

EFFECT OF PIPERONYL BUTOXIDE ON PERMETHRIN TOXICITY IN THE AMPHIPOD
HYALELLA AZTECA

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Abstract—Piperonyl butoxide (PBO) is a synergist of pyrethroid pesticides found in many products for structural pest control, mosquito control, and home and garden uses. Because both PBO and pyrethroid residues potentially co-occur in urban creeks, this study determined if environmental levels of PBO were capable of synergizing pyrethroids in the environment. Three types of toxicity tests were conducted with the amphipod *Hyaella azteca* to determine the minimum PBO concentration required to increase toxicity of the pyrethroid permethrin: Sediment was spiked with permethrin only; permethrin and overlying water spiked with PBO; and permethrin, PBO, and overlying water spiked with PBO. In tests with PBO added to both water and sediment, PBO concentrations of 2.3 µg/L in water and 12.5 µg/kg in sediment reduced the permethrin median lethal concentration (LC50) nearly 50% to 7.3 mg/kg organic carbon (OC). Higher concentrations of PBO increased permethrin toxicity up to sevenfold. In exposures with PBO in water alone, 11.3 µg/L was required to increase permethrin toxicity. Urban creek sediments from California and Tennessee, USA, had PBO concentrations in the low µg/kg range; only one water sample was above the detection limit of 0.05 µg/L. Wetlands in northern California also were sampled after application of pyrethrins and PBO for mosquito abatement. Sediment and water PBO concentrations within 12 h of abatement spraying peaked at 3.27 µg/kg and 0.08 µg/L, respectively. These results suggest that environmental PBO concentrations rarely, if ever, reach concentrations needed to increase pyrethroid toxicity to sensitive organisms, though available data on environmental levels are very limited, and additional data are needed to assess definitively the risk.

Keywords—Pyrethroid Piperonyl butoxide *Hyaella azteca* Synergism

INTRODUCTION

Piperonyl butoxide (PBO) was first reported as a pyrethrin synergist in 1947. Today, it also is used to enhance toxicity of the newer pyrethroid pesticides, which increasingly are used for many applications since the recent withdrawal of nearly all products for residential use in the United States containing the organophosphates diazinon and chlorpyrifos. Reported commercial use of PBO (excludes retail sales) in California, USA, in 2003 totaled over 18,000 kg (www.cdpr.ca.gov). This total includes applications for structural pest control (6,900 kg), mosquito control (7,330 kg), and landscape maintenance (200 kg). For example, Drione® is used widely for structural pest control, and contains 1% pyrethrins and 10% PBO. Scourge® is used for control of adult mosquitoes, and contains 18% resmethrin and 54% PBO. Piperonyl butoxide also is used to synergize agricultural pyrethroids. However, there was 127,500 kg of pyrethroids used in California agriculture in 2003, but only 2,700 kg of PBO, so it is clear that most of the agricultural pyrethroids are applied without a PBO synergist.

Piperonyl butoxide itself is not particularly toxic to animals; typical 96-h median lethal concentrations (LC50s) are in the low ppm range: 0.53 mg/L for *Hyaella azteca*, 2.74 mg/L for *Chironomus tentans*, and 3.54 mg/L for *Lumbriculus varie-*

gatus [1]. Erickson [2] reported a 96-h LC50 of 11.2 mg/L for rainbow trout, and 4.2 mg/L was reported for bluegill over the same test duration [3]. The toxicological significance of PBO lies in its ability to inhibit the mixed function oxidase enzymes, blocking natural detoxification pathways. Thus, PBO can enhance toxicity of compounds, such as pyrethroids, that are degraded by this pathway [4,5] and reduce toxicity of some organophosphate pesticides that require activation by mixed function oxidases [6]. Due to these distinctive effects, PBO also is used as a tool to identify certain types of pesticide toxicity in toxicity identification evaluations [7].

Technical-grade PBO is a yellow oil, soluble in water, with a log K_{ow} of 4.75. It is relatively stable in water-sediment systems: In the dark, 91% of the parent compound remained after 181 d under anaerobic conditions. In the presence of oxygen, 72% remained after 30 d in the dark. Soil degradation rates were much faster, however, with half-lives ranging from 1 to 4 d. Exposure to sunlight also rapidly increases degradation of PBO. In a neutral, aqueous solution exposed to sunlight, the half-life of PBO is just a few hours ([8]; www.ipfsaph.org). Piperonyl butoxide is detectable in surface waters, though there have only been a few attempts to measure concentrations in the environment. Piperonyl butoxide concentrations ranging from below the detection limit of 3.3 ng/L to 48.4 ng/L were measured in California rivers [9–11]. A concentration of 1,600 ng/L was reported from a single sample in a region of Spain known for rice and fruit production, though

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all other samples collected in that study were below the 50-ng/L detection limit [12]. In suspended and bed sediments, PBO was undetectable at 1.2 $\mu\text{g}/\text{kg}$ in rivers from the Salton Sea watershed [13]. The only other known published PBO sediment data are from application to an experimental soil plot. Piperonyl butoxide concentrations dropped rapidly from 840 $\mu\text{g}/\text{kg}$ immediately after application to 3 $\mu\text{g}/\text{kg}$ in 30 d [14].

Data also are emerging on environmental concentrations of pyrethroid pesticides. In a survey throughout the Central Valley of California, an area of intensive agriculture, pyrethroids were found in 86% of the sediment samples [15]. In nearly 70% of those samples showing acute toxicity to *Hyalella azteca*, pyrethroid concentrations were high enough to account for it, even without considering potential synergism of PBO. In the Salton Sea watershed, permethrin, lambda-cyhalothrin, and bifenthrin were detected occasionally in suspended and bed sediments at concentrations ranging from above the detection limit (0.5–1.4 $\mu\text{g}/\text{kg}$) to 18.7 $\mu\text{g}/\text{kg}$ [13]. Since 1991, the U.S. Geological Survey's National Water Quality Assessment has analyzed bed sediments from over 2,600 sites for permethrin; nine sites across the USA were above the detection limit (usually 5 $\mu\text{g}/\text{kg}$) with permethrin concentrations reaching 33.6 $\mu\text{g}/\text{kg}$ (www.usgs.gov). Data are minimal on the distribution of pyrethroid pesticides in urban systems. Recent sampling around suburbs of Sacramento, California has found several pyrethroids, and particularly bifenthrin, in sediments of urban creeks often at concentrations sufficient to cause toxicity to sensitive invertebrates [16]. In addition, the amount of pyrethroids currently used in California for structural pest control, landscape maintenance, and public health applications is nearly twice that used in agriculture. This total does not include consumer home and garden use for pest control (it is not tracked by the State's Pesticide Use Reporting database), and therefore it seems likely that sediments in urban and suburban neighborhoods throughout California and potentially other portions of the United States may contain detectable pyrethroid concentrations.

This study was designed to determine PBO concentrations required to synergize toxicity of pyrethroids and whether or not these PBO concentrations are found in the environment. We address these issues by establishing the minimal PBO concentration required to increase permethrin toxicity to the amphipod *H. azteca*. Sediment LC50s for permethrin in spiked reference sediment were determined under three PBO exposure scenarios: No PBO, PBO exposure via water alone, and PBO spiked into both water and sediment at steady state concentrations. Additionally, sediment and water samples from urban creeks in northern California and Tennessee, USA, were collected and analyzed for PBO to determine typical urban concentrations. Wetlands in northern California sprayed with a mosquito adulticide containing PBO also were sampled shortly after application to determine PBO concentrations in water and sediment.

MATERIALS AND METHODS

Sample collection

Reference sediment for use in spiking experiments was collected in October 2003 from the South Fork of the American River (CA) about 2 km west of the confluence with Weber Creek, in Placer County near Folsom Lake. Sediment was collected by skimming the top 2 to 3 cm of sediment and sieving the material on a 1-mm screen. Sediment passing

through the screen was homogenized and frozen until use. Chemical analysis showed no detectable concentrations (<1 $\mu\text{g}/\text{kg}$) of pyrethroids and the material had an organic carbon (OC) content of 1.87%.

Sediment and water samples were collected for PBO analysis from six urban creeks in the San Francisco Bay (CA, USA) area and sediments were collected from 16 sites in Sacramento, California, and Nashville, Tennessee, USA. In the San Francisco Bay region, water samples were collected in April 2004 by filling 4-L glass jars after an initial rinse with creek water. Sediment was collected at least twice: Once in late spring (April 2004) and again after the first fall storm event (October 2004). Two Sacramento, California, creeks and 14 sites from 12 creeks in Nashville also were sampled once in 2004. The surficial 2 to 3 cm of sediment was collected using a stainless steel scoop. Sediments and water samples were transported on ice to the laboratory. Water samples were extracted immediately onto C18 solid-phase extraction cartridges (Agilent Technologies, Palo Alto, CA, USA) using 500 ml of unfiltered sample. Sediments were stored at 4°C for up to one week before homogenization using a stainless steel bowl and spoon. The C18 cartridges and sediment samples were frozen at -30°C until chemical analysis. The same procedures were used for laboratory-spiked water and sediment samples.

Sediment and water samples also were collected from wetland sites in the Colusa and Delevan National Wildlife Refuges (NWRs), located 110 and 130 km north of Sacramento, respectively. The wetlands at Colusa NWR (39.13427°N, 122.04473°W and 39.13658°N, 122.04253°W) were sprayed biweekly in the late summer and early fall of 2004 to control mosquito populations. The Delevan NWR wetlands (39.19324°N, 122.05912°W and 39.19157°N, 122.05924°W) were not sprayed in 2004 or in any previous years. The Colusa Mosquito Abatement district applied Pyrenone 5–25, an adulticide mixture of 5% pyrethrin I and II and 25% PBO, at dusk using ultralow-volume foggers. The U.S. Fish and Wildlife Service collected samples the morning after mosquito adulticide application in order to evaluate possible accumulation of Pyrenone after repeated applications. Initial water and sediment samples were collected from the wetlands one week prior to the treatment period to obtain background concentrations. Additionally, water and sediment samples from the wetland inflow pipe were collected one week prior to, and also during, the initial adulticide application to obtain background PBO concentrations entering the wetlands. Sediments were collected with an Ekman hand grab (Forestry Suppliers, Jackson, MS, USA), and the upper 1 cm of sediment was retained. Occasionally, 1-L water samples were collected. At each site, three water and sediment samples were collected in individual containers and composited prior to analysis. Sampling continued throughout the 6-week abatement treatment period from September through October 2004.

Spiking of water and sediment

Permethrin (20% *cis*, 78% *trans*) was purchased from Chem Service (West Chester, PA, USA). It was chosen as a model pyrethroid because it is one of the most widely used pyrethroids in California. It also is useful for study because it has relatively low toxicity compared to other pyrethroids, allowing accurate analytical quantification of permethrin concentrations when synergized with PBO. Many other pyrethroid LC50s are near the analytical detection limit even without synergism with

PBO; exposure to PBO with more potent pyrethroids potentially could result in an LC50 below the method detection limit. Permethrin was dissolved in an acetone carrier and spiked into sediments using <200 μl acetone/kg wet sediment. This concentration of acetone previously has been shown to have no effect on pyrethroid toxicity to *H. azteca* [17]. Nominal sediment permethrin concentrations ranged from 31 to 540 ng/g.

Technical-grade PBO (90% purity) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Piperonyl butoxide was dissolved in methanol and spiked into the sediment using <75 μl methanol/kg wet sediment. Nominal sediment PBO concentrations ranged from 0.5 to 313 ng/g, and a solvent control for methanol at 75 μl methanol/kg wet sediment (and 25 μl methanol/L) was included among the treatments.

After spiking, the sediments were mixed with a steel paint mixing attachment in an electric drill and aged at 4°C for 11 to 12 d. Aliquots then were removed for chemical verification. Water was spiked with PBO in methanol using 25 μl methanol/L immediately before use, and the solution was mixed with a magnetic stirrer.

Some treatments involved spiking PBO into overlying water only. Piperonyl butoxide concentrations ranged from 0.1 to 56.3 $\mu\text{g/L}$, and the overlying water was replaced with a fresh solution containing PBO at the desired concentration every 24 h. Other treatments involved spiking PBO into sediment, and then changing the water daily with water to which PBO had been added. The treatments in which PBO was added to both the sediment and water were intended to begin the toxicity testing exposures with approximately steady state conditions so that PBO would be less likely to partition into the sediments, reducing the aqueous exposure concentration. In these cases, nominal PBO water concentrations were the same as water-only PBO exposures, and the PBO sediment spiking concentrations were determined assuming a PBO $K_{oc} = 400$ [18]. A 1.39% organic carbon content of the sediments was assumed based on analysis of a previous batch of sediment. When organic carbon data for the batch of sediments actually used in these experiments became available, it was determined to be 1.87%; thus, the PBO sediment concentrations may have been slightly below steady state levels. However, this error is not thought to be significant given the uncertainty in actual PBO K_{oc} values, for which estimates range from 400 to 1,800 ([8,18]; www.pesticideinfo.org).

Chemical analyses

Spiked sediment samples were analyzed for permethrin and PBO, but water samples were analyzed only for PBO. The sediment was analyzed for permethrin following the methods of You et al. [19] on an Agilent 6890 series gas chromatograph equipped with an Agilent 7683 autosampler and an electron capture detector (Agilent Technologies). Two columns from Agilent, a HP-5MS (30 m \times 0.25 mm; 0.25- μm film thickness) and a DB-608 (30 m \times 0.25 mm; 0.25- μm film thickness) were used for confirmation. Sediment was sonicated with acetone and methylene chloride and the extract was cleaned with deactivated Florisil. Ethyl ether and hexane were used as elution solvents, and the eluent was evaporated and redissolved in 2 ml of hexane. Half of the extract (1 ml) was used for permethrin analysis after dilution to a concentration within the calibration curve. Due to the hydrophobicity of permethrin, all pyrethroid sediment data are reported normalized to sediment organic carbon content.

The other half of sediment extract (1 ml) was used for PBO analysis that was performed on a high-performance liquid chromatography (HPLC) after further C18 solid-phase extraction cleanup. The hexane extract was solvent exchanged to 1 ml of methanol, and then diluted into 200 ml water. This solution, as well as a 6-ml water glassware rinse, was loaded on a previously conditioned solid-phase extraction cartridge, and the eluent was discarded. After drying the cartridge, PBO was eluted with 6 ml of methanol and the eluent was evaporated to 1 ml. In the case of the water samples, 500 ml of sample was loaded directly onto the solid-phase extraction cartridge.

Piperonyl butoxide concentrations then were determined on an Agilent 1100 series HPLC equipped with fluorescence detector (FLD) and ultraviolet detector (Agilent Technologies) and using a ZORBAX SB-C18 5 μm , 150 \times 4.6 mm column (Agilent Technologies). A mobile phase of methanol and water was used for PBO separation. The flow rate was 1.1 ml/min and the injection volume was 25 μl . The excitation and emissions wavelengths were 295 and 335nm for fluorescence detection, and a wavelength of 287 nm was used for ultraviolet detection. With method detection limits of 0.007 $\mu\text{g/L}$ and 0.3 $\mu\text{g/kg}$, the reporting limits were set at 0.05 $\mu\text{g/L}$ and 5 $\mu\text{g/kg}$ for water and sediment samples, respectively. The above procedure was followed for all Sacramento and San Francisco area urban creek samples. Piperonyl butoxide concentrations in a subset of these samples were verified by HPLC–mass spectroscopy (HPLC-MS) at the Marine Sciences Research Center, Stony Brook, New York, USA. Sediment and water samples collected from the Colusa and Delevan NWRs were analyzed via HPLC-MS by California Department of Fish and Game Water Pollution Laboratory, Rancho Cordova (CA, USA).

Test sediments were wet sieved to determine grain-size distribution and analyzed for organic carbon content on a CE-440 elemental analyzer from Exeter Analytical (Chelmsford, MA, USA), following acid vapor treatment to remove inorganic carbon.

Toxicity testing

Ten-day toxicity tests with the freshwater amphipod *H. azteca* were performed on sediments using standard U.S. Environmental Protection Agency protocols [20]. All toxicity tests were conducted at 23°C with a 16:8-h light:dark cycle in 400-ml beakers (four replicates per concentration) containing 50 to 75 ml of spiked sediment and 300 ml of moderately hard water reconstituted from Milli-Q® purified water (Millipore, Billerica, MA, USA). Ten amphipods, 7 to 10 d in age, were added to each beaker at test initiation. A yeast, cerophyll, and trout chow mixture was fed daily during the 10-d tests. Water changes (80%) using freshly prepared PBO-spiked water were performed every day, and water samples were taken prior to water renewal after 24 h and again on day 10 for analysis of temperature, dissolved oxygen, pH, conductivity, alkalinity, hardness, and ammonia. Sediment samples were taken for permethrin and PBO analysis at test initiation. Water samples were taken for PBO analysis at test initiation, again after 24 h before water exchange, and finally on day 10 at the conclusion of the test. All beakers were aerated gently and continuously. Tests were terminated by sieving contents of the beakers over a 425- μm screen and counting the surviving amphipods.

Table 1. Piperonyl butoxide (PBO) and permethrin concentrations and recoveries measured by high-performance liquid chromatography with fluorescence detection in spiked water and sediments. Data shown are from experiments in which PBO was spiked into both the sediment and overlying water. NA = Not available

Compound	Nominal concn.	Measured concn. (initial)	Measured concn. (24 h)	Measured concn. (10 d)	% Recovery initial	% Recovery 24 h	% Recovery 10 d
PBO in water ($\mu\text{g/L}$)	56.3	59.4	39.1	38.7	105.6	65.8	65.2
	11.3	11.6	7.2	7.8	103.1	62.1	67.2
	11.3	12.9	8.2	5.9	114.4	63.5	45.8
	2.3	2.7	2.3	1.7	120.0	85.2	63.0
	0.5	0.5	0.4	NA	117.8	81.1	NA
PBO in sediment ($\mu\text{g/kg}$)	312.7	135.0	NA	NA	43.2	NA	NA
	62.6	26.6	NA	NA	42.5	NA	NA
	12.5	10.8	NA	NA	86.3	NA	NA
	2.5	<5	NA	NA	NA	NA	NA
	0.5	<5	NA	NA	NA	NA	NA
Permethrin in sediment ($\mu\text{g/kg}$)	352.0	217.7	NA	NA	61.8	NA	NA
	235.0	144.1	NA	NA	61.3	NA	NA
	157.0	110.7	NA	NA	70.5	NA	NA
	157.0	117.1	NA	NA	74.6	NA	NA
	104.0	80.2	NA	NA	77.1	NA	NA

Statistics

Toxicity data were analyzed using ToxCalc 5.0 software (Tidepool Scientific Software, McKinleyville, CA, USA). Survival data were arc-sin transformed prior to analysis. The Spearman-Kärber method was used to determine LC50s and associated confidence intervals for each permethrin/PBO mixture. Abbott's correction was applied in a few cases to account for control mortality.

RESULTS

Spiking recovery

Mean recovery of PBO spiked into water in the toxicity tests was $112 \pm 11\%$ of the initial nominal concentrations (Table 1). Over the 24-h period between water renewal in the beakers, PBO declined to $70 \pm 10\%$ of the initial concentrations. Because PBO is susceptible to photodegradation, this loss most likely is due to breakdown in the test water, although additional losses to adsorption, evaporation, or other loss mechanisms have not been evaluated.

Sediments spiked with permethrin and PBO had recoveries lower than nominal concentrations. In five samples, mean permethrin recovery at test initiation was $69 \pm 7\%$. Low recoveries may have been due to degradation during the 12-d aging period, adsorption on container walls (glass), or incomplete chemical extraction from the sediments. Recoveries in this range have been typical of past uses of the same spiking and analytical techniques [17]. Piperonyl butoxide recovery in sediments averaged $58 \pm 25\%$, with loss possibly due to degradation during sediment aging. All data reported are based on nominal concentrations.

Toxicity testing

Control survival in all tests averaged $96 \pm 4\%$, and all water-quality parameters were acceptable. Addition of PBO in the absence of permethrin had no toxic effect at any concentration used (up to $56 \mu\text{g/L}$); mean survival in all tests with PBO in sediment and/or water, but with no permethrin was $96 \pm 3\%$. Permethrin LC50s for *H. azteca* in this study were 14.2 mg/kg OC (confidence interval = $11.8\text{--}17.1$) and 21.3 mg/kg OC ($14.7\text{--}30.5$) as determined in two independent tests. No

statistically significant differences were found between these values and permethrin LC50 determined in the acetone solvent control (13.2 mg/kg OC [$11.5\text{--}15.2$]) or the permethrin LC50 reported by Amweg et al. [17] in similar American River test sediment (17.9 mg/kg OC [$14.8\text{--}19.9$]). Because no difference existed between solvent control and control LC50s determined in this study, the median test LC50 (14.2 mg/kg OC) was used for statistical evaluation of PBO effects.

Hyalella azteca exposed to PBO and permethrin in laboratory toxicity tests exhibited higher mortality than those exposed to permethrin alone. In the tests conducted with PBO-spiked sediment and water, permethrin was approximately twice as toxic if the organisms were concurrently exposed to PBO at $12.5 \mu\text{g/kg}$ and $2.3 \mu\text{g/L}$ (permethrin LC50 reduced from 14.2 to 7.3 mg/kg OC ; Table 2, Figure 1A). Higher concentrations of PBO reduced the LC50 by even greater amounts. A 3.5-fold increase in toxicity was noted for permethrin when

Table 2. Nominal water and sediment piperonyl butoxide (PBO) concentrations in all laboratory exposures and the resulting permethrin 10-d median lethal concentration (LC50) to *Hyalella azteca*. The PBO treatments with asterisks indicate LC50s significantly lower ($p < 0.05$) than the permethrin-only LC50. OC = Organic carbon; CI = 95% confidence interval

Aqueous PBO ($\mu\text{g/L}$)	Sediment PBO ($\mu\text{g/kg}$)	Permethrin LC50 (CI) (mg/kg OC)
Permethrin with no PBO		
Permethrin (1)–0.0	0	14.2 (11.8–17.1)
Permethrin (2)–0.0	0	21.3 (14.7–30.5)
Permethrin, solvent control–0.0	0	13.2 (11.5–15.2)
Permethrin with PBO in water and sediment		
0.1	0.5	13.3 (11.7–15.2)
0.5	2.5	14.3 (13.0–15.8)
2.3	12.5	*7.3 (6.5–8.0)
11.3	62.6	*5.5 (5.0–6.1)
56.3	312.7	*2.0 (1.9–2.1)
Permethrin with PBO in water only		
2.3	0	10.5 (9.3–11.8)
11.3	0	*8.6 (7.7–9.6)
56.3	0	*4.1 (2.6–5.0)

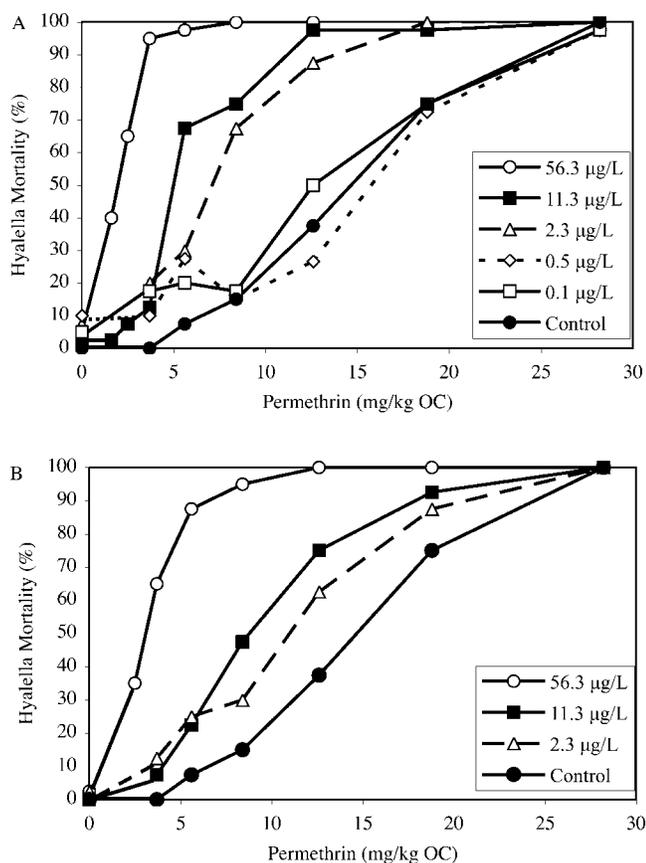


Fig. 1. Permethrin median lethal concentration (LC50) curves for *Hyalella azteca*. Exposures with piperonyl butoxide (PBO) in sediment and water are shown in (A), and exposures with PBO in water only are shown in (B). The legend indicates water concentrations of PBO; concurrent sediment concentrations are shown in Table 2. OC = organic carbon.

also exposed to PBO at 11.3 $\mu\text{g/L}$ and 62.6 $\mu\text{g/kg}$. Piperonyl butoxide concentrations of 56.3 $\mu\text{g/L}$ and 313 $\mu\text{g/kg}$ resulted in a permethrin LC50 of just 2.0 mg/kg OC, a sevenfold increase in toxicity. The synergistic effect of PBO on permethrin toxicity is seen clearly by a dose-dependent shift in the dose-response curve to the left and an increase in the slope of the curve at higher PBO concentrations.

In toxicity tests conducted with only PBO-spiked water, permethrin toxicity also was increased (Table 2, Figure 1B). A PBO concentration of 2.3 $\mu\text{g/L}$, which significantly changed permethrin toxicity in the spiked sediment and water treatment, resulted in a slight, but not statistically significant, increase in permethrin toxicity in the spiked water-only tests. A PBO concentration of 11.3 $\mu\text{g/L}$ was necessary to increase significantly permethrin toxicity, reducing the LC50 from 14.2 to 8.6 mg/kg OC. Permethrin toxicity was increased by a greater amount in tests where PBO was spiked into both sediment and water, as compared to tests with PBO exposure via water alone. Because both types of tests were conducted using the same nominal PBO water concentrations, this effect probably was due to two factors. First, PBO in the water-only tests would partition into sediment, reducing the bioavailable fraction in the overlying water and pore water. Sediments were spiked with steady state concentrations of PBO to eliminate PBO flux to sediments in tests with PBO in sediment and water. Addition-

ally, *H. azteca* probably is exposed to a mixture of overlying and pore water [21–23]. When only the overlying water is spiked, dilution of PBO with pore water in the surficial sediments occupied by *H. azteca* would reduce the exposure concentration below that measured in the overlying water.

Urban creek sampling

Sediment collected from urban creeks in the San Francisco Bay area contained detectable PBO at least once at every site (Table 3). In April 2004, the reporting limit for PBO was 1 $\mu\text{g/kg}$; concentrations measured by HPLC-FLD ranged from 2.6 to 8.9 $\mu\text{g/kg}$. In October 2004, only two of eight sediments contained PBO above the reporting limit of 5 $\mu\text{g/kg}$. Piperonyl butoxide concentrations in Kirker and San Leandro sediments were verified by HPLC-MS, a more accurate method for determining compound identity. Both were found to contain 1.8 $\mu\text{g/kg}$ PBO, compared to the 3.6 and 8.9 $\mu\text{g/kg}$ reported by HPLC-FLD. Because HPLC-MS offers compound identification, the lower values of 1.8 $\mu\text{g/kg}$ PBO are considered more accurate and suggest that other contaminants may be misidentified as PBO by the HPLC-FLD at times. With the only two verified samples containing 20 and 50% PBO of that reported by HPLC-FLD, it is not possible to generalize whether these ratios are typical of the other environmental sediment samples.

Water samples typically were below the detection limit for both methods, although Lauterwasser Creek did contain 0.13 $\mu\text{g/L}$ PBO as measured by HPLC-MS (0.068 $\mu\text{g/L}$ by HPLC-FLD). It is unclear why HPLC-FLD detected a lower concentration than HPLC-MS in this sample. Theoretically, peaks detected by HPLC-FLD could contain both PBO and other co-eluting contaminants, whereas HPLC-MS offers more accurate compound identification, resulting in PBO concentrations typically lower than determined by HPLC-FLD. However, in the absence of co-eluting contaminants, HPLC-FLD performs quite well: Recoveries were 112% of nominal in laboratory water spiked with PBO (Table 1). Taken together, these data suggest that HPLC-FLD would be an appropriate screening tool for detecting PBO in environmental samples, with PBO detection verified by HPLC-MS.

Out of the 14 urban creek sediments sampled in Nashville, only three sediment samples contained PBO in sediment above the reporting limit of 5 $\mu\text{g/kg}$. These concentrations were 5.5, 7.7, and 26.2 $\mu\text{g/kg}$ PBO. This range overlaps with effective synergistic concentrations in laboratory tests with PBO-spiked sediment and water. Neither of the two creek sediments collected from Sacramento contained detectable concentrations of PBO.

Mosquito abatement sampling

None of 20 sediment samples or 12 water samples collected at the control site, Delevan NWR, contained detectable levels of PBO (data not shown). In Colusa NWR wetlands, PBO was detected in 10 of 18 sediment samples taken during mosquito abatement activities, although only two samples contained PBO above the 2.0 $\mu\text{g/kg}$ reporting limit (Table 4). Sediment PBO concentrations 12 h following ultralow-volume fogger application of Pyrenone 5–25 peaked at 3.3 $\mu\text{g/kg}$. Prior to the initial Pyrenone 5–25 application and one week after applications had ceased, sediment PBO concentrations were below the reporting limit. In water samples, PBO was detectable in seven of eight water samples collected within 12 h of abate-

Table 3. Piperonyl butoxide (PBO) concentrations in sediment and water collected from urban creeks in California and Tennessee, USA. The reporting limit in sediments for high-performance liquid chromatography with fluorescence detection (HPLC-FLD) is 1 $\mu\text{g}/\text{kg}$ for April 2004, 5 $\mu\text{g}/\text{kg}$ for October 2004, and 0.050 $\mu\text{g}/\text{L}$ for water samples. For samples verified by HPLC–mass spectroscopy, the results of these confirmatory analyses are shown in parentheses after the HPLC-FLD values. OC = organic carbon

Site	Location	Date	Latitude	Longitude	% OC	Sediment ($\mu\text{g}/\text{kg}$)	Sediment (mg/kg OC)	Water ($\mu\text{g}/\text{L}$)
San Francisco Bay Area, CA								
Glen Echo Creek	Oakland	04/20/2004	37.97500	-122.50833	1.55	7.7	0.50	<0.050
		10/26/2004			0.52	5.9	1.13	
Kirker Creek	Pittsburg	04/20/2004	38.01655	-121.83914	2.12	3.6 (1.8)	0.17 (0.08)	<0.050 (<0.005)
		10/18/2004			1.35	<5.0	<0.37	
Lion Creek	Oakland	10/25/2004			2.91	<5.0	<0.17	
		04/21/2004	37.76037	-122.19512	4.54	6.8	0.15	<0.050
Lauterwasser Creek	Orinda	10/28/2004			7.81	12.7	0.16	
		04/21/2004	37.89754	-122.19238	0.36	2.6	0.73	0.068 (0.130)
		10/18/2004			1.13	<5.0	<0.44	
		10/25/2004			1.60	<5.0	<0.31	
San Pablo Creek	Richmond	04/20/2004	37.88611	-122.25500	0.92	2.9	0.31	<0.050
San Leandro Creek	Oakland	10/26/2004			0.94	<5.0	<0.53	
		04/20/2004	37.72547	-122.18278	1.95	8.9 (1.8)	0.46 (0.09)	<0.050
		10/29/2004			2.16	<5.0	<0.23	
Sacramento area, CA								
Arcade Creek	Sacramento	10/15/2004	38.64217	-121.36695	1.40	<5.0	<0.36	
Curry Creek	Roseville	10/15/2004	38.75813	-121.35860	3.64	<5.0	<0.14	
Nashville area, TN								
Cedar Creek	Nashville	06/28/2004	36.23131	-86.50636	1.34	<5.0	<0.37	
Mill Creek	Nashville	06/29/2004	36.09185	-86.68623	1.58	<5.0	<0.32	
Mill Creek	Nashville	06/29/2004	36.11757	-86.71921	3.16	26.2	0.83	
West Fork Hamilton Creek	Nashville	06/29/2004	36.08896	-86.62771	1.88	5.5	0.29	
Little Harpeth River	Nashville	06/29/2004	36.01928	-86.82078	5.17	<5.0	<0.10	
Harpeth River	Nashville	06/29/2004	36.02869	-86.92424	1.80	<5.0	<0.28	
Harpeth River	Nashville	06/29/2004	36.07711	-86.95721	1.72	<5.0	<0.29	
Dry Creek	Nashville	06/28/2004	36.28455	-86.70625	3.08	7.7	0.25	
Madison Creek	Nashville	06/28/2004	36.31434	-86.66566	1.46	<5.0	<0.34	
Drake Creek	Nashville	06/28/2004	36.31261	-86.60865	1.74	<5.0	<0.29	
Station Camp Creek	Nashville	06/28/2004	36.34674	-86.52545	3.07	<5.0	<0.16	
East Fork Station Camp Creek	Nashville	06/28/2004	36.38684	-86.48183	2.94	<5.0	<0.17	
Gills Creek	Nashville	06/28/2004	36.34583	-86.44000	4.43	<5.0	<0.11	
Hays Branch	Nashville	06/28/2004	36.25513	-86.55940	1.27	<5.0	<0.39	

ment spraying. Three of these samples were above the 0.01 $\mu\text{g}/\text{L}$ reporting limit with concentrations ranging from 0.04 to 0.08 $\mu\text{g}/\text{L}$. Water PBO concentrations were below the reporting limit one week after abatement activities.

DISCUSSION

The results of this study suggest that PBO synergism in the environment is possible, although not likely under most conditions. In laboratory tests, 2.3 $\mu\text{g}/\text{L}$ PBO in water and 12.5 $\mu\text{g}/\text{kg}$ in sediment significantly increased 10-d permethrin toxicity. The synergistic sediment concentration of PBO was exceeded by concentrations in two of the 30 urban creek samples: 26.2 $\mu\text{g}/\text{kg}$ in a Nashville creek and 12.7 $\mu\text{g}/\text{kg}$ in Lion Creek, but considering the possibility of overestimation by HPLC-FLD, urban sediment concentrations actually may be lower. Peak sediment concentrations of PBO following mosquito abatement activities were fourfold below effective synergistic concentrations. No tests were conducted with PBO-spiked sediment only; however, assuming *H. azteca* is exposed to a mixture of pore and overlying water [21], in the absence of overlying water PBO exposure, sediment PBO concentrations nec-

essary to cause synergy are probably far higher than any seen in these environmental samples.

Only one of the six urban creek water samples contained PBO concentrations above the 0.050 $\mu\text{g}/\text{L}$ reporting limit. This sample, from Lauterwasser Creek, contained 0.068 $\mu\text{g}/\text{L}$ (by HPLC-FLD) or 0.13 $\mu\text{g}/\text{L}$ (by HPLC-MS); the concentration was at least 18 times too low to affect permethrin toxicity based on these laboratory experiments. The concentration of PBO necessary to synergize other pyrethroids was not determined, but presumably would be comparable to that for permethrin, because a PBO-induced inhibition of p450 activity would affect detoxification of all pyrethroids.

Given the results from this study, higher environmental PBO concentrations would be necessary in order to exert a synergistic effect with pyrethroids, perhaps in the case of pulses of PBO carried in to surface waters by storm events, or more likely when surface waters are contaminated directly during mosquito spraying. However, even 12 h following mosquito abatement applications of PBO using ultralow-volume foggers, peak water concentrations were just 0.08 $\mu\text{g}/\text{L}$, about 3% of synergistic levels. Although PBO frequently was de-

Table 4. Piperonyl butoxide (PBO) concentrations in sediment and water collected from the Colusa National Wildlife Refuge (CA, USA) following application of Pyrenone 5-25 for mosquito abatement purposes. The reporting limit for sediment and water was 2.0 $\mu\text{g}/\text{kg}$ and 0.01 $\mu\text{g}/\text{L}$, respectively; the detection limit was 1.0 $\mu\text{g}/\text{kg}$ and 0.005 ng/L , respectively. <RL = detected but less than reporting limit; ND = not detected; NA = not available because no sample was collected

	Location	Date	Sediment PBO concn. ($\mu\text{g}/\text{kg}$)	Water PBO concn. ($\mu\text{g}/\text{L}$)
1 Week prior to Pyrenone 5-25 application	Inflow	9/2/04	ND	ND
	Wetland	9/2/04	ND	ND
	Wetland	9/2/04	ND	ND
Mosquito abatement treatments with Pyrenone 5-25 (samples collected 12 h following each abatement treatment)	Inflow	9/10/04	<RL	NA
	Wetland	9/10/04	<RL	NA
	Wetland	9/10/04	<RL	NA
	Wetland	9/15/04	ND	0.08
	Wetland	9/15/04	ND	<RL
	Wetland	9/17/04	ND	<RL
	Wetland	9/17/04	ND	0.04
	Wetland	9/22/04	ND	NA
	Wetland	9/22/04	ND	NA
	Wetland	9/24/04	<RL	<RL
	Wetland	9/24/04	ND	<RL
	Wetland	9/29/04	<RL	NA
	Wetland	9/29/04	3.00	NA
	Wetland	10/1/04	<RL	NA
	Wetland	10/1/04	3.27	NA
1 Week after abatement applications concluded	Wetland	10/13/04	ND	ND
	Wetland	10/13/04	<RL	0.06
	Wetland	10/20/04	<RL	<RL
	Wetland	10/20/04	ND	<RL
	Wetland	10/20/04	ND	<RL

ected in water samples following mosquito abatement application of Pyrenone 5–25, concentrations were not elevated above those found in urban creek samples. Sediment pyrethroid residues that co-occurred with PBO contamination also would be required in order for increased toxicity to invertebrates with similar sensitivities to *H. azteca*. Piperonyl butoxide synergism could occur only under a limited set of environmental circumstances, and it seems likely environmental PBO concentrations typically would not alter pyrethroid toxicity. However, this conclusion must be somewhat qualified by the relatively small data set available for this study on environmental PBO concentrations.

It is important to bear in mind that only the amphipod *H. azteca* was tested in this study. It is one of the more sensitive species to pyrethroids tested to date [15], and therefore the effect of PBO and pyrethroids on other aquatic organisms is not immediately clear. Insect populations are known to develop insecticide resistance by increasing detoxification enzymes, including increased p450 metabolism. In a pyrethroid and organophosphate pesticide resistant tobacco budworm population, PBO was two to 11 times more effective as a synergist than in a nonresistant population [24]. In beet army-worms, PBO was 14 times more effective as a synergist in pyrethroid-resistant populations than nonresistant populations [25]. Thus, if pesticide resistance exists in wild populations, exposure to PBO may enhance pyrethroid toxicity at concentrations lower than found in the present study.

Pyrethroid resistance in insects also has been shown to confer crossresistance among pyrethroids and DDT, with partial reversal to susceptibility with exposure to PBO [26–28]. Although several molecular resistance mechanisms are known, the efficacy of PBO in these cases suggests the mechanism involves enhanced p450-mediated detoxification [29,30]. Pes-

ticide-resistant populations, including aquatic invertebrates, with increased p450 metabolism therefore also could face PBO synergism of DDT if these two compounds co-occurred at biologically relevant concentrations. This effect has been shown in laboratory exposures with DDT-resistant *Drosophila* populations capable of metabolizing DDT via a p450-mediated pathway [31,32].

In addition to resistance, species differences in p450 metabolism also appear to have a dramatic influence on PBO efficacy. Exposure to PBO did not alter organophosphate toxicity to *L. variegatus*, whereas PBO exposure in *C. tentans* and *H. azteca* caused the expected mitigation of toxicity [1]. The authors postulate that *L. variegatus* has a relatively low p450 metabolic activity and pesticides and xenobiotics are detoxified via other pathways, such as carboxylesterases or glutathione-S-transferases. Oligochaetes and other organisms with low p450 activity will be much less sensitive to the synergistic effects of PBO under environmental conditions as well.

Regardless, populations of *H. azteca* do exist in creeks throughout the United States. In order to protect this sensitive species and others, effective monitoring programs should consider the cumulative effect of exposure to contaminant mixtures, and additional information on environmental PBO levels should be acquired before dismissing potential synergism of pyrethroids under realistic environmental conditions.

CONCLUSION

Laboratory toxicity tests conducted with the amphipod, *H. azteca*, show that PBO effectively synergizes toxicity of the pyrethroid pesticide permethrin. Permethrin sediment 10-d LC50s conducted with exposure to PBO via spiked water and sediment caused increased toxicity at concentrations as low as 2.3 $\mu\text{g}/\text{L}$ and 12.5 $\mu\text{g}/\text{kg}$. In permethrin sediment toxicity tests

with PBO exposure via water only, 11.3 $\mu\text{g/L}$ PBO was required before permethrin toxicity to *H. azteca* was enhanced.

In field samples from urban creeks in California and Tennessee, and samples from a California wetland following mosquito abatement treatments applied with ultralow-volume foggers, PBO water concentrations were far below those necessary to synergize permethrin. In a small proportion of the samples, sediment PBO concentrations did approach such levels, although PBO concentrations in overlying water were not measured or were below synergistic levels at these times. Given that synergistic levels of PBO appear to occur infrequently, and they would have to co-occur with toxicologically relevant levels of pyrethroids in the environment to be of concern, it appears environmental synergism of pyrethroids by PBO would be unlikely, except perhaps under exceptional circumstances.

Pyrethroid use has been increasing in recent years, especially in urban areas. Given the recent concern over West Nile virus and the withdrawal of some alternative pesticides from the consumer market, this trend likely will continue. Although, when based on the limited data the risk of environmental synergy appears low, more information on pyrethroid distribution in urban creeks, as well as additional information on PBO concentrations in surface waters and sediments, is necessary to assess properly risk from pesticide residues.

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REFERENCES

- Ankley GT, Collyard SA. 1995. Influence of piperonyl butoxide on the toxicity of organophosphate insecticides to three species of freshwater benthic invertebrates. *Comp Biochem Physiol C: Toxicol Pharmacol* 110:149–155.
- Erickson DA, Goodrich MS, Lech JJ. 1988. The effect of piperonyl butoxide on hepatic cytochrome P-450-dependent monooxygenase activities in rainbow trout *Salmo gairdneri*. *Toxicol Appl Pharmacol* 94:1–10.
- Mayer FLJ, Ellersieck MR. 1986. Manual of acute toxicity: Interpretation and database for 410 chemicals and 66 species of freshwater animals. Resource Publication 160. U.S. Fish and Wildlife Service, U.S. Department of the Interior, Washington, DC.
- Kakko I, Toimela T, Tahti H. 2000. Piperonyl butoxide potentiates the synaptosome ATPase inhibiting effect of pyrethrin. *Chemosphere* 40:301–305.
- Casida JE, Quistad GB. 1995. Metabolism and synergism of pyrethrins. In Casida JE, Quistad GB, eds, *Pyrethrum Flowers: Production, Chemistry, Toxicology, and Uses*. Oxford University, New York, NY, USA, pp 258–276.
- Ankley GT, Dierkes JR, Jensen DA, Peterson GS. 1991. Piperonyl butoxide as a tool in aquatic toxicological research with organophosphate insecticides. *Ecotoxicol Environ Saf* 21:266–274.
- Bailey HC, Digiorgio C, Kroll K, Miller JL, Hinton DE, Starrett G. 1996. Development of procedures for identifying pesticide toxicity in ambient waters: Carbofuran, diazinon, chlorpyrifos. *Environ Toxicol Chem* 15:837–845.
- Food and Agriculture Organization of the United Nations. 2001. Piperonyl butoxide. Scientific Evaluation Report from the Joint FAO/WHO Meeting on Pesticide Residues. Plant Production and Protection Division of the FAO, Rome, Italy.
- Orlando JL, Kuivila KM, Whitehead A. 2003. Dissolved pesticide concentrations detected in storm-water runoff at selected sites in the San Joaquin River basin, California, 2000–2001. Scientific Investigations Report 2003–101. U.S. Geological Survey, Reston, VA.
- Orlando JL, Jacobson LA, Kuivila KM. 2004. Dissolved pesticide and organic carbon concentrations detected in surface waters, Northern Central Valley, California, 2001–2002. Open File Report 2004–1214. U.S. Geological Survey, Reston, VA.
- LeBlanc LA, Orlando JL, Kuivila KM. 2003. Pesticide concentrations in water and in suspended and bottom sediments in the New and Alamo rivers, Salton Sea Watershed, California, April 2003. DS/DDS 104. U.S. Geological Survey, Reston, VA.
- Planas C, Caixach J, Santos FJ, Rivera J. 1997. Occurrence of pesticides in Spanish surface waters. Analysis by high-resolution gas chromatography coupled to mass spectrometry. *Chemosphere* 34:2393–2406.
- LeBlanc LA, Orlando JL, Kuivila KM. Occurrence, distribution, and transport of pesticides, trace elements, and selected inorganic constituents into the Salton Sea Basin, California, 2001–2002. Scientific Investigations Report 2004–5117. U.S. Geological Survey, Reston, VA.
- Antonious GF, Byers ME, Kerst WC. 1997. Residue levels of pyrethrins and piperonyl butoxide in soil and runoff water. *J Environ Sci Health Part B: Pestic Food Contam Agric Wastes* 32:621–644.
- Weston DP, You J, Lydy MJ. 2004. Distribution and toxicity of sediment-associated pesticides in agriculture-dominated water bodies of California's Central Valley. *Environ Sci Technol* 38:2752–2759.
- Weston DP, Holmes RW, You J, Lydy MJ. 2005. Aquatic toxicity due to residential use of pyrethroid insecticides. *Environ Sci Technol* 39:9778–9784.
- Amweg EL, Weston DP, Ureda NM. 2005. Use and toxicity of pyrethroid pesticides in the Central Valley, California, USA. *Environ Toxicol Chem* 24:966–972. Correction. 1300–1301.
- Tomlin CDS. 1997. *The Pesticide Manual*, 11th ed. British Crop Protection Council, Farnham, UK.
- You J, Weston DP, Lydy MJ. 2004. A sonication extraction method for the analysis of pyrethroid, organophosphate, and organochlorine pesticides from sediment by gas chromatography with electron-capture detection. *Arch Environ Contam Toxicol* 47:141–147.
- U.S. Environmental Protection Agency. 2000. Methods for measuring the toxicity and bioaccumulation of sediment-associated contaminants with freshwater invertebrates, second edition. EPA/600/R-99/064. Washington, DC.
- Ankley GT, Call DJ, Cox JS, Kahl MD, Hoke RA, Kosian PA. 1994. Organic carbon partitioning as a basis for predicting the toxicity of chlorpyrifos in sediments. *Environ Toxicol Chem* 13:621–626.
- Ankley GT, Mattson VR, Leonard EN, West CW, Bennett JL. 1993. Predicting the acute toxicity of copper in freshwater sediments: Evaluation of the role of acid-volatile sulfide. *Environ Toxicol Chem* 12:315–320.
- Di Toro DM, Zarba CS, Hansen DJ, Berry WJ, Swartz RC, Cowan CE, Pavlou SP, Allen HE, Thomas NA, Paquin PR. 1991. Technical basis for establishing sediment quality criteria for nonionic organic chemicals using equilibrium partitioning. *Environ Toxicol Chem* 10:1541–1584.
- Zhao G, Rose RL, Hodgson E, Roe RM. 1996. Biochemical mechanisms and diagnostic microassays for pyrethroid, carbamate, and organophosphate insecticide resistance/crossresistance in the tobacco budworm, *Heliothis virescens*. *Pestic Biochem Physiol* 56:183–195.
- Liu YJ, Shen JI. 2003. Biochemical mechanism and genetics of resistance to lambda-cyhalothrin in the beet armyworm, *Spodoptera exigua*, and the relative fitness of the resistant strain. *Acta Entomol Sin* 46:567–572.
- Kumar S, Thomas A, Sahgal A, Verma A, Samuel T, Pillai MKK. 2004. Variations in the insecticide-resistance spectrum of *Anopheles stephensi* after selection with deltamethrin or a deltamethrin-piperonyl butoxide combination. *Ann Trop Med Parasitol* 98:861–871.
- Yoon KS, Gao JR, Lee SH, Coles GC, Meinking TL, Taplin D, Edman JD, Takano-Lee M, Clark JM. 2004. Resistance and cross-resistance to insecticides in human head lice from Florida and California. *Pestic Biochem Physiol* 80:192–201.
- McAbee RD, Kang KD, Stanich MA, Christiansen JA, Wheelock CE, Inman AD, Hammock BD, Cornel AJ. 2004. Pyrethroid tol-

- erance in *Culex pipiens pipiens* var *molestus* from Marin County, California. *Pest Manag Sci* 60:359–368.
29. Soderlund DM, Knipple DC. 2003. The molecular biology of knockdown resistance to pyrethroid insecticides. *Insect Biochem Mol Biol* 33:563–577.
 30. French-Constant RH, Daborn PJ, Le Goff G. 2004. The genetics and genomics of insecticide resistance. *Trends Genet* 20:163–170.
 31. Amichot M, Tares S, Brun-Barale A, Arthaud L, Bride JM, Berge JB. 2004. Point mutations associated with insecticide resistance in the *Drosophila* cytochrome P450 Cyp6a2 enable DT metabolism. *Eur J Biochem* 271:1250–1257.
 32. Brandt A, Scharf M, Pedra JHF, Holmes G, Dean A, Kreitman M, Pittendrigh BR. 2002. Differential expression and induction of two *Drosophila* cytochrome P450 genes near the Rst(2)DDT locus. *Insect Mol Biol* 11:337–342.