

Aquatic Effects of Aerial Spraying for Mosquito Control over an Urban Area

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In an effort to combat West Nile Virus, planes dispersed insecticide over Sacramento, CA, treating nearly 50,000 hectares with pyrethrins and the synergist piperonyl butoxide (PBO). Widespread dispersal of insecticide over a metropolitan area, coupled with extensive pretreatment data on the area's urban creeks, provided a unique opportunity to study effects of mosquito control agents on aquatic habitats within an urban setting. There was no evidence of aquatic toxicity from the two active ingredients in the product applied. However, PBO concentrations were high enough to enhance toxicity of pyrethroids already existing in creek sediments from general urban pesticide use. PBO concentrations of 2–4 $\mu\text{g/L}$ were high enough to nearly double the toxicity of sediments to the amphipod *Hyalella azteca*. Though the increase in toxicity was modest, it was unexpected to find environmental synergy at all. Risk assessments for mosquito control agents have focused on the active ingredients but have failed to recognize the potential for interactions with pesticides previously existing in the environment, which in this case appeared to represent a risk to aquatic life greater than that of the active ingredients themselves.

Introduction

Mosquito control usually involves monitoring, public education, elimination of breeding sites, or application of larvicides (1). When these measures prove inadequate, spraying or fogging of adulticides may be done, typically using truck-mounted equipment. Adulticides most commonly used in the United States contain pyrethrins, any of several pyrethroids (permethrin, sumithrin, resmethrin), or organophosphates (malathion, naled). When adulticides contain pyrethrins or pyrethroids, they often also contain piperonyl butoxide (PBO), a compound that is relatively nontoxic but

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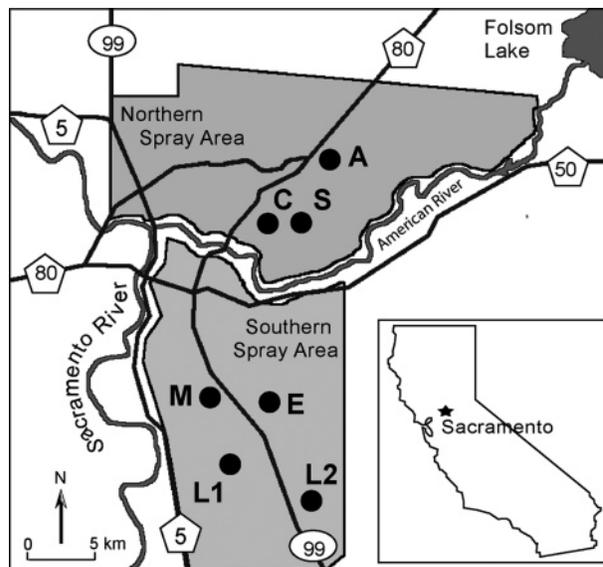


FIGURE 1. Map of the northern and southern spray areas and locations of sampling sites. Inset shows the general location of the study area within California. Sites are labeled as follows: A = Arcade Creek, C = Chicken Ranch Slough, S = Strong Ranch Slough, M = Morrison Creek, E = Elder Creek, L1 = Laguna Creek site 1, and L2 = Laguna Creek site 2.

is a synergist. The synergistic effect of PBO is due to its inhibition of mixed function oxidase activity in the target organism that would otherwise detoxify the pyrethrin or pyrethroid (2). With enzymatic detoxification inhibited, the parent compound persists longer at the site of action, resulting in greater efficacy of the product.

Mosquito control efforts have taken on added significance since the appearance of West Nile Virus in the United States in 1999. In 2005, 3000 human cases in the U.S. were reported to the Centers for Disease Control and Prevention, with 29% of these cases from California alone (3). Efforts to combat West Nile Virus have led to aggressive mosquito control efforts, with spraying where it had not previously been necessary, and use of aircraft to treat broader areas. Though authorities generally reserve widespread aerial application of adulticides for situations with clear public health risks (1), there is often public debate about the relative risks of the virus versus health and environmental risks associated with pesticide exposure.

In August 2005, mosquito control authorities took the unusual step of applying adulticide by air over the densely populated metropolitan area of Sacramento, CA. This effort was sparked by about 2 dozen confirmed cases of West Nile Virus in Sacramento County by early August. This aerial application of insecticide provided an unusual opportunity to assess aquatic effects, since rarely have such studies been performed in urban areas or on applications of this scale.

Materials and Methods

Aerial Application. The insecticide applied over Sacramento was Evergreen Crop Protection EC 60-6, containing 60% PBO and 6% pyrethrins. Planes dispersed 2.8 g pyrethrin per hectare from about 8 p.m. to midnight. North of the American River (Figure 1), 22,000 hectares were repeatedly treated on three consecutive nights (August 8, 9, and 10, 2005), with the same amount of product applied over the same area each night. South of the American River, 27,000 hectares were

partially treated on August 11, but treatment was terminated due to winds after only half the area was covered. Treatment of the southern area resumed, with full coverage of the area on three successive nights (August 20, 21, and 22). The areas treated were primarily residential and commercial.

Field Sampling. Three creeks draining the northern treatment area (Arcade Creek at Auburn Ave., Strong Ranch Slough at Cottage Park, Chicken Ranch Slough at Howe Park; Figure 1) and three creeks draining the southern treatment area (Elder Creek at Gerber Rd., Morrison Creek at Franklin Blvd., Laguna Creek site 1 at Franklin Blvd., and Laguna Creek site 2 at Bond Rd.) were sampled. All creeks were flowing at the time of sampling, except Laguna which was ponded. Mosquito control authorities provided little public notice prior to spraying the northern area, thus it was not possible to obtain preapplication samples. Postapplication sampling of the northern creeks occurred on the morning of August 12, about 34 h after the third application. In the southern creeks, preapplication sampling occurred on August 14, 6 days before the primary insecticide application. Postapplication sampling occurred the morning of August 22, about 10 h after the second of three applications.

Amber glass water sampling bottles were submerged below the surface, collecting some of the surface film as the bottle opening passed through the air–water interface but were primarily filled about 10–20 cm below the surface. Sediments were sampled using a stainless steel scoop to skim the upper 2 cm of sediment, and the samples were stored in glass bottles. Water and sediment samples were held on ice until return to the laboratory where they were stored at 4 °C.

Over the previous year, we had sampled sediments at all of the sites using the same procedures as part of a separate study on pyrethroids in Sacramento creeks (4), and we present some of this historical data for comparison.

Toxicity Testing. Water samples were tested using the U.S. Environmental Protection Agency (EPA) short-term chronic *Ceriodaphnia dubia* test (5). This test consists of exposing test organisms to water samples for the length of time it takes for control treatment females to produce 3 broods (typically 6–8 d), after which effects on survival and reproduction are evaluated. Control water for these tests consisted of a mixture of commercial waters (80% Arrowhead; 20% Evian). Test solutions were replaced daily, and organisms were fed *Selenastrum capricornutum* and Yeast–Cerophyll–Trout Food (YCT). There were 10 15-ml replicates per treatment. Tests were done at 25 °C, on a 16:8 h light–dark cycle. Test data were compared to the control using CETIS statistical software (TidePool Scientific Software, McKinleyville, CA).

Sediment toxicity tests were performed using the amphipod *Hyalella azteca* in 10-d exposures with survival as the endpoint. Testing using standard methods (6) was done in 400 mL beakers containing 75 mL of sediment and 250 mL of water (8 replicates per sample) using 7- to 10-d old individuals. Tests were done at 23 °C, with a 16:8 h light–dark cycle, and daily feeding with YCT. Two volume additions (500 mL) of water were supplied daily to each beaker. All tests included control sediment from a drinking water reservoir containing 0.21% organic carbon.

Two laboratory experiments were done to determine the effect of PBO on toxicity of sediment-sorbed pyrethroids. Sediment collected from Strong Ranch Slough in the northern spray area was tested and found to cause near total mortality of *H. azteca*. A dilution series was done with five dilution levels between 6% and 0.4% Strong Ranch Slough sediment, using control sediment as the diluent, and four replicate beakers per dilution step. The two sediments were thoroughly mixed by hand and held 24 h before use. LC₅₀s were determined concurrently with 0, 4, and 25 µg/L PBO (Sigma

Chemical, St. Louis, MO) in overlying water. Approximately 80% of the overlying water was removed daily and replaced with fresh PBO solution at the appropriate concentration. PBO additions done this way yield concentrations that match the nominal values immediately after water exchange, but over the 24 h between PBO renewals, approximately 30% of the PBO is lost, presumably to photodegradation (7). Thus, for example, the nominal 4 µg/L PBO treatment fluctuated between about 2.7 and 4 µg/L daily. These tests included a solvent control containing the methanol carrier solvent (10 µL/L) for PBO.

In a second laboratory experiment, control sediment was spiked with bifenthrin (Chem Service, West Chester, PA). Bifenthrin in acetone was added to the sediment (0.9 mL/kg) and blended using a paint mixing attachment in an electric drill (7, 8). The material was aged at 4 °C for 4 weeks before use. Five concentrations were used from 0.25 to 3.3 µg/kg, with four replicates per concentration, to determine the bifenthrin 10-d LC₅₀ to *H. azteca*. The concentration of the bifenthrin spike was analytically verified (methods below), and was 95% of nominal. Toxicity tests with bifenthrin-spiked sediment were performed concurrently with 0, 4, and 25 µg/L PBO in the overlying water, with daily water exchanges. The solvent control contained the methanol solvent for PBO and the acetone carrier for bifenthrin.

Toxicity data were analyzed using ToxCalc Version 5.0 (Tidepool Scientific Software). Dunnett's Multiple Comparison test was used with arcsin squareroot transformation when necessary to meet parametric assumptions. If assumptions were not met after transformation, comparison to the control was done using Steel's test. LC₅₀ values were determined using the probit method.

Analytical Chemistry. Water samples were extracted by two methods. In the first method, following Antonious et al. (9), a 500–1000 mL aliquot was extracted on an AccuBond II ODS-C18 cartridge (Agilent Technologies, Palo Alto, CA) conditioned with 5 mL of methanol and 5 mL of deionized water. Extraction occurred within 24 h of collection. Cartridges were held at –20 °C until analysis, then eluted with methanol. The remaining sample was held in the dark at 4 °C for 10–20 days (awaiting results from the C18 fraction), and then liquid–liquid extracted using dichloromethane. The dichloromethane extract was concentrated and solvent exchanged to methanol. Both extract types were analyzed for pyrethrins and PBO, with results nearly identical by the two approaches, and very similar to independent data collected by the Mosquito Control District, as discussed below. We present data from the liquid–liquid extraction because it provided better pyrethrin recoveries in quality assurance samples. Analysis was done using an Agilent 1100 liquid chromatograph–mass spectrometer (LC–MS) quadrupole system coupled to a diode array UV–Vis detector. Target analytes were separated with a Phenomenex C18 column (125 mm × 4.6 mm i.d. × 5 µm) using gradient elution with methanol/water fortified with 5 mM formic acid. Selected ion monitoring and total ion chromatograms were simultaneously collected. A multi-point calibration curve for quantitation ranged from 5 to 1000 µg/L, using certified standards from Chem Service. Positively identified PBO in extracts were further confirmed with a Varian Saturn 2000 gas chromatograph–mass spectrometer–ion trap detector (Varian, Palo Alto, CA) operated in MS–MS mode. Lab control spikes and matrix spikes yielded recoveries of pyrethrins and PBO ranging from 87 to 112% (liquid–liquid extraction). Duplicates were within 5% of mean values.

We report concentrations of pyrethrins I as the sum of the esters of chrysanthemic acid (pyrethrin I, cinerin I, and jasmolin I). Similarly, pyrethrins II is the sum of the three esters of pyrethric acid. The general term “pyrethrins” is used to refer to the sum of pyrethrins I and II.

TABLE 1. Concentrations ($\mu\text{g/L}$) of Insecticides in Water Samples Before and After Aerial Insecticide Application^a

sampling site	PBO		Diazinon	
	before	after	before	after
Northern				
Arcade Creek	ns ^b	0.98	ns	0.02
Strong Ranch Slough	ns	2.10	ns	U ^c
Chicken Ranch Slough	ns	1.25	ns	U
Southern				
Morrison Creek	0.20	2.52	U	U
Elder Creek	0.20	0.44	U	U
Laguna Creek site 1	U	3.92	U	U
Laguna Creek site 2	U	0.56	0.07	0.04

^a Pyrethrins I, pyrethrins II, and chlorpyrifos were not detected above the reporting limits (0.01, 0.01, and 0.05 $\mu\text{g/L}$, respectively). ^b The designation "ns" indicates no sample available. ^c U indicates concentrations below reporting limits: PBO = 0.01 $\mu\text{g/L}$, diazinon = 0.02 $\mu\text{g/L}$.

Water samples were also analyzed for the organophosphates chlorpyrifos and diazinon using the methanol eluent from the C18 cartridge described above. Analysis was on an Agilent 6890 gas chromatograph with dual capillary columns (DB5 and DB 17MS, Agilent Technologies) and dual flame photometric detectors in phosphorus mode. Samples were injected with an Agilent 7683 autosampler. Instrument calibration was based on peak area using external standard mode with concentrations from 5 to 500 $\mu\text{g/L}$.

Sediments were analyzed for seven pyrethroids, chlorpyrifos, pyrethrins, and PBO. Sediment samples were sonicated with a solution of acetone and dichloromethane and the extracts were cleaned with deactivated Florisil. Pyrethroid and chlorpyrifos analysis, following You et al. (10), was performed on an Agilent 6890 gas chromatograph with an Agilent 7683 autosampler, an electron capture detector, and two columns (HP-5MS, DB-608; Agilent Technologies). Qualitative identity was established using a 1% retention window with confirmation on a second column, and calibration was based on area using external standards at concentrations from 10 to 100 $\mu\text{g/L}$. Pyrethroid and chlorpyrifos recoveries for the technique were 72–130%, with relative standard deviation of <11% (9). The sediment extract was also used for PBO and pyrethrins analysis by LC–MS as described above.

Total organic carbon was determined on a CE-440 elemental analyzer (Exeter Analytical, Chelmsford, MA), following acid vapor treatment to remove inorganic carbon.

Results

Water Column Toxicity. No water samples caused significant mortality of *C. dubia* after 7 d exposure. Survival in controls was 100% and >90% in each creek sample. Three of eleven samples caused a significant reduction in reproduction. Laguna site 1 reduced neonate production by 21–38% relative to controls, both before and after insecticide application. Elder Creek caused a slight reduction (18%) in neonate production in the postapplication sample.

Water Column Chemistry. The insecticide applied contained two active ingredients, pyrethrins and PBO. No water samples collected before or 10–34 h after spraying had detectable pyrethrins (Table 1). Results are comparable to data collected by the local Mosquito Control District (D. Brown, unpublished data) in which none of 14 water samples prior to spraying had detectable pyrethrins (<0.2 $\mu\text{g/L}$). A few hours after spraying, 35% of the samples contained measurable residues (up to 3.8 $\mu\text{g/L}$), but pyrethrin was undetectable 16 h after treatment at all sites.

PBO occurred in all water samples except for the preapplication samples from Laguna Creek. Preapplication

TABLE 2. Percent Survival (Mean and Standard Deviation) of *H. azteca* in 10-d Toxicity Tests Before and After Aerial Application of Insecticide^a

sample site	preapplication survival (%)	postapplication survival (%)	historical survival (%)
control sediment	99 ± 4	99 ± 4, 98 ± 7	
Northern			
Arcade Creek	ns ^b	71 ± 15*	2*, 47*, 62*
Strong Ranch Slough	ns	10 ± 16*	19*, 21*, 83*
Chicken Ranch Slough	ns	83 ± 21	61*, 67*, 90
Southern			
Morrison Creek	63 ± 23*	59 ± 19*	64*, 87, 92
Laguna Creek site 1	84 ± 7	99 ± 3	89, 89, 92

^a Asterisk indicates statistically significant difference from control. "Historical survival", taken from Amweg et al. (4), is intended to illustrate typical conditions for the site, and refers to three samples at each site collected between August 2004 and March 2005, at least five months before aerial treatment. No data are available for Elder Creek or Laguna Creek site 2 due to lack of sufficient quantities of soft sediment. ^b The designation "ns" indicates no sample available from the northern area prior to insecticide application.

samples from Elder and Morrison Creeks contained 0.2 $\mu\text{g/L}$ PBO. It is unknown if these low levels in the southern creeks were from the aborted partial treatment of the area 3 days earlier, or if they originated from general urban use of PBO-containing products. The Mosquito Control District's own sampling (D. Brown, unpublished data) found no PBO in 14 samples collected prior to aerial spraying, but their reporting limit was relatively high (1 $\mu\text{g/L}$).

After aerial spraying, PBO was detected in every creek sample, with concentrations ranging from 0.44 to 3.92 $\mu\text{g/L}$. There were no obvious differences between samples collected 34 h after spraying (northern creeks; 0.98–2.10 $\mu\text{g/L}$) and samples collected only 10 h after spraying (southern creeks; 0.44–3.92 $\mu\text{g/L}$). These results are similar to the District's postapplication sampling that reported PBO concentrations of about 4 $\mu\text{g/L}$ in four of 10 creeks, and 20 $\mu\text{g/L}$ in one creek (D. Brown, unpublished data).

Diazinon and chlorpyrifos were analyzed because they have reached levels toxic to *C. dubia* in some of the creeks under study (11). Chlorpyrifos was below the reporting level in all samples (<0.05 $\mu\text{g/L}$). Diazinon was found at low levels (0.02–0.07 $\mu\text{g/L}$) in 3 of 11 samples. These levels are 5–15% of the LC₅₀ of 0.44 $\mu\text{g/L}$ for *C. dubia* (based on 48–96 h exposures; (12)).

Sediment Toxicity. Survival in control sediments exceeded 97%. Significant mortality to *H. azteca* was seen in many posttreatment sediment samples (Table 2), but historical data (4) showed toxicity at some sites prior to mosquito spraying. In the northern spray area, sediments in Arcade Creek and Strong Ranch Slough were acutely toxic after aerial spraying, but historical data indicate that these sites were toxic in previous samplings. Chicken Ranch Slough sediments were not toxic, but had been intermittently toxic prior to spraying.

In the southern spray area, soft sediments were only available at two sites: Morrison Creek and Laguna site 1. The Morrison site caused significant mortality after aerial spraying, but had also been toxic before spraying. No toxicity was observed at Laguna site 1, before or after spraying.

Sediment Chemistry. Six pyrethroids from urban pesticide use unrelated to aerial spraying were found in creek sediments (Table 3). Bifenthrin concentrations alone were high enough to explain observed *H. azteca*. The 10-d LC₅₀ of bifenthrin to *H. azteca* is 0.52 $\mu\text{g/g}$ organic carbon (8), and the three sites showing post-spraying toxicity (Arcade, Strong Ranch, Morrison) contained bifenthrin at 0.8, 2.3, and 0.7 times the LC₅₀, respectively. Chicken Ranch Slough contained bifenthrin at 1.1 times the LC₅₀ and showed depressed survival

TABLE 3. Concentration of Pyrethroids, Pyrethrins I, and PBO in Creek Sediments ($\mu\text{g}/\text{kg}$ Dry Weight)^a

sampling site	Bifenthrin	Cyfluthrin	Cypermethrin	Deltamethrin	Permethrin	Pyrethrins I		PBO	
						before	after	before	after
Northern									
Arcade Creek	3.7 (2.5–15.0)	3.8 (1.1–5.9)	U ^c (U-1.1)	U (U-2.8)	7.0 (5.6–16.9)	ns ^b	U	ns	U
Strong Ranch Slough	48.0 (8.0–89.8)	26.1 (2.0–26.3)	4.3 (U-15.0)	4.6 (U-6.5)	35.7 (9.5–93.9)	ns	403	ns	34.0
Chicken Ranch Slough	45.1 (2.9–9.7)	6.6 (U-4.9)	U (0–4.1)	6.3 (U-1.6)	33.2 (U-15.8)	ns	239	ns	61.4
Southern									
Morrison Creek	13.4 (14.8–25.9)	9.1 (U-19.2)	4.3 (6.5–9.5)	4.5 (3.4–5.4)	31.0 (26.1–73.0)	U	93.1	U	19.2
Laguna Creek site 1	U (3.2–4.1)	U (U)	U (U)	U (U)	1.0 (U-5.6)	U	372	U	15.9
Laguna Creek site 2	1.6 (13.8)	U (3.9)	U (7.2)	U (2.7)	U (30.0)	ns	U	ns	U

^a Pyrethroid concentrations, which were unaffected by the aerial spraying, are shown for the postapplication samples, and in parentheses, the range of about four previous samples at the site, in the year prior to treatment (this study and Amweg et al. (4)). Data for pyrethrin and PBO, the active ingredients in the spray, are provided both before and after aerial insecticide application. Esfenvalerate and pyrethrins II were not detected above the reporting limits ($1 \mu\text{g}/\text{kg}$), lambda-cyhalothrin was found in only one sample ($6.6 \mu\text{g}/\text{kg}$ in Strong Ranch Slough), and chlorpyrifos was just above the reporting limit in a few samples. No data are available for Elder Creek due to lack of sufficient quantities of soft sediment. ^b The designation “ns” indicates no sample available. ^c U indicates pyrethroid, pyrethrin or PBO concentrations below reporting limit of $1 \mu\text{g}/\text{kg}$.

in some replicates, but was not statistically different from the control.

Pyrethrins were not detectable before spraying in the two creeks for which pre-spray data were available (Morisson and Laguna; Table 3). However, 8 days later, immediately following aerial pyrethrin application, sediments in these creeks contained 93 and 372 $\mu\text{g}/\text{kg}$, respectively. Two of three northern creeks also contained $>200 \mu\text{g}/\text{kg}$ pyrethrins in posttreatment samples. These data are for pyrethrins I, which are 10 times more hydrophobic than pyrethrins II (9). Pyrethrins II were not detected in sediment.

PBO was not detected in sediments collected just before aerial spraying (Morisson and Laguna), and was also not detected at the Arcade site 10 months earlier (4). After spraying, PBO was detected at 16–61 $\mu\text{g}/\text{kg}$ in 4 of 6 samples; these 4 samples also contained pyrethrins.

Synergistic Effects of PBO. We found a maximum PBO concentration of 3.9 $\mu\text{g}/\text{L}$ (Table 1). The Mosquito Control District reported similar concentrations in five creeks after spraying (D. Brown, unpublished data). Therefore, 4 $\mu\text{g}/\text{L}$ was selected as an environmentally relevant concentration to test the potential for PBO to synergize pyrethroids present in creek sediments. As noted above, this nominal concentration produces actual concentrations in toxicity tests ranging from 2.7 to 4 $\mu\text{g}/\text{L}$.

A toxicity test with the postapplication sample from Strong Ranch Slough showed high toxicity (10% survival; 0% in a retest). A dilution series with control sediments indicated a 10-d LC_{50} to *H. azteca* slightly above the highest concentration (6%) of Strong Ranch sediment used ($62 \pm 10\%$ survival at 6% Strong Ranch sediment). When the same dilution series was tested with 4 $\mu\text{g}/\text{L}$ PBO in the overlying water, the LC_{50} decreased to 3.0% (C.I. = 2.4–3.6%; $15 \pm 13\%$ survival at 6% Strong Ranch sediment). Thus, it appears the concentration of PBO in creek waters was capable of approximately doubling toxicity of the sediments. An increase in PBO concentration to 25 $\mu\text{g}/\text{L}$ (slightly above the highest level of 20 $\mu\text{g}/\text{L}$ observed in Mosquito Control District’s sampling) caused a further reduction in the LC_{50} to 1.7% Strong Ranch Slough sediment (C.I. = 1.3–2.0%; $3 \pm 6\%$ survival at 6% Strong Ranch sediment).

Bifenthrin has been shown to be the major pyrethroid contributor to *H. azteca* toxicity in these creeks (4). To quantify the interaction between PBO and bifenthrin, control sediment was spiked with bifenthrin over a range of concentrations. With no PBO present, a 10-d LC_{50} was 1.3 $\mu\text{g}/\text{kg}$ (C.I. = 1.1–1.5). When expressed relative to the organic carbon (OC) content of the sediment, this value is equivalent to 0.62 $\mu\text{g}/\text{g}$ OC (C.I. = 0.52–0.71), comparable to 0.52 $\mu\text{g}/\text{g}$ OC reported in the literature (8). Addition of 4 $\mu\text{g}/\text{L}$ PBO to the overlying water produced a reduction in the LC_{50} to 0.38 $\mu\text{g}/\text{g}$ OC (C.I.

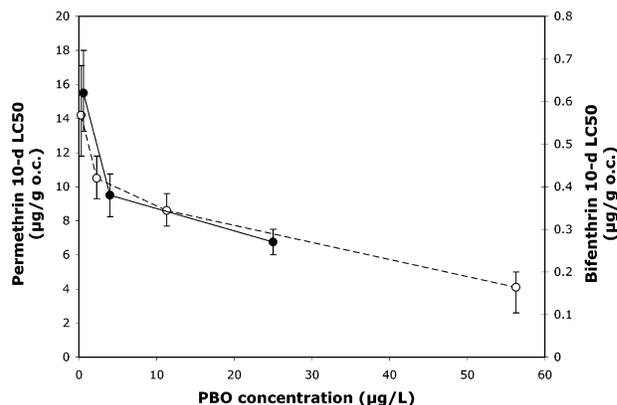


FIGURE 2. Influence of PBO concentration in the water column on sediment LC_{50} s of permethrin (open circles) and bifenthrin (filled circles) using 10-d *H. azteca* toxicity tests. Error bars indicate 95% confidence interval of the LC_{50} values. LC_{50} values at 0 $\mu\text{g}/\text{L}$ PBO have been moved slightly off the y-axis so as not to obscure the error bars. Permethrin data from Amweg et al. (7).

= 0.33–0.43). An additional increase in PBO concentration in the overlying water to 25 $\mu\text{g}/\text{L}$ further increased toxicity, reducing the LC_{50} to 0.27 $\mu\text{g}/\text{g}$ OC (C.I. = 0.24–0.30). The effect of PBO on toxicity of bifenthrin closely parallels the previously reported response of *H. azteca* to permethrin in the presence of PBO (Figure 2) (7).

Toxicity units (TU) have proven useful in assessing the potential for pyrethroid-associated toxicity (13) and can also be used to illustrate the potential effect of PBO in Sacramento creeks on pyrethroid toxicity of sediments. TUs were calculated as follows:

$$\text{TU} = \frac{\text{actual pyrethroid concentration on a sediment OC-normalized basis}}{H. azteca \text{ LC}_{50} \text{ for the pyrethroid on an OC-normalized basis}}$$

TUs for each pyrethroid were determined using published LC_{50} values (8, 14), and then summed assuming additivity since pyrethroids all have a similar mode of neurotoxic action. The bifenthrin data of Figure 2 were used to estimate how actual PBO concentrations observed in creek waters could have altered the apparent TUs. Given the similarity observed in the permethrin and bifenthrin toxicity response to PBO, the synergistic effect of PBO on bifenthrin (Figure 2) was assumed to be similar for all other pyrethroids found in creek sediments. This approach should be viewed as an approximation since it includes these assumptions: the nominal 4 $\mu\text{g}/\text{L}$ PBO concentration in laboratory tests yielded a

TABLE 4. Estimated Effect of the Measured PBO Concentrations on the Toxicity of Sediment-Associated Pyrethroids in the Sacramento Creeks^a

sampling site	observed PBO ($\mu\text{g/L}$)	observed <i>H. azteca</i> toxicity	Pyrethroid TUs without PBO	Pyrethroid TUs adjusted for observed PBO
Northern				
Arcade Creek	0.98	yes	1.2	1.4
Strong Ranch Slough	2.10	yes	3.4	4.5
Chicken Ranch Slough	1.25	no	1.4	1.7
Southern				
Morrison Creek	2.52	yes	1.5	2.3
Laguna Creek site 1	3.92	no	0.1	0.2
Laguna Creek site 2	0.56	no data	0.1	0.1

^a Pyrethroid toxicity units (TUs) are an average of data before and after insecticide application, when available. The analysis does not include pyrethrin TUs and any effect of PBO on its toxicity.

constant actual concentration of 3 $\mu\text{g/L}$; PBO measured in creeks persisted at that level for 10 d (discussed below); and the effect of PBO on bifenthrin toxicity was linear between 0 and 4 $\mu\text{g/L}$, the concentrations used. This approach does not include TUs due to pyrethrins in the sediment since their *H. azteca* LC_{50} is unknown.

This analysis (Table 4) indicates that most sediments contained concentrations of pyrethroids acutely lethal to *H. azteca* due to urban usage of pyrethroids unrelated to mosquito control actions (4). In 4 of 5 samples, the observed presence or absence of toxicity could be predicted based on pyrethroid concentration alone. The one exception, Chicken Ranch Slough, with 1.4 TU but no toxicity, cannot be explained. The TU approach is highly predictive of pyrethroid-related toxicity although outliers exist (4), presumably due to unquantified factors affecting bioavailability.

The presence of PBO in the overlying water was likely sufficient to increase toxicity at most sites. At Laguna site 1 PBO concentrations were high enough to double the TUs, however this site had low initial pyrethroid TUs in the sediment, so the actual increase in toxicity was negligible. The two sites where PBO synergy may have been most significant were Strong Ranch Slough and Morrison Creek, both of which had substantial levels of pyrethroids in the sediment and enough PBO in the overlying water to increase pyrethroid toxicity by about 1.5 times.

Discussion

The low risk for pyrethrin toxicity in the water column is indicated by the absence of pyrethrins in water as little as 10 h after aerial application and by the lack of *C. dubia* mortality. Pyrethrin absence in water may in part have been due to photodegradation, which is quite rapid for pyrethrins (15). However, adsorption to bed sediments was an additional route of loss. Sediment concentrations of pyrethrins in Sacramento creeks increased from <1 $\mu\text{g/kg}$ before treatment to about 400 $\mu\text{g/kg}$ in some samples after spraying. Pyrethrins I were present in sediment at far higher concentrations than pyrethrins II, consistent with the reported K_{oc} values of 26 915 for pyrethrin I and 2042 for pyrethrin II (9). The sediment concentration of pyrethrins that is acutely lethal to *H. azteca* is unknown, but some of the higher pyrethrin concentrations (239 and 372 $\mu\text{g/kg}$) were seen in sediments with no toxicity. When toxicity occurred, pyrethroids in the creek unrelated to mosquito abatement were sufficient to explain it. While we have no evidence of pyrethrin-related sediment toxicity, our data indicate that monitoring for pyrethrins after application of mosquito adulticides should include bed sediments.

Aerial spraying resulted in the appearance of PBO in water (to about 4 $\mu\text{g/L}$) and in sediments (to about 60 $\mu\text{g/kg}$). These concentrations do not appear to represent a direct risk to organisms in the creeks. PBO concentrations up to 375 $\mu\text{g/L}$ have been used with *H. azteca* (16), and concentrations of 200 $\mu\text{g/L}$ have been used with *C. dubia* (17) without apparent effects. PBO LC_{50} s for aquatic life are generally in the low ppm range (16, 18), 1000-fold higher than those seen in Sacramento creek waters. Toxicity thresholds for PBO in sediment are less documented, although 313 $\mu\text{g/kg}$, about five times higher than those seen in the Sacramento sediments, has been used with *H. azteca* without acute effects (7).

The greatest aquatic risk of aerial application of insecticide was not toxicity of pyrethrins or PBO individually, but was the synergy between PBO and preexisting pyrethroids in creek sediments. PBO concentrations of 2–4 $\mu\text{g/L}$ were widespread in Sacramento creeks after aerial spraying. In laboratory tests, a nominal concentration of 4 $\mu\text{g/L}$ (2.7–4 $\mu\text{g/L}$ actual) was sufficient for a 1.6-fold increase in toxicity of bifenthrin, the pyrethroid primarily responsible for *H. azteca* toxicity in creek sediments. This PBO concentration was sufficient to approximately double the toxicity of Strong Ranch Slough sediment in laboratory tests.

The observed doubling of toxicity due to synergistic effects of PBO in the creeks is relatively modest. A greater synergistic effect would be expected with higher concentrations of PBO, because the synergistic effect is proportional to the logarithm of the PBO concentration (19). Work with a variety of insects using more synergist as would be employed in pesticide formulations have commonly shown two- to 20-fold increases in the toxicity of pyrethrins or pyrethroids in the presence of PBO (20). The important finding from Sacramento creeks is not the magnitude of the synergy, but that synergy was observed at all with environmentally realistic PBO concentrations.

Uncertainty about the duration of exposure results in uncertainty about the actual effects of PBO in Sacramento creeks. We used standard 10-d exposures for *H. azteca* sediment toxicity tests (6), but it is unknown how long PBO persisted in Sacramento creeks. Aerial spraying occurred over three successive nights, and there were no appreciable differences in concentrations between samples taken 10 h after spraying and those taken 34 h after spraying. While it is likely that concentrations of at least 2–4 $\mu\text{g/L}$ persisted for the 3 days of spraying and about 2 days afterward, no data are available over longer times. With time, PBO concentrations are likely to decline due to photodegradation and adsorption to sediment. Field applications have shown PBO half-lives in water of 0.55–1.64 d, and up to 24 d for sediment-associated residues (21).

An earlier assessment of the potential for PBO synergy of environmental pyrethroids (7) concluded it was unlikely, although the authors noted their conclusion was based on minimal data on environmental PBO concentrations. The concentrations we observed in Sacramento creeks were far greater than those in the earlier study. The earlier study's conclusion of low potential for synergy probably remains valid in most cases, but several factors combined to increase the risk in the Sacramento instance. First, the scale of application was atypical. Truck-mounted foggers often disperse adulticides, a method that limits the area treated. In Sacramento, dispersal by plane allowed treatment of 50,000 hectares, and reapplication over the area for three successive nights. Second, the area treated was heavily urbanized, and runoff from landscape irrigation and washing of outdoor furniture and similar surfaces (as residents were advised to do by the Mosquito Control District) may have increased PBO in the creeks beyond that deposited directly on the water surface. Finally, a synergistic effect requires a

synergizable insecticide in the aquatic environment. Most Sacramento creek sediments were already acutely toxic to *H. azteca* due to pyrethroids before aerial spraying, but addition of PBO may have increased the risk to less sensitive species.

Not surprisingly, the primary emphasis in risk assessments for mosquito adulticides has been toxicity of the insecticide to nontarget organisms. Risk assessments of PBO have concluded it presents little aquatic risk due to its low toxicity and persistence. However, published risk assessments (22) and EPA's PBO risk assessment (23) have failed to consider the potential for PBO to enhance toxicity of insecticides already in the environment. It has recently become apparent that pyrethroids or other compounds synergized by PBO (e.g. carbamates, (24)) can be found in urban creeks (4, 25). Our data indicate the potential for aquatic toxicity resulting from the synergistic effect of PBO on pyrethroids that were already in creek sediments. Sacramento creeks are among the few in the United States that have been analyzed for pyrethroids. Further study is required to determine if pyrethroid residues are present in other urban creeks and whether synergy could be expected elsewhere.

Acknowledgments

This work was partially supported by the Sacramento River Watershed Program and the National Institutes for Water Resources and U.S. Geological Survey National Competitive Grants Program.

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Received for review January 23, 2006. Revised manuscript received June 19, 2006. Accepted June 21, 2006.

ES0601540