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IDENTIFYING THE CAUSE AND SOURCE OF SEDIMENT TOXICITY IN AN AGRICULTURE-INFLUENCED CREEK

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Abstract—Del Puerto Creek, an agriculturally influenced stream in northern California, USA, with a history of sediment toxicity, was used as a case study to determine the feasibility of using sediment toxicity testing and chemical analysis to identify the causative agent for the toxicity and its sources. Testing with the amphipod *Hyalella azteca* confirmed historical toxicity and identified a point along the creek at which there was an abrupt increase in sediment toxicity that persisted for at least 6 km downstream. Three recently developed whole sediment toxicity identification evaluation manipulations, temperature reduction, piperonyl butoxide addition, and esterase addition, were applied to sediment from one site and were suggestive of a pyrethroid as the cause for toxicity. Utilizing published median lethal concentration (LC50) values in a toxic unit analysis, the pyrethroid insecticide bifenthrin was identified as the primary contributor to toxicity in nearly all sites at which toxicity was observed, with occasional additional contributions from the pyrethroids lambda-cyhalothrin, esfenvalerate, and cyfluthrin. Most agricultural drains discharging to Del Puerto Creek contained bifenthrin in their sediments at concentrations near or above acutely toxic concentrations. However, only one drain contained sediments with bifenthrin concentrations approaching the concentrations measured in creek sediments. This fact, along with the proximity of that particular discharge to the location in the creek with the highest concentrations, suggested that one drain may be responsible for much of the toxicity and pyrethroid residues in creek sediments. The methods employed in this study are likely to be of considerable value in total maximum daily load efforts in Del Puerto Creek or other California surface water bodies known to have pyrethroid-related aquatic toxicity.

Keywords—Pyrethroids Bifenthrin Toxicity identification evaluation Sediment toxicity

INTRODUCTION

Sediment toxicity testing is a well-established and widely used technique for monitoring environmental quality in aquatic systems. Toxicity to the test organism provides an indication that resident biota may be at risk, if not already affected [1], indicating the need for further investigation or mitigation actions. The presence of toxicity may also serve as the basis for regulatory actions such as listing of the water body as impaired under the Clean Water Act, Section 303(d) (http://www.epa.gov/waterscience/standards/303.htm). Numerous studies have used sediment toxicity testing in a monitoring context, either alone or in conjunction with monitoring of resident fauna.

While sediment toxicity testing has been a valuable tool for identifying instances of environmental degradation, it can be of limited value in guiding efforts to correct such problems. There are usually two obstacles. First, the substance or substances causing the toxicity may not be identifiable. The typical approach for identifying the cause of toxicity is through a toxicity identification evaluation (TIE) [2–4], but TIE procedures for whole sediments are less developed than those for water. Most of the published sediment TIE work has focused on procedures to determine if the contaminant falls within broad categories, such as an organic contaminant [5,6], trace metal [7], or ammonia [8], and such procedures may not identify the toxicant with sufficient specificity for source identification. There has been recent progress in developing more specific procedures for pyrethroids [9–12], as well as addressing sediment toxicity through application of the better-established water-based TIE methods to interstitial water [12,13]. Second, even if the substance causing toxicity can be identified, correcting the problem may be difficult if its source to the water body can not be located, perhaps because sources are too numerous and diffuse, or the contamination represents a legacy of past practices.

The present study focused on an agriculture-dominated creek with a history of acute sediment toxicity. By utilizing newly developed tools for bulk sediment TIEs [9–12], experience gained from extensive sediment monitoring in the region [14,15], and a unique pesticide use database (http://www.cdpr.ca.gov/docs/pur/purmain.htm), the present study was designed to determine the toxicity's cause and the location of the contaminant sources.

MATERIALS AND METHODS

Study area

Del Puerto Creek originates in the hills east of San Jose, California, USA, with little development in its upper watershed. It flows northeastward, entering the heavily agricultural Central Valley near Patterson, California, and flows 13 km across the valley floor before its confluence with the San Joaquin River. The agricultural lands in the lower watershed produce a wide variety of crops, including almonds, walnuts, apricots, tomatoes, beans, and lawn turf.

Del Puerto Creek varies in width from 1 to 4 m, and is typically 0.5 to 1 m deep. The creek bed is frequently scoured

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by high flow events, and the substrate in most reaches of the creek is predominantly gravel and cobble. Like all creeks in the region, flow is highly variable. During the winter months when the majority of annual rain falls, flow is intermittent and highly rainfall dependent. The creek is often dry between winter rain events. During the summer growing season flow is less variable, but supplied entirely by irrigation water, including return flow from the surrounding croplands.

The main irrigation channels in the area are concrete-lined canals, known as laterals, and carry water pumped from the San Joaquin River. Growers utilize water from these laterals for irrigation, and return excess irrigation runoff to the lateral system. The laterals eventually discharge to Del Puerto Creek, carrying a mix of supply and irrigation return water. Some of the laterals pass over or under Del Puerto Creek to reach farmland on the other side, but even in these cases, there are gates at the creek crossing that can be opened to allow all or part of the water in the lateral to enter Del Puerto Creek. While the majority of irrigation runoff reaching Del Puerto Creek would enter via the laterals, some individual growers cultivating land along the creek banks have their own discharges of irrigation runoff directly to the creek.

Past monitoring in Del Puerto Creek has shown pesticide contamination of sediments and/or sediment toxicity due to agricultural activities. Sediment in the creek contains pyrethroid pesticides and DDT at high concentrations compared to other agricultural streams in the region [14,15]. Toxicity of creek sediments has been tested repeatedly and found to be consistently acutely toxic to the amphipod *Hyalella azteca*. From 2001 to 2005 nine samples were collected from the creek and all were found to cause significant toxicity, usually in the range of 60 to 100% mortality ([14]; D. Weston, unpublished data; J. Rowan, Central Valley Regional Water Quality Control Board, Rancho Cordova, CA, USA, unpublished data).

Field sampling

The present sediment sampling was conducted on four occasions extending from December 2005 through March 2006. The multiple sampling trips allowed analysis of early samples and use of that data to guide source tracking efforts in subsequent visits. Sampling in the mainstem of Del Puerto Creek was done almost entirely in the first two visits (eight samples in December and four samples in January). The creek contained only isolated pools of standing water on both of these occasions. Sampling in the laterals occurred in January (four samples), February (five samples), and March (four samples plus coring), with the later visits tending to be used for further investigations farther up laterals where contamination was confirmed in earlier visits. During winter sampling the drains were dry or contained only minimal standing water since there is no irrigation activity during this time of the year.

Sampling efforts focused on fine-grained sediments since they would be more likely to contain measurable concentrations of hydrophobic pesticides, however, such sediments are not widespread in Del Puerto Creek and its tributary drains. In the creek mainstem there are a few areas of extensive depositional soft sediments that were sampled, but for the most part the creek bottom is gravel and cobble. In these areas, limited soft sediment could usually be obtained on the channel flanks where it had deposited in a previous high-flow event or in low areas on the channel bottom where it was deposited as the previous high-flow event diminished and eventually ceased. The irrigation laterals draining to Del Puerto Creek are, in most reaches, lined with concrete. However, it was possible to collect a thin layer (~1 cm) of soft sediment that had deposited in the bottoms of these channels.

Sediment was collected using a stainless steel scoop to skim the upper 1 cm of the sediment, and approximately 3 L of material was placed in a 4-L glass jar that had been solvent-cleaned for pesticide analyses. The sediment was kept at 4°C for up to a week, and then homogenized by hand mixing in a large stainless steel bowl. Subsamples were removed for toxicity testing and grain size analysis (both kept at 4°C) and for pesticide analysis and total organic carbon (OC) (both kept at −20°C).

In March of 2006 core samples were taken for vertical contaminant profiling in three areas of extensive soft-sediment deposition. Two acrylic cores, each 5 cm in diameter, were pushed into the bed sediments by hand at each site until underlying impenetrable hard clay was reached at a depth of approximately 28 cm. The cores were returned to the lab where they were extruded and sectioned into strata of 0 to 2, 2 to 10, 10 to 20, and 20 to 28 cm. Material from the two cores at each site were composited.

Toxicity testing

Sediment toxicity testing was done using 7- to 14-d-old amphipods, *H. azteca*, following standard protocols [16]. Approximately 75 ml of sediment was placed in 400-ml glass beakers, and covered with 250 ml moderately hard water prepared by addition of salts to Milli-Q® purified water (Millipore, Billerica, MA, USA). Five to eight replicates were prepared per sample, and placed in a constant temperature water bath at 23°C. Ten amphipods were added per replicate at test initiation. Fresh water was added by an automatic water delivery system at a rate of 500 ml/beaker/d. Test organisms were fed 1 ml/beaker/d of yeast–cerophyll–trout chow, and maintained on a 16:8 h light:dark cycle. Conductivity, pH, ammonia, hardness, and alkalinity were measured at the beginning and end of the test; temperature and dissolved oxygen were measured throughout the test and were within permissible limits (22–24°C, >2.5 mg/L dissolved oxygen). After a 10-d exposure, test organisms were recovered on a 425-μm screen and enumerated to determine survival. Each test batch was accompanied by control sediment (2.0% OC) obtained by blending sediments from San Pablo Dam Reservoir, Orinda (CA, USA) and Lake Anza, Berkeley (CA, USA). Control survival among all the tests ranged from 86 to 98% (average = 93%).

Toxicity identification evaluation procedures specifically developed for identification of toxicity related to pyrethroid pesticides were used on sediment from one site, including temperature manipulation, piperonyl butoxide (PBO) addition, and esterase addition. The general approach of the temperature and PBO TIE procedures was to use a dilution series to determine how the TIE manipulation changed the 10-d sediment median lethal concentration (LC50) of the test sediment. The sediment of interest was diluted in half-steps (e.g., 25, 12, 6, 3, 1.5%) using control sediment as the diluent. Control and test sediments were thoroughly blended by hand mixing, and the tests started within 24 h. The esterase TIE procedure used a sediment that had been diluted to two concentrations (6 and 20%), and the effect of esterase on mitigating toxicity at both these concentrations was determined.

Pyrethroids are unlike many other toxicants in that they show an inverse temperature coefficient (i.e., greater toxicity at colder temperature) [11,17,18]. Parallel LC50 determina-

tions were performed at both 18 and 23°C using the standard toxicity test methods as described above. Test mortality due to pyrethroids is usually manifested by an 18°C LC50 approximately half that at 23°C (D. Weston, unpublished data). Piperonyl butoxide is known to increase the toxicity of pyrethroid pesticides by inhibiting their enzymatic degradation. Toxicity tests were performed with and without 25 mg/L PBO in the overlying water, a concentration that typically more than doubles the toxicity of pyrethroids [9,19]. The PBO (Sigma, St. Louis, MO, USA) was dissolved in methanol and the methanol added to the test solution at a rate of 10 μl/L. A control with PBO added to control sediments was also included. The general toxicity testing procedures were followed except that water was changed daily by removing approximately 80% of the water and replacing it with fresh PBO solution. Finally, esterase addition was used, as it has been shown to hydrolyze pyrethroids and thereby decrease their toxicity to *H. azteca* [10,20]. Ten-day tests following standard protocols were run, except that esterase (as a lyophilized powder, Sigma E3019, lot 026K7029) was added to the overlying water at a concentration of 27 units/ml, equivalent to 46 mg/L given the activity of the available lot. Esterase was added daily to restore the nominal starting concentration, replacing that which had been lost from the system through the automatic water changes over the previous 24 h. A bovine serum albumin (BSA) treatment at the same concentration as the esterase (46 mg/L) was also used. Esterase addition can alleviate pyrethroid toxicity either by the catalytic activity of the enzyme, or simply by complexation of the pyrethroid with the dissolved organic matter that the esterase represents, thus use of a noncatalytic enzyme such as BSA provides a control for the latter mechanism, and pyrethroid-related mortality is indicated only by a reduction in toxicity above that achieved by BSA [10].

Toxicity test statistics were calculated using ToxCalc 5.0 (Tidepool Scientific Software, McKinleyville, CA, USA). Test sediments were compared to controls using equal variance *t*-tests when possible, or by unequal variance *t* tests if *F* tests showed variance assumptions were not met. The LC50 values were calculated by the trimmed Spearman–Karber method.

Measured sediment concentrations were reported in relation to those concentrations known to be acutely toxic to *H. azteca* using toxic units (TU). Given the strong hydrophobicity of pyrethroids, and thus the importance of sediment OC content in determining their bioavailability and toxicity, TUs are best calculated on an OC basis as

$$\text{TU} = (\text{Actual contaminant concentration on an OC basis}) \div (\textit{H. azteca} \text{ sediment LC50 on an OC basis})$$

Values for the LC50s were obtained from previously published work [21,22].

Chemical analysis

Sediment analysis was done following the methods of You et al. [23]. Sediment was thawed, centrifuged to remove excess water and homogenized. Two surrogates, 4,4′-dibromooctafluorobiphenyl and decachlorobiphenyl, were added to the sediment prior to the extraction to verify extraction and cleanup efficiency. Approximately 20 g of sediment (wet wt) was mixed with anhydrous $MgSO_4$ and sonicated with 50 ml of 50:50 acetone:methylene chloride (v/v) for 3 min. The extract was centrifuged, decanted, and filtered. This procedure was repeated twice more. Extracts were combined, solvent exchanged with hexane, and the volume reduced to 2 ml. Extract cleanup was done with Florisil® (Floridin, Warren, PA, USA), deactivated by mixing with distilled water (6% w/v). The pesticides were eluted from the column with 50 ml of 30% diethyl ether in hexane (v/v). The eluent was evaporated, redissolved in 2 ml of hexane, and analyzed.

Analysis was performed on an Agilent 6890 series gas chromatograph equipped with an Agilent 7683 autosampler and an electron capture detector (Agilent Technologies, Palo Alto, CA, USA). Two columns from Agilent, an HP-5MS (30-m length, 0.25-mm diameter, 0.25-μm film thickness) and a DB-608 (30-m length, 0.25 mm diameter, 0.25-μm film thickness) were used. Five external standards solutions ranged from 5 to 250 ng/ml were used for calibration. The calibration curves were linear within this concentration range. Qualitative identity was established using a retention window of 1% with confirmation on a second column. All the sediment samples were analyzed for seven pyrethroids (bifenthrin, cyfluthrin, cypermethrin, deltamethrin, esfenvalerate, lambda-cyhalothrin, and permethrin), one organophosphate insecticide (chlorpyrifos), and 20 organochlorine insecticides or their degradation products. With method detection limits of 0.22 to 0.85 ng/g dry weight, the method reporting limits were set at 1 ng/g for all analytes.

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

Pesticide use

The California Department of Pesticide Regulation (CDPR) maintains a unique database to track pesticide use within the state. Any agricultural and most commercial nonagricultural applications of a pesticide (but not use by homeowners) is required to be reported to CDPR. For agricultural applications, the report contains specifics on the chemical and product name applied, the amount used, the date of application, and the crop treated. The location of application is identified with resolution of 1.6 by 1.6 km (1 $mile^2$) or better. These data are compiled by CDPR in the Pesticide Use Reporting (PUR) database and are publicly available (http://www.cdpr.ca.gov/docs/pur/purmain.htm). The database was accessed for the present study to identify areas to which the pesticide of interest was reported to have been applied.

RESULTS AND DISCUSSION

Toxicity tests

The agricultural portion of the Del Puerto Creek watershed begins approximately at Interstate Highway 5 and extends all the way to the San Joaquin River, a distance of approximately 13 km along the river. There was no appreciable mortality in the undeveloped headwaters (only 2% mortality), nor was there significant toxicity for approximately the first half of the agricultural reach of the creek (Fig. 1). There was an abrupt transition from nontoxic to toxic sediments that occurred slightly upstream of Highway 33. From this point to the San Joaquin River, a distance of approximately 6 km, every sediment sample proved to be significantly toxic to *H. azteca*, causing total or near total mortality.

Sediment toxicity was observed in five of seven tributaries sampled (six laterals and one unnamed drain). The only two tested laterals lacking acutely toxic sediments were the most downstream drains of lateral 3N and lateral B. The unnamed drain west of Highway 33 was noteworthy in having four

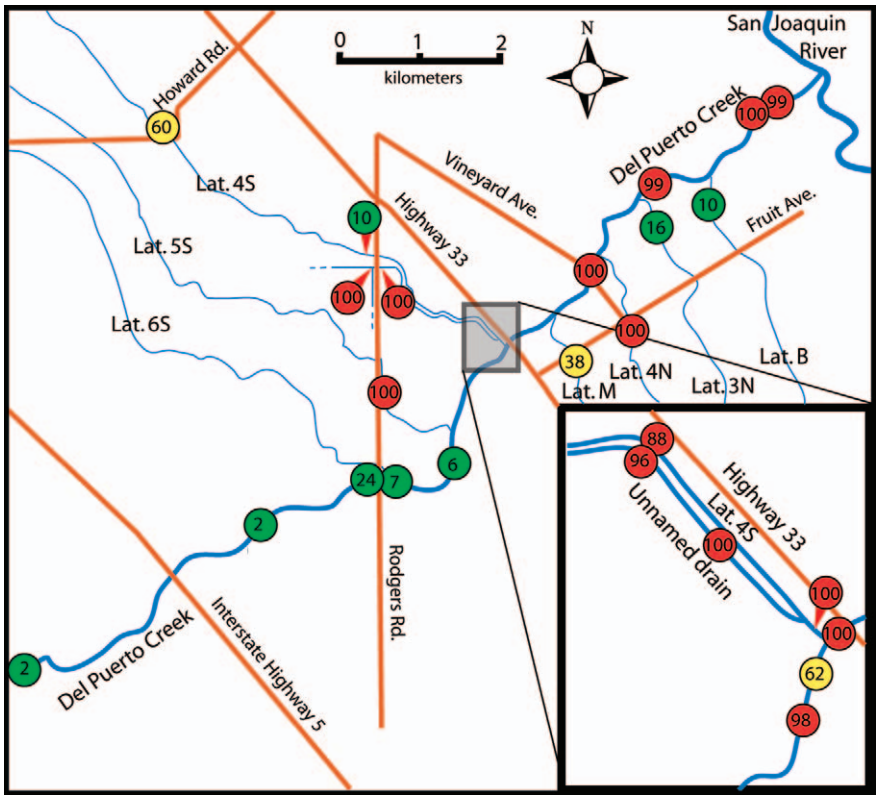

Fig. 1. Percent mortality of *Hyalella azteca* exposed to sediments in 10-d tests. Green indicates sites with mortality not significantly different from control. Yellow indicates statistically significant but <70% mortality. Red indicates >70% mortality. The irrigation laterals are abbreviated as Lat. The area at the intersection of Del Puerto Creek and Highway 33 (near Patterson, CA, USA), where sample density is high, is enlarged in the lower right of the figure.

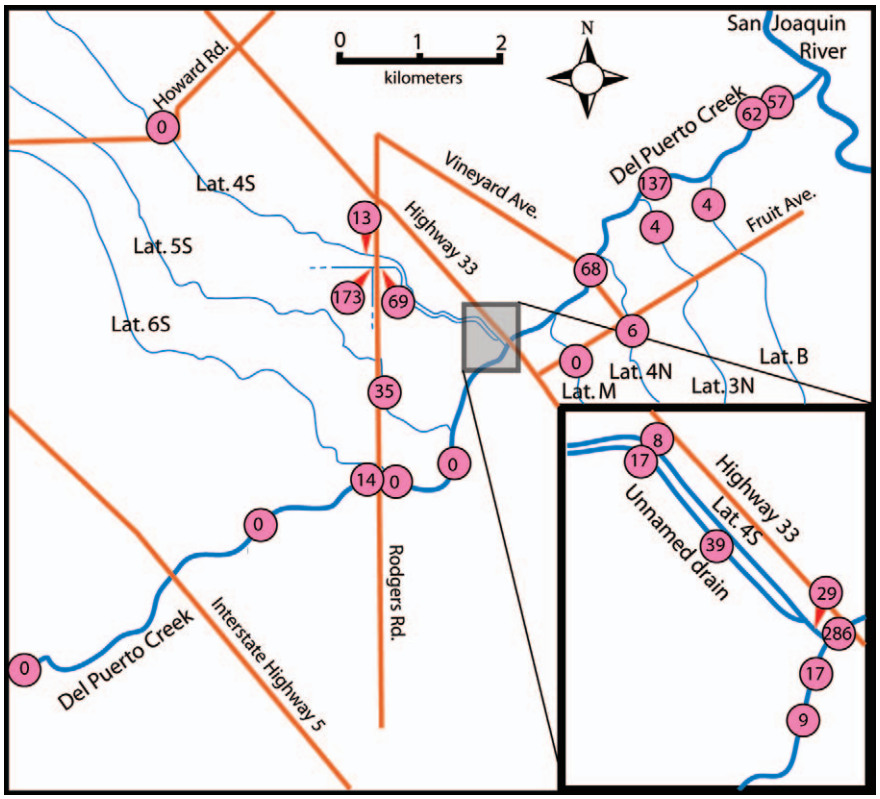

Fig. 2. Concentration of bifenthrin (ng/g) in surficial sediments throughout the study area (near Patterson, CA, USA). If undetected (<1 ng/g), a value of zero is shown. The irrigation laterals are abbreviated as Lat.

Table 1. Concentrations of all detected pesticides in the sediments at the site tested with toxicity identification evaluation procedures (Del Puerto Creek mainstem, first site upstream of Highway 33, Patterson, CA, USA). The site was sampled in both December 2005 and January 2006. The estimated 10-d sediment median lethal concentration (LC50) to *Hyalella azteca* for each pesticide is shown, based on published LC50 values [9,14,22], adjusted for the 1.24% organic carbon present in the December sample at this site. ND = not detected (<1 ng/g). DDT, DDE, and DDD refer to dichlorodiphenyltrichloroethane and its breakdown products, dichlorodiphenyldichloroethylene and dichlorodiphenyldichloroethane, respectively

Pesticide	Concentration in December sample (ng/g)	Concentration in January sample (ng/g)	10-d LC50 for *H. azteca* in sediment containing 1.24% organic carbon
Bifenthrin	286	199	6.4
Lambda-cyhalothrin	13.1	4.0	5.6
Cyfluthrin	2.4	ND	13.4
Permethrin	20.2	11.8	134
Esfenvalerate	2.2	1.5	19.1
DDT	17.1	6.9	3,200
DDE	42.0	35.7	16,000
DDD	4.3	3.0	103,000
Dieldrin	1.4	ND	25,000
Chlorpyrifos	2.5	1.0	36.8

samples taken, all of which showed 96 to 100% mortality. Clearly there were multiple sources of toxicity to Del Puerto Creek, but the toxicity data alone did not help to identify a dominant source. The toxicity data alone could not establish if the toxicity was all due to the same toxicant. Also, with total or near total mortality at many sites, it was difficult to determine if conditions worsened as each successive drain entered the creek.

Toxicity identification evaluations

Pyrethroids have been implicated in approximately two-thirds of the instances of *H. azteca* sediment toxicity in agricultural areas of the Central Valley of California [15], and sediments from Del Puerto Creek at Vineyard Avenue had been shown to contain pyrethroid concentrations toxic to *H. azteca* when tested several years previously [14]. Therefore, an abbreviated TIE was done, using PBO addition, temperature manipulation, and esterase addition, given that these procedures have demonstrated success when toxicity due to pyrethroid pesticides is suspected [9–12].

These TIE procedures were performed on sediment collected from the first site (when moving in a downstream direction) at which 100% mortality was observed (site on the mainstem of Del Puerto between the lateral 4S/unnamed drain combined input and Highway 33). Without PBO in the overlying water, the sediment was highly toxic with a 10-d LC50 of 1.6% (95% confidence interval [CI] = 1.5–1.8%). In a concurrent test with 25 μg/L PBO in the overlying water, the same sediment was even more toxic, with an LC50 of 1.2% (CI = 1.0–1.3%). The most dramatic difference was observed in the 1.5% Del Puerto sediment dilution in which 57% of the individuals survived without PBO, but only 23% survived with PBO addition. Control performance, both with and without PBO in the overlying water, ranged from 90 to 97% survival, indicating no toxicity due to the PBO addition itself. Piperonyl butoxide inhibits cytochrome P450 activity that plays an important role in the detoxification of pyrethroids and some other toxicants. The increase in toxicity seen with addition of PBO does not conclusively prove causality, but is consistent with a pyrethroid as the causative agent though the magnitude of the response was somewhat muted. In tests with eight pyrethroid-contaminated sediments [9], the increase in toxicity due to PBO was usually a factor of two (ratio of LC50 without PBO to LC50 with PBO) rather than the 1.3 ratio observed in the Del Puerto Creek sediment.

The same sediment later was tested using temperature manipulation as a TIE approach. When tested at the standard test temperature of 23°C the sediment's LC50 was 2.8% of the initial sediment (CI = 2.5–3.2). When tested at 18°C, the sediment was more than twice as toxic, with an LC50 of 1.1% (CI = 1.0–1.3%). When the same 1.5% Del Puerto dilution noted above was tested in connection with the temperature TIE, survival was 83% at 23°C, but only 13% survived at 18°C. Controls showed 97% survival at 23°C and 90% survival at 18°C. These results are consistent with pyrethroids as the cause of toxicity. The magnitude of response observed, approximately a factor of two change in the *H. azteca* LC50 over the temperature range used of 18 to 23°C, is comparable to that observed in many other instances of pyrethroid-induced sediment toxicity (D. Weston, unpublished data).

The same site as was used for the temperature and PBO tests was resampled one month after the original collection, and this sediment tested with unamended water, with BSA in the overlying water, and with esterase in the overlying water. In these experiments the sediment was tested at two concentrations, 6 and 20%, diluted with control sediment. Control survival exceeded 90% in the water, BSA, and esterase treatments. In the 6% trial, survival (mean and standard deviation) in the water, BSA, and esterase treatments were 56 ± 5%, 76 ± 15%, and 90 ± 10%, respectively. In the 20% trial the survival rates were 2 ± 4%, 30 ± 19%, and 70 ± 19%, respectively. Though addition of BSA mitigated toxicity to some extent (significant difference in survival from the water only treatment, t test, $p < 0.05$ in both trials), the fact that esterase provided even greater reduction of toxicity ($p < 0.05$ in the 20% trial) indicated the toxicant was susceptible to ester hydrolysis, consistent with a pyrethroid as the causative agent.

Sediment chemistry

All sediments were analyzed for seven pyrethroids, chlorpyrifos, and 20 organochlorine pesticides or their degradation products. The sediments that had been used for the TIE procedures discussed above contained two analytes present at potentially toxic concentrations, both pyrethroids (Table 1). Bifenthrin was found at a concentration of 286 ng/g in the first sample collected at the site. Repeat sampling at the same location one month later confirmed this high value, with 199 ng/g found in the second sample. Given the published *H. azteca* sediment LC50 for bifenthrin and the OC content of 1.24% in this sediment, the observed 286 ng/g represents near-

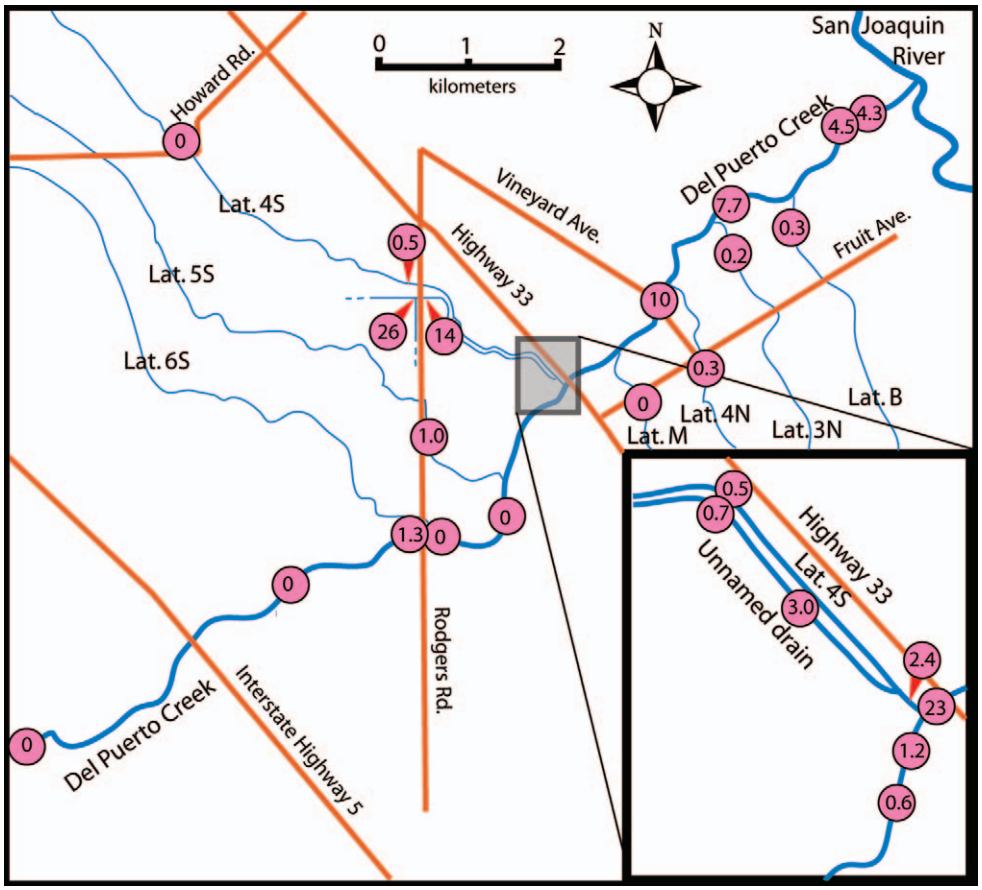

Fig. 3. Concentration of bifenthrin in surficial sediments throughout the study area (near Patterson, CA, USA) on an OC-normalized basis (μg/g OC). If undetected (<1 ng/g), a value of zero is shown. For comparison, a value of 0.52 μg/g OC is the reported 10-d median lethal concentration for *Hyalella azteca* [22]. The irrigation laterals are abbreviated as Lat.

ly 45 TU of bifenthrin in the sediment. Lambda-cyhalothrin was present in the same sample at a concentration of 13 ng/g, equivalent to approximately 2 TU. This result suggests that while bifenthrin was predominantly responsible for the toxicity of the sediment to *H. azteca*, the sediment would likely still be toxic, though substantially less so, in the absence of bifenthrin. Three other pyrethroids, four organochlorines, and chlorpyrifos were detected in the sediment, though none were at concentrations likely to cause acute toxicity to *H. azteca*. Thus, the chemistry data supports the inference of pyrethroid-related toxicity obtained through the TIE procedures, but provides greater specificity, indicating bifenthrin, and secondarily lambda-cyhalothrin, as the primary toxicants.

Bifenthrin was commonly found throughout Del Puerto Creek and its tributary drains (Fig. 2). The data are also shown on an OC-normalized basis (Fig. 3) so that concentrations can be interpreted in a toxicity context, recognizing that the published bifenthrin LC50 to *H. azteca* averaged 0.52 μg/g OC when tested in multiple sediments, and was 0.57 μg/g OC when determined by spiking Del Puerto Creek sediments from the creek headwaters with bifenthrin [22].

In the upper reaches of Del Puerto Creek above areas of agricultural development, bifenthrin was undetected (<1 ng/g). From Rodgers Road to the combined input of lateral 4S and the unnamed drain, concentrations ranged from <1 to 17 ng/g. From the combined input of those drains to the San Joaquin River, bifenthrin concentrations ranged from 57 to 286 ng/g at five sites in the mainstem of the creek. On an OC basis, all of the sediments tested from Highway 33 to the San Joaquin River contained at least eight times the reported *H. azteca* sediment LC50 of bifenthrin.

Bifenthrin was detected in the sediments of six out of seven tributaries. Lateral 5S sediments contained elevated concentrations of bifenthrin (35 ng/g), though well below the concentrations measured further downstream in Del Puerto Creek. In addition, the two sites in the mainstem of Del Puerto immediately downstream of lateral 5S had relatively low bifenthrin concentrations (9 and 17 ng/g), further suggesting lateral 5S was not responsible for most of the contamination present still further downstream, east of Highway 33. The unnamed drain was the only tributary that contained bifenthrin concentrations in the sediments comparable to the magnitude of concentrations measured in the lower reaches of the creek mainstem. Surficial sediment concentrations in the unnamed drain ranged from 17 to 173 ng/g. The latter value was in a composite sample of the two branches of the drain from just above Rodgers Road, suggesting that one of these branches contained even more than 173 ng/g bifenthrin. On an OC basis, the sediments in this unnamed drain were slightly more contaminated than in the mainstem creek (26 vs 23 μg/g OC), and 50 times the *H. azteca* sediment LC50.

Bifenthrin is primarily transported from its point of use adsorbed to sediment particles [24]. Therefore, a source drain is likely to contain sediment residues of equal or greater concentration than those measured in sediments of the creek to which it discharges. Only the unnamed drain contained sedi-

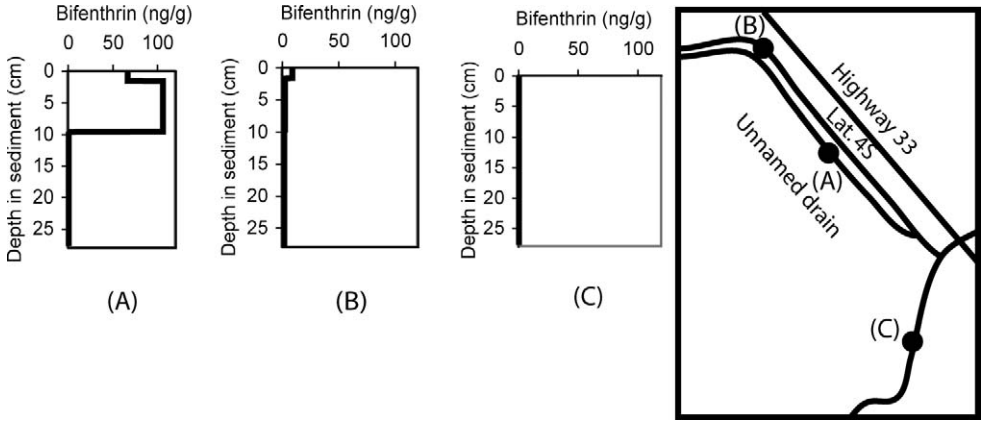

Fig. 4. Concentration of bifenthrin (ng/g) with depth in the sediment column. The highest bifenthrin concentrations were found in surficial sediments near the juncture of Del Puerto Creek and Highway 33 near Patterson, California, USA, so cores were taken in each of the three potential sources upstream of this point. The location of cores (A), (B), and (C) are shown on the inset map. Concentrations in the uppermost strata do not necessarily correspond with those in Figure 2, as the vertical cores represent a different sampling event. The irrigation laterals are abbreviated as Lat.

ments with bifenthrin concentrations approaching those found in the creek mainstem. In addition, the drain discharges to the creek only approximately 10 m upstream of the point where the high bifenthrin concentrations first appeared.

Lateral 4S contained acutely toxic concentrations of bifenthrin at some points (<1–13 ng/g) and most of the laterals east of Highway 33 contained low concentrations of bifenthrin (4–6 ng/g), at or just below the *H. azteca* sediment LC50. There are three irrigation laterals east of Highway 33 that flow towards Del Puerto Creek, but go underground 1 to 2 km from the creek and are not shown in Figure 2. They were not sampled and their points of entry into the creek, if they exist, are not known. In addition, individual growers may release irrigation runoff to the creek at publicly inaccessible points, so it is likely that there are other discharges of bifenthrin to the creek beyond those sampled and shown on Figure 2. With increasing downstream distances, as the potential for unidentified and unsampled drain inputs increases, it becomes more difficult to establish if the unnamed drain just upstream of Highway 33 remains the dominant source for the bifenthrin residues. Additional inputs could be masked by the more upstream source.

The bifenthrin concentrations in the surficial sediment seemed to indicate that the unnamed drain was the only source with sufficient bifenthrin concentrations to account for the 286 ng/g bifenthrin near Highway 33, and possibly for much of the bifenthrin in the downstream reaches. However, vertical cores were taken to determine whether the contribution of other inputs may have been obscured by burial of contaminated material beneath cleaner surficial sediments. The vertical core data confirmed the dominant contribution of the unnamed drain (Fig. 4). A core in the mainstem of Del Puerto Creek (core C), at the site were 9 ng/g bifenthrin was detected in the surficial sediments on a previous visit (Fig. 2), contained no measurable bifenthrin throughout the entire length of the core to the maximum depth of penetration (28 cm). A core in lateral 4S (core B), at the site where 8 ng/g had previously been measured in the surficial sediments (Fig. 2), contained 11 ng/g in the 0- to 2-cm stratum, but little bifenthrin at deeper depths (1.6–4.8 ng/g). A core in the unnamed drain (core A) at the site where 39 ng/g had been previously found in surficial sediments contained 69 ng/g in the 0- to 2-cm stratum, and 109 ng/g in the 2- to 10-cm stratum. This finding supports the contribution of the unnamed drain as a major bifenthrin source, and indicates that cleaner sediments, relatively speaking, have overlain more contaminated material.

If the number of bifenthrin toxic units in each sample is compared to the *H. azteca* mortality found in toxicity testing of that sediment (Fig. 5), it is apparent that bifenthrin alone can explain the majority of the toxicity found throughout Del Puerto Creek and its tributaries. When there was less than approximately 0.5 TU of bifenthrin present, the sediments were usually not toxic. There were two exceptions to this generalization (0.1 TU; 38 and 60% mortality on Fig. 5), and the cause for toxicity at these sites is unknown, though both sites also contained 0.2 to 0.3 TU esfenvalerate. When bifenthrin concentrations exceeded 1 TU, 14 out of 15 sediments were toxic, in most cases causing complete mortality.

Lambda-cyahlothrin was present in Del Puerto Creek sediments at a few sites at concentrations at which *H. azteca* toxicity would be expected. Three sites just upstream of Highway 33 and one sample at Rodgers Road contained 13 to 63 ng/g. The acutely toxic concentration of lambda-cyhalothrin would be expected to be approximately 7 ng/g in Del Puerto Creek sediments (range 2.2–19.0 ng/g) given the reported

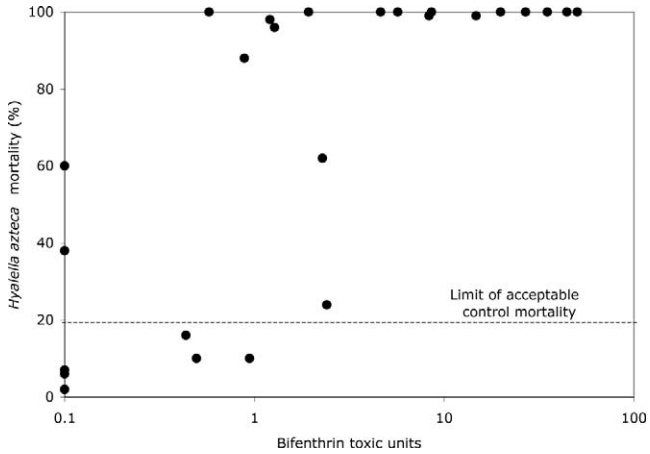

Fig. 5. Relationship between bifenthrin toxic units and mortality to *Hyalella azteca* in the toxicity tests conducted for this study. Sites with undetected bifenthrin are arbitrarily plotted on the figure at 0.1 toxic units. Limit of acceptable control mortality obtained from the U.S. Environmental Protection Agency [16].

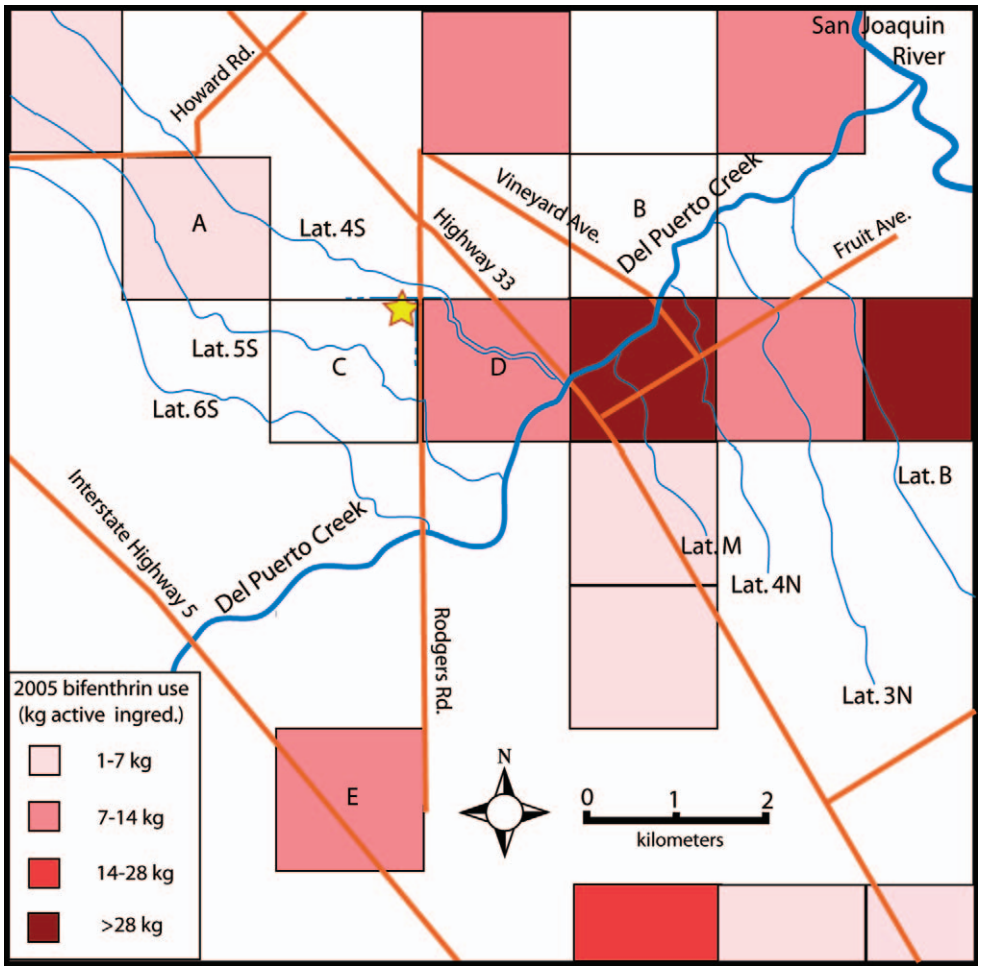

Fig. 6. Reported agricultural use of bifenthrin in 2005 in the Del Puerto Creek region (near Patterson, CA, USA). There was no reported use of bifenthrin in areas of the map that are white. The parcels shown represent areas of 1.6 by 1.6 km (1 mile^2). Parcels discussed in the text are labeled A through E. The yellow star indicates the most upstream sampling site with high bifenthrin concentrations in the sediment (173 ng/g), suggesting a source at or upstream of this location. The irrigation laterals are abbreviated as Lat.

10-d LC50 of 0.45 μg/g OC [22] and the OC content of the sediments in the agricultural portion of Del Puerto Creek and its laterals (median = 1.44% OC; range 0.49–4.23%). The source(s) of these scattered high concentrations of lambda-cyhalothrin could not be determined. None of the laterals contained comparable concentrations, nor was the compound present in any of the core samples in any depth stratum above 8 ng/g.

Cyfluthrin and esfenvalerate were the only other pyrethroids found at potentially toxic concentrations, but each only at a single site. Cyfluthrin was present at 7.5 ng/g at the site immediately upstream of the combined lateral 4S/unnamed drain input. This concentration would represent 0.5 TU given the 1.44% OC content of the sediments at that site. Esfenvalerate reached a maximum concentration of 22.3 ng/g in lateral 4N, corresponding to 0.7 TU given the OC content of 2.13% at that site. Permethrin, cypermethrin, and deltamethrin were occasionally found in some samples, though concentrations never exceeded about one-third of their reported *H. azteca* LC50s [21,22].

Chlorpyrifos was not a significant contributor to toxicity, as it never exceeded 7.9 ng/g, corresponding to 0.2 TU given a sediment 10-d *H. azteca* LC50 of 2.97 μg/g OC [9]. The organochlorine pesticide DDT and its degradation products were present in all samples, with maximum concentrations of 135, 148, and 19 ng/g for DDT, dichlorodiphenyldichloroethylene, and dichlorodiphenyldichloroethane, respectively. The only other organochlorines detected were alpha-chlordane (maximum 2.6 ng/g), dieldrin (maximum 3.7 ng/g), endrin (maximum 2.4 ng/g), and endosulfan sulfate (maximum 9.7 ng/g). No organochlorines were present at concentrations exceeding 0.01 TU (using the LC50 values of Weston et al. [14]), and thus were unlikely to have played any role in the observed toxicity.

Bifenthrin use in the Del Puerto Creek watershed

Using the CDPR's PUR database, bifenthrin applications in the Del Puerto Creek region were mapped, using all reported agricultural applications in 2005 (given that field sampling occurred in December 2005 through March 2006). Nonagricultural use was not included as there was no residential or commercial development in the likely source area. Agricultural bifenthrin use was scattered throughout the watershed, though some of the parcels receiving the largest amounts of bifenthrin (up to ~30 kg in 2005) were located south of the creek and east of Highway 33 (Fig. 6). Within the area of Figure 6, approximately 160 kg of bifenthrin was reported to have been used. The multiple reported uses throughout the watershed support the environmental data in which bifenthrin was found in the sediments of nearly all tributary drains.

The map of reported uses is of less value in confirming the unnamed drain as a dominant bifenthrin source. Applications within parcel D (as labeled on Fig. 6) may contribute to bifenthrin within portions of the unnamed drain near Del Puerto Creek, depending on precisely where within parcel D the application occurred and the drainage patterns from those specific fields. However, parcel D applications would be less likely to explain the most upstream site in the drain at which 173 ng/g bifenthrin was found. This site was west of parcel D, and since surface flow is primarily to the northeast given regional topography, irrigation runoff from parcel D would have to be pumped to the west. The bifenthrin application within parcel A may have been a contributor to the site in question though local drainage patterns in that area and the precise location of application within parcel A are unknown. While the reported agricultural uses of bifenthrin in 2005 are not consistent with the environmental chemistry data collected, the possibility exists that application of bifenthrin was made without the required reporting to the CDPR, in which case the source parcel could appear in Figure 6 as having no bifenthrin use, or the residues reflect use in prior years.

Bifenthrin has a half-life in aerobic soils of three months [25] and a half-life in aquatic sediments of 8 to 17 months at 20°C [24]. Thus, it is possible that sediments in the unnamed drain reflected bifenthrin use prior to the 2005 data of Figure 6. However, an assessment of 2004 applications provided no additional useful information. Within the area of Figure 6 the only reported 2004 bifenthrin applications were in parcels A, B, and E.

Parcel C was of particular interest given it was the location of the drain samples with the highest sediment bifenthrin concentrations (173 ng/g), therefore PUR records for this parcel were examined every year from 2005 to 1995. During this decade there were only three years of reported bifenthrin use in this parcel, all on melons: 28 kg in 2000, 6 kg in 1997, and 3 kg in 1996. Thus, if the source lies within Parcel C, as would be most likely given proximity to the point of high bifenthrin concentrations, the presence of bifenthrin in the unnamed drain could only result from nonreporting of recent use or persistence of residues in the sediment for at least five and a half years.

Based on data in the PUR database, much of the agricultural land in the Del Puerto Creek watershed is treated with an average of 20 to 40 g pyrethroids per hectare of cultivated farmland annually, with up to six times this amount applied in some portions of the watershed. The majority of this usage is comprised of lambda-cyhalothrin (37% of total pyrethroid use), with lesser amounts of bifenthrin (22%), permethrin (18%), esfenvalerate (16%), and several minor use pyrethroids (7%). Despite the proportionally lesser use of bifenthrin, the compound is often the dominant contributor among the pyrethroids to *H. azteca* toxicity. In a geographically broad survey of agricultural areas in central California, bifenthrin was responsible for toxicity to *H. azteca* about twice as often as any other pesticide [15]. There are at least two potential explanations for the disproportionate role of the compound in aquatic toxicity. First, it may be more persistent in sediments than the other pyrethroids. It is known to be somewhat more persistent in soils [25], and though there are minimal data on the persistence of pyrethroids in sediments, bifenthrin does appear to at least be considerably more persistent than permethrin [24]. A greater environmental persistence has also been suggested as a possible reason for its prevalence in urban streams where it is also found in concentrations disproportionate to its relative use among the pyrethroids [26].

A second possible explanation may lie in the types of crops to which bifenthrin is applied. In Stanislaus County, California, the county in which the study area lies, the PUR database for 2004 indicates that bifenthrin was used primarily on corn (78% of total agricultural use). In the Del Puerto Creek watershed specifically, bifenthrin applications were largely on cauliflower, cantaloupe, and beans. All bifenthrin usage in the region is on row crops, whereas other pyrethroids have substantial use on orchard crops. Lambda-cyhalothrin usage in Stanislaus County is 44% in orchards. Permethrin and esfenvalerate use are 69 and 84% in orchards, respectively. Off-site transport of soil and associated pyrethroids may be greater for row crops than for orchards. Though some California orchards are flood irrigated, an increasing number of orchard growers are relying upon drip irrigation or microsprinklers, virtually eliminating irrigation runoff. Row crops in the region, however, are usually irrigated by flooding the furrows. This practice has the potential to generate much larger volumes of runoff, and given the freshly tilled soil in the furrows, this runoff often carries substantial suspended sediment loads. Thus, the dominance of bifenthrin in the sediments of agricultural water bodies could also be a consequence of the fact that it is applied to crops with a greater potential for off-site sediment transport.

Bifenthrin or products containing the compound are registered for agricultural use at both the federal and state (California) levels, and while we have no specific information from the Del Puerto Creek watershed, there is no reason to believe its use is occurring in a manner inconsistent with its labeling. Yet residues are clearly reaching surface waters. While one drain in particular appeared to be the dominant source for much of the bifenthrin-contaminated sediment in the creek, the ubiquity of the compound in the other tributary drains indicated the presence of multiple sources. Control of soil loss from farmland is the key to preventing the sediment degradation observed, and that has long persisted in Del Puerto Creek given the frequent reports of sediment toxicity in the creek over at least the past several years. Recovery and reuse of irrigation runoff, with no discharge to surface waters would be a definitive solution, but substantial improvement may also be possible with relatively simple changes in management practices, such as the use of vegetated drainage ditches [27,28]. Another possible approach is addition of polyacrylamide to irrigation water to minimize erosion and promote flocculation of suspended material [29], a technique that has been shown to be very effective in mitigating soil transport in testing in the vicinity of Del Puerto Creek [30].

Del Puerto Creek is currently listed as an impaired water body due to chlorpyrifos, diazinon, and pyrethroid contamination under section 303(d) of the Clean Water Act (http://www.swrcb.ca.gov/tmdl/docs/303dlists2006/approved/r5_06_303d_reqtmdls.pdf). The listing identifies bifenthrin, lambda-cyhalothrin, esfenvalerate/fenvalerate, and permethrin as pyrethroids of concern, with their presence attributed to unknown sources. Though no effort to develop a total maximum daily load allocation has yet been scheduled, the data of the present study could be of considerable value to that effort in better defining the contaminants of concern and locating sources. In addition, under the current 303(d) listing cycle many more California streams are being added to the impaired water body list due to previously documented pyrethroid-associated sediment toxicity [14,26,31], and the techniques employed in Del

Puerto Creek are likely to prove useful in addressing similar problems elsewhere.

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