# Analysis, occurrence, and toxic potential of pyrethroids and fipronil in sediments from the Ballona Creek estuary

*Wenjian Lao, David Tsukada, Darrin J. Greenstein, Steven M. Bay and Keith A. Maruya*

## 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, 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 lower levels (≤0.18 to 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 2008 dry season.

## Introduction

Current use pesticides are a category of largely unregulated chemicals that are cause for concern due to their toxicity to non-target species and/or persistence in the environment. As many organophosphate insecticides were phased out in the United States (US) over the past two decades, synthetic pyrethroids and the phenylpyrazole compound known as fipronil have been substituted for use in urban and suburban landscapes (Amweg *et al*. 2006a). 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 (Holmes *et al*. 2008) with a single compound (bifenthrin) found in all 30 waterways sampled. In addition to bifenthrin, cypermethrin and cyfluthrin were detected in residential runoff (Weston *et al*. 2009) and fenpropathrin was reported as one of the most frequently detected pyrethroids in a mixed land use watershed in southern California (Budd *et al*. 2007). While fipronil and its degradates have been found at much lower concentrations than pyrethroids (Gunasekara *et al*. 2007, Hintzen *et al*. 2009), their potential for toxicity to non-target organisms is relatively high (Gunasekara *et al*. 2007).

In most studies published to date, the non-specific electron capture detector (ECD) was employed to detect and quantify pyrethroids and fipronil in the environment (Holmes *et al*. 2008, Maul *et al*. 2008). Proper identification and thus accurate quantitation using ECD becomes difficult at trace levels, particularly for chiral pyrethroids whose multiple stereoisomers may co-elute with other constituents present, even when employing dual column analysis. Conversely, not accounting for all chiral components 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 (Esteve-Turrillas *et al.* 2004, 2006; You *et al.* 2008).

Ballona Creek is a relatively short, channelized waterway that drains highly urbanized landscapes within the city of Los Angeles, CA. In a recent study, sediments from the tidally influenced creek mouth exhibited a spatially distinct pattern of toxicity to the estuarine amphipod, *E. estuarius*. Analysis of priority pollutants including chlordanes, DDTs and PCBs that are regulated under the Clean Water Act could not explain the observed toxicity associated with these samples. The goals of this 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*.

## METHODS

Bifenthrin (99%), permethrin (97%), and cypermethrin (95%) were obtained from FMC (Princeton, PA, USA). Cyfluthrin (92%) and deltamethrin (99%) were obtained from Bayer CropScience (Stilwell, KS, USA). Lambda-cyhalothrin (99%) and fenpropathrin (>99%) were supplied by Syngenta (Bracknell, Berks, UK) and Valent (Walnut Creek, CA, USA), respectively. Esfenvalerate (98%) was purchased from Chem Service (West Chester, PA, USA). Solutions of fipronil and fipronil desulfinyl, sulfide and sulfone were purchased from AccuStandard (New Haven, CT, USA). PCB congeners used as recovery surrogates and internal standards were purchased from Ultra Scientific (Kingstown, RI, USA). Florisil (60 - 100 mesh) was supplied by Supelco (Bellefonte, PA, USA). Pesticide grade hexane and granular copper (20 - 30 mesh) were purchased from J.T. Baker (Phillipsburg, NJ, USA). All glassware and sodium sulfate (Mallinckrodt, Phillipsburg, NJ, USA) were baked at 500ºC for 4 hours before use.

### Study Area, Sample Collection and Processing

Ballona Creek is a nine-mile long flood-control channel that drains a highly urbanized 329 km² watershed in Los Angeles, CA, USA, 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 (Stein and Tiefenthaler 2005). Historic and reclaimed wetlands adjacent to lower Ballona Creek and the estuary have limited connectivity to the flood-control channel. Six stations from the upper estuary to the rivermouth were selected for this study (Figure 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. Between 1 and 5 g 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-minute cycles) using a Dionex Accelerated Solvent Extraction (ASE) 300 system (Sunnyvale, CA, USA). After concentration and solvent exchange to hexane, sediment extracts were treated with acid-activated copper to remove sulfur prior to 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 ~1 ml. Internal quantitation standards PCB 30 and PCB 205 were added to final extracts prior to GC analysis.
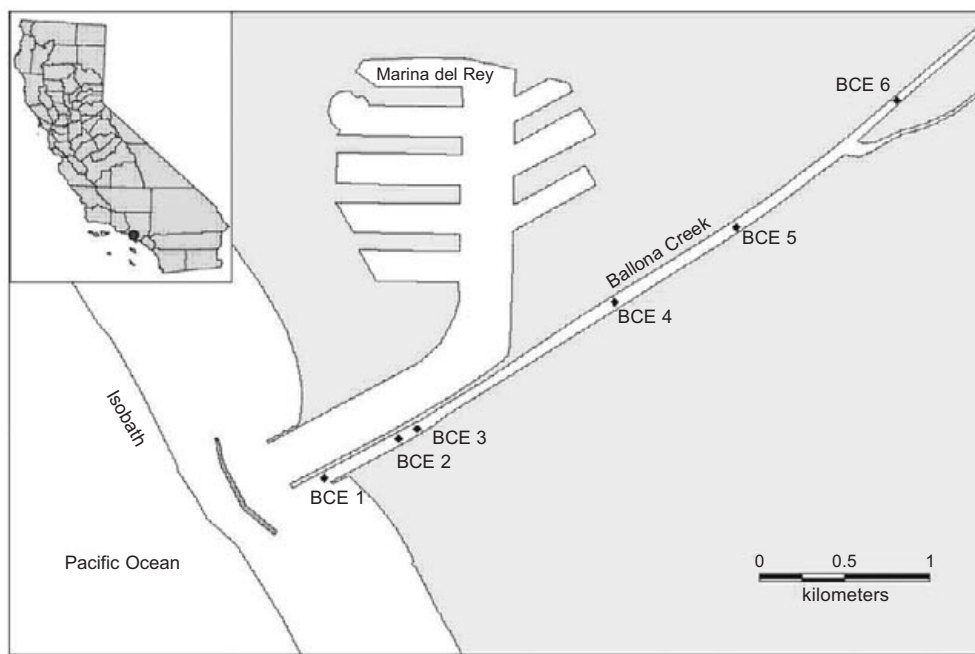
**Figure 1. Sediment sampling stations in the Ballona Creek estuary, Los Angeles, CA.**

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-day survival toxicity tests with the *E. estuarius* were performed in accordance with standard US Environmental Protection Agency (USEPA) protocols (USEPA 1994) on samples from the same 6 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 10-day test.

## Gas Chromatography Analysis

Extracts of sediments collected in September 2007 were analyzed using a Hewlett Packard 5890 Series II plus gas chromatograph with two electron capture detectors and a 7683 autosampler (Palo Alto, CA, USA) outfitted with DB-5MS "quantitation" and DB-1701 "confirmation" columns (J&W Scientific, Folsom, CA, USA). Both GC columns were 60 m L x 0.25 mm id with a 0.25-μm film thickness. The injector and detector temperatures were 280˚C and 300˚C, respectively. Two ml of sample extract was injected in the splitless mode

with two-minute solvent delay. The GC oven was programmed at 170˚C for 20 minutes, ramped to 290˚C at 4˚C/minutes (20-minute hold). Quantitation of target analytes was based on a 6-point calibration curve (2 - 200 mg/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 digitial gas chromatograph with a micro-electron capture detector (μ-ECD) and a 5975C quadrupole mass spectrometer (Wilmington, DE, USA). The two detectors were coupled to a single GC column via a two-way effluent splitter (split ratio of 18:0.3, MS: μ-ECD). A J&W Scientific DB-XLB capillary column (30 m x 0.25 mm x 0.25 mm) and ultrahigh purity helium (flow rate = 1.9 ml/minute) was used to separate target analytes. The GC oven temperature program was as follows: 90°C (1-minute hold); ramp to 150°C at 5°C/minute, ramp to 260°C at 3°C/minute, and final ramp to 320°C at 20°C/minute (5-minute hold). A 10-minute post run at 330°C with helium backflush was pre-programmed to remove high-boiling components through the split vent to minimize carryover and retention time shift (Gomez *et al*. 2009). 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 negative chemical ionization (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 selected ion monitoring (SIM) 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 hours 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 $74 \pm 8\%$ ($n = 3$), $95 \pm 15\%$ ($n = 2$), and $95 \pm 4\%$ ($n = 2$) for the 8 pyrethroid analytes, respectively, and $84 \pm 7\%$, $99 \pm 13\%$, and $96 \pm 4\%$ for the 4 fipronil analytes, respectively. Surrogate recoveries for the September 2007 samples ranged from $66 \pm 6\%$ to $95 \pm 5\%$ for PCB 65 and 209. Using GC-NCI-MS and m-ECD for the 2008 samples, recoveries for sediment spiked at 20 and 50 ng/g dry wt were $99 \pm 11\%$ and $96 \pm 8\%$ ($n = 2$) for pyrethroids, respectively; and $104 \pm 9\%$ and $95 \pm 17\%$ for fipronil, respectively. Surrogate recoveries of PCB 65 and PCB 209 were $96 \pm 3\%$ and $93 \pm 3\%$. Final results were not corrected for surrogate recovery.

The relative percent difference (RPD; mean $\pm$ standard deviation) of target analyte concentrations in sample duplicates was $13 \pm 14\%$ for dual column GC-ECD and $8.3 \pm 5.0\%$ ($n = 4$) for GC-NCI-MS/μ-ECD. Limits of detection (LODs) were calculated by dividing the lowest detectable target analyte mass (minimum S/N of 3) by the mass of sediment extracted. LODs 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/μ-ECD, 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 (Version 2.03, SPSS Inc. Chicago, IL, USA).

Individual and total pyrethroid and fipronil (ΣPYR and ΣFIP) concentrations in sediment were expressed on a dry weight basis. Toxic units (TUs) for individual and total pyrethroids or fipronil were calculated as follows:

$$TU = \frac{\text{Sediment concentration (μg/g OC)}}{LC_{50} \text{ (μg/g OC)}}$$

using 10-day whole sediment $LC_{50}$ values of *E. estuarius*, and the freshwater amphipod *Hyallela azteca*, respectively1.05 and 0.52 μg/g OC for bifenthrin, and 18.7 and 10.8 μg/g OC for permethrin, (Anderson *et al*. 2007). For the remaining six pyrethroids, $LC_{50}$ 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 (Amweg *et al*. 2005, Hintzen *et al*. 2009).

## RESULTS

GC-NCI-MS/μ-ECD analysis using a 30-m DB-XLB column achieved excellent separation for all target compounds (Figure 2A), including the four diastereomeric isomers each of cyfluthrin and cypermethrin. Although PCB 209 used as a recovery surrogate co-eluted with three of four cypermethrin isomers in total ion chromatograms (Figure 2B), selective ion monitoring (SIM) with NCI-MS was able to separate these overlapping signals to allow quantitation of all four cypermethrin stereoisomers (Figure 2B). Agreement between the two detection methods was excellent (<27% RPD) for bifenthrin, which was present in moderate concentrations in Ballona sediments (5 - 50 ng/g; Figure 3). For λ-cyhalothrin at lower levels (0.5 - 5 ng/g), μ-ECD invariably gave higher estimates than NCI-MS, a trend that was consistent among other pyrethroids detected at lower levels.

The 18 sediment samples analyzed in this study were fine-grained to mostly sand, with 2 of the 3 samples collected at Station 6 containing more than 80% gravel (Table 1). Percent fines (= silt+clay fractions) ranged between 0 (Station 5, September 2007) and 56.8% (Station 3, September 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
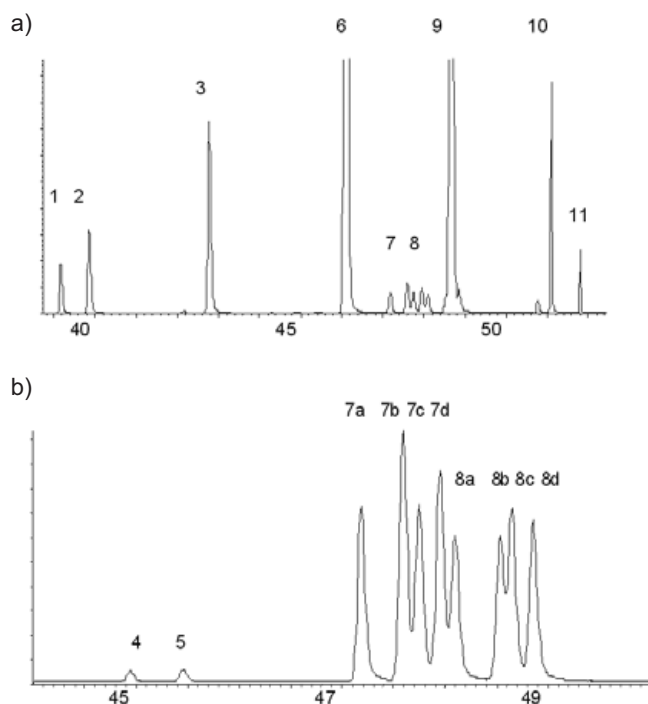
a)

b)

**Figure 2. Chromatographic separation of eight pyrethroids with a 30 m DB-XLB using GC-NCI-MS in selected ion monitoring (SIM) mode (m/z 207) (a); and for stereoisomers of cyfluthrin and cypermethrin (b). Peak identification: 1-bifenthrin, 2-fenpropathrin, 3-λ-cyhalothrin, 4-*cis*-permethrin, 5-*trans*-permethrin, 6-PCB 205 (int. std.), 7-cyfluthrin (4 pks), 8-cyperme-thrin (4 pks), 9-PCB 209 (int. std.), and 10-esfenvalerate, 11-deltamethrin.**

events was observed for Stations 3 and 5. Grain size was not measured for sediments collected in October 2008. Total pyrethroids (ΣPYR) was highly correlated with percent fines ($R_s = 0.77$; $p < 0.01$; $n = 12$) and TOC ($R_s = 0.64$; $p < 0.01$; $n = 18$).

Of the eight pyrethroids targeted, only fen-propathrin was not detected. Whereas only bifen-thrin and permethrin were detected by ECD at all six stations for September 2007, the six remaining pyrethroids were detected in all samples from June and October 2008 using GC-NCI-MS (Figure 4). Permethrin (sum of *cis*- and *trans*-isomers) was the most abundant pyrethroid (Table 2), with a maxi-mum concentration of (190 ng/g). Bifenthrin was the next most abundant pyrethroid, with concentrations ranging between 3.0 and 80 ng/g. 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 ΣPYR.
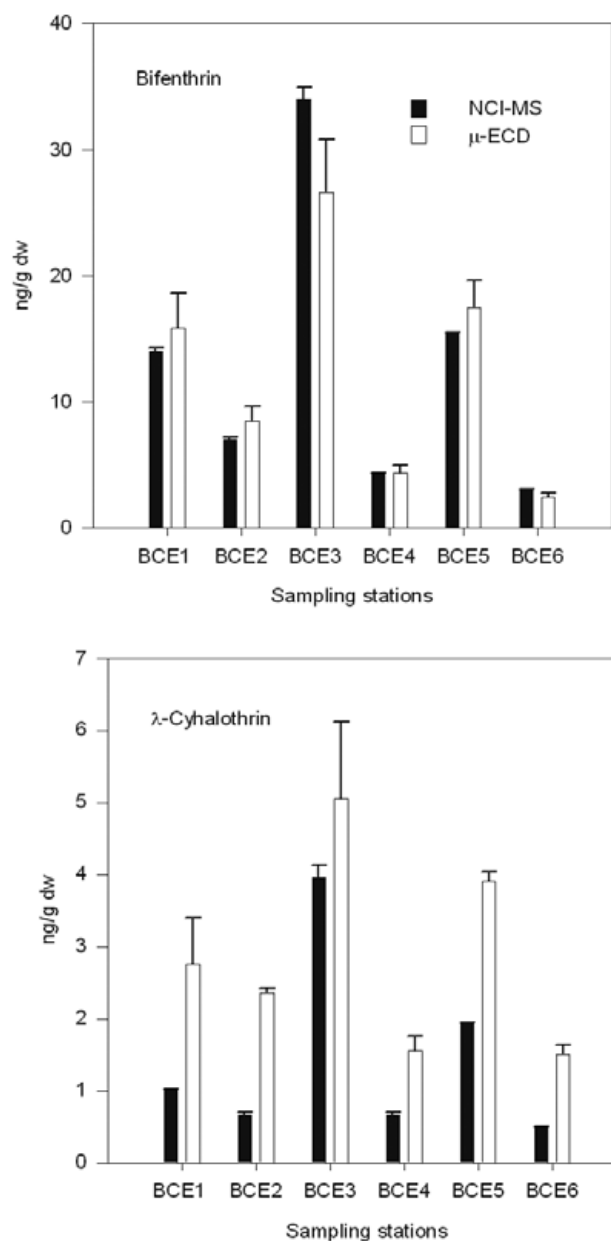


**Figure 3. Comparison of bifenthrin (top) and λ-cyhalothrin (bottom) concentrations in Ballona estu-ary sediments determined by GC-NCI-MS and GC-ÌECD. Values are means for duplicate injections; error bars represent standard deviation.**

The sediment pyrethroid distribution was signifi-cantly 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; www.cdpr.ca.gov). Percent contribution in sediment and usage were 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

**Table 1. Total pyrethroid and fipronil concentration (ng/g dry wt) for Ballona estuary sediments. NA = data not available; < = not detected; and ≤ = detected at level lower than limit of detection.**

| Station | Date | % Fines | %TOC | ΣPYR[a] (ng/g) | ΣFIP[b] (ng/g) |
|---------|------|---------|------|----------------|----------------|
| 1 | Sep 2007 | 19.9 | 0.57 | 10 (4) | ≤ 2.2 (5) |
|   | Jun 2008 | 15.2 | 0.44 | 47 (3) | ≤ 0.6 (4) |
|   | Oct 2008 | NA | 1.15 | 107 (2) | ≤ 0.8 (2) |
| 2 | Sep 2007 | 35.0 | 0.97 | 212 (2) | 4.7 (2) |
|   | Jun 2008 | 33.6 | 1.17 | 143 (2) | 2.9 (2) |
|   | Oct 2008 | NA | 1.15 | 41 (4) | ≤ 0.2 (5) |
| 3 | Sep 2007 | 56.8 | 1.38 | 473 (1) | 17.0 (1) |
|   | Jun 2008 | 2.4 | 0.55 | 43 (4t) | ≤ 0.3 (5) |
|   | Oct 2008 | NA | 2.49 | 233 (1) | ≤ 0.7 (3) |
| 4 | Sep 2007 | 7.0[c] | 0.53 | <12 (6) | ≤ 3.2 (3t) |
|   | Jun 2008 | 6.6 | 0.32 | 19 (6) | 0.3 (6) |
|   | Oct 2008 | NA | 0.74 | 21 (5) | ≤ 0.2 (6) |
| 5 | Sep 2007 | 0[d] | 0.38 | ≤ 14 (3) | ≤ 3.2 (3t) |
|   | Jun 2008 | 53.6 | 1.74 | 333 (1) | 7.1 (1) |
|   | Oct 2008 | NA | 2.95 | 60 (3) | ≤ 0.9 (1) |
| 6 | Sep 2007 | 0.4[e] | NA | ≤ 10 (5) | 2.0 (6) |
|   | Jun 2008 | 6.2[f] | 0.33 | 43 (4t) | 1.1 (3) |
|   | Oct 2008 | NA | 0.31 | 17 (6) | ≤ 0.3 (4) |

[a] sum of eight pyrethroids (station ranking in parentheses. t = tie)

[b] sum of fipronil, fipronil desulfinyl, sulfide, and sulfone (station ranking in parentheses. t = tie)

[c] 46.8% gravel

[d] 8.7% gravel, 91.3% sand

[e] 82.1% gravel

[f] 82.4% gravel

(16 - 18% vs. 4.9%). The opposite was true for cypermethrin where its average contribution in sediment was approximately half of its average usage.

The ratio of *cis*- to *trans*-permethrin in commercial products has been reported to be approximately 0.7 (You and Lydy 2007), which is similar to the average value of 0.85 ± 0.28 observed in runoff from two commercial nurseries in Orange County, CA (Bondarenko *et al*. 2007). In comparison, previous investigators reported higher average ratios for sediments, e.g., 2.63 ± 0.92 (Yasin *et al*. 1995), 3.26 ± 1.33 (Yasin *et al*. 1996), and 2.64 (You and Lydy 2004b). The average *cis*- to *trans*-permethrin ratio for sediments in this study was 1.67 ± 0.19 (June 2008), and 1.89 ± 0.23 (October 2008). Assuming a "starting" value that is less than unity (i.e., a non-degraded condition), this parameter can indicate the degree of weathering in environmental samples (Yasin *et al*. 1995).

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 as high as 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 ΣFIP in Ballona sediments, com-
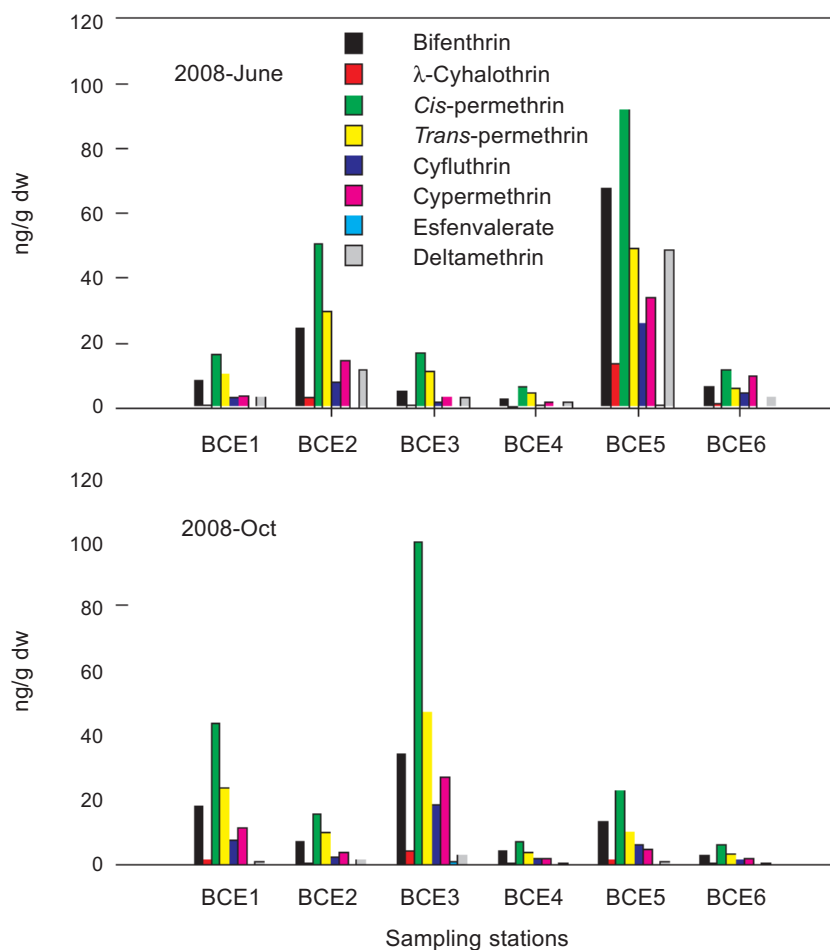
**Figure 4. Concentration (ng/g dry) of individual pyrethroids in Ballona estuary sediments collected in June 2008 (top) and October 2008 (bottom).**

**Table 2. Percent contribution of eight common pyrethroids (mean ± standard deviation) in Ballona estuary sediments[a] compared with local usage statistics. NA = data not available; ND = not detected.**

| Compound | Total Pyrethroids (%) | | |
|---|---|---|---|
| | 2008-June | 2008-Oct | Usage[b] |
| bifenthrin | 16 ± 3 | 18 ± 3 | 4.9 ± 3 |
| fenpropathrin | ND | ND | NA |
| l-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 Oct 2008

[b] average annual use for structural pest control in Los Angeles County, 1999-2006 (www.cdpr.ca.gov)

pared to approximately 20% each for the desulfinyl and sulfide degradates.

Individual and total concentrations of fipronil were at least 10 times lower than for pyrethroids (Table 1). The spatial pattern of fipronil contamination also coincided with pyrethroids, with the highest ΣFIP occurring at: Stations 2 and 3 (September 2007); Stations 2 and 5 (June 2008); and at Stations 1 and 3 (October 2008). Total fipronil (ΣFIP) was highly correlated with sediment fines ($R_s = 0.79$; $p < 0.01$; $n = 12$), but was not correlated with TOC ($R_s = 0.31$; $p > 0.2$; $n = 18$).

Toxic units (TUs) for individual pyrethroids based on *H. azteca* for all sediment samples are summarized in Table 3. The summation of pyrethroid TUs ($\Sigma TU_{pyrethroids}$) by station revealed that Stations 2 and 3 (September 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,

**Table 3.** Toxic units (TUs) for pyrethroids in Ballona creek sediments based on $LC_{50}$s for *Hyalella azteca* from Amweg *et al.* (2005) and Hintzen *et al.* (2009).

| Station | 1 | 2 | 3 | 4 | 5 | 6 | No. TU ≥1 |
|---|---|---|---|---|---|---|---|
| **2007-Sept** | | | | | | | |
| bifenthrin | 1 | 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 |
| **2008-June** | | | | | | | |
| bifenthrin | 3.6 | 4 | 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 | 1.3 | 0.7 | 0.6 | 3.5 | 1.3 | 4 |
| $\Sigma TU_{pyrethroids}$ | 8.7 | 10 | 5.3 | 4.5 | 20 | 16 | 19 |
| **2008-Oct** | | | | | | | |
| bifenthrin | 3 | 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 |
| deltamethrin | 0.1 | 0.2 | 0.2 | 0.1 | 0 | 0.2 | 0 |
| $\Sigma TU_{pyrethroids}$ | 7.1 | 2.8 | 7.2 | 2.5 | 1.7 | 4.9 | 8 |

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 (October 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 $\Sigma TU_{pyrethroids}$ ($R_s = 0.471$, $p = 0.04$; Figure 5).

For bifenthrin, TUs based on *E. estuarius* were greater than unity for the same nine of 18 samples which contributed 49% of $\Sigma TU_{pyrethroids}$ 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 $\Sigma TU_{pyrethroids}$ (Table 3). For cypermethrin, $\Sigma TU_{pyrethroids}$ was >2 for 8 of 18 samples, contributing 46 ± 11% to this sum. Cyfluthrin TUs were >5 for 2 samples (Stations 2 and 3, September 2007), but its overall average contribution to summed TUs (~17%) was lower than for bifenthrin or cypermethrin. The TUs for individual and total fipronil based on *H. azteca* were all less than 0.1 (computations not shown).

## DISCUSSION

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 (You and Lydy 2004a, Barro *et al*. 2006, Yoshida 2009). The complexity associated with chromatographic separation of pyrethroids is particularly important when using
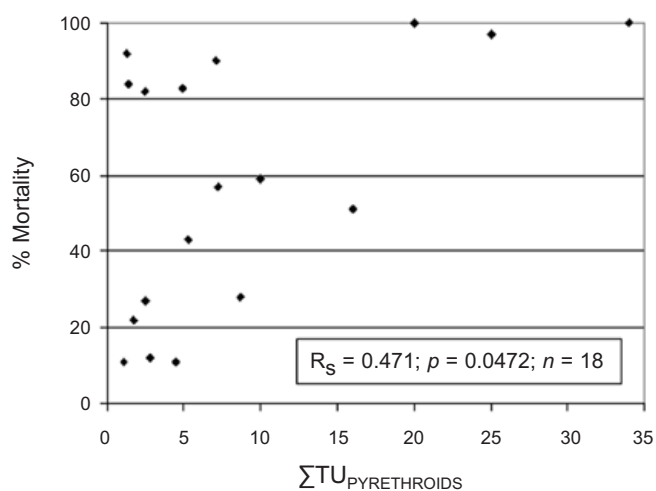
**Figure 5. Percent mortality of *Eohaustorius estuarius* in 10-day whole sediment toxicity tests vs. the sum of toxic units for detectable pyrethroids in Ballona estuary sediments ($\sum TU_{pyrethroids}$). Toxic units are based on $LC_{50}$s for the freshwater amphipod *Hyallela azteca*. ($R_s$ = Spearman's rank correlation coefficient).**

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 have been observed (Bondarenko *et al*. 2007). 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 non-specific detectors.

The unique configuration of our GC-NCI-MS/μ-ECD instrument also allowed for simultaneous comparison of detector response, as was reported previously for analysis of organic contaminants in water (Gomez *et al*. 2009). Good agreement for sediments with intermediate pyrethroid concentrations (Figure 3) indicated 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. The deviation between NCI-MS and ECD quantitation for samples with low (i.e., near LOD) and/or higher pyrethroid concentrations is more problematic. It is possible 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 (Esteve-Turrillas *et al*. 2004, 2006; Barro *et al*. 2006; Garcia-Rodriguez *et al*. 2008; You *et al*. 2008; Vonderhee *et al*. 2009). No such comparisons for fipronil were found in the literature.

The maximum pyrethroid levels in this study were greater than or equal to those previously reported for several urban creeks in California (Amweg *et al*. 2006a). For permethrin and bifenthin, the observed concentrations were less than the maximum concentrations reported in agriculturally dominated areas, a mixed land use watershed and a statewide sediment investigation (Weston *et al*. 2004, Budd *et al*. 2007, Holmes *et al*. 2008). These levels, however, were higher than reported previously for the Sacramento urban area (Weston *et al*. 2006).

The correlation between percent contribution of individual pyrethroids in Ballona sediments and local usage illustrated the linkage between application and environmental persistence (Table 2). The higher relative contribution of bifenthrin in sediments confirmed the high degree of persistence for this compound reported previously ($t_{1/2}$ of 251 - 498 days under anaerobic conditions; Gan *et al*. 2005). In contrast, the percent contribution of cypermethrin (Table 2) suggested a lower relative degree of persistence or attenuated transport into this waterway for this active ingredient. The fact that fenpropathrin, another high use pyrethroid, was not detected was in agreement with a 2008 investigation of sediment in California urban waterways (Holmes *et al*. 2008), and is indicative of its short half-life in the environment. The intermediate values for *cis*- to *trans*-permethrin ratio in our samples 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 to the June samples.

The lower concentrations of fipronil and its degradates compared with pyrethroids is consistent with findings from previous field surveys, including an investigation of freshwater sediments by the US Geological Survey (USGS; Gunasekara *et al*. 2007, Hintzen *et al*. 2009). In the absence of usage data to assess occurrence and persistence, the lower hydrophobicity and sediment-water partition coeffi-

cients for fipronil and its degradates compared with pyrethroids (Bondarenko *et al*. 2006, Lin *et al*. 2008, Lin *et al*. 2009) can explain these trends. Less than 10% of ΣFIP was attributable to the parent compound, indicating a short half life in the aquatic environment. Indeed, fipronil 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 (Demcheck and Skrobialowski 2003). In contrast, fipronil sulfone was more abundant in sediments of an urban stream in central Texas, which were reported to be largely aerobic (Hintzen *et al*. 2009). 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 samples in this study would suggest aerobic conditions were predominant.

A TU exceeding one indicates a concentration that exceeds the established $LC_{50}$, normalized to TOC. Because $LC_{50}$ values for the estuarine amphipod *E. estuarius* are currently limited to bifenthrin and permethrin (Anderson *et al*. 2007), $LC_{50}$ values for *H. azteca*, a commonly used freshwater amphipod test species, were employed 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*. The elevated $ΣTU_{pyrethroids}$ for several Ballona sediment samples (Table 3) and the significant correlation between this parameter and mortality of *E. estuarius* in 10-day sediment toxicity tests (Figure 5) is a clear indication that pyrethroids likely play a significant role in explaining toxicity.

Conversely, the TUs for fipronil suggested 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* (Schlenk *et al*. 2001). Considering a test species that is more sensitive to fipronil (e.g. the freshwater midge *Chironomous*

*tentans*; Maul *et al*. 2008), average summed TUs for fipronil and its sulfide and sulfone metabolites exceed unity for 8 of the 18 samples, including Stations 1 through 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.

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. 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 via runoff during storm events in the months preceding the dry period (Stein and Tiefenthaler 2005). 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 (Figure 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 (October 2008; Figure 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; Figure 5), samples with high levels of permethrin do not correspond with observed sediment toxicity. This is due to its lower intrinsic toxicity, about 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. On the other end of the spectrum, physical characteristics of selected samples may have contributed to low survival of *E. estuarius* in some cases. Greater than 90% coarse grained substrate was present in at least 3 of the 5 samples with high mortality but relatively low ΣTU for pyrethroids or fipronil (Figure 5).

Another factor affecting the distribution of fine-grained sediments and associated contaminants in this system is sediment scour due to ener-

getic 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 (TIE) 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 (PBO), a synergist of pyrethroid pesticides, may increase the toxicity of pyrethroids already present (Weston *et al*. 2006, Amweg *et al*. 2006b). Modulation of response by PBO, one of the TIE treatments, 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 non-target organisms. An extremely high concentration of methoprene (440 ng/g) was detected in the sample from Station 3 for September 2007 (K. Smalling, USGS, pers. comm.), which proved to be extremely toxic to *E. estuarius* (Figure 5). However, methoprene was not detected in the 2 other highly toxic samples where summed pyrethriod TUs for *H. azteca* were 25 and 20 (Table 3; Station 2, September 2007 and Station 5, June 2008).

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