Journal of the American Mosquito Control Association, 26(1):57–66, 2010

EVALUATION OF EFFICACY AND HUMAN HEALTH RISK OF AERIAL ULTRA-LOW VOLUME APPLICATIONS OF PYRETHRINS AND PIPERONYL BUTOXIDE FOR ADULT MOSQUITO MANAGEMENT IN RESPONSE TO WEST NILE VIRUS ACTIVITY IN SACRAMENTO COUNTY, CALIFORNIA

PAULA A. MACEDO,[1] JEROME J. SCHLEIER III,[2] MARCIA REED,[1] KARA KELLEY,[1]
GARY W. GOODMAN,[1] DAVID A. BROWN[1] AND ROBERT K. D. PETERSON[2]

ABSTRACT. The Sacramento and Yolo Mosquito and Vector Control District (SYMVCD, also referred to as "the District") conducts surveillance and management of mosquitoes in Sacramento and Yolo counties in California. Following an increase in numbers and West Nile virus (WNV) infection rates of *Culex tarsalis* and *Culex pipiens*, the District decided on July 26, 2007, to conduct aerial applications of Evergreen® EC 60-6 (60% pyrethrins: 6% piperonyl butoxide) over approximately 215 km^2 in the north area of Sacramento County on the nights of July 30, July 31, and August 1, 2007. At the same time, the District received notification of the first human WNV case in the area. To evaluate the efficacy of the applications in decreasing mosquito abundance and infection rates, we conducted pre- and post-trapping inside and outside the spray zone and assessed human health risks from exposure to the insecticide applications. Results showed a significant decrease in abundance of both *Cx. tarsalis* and *Cx. pipiens*, and in the minimum infection rate of *Cx. tarsalis*. Human-health risks from exposure to the insecticide were below thresholds set by the US Environmental Protection Agency.

KEY WORDS West Nile virus, aerial spraying, insecticide, mosquito control, risk assessment

INTRODUCTION

West Nile virus (WNV, family Flaviridae, genus *Flavivirus*) was first detected in the United States in 1999 in New York City, and reached California in the summer of 2003 (Reisen et al. 2004). In 2004, WNV amplified to epidemic levels and dispersed to all 58 counties in the state, and was associated with low-level transmission to humans and horses in Sacramento and Yolo counties that year (Armijos et al. 2005, Hom et al. 2005). In 2005, there was a severe outbreak in Sacramento County, with 177 human cases and 40 equine cases (Elnaiem et al. 2006).

The Sacramento and Yolo Mosquito and Vector Control District (SYMVCD, also referred to as "the District") conducts routine surveillance and management of mosquito populations in Sacramento and Yolo counties. The District monitors weekly mosquito abundance and West Nile, western equine encephalitis (WEE), and St. Louis encephalitis (SLE) viral infection. The District follows the California Mosquito-Borne Virus Surveillance and Response Plan (Kramer 2005) and its own Mosquito and Mosquito-Borne Disease Management Plan (SYMVCD 2005), and applies the principles of integrated pest management (IPM) in its program. When WNV reached epidemic levels in 2005 despite SYMVCD's intensive larviciding and public education efforts, the District intervened by aerially applying a formulation of pyrethrins and piperonyl butoxide (PBO) over an urban/suburban area in Sacramento County (Elnaiem et al. 2008), which most likely interrupted the WNV transmission cycle (Carney et al. 2008).

Although traditionally used in response to epidemics and as part of a sustainable public health program (Rose 2001), the application of pesticides often generates public concerns and controversy about the safety of these chemicals to people and the environment as well as the efficacy of such practice (Thier 2001, Roche 2002, Hodge and O'Connell 2005). A human health risk assessment conducted by Peterson et al. (2006) for truck-mounted ultra-low volume (ULV) applications of adulticides commonly used in mosquito management programs determined risks to be below levels established by the US Environmental Protection Agency (USEPA), which agrees with the current scientific weight of evidence (NYCDOH 2001, Karpati et al. 2004, Currier et al. 2005, O'Sullivan et al. 2005). The results from Peterson et al. (2006) indicated that potential health risks from WNV exceed risks from exposure to these pesticides when used at label rates to control adult mosquitoes. Their study used extremely conservative assumptions and estimated exposure after truck-mounted ULV applications as a worse-case scenario, used application rates greater than the ones used by SYMVCD, and therefore likely overestimated the exposure that would be seen for the application

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[1] Sacramento-Yolo Mosquito and Vector Control District, 8631 Bond Road, Elk Grove, CA 95757.

[2] Department of Land Resources and Environmental Sciences, Montana State University, 334 Leon Johnson Hall, Bozeman, MT 59717.

by the District. Schleier et al. (2009b) evaluated probabilistically the deterministic risk estimates presented by Peterson et al. (2006) and found them to be very conservative. Davis et al. (2007) evaluated ecological risks posed by adult mosquito management programs and concluded that risks to nontarget organisms from pesticides applied for adult mosquito management are low and not likely to exceed regulatory levels of concern.

Although vector control strategies and their effectiveness have generated concern in past years, there have been few published studies addressing the efficacy of these operations in reducing mosquito populations, infection rates, and virus transmission. Sacramento County experienced a WNV epidemic for the first time in 2005. Although the evaluation of the aerial adulticiding conducted during that year suggested interruption of transmission (Carney et al. 2008) and reduction of vector abundance (Elnaiem et al. 2008), some uncertainties were identified by SYMVCD staff to be addressed in future evaluations, particularly the absence of fixed locations for trapping mosquitoes before and after the applications, the small number of mosquito pools collected from those areas, and other confounding factors such as the effect of wind shadow at some of the locations.

In 2006, WNV reached epidemic levels in the cities of Davis and Woodland in Yolo County, and the SYMVCD, with the collaboration of the Center for Vectorborne Diseases at the University of California–Davis, monitored abundance and infection rates and conducted aerial applications of pyrethrins and PBO on the nights of August 8 and 9, 2006 (Macedo et al. 2007a, Nielsen et al. 2007). Analysis of data from 2005 and 2006 showed that the aerial applications could have been more successful in interrupting virus transmission to people if they had been conducted 1 wk or 2 wk before, when mosquito abundance and infection rates were higher (Macedo et al. 2008).

The first indication of active WNV transmission in the District in 2007 was the detection of WNV in a dead American crow on May 31. The first positive mosquito pool was obtained on July 4, 2007, in a pool of *Culex pipiens* L. West Nile virus continued to amplify during the month of July, and mosquito abundance and maximum likelihood estimates of minimum infection rates continued to be monitored. The District identified an area of approximately 215 km^2 in the north part of Sacramento County as higher risk and intensified all the aspects of its IPM program in an attempt to reduce mosquito populations. On the week of July 24, 2007, infection rates had reached 10.85 and 7.87 per 1,000 mosquitoes for *Culex tarsalis* Coquillett and *Cx. pipiens*, respectively. Following the guidelines of the California Mosquito-Borne Virus Surveillance and Response Plan (Kramer 2005) and its Mosquito and Mosquito-Borne Disease Management Plan (SYMVCD 2005), the District made the decision on July 26, 2007, to aerially apply pyrethrins and PBO (Evergreen EC-60-6®) over the area of concern. On the same day that this decision was made, the District received notification of the first human case in the area. The insecticide applications took place on the nights of July 30 and 31, and August 1, 2007. The objectives of this study were to 1) evaluate the efficacy of the aerial applications in reducing adult mosquito populations and infection rates of the two main species implicated in WNV transmission in Sacramento County and 2) assess human health risks for the aerial application of adulticide conducted in Sacramento County in 2007 in response to the WNV activity.

MATERIALS AND METHODS

Aerial applications and study area

The spray zone was a 215 km^2 area in northern Sacramento County, located in the Central Valley of California (Fig. 1). The insecticide Evergreen EC 60-6 (60% PBO and 6% pyrethins; McLaughlin Gormley King, Golden Valley, MN) was applied by a fixed-wing Piper Aztec and a Cessna 402 aircraft (VDCI/ADAPCO Vector Control Services, Greenville, MS) for three consecutive nights on July 30 and 31, and August 1, 2007. The application rate was 2.8 g/ha (0.0025 lb/ac) of pyrethins and 28 g/ha (0.025 lb/ac) of PBO. The release altitude was 91 m (300 ft), the wind speed ranged from 3.7 km/h to 18.5 km/h, and the temperature at the time of the applications ranged from 34°C to 36°C. Application start times ranged from 7:34 p.m. to 7:55 p.m. and application end times ranged from 9:20 p.m. to 9:51 p.m.

Mosquito abundance

To evaluate the effect of the aerial applications on mosquito abundance, the District used encephalitis virus surveillance traps (EVS) baited with dry ice, herein referenced to as CO_2 traps (Rohe and Fall 1979), and gravid-female traps (Cummings 1992) to collect mosquitoes inside and outside of the aerial spray zone for 3 days before and 3 days after the application events. Trap collections were brought to the laboratory, where mosquitoes were anesthetized with triethylamine, identified to species and counted, and then frozen at -80°C for later virus testing. Three CO_2 traps and one gravid trap were placed at each of the 12 fixed sites in the aerial spray zone and six fixed sites in the untreated control zone (Fig. 1). Counts of females per trap-night were transformed by $\ln(y + 1)$ and expressed as

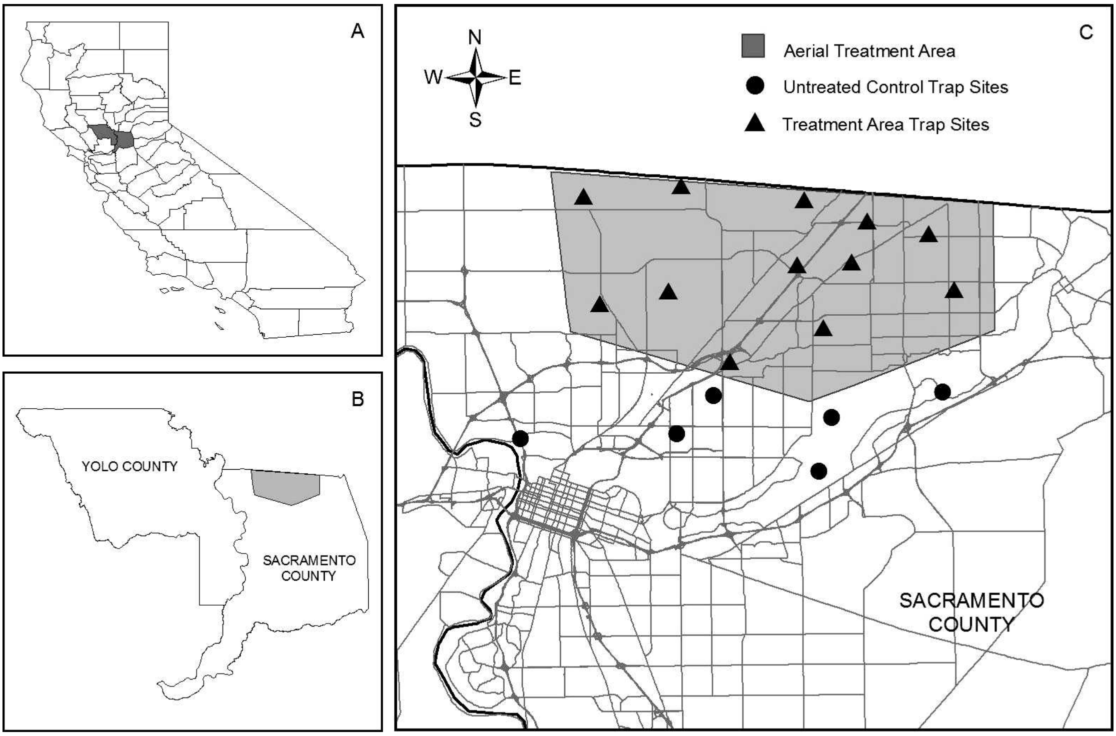

Fig. 1. Map of California showing (A) the location of Sacramento and Yolo counties, (B) the 2007 spray zone in north Sacramento, and (C) the locations of trapping sites used in the spray zone and untreated control area during 3 days before and 3 days after the aerial applications of pyrethrins and piperonyl butoxide in 2007.

geometric means (Reisen and Lothrop 1999), and analyzed by two-way analysis of variance (ANOVA) and paired *t*-tests (SAS, version 9.1, SAS Institute, Cary, NC). Percentage of reduction of *Cx. tarsalis* and *Cx. pipiens* abundance was estimated by the formula described by Mulla et al. (1971).

West Nile virus infection rates

Mosquitoes from trapping conducted in the 18 fixed sites 3 days before and 3 days after the aerial applications, as well as from other traps set inside the spray zone during the week before and after the adulticide applications were collected and brought to the laboratory where they were then anesthetized with triethylamine, identified to species, pooled in groups of one to 50 females, frozen at -80°C, and tested for arboviral RNA (WN, SLE, and WEE viruses) by multiplex real-time reverse transcriptase–polymerase chain reaction (Shi et al. 2001), using WNV primers published previously (Lanciotti et al. 2000). Infection rates were calculated for the week before and the week after the aerial application events using bias-corrected maximum likelihood estimation (Biggerstaff 2006). In addition, percentage of reduction of minimum infection rates was estimated by the formula described by Mulla et al. (1971), which accounts for reductions or increases in the untreated areas.

Sentinel cages

Sentinel mosquitoes were exposed within disposable bioassay cylindrical cardboard cages, 15 cm diam and 4.5 cm deep, with 14 × 18–mesh polyester screens on both vertical circular surfaces (modified from Townzen and Natvig 1973), with a hole in the cardboard for cotton pads moistened with 10% sugar water. One cage containing approximately 25 wild-caught adult *Cx. tarsalis* and another containing 25 wild-caught *Cx. pipiens* were placed at each of the 12 sites within the aerial spray zone and at the six control sites during each application. Cages were placed vertically at a 1-m height with a screened surface positioned to face the prevailing wind direction. Mosquito mortality was evaluated at the time of the placement of the cages (before the insecticide application), and at 1, 2, and 12 h after each application. Results were expressed as percentage of mortality.

Human health risk assessment

Human health risk assessments had been previously conducted for truck-mounted applications of pyrethrins and PBO using greater application rates than the ones used by SYMVCD for adult mosquito control, and for a different application schedule (Peterson et al. 2006). We modified the risk assessments by Peterson et al. (2006) and Schleier et al. (2009b) to more accurately represent the application type, rate, and schedule used by SYMVCD in 2007. In addition to those modifications to the previous risk assessments, we incorporated more recent deposition data from Schleier et al. (2008). We estimated the human health risk from exposure to 3 days of aerial applications of pyrethrins and PBO at the rates specified above. To account for age-related differences, exposures were estimated for adult males and adult females (18–65 years of age), youth (10–12 years of age), children (5–6 years of age), toddlers (2–3 years of age), and infants (0.5–1.5 years of age).

Toxicity and dose-response relationships: Dose-response information for each compound was reviewed and endpoints were chosen based on acute and subchronic exposures. The toxicity thresholds used in this assessment were ingestion reference doses (RfD) established by the USEPA. Ingestion RfDs were based on the no-observed-adverse-effect level (NOAEL) with a 100-fold safety factor for intra- and interspecies extrapolation uncertainties. The acute oral RfD for pyrethrins and PBO are 0.07 and 6.3 mg/kg body weight (BW)/day, respectively (USEPA 2006a, 2006b).

Risk characterization: Total acute exposure to each active ingredient for each group was estimated by summing inhalation, dermal, hand-to-mouth, turf-dislodgeable, and ingestion exposure routes which are outlined below. The risk quotient (RQ) was calculated by dividing the total potential exposure for each group and chemical by its respective ingestion toxic endpoint value (RfD). The multi-route exposure was compared to the ingestion RfD because it provided a conservative endpoint, which is based on the most sensitive NOAEL. Estimated RQs were compared to a RQ level of concern (LOC), which is set by the USEPA or other regulatory agencies to determine if regulatory action is needed. The RQ LOC used in the assessment was 1.0. An RQ >1.0 means that the estimated exposure was greater than the relevant RfD.

Probabilistic analysis: Monte Carlo simulation (Crystal Ball® 7.3; Decisioneering, Denver, CO) was used to generate the exposures and RQs. Probabilities of occurrence of RQ values were determined by incorporating sampling from the statistical distribution of each input variable used to calculate the RQs. Each of the input variables was sampled so that its distribution shape was reproduced. Then, the variability for each input was propagated into the output of the model so that the model output reflected the probability of values that could occur. This was performed by using 20,000 iterations with the assumptions outlined below and in Table 1. Respiratory rate, BW, percentage of surface area of two hands, air concentrations, and spray deposition were truncated at zero because it is not possible for these quantities to have negative values.

Environmental concentrations: We used the environmental concentration data from Schleier et al. (2008) at ground level using the same application rates listed above (see Schleier et al. 2008 for details of the applications). To model the deposition of pyrethrins and PBO onto surfaces, we created distributions using concentrations measured 1 h and 12 h after application because the concentrations at these times were not significantly different (Schleier et al. 2008). Distributions for deposition onto surfaces were chosen based on the Anderson–Darling goodness-of-fit test, which for non-normalized data weights the differences between two distributions at their tails (Pettitt 1977, Oracle 2007). The distribution fit of the environmental concentration data for PBO was log-normal with a mean 0.01 $\mu g/cm^2$ and a standard deviation of 0.01. To model air concentrations of PBO we assumed the same amount that deposited on the ground would be available in 1 m^3 of air. We used the same distribution for air concentrations as we did for ground deposition. Schleier et al. (2008) did not detect any pyrethrins during their study; therefore we modeled deposition and air concentrations using the same assumptions as for PBO, scaling the distributions based on the application rate. Pyrethrins were applied at an application rate 10% of that for PBO.

Acute exposure: We assumed that acute multi-route exposures immediately after a single-spray event were limited to 24 h. Routes of insecticide exposure to each group were inhalation, dermal, and dietary and non-dietary ingestion. Assumptions of body weight, respiration rate, and frequency of hand-to-mouth activity are presented in Table 1.

Because the data from Schleier et al. (2008) demonstrated that the inhalation exposure is most likely limited to 1 h, we assumed that each group would be outside when the aerial spray began and that the duration of the exposure was 1 h. Instead of using modeled environmental concentrations we incorporated the deposition rates of Schleier et al. (2008). The exposure modeling assumptions for dermal, hand-to-mouth, and turf-dislodgeable residues follow the assumptions of Schleier et al. (2009b), except actual environmental concentrations were used instead of modeled environmental concentrations. The modifications to inhalation and ingestion exposure are outlined below.

Table 1. Assumptions for body weight, respiratory rate, and frequency of hand-to-mouth activity for each group assessed.

Input Variables	Group	Parameter[1]	Values	Units	Distribution	Source
Body weight	Adult males[2]	Mean	78.65	kg	Log-normal (truncated)	Portier et al. (2007)
		SD	13.23			
	Adult females[3]	Mean	65.47	kg		
		SD	13.77			
	Youth[4]	Mean	36.16	kg		
		SD	7.12			
	Children[5]	Mean	19.67	kg		
		SD	2.81			
	Toddlers[6]	Mean	13.27	kg		
		SD	1.62			
	Infants[7]	Mean	9.1	kg		
		SD	1.24			
Respiratory rate	Adult males	Mean	17.53	m^3/day	Log-normal (truncated)	Brochu et al. (2006)
		SD	2.8			
	Adult females	Mean	13.78	m^3/day		
		SD	2.1			
	Youth	Mean	11.3	m^3/day		
		SD	2.14			
	Children	Mean	7.74	m^3/day		
		SD	1.04			
	Toddlers	Mean	5.03	m^3/day		
		SD	0.94			
	Infants	Mean	3.72	m^3/day		
		SD	0.81			
Hand-to-mouth frequency	Toddlers	Location	5.3	events/h	Weibull (truncated)	Xue et al. (2007)
		Scale	3.41			
		Shape	0.56			
	Infants	Location	14.5	events/h		
		Scale	15.98			
		Shape	1.39			

[1] SD = standard deviation.
[2] 18–65 years of age.
[3] 18–65 years of age.
[4] 10–12 years of age.
[5] 5–6 years of age.
[6] 2–3 years of age.
[7] 0.5–1.5 years of age.

Inhalation exposure was estimated by

$$PE_{Inhalation} = (AEC \times RR \times D)/BW \qquad (1)$$

where $PE_{Inhalation}$ is potential exposure from inhalation (mg/kg BW), AEC is actual environmental air concentrations (μg/m^3), RR is respiratory rate for each group (m^3/h), D is duration of exposure, and BW is body weight (kg) for each group (Table 1).

For acute ingestion exposure from tomatoes that were exposed to the pesticide, we assumed that all foods containing tomatoes eaten per day were consumed from tomatoes grown in a home garden without being washed. In addition, we assumed there would be no degradation in the preparation process. Acute ingestion was estimated by

$$PE_{Ingestion} = [(AEC \times CF) \times SAT]/BW \qquad (2)$$

where $PE_{Ingestion}$ is potential exposure from consuming exposed produce (mg/kg BW), AEC is the actual environmental concentration of insecticide that settles onto surfaces (μg/cm^2), CF is the conversion from μg/cm^2 to mg/m^2, SAT is the surface area of tomatoes consumed as estimated by Eifert et al. (2006) (m^2), and BW is body weight (kg). The average amount plus the standard error of tomatoes consumed per day by adult males and females, youth, children, toddlers, and infants is 0.804, 0.804, 0.874, 1.19, 1.77, and 1.21 g/kg BW, respectively (USEPA 1997).

RESULTS

Two-way ANOVA showed significant differences in *Cx. tarsalis* and *Cx. pipiens* abundance in the CO_2-baited traps before and after the applications (F = 14.59; df 1, 16; P = 0.0015; and F = 8.49; df 1, 13; P = 0.0121 respectively). There was no interaction between time and treatment, so a paired *t*-test was used to compare

Table 2. Mean female mosquitoes per trap night and standard deviation before and after the insecticide applications inside (spray zone) and outside (control) of the spray area.[1]

	Spray zone		Control	
Species, trap type	Before	After	Before	After
Culex pipiens, CO_2 traps	4.94 (4.70) a	2.46 (2.24) b	10.33 (13.59) a	8.48 (13.68) a
Culex pipiens, gravid traps	14.79 (12.27) a	9.79 (9.75) a	12.33 (12.50) a	14.47 (9.65) a
Culex tarsalis, CO_2 traps	8.75 (7.44) a	3.00 (1.61) b	17.78 (19.08) a	14.28 (20.00) a

[1] Means within a column in spray zone or control followed by the same letter were not significantly different ($P > 0.05$).

abundance before and after in the spray zone and control areas separately. There was a significant reduction in abundance of host-seeking *Cx. tarsalis* and *Cx. pipiens* inside of the spray zone and not in the untreated control areas (Table 2). There was no significant difference in the number of *Cx. pipiens* females captured by the gravid traps before and after the aerial adulticide applications in the spray zone or in the control areas. Percentage of reduction calculated by Mulla's formula was estimated to be 57.33% for *Cx. tarsalis* and 40.81% for *Cx. pipiens*.

Maximum likelihood estimates of WNV infection rates for *Cx. pipiens* and *Cx. tarsalis* before and after the application events are shown in Table 3. Infection rates for *Cx. tarsalis* decreased significantly in the spray zone after the aerial adulticide applications, but not for *Cx. pipiens*. In contrast, infection rates for both species increased in the untreated control area after the applications. Percentage of reduction of the minimum infection rates calculated by Mulla's formula was estimated to be 77.41% for *Cx. tarsalis* and 21.56% for *Cx. pipiens*.

Sentinel cage bioassay data showed that average mortality 1 h after application was 40% (range 0% to 91%) for *Cx. pipiens* and 51% (range 0% to 94%) for *Cx. tarsalis*. Results for mortality at 1, 2, and 12 h are shown in Table 4. We observed a high variability among sentinel cage mortality, suggesting that the insecticide application did not reach all sites. Nonetheless, mortality of mosquitoes in the sentinel cages in the spray zone was significantly different than mortality at the untreated control area ($F = 142.91$; $P < 0.0001$ and $F = 185.34$; $P < 0.0001$ for *Cx. pipiens* and *Cx. tarsalis*, respectively).

The human health risk assessment from exposure to three aerial ULV applications of pyrethrins and PBO at rates used by the SYMVCD indicated that total acute exposure for pyrethrins at the 95th percentile of exposure ranged from 0.000004 to 0.0003 mg/kg BW/day for the groups assessed (Table 5). Risk quotients for pyrethrins at the 95th percentile ranged from 0.00003 to 0.002 for all groups (Table 6). Total acute exposure for PBO at the 95th percentile ranged from 0.00008 to 0.003 mg/kg BW/day for the groups assessed (Table 5). Risk quotients for PBO at the 95th percentile ranged from 0.00001 to 0.0005 for all groups (Table 6). No chemical or group exceeded the RQ LOC. Toddlers and infants were the highest-risk groups whereas adult males were the lowest-risk group assessed in this study (Table 6).

Our results showed that median inhalation exposure contributed <0.01% to the overall exposure of all groups. Median dermal exposure contributed about 53% to the overall exposure of adult males and females, youth, and children; however, the median dermal exposure only contributed 18% to the overall exposure of toddlers and infants. Median exposure from hand-to-mouth exposure from insecticide settling onto their hand contributed about 17% to the overall exposure of toddlers and infants. Median exposure from hand-to-mouth turf-dislodgeable residue contributed about 16% to the overall

Table 3. Maximum likelihood estimates of WNV infection rates for mosquito pools collected in the spray zone and untreated control area before and after the application events.

Area	Time	Species	MLE[1] (95% CI)	No. females	No. pools	No. positive pools	% positive pools
Spray zone	Before	*Culex pipiens*	7.87 (5.02–11.86)	2,968	188	21	11.17
		Culex tarsalis	10.85 (6.54–17.09)	1,605	118	16	13.56
	After	*Culex pipiens*	7.51 (2.82–16.65)	705	69	5	7.25
		Culex tarsalis	3.42 (0.20–16.54)	292	50	1	2.00
Control	Before	*Culex pipiens*	5.31 (1.76–12.70)	781	45	4	8.89
		Culex tarsalis	4.53 (1.51–10.81)	910	37	4	10.81
	After	*Culex pipiens*	6.46 (2.84–12.92)	1,205	68	7	10.29
		Culex tarsalis	6.32 (2.38–14.05)	855	47	5	10.64

[1] MLE, bias-corrected maximum likelihood estimate of infection rate in 1,000 mosquitoes (Biggerstaff 2006); CI, confidence interval.

Table 4. *Culex pipiens* and *Culex tarsalis* average percentage of mortality (standard deviation) in bioassay cages.

	Spray zone		Control	
Time (hr)	*Cx. pipiens*	*Cx. tarsalis*	*Cx. pipiens*	*Cx. tarsalis*
1	40.07 (25.83)[1]	51.41 (29.03)[2]	2.94 (12.13)	8.51 (23.95)
2	56.47 (26.3)[1]	72.27 (29.43)[2]	6.96 (18.28)	9.14 (24.8)
12	68.48 (28.48)[1]	86.56 (21.74)[2]	7.55 (18.2)	9.14 (24.8)

[1] Significantly different than *Cx. pipiens* control ($P < 0.05$).
[2] Significantly different than *Cx. tarsalis* control ($P < 0.05$).

exposure of toddlers and infants. Mean ingestion exposure contributed about 43% to the overall exposure of all groups.

DISCUSSION

Although evaluation of efficacy is an essential component of assessing pesticide applications (Carney et al. 2008) and vector abundance is an important measure of efficacy of control strategies (Nielsen et al. 2007), it remains a difficult task for mosquito control districts because there are many variables that cannot be controlled. In 2007, when the decision was made to conduct aerial applications of pyrethrins and PBO to manage adult populations of mosquitoes in the north Sacramento area, the District selected areas outside of the aerial spray zone to be the untreated control sites, and as such, they should not have received any insecticide application. But as a vector control agency, once one of these control sites shows either high abundance of mosquitoes or positive mosquito pools, it is the District's responsibility to respond to those surveillance parameters and follow its management plan. Therefore, although the control sites were not sprayed aerially, some did receive ground treatments with trucks and backpack foggers in response to high trap counts, positive dead birds, and positive mosquito pools. Although this may be a confounding factor when comparing abundance of mosquitoes and infection rates inside and outside of the aerial spray zone, our results showed significant differences in abundance between the two areas even in the presence of ground treatments at the control sites. Another variable out of our control is that mosquito abundance tends to vary markedly among trap sites. Moreover, routine vector control strategies continued to be conducted by SYMVCD following its IPM program, and source reduction and larvicide applications were performed before, during, and after the aerial adulticide applications throughout all areas of Sacramento and Yolo counties.

Our analysis indicates that the aerial applications were made at a time when *Cx. pipiens* populations in Sacramento County were already declining, but *Cx. tarsalis* populations were increasing. Analysis of the data and population trends indicate that most *Cx. pipiens* collected at the time of the aerial adulticide applications were gravid females, suggesting that the population of these mosquitoes was composed of older, blood-fed females, presenting a different behavior than the host-seeking mosquitoes. That may explain why there was a greater reduction in host-seeking *Cx. tarsalis* than *Cx. pipiens*. Nonetheless, there was still a significant reduction in host-seeking *Cx. pipiens* abundance in the spray zone. Although not statistically significant, a reduction of 33.8% in *Cx. pipiens* captured in gravid traps was also observed in the spray zone. That is important because, at the same time, *Cx. pipiens* captured in gravid traps in the untreated control area increased 17.4%.

Sentinel mosquitoes were used to evaluate the deposition of the pesticide into the target areas.

Table 5. Acute total potential exposure means at 50th and 95th percentile confidence intervals for each group and chemical assessed.

Chemical	PE[1]	Adult males[2]	Adult females[3]	Youth[4]	Children[5]	Toddlers[6]	Infants[7]
Pyrethrins	50th	0.000001	0.000001	0.000002	0.000003	0.00001	0.00002
	95th	0.000005	0.000005	0.000008	0.00001	0.00005	0.00009
Piperonyl butoxide	50th	0.00003	0.00003	0.00004	0.00006	0.0001	0.0003
	95th	0.0001	0.0001	0.0002	0.0002	0.0006	0.001

[1] PE, potential exposure. Total acute exposure to each active ingredient for each group was estimated by adding together inhalation, dermal, hand-to-mouth, turf-dislodgeable, and ingestion exposure routes (mg/kg body weight/day).
[2] 18–65 years of age.
[3] 18–65 years of age.
[4] 10–12 years of age.
[5] 5–6 years of age.
[6] 2–3 years of age.
[7] 0.5–1.5 years of age.

Table 6. Acute risk quotient means at 50th and 95th percentile confidence intervals for each group and chemical assessed.

Chemical	RQ[1]	Adult males[2]	Adult females[3]	Youth[4]	Children[5]	Toddlers[6]	Infants[7]
Pyrethrins	50th	0.000008	0.00001	0.00001	0.00002	0.00008	0.0002
	95th	0.00003	0.00004	0.00006	0.0001	0.0004	0.0007
Piperonyl butoxide	50th	0.000004	0.000005	0.000006	0.000009	0.00002	0.00004
	95th	0.00002	0.00002	0.00003	0.00004	0.0001	0.0002

[1] Risk quotient.
[2] 18–65 years of age.
[3] 18–65 years of age.
[4] 10–12 years of age.
[5] 5–6 years of age.
[6] 2–3 years of age.
[7] 0.5–1.5 years of age.

Deposition may be markedly altered by local meteorological conditions that are affected by the presence of heavy vegetation (Barber et al. 2007, Elnaiem et al. 2008), which also filters out the pesticide (Taylor and Schoof 1971). To penetrate the canopy, wind direction must be perpendicular to the spray line and wind speeds must move the pesticide through the canopy (Barber et al. 2007). Wind direction and speed are usually measured at the application point, but may be different from conditions at vegetated locations. Mortality results from our bioassay cages varied significantly, with different locations presenting very low mortality at different application events. Environmental conditions may have been responsible for the reduced spray movement through some of these target zones in different days.

The probabilistic risk assessment showed that RQs for a single truck-mounted application are about 10-fold greater than those estimated for three applications of aerial ULV. Although the rates used for aerial applications may be greater than for truck-mounted, deposition on the ground is lower after aerial ULV (Lothrop et al. 2007, Schleier and Peterson 2009, Schleier et al. 2009b). These results support the findings of previous risk assessments and regulatory documents that the risks from aerial ULV are lower than those of truck-mounted ULV (NYCDOH 2005, Peterson et al. 2006). Our results are supported by biomonitoring studies that showed no increase in urinary metabolites after aerial ULV applications of naled (Kutz and Strassman 1977, Duprey et al. 2008). Our assessment determined that exposures after three aerial ULV applications are 0.001% of the acute RfD, and are below regulatory LOCs.

The main objective of the aerial adulticide applications conducted by SYMVCD in 2007 was to decrease the number of infected and infective adult mosquitoes in the target area. Infection rates were significantly lower for *Cx. tarsalis* in the spray zone after the aerial adulticide applications, but not in the control areas. Even though we did not observe a significant decrease in the maximum likelihood estimate of minimum infection rates for *Cx. pipiens* in the spray zone, rates for this species were also higher in the control areas after the application events, and more positive mosquito pools were found in the control area after the adulticide applications than before. Therefore, our data indicate that the aerial ULV treatments conducted by the SYMVCD in 2007 may have reduced the risk of WNV transmission to humans by effectively reducing the population of infected adult mosquitoes at the target area. The probabilistic risk assessment suggests that human risk from exposure to the insecticide applications was below regulatory levels of concern, so the benefits likely exceeded risks. The current weight of evidence from biomonitoring, epidemiology, risk assessments, and reduction in disease incidence rates after ULV applications (Kutz and Strassman 1977; Karpati et al. 2004; Currier et al. 2005; O'Sullivan et al. 2005; Carr et al. 2006; Peterson et al. 2006; Macedo et al. 2007b; Duprey et al. 2008; Schleier et al. 2009a, 2009b) demonstrate that the benefit of reducing WNV incidence rates outweigh public health risks from insecticide applications to manage adult mosquitoes.

ACKNOWLEDGMENTS

The authors thank the County of Sacramento Department of Health and Human Services Public Health Laboratory for providing Biosafety Laboratory–3 containment space; Leslie Shama for assistance in an earlier risk assessment; Rhonda Laffey for assistance with mapping; and Vicki Kramer, William K. Reisen, and Mark Novak for critically reviewing the manuscript. We are grateful for the support from the Center for Vectorborne Diseases (University of California, Davis, CA).

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