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ENVIRONMENTAL CONCENTRATIONS, FATE, AND RISK ASSESSMENT OF PYRETHRINS AND PIPERONYL BUTOXIDE AFTER AERIAL ULTRALOW-VOLUME APPLICATIONS FOR ADULT MOSQUITO MANAGEMENT

JEROME J. SCHLEIER III,*† ROBERT K.D. PETERSON,† PAULA A. MACEDO,‡ and DAVID A. BROWN‡
†Department of Land Resources and Environmental Sciences, Montana State University, 334 Leon Johnson Hall, Bozeman, Montana 59717, USA
‡Sacramento-Yolo Mosquito and Vector Control District, 8631 Bond Road, Elk Grove, California 95624, USA

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Abstract—One of the most effective ways of managing adult mosquitoes that vector human and animals diseases is the use of ultralow-volume insecticides. Because of concerns about the safety of the insecticides used for the management of adult mosquitoes, we conducted an environmental fate and efficacy study in Princeton and Colusa (both CA, USA) after aerial applications of pyrethrins and piperonyl butoxide (PBO). One hour before application, PBO concentrations in water were 0.008 and 0.2175 μg/L for Princeton and Colusa, respectively. One hour after the spray event in Princeton, the average PBO concentrations were 0.0125 μg/cm^2 on ground-deposition pads and 0.1723 μg/L in water samples, with concentrations decreasing significantly over time. One hour after the spray event in Colusa, the average PBO concentrations were 0.0199 μg/cm^2 on deposition pads and 1.274 μg/L in water samples, with concentrations decreasing significantly over time. A significant time and location effect for both deposition pads and water samples in Princeton and Colusa was observed ($p < 0.001$ and $p = 0.014$, respectively). Pyrethrins were not detected in nearly all ground and water samples. One hour after application, mortality of *Culex tarsalis* and *Culex pipiens* in sentinel cages was significantly higher than at the control site for both locations ($p < 0.001$). Risk quotients for aquatic surrogate species in Princeton and Colusa were 0.002 or less at 1 h after application, which did not exceed the U.S. Environmental Protection Agency risk quotient level of concern for endangered aquatic organisms of 0.05. Our results suggest that the amounts of pyrethrins and PBO deposited on the ground and in water after aerial ULV insecticide applications are lower than those estimated by previous exposure and risk assessments.

Keywords—*Culex* Mosquito control Pest management Pesticide Risk analysis

INTRODUCTION

One of the most effective ways to manage high densities of adult mosquitoes that vector human and veterinary pathogens is using ultralow-volume (ULV) aerosol applications of insecticides, which have been the worldwide standard for adult mosquito management for more than 30 years [1,2]. Ultralow volume is the minimum effective volume of insecticide that is used as a space spray for adult mosquitoes. Small droplets from 5 to 25 μm are the optimum size for ULV applications. Smaller droplets tend to travel farther and not to settle out of the air column as quickly as larger droplets, making ULV applications an effective measure for the control of adult mosquitoes seeking a blood meal.

Since West Nile virus was accidentally introduced into the United States in 1999, more areas of the country have experienced active mosquito-control programs than before, and subsequently, there has been greater public attention to the human health and environmental risks associated with ULV insecticide applications [3]. In response to these concerns, tier I/II risk assessments have been performed to quantify reasonable worst-case estimates of risk. Peterson et al. [4] performed a reasonable worst-case human health risk assessment for six mosquito insecticide active ingredients, including pyrethrins and the synergist piperonyl butoxide (PBO), after ground ULV applications. Those researchers demonstrated that the risks to humans most likely are negligible. Davis et al. [5] and Schleier et al. [6] examined pyrethrins and PBO as well and found similar results, demonstrating that ecological and equine risks from truck-mounted ULV applications most likely are negligible. Carr et al. [7] showed that the use of aerially applied ULV resmethrin above agricultural fields as a result of a public health emergency would result in negligible human dietary risk. Other biomonitoring and epidemiological studies, reports, and regulatory assessments also have concluded that risks to humans and other nontarget organisms from exposure to mosquito insecticides most likely are negligible [8–12].

Although risk assessments have been performed, they have relied on estimates of environmental concentrations of adulticides from models that do not have algorithms for ULV-type application methods. Few data are available regarding actual environmental concentrations of ULV insecticides after application. Jensen et al. [13] found nondetectable (ND) concentrations of pyrethrins and permethrin in water samples from wetlands before and after truck-mounted ULV applications. Weston et al. [14] examined water concentrations of pyrethrins and PBO 10 h after the second aerial ULV application of mosquito adulticides and 34 h after the third aerial ULV application of mosquito adulticides over Sacramento (CA, USA). They observed concentrations of 0.44 to 3.92 μg/L of PBO but did not detect pyrethrins. Lothrop et al. [15] measured concentrations of pyrethrins and PBO directly under the aircraft and out to 300 m from the flight path to maximize efficacy while minimizing evaporation of the insecticide in a desert environment. They observed concentrations of pyrethrins

* To whom correspondence may be addressed (jerome.schleier@myportal.montana.edu).
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ranging from ND to 0.0791 μg/cm^2, and for PBO, they observed concentrations ranging from ND to 1.07 μg/cm^2.

Because of concerns about the safety of adulticides used for the control of adult mosquitoes and the lack of actual environmental concentration data, we conducted an environmental fate and efficacy study. The objective of the present study was to characterize terrestrial surface residues and surface-water concentrations of pyrethrins and PBO in an urban setting after a typical aerial application. To accomplish our objective, we examined the concentrations of insecticide present before and after a single ULV application, and we related those to refining risk assessments for ULV insecticides.

MATERIALS AND METHODS

The insecticide Evergreen® EC 60-6 (60% PBO and 6% pyrethrins; McLaughlin Gormley King MGK, Golden Valley, MN, USA) was applied in Princeton (39°24′17.58″N, 122°0′38.58″W; CA, USA) and Colusa (39°12′23.76″N, 122°0′24.44″W; CA, USA) on June 26 and 28, 2007, respectively. Application was at 8:22 PM for Princeton and 8:52 PM for Colusa. Undiluted Evergreen EC 60-6 was applied aerially at a rate of 2.8 g/ha of pyrethrins and 28 g/ha of PBO using a fixed-wing Piper Aztec aircraft by ADAPCO Vector Control Services (Greenville, MS, USA) in conjunction with the Sacramento–Yolo Mosquito and Vector Control District. The release height of insecticide from the aircraft for Princeton and Colusa was 61 and 91 m, respectively, above the ground. Temperature, wind speed, and relative humidity at ground level were recorded with a Kestrel® 4000 pocket weather tracker (Nielsen-Kellerman, Boothwyn, PA, USA).

At each location (Colusa and Princeton), we used four sampling sites, with two subsamples placed 1 m apart at each sampling site. Samples were taken from both surface water and ground deposition at four time intervals (1, 12, 24, and 36 h) following the spray event. Ground-deposition samples and water samples were collected 1 h before spraying to determine background levels of pyrethrins and PBO. At each spray location, 76 samples were collected, with four sites, two subsamples, two media (surface water and ground deposition), four collection times (1, 12, 24, and 36 h postapplication), four background samples, one spike (positive control), and one negative control.

Collection of surface residues at ground level was on 10- × 10-cm (100-cm^2) cotton dosimeters pinned to a cardboard backing. The cardboard backing was covered with plastic wrap to prevent contact between the cardboard and cotton pads. Four cotton pads were pinned to each cardboard backing before the spray event at each location. One pad was collected from each subsample at each of the times listed above.

Four blank and four spiked cotton pads were placed in a control (untreated) area. Controls for Princeton and Colusa were located 4 and 6 km, respectively, upwind of the spray zones. In Princeton and Colusa, deposition pads were spiked with 0.133 and 0.325 μg/cm^2, respectively, of pyrethrins and 0.07 and 0.023 μg/cm^2, respectively, of PBO. Spikes and controls were set out when the aircraft finished spraying, and one pad from each was collected at the times listed above. Cotton pads were placed in open areas with minimal vegetation to represent a worst-case assessment of ground deposition. Cotton pads were collected with tweezers that were rinsed with high-pressure liquid chromatography grade acetone between pads to prevent cross-contamination. Individual samples were stored in separate 120-ml I-Chem™ glass jars with Teflon® lids (Chase Scientific Glass, Vineland, NJ, USA). After collection, jars were immediately placed on dry ice for transport to the Caltest Analytical Laboratory (Napa, CA, USA) for analysis.

Water samples were collected from flowing irrigation ditches in Princeton and static golf course ponds in Colusa, which were the only available bodies of water in each town. Water samples were taken approximately 2 to 6 cm under the surface in 1-L, amber I-Chem glass jars with Teflon lids, immediately placed on dry ice, and transferred to liquid ice after returning to the Sacramento–Yolo Mosquito and Vector Control District Laboratory.

The analytical laboratory analyzed the six active chemical components of pyrethrins (cinerin I and II, jasmolin I and II, and pyrethrins I and II) as well as PBO. Both cotton pads and water were extracted with dichloromethane. Extraction was performed using the U.S. Environmental Protection Agency SW846 method 3550B [16] for deposition pads and method 3510C [17] for water. Analysis was done by gas chromatography–mass spectrometry using U.S. Environmental Protection Agency SW846 method 8270C [18].

We used Statistical Analysis System 9.1 (SAS Institute, Cary, NC, USA) to run repeated-measures analysis of variance ($\alpha = 0.05$) using a mixed model on log-transformed concentrations to determine differences between times and treatments, and we performed regression analysis of chemical components with respect to time using SigmaPlot® 8 (SPSS, Chicago, IL, USA) [19]. For ND concentrations, we substituted half the detection limit when the NDs were less than 10% of the PBO data [20].

Disposable bioassay cylindrical cardboard cages (diameter, 15 cm; depth, 4.5 cm) were used with 14 × 18 cm polyester mesh screens covering the vertical circular surfaces of the cage. A hole was placed in the side of the cardboard where cotton pads moistened with sugar water were used [21]. One cage containing approximately 25 laboratory-reared adult *Culex tarsalis* and one cage containing approximately 25 laboratory-reared adult *Culex pipiens* was placed at each of nine sites within the spray zone. Eighteen additional cages (one of each mosquito species per site) were placed on nine sites outside of the spray zone in Williams (CA, USA) to serve as controls during each spray event. Cages were placed vertically at 1 m with a screened surface positioned to face the predominant wind direction at a 45° angle [22]. Mosquito mortality was evaluated at the time of placement and at 1 h after the spray event. Cages were then brought to the Sacramento–Yolo Mosquito and Vector Control District Laboratory and reevaluated at 2, 12, and 24 h after the spray event. We ran mixed-model, repeated-measures analysis of variance ($\alpha = 0.05$) on log-transformed mortality percentages to determine significant differences between times, treatments, locations, and species.

RESULTS

The analytical detection limits for both water and deposition pads are presented in Table 1. The average ambient air temperature at ground level at the time of the spray was 31°C at Princeton and 24°C at Colusa. Average wind speed for Princeton at ground level was 8.6 km/h, with a peak wind gust of 15.8 km/h out of the southeast and a relative humidity of 35.5%. Average wind speed for Colusa was 2.7 km/h, with a peak wind gust of 9.3 km/h from the west-southwest and a relative humidity of 55.9%. At the release height in Princeton

Table 1. Analytical detection limits for water and deposition pads for each compound

Compound	Water (μg/L)	Pad (μg/cm²)
Cinerin I	0.05	0.02
Cinerin II	0.05	0.02
Jasmolin I	0.05	0.02
Jasmolin II	0.05	0.02
Piperonyl butoxide	0.001	0.0007
Pyrethrin I	0.1	0.05
Pyrethrin II	0.5	0.2

and Colusa, winds were variable, at 11 to 19 km/h and at 22 km/h, respectively.

A significant time and location (Princeton vs Colusa) effect for deposition pads was found ($F = 13.7, 15.35$; $p < 0.001$). In Princeton, pyrethrins were not detected on the deposition pads. Pyrethrins and PBO were not detected on any of the background samples taken 1 h before the spray event. One hour after the spray event, the average concentration of PBO was 0.0125 μg/cm² (Table 2). On the spiked pads, pyrethrins were ND after 12 h, with PBO decreasing with time (Table 2). Control pads had no detectable amounts of any compound. A significant exponential decay relationship for the deposition pads was found ($F = 7.23$, $p = 0.018$, $r^2 = 0.341$, $y = 0.0138e^{-0.0437x}$) (Fig. 1).

Pyrethrins were not detected on any deposition pads in Colusa, nor were pyrethrins detected on any of the background samples taken 1 h before the spray event. However, PBO was detected in one background sample at a concentration of 0.031 μg/cm². On the spiked pads, pyrethrins were ND on all pads, with PBO decreasing with time (Table 2). Control pads had no detectable amounts of any compound. One hour after the spray event, average concentrations of PBO were 0.02 μg/cm², but concentrations decreased 65% to 0.007 μg/cm² at 36 h after the spray (Table 2). A significant exponential decay relationship for the deposition pads was found ($F = 14.66$, $p = 0.002$, $r^2 = 0.51$, $y = 0.0218e^{-0.0352x}$) (Fig. 1).

Pyrethrins were not detected in any water samples in Princeton before or after the spray event. Background PBO was found in the water at an average concentration of 0.008 μg/L (Table 3). One hour after application, the average concentration was 0.172 μg/L (Table 3). Concentrations decreased approximately

Table 2. Mean piperonyl butoxide (PBO) ground-deposition concentrations after application in Princeton and Colusa (both CA, USA)

	PBO concn. (μg/cm²)	
Sample time	Princeton	Colusa
1 h	0.0125 (±0.004)[a]	0.0199 (±0.003)
12 h	0.0105 (±0.006)	0.0182 (±0.002)
24 h	0.0028 (±0.0009)	0.0055 (±0.0007)
36 h	0.003 (±0.001)	0.0072 (±0.0009)
Control, 1 h	ND[b]	ND
Spike, 1 h	0.11	0.056
Control, 12 h	ND	ND
Spike, 12 h	0.19	0.02
Control, 24 h	ND	ND
Spike, 24 h	0.012	0.0095
Control, 36 h	ND	ND
Spike, 36 h	0.025	0.011

[a] Values in parentheses are the standard error.
[b] ND = nondetectable concentrations.

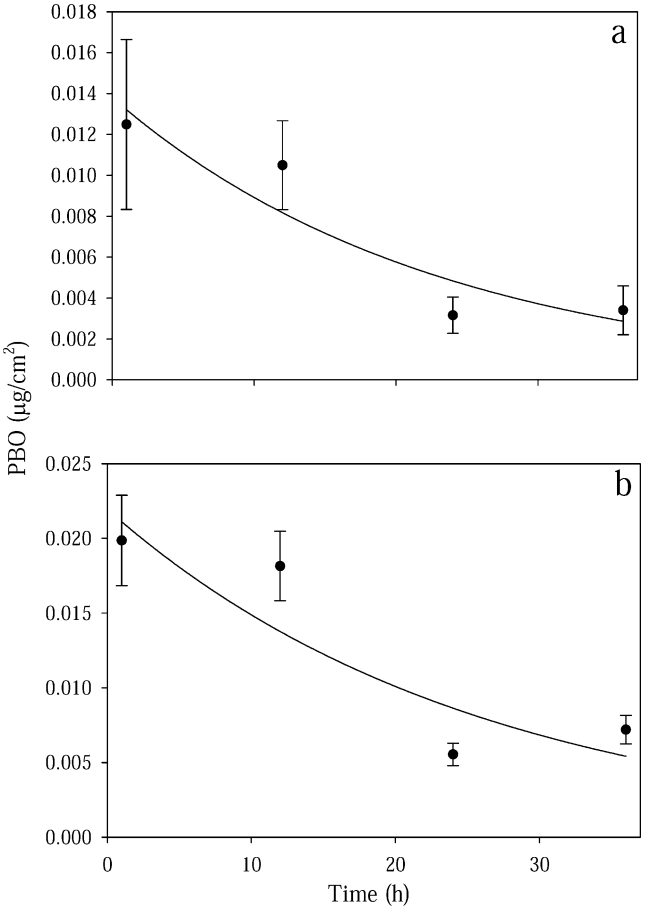

Fig. 1. Change in piperonyl butoxide (PBO) concentrations on deposition pads from 1 to 36 h after the spray with exponential decay curve for Princeton (**a**) (CA, USA; $F = 7.23$, $p = 0.018$, $r^2 = 0.341$, $y = 0.0138e^{-0.0437x}$) and Colusa (**b**) (CA, USA; $F = 14.66$, $p = 0.002$, $r^2 = 0.51$, $y = 0.0218e^{-0.0352x}$).

77% between 1 and 12 h (Fig. 2 and Table 2). Concentrations 1 h after the spray event were significantly higher than those in background samples taken 1 h before the spray event ($F = 28.25$, $p = 0.0001$). No significant difference was found between the background sample concentrations and the concentrations at 36 h after application ($F = 0.43$, $p = 0.52$). A significant exponential decay relationship for the water samples was found ($F = 21.37$, $p = 0.0005$, $r^2 = 0.622$, $y = 0.5661e^{-1.2039x}$) (Fig. 2).

In Colusa, the background concentrations of PBO in water averaged 0.218 μg/L (Table 3). One hour after application, the

Table 3. Mean piperonyl butoxide (PBO) water concentrations from irrigation ditches located in Princeton and static ponds in Colusa (both CA, USA) before and after the spray event

	PBO concn. (μg/L)	
Sample time	Princeton	Colusa
1 h before (background)	0.008 (±0.002)[a]	0.218 (±0.012)
1 h	0.172[b] (±0.036)	1.274[b] (±0.328)
12 h	0.039[c] (±0.012)	0.791 (±0.067)
24 h	0.029[c] (±0.014)	0.366[c] (±0.046)
36 h	0.012[c] (±0.002)	0.073[c] (±0.009)

[a] Values in parentheses are the standard error.
[b] Significantly different than 1 h before application ($p < 0.05$).
[c] Significantly different than 1 h after application ($p < 0.05$).

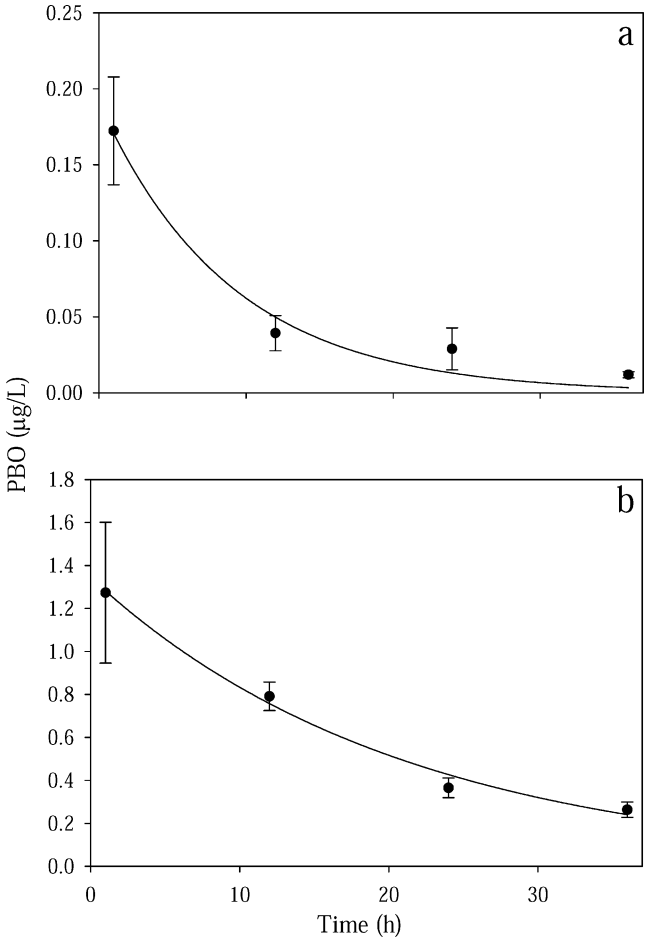

Fig. 2. Change in piperonyl butoxide (PBO) concentrations in water from 1 to 36 h after the spray with exponential decay curve for Princeton (**a**) (CA, USA; $F = 21.37$, $p = 0.0005$, $r^2 = 0.622$, $y = 0.5661e^{-1.2039x}$) and Colusa (**b**) (CA, USA; $F = 11.53$, $p = 0.004$, $r^2 = 0.452$, $y = 1.3446e^{-0.0478x}$).

average concentration was 1.274 µg/L, with the average 36-h concentrations dropping below the background concentration (Table 3 and Fig. 2). Concentrations 1 h after the spray event were significantly higher than those in the background samples taken 1 h before the spray event ($F = 26.25$, $p = 0.0001$). No significant difference was found between background samples and concentrations at 24 h after application ($F = 2.71$, $p = 0.12$). A significant exponential decay relationship for the water samples was found ($F = 11.53$, $p = 0.004$, $r^2 = 0.452$, $y = 1.3446e^{-0.0478x}$) (Fig. 2). A significant time and location (Princeton vs Colusa) effect was found for water samples ($F = 16.85$, 233.5; $p < 0.0001$).

Table 5. Average percentage mortality for *Culex pipiens* and *Culex tarsalis* in bioassay cages in Colusa (treated) and Williams (control; both CA, USA)

Time (h)	Colusa (%)		Williams (%)	
	C. pipiens	*C. tarsalis*	*C. pipiens*	*C. tarsalis*
1	77.16[a] (±9.54)[b]	72.63[c] (±12.86)	0	0
2	81.31[a] (±8.42)	83.57[c] (±7.34)	1.30 (±0.67)	0
12	93.78[a] (±3.49)	91.31[c] (±7.30)	1.30 (±0.67)	0
24	95.5[a] (±2.70)	95.47[c] (±4.53)	1.30 (±0.67)	0

[a] Significantly different than *C. pipiens* control ($p < 0.05$).
[b] Values in parentheses are the standard error.
[c] Significantly different than *C. tarsalis* control ($p < 0.05$).

Pyrethrins were not detected in the background water samples or at 1, 12, and 24 h after application; however, 36 h after application, detectable amounts of jasmolin II (0.213 µg/L) were found in five of the eight subsamples. The demonstrated rapid decline of pyrethrins observed during the present study and the magnitude of the measured concentrations make this unlikely to have been caused by the aerial application.

Sentinel-cage bioassay data for Princeton showed that mortality 1 h after application was 40% for *C. tarsalis* and 22% for *C. pipiens* (Table 4), and mortality at Colusa 1 h after application was 73% for *C. tarsalis* and 77% for *C. pipiens* (Table 5). At 24 h after application, Princeton showed a mortality rate of 86% for *C. tarsalis* and 88% for *C. pipiens* (Table 4), and Colusa showed a 96% mortality rate for both *C. tarsalis* and *C. pipiens* (Table 5). Control mortality at 1, 2, 12, and 24 h after application for both Princeton and Colusa did not exceed 5% (Tables 4 and 5). Mortality at 1, 2, 12, and 24 h after the applications in Princeton and Colusa for both *C. tarsalis* and *C. pipiens* was significantly different than mortality in the control town of Williams ($F = 1{,}404.14$, $p < 0.0001$). A significant time and location (Princeton vs Colusa) effect also was found ($F = 11.93$, $p < 0.0001$ and $F = 6.17$, $p = 0.014$, respectively). No significant difference in mortality between the two mosquito species was found ($F = 0.88$, $p = 0.348$).

DISCUSSION

Similarities were found between the ground and water deposition data from Princeton and Colusa, with concentrations peaking 1 h after application and then decreasing significantly over time in an exponential decay pattern. Pyrethrins were not detected on the deposition pads in either Princeton or Colusa. The present study found ND concentrations of pyrethrins and lower concentrations of PBO with higher mosquito mortality than the values reported by Lothrop et al. [15]. They measured

Table 4. Average percentage mortality for *Culex pipiens* and *Culex tarsalis* in bioassay cages at Princeton (treated) and Williams (control; both CA, USA)

Time (h)	Princeton (%)		Williams (%)	
	C. pipiens	*C. tarsalis*	*C. pipiens*	*C. tarsalis*
1	22.45[a] (±7.19)[b]	40[c] (±11.89)	0	0.34 (±0.34)
2	55.26[a] (±14.79)	57.25[c] (±13.86)	0	0.34 (±0.34)
12	78.37[a] (±9.18)	76.67[c] (±8.18)	1.18 (±0.78)	0.34 (±0.34)
24	85.63[a] (±6.95)	87.88[c] (±6.8)	4.5 (±1.33)	0.80 (±0.53)

[a] Significantly different than *C. pipiens* control ($p < 0.05$).
[b] Values in parentheses are the standard error.
[c] Significantly different than *C. tarsalis* control ($p < 0.05$).

concentrations and efficacy directly under the aircraft and at distances out to 300 m, while we measured concentrations within the spray zone that were 100 to more than 300 m from the aircraft spray path. Lothrop et al. observed an average mortality ranging from only 1.5 to 12% at 1 h after application. Their observed 2-h depositions of PBO were 17- and 9-fold greater than what we observed in Princeton and Colusa, respectively. Lothrop et al. did not report the analytical detection limits, however, and obtained samples at only one time after application.

The significant location effect in water can be explained by the two different water sources sampled in each town. Our results support the findings of Weston et al. [14] of no detectable amounts of pyrethrins in the water after a spray event. Both in the present study and in that of Weston et al., detectable levels of PBO were found in all background samples. The PBO concentrations at Princeton 1 h after application were lower than any concentration that Weston et al. reported in their study. In the Colusa static ponds, 1-h concentrations were lower than those in four of the seven sites sampled by Weston et al., which were sampled from flowing creeks. After 12 h in the Colusa ponds, concentrations were less than what Weston et al. reported for 34 h after a third application by aircraft. Concentrations from Princeton 12 h after application were 11-fold lower than the lowest concentration that Weston et al. reported for 10 h after application. Thirty-six hours after application in Princeton and Colusa, concentrations were 82- and 13-fold less, respectively, than the lowest concentration that Weston et al. reported.

The amount of pyrethrins and PBO deposited on the ground after aerial ULV application was much lower than what was estimated by previous risk assessments using modeled environmental concentrations after truck-mounted ULV applications. The amount of PBO deposited on the ground was 47- to 76-fold less than that estimated by Peterson et al. [4] and by Davis et al. [5] using a tier I model, ISCST3 (http://www.epa.gov/scram001/tt22.htm#screen). Amounts of PBO were two- to threefold less than what Schleier et al. [6] estimated using the model AgDrift® (Stewart Agricultural Research Services, Macon, MO, USA) [23]. This indicates that exposures and concomitant human and ecological risks from aerial applications most likely are much lower than those from ground applications of pyrethrins and PBO.

The insecticides used for adult mosquito management are most toxic to aquatic invertebrates and vertebrates. Using the same toxic end points and surrogate species as Davis et al. [5], the risk quotients (RQs; i.e., environmental concentration/toxic end point) for PBO samples taken 1 h before the application for *Daphnia magna*, rainbow trout (*Oncorhynchus mykiss*), and bluegill sunfish (*Lepomis macrochirus*) were all less than 0.0001 in Princeton and were 0.0004, less than 0.0001, and 0.0001, respectively, in Colusa. The RQs for PBO at 1 h after application in Princeton for *D. magna*, rainbow trout, and bluegill sunfish were 0.0003, less than 0.0001, and less than 0.0001, respectively, whereas in Colusa, the RQs were 0.002, 0.0003, and 0.0007, respectively. The increase in RQs for PBO was well below the U.S. Environmental Protection Agency RQ level of concern of 0.05 for endangered aquatic organisms.

Our results as well as those of previous studies suggest that the risk of PBO and pyrethrins applied aerially for mosquito control is negligible for aquatic vertebrates and invertebrates. Furthermore, our results suggest that levels of PBO in water return to baseline levels by 36 h after application. Because PBO is a synergist for pyrethroid insecticides, however, a potential exists for it to synergize with pyrethroids already present in the aquatic sediment and water column [14,24,25]. Weston et al. [26] found seven different pyrethroid insecticides in creek sediments in Sacramento, most likely occurring from residential use and not from agricultural or mosquito-control practices.

Pyrethrins are unstable compounds, especially in sunlight. Even with spikes above the detection limits of the analytical laboratory, little was recovered from the Princeton spikes, and ND levels were found in Colusa. If PBO is used as a surrogate for potential concentrations of pyrethrins, we would have seen 0.002 and 0.001 $\mu g/cm^2$ at 1 h after application in Princeton and Colusa, respectively. These values are 103- and 205.8-fold less, respectively, than the concentrations modeled in ISCST3 and 6- and 12-fold smaller, respectively, than the concentrations modeled in AgDrift [4–6].

Our results show that the amount of pyrethrins and PBO deposited on the ground and in the water after aerial ULV application is lower than what was estimated by previous risk assessments using modeled environmental concentrations after truck-mounted ULV application. These concentrations decreased with time and, in the case of water, were not significantly different from background levels by 24 to 36 h after the application. Future research should be directed toward developing a model that can better predict both truck-mounted and aerial ULV insecticide applications for adult mosquito management.

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