Water Ambient Monitoring Program (SWAMP, 2008). All sample collection was conducted by the UC Davis Granite Canyon Laboratory, Michael L. Johnson LLC, or URS Corporation.

Water toxicity for organophosphate applications was assessed using the 7-day survival and reproduction protocol for *Ceriodaphnia dubia* (U.S. EPA, 2002b). Water toxicity for pyrethrins and pyrethroid applications was assessed with the 96-hour survival protocol for *Hyalella azteca* (U.S. EPA, 2002a). Sediment toxicity for pyrethrin and pyrethroids applications was assessed using the 10-day survival and growth protocol for *H. azteca* (U.S. EPA, 2000). All protocols are compatible with the Surface Water Ambient Monitoring Program (SWAMP) and are summarized in the SWAMP Quality Assurance Program Plan (SWAMP, 2008). All toxicity tests were conducted at the Granite Canyon Laboratory.

Water samples from both monitoring years and sediment samples from the first year were analyzed for a suite of pesticides that included the active ingredient applied during each event. Year 2 sediment samples and T12 water samples from Events 12-15 were not immediately analyzed for chemistry because of budget constraints, but were frozen and archived for later analysis. Archived samples had a one year maximum storage time. Samples were analyzed by either the California Department of Fish and Wildlife's Water Pollution Control Laboratory (WPCL, Year 1) or Caltest Analytical Laboratory (Caltest, Year 2). Organophosphate pesticides in water were analyzed for a suite of organophosphate pesticides using U.S. EPA Methods 8141 or 641. Pyrethrins and pyrethroid pesticides in water and sediment samples were analyzed using a specific WPCL method (SOP 67) or by U.S. EPA Method 625M. Samples analyzed for pyrethrins and pyrethroids were also analyzed for piperonyl butoxide (PBO).

All toxicity and chemistry data were compiled in a format compatible with SWAMP. Concentrations of active ingredients were compared to test organism toxicity thresholds when available. If organism-specific median lethal concentrations (LC50s) were not available, then chemistry results were evaluated using LC50s from closely related organisms. Concentrations of active ingredients were also compared to the U.S. EPA OPP benchmark values, Vector Control Permit receiving water monitoring trigger values, and in the case of malathion, the water quality criterion established by the U.S. EPA (Table 2). OPP benchmark values can be found at www.epa.gov/oppefed1/ecorisk_ders/aquatic_life_benchmark.htm#benchmarks and includes values for acute and chronic fish and invertebrate species, as well as vascular and non-vascular plant species. Statistically-significant toxicity of all test results were determined using the Test of Significant Toxicity (TST), following U.S. EPA procedures (U.S. EPA, 2010).

Table 2. U.S. EPA Office of Pesticide Protection benchmark values and median lethal concentrations for test organisms or alternate organisms.

Chemical	Permit Trigger (ng/L)	OPP Benchmark Acute Invertebrate (ng/L)	OPP Benchmark Chronic Invertebrate (ng/L)	LC50 (ng/L)	Organism	Reference
Dichlorvos	NA	35	5.8	130	*C. dubia*	(Ankley et al., 1991)
Etofenprox	1.9	400	170	800	*D. magna*	Based on U.S. EPA OPP Acute Benchmark
Malathion	100	300	35	2,120	*C. dubia*	(Ankley et al., 1991)
Naled	14	NA	45	360	*D. magna*	(Frear and Boyd, 1967)
PBO	49,000	225,000	30,000	530,000	*H. azteca*	(Ankley and Collyard, 1995)
Permethrin	30	10	1.4	21	*H. azteca*	(Anderson et al., 2006)
Pyrethrin	140	5,800	860	17,000	*D. magna*	(Oikari et al., 1992) for pyrethrin II
Sumithrin	2.5	2,200	470	7,100	*D. magna*	(Paul, 2004)

Application Areas

Six active ingredients were applied during fifteen events that spanned 2011 and 2012 (Table 3). Up to six sites for each of the environmental settings were to be sampled, but full sets of samples were collected in only seven of the combinations (Table 4). Application areas from seven vector control districts were represented, and the geographical areas ranged from Los Angeles County in the south to Tehama County in the north. Google Earth™ images of the application areas are provided in Appendix B. The high priority pesticides temephos, resmethrin, prallethrin, and cyfluthrin were not monitored because they weren't used during the study period. Because decisions to apply adulticides were often made less than 24-hours before the application, it was not always feasible to sample a given application event due to the coordination required by all of the parties involved.

Table 3. Sample event and location information.

Station ID	Station Name	Latitude	Longitude	District	Env. Setting	App. Method	Active Ingredient
Event 1	7/28/2011						
SHK_W	Shin Kee Wetlands	38.10521	-121.41904	San Joaquin	Wetland	Aerial	Naled
WSL_A	White Slough	38.08735	-121.40138	San Joaquin	Agriculture	Aerial	Naled
ELV_A	Elverta Canal	38.71441	-121.52140	Sac-Yolo	Agriculture	Aerial	Sumithrin
NBC_W	Natomas Basin Conservancy	38.72953	-121.50694	Sac-Yolo	Wetland	Aerial	Sumithrin
Event 2	8/9/2011						
ELV_A	Elverta Canal	38.71441	-121.52140	Sac-Yolo	Agriculture	Aerial	Sumithrin
NBC_W	Natomas Basin Conservancy	38.72953	-121.50694	Sac-Yolo	Wetland	Aerial	Sumithrin
YBW_A	Yolo Basin Wildlife Area Ag Drain	38.55234	-121.62917	Sac-Yolo	Agriculture	Aerial	Sumithrin
YBW_W	Yolo Basin Wildlife Area Wetland	38.55113	-121.62769	Sac-Yolo	Wetland	Aerial	Sumithrin
Event 3	8/23/2011						
UHC_U	Union House Creek	38.46350	-121.44724	Sac-Yolo	Urban	Aerial	Pyrethrin
SBC_U	Strawberry Creek	38.44890	-121.38480	Sac-Yolo	Urban	Aerial	Pyrethrin
LGC_U	Laguna Creek at Jack Hill Park	38.41700	-121.35800	Sac-Yolo	Urban	Aerial	Pyrethrin
CDL_U	Camden Lake	38.42396	-121.37510	Sac-Yolo	Urban	Aerial	Pyrethrin
EGC_U	Elk Grove Creek	38.42832	-121.40810	Sac-Yolo	Urban	Aerial	Pyrethrin
LGL_U	Laguna Lake at Ayr Drive	38.41493	-121.43151	Sac-Yolo	Urban	Aerial	Pyrethrin
Event 4	9/29/2011						
ELV_A	Elverta Canal	38.71441	-121.52140	Sac-Yolo	Agriculture	Aerial	Sumithrin
NBC_W	Natomas Basin Conservancy	38.72953	-121.50694	Sac-Yolo	Wetland	Aerial	Sumithrin
YBW_A	Yolo Basin Wildlife Area Ag Drain	38.55234	-121.62917	Sac-Yolo	Agriculture	Aerial	Sumithrin
YBW_W2	Yolo Basin W.A. Wetland #2	38.55077	-121.62625	Sac-Yolo	Wetland	Aerial	Sumithrin
Event 5	5/16/2012						
PIG_W	Pig Lake	38.15284	-121.28674	San Joaquin	Wetland	Truck	Sumithrin
LOD_U	Lodi Lake	38.14852	-121.29692	San Joaquin	Urban	Truck	Sumithrin
COW_A	Cow pasture pond	38.15529	-121.28364	San Joaquin	Agriculture	Truck	Sumithrin
Event 6	5/25/2012						
ETD_A	Empire Tract Drain	38.06016	-121.49755	San Joaquin	Agriculture	Truck	Malathion
EMP_W	Empire Tract Drain	38.06506	-121.48705	San Joaquin	Wetland	Truck	Malathion
Event 7	6/11-12/2012						
LGC_U	Laguna Creek at Jack Hill Park	38.41700	-121.35800	Sac-Yolo	Urban	Aerial	Sumithrin
CDL_U	Camden Lake	38.42396	-121.37510	Sac-Yolo	Urban	Aerial	Sumithrin
ECP_U	Elder Creek @ Cedar Point	38.48194	-121.34495	Sac-Yolo	Urban	Aerial	Sumithrin
UHH_U	Union House @ Halbrite Way	38.47433	-121.39939	Sac-Yolo	Urban	Aerial	Sumithrin
LGC_U	Laguna Creek at Jack Hill Park	38.41700	-121.35800	Sac-Yolo	Urban	Aerial	Naled
CDL_U	Camden Lake	38.42396	-121.37510	Sac-Yolo	Urban	Aerial	Naled
ECP_U	Elder Creek @ Cedar Point	38.48194	-121.34495	Sac-Yolo	Urban	Aerial	Naled
UHH_U	Union House @ Halbrite Way	38.47433	-121.39939	Sac-Yolo	Urban	Aerial	Naled
SBC_U	Strawberry Creek	38.44890	-121.38480	Sac-Yolo	Urban	Aerial	Naled
WAE_W	Wetland along Excelsior Rd	38.48692	-121.29752	Sac-Yolo	Wetland	Aerial	Naled
EGK_U	Elk Grove Creek near Kiawah Ct.	38.41485	-121.39857	Sac-Yolo	Urban	Aerial	Naled
Event 8	6/26/2012-6/30/2012						
NSH_W	North Shore Fish Pond	33.54056	-116.06822	Coachella Valley	Wetland		Pyrethrin
DMB_W	Dos Hombres Fish Pond	33.55369	-116.07136	Coachella Valley	Wetland		Pyrethrin
SUN_W	Sunset Duck Pond	33.54461	-116.07810	Coachella Valley	Wetland		Pyrethrin
Event 9	7/18/2012						
HAR_U	Harbor Lake	33.78237	-118.29312	Greater LA	Urban		Sumithrin
Event 10	7/23/2012-7/27/2012						
DMB_W	Dos Hombres Fish Pond	33.55369	-116.07136	Coachella Valley	Wetland		Permethrin
76A_A	76th Avenue	33.49878	-116.09555	Coachella Valley	Agriculture		Permethrin
Event 11	8/1/2012						
TCV_A	Toomes Creek @ Tehama Vina	39.97964	-122.06913	Tehama County	Agriculture	Truck	Permethrin
MCS_A	Mills Creek at Shasta Blvd	40.04615	-122.09555	Tehama County	Agriculture	Truck	Permethrin

Station ID	Station Name	Latitude	Longitude	District	Env. Setting	App. Method	Active Ingredient
DYC_A	Dye Creek at Shasta Blvd	40.08837	-122.09120	Tehama County	Agriculture	Truck	Permethrin
ACG_A	Antelope Creek at Cone Grove	40.16717	-122.13590	Tehama County	Agriculture	Truck	Permethrin
CGS_A	Cone Grove Slough	40.16983	-122.14729	Tehama County	Agriculture	Truck	Permethrin
Event 12	9/13/2012						
GSU_U	Gilsizer Slough	39.11259	-121.63643	Sutter/Yuba	Urban	Truck	Permethrin
PLU_U	Plumas Lake	39.00609	-121.55292	Sutter/Yuba	Urban	Truck	Permethrin
Event 13	9/20/2012						
GSU_U	Gilsizer Slough	39.11259	-121.63643	Sutter/Yuba	Urban	Truck	Permethrin
PLU_U	Plumas Lake	39.00609	-121.55292	Sutter/Yuba	Urban	Truck	Permethrin
TBU_U	Tierra Buena	39.14610	-121.67179	Sutter/Yuba	Urban	Truck	Permethrin
Event 14	9/26/2012						
HAR_U	Harbor Lake	33.78237	-118.29312	Greater LA	Urban		Etofenprox
Event 15	10/2/2012						
NG1_W	North Grasslands 1	37.05069	-120.78326	Merced County	Wetland	Aerial	Pyrethrin
NG2_W	North Grasslands 2	37.04201	-120.77711	Merced County	Wetland	Aerial	Pyrethrin
NG3_W	North Grasslands 3	37.03594	-120.77993	Merced County	Wetland	Aerial	Pyrethrin

Table 4. Number of sites sampled for each combination of active ingredient and environmental setting.

	Environmental Setting		
Active Ingredient	Agriculture	Urban	Wetland
Etofenprox	0	1	0
Malathion	1	0	1
Naled	1	6	2
Permethrin	6	5	1
Pyrethrin	0	6	6
Sumithrin	6	6	6

Results

Quality Assurance

All toxicity tests met test acceptability criteria and all water quality parameters were within acceptable ranges for the test organisms (U.S. EPA, 2000, 2002a, b).

All analytical chemistry laboratory control standards (LCS), surrogate spikes (SS), matrix spikes, and matrix spike duplicates from samples conducted during the first year of the project were within the acceptable range of 50% to 150%, with the exception of the etofenprox LCS from Event 1, which was outside of the range. This QA result did not affect the accurate measurement of sumithrin, the active ingredient that was applied during this event.

During the second year of the project there were a number of QA samples outside the acceptable ranges. The majority of these results did not affect the accurate measurement of the active ingredient, or other chemicals that might have influenced toxicity, but the results of several QA samples had potential to influence the final interpretation of the data. Most often, one of the surrogate spikes did not fall within the acceptable range. Caltest Laboratories considers batches with one out-of-range surrogate spike to be acceptable for interpretation. There were several instances where both surrogate spikes were out of range. These included three samples from Event 6, one sample from Event 7, and one sample from Event 10. These results were generally greater than the range of acceptable recoveries, and only one sample from Event 6 had detectable active ingredient. Several active ingredient matrix spikes were also out of range. These include sumithrin in Event 5, pyrethrin in Event 8 and permethrin in Event 12. The only active ingredient detected in these events was permethrin. Caltest reported the results as acceptable based on the outcome of the other QA samples.

The method detection limits and reporting limits for the participating chemistry laboratories differed considerably (Table 5). The Caltest reporting limits were designed only for the specific active ingredients listed in the permit, and do not apply to other reporting limits for other chemicals not listed in the permit. Although the Caltest reporting limits were adequate for the purposes of the permit, the limits for some pyrethroids were close to or above the toxicity thresholds for the test organisms. The significance of this is discussed in the context of results for the individual spray events.

Table 5. Method detection limits (MDL) and reporting limits (RL) for the California Department of Fish and Wildlife Water Pollution Control Laboratory (WPCL) and Caltest. Median lethal concentrations (LC50s) for test organisms or alternate organisms are presented for reference.

	WPCL		Caltest				
	MDL (ng/L)	RL (ng/L)	MDL (ng/L)	RL (ng/L)	LC50 (ng/L)	Organism	Reference
Chlorpyrifos	18	40	5	10	54	*C. dubia*	(Bailey et al., 1997)
Diazinon	14	40	7	20	320	*C. dubia*	(Bailey et al., 1997)
Dichlorvos	30	50	5	100	130	*C. dubia*	(Ankley et al., 1991)
Malathion	18	100	5	50	2,120	*C. dubia*	(Ankley et al., 1991)
Naled	2	5	5	50	360	*D. magna*	(Frear and Boyd, 1967)
Bifenthrin	0.5	1	10	50	9.3	*H. azteca*	(Anderson et al., 2006)
Cyfluthrin	2	5	5	20	2.3	*H. azteca*	(Weston and Jackson, 2009)
Cypermethrin	2	5	5	50	2.3	*H. azteca*	(Weston and Jackson, 2009)
Etofenprox	1	2	1.6	10	800	*D. magna*	Based on U.S. EPA OPP Acute Benchmark
Permethrin	2	5	5	20	21	*H. azteca*	(Anderson et al., 2006)
Sumithrin	2	5	2.4	10	7,100	*D. magna*	(Paul, 2004)
PBO	1	2	5	10	530,000	*H. azteca*	(Ankley and Collyard, 1995)
Pyrethrin II	1	2	10	50	17,000	*D. magna*	(Oikari et al., 1992)
Jasmolin II	2	5	5	20			
Cinerin II	4	5	5	20			

Naled

This active ingredient was applied during Events 1 and 7. Event 1 took place west of the City of Stockton (Figure B1). Two sites were sampled for water toxicity and chemistry: one from an agricultural area and one from a wetland. Naled was also applied during Event 7 south of Sacramento in the City of Elk Grove (Figure B2). Six urban sites and one wetland site were sampled for water toxicity and chemistry.

Event 1

No toxicity was observed in Event 1 at either site prior to the application of naled. Significant toxicity to *C. dubia* was observed at the Shin Kee Wetland at T12, but not at White Slough (Table 5). Although no naled was measured in this water sample, dichlorvos, a naled breakdown product, had a concentration of 169 ng/L which exceeded the LC50 of 130 ng/L for *C. dubia* (Ankley et al., 1991). The concentration of dichlorvos also exceeded the OPP chronic benchmark value of 0.0058 µg/L. A significant reduction of *C. dubia* offspring was also observed in the T0 sample from White Slough.

At the time of sampling, the water level of the Shin Kee Wetland was tidally influenced by the waters of White Slough. The banks of the wetland were lined with reeds and the water samples were collected from within these reeds. Water exchange during the application was significantly less than that observed in White Slough, where there was a noticeable current.

Table 6. Toxicity results for tests conducted before and after applications of naled during Event 1. Concentrations of detected organophosphate chemicals are listed. Shading indicates significant toxicity or chemical concentration exceeding the dichlorvos LC50 value for *C. dubia* (130 ng/L) or the OPP chronic benchmark value (5.8 ng/L). The permit trigger value for naled was not exceeded (14 ng/L). SD indicates standard deviation.

Station	Station ID	Setting	Time (h)	*C. dubia* Mean % Survival	*C. dubia* Mean Offspring	SD	Naled (ng/L)	Dichlorvos (ng/L)
Shin Kee Wetland	SHK_W	Wetland	0	100	25	4	ND	ND
			12	0	NA	NA	ND	169
			24	100	24	3	ND	88
White Slough	WSL_A	Agriculture	0	90	20	8	ND	ND
			12	80	23	8	ND	ND
			24	100	24	5	ND	ND

Event 7

No toxicity was observed in the T0 samples from Event 7, but significant toxicity was observed in all of the T12 samples and three of the T24 samples. Of the ten samples that were significantly toxic, two samples had measurable concentrations of naled that were just above the reporting limit, but not at concentrations that would be toxic to *Daphnia magna* (<LC50 value of 0.36 ug/L, (Frear and Boyd, 1967)). These detected concentrations were greater than the Vector Control Permit's receiving water monitoring trigger of 0.014 ug/L and the OPP chronic benchmark for naled (0.045 µg/L). Eight samples contained concentrations of dichlorvos that exceeded the *C. dubia* LC50 (0.13 µg/L (Ankley et al., 1991), Table 6). Toxicity of the application to the receiving water was readily apparent when it was noted that

the pre-application sample from Strawberry Creek contained a number of native daphnids and other organisms, whereas the post-application samples did not contain any live native organisms. These samples also caused complete mortality to *C. dubia*, the laboratory test organisms. The use of toxicity testing with naled applications provided additional information regarding the potential for receiving water impacts, beyond that provided by only measuring the concentration of the parent compound. Additional information was also provided by testing samples collected at 12 hours vs. 24 hours.

Table 7. Toxicity results for tests conducted before and after applications of naled during Event 7. Concentrations of detected organophosphate chemicals are listed. Shading indicates significant toxicity or chemical concentration exceeding the dichlorvos LC50 value for *C. dubia* (130 ng/L) or the OPP chronic benchmark value (5.8 ng/L), or the spray permit trigger value for naled (14 ng/L). Concentrations in bold are below reporting limits, and therefore estimated.

Station	Station ID	Setting	Time (h)	*C. dubia* Mean % Survival	*C. dubia* Mean Offspring	SD	Naled (ng/L)	Dichlorvos (ng/L)
Camden Lake	CDL_U	Urban	0	100	24	9	ND	ND
			12	0	NA	NA	50	300
			24	100	28	6	ND	**30**
Elder Creek	ECP_U	Urban	0	100	19	6	ND	ND
			12	50	7	9	ND	ND
			24	100	19	6	ND	ND
Elk Grove Creek	EGK_U	Urban	0	100	25	7	ND	ND
			12	0	NA	NA	ND	7800
			24	0	NA	NA	ND	1600
Laguna Creek	LGC_U	Urban	0	100	21	9	ND	ND
			12	0	NA	NA	70	200
			24	0	NA	NA	ND	200
Strawberry Creek	SBC_U	Urban	0	100	32	4	ND	ND
			12	0	NA	NA	ND	900
			24	0	NA	NA	ND	600
Union House Creek	UHH_U	Urban	0	100	31	12	ND	ND
			12	0	NA	NA	ND	**80**
			24	100	31	9	ND	ND
Wetland at Excelsior	WAE_W	Wetland	0	100	19	9	ND	ND
			12	0	NA	NA	ND	200
			24	90	20	9	ND	**60**

Sumithrin

Sumithrin was applied during six spray events conducted primarily in the Central Valley. One event occurred in Los Angeles County. All sites were sampled for water and sediment toxicity and water chemistry. Sediment samples collected in the first year were also analyzed for chemistry. Sediment samples collected in Year 2 were archived for later analysis if significant toxicity was observed. Events 1, 2 and 4 occurred north and west of Sacramento near Natomas and the Yolo Bypass, respectively. One agriculture site and one wetland site near Natomas were sampled in the first event (Figure B3). These sites were also sampled in Events 2 and 4, along with an additional agriculture site and an additional wetland site in the Yolo Bypass area (Figure B4). Sumithrin was also applied near the City of Lodi during Event 5 (Figure B5). One site from each environmental setting was sampled. Four urban sites were sampled during Event 7, which took place in the City of Elk Grove (Figure B6), and one urban sample was collected during Event 9 in Los Angeles County (Figure B7).

Events 1, 2 and 4

Sumithrin was applied to the area surrounding Natomas Basin Conservancy and Elverta Channel three times in 2011. Sumithrin was also applied in the Yolo Bypass Wildlife Area twice in 2011. No significant water toxicity to *H. azteca* was observed in any of the samples. A number of pyrethroid pesticides were detected in these water bodies, but none at toxic concentrations (Table 7). Sumithrin was detected in only one sample (YBW_W at T24), but the concentration of 4.3 ng/L was below the laboratory reporting limit of 5 ng/L and, therefore, estimated. This concentration was also well below the toxicity threshold for *D. magna* (Paul, 2004), and below the OPP acute and chronic benchmarks (2,200 ng/L and 470 ng/L, respectively). However, it exceeded the Vector Control Permit's receiving water monitoring trigger of 2.5 ng/L.

It was clear from theT12 and T24 concentrations of the synergist PBO in the water that the spray events increased PBO in the receiving waters. PBO was detected in most T0 samples, and showed an increase in all T12 samples. Most of the T24 samples were lower than T12, but concentrations generally did not return to background levels. Concentrations of PBO were below toxic concentrations for *H. azteca* (Ankley and Collyard, 1995), OPP acute and chronic benchmarks (225,000 ng/L and 30,000 ng/L, respectively), and the Vector Control Permit's receiving water monitoring trigger (49,000 ng/L).

There was no significant toxicity observed in any of the sediment samples collected prior to sumithrin applications, or within four to seven days of the applications (Table 9). A number of pyrethroids were measured in the sediment samples, but none at concentrations likely to contribute to toxicity. Sumithrin was not detected in the sediments from Event 1, but four samples from Event 2 contained sumithrin (two at estimated concentrations), and three samples from Event 4 contained the active ingredient. During Event 2 the post-application samples from Natomas Basin Wetland and Yolo Basin Agriculture Drain contained 2.40 and 2.33 ng/g, respectively, whereas the pre-application samples did not contain sumithrin. Similar results were observed in Event 4 at Elverta Road and Yolo Basin Wetland 2. The opposite was true of the sample from Natomas Basin. The pre-application sample contained 2.40 ng/g sumithrin, but none was detected in the post-application sample. Event 4 was the second application of sumithrin in the Yolo Bypass Wildlife Area, and it is possible that the detected sumithrin was residual pesticide from the previous application. Concentrations of PBO did not track with the applications. There was often a higher concentration of PBO in the sediments prior to the applications (Table 9).

Table 8. Water toxicity results for tests conducted before and after applications of sumithrin. Concentrations of detected pyrethroid pesticides are listed. Shading indicates a chemical concentration exceeding the spray permit trigger value (2.5 ng/L). BIF = bifenthrin, CYH = cyhalothrin, CYP = cypermethrin, DEL/TRA = deltamethrin/tralomethrin, PER = permethrin, SUM = sumithrin, and PBO = piperonyl butoxide. Concentrations in bold are below reporting limits, and therefore estimated.

Station	Station ID	Setting	Time (h)	*H. azteca* Mean % Survival	SD	BIF (ng/L)	CYH (ng/L)	CYP (ng/L)	DEL/ TRA (ng/L)	PER (ng/L)	SUM (ng/L)	PBO (ng/L)
Event 1												
Elverta Canal	ELV_A	Agriculture	0	100	0	ND	2	ND	ND	ND	ND	32.8
			12	100	0	ND	1.8	ND	ND	ND	ND	168
			24	98	4	ND	ND	ND	ND	ND	ND	99.8
Natomas Basin Conservancy	NBC_W	Wetland	0	100	0	ND	1	ND	ND	ND	ND	10.2
			12	100	0	ND	2.3	ND	ND	ND	ND	155
			24	94	5	ND	ND	ND	ND	ND	ND	152
Event 2												
Elverta Canal	ELV_A	Agriculture	0	98	4	ND	ND	ND	ND	ND	ND	3.8
			12	100	0	ND	ND	ND	ND	ND	ND	166
			24	100	0	ND	ND	ND	ND	ND	ND	136
Natomas Basin Conservancy	NBC_W	Wetland	0	100	0	ND	ND	ND	ND	ND	ND	10.4
			12	98	4	ND	ND	ND	ND	ND	ND	129
			24	100	0	ND	ND	ND	ND	ND	ND	133
Yolo Basin W.A. Ag Drain	YBW_A	Agriculture	0	100	0	ND	ND	ND	ND	ND	ND	4.2
			12	98	4	ND	ND	ND	ND	ND	ND	82.2
			24	98	4	ND	ND	ND	ND	ND	ND	78.4
Yolo Basin W.A. Wetland	YBW_W	Wetland	0	100	0	ND	ND	ND	ND	ND	ND	14.8
			12	100	0	ND	ND	ND	ND	ND	ND	422
			24	96	5	ND	ND	ND	ND	ND	**4.3**	286
Event 4												
Elverta Canal	ELV_A	Agriculture	0	90	10	ND	ND	ND	ND	ND	ND	5
			12	96	5	ND	ND	ND	ND	ND	ND	10
			24	90	12	ND	ND	ND	ND	ND	ND	11
Natomas Basin Conservancy	NBC_W	Wetland	0	90	7	ND	ND	ND	ND	ND	ND	3
			12	100	0	3	ND	ND	ND	ND	ND	13
			24	92	4	ND	ND	ND	24	ND	ND	14
Yolo Basin W.A. Ag Drain	YBW_A	Agriculture	0	92	8	ND	ND	5	ND	ND	ND	ND
			12	86	11	3	ND	ND	ND	ND	ND	22
			24	96	5	3	ND	ND	ND	ND	ND	3
Yolo Basin W.A. Wetland 2	YBW_W2	Wetland	0	98	4	ND	ND	ND	ND	ND	ND	ND
			12	96	5	ND	ND	ND	ND	7	ND	4
			24	96	5	ND	ND	ND	ND	10	ND	2

Table 9. Sediment toxicity results for tests conducted before and after applications of sumithrin. Concentrations of detected pyrethroid pesticides are listed. BIF = bifenthrin, CYF = cyfluthrin, CYH = cyhalothrin, CYP = cypermethrin, DEL/TRA = deltamethrin/tralomethrin, FEP = fenpropathrin, PER c= permethrin (cis), PERt = permethrin (trans), SUM = sumithrin, and PBO = piperonyl butoxide. Concentrations in bold are below reporting limits, and therefore estimated.

Station	Station ID	Setting	Time (d)	*H. azteca* Mean % Survival	SD	*H. azteca* Growth (mg/ind)	SD	BIF (ng/g)	CYF (ng/g)	CYH (ng/g)	CYP (ng/g)	DEL/ TRA (ng/g)	FEP (ng/g)	PERc (ng/g)	PERt (ng/g)	SUM (ng/g)	PBO (ng/g)
Event 1																	
Elverta Canal	ELV_A	Agriculture	0	95	8	0.237	0.036	0.215	ND	1.55	ND	ND	ND	ND	ND	ND	3.69
			4-7	94	7	0.203	0.023	**0.145**	ND	2.97	ND	ND	ND	ND	ND	ND	2.06
Natomas Basin Conservancy	NBC_W	Wetland	0	99	4	0.271	0.035	**0.175**	ND	2.03	ND	ND	ND	ND	ND	ND	6.24
			4-7	91	6	0.320	0.028	ND	ND	0.733	ND	ND	ND	ND	ND	ND	7.29
Event 2																	
Elverta Canal	ELV_A	Agriculture	0	94	7	0.331	0.146	**0.097**	ND	ND	ND	ND	ND	ND	ND	ND	2.11
			4-7	99	4	0.332	0.092	0.284	ND	3.29	ND	ND	ND	ND	ND	ND	2.86
Natomas Basin Conservancy	NBC_W	Wetland	0	90	8	0.376	0.050	**0.224**	ND	2.64	ND	ND	ND	ND	ND	ND	10.7
			4-7	93	9	0.270	0.037	ND	ND	0.950	ND	ND	ND	ND	ND	2.40	8.11
Yolo Basin W.A. Ag Drain	YBW_A	Agriculture	0	89	6	0.169	0.036	0.278	ND	7.01	ND	ND	0.930	ND	ND	**0.548**	3.69
			4-7	90	8	0.168	0.037	0.626	ND	5.63	ND	ND	ND	ND	ND	**0.447**	3.49
Yolo Basin W.A. Wetland	YBW_W	Wetland	0	91	6	0.379	0.046	**0.181**	ND	ND	ND	ND	ND	ND	ND	ND	**1.58**
			4-7	84	16	0.382	0.070	ND	ND	ND	ND	ND	ND	ND	ND	ND	4.51
Event 4																	
Elverta Canal	ELV_A	Agriculture	0	90	8	0.218	0.032	0.657	ND	1.88	ND	ND	ND	ND	ND	ND	2.59
			4-7	93	7	0.230	0.074	2.30	ND	12.0	ND	10.4	ND	ND	ND	7.80	8.08
Natomas Basin Conservancy	NBC_W	Wetland	0	90	9	0.185	0.032	0.479	ND	0.187	ND	ND	ND	ND	ND	2.71	4.13
			4-7	95	8	0.279	0.043	1.14	ND	ND	ND	7.31	ND	ND	ND	ND	3.20
Yolo Basin W.A. Ag Drain	YBW_A	Agriculture	0	89	14	0.285	0.055	1.08	**0.608**	4.70	**0.442**	ND	ND	**0.671**	**0.869**	ND	5.38
			4-7	93	10	0.245	0.022	1.93	ND	7.63	ND	ND	ND	1.29	2.62	2.33	3.65
Yolo Basin W.A. Wetland 2	YBW_W2	Wetland	0	89	10	0.227	0.036	0.508	ND	**0.158**	ND	ND	ND	ND	ND	ND	7.87
			4-7	95	8	0.264	0.066	1.88	ND	ND	ND	3.50	ND	ND	3.65	3.86	7.43

Events 5, 7 and 9

Sumithrin was applied to three different areas in Lodi, Elk Grove and Los Angeles. The only significant water toxicity was observed in the pre-application sample from Camden Lake in Elk Grove (Table 10). There was no significant toxicity observed in any post-application samples. Sumithrin was not detected in any of the water samples, but one urban sample contained an estimated concentration of bifenthrin. As with the previous sumithrin applications, PBO concentrations were low in the pre-application sample, highest immediately post application, and then generally moving toward background levels after 24 hours. All PBO concentrations were below toxicity thresholds and benchmark values.

Table 10. Water toxicity results for tests conducted before and after applications of sumithrin in Events 5, 7 and 9. Concentrations of detected pyrethroid pesticides are listed. Shading indicates significant toxicity. BIF = bifenthrin, CYH = cyhalothrin, CYP = cypermethrin, DEL/TRA = deltamethrin/tralomethrin, PER = permethrin, SUM = sumithrin, and PBO = piperonyl butoxide. Concentrations in bold are below reporting limits, and therefore estimated.

Station	Station ID	Setting	Time (h)	*H. azteca* Mean % Survival	SD	Sumithrin (ng/L)	Bifenthrin (ng/L)	Piperonyl Butoxide (ng/L)
Event 5								
Cow Pasture Pond	COW_A	Agriculture	0	98	4	ND	ND	ND
			12	98	4	ND	ND	50
			24	98	4	ND	ND	50
Lodi Lake	LOD_U	Urban	0	96	5	ND	ND	ND
			12	100	0	ND	ND	30
			24	98	4	ND	ND	30
Pig Lake	PIG_W	Wetland	0	98	4	ND	ND	10
			12	94	5	ND	ND	20
			24	96	5	ND	ND	20
Event 7								
Camden Lake	CDL_U	Urban	0	76	23	ND	ND	**6**
			12	94	5	ND	**30**	70
			24	100	0	ND	ND	40
Elder Creek	ECP_U	Urban	0	100	0	ND	ND	40
			12	98	4	ND	ND	100
			24	94	5	ND	ND	200
Laguna Creek	LGC_U	Urban	0	94	5	ND	ND	**9**
			12	98	4	ND	ND	60
			24	94	5	ND	ND	**40**
Union House Creek	UHH_U	Urban	0	100	0	ND	ND	ND
			12	100	0	ND	ND	200
			24	100	0	ND	ND	90
Event 9								
Harbor Lake	HAR_W	Urban	0	98	4	ND	ND	20
			12	98	4	ND	ND	70
			24	98	4	ND	ND	40

Significant sediment toxicity was observed in pre- and post-application samples from Elk Grove (Table 10). Survival results for both samples were lower after the application, but were not significantly different from the pre-application results. Chemical analyses were not conducted on these samples because the pre-event samples showed significant amphipod mortality, so it is impossible to determine the cause of the observed toxicity. These four samples were also significantly toxic for the *H. azteca* growth endpoint, as was the pre-application sample from Los Angeles.

Table 11. Sediment toxicity results for tests conducted before and after applications of sumithrin. Shading indicates significant toxicity. No chemical analyses for sediments were conducted for these events.

Station	Station ID	Setting	Time (d)	*H. azteca* Mean % Survival	SD	*H. azteca* Growth (mg/ind)	SD
Event 5							
Cow Pasture Pond	COW_A	Agriculture	0	93	8	0.244	0.020
			4-7	89	8	0.273	0.030
Lodi Lake	LOD_U	Urban	0	96	7	0.211	0.020
			4-7	88	10	0.279	0.015
Pig Lake	PIG_W	Wetland	0	96	7	0.347	0.024
			4-7	95	5	0.352	0.024
Event 7							
Camden Lake	CDL_U	Urban	0	70	17	0.110	0.024
			4-7	54	25	0.079	0.012
Elder Creek at Cedar Point	ECP_U	Urban	0	88	10	0.268	0.029
			4-7	90	11	0.278	0.020
Laguna Creek at Jack Hill Park	LGC_U	Urban	0	94	7	0.231	0.027
			4-7	98	7	0.231	0.008
Union House Creek	UHH_U	Urban	0	59	21	0.094	0.022
			4-7	48	23	0.120	0.018
Event 9							
Harbor Lake	HAR_W	Urban	0	84	13	0.134	0.013
			4-7	85	19	0.197	0.033

Pyrethrin

Pyrethrin was applied during Events 3, 8 and 15. Event 3 sites were sampled for water and sediment toxicity and chemistry, whereas Events 8 and 15 were sampled for water and sediment toxicity and water chemistry, but not sediment chemistry. Event 3 covered six urban sites within the City of Elk Grove, south of Sacramento (Figure B8), and included two consecutive nights of pyrethrin application. Some of the Event 3 sites overlapped with sumithrin applications that occurred during Event 7. Event 8 occurred in the Coachella Valley area and consisted of three wetland sites (Figure B9). Three wetland sites were also sampled for Event 15, which took place in the central valley near the City of Los Banos (Figure B10).

Events 3, 8 and 15

Significant post-application toxicity was observed during Event 3 in Union House Creek at T12 and T24 (Table 12). Although these water samples contained the highest concentrations of pyrethrins, these chemicals alone were probably not at high enough concentrations to cause the observed toxicity based on the LC50 of 17,000 ng/L for *Daphnia magna* (Oikari et al., 1992). Pyrethrin concentrations also did not exceed the OPP acute or chronic benchmark values. Concentrations of PBO were also well below toxic concentrations for *H. azteca* (Ankley and Collyard, 1995), and well below the OPP acute and chronic benchmarks (225,000 ng/L and 30,000 ng/L, respectively), and the Vector Control Permit's receiving water monitoring trigger (49 ug/L). Pyrethroid pesticides were not measured in the water samples because only the sprayed active ingredient was targeted. Pyrethroids were analyzed along with pyrethrins in the sediment samples collected during this event. Concentrations of pyrethroids, particularly bifenthrin, were highest in sediment samples from Union House Creek (Table 12). Water samples from Union House Creek also had the highest post-application concentrations of PBO. Because pyrethroids were present in this system, it is possible that PBO synergized pyrethroids present in the water column. Concentrations of PBO were highest in the first post-application sample, and had generally not returned to background concentrations within 24 hours. Water toxicity was not observed in any of the samples collected as part of Events 8 and 15.

Significant sediment toxicity was also observed during Event 3, but only in a pre-application sample (Table 13). Although the percent survival in Strawberry Creek was similar to the responses in other samples, this site was determined to be significantly toxic because of the high variability among laboratory replicates. Minimal toxicity was observed despite the elevated pyrethroid concentrations measured in the sediments. Six samples from Event 3 contained organic carbon-corrected concentrations of bifenthrin that were up to five times the LC50 for *H. azteca*, but only one of these samples was significantly toxic. The samples with the highest concentrations of bifenthrin had 79% and 74% survival, respectively. Although the organic carbon content of these sediments was higher than average, the carbon-corrected concentrations were still high enough to cause toxicity. The relationship between organic carbon content and bioavailability of hydrophobic organic compounds is well established. Recent studies have shown that organic carbon quality also affects chemical bioavailability and thus, toxicity. For example intact reeds and leaves are likely less effective at binding hydrophobic chemicals than more humic plant material (Gunnarson et al., 1999; Cornelissen et al., 2000). Variance in the type of TOC present in this sample may explain the lack of toxicity.

Post-application sediment toxicity was observed in one sample during Event 8 (Table 13). The samples were analyzed for active ingredients, but none were detected. No toxicity was observed during Event 15, and sediment chemistry was not measured as part of this event.

Table 12. Water toxicity results for tests conducted before and after applications of pyrethrin. Concentrations of the detected components of pyrethrins are listed (pyrethrins 1 and 2, jasmolin 2 and cinerin 2). Shading indicates significant toxicity. PBO = piperonyl butoxide. Concentrations in bold are below reporting limits, and therefore estimated.

Station	Station ID	Setting	Time (h)	*H. azteca* Mean % Survival	SD	Pyrethrin 1 (ng/L)	Pyrethrin 2 (ng/L)	Jasmolin 2 (ng/L)	Cinerin 2 (ng/L)	PBO (ng/L)
Event 3										
Camden Lake	CDL_U	Urban	0	96	5	ND	ND	ND	ND	ND
			12	96	5	ND	ND	ND	ND	1,160
			24	96	5	ND	ND	ND	ND	660
Elk Grove Creek	EGC_U	Urban	0	100	0	ND	ND	ND	ND	ND
			12	100	0	ND	8	ND	5	11,200
			24	98	4	ND	ND	ND	ND	3,130
Laguna Creek	LGC_U	Urban	0	98	4	ND	ND	ND	ND	ND
			12	98	4	ND	ND	ND	ND	166
			24	92	8	ND	ND	ND	ND	25
Laguna Lake	LGL_U	Urban	0	100	0	ND	ND	ND	ND	ND
			12	98	4	ND	1	ND	ND	1,430
			24	100	0	ND	ND	ND	ND	1,240
Strawberry Creek	SBC_U	Urban	0	98	4	ND	ND	ND	ND	ND
			12	92	18	ND	7	ND	ND	2,060
			24	90	7	ND	4	ND	ND	2,290
Union House Creek	UHC_U	Urban	0	100	0	ND	ND	ND	ND	ND
			12	0	0	3	14	**2**	5	9,200
			24	0	0	ND	**1**	ND	ND	5,200
Event 8										
North Shore Fish Pond	DMB_W	Wetland	0	98	4	ND	ND	ND	ND	40
			12	100	0	ND	ND	ND	ND	100
			24	98	4	ND	ND	ND	ND	70
Dos Hombres Fish Pond	NSH_W	Wetland	0	98	4	ND	ND	ND	ND	ND
			12	100	0	ND	ND	ND	ND	20
			24	98	4	ND	ND	ND	ND	20
Sunset Duck Pond	SUN_W	Wetland	0	98	4	ND	ND	ND	ND	60
			12	96	9	ND	ND	ND	ND	80
			24	98	4	ND	ND	ND	ND	**40**
Event 15										
North Grasslands 1	NG1_W	Wetland	0	92	8	ND	ND	ND	ND	100
			12	96	5					
			24	98	4	ND	ND	ND	ND	200
North Grasslands 2	NG2_W	Wetland	0	98	4	ND	ND	ND	ND	ND
			12	98	4					
			24	98	4	ND	ND	ND	ND	200
North Grasslands 3	NG3_W	Wetland	0	100	0	ND	ND	ND	ND	10
			12	98	4					
			24	96	5	ND	ND	ND	ND	200

Table 13. Sediment toxicity results for tests conducted before and after applications of pyrethrin. Concentrations of detected pyrethroid pesticides are listed. BIF = bifenthrin, CYF = cyfluthrin, CYH = cyhalothrin, CYP = cypermethrin, DEL/TRA = deltamethrin/tralomethrin, ESF/FEN = esfenvalerate/fenvalerate, FEP = fenpropathrin, PER c= permethrin (cis), PERt = permethrin (trans), and PBO = piperonyl butoxide. Concentrations in bold are below reporting limits, and therefore estimated.

Station	Station ID	Setting	Time (d)	*H. azteca* Mean % Survival	SD	*H. azteca* Growth (mg/ind)	SD	BIF (ng/g)	CYF (ng/g)	CYH (ng/g)	CYP (ng/g)	DEL/TRA (ng/g)	ESF/FEN (ng/g)	FEP (ng/g)	PERc (ng/g)	PERt (ng/g)	PBO (ng/g)
Event 3																	
Camden Lake	CDL_U	Urban	0	91	16	0.248	0.037	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
			4-7	96	5	0.215	0.055	31.1	0.855	2.80	1.05	0.629	0.301	ND	3.72	4.14	1.40
Elk Grove Creek	EGC_U	Urban	0	98	5	0.332	0.090	1.14	ND	ND	ND	ND	ND	ND	ND	ND	ND
			4-7	100	0	0.365	0.031	4.02	ND	0.100	ND	ND	ND	ND	ND	ND	4.23
Laguna Creek	LGC_U	Urban	0	99	4	0.317	0.101	3.96	ND	ND	ND	ND	ND	ND	ND	**0.790**	**1.46**
			4-7	96	5	0.290	0.030	17.1	ND	0.334	ND	ND	0.122	ND	0.795	ND	ND
Laguna Lake	LGL_U	Urban	0	78	21	0.262	0.200	13.4	ND	ND	ND	ND	ND	ND	ND	4.64	4.98
			4-7	76	13	0.311	0.069	14.6	ND	0.172	1.53	0.293	0.267	ND	204	68.8	4.08
Strawberry Creek	SBC_U	Urban	0	74	23	0.135	0.043	42.2	ND	ND	ND	ND	ND	4.68	14.4	13.5	ND
			4-7	74	11	0.202	0.043	149	7.94	2.17	2.43	1.53	0.604	ND	21.8	8.02	1.24
Union House Creek	UHC_U	Urban	0	79	12	0.310	0.152	19.6	ND	ND	ND	ND	ND	ND	ND	ND	**1.58**
			4-7	79	10	0.226	0.034	88.0	6.07	9.66	8.27	1.38	0.883	ND	10.9	8.30	24.1
Event 8																	
North Shore Fish Pond	DMB_W	Wetland	0	99	4	0.215	0.030	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
			4-7	95	8	0.220	0.014	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Dos Hombres Fish Pond	NSH_W	Wetland	0	99	4	0.245	0.016	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
			4-7	90	8	0.309	0.046	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Sunset Duck Pond	SUN_W	Wetland	0	99	4	0.217	0.037	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
			4-7	70	11	0.155	0.022	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Event 15																	
North Grasslands 1	NG1_W	Wetland	0	96	7	0.225	0.022	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
			4-7	95	8	0.274	0.024	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
North Grasslands 2	NG2_W	Wetland	0	96	5	0.295	0.021	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
			4-7	94	5	0.270	0.032	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
North Grasslands 3	NG3_W	Wetland	0	95	5	0.382	0.046	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
			4-7	96	5	0.330	0.040	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA

Malathion

This active ingredient was only applied during Event 6 in San Joaquin County northwest of the City of Stockton (Figure B11). Two sites were sampled for water toxicity and chemistry: one from an agricultural area and one from a wetland.

No significant toxicity was observed during this event (Table 14). Malathion was detected in three of the four post-application samples, but all concentrations were well below the toxicity threshold for *C. dubia* (2,120 ng/L, (Ankley et al., 1991)). The detected concentrations were greater than the OPP chronic benchmark of 35 ng/L, and two concentrations were greater than the U.S. EPA ambient water quality chronic criterion of 100 ng/L.

Table 14. Toxicity results for tests conducted before and after applications of malathion. Shading indicates a chemical concentration exceeding the water quality numeric limitation (100 ng/L) or the OPP chronic benchmark (35 ng/L). Concentrations of detected organophosphate chemicals are listed.

Station	Station ID	Setting	Time (h)	*C. dubia* Mean % Survival	*C. dubia* Mean Offspring	SD	Malathion (ng/L)
Empire Tract Drain	EMP_W	Wetland	0	90	21	14	ND
			12	100	32	5	160
			24	100	30	13	90
Empire Tract Drain	ETD_A	Agriculture	0	100	29	6	ND
			12	100	30	14	ND
			24	100	23	14	110

Permethrin

Permethrin was applied during four spray events (10-13) in three areas. All sites were sampled for water and sediment toxicity and water chemistry. Event 10 took place in Coachella Valley at the north end of the Salton Sea and included one agricultural site and one wetland site (Figure B12). Dos Hombres Fish Pond was also monitored during the Event 8. Five agricultural sites were sampled as part of Event 11, which occurred in Tehama County near the City of Red Bluff (Figure B13). The application for Events 12 and 13 took place in Sutter/Yuba Counties around Yuba City (Figure B14). Two urban sites were sampled as part of both events, and an additional urban site was sampled for Event 13.

Events 10-13

No water toxicity was observed during Event 10, and no permethrin was detected in the water samples (Table 15). Dos Hombres Fish Pond also had pyrethrin applied the previous month (June 2012), but did not show any cumulative effects from the additional permethrin application.

One 24-hour post-application sample from Event 11 was significantly toxic (Table 15). Although the 24-hour sample from Dip Dye Creek was toxic, the 12-hour sample had acceptable survival. Permethrin was detected in the post-application samples from both Dip Dye Creek and Toomes Creek. The 12-hour sample from Dip Dye Creek and the 24-hour sample from Toomes Creek contained 30 ng/L of permethrin, and although these concentrations were greater than the *H. azteca* LC50 of 21 ng/L (Anderson et al., 2006), no toxicity was observed in these samples. The other measured concentrations were below the reporting limit, and therefore estimated. All of the detected concentrations from this event were greater than the OPP acute and chronic benchmark values of 10 ng/L and 1.4 ng/L, respectively. However, they did not exceed the Vector Control Permit's receiving water monitoring trigger of 0.03 ug/L.

Events 12 and 13 were conducted one week apart and received the same active ingredient. Twelve-hour toxicity samples were collected, but these samples were not analyzed for chemistry. Statistically significant toxicity was observed in 24-hour sample from Plumas Lake, but because the survival was 90%, the statistical result, which was caused by high variability among the laboratory replicates, is not considered biologically significant (Table 15). Plumas Lake was the only sample from Event 12 that contained detectable concentrations of permethrin, and this concentration was above the LC50 value for *H. azteca*. However, it is at the same concentration as the Vector Control Permit's receiving water monitoring trigger of 30 ng/L. The 24-hour sample from Plumas Lake was also significantly toxic in Event 13, but had much lower survival. This sample contained a measurable concentration of permethrin and a toxic concentration of the pyrethroid bifenthrin (Anderson et al., 2006). Bifenthrin was also detected in water samples from Tierra Buena, but concentrations were below the reporting limit. Both detected concentrations of permethrin were greater than the OPP acute and chronic benchmark values.

Table 15. Water toxicity results for tests conducted before and after applications of permethrin in Events 10-13. Concentrations of detected bifenthrin, permethrin and PBO are listed. Shading indicates significant toxicity or a chemical concentration exceeding the LC50 for *H. azteca* (permethrin – 21 ng/L) or the OPP chronic benchmark (1.4 ng/L). Concentrations in bold are below reporting limits, and therefore estimated.

Station	Station ID	Setting	Time (h)	*H. azteca* Mean % Survival	SD	Bifenthrin (ng/L)	Permethrin (ng/L)	Piperonyl Butoxide (ng/L)
Event 10								
76th Avenue	76A_A	Agriculture	0	100	0	ND	ND	**6**
			12	100	0	ND	ND	ND
			24	100	0	ND	ND	ND
Dos Hombres Fish Pond	DMB_W	Wetland	0	98	4	ND	ND	60
			12	100	0	ND	ND	20
			24	98	4	ND	ND	20
Event 11								
Antelope Creek	ACG_A	Agriculture	0	100	0	ND	ND	ND
			12	100	0	ND	ND	ND
			24	94	9	ND	ND	ND
Cone Grove Slough	CGS_A	Agriculture	0	100	0	ND	ND	ND
			12	100	0	ND	ND	ND
			24	100	0	ND	ND	ND
Dip Dye Creek	DYC_A	Agriculture	0	100	0	ND	ND	ND
			12	94	5	ND	30	20
			24	8	8	ND	**7**	ND
Mill Creek	MCS_A	Agriculture	0	100	0	ND	ND	ND
			12	92	4	ND	ND	ND
			24	98	4	ND	ND	ND
Toomes Creek	TCV_A	Agriculture	0	94	5	ND	ND	ND
			12	98	4	ND	**20**	100
			24	98	4	ND	30	ND
Event 12								
Gilsizer Slough	GSU_U	Urban	0	100	0	ND	ND	ND
			12	98	4			
			24	98	4	ND	ND	ND
Plumas Lake	PLU_U	Urban	0	100	0	ND	ND	ND
			12	98	5			
			24	90	14	ND	30	200
Event 13								
Gilsizer Slough	GSU_U	Urban	0	100	0	ND	ND	ND
			12	98	4			
			24	100	0	ND	ND	**5**
Plumas Lake	PLU_U	Urban	0	100	0	ND	ND	ND
			12	100	0			
			24	2	4	50	20	100
Tierra Buena	TBU_U	Urban	0	100	0	**20**	ND	ND
			12	100	0			
			24	100	0	**20**	ND	ND

Table 16. Sediment toxicity results for tests conducted before and after applications of permethrin. Shading indicates significant toxicity, and * indicates significantly greater post-application toxicity.

Station	Station ID	Setting	Time (d)	*H. azteca* Mean % Survival	SD	*H. azteca* Growth (mg/ind)	SD
Event 10							
76th Avenue	76A_A	Agriculture	0	78	16	0.245	0.050
			4-7	99	4	0.315	0.052
Dos Hombres Fish Pond	DMB_W	Wetland	0	96	5	0.203	0.024
			4-7	98	7	0.228	0.072
Event 11							
Antelope Creek	ACG_A	Agriculture	0	91	14	0.218	0.032
			4-7	98	5	0.216	0.034
Cone Grove Slough	CGS_A	Agriculture	0	58	32	0.137	0.055
			4-7	84	18	0.180	0.041
Dip Dye Creek	DYC_A	Agriculture	0	91	8	0.223	0.020
			4-7	93	7	0.270	0.026
Mill Creek	MCS_A	Agriculture	0	73	36	0.160	0.036
			4-7	93	5	0.189	0.028
Toomes Creek	TCV_A	Agriculture	0	95	5	0.204	0.023
			4-7	94	9	0.225	0.037
Event 12							
Gilsizer Slough	GSU_U	Urban	0	90	8	0.134	0.020
			4-7	80	13	0.107	0.013
Plumas Lake	PLU_U	Urban	0	66	17	0.083	0.007
			4-7	89	11	0.135	0.020
Event 13							
Gilsizer Slough	GSU_U	Urban	0	80	13	0.107	0.013
			4-7	93	9	0.132	0.037
Plumas Lake	PLU_U	Urban	0	89	11	0.135	0.020
			4-7	66	13	0.088	0.017
Tierra Buena	TBU_U	Urban	0	79	10	0.072	0.009
			4-7	66*	16	0.086	0.022

Significant sediment toxicity was observed in a number of pre- and post-application samples associated with permethrin spray events (Table 16). Two samples from Event 11 had significant pre-application toxicity and no observed toxicity in the post-application sample. Events 12 and 13 occurred one week apart at the same stations. The Day 4-7 samples for Gilsizer Slough and Plumas Lake in Event 12 were the T0 samples for Event 13, meaning these two sites were sampled three times in as many weeks. Although the second sample from Gilsizer Slough was mildly toxic (80% survival), and had a significantly different response from the first sample, sediment chemistry was not analyzed because the third sample in the series was not significantly toxic. The reverse occurred at Plumas Lake. The first sample was significantly toxic (66% survival), but the second sample was not, and the third sample had the same organism response as the first. Chemical analysis was not performed on these samples because there was not significantly greater post application toxicity. The third site (Tierra Buena) was only tested twice. Both the pre- and post-application samples were significantly toxic, but the second sample was significantly more toxic than the first. Sediment pyrethroids for these samples were analyzed. Both samples contained similar concentrations of bifenthrin, cyfluthrin and lambda cyhalothrin that were high enough to cause the observed toxicity. Concentrations of permethrin in both samples were less than one-third of the organism LC50. The concentration of permethrin in the post-application sample was less than that of the pre-application sample.

Etofenprox

Etofenprox was applied in Los Angeles County during Event 14 (Figure B7). The Harbor Lake urban site was sampled for water toxicity and chemistry, and sediment toxicity. Harbor Lake had also received an application of sumithrin approximately nine weeks prior to the application of etofenprox as part of Event 9. No acute water or sediment toxicity was observed before or after the sumithrin application, but significant toxicity was observed in the 24-hour water sample after the etofenprox application (Table 17). Etofenprox was detected in this sample, but it was below the reporting limit, and therefore estimated. Based on the minimal available literature, etofenprox can be toxic to invertebrates at concentrations of approximately 800 ng/L (based on U.S. EPA OPP Benchmark). The estimated concentration was well below reported LC50 values for invertebrates, and was also below the OPP acute and chronic benchmark concentrations of 400 ng/L and 170 ng/L, respectively, but was not below the permit trigger value of 1.9 ng/L. Significant sediment toxicity was observed in the pre-application samples, but no toxicity was observed in the post-application sample (Table 18). Sediment chemistry was not measured as part of this event because no toxicity was observed after the application.

Table 17. Water toxicity results for tests conducted before and after applications of etofenprox in Event 14. Shading indicates significant toxicity. Concentrations in bold are below reporting limits, and therefore estimated.

Station	Station ID	Setting	Time (h)	*H. azteca* Mean % Survival	SD	Etofenprox (ng/L)
Harbor Lake	HAR_U	Urban	0	100	0	ND
			12	98	4	NA
			24	82	15	**20**

Table 18. Sediment toxicity results for tests conducted before and after applications of etofenprox. Shading indicates significant toxicity.

Station	Station ID	Setting	Time (d)	*H. azteca* Mean % Survival	SD	*H. azteca* Growth (mg/ind)	SD
Harbor Lake	HAR_U	Urban	0	78	10	0.204	0.034
			4-7	91	8	0.163	0.023

Toxicity Summary

Table 19 summarizes the detections of active ingredients in water and the amount of significant post-application water toxicity during monitoring of all of the 2011 – 2012 adult mosquito spray events. Every monitoring event consisted of three samples: T0 (pre-application background), T12 (12 hours post-application), and T24 (24 hours post-application). In some cases the active ingredient was present in the 24-hour sample, but not in the 12-hour sample. In the case of the naled applications, more dichlorvos was detected at 12 hours than at 24 hours, but in the case of the pyrethroids, there were more detections at 24 hours. Some of the detections of sumithrin, permethrin, pyrethrin and etofenprox were below the reporting limit for the laboratory and therefore, estimated concentrations were reported. There was more toxicity observed at 12 hours for naled/dichlorvos, but in toxicity was only observed in the 24-hour samples for etofenprox and permethrin. It appears that the organophosphates, which are more water soluble than pyrethroids, were more readily detected in the 12-hour samples, whereas the less soluble pyrethroids were more readily detected at 24 hours. The solubility of the active ingredients might be affecting their rates of transport.

Table 19. Detection of active ingredients and occurrence toxicity in water samples.

Active Ingredient	Number of Sites Tested	Number of Samples with Active Ingredient Detection			Number of Samples with Significant Increase in Toxicity	
		Pre-App	Post-12	Post-24	Pre- to Post-12	Pre- to Post-24
Etofenprox	1	0	0	1	0	1
Malathion	2	0	1	2	0	0
Naled	9	0	2	0	8	3
Permethrin	12	0	2	4	0	2
Pyrethrin	12	0	4	2	1	1
Sumithrin	18	0	0	1	0	0

Considerable receiving water toxicity occurred after applications of naled at nine sites (Table 19). While none of the pre-application samples were toxic, 61% of the post-application samples were toxic to *C. dubia*. Naled was detected in only 7% of the samples, but the breakdown product dichlorvos was detected in 72% of post-application samples. Dichlorvos was detected at greater than toxic concentrations in 50% of the post-application samples, and all of these samples were significantly toxic. Sediment toxicity and analysis for naled/dichlorvos was not conducted for the naled application events.

Active ingredient detections in sediment and sediment toxicity are summarized in Table 20. Sediment chemical analyses were only performed during the first year of the project, so there are no measurements for etofenprox or permethrin. There were only four instances where there was significantly increased toxicity between the pre- and post-application samples.

Table 20. Detection of active ingredients and occurrence of toxicity in sediment samples.

Active Ingredient	Number of Sites Tested	Number of Samples with Active Ingredient Detection		Number of Samples with Significant Increase in Toxicity
		Pre-App	Post-App	Pre- to Post
Etofenprox	1	NA	NA	0
Permethrin	12	NA	NA	3
Pyrethrin	12	0	0	1
Sumithrin	18	2	5	0

Water and sediment samples were collected from twelve sites before and after applications of pyrethrin. None of the pre-application samples were significantly toxic, but complete mortality was observed in two post-application samples from Event 3. Pyrethrins were detected at four of the six sites from the third event, but not at concentrations that would significantly contribute to toxicity. Pyrethrins were not detected in any other samples. It is hypothesized that the PBO added as part of the pyrethrin applications synergized concentrations of pyrethroids that were already present in the water bodies, as evidenced by the presence of pyrethroids in the sediments. This could not be confirmed because water samples were not analyzed for pyrethroids. Two sediment samples were significantly toxic, one pre-application and one post-application. Although a number of pyrethroids were detected in sediments from Event 3, pyrethrins were not detected. Pyrethrins were also not detected in sample from Event 8.

Water and sediment samples from twelve sites were tested before and after applications of permethrin. None of the pre-application water samples were toxic, but three of the 24-hour post-application samples were significantly toxic. Permethrin was detected in 32% of the post-application water samples. Three samples contained permethrin at a concentration that was higher than the test organism LC50, but none of these samples were significantly toxic. Bifenthrin was detected in one of the other two toxic water samples at a concentration high enough to cause the observed toxicity.

Of the 24 sediment samples that were collected during permethrin applications, seven were significantly toxic for the survival endpoint (four pre-application samples and three post-application samples). Six samples at two sites alternated between toxic and not toxic during two application events. At another site, both pre- and post-application samples were toxic, and the post-application sample had a significantly lower organism response than the pre-application sample. The observed toxicity in these samples was probably caused by elevated concentrations of bifenthrin, cyfluthrin and lambda cyhalothrin. The concentrations of permethrin in the pre- and post-application samples did not differ. Significant amphipod growth toxicity was observed in all of the samples from Events 12 and 13.

Eighteen sites sprayed during six events were monitored for toxicity as part of applications of sumithrin. Only one pre-application water sample was significantly toxic. Sumithrin was detected in only one post-application water sample, and this was at a concentration below the reporting limit. Sediment toxicity in both pre- and post-application samples was observed at sites from Event 7. Although the percent survival was lower, neither post-application sample had significantly greater toxicity than the pre-application sample. Sediment concentrations of sumithrin were analyzed as part of the first three application events. The active ingredient was detected in 35% of the samples. The sites monitored during these events were exposed to multiple applications of sumithrin over two months, and it is possible that some of these detections were a product of build up through more than one application.

Malathion was only applied during one event, and two sites were monitored. No water toxicity was observed in any of the samples. Pre-application samples did not contain any malathion, but three of the four post-application samples contained detectable concentrations. These concentrations were well below the published toxicity threshold for the test organism, but two exceeded the ambient water quality chronic criterion of 100 ng/L. Sediment toxicity and analysis for malathion was not conducted for this event.

Etofenprox was also applied during a single event, and only one site was monitored. No toxicity was observed in the pre-application sample, but significant toxicity was observed in the 24-hour water sample. This sample also contained a detectable concentration of the active ingredient, but this was below the reporting limit and OPP acute and chronic benchmark values, and thus likely did not contribute to the observed water toxicity. Acute sediment toxicity was observed in the pre-application sample, but there was no toxicity in the post-application sample. There was no toxicity to the growth endpoint in either sample. Sediment chemistry was not measured as part of this event.

Summary of Active Ingredient Detections

Concentrations of detected active ingredients were evaluated against several different water quality thresholds. Concentrations listed in the spray pesticide NPDES permit included the malathion receiving water limitation as well as receiving water monitoring triggers. Active ingredient detections were also compared to U.S. EPA OPP benchmarks and test organism LC50 values. If an LC50 was not available for the test organism, then an LC50 from a closely-related organism was used. Malathion concentrations were also compared to the U.S. EPA's Water Quality Chronic Criterion.

Active ingredients were detected to varying degrees in post-application samples (Table 21). Pyrethrin concentrations did not exceed any of the evaluation thresholds. There were single detections of etofenprox and sumithrin that exceeded the spray permit trigger values.

There is no acute benchmark for naled, but in both circumstances where this compound was detected, the concentrations exceeded the receiving water trigger value and the OPP chronic benchmark, but not the LC50. The naled breakdown product dichlorvos was detected in 13 of 18 post-application samples. All of these detections for dichlorvos were greater than the OPP chronic benchmark and nine of these detections were greater than the *C. dubia* LC50. There is no receiving water trigger value for dichlorvos listed in the permit, but a value could be calculated using the *C. dubia* LC50 and an appropriate safety factor. As an example, if the LC50 was divided by 10, the resulting value of 13 ng/L would have been exceeded in every sample in which dichlorvos was detected. Dichlorvos, a breakdown product of naled, may be causing toxicity associated with the naled spray applications because toxicity testing results showed a high magnitude of toxicity in the absence of any other obvious cause. This is an example where the implementation of receiving water toxicity testing provided additional useful information to the spray event monitoring beyond that provided through analysis of the spray pesticide active ingredient.

Two of the three detected concentrations of malathion exceeded the U.S. EPA water quality criterion of 100 ng/L. All three concentrations exceeded the OPP chronic benchmark, but not the corresponding acute benchmark or the LC50.

Five of six post-application permethrin detections exceeded the OPP acute benchmark and all detections exceeded the chronic benchmark. Three detections also exceeded the LC50 for *H. azteca*, but only one of these samples exhibited significant toxicity. Two detections of permethrin that did not exceed the LC50 exhibited significant toxicity, but in one of these samples, bifenthrin was also detected at a level exceeding the LC50 for *H. Azteca*.

Table 21. A summary of post-application detections of active ingredients and numbers of samples exceeding numeric limitations, trigger values, OPP benchmarks and LC50s.

Active Ingredient	Number of Post-Application Detections	Numeric Limitation or Trigger Value	Number Exceeding Value	OPP Acute Benchmark	Number Exceeding Benchmark	OPP Chronic Benchmark	Number Exceeding Benchmark	LC50 (ng/L)	Number Exceeding LC50
Etofenprox	1	0.0019	1	0.4	0	0.17	0	890	0
Malathion	3	0.1	2	0.3	0	0.035	3	2,120	0
Naled	2	0.014	2	NA	NA	0.045	2	360	0
Permethrin	6	0.03	0	0.01	5	0.0014	6	21	3
Pyrethrin	6	0.14	0	5.8	0	0.86	0	17,000	0
Sumithrin	1	0.0025	1	2.2	0	0.47	0	7,100	0
PBO (in PYR)	25	0.014	24	225	0	30	0	530	0

Multiple Applications of Spray Pesticides within an Event

Two events included multiple nightly applications of adulticides. Event 3 consisted of two consecutive nights of pyrethrin applications. Pyrethrin was applied during three events, but significant water toxicity only occurred during Event 3. Similarly, pyrethrins were detected during Event 3, but not in water samples from the other events. WPCL conducted the chemical analysis for Event 3 and Caltest conducted subsequent analysis. Eighty percent of the concentrations detected during Event 3 were below the Caltest reporting limits.

Events 8 and 10 consisted of five consecutive nights of pyrethrin and permethrin applications, respectively. Monitoring of these events did not result in any detection of water toxicity or active ingredients. Detected concentrations of PBO during these events were generally less than concentrations detected in other pyrethrin and permethrin application events.

Multiple Applications of Spray Pesticides over Several Events

There were repeated applications of sumithrin to blocks over Elverta Canal and Natomas Basin Conservancy during Events 1, 2 and 4. The first two events occurred approximately two weeks apart, and Event 4 occurred approximately seven weeks after Event 2. No significant water or sediment toxicity was observed during these events. Sumithrin was detected in one water sample from Event 2, and the active ingredient was detected in one sediment sample from Event 2 and two samples from Event 4. The concentrations detected during Event 4 were higher than the concentration detected during Event 2. None of the detected concentrations in water or sediment were high enough to cause toxicity.

The Yolo Basin Wildlife Area sites also underwent sumithrin applications during Events 2 and 4. Similar results were observed. No water or sediment toxicity was detected, and no sumithrin was detected in any of the water samples. Two sediment samples each from Events 2 and 4 contained sumithrin. The concentrations measured during Event 4 were approximately 5-7 times higher than those measured during Event 2.

Two sites in the City of Elk Grove underwent three applications of different chemicals. During 2011 blocks over Camden Lake and Laguna Creek underwent an aerial application of pyrethrin. These sites were part of a two night application of sumithrin and naled in 2012. No water toxicity was observed in these samples until the application of naled. Toxic concentrations of the naled breakdown product dichlorvos were measured in the toxic samples. Neither of the sediment samples from these sites had significantly greater post-application toxicity.

Dos Hombres Fish Pond in the Coachella Valley underwent applications of pyrethrin and permethrin approximately one month apart in the summer of 2012. No toxicity or active ingredients were observed in the water samples, and there was no significant toxicity in the sediment samples.

Harbor Lake in Los Angeles County underwent applications of sumithrin and etofenprox approximately ten weeks apart. No toxicity or active ingredient was measured after the sumithrin application, but the 24-hour post-application water sample was toxic after the etofenprox application. Etofenprox was detected in this sample, but at a concentration that was less than what would be considered toxic. Neither post-application sediment sample had significantly greater toxicity than the pre-application sample.

Two sites from Yuba City (Gilsizer Slough and Plumas Lake) underwent permethrin applications one week apart in 2012. Significant post-application toxicity was observed in both 24-hour water samples from Plumas Lake. One sample contained permethrin at a concentration greater than the LC50, and the other sample contained a concentration of permethrin at slightly less than the LC50. One post-application sediment sample from Gilsizer Slough and one sample from Plumas Lake caused significantly greater toxicity than the pre-application samples.

Conclusions

Sixteen (approximately 16%) post-application water samples were significantly toxic. Of the 16 toxic post-application samples, the toxicity of nine samples can be attributed to the naled breakdown product dichlorvos. One of two toxic samples that were observed after permethrin applications could be explained by the presence of bifenthrin, which was not applied as part of vector spray pesticide activities. Two toxic samples observed after the application of pyrethrin could have been caused by PBO synergizing ambient concentrations of pyrethroids in the urban setting, but the concentrations of pyrethrins were well below toxic levels. Four water samples had toxicity that could not be attributed to the measured chemicals.

Four of the 43 post-application sediment samples were significantly more toxic than their corresponding pre-application sample. Two of these samples were collected as part of repeated applications, and demonstrated a return to background toxicity. The measured active ingredients in the other samples were not significantly higher in the post-application samples, nor were they high enough to cause the observed toxicity. One sample contained high enough concentrations of other pyrethroids to cause the observed toxicity.

This study was designed to determine if toxicity testing provided additional important information beyond the analysis of the active ingredient regarding the potential for impacts in the receiving water system. In the case of naled, analysis of the active ingredient would have underestimated potential impacts to the receiving system. Toxicity testing provided information that led to the inclusion of dichlorvos to the analyte list. Because of the strong relationship between the concentration of dichlorvos and toxicity, it is likely that including monitoring for dichlorvos under the permit would provide similar information as toxicity testing.

Toxicity was also linked to the active ingredient in a post-application sample that contained permethrin. Although the permethrin concentration in this sample was greater than the toxicity threshold of the organism, the sample was only mildly toxic. Two other samples contained the same concentration of permethrin, but were not toxic. Because the laboratory reporting limit for permethrin in these samples was approximately the same concentration as the toxicity threshold, it is possible that small influences in bioavailability could have affected the toxicity of each sample.

Two toxic samples were observed after the application of pyrethrin. The elevated concentrations of PBO measured in these samples suggested the possibility of a synergistic effect with pyrethroids already present in these urban receiving waters. Pyrethroids were not measured in the water samples, but if this situation were to occur, then toxicity testing or additional chemical analysis would be necessary to demonstrate the ancillary effects.

Sediment toxicity results did not provide significant additional information during the first year of the study, but sediment chemical analysis demonstrated that sumithrin concentrations increased over multiple applications at individual sites, although concentrations were still below toxic levels. During the second year of the study, four sediments had significantly higher post-application toxicity, but the toxicity of these samples was not related to the spray application.

Additional Research

Based on the results of the research to date, naled is the primary active ingredient that causes receiving water impacts. Post-application toxicity was observed in many of the naled samples, and in one instance it was clear that resident organisms in a post application sample had been affected. Because naled is one of the few organophosphate pesticides available for vector control, it would be beneficial to apply some best management practices to naled applications. These practices might include reducing the dose based on label rate or adjusting droplet size to reduce loading of naled and the subsequent occurrence of dichlorvos in receiving waters. The effectiveness of these practices should be evaluated through chemical analysis and possibly toxicity testing.

Another potential issue involves the application of products containing PBO. If applied PBO is synergizing resident pyrethroids, then unforeseen impacts could be occurring. This could be accounted for through toxicity testing and measurements of pyrethroid pesticides in the receiving systems.

Several active ingredients were only monitored a few times, or not at all. Additional monitoring should be conducted on malathion, temephos, etofenprox, prallethrin and resmethrin.

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Appendix A

Table A1. Complete list of high, moderate and low priority active ingredients for the pesticide spray permit toxicity study.

Active Ingredients	Vector Control	Spray Application	Weed Control	Chemical Type
High Priority				
Malathion	√	√		Organophosphate Insecticide
Naled	√	√		Organophosphate Insecticide
Temephos	√			Organophosphate Insecticide
Pyrethrins	√	√		Pyrethrin Insecticide
Cyfluthrin		√		Pyrethroid Insecticide
Ethofenprox	√			Pyrethroid Insecticide
Permethrin	√			Pyrethroid Insecticide
Prallethrin	√			Pyrethroid Insecticide
Resmethrin	√			Pyrethroid Insecticide
Sumithrin	√			Pyrethroid Insecticide
Piperonyl Butoxide (PBO)	√	√		Pyrethrin and Pyrethroid Synergist
Carbaryl		√		Carbamate Insecticide
Imidacloprid		√		Neonicitinoid Insecticide
Moderate Priority				
Diquat			√	Herbicide
Methoprene	√			Insecticide
N-octyl bicycloheptene dicarboximide (or MGK-264)	√			Insecticide
Triclopyr Butoxyethyl Ester (BEE)		√		Herbicide
Lower Priority				
Acetamiprid		√		Neonicitinoid Insecticide
Acid Blue			√	Herbicide
Acid Yellow			√	Herbicide
Aminopyralid		√		Herbicide
Chlorsulfuron		√		Herbicide
Clomazone			√	Herbicide
Clopyralid		√		Herbicide
Dinotefuran		√		Neonicitinoid Insecticide
Endothall			√	Herbicide
Imazapyr		√	√	Herbicide
Penoxsulam			√	Herbicide
Petroleum Distillates	√			Insecticide
Pheromone		√		Insecticide
Sodium Carbonate Peroxyhydrate			√	Herbicide
Spinosad A and D	√	√		Insecticide
Triclopyr Triethylamine Salt (TEA)		√	√	Herbicide

Appendix B – Application Areas listed by event number

Figure B1. Event 1 – Naled

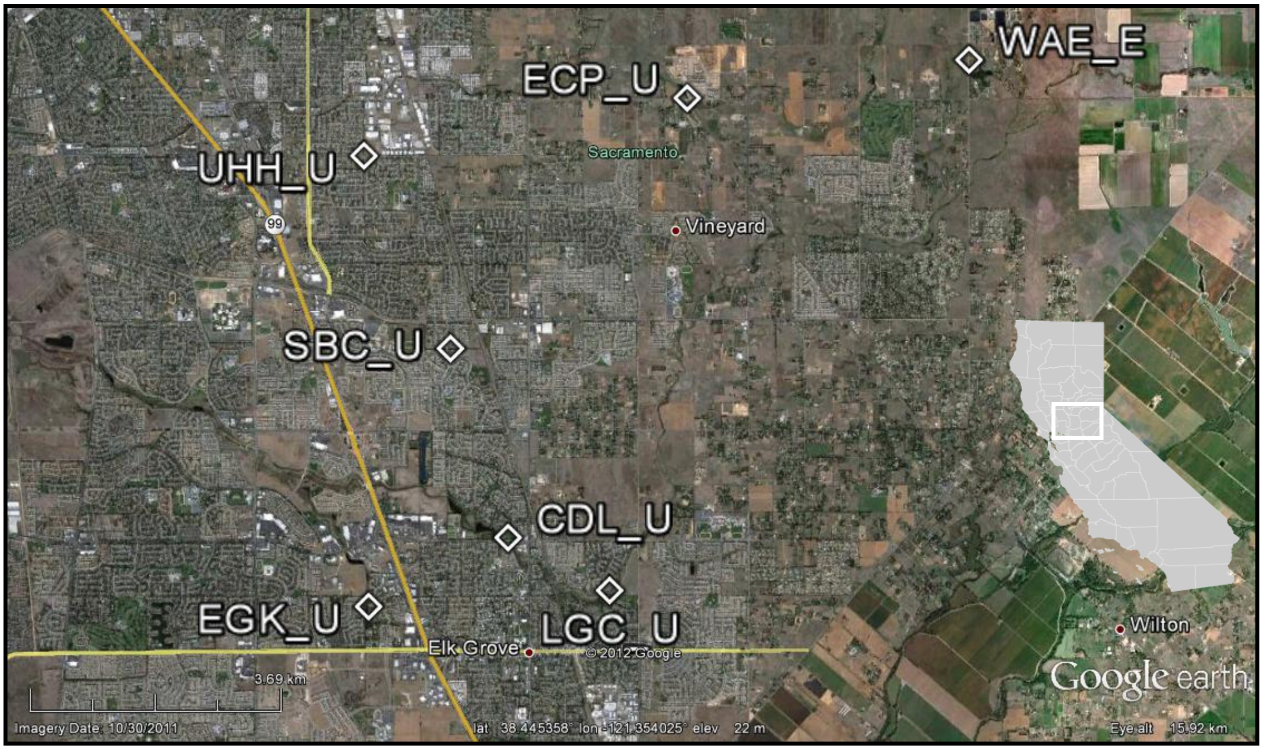

Figure B2. Event 7 – Naled

Figure B3. Events 1, 2 and 4 - Sumithrin

Figure B4. Events 2 and 4 – Sumithrin

Figure B5. Event 5 – Sumithrin

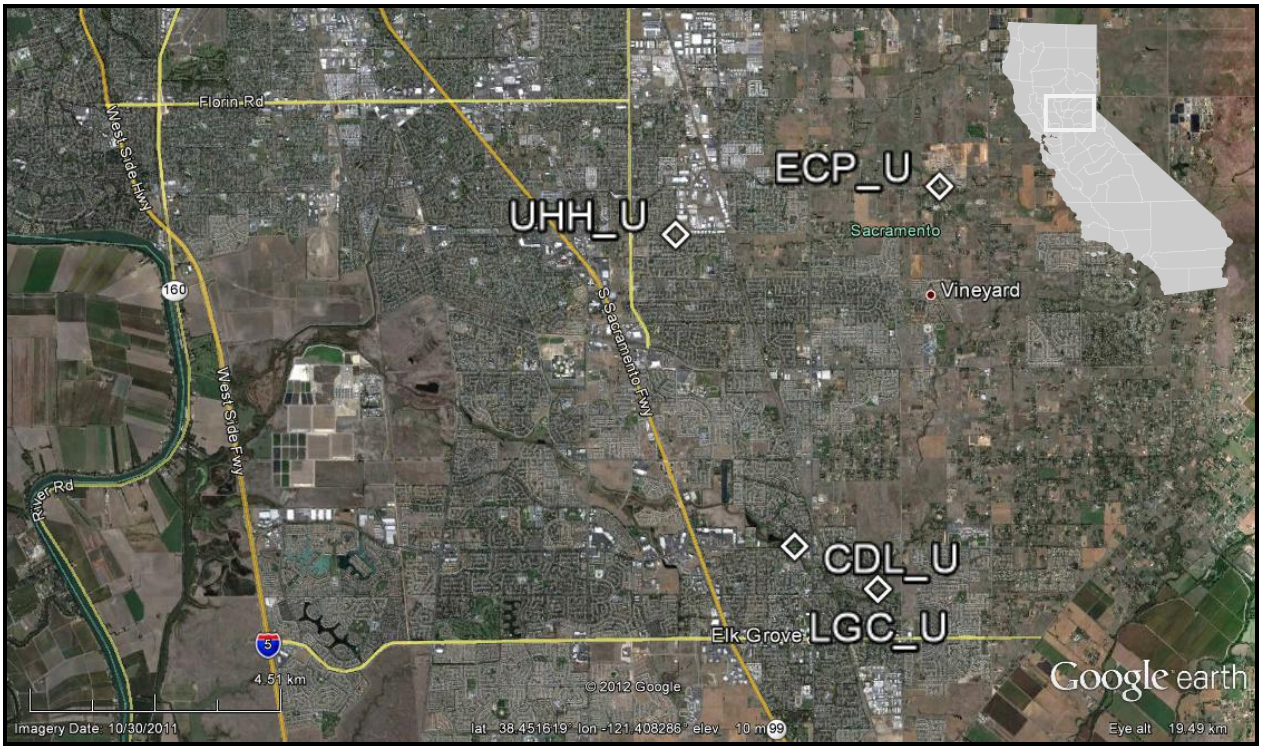

Figure B6. Event 7 – Sumithrin

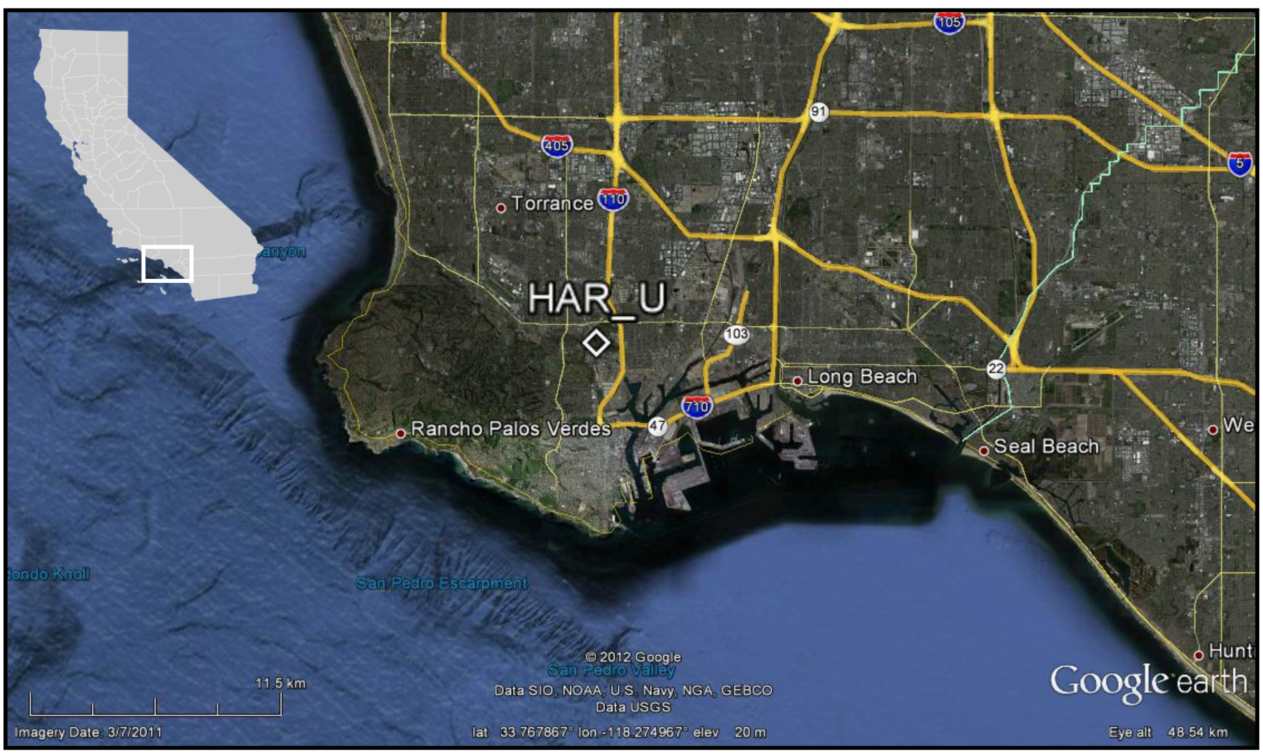

Figure B7. Event 9 – Sumithrin and Event 14 – Etofenprox

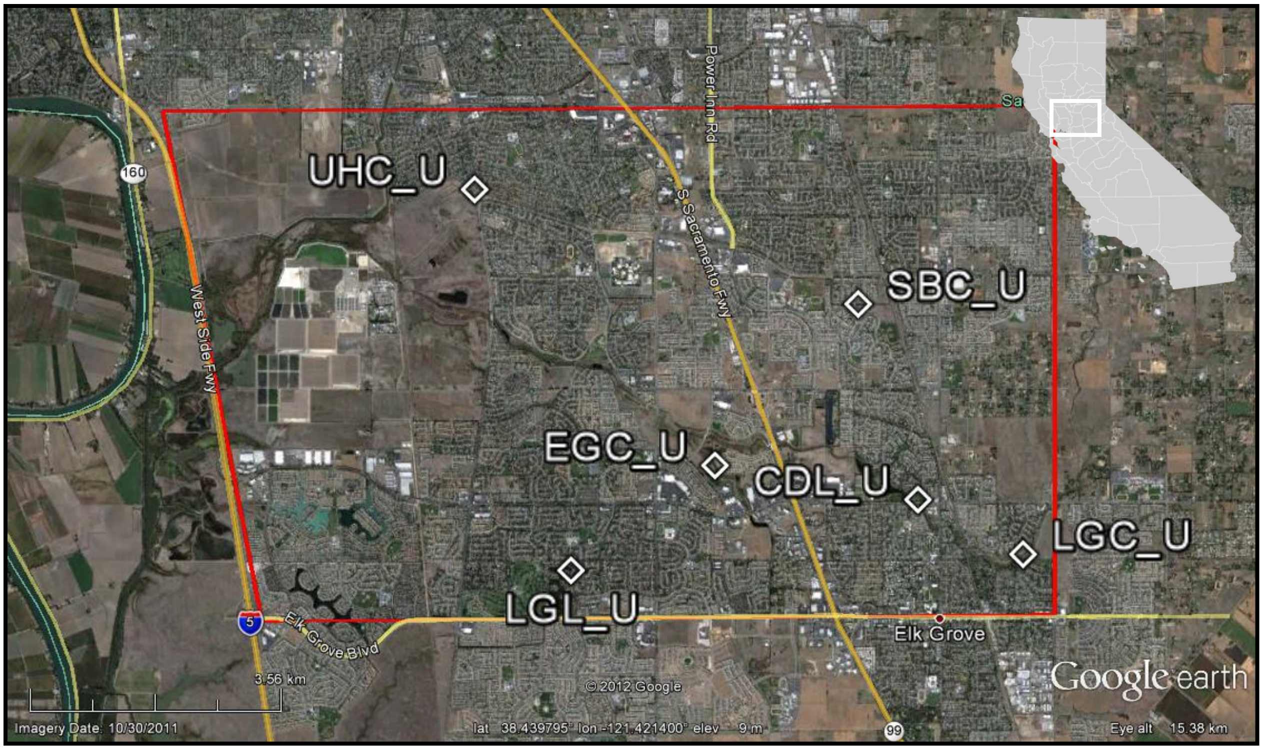

Figure B8. Event 3 – Pyrethrin

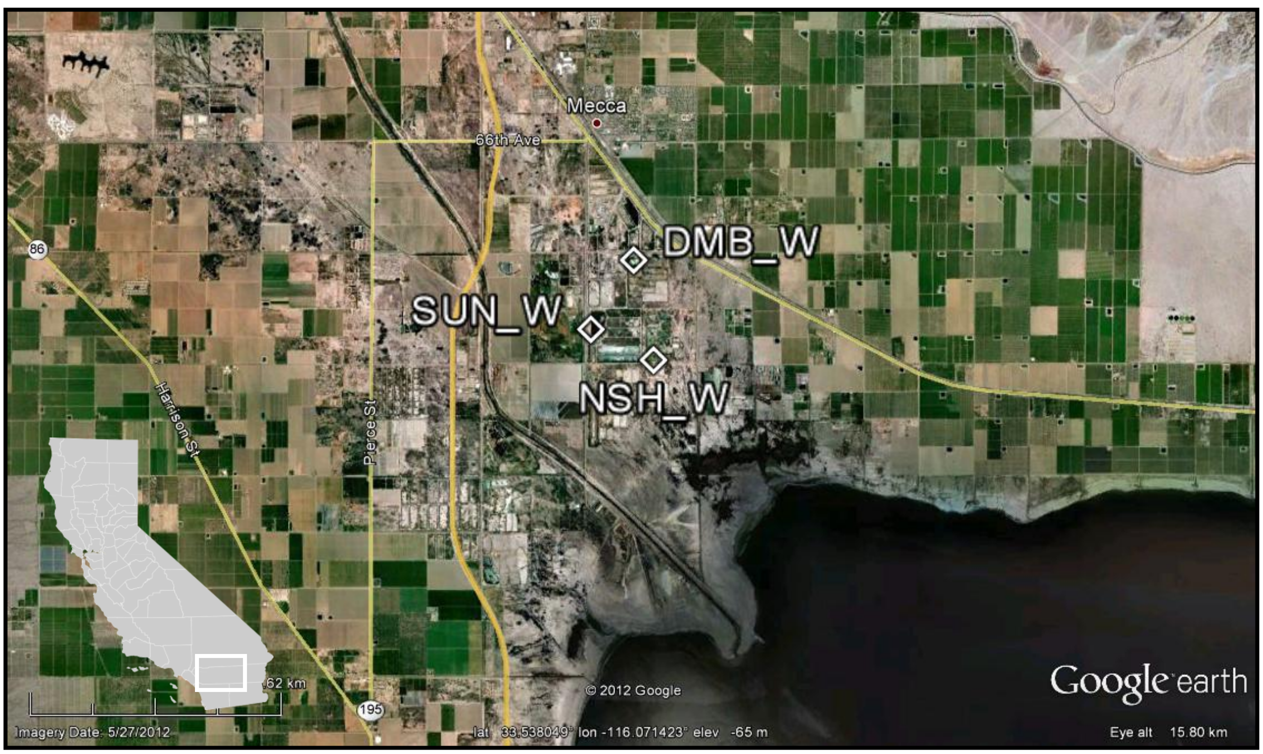

Figure B9. Event 8 – Pyrethrin

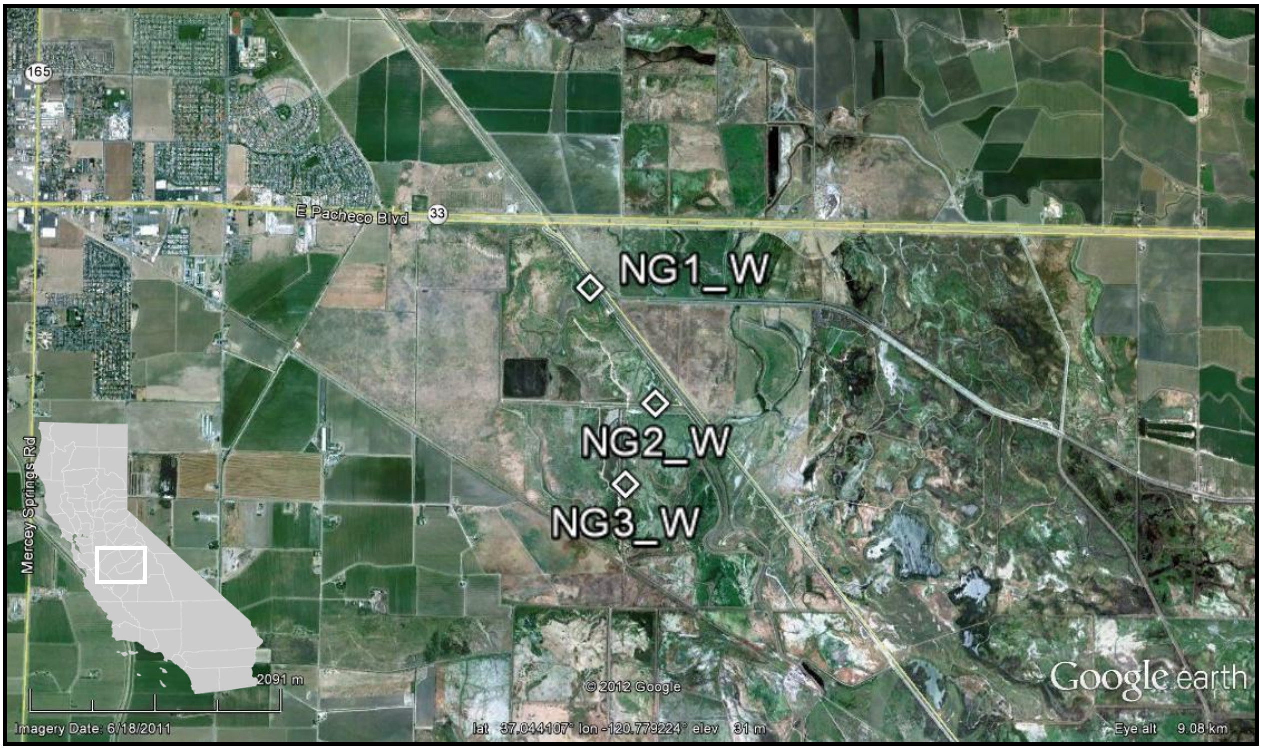

Figure B10. Event 15 – Pyrethrin

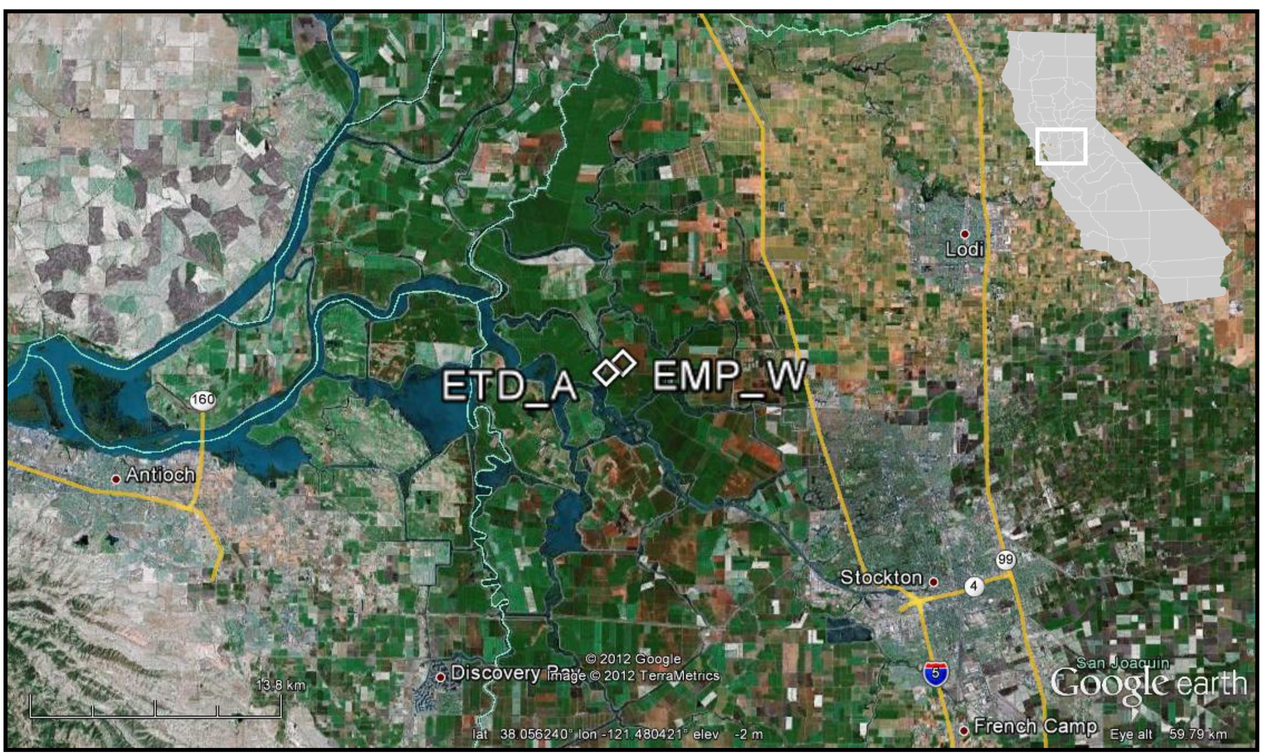

Figure B11. Event 6 - Malathion

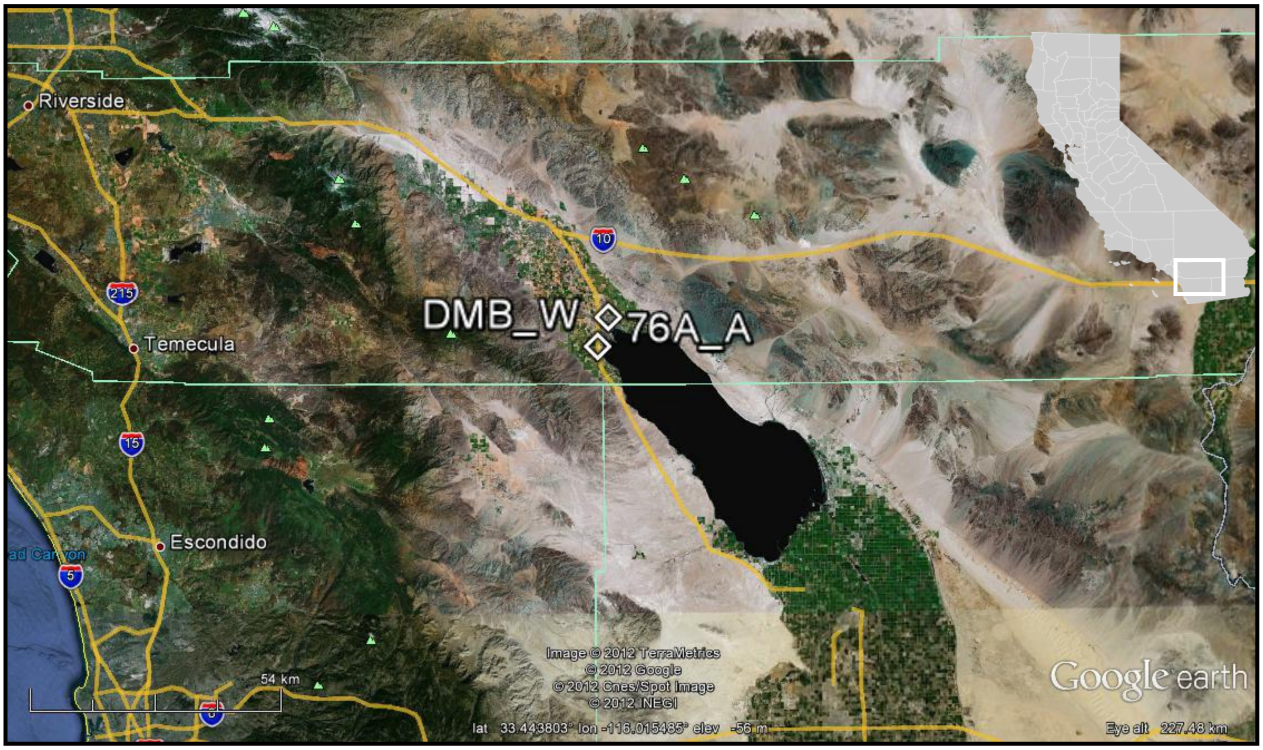

Figure B12. Event 10 – Permethrin

Figure B13. Event 11 – Permethrin

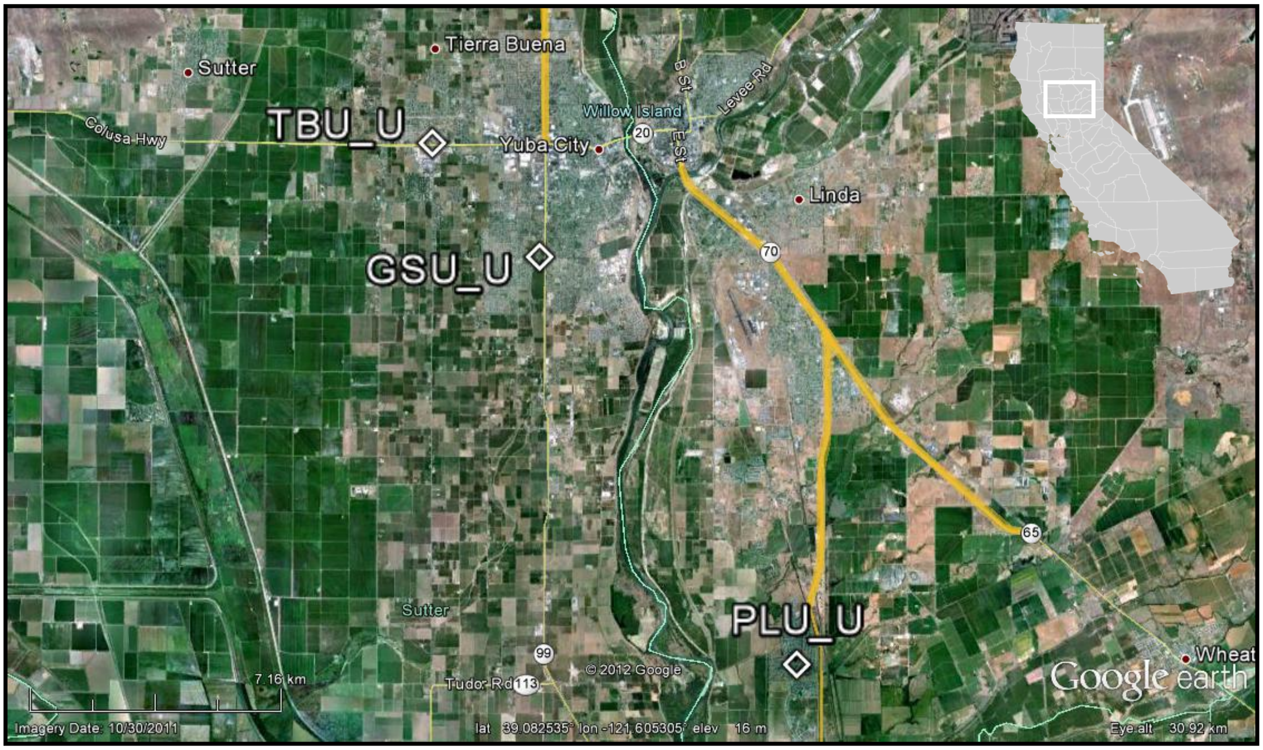

Figure B14. Events 12 and 13 – Permethrin