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ANALYSIS, OCCURRENCE, AND TOXIC POTENTIAL OF PYRETHROIDS, AND FIPRONIL IN SEDIMENTS FROM AN URBAN ESTUARY

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Abstract—Eight pyrethroids and fipronil and its three major degradates were analyzed in urban estuarine sediments that exhibited a range of toxic effects to an amphipod test species. Sediments from Ballona Creek, an urban estuary in Southern California (USA), collected during three dry season events were analyzed by gas chromatography with electron capture and negative chemical ionization mass spectrometric detection (GC-ECD and GC-NCI-MS). The two detection methods were in agreement for intermediate levels of pyrethroid contamination (10–50 ng/g dry wt) but deviated for both low and high concentrations (< 5 and > 50 ng/g). Sediments contained total pyrethroids as high as 473 ng/g with permethrin, bifenthrin, and cypermethrin as the most abundant compounds. In contrast, fipronil and its desulfinyl, sulfide, and sulfone degradates were detected at much lower levels ($\leq$ 0.18–16 ng/g). Toxic units estimated for these compounds revealed that bifenthrin and cypermethrin were likely contributors to the mortality observed in tests with the estuarine amphipod *Eohaustorius estuarius*. Although fipronil was not a likely contributor to the observed mortality, the concentrations detected may be of concern for more sensitive crustacean species. Furthermore, the spatial pattern of pyrethroid contamination and potential toxicity was highly correlated with fine-grained substrate, which shifted to downstream stations within a three-month period during the dry season. Environ. Toxicol. Chem. 2010;29:843–851. © 2010 SETAC

Keywords—Pyrethroids Fipronil Sediment toxicity Estuarine sediment Toxicant identification

INTRODUCTION

Current-use pesticides are a category of largely unregulated chemicals that are a cause for concern due to their toxicity to nontarget species and/or persistence in the environment. As many organophosphate insecticides were phased out in the United States over the past two decades, synthetic pyrethroids and the phenylpyrazole compound known as fipronil have been substituted for use in urban and suburban landscapes [1]. Due to their hydrophobicity, pyrethroids and, to a lesser extent, fipronil associate with soils and sediments. In California, pyrethroids were detected in sediment of 30 urban creeks [2], with a single compound (bifenthrin) found in all 30 waterways sampled. In addition to bifenthrin, cypermethrin and cyfluthrin were detected in residential runoff [3], and fenpropathrin was reported as one of the most frequently detected pyrethroids in a mixed land use watershed in southern California [4]. While fipronil and its degradates have been found at much lower concentrations than pyrethroids [5,6], their potential for toxicity to nontarget organisms is relatively high [5].

In most studies published to date, the nonspecific electron capture detector (ECD) was used to detect and quantify pyrethroids and fipronil in the environment [2,7]. Proper identification and thus accurate quantitation using ECD becomes difficult at trace levels, particularly for pyrethroids with multiple isomers, which may co-elute with other constituents present, even when using dual column analysis. Conversely, not accounting for all isomers of a given analyte may result in underestimation of its concentration. The presence of halogens on fipronil and on most pyrethroid compounds makes them suitable for detection by negative chemical ionization mass spectrometry (NCI-MS), a highly selective technique that can eliminate these issues. However, few if any studies have compared ECD and NCI-MS results for complex matrices such as sediment [8–10].

Ballona Creek is a relatively short, channelized waterway that drains highly urbanized landscapes within the city of Los Angeles (CA, USA). In a recent study, sediments from the tidally influenced creek mouth exhibited a spatially distinct pattern of toxicity to the estuarine amphipod, *Eohaustorius estuarius*. Analysis of priority pollutants including chlordanes, DDTs and polychlorinated biphenyls (PCBs) that are regulated under the Clean Water Act could not explain the observed toxicity associated with these samples. The goals of the present study were to compare GC-ECD and GC-NCI-MS as detection methods for pyrethroids, examine the spatial and temporal patterns in pyrethroid and fipronil contamination in Ballona Creek sediment, and estimate their toxic potential to benthic invertebrates including *E. estuarius*.

MATERIALS AND METHODS

Bifenthrin (99%), permethrin (97%), and cypermethrin (95%) were obtained from FMC. Cyfluthrin (92%) and deltamethrin (99%) were obtained from Bayer CropScience. Lambda-cyhalothrin (99%) and fenpropathrin (>99%) were supplied by Syngenta and Valent, respectively. Esfenvalerate (98%) was purchased from Chem Service. Solutions of fipronil and fipronil desulfinyl, sulfide, and sulfone were purchased from AccuStandard. Polychlorinated biphenyls used as recovery surrogates and internal standards were purchased from Ultra Scientific. Florisil (60–100 mesh) was supplied

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by Supelco. Pesticide grade hexane and granular copper (20–30 mesh) were purchased from J.T. Baker. All glassware and sodium sulfate (Mallinckrodt) were baked at 500°C for 4 h before use.

Study area, sample collection, and processing

Ballona Creek is a 14-km-long flood-control channel that drains a highly urbanized 329 km^2 watershed in Los Angeles, a city of more than 4,000,000 residents. As no industrial or municipal discharges are permitted, this waterway conveys stormwater and dry season runoff to the Pacific Ocean near Marina del Rey, a large residential seaside complex. The local Mediterranean climate features distinct wet and dry seasons, with the majority of rainfall occurring in a four-month period (December through March). As a result, the bulk of the sediment load originating upstream of the 4-km tidally influenced estuary is deposited during the wet season [11]. Historic and reclaimed wetlands adjacent to the lower Ballona creek and the estuary have limited connectivity to the flood-control channel. Six stations along a 4-km tidally influenced stretch of this waterway were selected for the present study (Fig. 1).

Sediment samples were collected at each station during three dry season events: September 2007, June 2008, and October 2008. Samples were collected to a depth of 5 cm using a petite Ponar grab (subtidal stations) or a stainless steel shovel (intertidal stations), which were cleaned with site water and rinsed with methanol between stations. Sediment grabs from each station were composited and homogenized in a 19-L plastic tub with a methanol rinsed stainless steel spoon. Approximately 10 L of sediment was collected at each station for various physical, chemical, and biological measures. A well mixed aliquot was transferred to a 500-ml I-Chem glass jar for pyrethroid and fipronil analysis and 4 L were transferred to polyethylene jars for grain size analysis and toxicity testing. Sediment jars were stored and transported to the laboratory in an ice chest where the chemistry sample was immediately frozen at −20°C and the grain size/toxicity sample was stored at 4°C.

After thawing, sediment aliquots were freeze dried to a constant weight. One to five grams of freeze dried sediment was spiked with PCB 65 and PCB 209 (as recovery surrogates) and extracted with dichloromethane at 100°C and 1500 psi (four 5-min cycles) using a Dionex Accelerated Solvent Extraction 300 system. After concentration and solvent exchange to hexane, sediment extracts were treated with acid-activated copper to remove sulfur before chromatographic cleanup with 10 g of 6% water-deactivated Florisil. Target pyrethroid and fipronil analytes were eluted with 60 ml hexane/ethyl ether (7:3, v/v), which was then exchanged to hexane and concentrated to approximately 1 ml. Internal quantitation standards PCB 30 and PCB 205 were added to final extracts before GC analysis.

Percent solids, grain size (gravel, sand, silt, and clay) and total organic carbon (TOC) in sediment samples were determined using standardized methods. In addition, whole sediment, 10-d survival toxicity tests with the estuarine amphipod *Eohaustorius estuarius* were performed in accordance with standard protocols [12] on samples from the same six stations in the Ballona estuary. Toxicity testing was performed by the City of Los Angeles, Environmental Monitoring Division (September 2007 and June 2008 samples), and by the authors (October 2008 samples). Samples for toxicity testing were passed through a 2-mm sieve and homogenized before testing to remove potential predators and very coarse sediment. Percent survival was computed as the number of viable amphipods recovered at the end of the test.

GC analysis

Extracts of sediments collected in September 2007 were analyzed using a Hewlett-Packard 5890 Series II plus gas

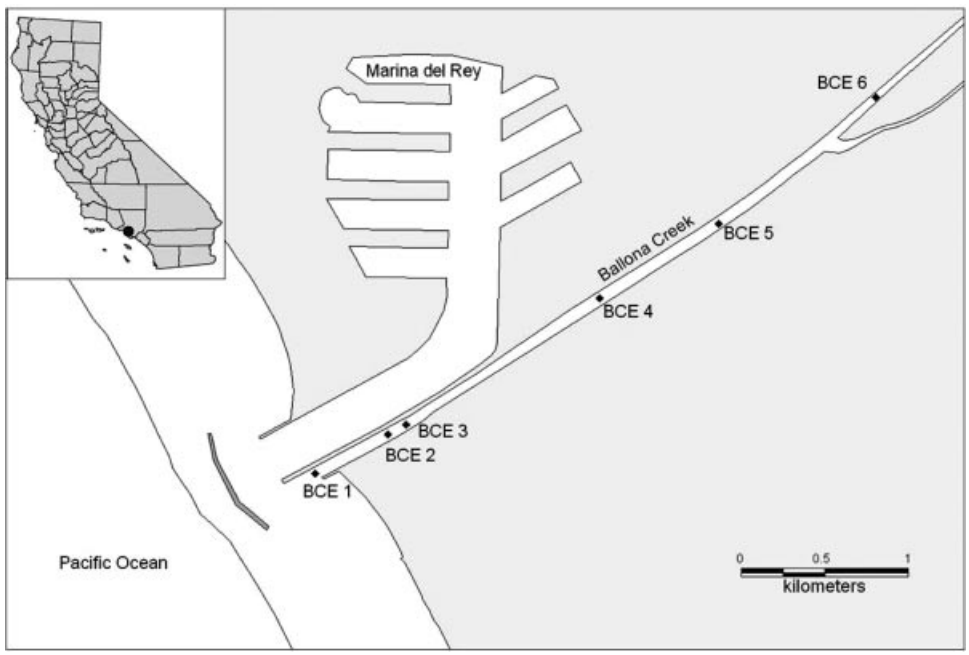

Fig. 1. Sediment sampling stations in the Ballona Creek estuary, Los Angeles (CA, USA).

chromatograph with two electron capture detectors and a 7683 autosampler outfitted with DB-5MS quantitation and DB-1701 confirmation columns (J&W Scientific. Both GC columns were 60 m × 0.25 mm internal diameter with a 0.25-μm film thickness. The injector and detector temperatures were 280 and 300°C, respectively. Two microliters of sample extract was injected in the splitless mode with a 2-min solvent delay. The GC oven was programmed at 170°C for 20 min, then ramped to 290°C at 4°C/min (20-min hold). Quantitation of target analytes was based on a six-point calibration curve (2–200 μg/L range) with PCB 30 and PCB 205 serving as internal standards.

Sediment extracts from the June and October 2008 events were analyzed using an Agilent 7890 digital gas chromatograph with a micro-electron capture detector (μ-ECD) and a 5975C quadrupole mass spectrometer. The two detectors were coupled to a single GC column by means of a two-way effluent splitter (split ratio of 18:0.3, MS: μ-ECD). A J&W Scientific DB-XLB capillary column (30 m × 0.25 mm × 0.25 μm) and ultrahigh purity helium (flow rate = 1.9 ml/min) was used to separate target analytes. The GC oven temperature program was as follows: 90°C (1 min hold); ramp to 150°C at 5°C/min, ramp to 260°C at 3°C/min, and final ramp to 320°C at 20°C/min (5 min hold). A 10-min post run at 330°C with helium backflush was preprogrammed to remove high-boiling components through the split vent to minimize carryover and retention time shift [13]. The split/splitless injector and μ-ECD were maintained isothermal at 280°C and 350°C, respectively. The injection volume was 1 μl and the MS was operated in the NCI mode with methane as the moderating gas. Transfer line, ion source, and quadrupole MS temperatures were 320, 150, and 150°C, respectively. Primary and secondary target ions determined from injections of authentic standards were acquired using the selected ion monitoring mode. Quantitation was performed using a multi-point calibration curve with PCB 205 as the internal standard for all target analytes.

Quality assurance and quality control

The analytical methods described above were validated using a performance-based approach that included analysis of procedural blanks, matrix spikes, replicate samples, and assurance of quantitative target analyte recovery using spiked surrogates. Frozen sediment samples were processed within 72 h of sample collection. No target analytes were detected in blanks consisting of organic solvents or sodium sulfate extracted in parallel with the sediment samples. Mean recoveries of target analytes spiked into sediment at 0.5, 10, and 100 ng/g dry weight and analyzed by dual column GC-ECD were 74 ± 8% ($n = 3$), 95 ± 15% ($n = 2$), and 95 ± 4% ($n = 2$) for the eight pyrethroid analytes, respectively, and 84 ± 7%, 99 ± 13%, and 96 ± 4% for the four fipronil analytes, respectively. Surrogate recoveries for the September 2007 samples ranged from 66 ± 6% to 95 ± 5% for PCB 65 and 209. Using GC-NCI-MS and μECD for the 2008 samples, recoveries for sediment spiked at 20 and 50 ng/g dry weight were 99 ± 11% and 96 ± 8% ($n = 2$) for pyrethroids, respectively; and 104 ± 9% and 95 ± 17% for fipronil, respectively. Surrogate recoveries of PCB 65 and PCB209 were 96 ± 3% and 93 ± 3%. Final results were not corrected for surrogate recovery.

The relative percent difference (mean ± standard deviation) of target analyte concentrations in sample duplicates was 13 ± 14% for dual column GC-ECD and 8.3 ± 5.0% ($n = 4$) for GC-NCI-MS/μECD. Limits of detection were calculated by dividing the lowest detectable target analyte mass (minimum signal to noise ratio of 3) by the mass of sediment extracted. Limits of detection for fipronil were 0.05 to 1.0 ng/g and 0.02 to 0.20 ng/g using dual column GC-ECD and GC-NCI-MS, respectively. Corresponding LODs for pyrethroids were 0.04 to 1.8 ng/g and 0.015 to 1.5 ng/g.

Data analysis

Univariate statistics and Spearman's rank correlational analyses were computed using SigmaStat (Ver 2.03, SPSS). Individual and total pyrethroid and fipronil ($\sum$PYR and $\sum$FIP) concentrations in sediment were expressed on a dry weight basis. Toxic units (TUs) for individual and total pyrethroids or fipronil were calculated on an organic carbon (OC) basis as follows:

$$\mathrm{TU} = \frac{\text{Sediment concentration } (\mu g/g\ \mathrm{OC})}{\mathrm{LC}\,50(\mu g/g\ \mathrm{OC})}$$

using 10-d whole sediment median lethal concentration values (LC50s) of 1.05 and 0.52 μg/g OC for bifenthrin and 18.7 and 10.8 μg/g OC for permethrin for *E. estuarius* and the freshwater amphipod *Hyallela azteca*, respectively [14]. For the remaining six pyrethroids, LC50 values for *H. azteca* were between 0.38 and 1.54 μg/g OC; for fipronil, fipronil sulfide and fipronil sulfone, the reported values were 4.1, 7.7, and 9.7 μg/g OC, respectively [6,15].

RESULTS AND DISCUSSION

Comparison of analytical methods

Accurate quantitation of low-level pesticides is predicated on chromatographic resolution of target peaks, which becomes more complex for pyrethroids that exist as diastereomeric isomers. To date, most investigators have used 30-m columns with nonpolar (DB-1 or DB-5) stationary phases [16–18]. In the present study, a 60-m DB-5 column achieved excellent separation for all target compounds, including the four diastereomeric isomers each of cyfluthrin and cypermethrin (Fig. 2) [19,20]. With the 30-m DB-XLB column coupled to the NCI-MS/μECD system, PCB 209 used as a recovery surrogate co-eluted with three of four cypermethrin isomers in total ion chromatograms (Fig. 2). However, selective ion monitoring with NCI-MS was able to separate these overlapping signals to allow quantitation of all four cypermethrin stereoisomers.

The complexity associated with chromatographic separation of pyrethroids is particularly important when using different stationary phases and a nonselective detector like ECD. For example, bifenthrin and fenpropathrin co-elute using a DB-1 column, and multiple co-elutions of cyfluthrin and cypermethrin stereoisomers, and fipronil with fipronil sulfide using a 60-m DB-1701 column has been observed [21]. This latter stationary phase, which is commonly used to confirm analytes in a dual channel system, also does not completely resolve λ-cyhalothrin and *cis*-permethrin. In extracts of environmental samples, the selectivity of NCI-MS and its ability to resolve co-eluting peaks by mass provides a key advantage over nonspecific detectors.

The unique configuration of our GC-NCI-MS/μ-ECD instrument allowed for simultaneous comparison of detector

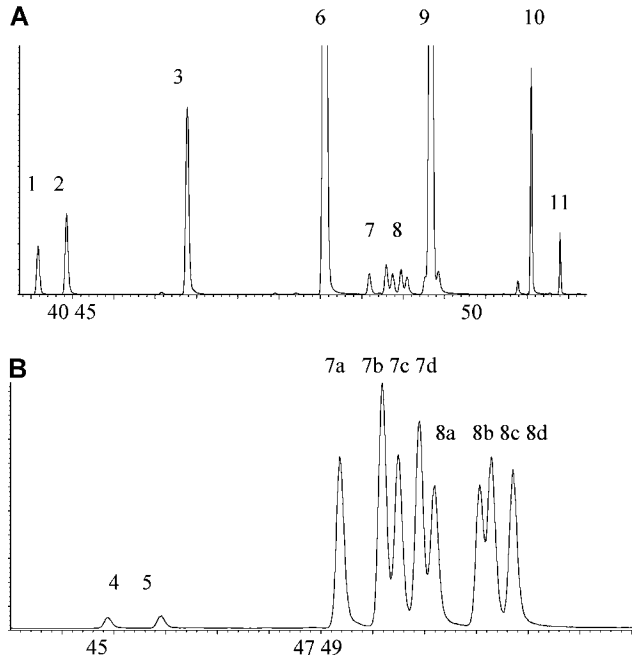

Fig. 2. Chromatographic separation of (**A**) eight pyrethroids with a 30 m DB-XLB using negative chemical ionization mass spectrometry in selected ion monitoring mode (*m/z* 207); and (**B**) for stereoisomers of cyfluthrin and cypermethrin. Peak identification: 1-bifenthrin, 2-fenpropathrin, 3-λ-cyhalothrin, 4-*cis*-permethrin, 5-*trans*-permethrin, 6-PCB 205 (internal standard), 7-cyfluthrin (4 peaks), 8-cypermethrin (4 peaks), 9-PCB 209 (recovery surrogate), 10-esfenvalerate, 11-deltamethrin.

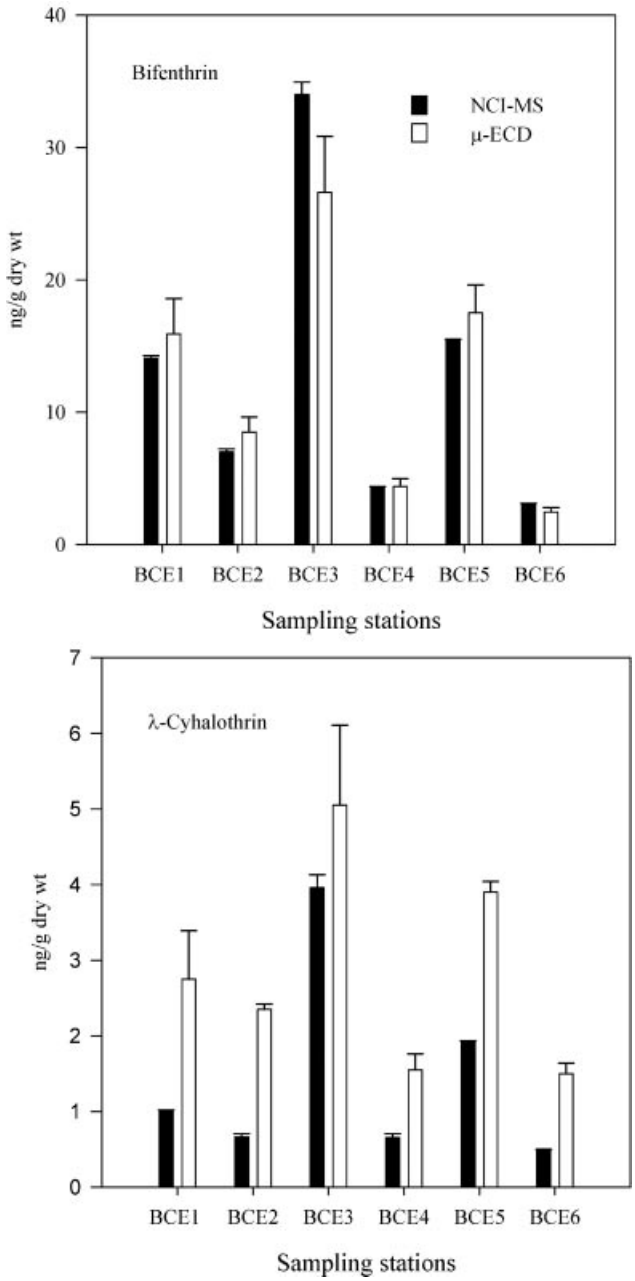

Fig. 3. Comparison of bifenthrin (top) and λ-cyhalothrin (bottom) concentrations in Ballona estuary sediments determined by negative chemical ionization mass spectrometry (NCI-MS) and electron capture detection (ECD). Values are means for duplicate injections; error bars represent standard deviation.

response, as was reported previously for analysis of organic contaminants in water [13]. Agreement between the two detection methods was excellent (< 27% relative percent difference) for bifenthrin, which was present in moderate concentrations in Ballona sediments (5–50 ng/g) (Fig. 3). This level of agreement also indicates stable and reproducible operation of the two-way effluent splitter configuration, which incorporates a post-run helium backflush at 330°C to minimize response and retention time fluctuations due to contamination within the instrument. For λ-cyhalothrin at lower levels (0.5 to 5 ng/g), μ-ECD invariably gave higher estimates than NCI-MS, a trend that was consistent among other pyrethroids detected at lower levels. It follows that the background associated with the sample matrix and the aforementioned co-elution issues account for the positive bias for ECD. Previous studies comparing different mass spectrometric configurations and operating modes (e.g., EI, NCI, and MS/MS) and ECD for analysis of pyrethroids concluded that sensitivity was similar but that the selectivity of NCI-MS and MS/MS was superior to ECD [8–10,17,22,23]. No such comparisons for fipronil were found in the literature.

Pyrethroid concentrations

The 18 sediment samples from the Ballona estuary were fine-grained to mostly sand, with two of the three samples collected at station 6 containing more than 80% gravel (Table 1). Percent fines (= silt+clay fractions) ranged between 0 (station 5; Sept. 2007) and 56.8% (station 3, Sept. 2007). Total organic carbon ranged between 0.32 (station 4; June 2008) to 2.95 % (station 5, October 2008) but was not determined for station 6 (September 2007) due to the large amount of gravel. A high degree of variability in grain texture and TOC across sampling events was observed for stations 3 and 5. Grain size was not measured for sediments collected in October 2008. Total pyrethroids ($\sum$PYR) was highly correlated with percent fines ($R_s = 0.77$; $p < 0.01$; $n = 12$) and TOC ($R_s = 0.64$; $p < 0.01$; $n = 18$). The maximum pyrethroid levels in the present study were greater than or equal to previously reported for several urban creeks in California [1].

Of the eight pyrethroids targeted, only fenpropathrin was not detected, which is in agreement with a 2008 investigation of sediment in California urban waterways [2]. Whereas only bifenthrin and permethrin were detected by ECD at all six

Table 1. Physicochemical composition and total pyrethroid and fipronil concentration (ng/g dry wt) for Ballona estuary sediments (CA, USA)

Station	Date	% FINES[a]	%TOC[b]	ΣPYR[c] (ng/g)	ΣFIP[d] (ng/g)
1	September 2007	19.9	0.57	10 (4)	≤ 2.2 (5)
	June 2008	15.2	0.44	47 (3)	≤ 0.61 (4)
	Oct. 2008	NA	1.15	107 (2)	≤ 0.84 (2)
2	September 2007	35.0	0.97	212 (2)	4.7 (2)
	June 2008	33.6	1.17	143 (2)	2.9 (2)
	October 2008	NA	1.15	41 (4)	≤ 0.23 (5)
3	September 2007	56.8	1.38	473 (1)	17 (1)
	June 2008	2.4	0.55	43 (4t)	≤ 0.34 (5)
	October 2008	NA	2.49	233 (1)	≤ 0.74 (3)
4	September 2007	7.0[e]	0.53	<12 (6)	≤ 3.2 (3t)
	June 2008	6.6	0.32	19 (6)	0.32 (6)
	October 2008	NA	0.74	21 (5)	≤ 0.18 (6)
5	September 2007	0[f]	0.38	≤ 14 (3)	≤ 3.2 (3t)
	June 2008	53.6	1.74	333 (1)	7.1 (1)
	October 2008	NA	2.95	60 (3)	≤ 0.86 (1)
6	September 2007	0.4[g]	NA	≤ 10 (5)	2.0 (6)
	June 2008	6.21[h]	0.33	43 (4t)	1.1 (3)
	October 2008	NA	0.31	17 (6)	≤ 0.26 (4)

[a] %FINES = sum of silt and clay fractions.
[b] %TOC = total organic carbon.
[c] Sum of eight pyrethroids (station ranking in parentheses; t signifies a tie).
[d] Sum of fipronil, fipronil desulfinyl, sulfide, and sulfone (station ranking in parentheses; t signifies a tie).
[e] 46.8% gravel.
[f] 8.7% gravel, 91.3% sand.
[g] 82.1% gravel.
[h] 82.4% gravel.
< = not detected; ≤ = detected at level lower than limit of detection; NA = data not available.

stations for September 2007, the six remaining pyrethroids were detected in all samples from June and October 2008 using GC-NCI-MS (Fig. 4). Permethrin (sum of *cis* and *trans*-isomers) was the most abundant pyrethroid (Table 2), with a maximum concentration (190 ng/g) that was higher than reported previously in California for the Sacramento urban area (94 ng/g)[24] but lower than for the San Joaquin valley, an agriculturally-dominated area (460 ng/g) [25]. Bifenthrin was the next most abundant pyrethroid, with concentrations ranging between 3.0 and 80 ng/g. These levels constitute the lower to middle of the range reported in the aforementioned statewide sediment investigation [2] and were several fold lower than the highest concentrations reported in a mixed land use watershed in the same region [4].

Cypermethrin and cyfluthrin were the next most abundant compounds, accounting for roughly 10 and 7% of total pyrethroid concentrations (Table 2). Together with permethrin and bifenthrin, these compounds accounted for 90% or more of $\sum$PYR. The sediment pyrethroid distribution was significantly correlated with their usage for pest control in Los Angeles county ($R_s = 0.86$ and 0.94; $p < 0.01$, for June and October 2008, respectively), perhaps linking their application to sediments in this urban watershed (www.cdpr.ca.gov). Percent contribution in sediment and usage was similar for several pyrethroids, including cyfluthrin, λ-cyhalothrin, and permethrin (Table 2). In contrast, the average contribution of bifenthrin in sediments was three times greater than its corresponding usage (16–18% vs 4.9%), confirming the higher degree of persistence for this compound reported previously ($t_{½}$ of 251–498 d under anaerobic conditions) [26]. The opposite was true for cypermethrin where its average contribution in sediment was approximately half of its average usage, suggesting a lower relative degree of persistence or attenuated transport into this waterway for this active ingredient.

The ratio of *cis*- to *trans*-permethrin in commercial products has been reported to be approximately 0.7 [27], which is similar to the average value of 0.85 ± 0.28 observed in runoff from two commercial nurseries in Orange County, California [21]. In comparison, previous investigators reported higher average ratios for sediments, e.g., 2.63 ± 0.92 [28], 3.26 ± 1.33 [29], and 2.64 [30]. The average *cis*- to *trans*-permethrin ratio for sediments in the present study was 1.67 ± 0.19 (June 2008), and 1.89 ± 0.23 (Oct. 2008). Assuming a starting value that is less than unity (i.e., a nondegraded condition), this parameter can indicate the degree of weathering in sediment samples [28]. The intermediate values for Ballona sediments would suggest a shorter residence time than in other systems; moreover, the higher mean ratio for the October 2008 samples suggests an enrichment of the *cis* isomer (i.e., more weathering) compared with the June samples.

Fipronil and its degradates

Fipronil desulfinyl, sulfide, and sulfone were detected in samples from each of the three sampling events. In contrast, the parent compound was detected infrequently; with only four of 18 samples containing levels above the LOD. Fipronil sulfone was the most abundant degradate, detected in 100% of the samples at concentrations ranging up to 9.8 ng/g. Fipronil desulfinyl and sulfide were detectable in all 2008 samples but at lower concentrations, generally < 1.0 ng/g. For the September 2007 event, these degradates were detected at stations 3, 4, and 6, with a maximum concentration of 6.2 ng/g for desulfinyl (station 3) and 1.5 ng/g for sulfide (station 4).

Fipronil sulfone accounted for more than 50% of $\sum$FIP in Ballona sediments, compared with approximately 20% each for the desulfinyl and sulfide degradates. Less than 10% of $\sum$FIP was attributable to fipronil, indicating a short half-life in the aquatic environment. Indeed, the parent compound is susceptible to breakdown through reduction, oxidation, photolysis, hydrolysis, and biological transformation, but the extent and products of transformation are highly dependent on environmental conditions. In the rice-producing area of Louisiana (USA), fipronil was not detected in anoxic bed sediments of wetland tributaries but its degradates were found with sulfide > sulfone > desulfinyl [31]. In contrast, fipronil sulfone was more abundant in sediments of an urban stream in central Texas, USA, which were reported to be largely aerobic [6]. Although the actual depositional history and sedimentary conditions in this system are likely complex, the greater than unity ratios of fipronil sulfone to sulfide for most of our samples would suggest aerobic conditions were predominant.

Individual and total concentrations of fipronil were at least ten times lower than for pyrethroids (Table 1). This is consistent with findings from previous field surveys, including an investigation of freshwater sediments by the U.S. Geological Survey [5,6]. The spatial pattern of fipronil contamination also coincided with pyrethroids, with the highest $\sum$FIP occurring at stations 2 and 3 (Sept. 2007); stations 2 and 5 (June 2008); and at stations 1 and 3 (Oct. 2008). Total fipronil ($\sum$FIP) was highly

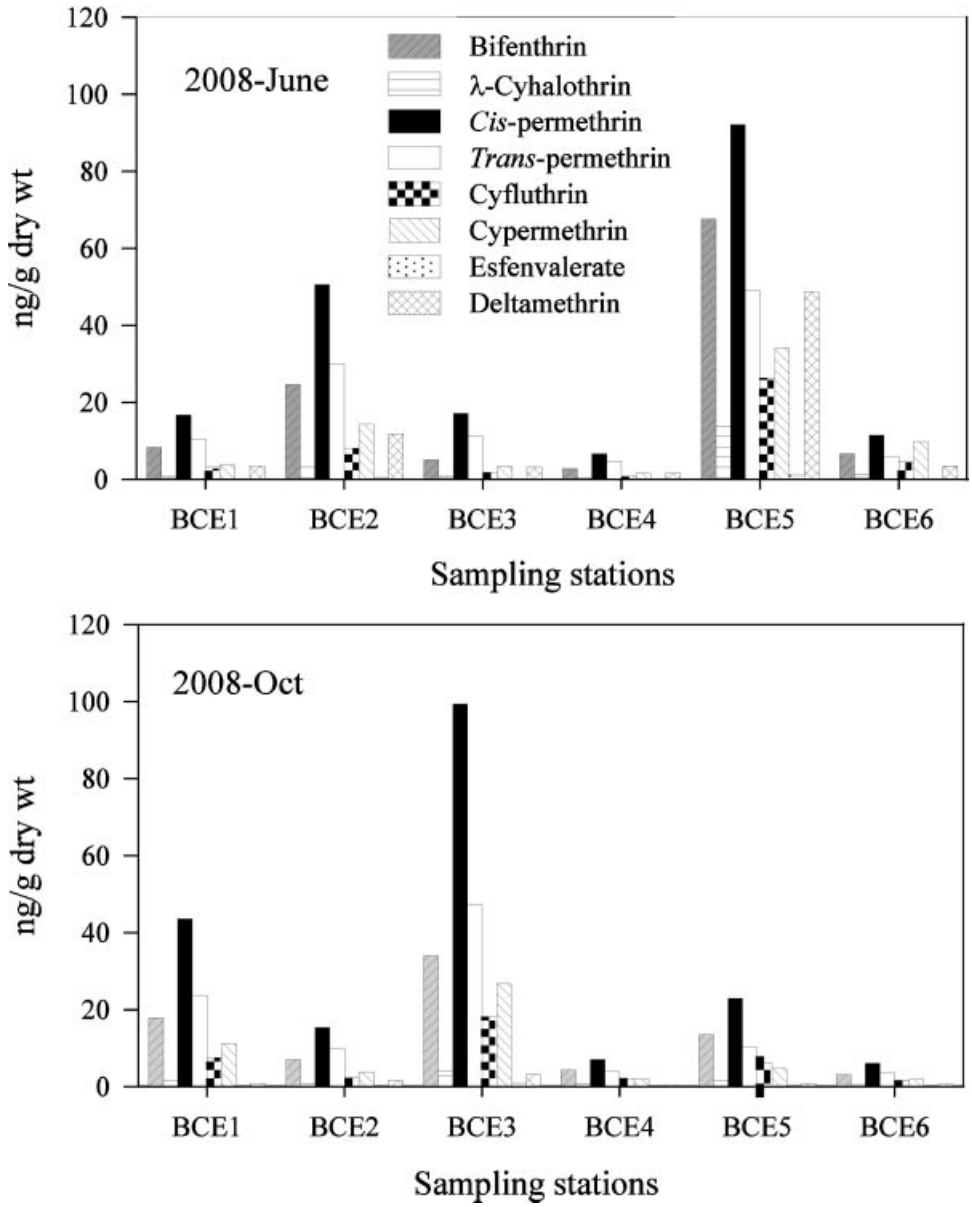

Fig. 4. Concentration of individual pyrethroids in Ballona estuary sediments (CA, USA) collected in June 2008 (top) and October 2008 (bottom).

Table 2. Percent contribution of eight common pyrethroids (mean ± standard deviation) in Ballona estuary (CA, USA) sediments[a] compared with local usage statistics

	Percentage of total pyrethroids (%)		
Compound	June 2008	October 2008	Usage[b]
Bifenthrin	16 ± 3	18 ± 3	4.9 ± 3
Fenpropathrin	ND	ND	NA
λ-Cyhalothrin	2.5 ± 0.8	2.4 ± 0.7	3.0 ± 2.0
Permethrin	54 ± 10	59 ± 4	67 ± 6
Cyfluthrin	6.7 ± 2.2	8.3 ± 1.7	4.2 ± 3.1
Cypermethrin	11 ± 5.7	9.9 ± 1.2	20 ± 9.3
Esfenvalerate	0.4 ± 0.1	0.4 ± 0.1	0.1 ± 0.05
Deltamethrin	8.9 ± 2.8	2.2 ± 1.2	0.8 ± 0.4

[a] Collected from six stations in June and October 2008.
[b] Average annual use for structural pest control in Los Angeles County (CA, USA; 1999–2006) (www.cdpr.ca.gov).
NA = data not available; ND = not detected.

correlated with sediment fines ($R_s = 0.79$; $p < 0.01$; $n = 12$), but was not correlated with TOC ($R_s = 0.31$; $p < 0.2$; $n = 18$). In the absence of usage data to assess occurrence and persistence, the lower hydrophobicity and sediment-water partition coefficients for fipronil and its degradates compared with pyrethroids [32–34] can explain these trends.

ESTIMATION OF TOXIC UNITS FOR BENTHIC INVERTEBRATES

A toxic unit exceeding one indicates a concentration that exceeds the established LC50, normalized to OC. Because LC50 values for the estuarine amphipod *E. estuarius* are currently limited to bifenthrin and permethrin [14], LC50 values for *Hyalella azteca*, a commonly used freshwater amphipod test species, were used to estimate the toxic potential for all target pyrethroids (except for fenpropathrin which was not detected in any sample). Based on available data, *H. azteca*

is approximately twice as sensitive to pyrethroids and thus a TU exceeding two is assumed to be toxicologically relevant for *E. estuarius*.

Toxic units for individual pyrethroids based on *H. azteca* for all sediment samples are summarized in Table 3. The summation of pyrethroid TUs ($\sum TU_{pyrethroids}$) by station revealed that stations 2 and 3 (Sept. 2007) would be predicted to exert the greatest toxicity, followed by stations 5 and 6 (June 2008). Corresponding percent survival of *E. estuarius* from toxicity tests were 3, 0, 0, and 49%, respectively. At the other end of the spectrum, sediments with the lowest predicted toxicity based on TUs were stations 1, 4, and 6 (September 2007); station 4 (June 2008) and stations 4 and 5 (Oct. 2008). Corresponding percent survival of *E. estuarius* for these samples were 89, 16, and 8%; 89%; and 73 and 78%, respectively. Using Spearman's rank correlation, percent mortality of *E. estuarius* was significantly correlated with $\sum TU_{pyrethroids}$ ($R_s = 0.471$; $p = 0.04$; Fig. 5), indicating that pyrethroids likely play a significant role in explaining toxicity.

For bifenthrin, TUs based on *E. estuarius* were greater than unity for the same nine of 18 samples which contributed 49% of $\sum TU_{pyrethroids}$ based on *H. azteca* (Table 3). In contrast, TUs for permethrin based on either invertebrate species were uniformly low (i.e., < 1 for *E. estuarius*; < 2 for *H. azteca*, Table 3), with a contribution of less than 7% of $\sum TU_{pyrethroids}$ (Table 3). For cypermethrin, $\sum TU_{pyrethroids}$ was > 2 for eight of 18 samples, contributing $46 \pm 11\%$ to this sum. Cyfluthrin TUs were > 5 for two samples (stations 2 and 3; Sept. 2007), but its overall average contribution to summed TUs ($\sim$17%) was lower than for bifenthrin or cypermethrin.

Toxic units for individual and total fipronil based on *H. azteca* were all less than 0.1 (computations not shown),

Table 3. Toxic units (TUs) for pyrethroids in Ballona Creek (CA, USA) sediments based on *Hyalella azteca* (supporting data from Hintzen et al. [6] and Amweg et al. [15])

Station	1	2	3	4	5	6	No. TU $\geq$1
September 2007							
Bifenthrin	1.0	5.3	11.1	1.3	2.3	1.2	6
λ-Cyhalothrin	0	1.1	1.8	0	0	0	2
Permethrin	0.1	0.6	1.3	0.1	0.2	0.1	1
Cyfluthrin	0	5.3	6.4	0	0	0	2
Cypermethrin	0	11	11	0	0	0	2
Esfenvalerate	0	0.8	1.2	0	0	0	1
Deltamethrin	0	1.1	1.3	0	0	0	2
$\Sigma TU_{pyrethroids}$	1.1	25	34	1.4	2.5	1.3	16
June 2008							
Bifenthrin	3.6	4.0	1.8	1.7	7.5	3.8	6
λ-Cyhalothrin	0.5	0.6	0.4	0.3	1.8	0.8	1
Permethrin	0.6	0.6	0.5	0.3	0.7	0.5	0
Cyfluthrin	0.7	0.6	0.3	0.3	1.4	1.3	2
Cypermethrin	2.3	3.2	1.6	1.3	5.2	7.8	6
Esfenvalerate	0	0	0	0	0	0	0
Deltamethrin	1.0	1.3	0.7	0.6	3.5	1.3	4
$\Sigma TU_{pyrethroids}$	8.7	10	5.3	4.5	20	16	19
October 2008							
Bifenthrin	3.0	1.2	2.6	1.1	0.9	1.9	5
λ-Cyhalothrin	0.3	0.1	0.4	0.2	0.1	0.4	0
Permethrin	0.5	0.2	0.5	0.1	0.1	0.3	0
Cyfluthrin	0.6	0.2	0.7	0.3	0.2	0.5	0
Cypermethrin	2.6	0.9	2.8	0.7	0.4	1.6	3
Esfenvalerate	0.0	0.0	0.0	0.0	0.0	0.0	0
Deltamethrin	0.1	0.2	0.2	0.1	0.0	0.2	0
$\Sigma TU_{pyrethroids}$	7.1	2.8	7.2	2.5	1.7	4.9	8

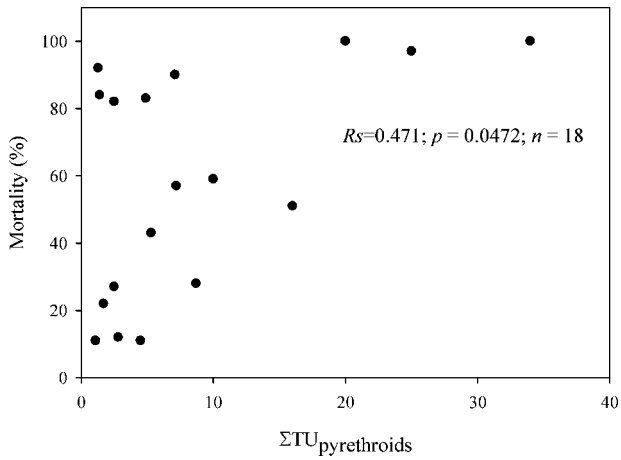

Fig. 5. Percent mortality of *Eohaustorius estuarius* in 10-d whole sediment toxicity tests versus the sum of toxic units for detectable pyrethroids ($\sum TU_{pyrethroids}$) in Ballona estuary sediments (CA, USA). Toxic units are based on the freshwater amphipod *Hyallela azteca*. (R_s = Spearman's rank correlation coefficient).

suggesting a low potential for sediment toxicity and indicating a negligible contribution to the observed toxicity to *E. estuarius*. This is due to their low concentrations and their intermediate toxicity to our surrogate species (*H. azteca*) relative to pyrethroids. However, fipronil and in particular its degradates have been shown to be extremely toxic to other crustacean species, including the crayfish *Procambarus clarkii* [35]. Considering a test species that is more sensitive to fipronil (e.g., the freshwater midge *Chironomous tentans*]) [7], average summed TUs for fipronil and its sulfide and sulfone metabolites exceed unity for eight of the 18 samples, including stations 1 to 5 for September 2007 and stations 2, 5, and 6 for June 2008. Thus, the species of concern plays a large role in determining risk of pyrethroid and fipronil contamination in sediments.

Spatial and temporal variability

The spatial pattern associated with elevated pyrethroid and fipronil sediment concentrations is readily explained by grain texture, i.e., the fine-grained sediments enriched with organic carbon contained the highest levels of these pesticides (Table 1). The dry season in southern California occurs from May through October [11]. The sampling events occurred at the end of the 2007 dry season (September) and at the beginning (June) and end (October) of the 2008 dry season. Greater than 90% of the annual sediment load transported within the watershed is carried by means of runoff during storm events in the months preceding the dry period [11]. These sediments can accumulate through the end of the wet season, resulting in fine-grained substrates being deposited in the upper estuary in a pattern similar to that observed in June 2008 (Fig. 4). During summer, low-flow conditions, tidal exchange drives sediment transport and resuspension/deposition cycles slowly transport the accumulated fine-grained sediments seaward, e.g., toward stations 2 and 3 (Oct. 2008; Fig. 4).

While the observed spatial pattern in fine-grained sediments and pesticide contamination also holds true in general for prediction of toxicity based on TUs (Table 3 and Fig. 5), samples with high levels of permethrin do not correspond with observed sediment toxicity. This is due to its lower intrinsic toxicity,

approximately 20 times less toxic than bifenthrin and other pyrethroids, to commonly used test invertebrates. To ameliorate concerns over sediment toxicity, measures to limit the input and concentrations of the more toxic and persistent pyrethroids such as bifenthrin, cyfluthrin, and cypermethrin should be considered. Elevated concentrations of pyrethroids could not, however, explain high mortality of toxicity to *E. estuarius* in all cases. At least three of the five samples with high mortality but relatively low $\sum$TU for pyrethroids (Fig. 5) or fipronil contained $> 90\%$ coarse grained substrate (Table 1). Other unmeasured toxicants may also be present in these samples.

Another factor affecting the distribution of fine-grained sediments and associated contaminants in this system is sediment scour due to energetic stormwater flows. Precipitation recorded by the California Irrigation Management Information System at the station nearest the study area during the two interceding wet seasons was 8.33 cm (2007) and 31.5 cm (2008). The greater amount of precipitation during the 2008 wet season suggests an increased channel flow and higher potential for scour of bottom sediments. Because the Ballona estuary is channelized, high stormwater flows will reduce sediment residence times within the estuary (i.e., sediment blowout). In summary, the fate of current use pesticides such as pyrethroids and fipronil within the Ballona estuary lie with fine-grained sediments, which in turn shift throughout the estuary depending on the time of collection and the intensity of preceding rainfall events.

Preliminary results from toxicity identification evaluation studies performed by the authors on these same set of samples did not indicate a metal contribution, and strongly supported the hypothesis that organic constituents were responsible for the observed response. Piperonyl butoxide, a synergist of pyrethroid pesticides, may increase the toxicity of pyrethroids already present [24,36]. Modulation of response after treating with this synergist gave further evidence that pyrethroids, and not legacy organics such as DDT or chlordanes, were contributing to the toxicity in these sediments. However, it remains possible that the presence of unmeasured toxicants, including several dozens of regulated pesticides that are routinely applied in California, may also contribute to the observed toxicity. Vector control agents such as methoprene used to combat mosquitoes in and around urban areas with artificial and natural wetlands may also exert toxic effects to nontarget organisms. An extremely high concentration of methoprene (440 ng/g) was detected in the sample from station 3 for September 2007 (K. Smalling, U.S. Geological Survey, personal communication), which proved to be extremely toxic to *E. estuarius* (Fig. 5). Methoprene was not detected, however, in the two other highly toxic samples where summed pyrethorid TUs for *H. azteca* were 25 and 20 (Table 3; station 2, Sept. 2007 and station 5, June 2008).

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