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Environmental Toxicology and Chemistry, Vol. 25, No. 6, pp. 1671–1676, 2006

Printed in the USA
0730-7268/06 $12.00 + .00

# SOLID-PHASE SEDIMENT TOXICITY IDENTIFICATION EVALUATION IN AN AGRICULTURAL STREAM

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(*Received* 30 *August* 2005; *Accepted* 29 *November* 2005)

**Abstract**—The lower Santa Maria River watershed provides important aquatic habitat on the central California coast and is influenced heavily by agricultural runoff. As part of a recently completed water quality assessment, we conducted a series of water column and sediment toxicity tests throughout this watershed. Sediment from Orcutt Creek, a tributary that drains agricultural land, consistently was toxic to the amphipod *Hyalella azteca*, which is a resident genus in this river. Toxicity identification evaluations (TIEs) were conducted to determine cause(s) of toxicity. We observed no toxicity in sediment interstitial water even though concentrations of chlorpyrifos exceeded published aqueous toxicity thresholds for *H. azteca*. In contrast to interstitial water, bulk sediment was toxic to *H. azteca*. In bulk-phase sediment TIEs, the addition of 20% (by volume) coconut charcoal increased survival by 41%, implicating organic chemical(s). Addition of 5% (by volume) of the carbonaceous resin Ambersorb 563® increased survival by 88%, again suggesting toxicity due to organic chemicals. Toxicity was confirmed by isolating Ambersorb from the sediment, eluting the resin with methanol, and observing significant toxicity in control water spiked with the methanol eluate. A carboxylesterase enzyme that hydrolyzes synthetic pyrethroids was added to overlying water, and this significantly reduced toxicity to amphipods. Although the pesticides chlorpyrifos, DDT, permethrin, esfenvalerate, and fenvalerate were detected in this sediment, and their concentrations were below published toxicity thresholds for *H. azteca*, additivity or synergism may have occurred. The weight-of-evidence suggests toxicity of this sediment was caused by an organic contaminant, most likely a synthetic pyrethroid.

**Keywords**—Sediment Toxicity identification evaluation *Hyalella azteca* Pyrethroid Carboxylesterase

## INTRODUCTION

The California State Water Resources Control Board supports ambient water-quality monitoring throughout the state, as well as special studies to determine the causes and sources of observed biological impacts. The current study investigated the causes of sediment toxicity observed in the lower Santa Maria River in central California, USA. The lower Santa Maria River and its estuary provide critical habitat for a number of threatened and endangered species and receive runoff from lands used for grazing, urban development, and intensive agriculture. An initial assessment of this watershed included monitoring of six stations for 12 months to identify tributaries that might convey toxic runoff to the lower river ecosystem. Consistent toxicity to *Ceriodaphnia dubia* in water column samples from several stations primarily was caused by the pesticide chlorpyrifos [1]. Toxicity to the amphipod *Hyalella azteca* also was observed in sediments from several stations, in particular Orcutt Creek, a tributary to the lower Santa Maria River. The weight-of-evidence from benthic macroinvertebrate surveys, laboratory toxicity tests, toxicity identification evaluations (TIEs), and chemical analysis indicated that pesticides entering the river were adversely affecting resident macroinvertebrate communities [1]. Data from this and other studies are being considered in the proposed listing of the Lower Santa Maria watershed as an impaired water body under section 303(d) of the Clean Water Act (http://www.epa.gov/region5/water/cwa.htm). Such listing would require determination of the total maximum daily load for chemicals entering this ecosystem.

Any total maximum daily load assessment would require identification of the runoff constituents responsible for the observed impairment. The TIE approach was used here for this purpose. Sediment TIEs can focus on interstitial water toxicity and use standard TIE methods for water [2–4], or can apply methods specifically designed for use with sediments in the solid-phase [5–15]. Anderson et al. [1] described a preliminary investigation of sediment toxicity in their study of the causes of water toxicity in this watershed. In the present study, we present a more detailed investigation of the causes of sediment toxicity in Orcutt Creek, which includes the use of published sediment TIE procedures, as well as newer methods developed both in our laboratory and in other laboratories at the University of California, Davis.

## METHODS

### *Test sediment*

Test sediment was collected on lower Orcutt Creek (ORC) approximately 0.5 km above the creek's confluence with the Santa Maria River. This station receives agriculture drain water, has variable flow, and generally is turbid (mean total suspended solids = 2,190 mg/L). As part of the overall watershed assessment, two sediment surveys were conducted in this creek in June 2002 and May 2003. Amphipod survival in sediment from these surveys was 6 and 0%, respectively. Sediment interstitial water from the first survey contained 231 ng/L chlor-

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pyrifos, a concentration approximately 2.5 times the median lethal concentration (LC50) for *H. azteca* (LC50 = 86 ng/L [16]). The sediment contained 43.3 ng/g of lambda-cyhalothrin and 23.1 ng/g of permethrin [1]. The concentration of lambda-cyhalothrin was above the mean sediment LC50 for toxic effects on *H. azteca* (5.6 ng/g [17]), but the permethrin concentration was below its mean sediment LC50 (201 ng/g [17]). Total DDT exceeded the consensus-based freshwater sediment threshold effect concentration guideline, but was considerably lower than the probable-effects concentration [1,18]. The concentration of chlorpyrifos in interstitial water from the second sediment sample was 459 ng/L. Because we measured significant toxicity and elevated concentrations of pesticides in both surveys, a third sediment sample was collected for TIE and chemical analyses. All TIE experiments in this study were conducted using sediment collected in October 2003.

*Toxicity testing and TIEs*

Sediment samples were collected to a depth of 5 cm using a polycarbonate core tube (7.5-cm diameter). Individual sediment cores were composited in 12 2-L glass jars. Samples were transported on ice and stored in the dark at 4 ± 3°C. The separate jars were not composited, and sediment for iterative TIE experiments conducted over the next six months used sediment sequentially from the original 12 jars, as needed. Ten-day solid-phase laboratory toxicity tests with *H. azteca* followed U.S. Environmental Protection Agency (U.S. EPA) [19]. Tests were conducted by placing 100 ml of sediment and 200 ml of overlying water into eight 300-ml beakers with screened overflows. Overlying water was renewed twice daily, and exposures were conducted at 23°C. Control sediment for the initial test and all TIEs consisted of a laboratory-formulated sediment composed of equal parts clean, kiln-dried sand (no. 60, RMC Pacific Materials, Monterey, CA, USA) and field sediment from a previously described reference site on the Salinas River [20,21], plus 15 g/kg organic peat (Uni-Gro, Chino, CA, USA). Formulated sediment was analyzed for organic contaminants, and only small concentrations of dacthal (5.6 ng/g) and the DDT metabolite dichlorodiphenyldichloroethylene (3.8 ng/g) were detected. Once initial toxicity of the Orcutt Creek sediment was observed, TIE investigations began with an initial interstitial water exposure. Interstitial water was extracted via refrigerated centrifuge (2,500 *g* at 4°C). The full-strength interstitial water exposure did not produce significant toxicity; therefore, solid-phase TIE treatments were used to investigate causes of toxicity in the Orcutt Creek sediment.

Solid-phase TIE treatments are designed to determine first whether toxicity is caused by ammonia, metals, or organic [14]. Because the site drains lands used for agriculture [1], all treatments were designed to evaluate the effects of organics toxicants. Ammonia concentrations measured in the initial tests were well below the tolerance threshold for *H. azteca*. Two amendments were added to the sample: Powdered coconut charcoal [14] and a carbonaceous resin [7,8,11]. Phase II TIE procedures consisted of separating the resin from the sediment, extracting it with solvent, and spiking control water with the methanol eluate as a toxicity add-back procedure. Two additional solid-phase TIE procedures also were employed. Piperonyl butoxide (PBO) and porcine carboxylesterase were added to the overlying water of the amphipod exposures alone and in combination to investigate whether toxicity was due to pyrethroid and/or organophosphate pesticides.

Powdered coconut charcoal (PCC) is pyrolized, activated coconut husk that has been ground to <45 μm (90–96%; Calgon Carbon, Pittsburgh, PA, USA; [14]). The PCC was hydrated with an excess of fresh well water in a 2,000-ml Erlenmeyer flask, then vacuum-filtered to form damp slurry. Fifteen percent (by wet wt) PCC was added to the sediment ([14]; T. Norberg-King, U.S. EPA, Duluth, MN, personal communication). Powdered coconut charcoal-treated sediment was homogenized for 24 h on a Wheaton Roller Apparatus (Wheaton Instruments, Millville, NJ, USA) and then loaded into exposure chambers, as described above (this section). A dilution blank was created to account for the effects of diluting ORC sediment with PCC. Orcutt Creek sediment was combined with 15% formulated sediment to account for the dilution. A PCC blank was created to account for the effects of adding PCC to test sediment by adding 15% PCC to formulated sediment. The PCC test followed U.S. EPA [19] with two renewals and one feeding per day.

Ambersorb 563® (Rohm and Haas, Spring House, PA, USA), a carbonaceous, nonpolar resin, was prepared by rinsing it thoroughly with Nanopure® (Barnstead International, Dubuque, IA, USA) water. For this experiment, test sediment was dry-sieved through 280-μm mesh before being treated and tested. This resulted in a smaller sediment size distribution that facilitated sieve-separation of the Ambersorb, which was removed from the sediment at the end of the experiment for phase II TIE elution procedures (discussed below, this section). Sixty-five percent of the original sediment passed through the mesh, and the survival of *H. azteca* in the sieved sediment was the same as unsieved sediment. Five percent Ambersorb by wet weight was added to sediment [7,11]. Treated sediment was homogenized for 24 h on the roller apparatus and then loaded into exposure chambers. A dilution blank was created by combining sieved test sediment with 5% formulated sediment, and an Ambersorb blank was created by adding 5% Ambersorb to formulated sediment. The Ambersorb test was conducted without overlying water renewal, with feeding every other day. At test termination, the sediment was screened through 280-μm mesh to retain the Ambersorb. The Ambersorb then was eluted by combining 5 g of resin with 2.5 ml of methanol in a 20-ml glass scintillation vial. The mixture was allowed to interact for 24 h. One milliter of methanol extract was combined with 100 ml of clean dilution water to create the eluate sample for toxicity testing with *H. azteca*. An Ambersorb elution blank was prepared by performing the above treatments on Ambersorb that had been combined with formulated sediment. A 1% methanol blank also was tested. One percent methanol concentrations were used because they are well within the methanol tolerance limit of *H. azteca* [2].

Additional solid-phase TIE treatments included addition of PBO and porcine carboxylesterase to the overlying water in a static solid-phase amphipod exposure. Piperonyl butoxide (Sigma-Aldrich, St. Louis, MO, USA) is used to block the metabolic activation of acetylcholinesterase-inhibiting organophosphate pesticides [22]. It is also a potent synergist of pesticide toxicity, because it inhibits their metabolism [23,24]. The enzyme carboxylesterase (Sigma-Aldrich) hydrolyzes ester-containing compounds such as pyrethroids to their corresponding acid and alcohol, which generally are not toxic [25]. Decreased toxicity with the addition of PBO suggests the presence of organophosphate pesticides. Increased toxicity with the addition of PBO or decreased toxicity with the addition of carboxylesterase suggests the presence of pyrethroids.

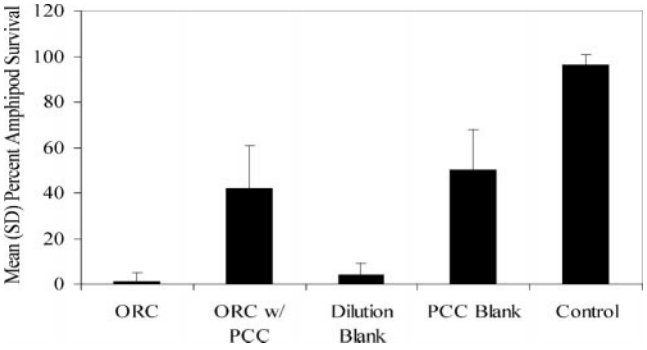


Fig. 1. Mean amphipod survival in solid-phase toxicity identification evaluation treatments using powdered coconut charcoal (PCC). ORC indicates untreated Orcutt Creek (CA, USA) sediment. Dilution blank consists of ORC sediment diluted with 15% formulated sediment. PCC blank consists of formulated sediment containing 15% PCC.

Carboxylesterase (500×) was added to the overlying water on the day of test initiation, 6 h before the addition of amphipods. This allowed for interaction between the enzyme and pyrethroids. The enzyme was added based on units of activity. One × of enzyme activity equals 0.0025 units of enzyme per ml of sample; therefore, at 500×, 1.25 units per ml were added. Enzyme strength is unique for each lot purchased [25]. In a separate treatment, 500 μg/L of PBO was added to overlying water. A combination treatment of enzyme and PBO also was conducted to help resolve toxicity due to combinations of organophosphate and pyrethroid pesticides. Enzyme was added and allowed to interact with the overlying water for 6 h before the addition of PBO and then amphipods. Eight replicates of each treatment were prepared. Two replicates were terminated and surviving amphipods were counted every 24 h for 4 d.

*Chemical measurements*

The initial interstitial water sample was analyzed for chlorpyrifos and diazinon with enzyme-linked immunosorbent assays. Measurements were compared to a five-point standard curve. The lowest detectable dose was calculated from analysis of laboratory standards according to the manufacturer's methodology (Strategic Diagnostic, Newark, DE, USA). The lowest detectable dose was the amount of pesticide required to achieve a ratio of 85% between the mean absorbance of the standard and the mean absorbance of a negative control or laboratory control water [26]. Absorbance is inversely proportional to concentration. The lowest detectable dose was 30 ng/L for diazinon and 50 ng/L for chlorpyrifos. External standards and sample duplicates were measured. Recovery of external standards was 98 and 142% for chlorpyrifos and diazinon, respectively, and duplicates had coefficients of variation less than 2%.

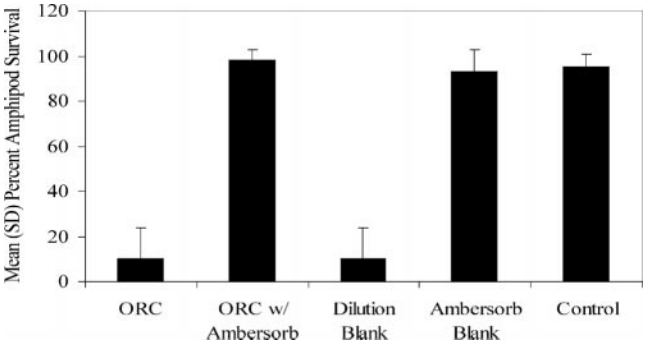


Fig. 2. Mean amphipod survival in solid-phase toxicity identification evaluation treatments using Ambersorb 563 (Rohm and Haas, Spring House, PA, USA). ORC indicates untreated Orcutt Creek (CA, USA) sediment. Dilution blank consists of ORC sediment diluted with 5% formulated sediment. Ambersorb blank consists of formulated sediment containing 5% Ambersorb.

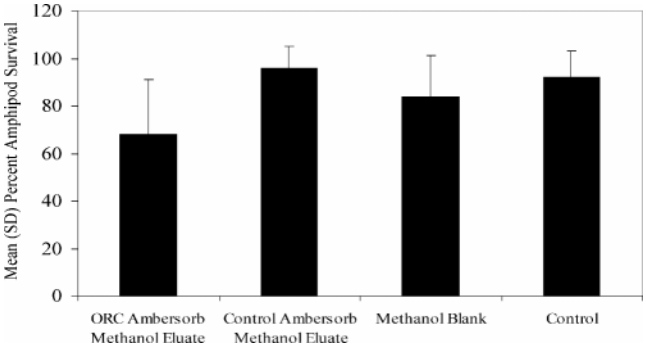


Fig. 3. Mean amphipod survival in solvent elution of Ambersorb resin (Rohm and Haas, Spring House, PA, USA).

Sediment samples from June 2002 were analyzed for organochlorine compounds (U.S. EPA method 8081 [27]) and pyrethroids (U.S. EPA method 1660 [28]). Sediments from May and October 2003 were analyzed for organochlorine compounds, organophosphates (U.S. EPA method 8141 [27]), carbamates (U.S. EPA method 531.1 [29]), and pyrethroids. All analyte identifications were confirmed by gas chromatography–mass spectrophotometer or liquid chromatograph–mass spectrophotometer. Standard quality-assurance procedures, including measurement of standard reference materials and quantification of surrogate recoveries and matrix spikes, were used in all analyses. Surrogate and spike recoveries ranged from 65 to 122%.

## RESULTS

Amphipod survival was 21% in the initial solid-phase test of sediment collected in October 2003. Survival in full-strength interstitial water was 73%. The interstitial water toxicity signal was considered too weak to pursue, and the TIE investigation focused on solid-phase treatments.

Both the Ambersorb and PCC sediment amendments significantly reduced sediment toxicity to *H. azteca*. Control survival in both tests was above 90%. Survival in ORC sediment conducted in conjunction with the PCC solid-phase TIE was 1% (Fig. 1). The addition of PCC significantly increased amphipod survival to 42%. However, survival in the PCC blank was only 50%, indicating an adverse reaction to PCC by the test amphipods. Survival in the dilution blank was not significantly different from the ORC sediment, indicating no dilution effect from adding PCC to ORC sediment. Addition of Ambersorb increased survival to 98% from the 10% observed in the unamended test sediment. Unlike the PCC, there was no toxic artifact observed in the Ambersorb blank consisting of control sediment treated with Ambersorb (Fig. 2). No dilution effects were in the Ambersorb TIE. The reduction of toxicity after treatment with Ambersorb indicated that toxicity was caused by one or more organic compound(s).

Amphipod survival was 68% in eluate from the Ambersorb recovered from test sediment and was significantly lower than the survival in eluate from Ambersorb recovered from the control sediment (Fig. 3). Survival in the methanol blank was 84%. Thus there appeared to be significant recovery of the

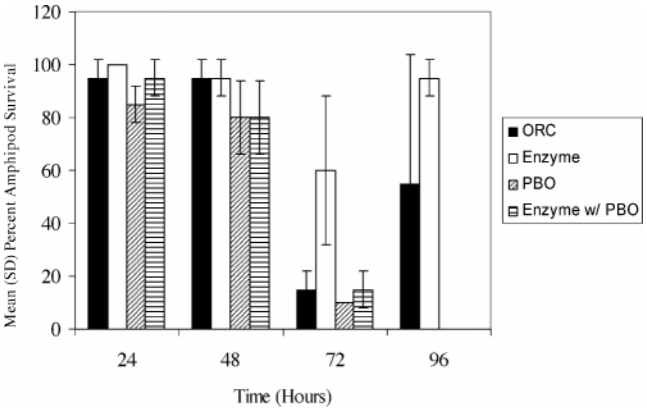


Fig. 4. Mean amphipod survival in solid-phase toxicity identification evaluation treatments with piperonyl butoxide (PBO) and carboxylesterase enzyme added to sediment overlying water. ORC indicates untreated Orcutt Creek (CA, USA) sediment.

toxic constituents removed by the Ambersorb from the test sediment.

Toxicity was reduced by addition of carboxylesterase to sediment overlying water and increased by addition of PBO (Fig. 4). Differences in amphipod survival between untreated test sediment and carboxylesterase-treated test sediment became apparent after 72 h (60 ± 28% in enzyme-treated sediment vs. 15 ± 7% in untreated sediment). The replicate test chambers that were terminated after 96 h had 55% survival in the untreated test sediment, because one of the two replicates had 90% survival. In the enzyme-treatment chambers that were terminated at 96 h, there was 95% survival. Survival in the PBO treatments did not differ from survival in ORC sediment until 96 h. At 96 h, there was complete mortality in the treatment with PBO and in the combination treatment containing PBO and enzyme. Reduction of toxicity with the addition of carboxylesterase coupled with increased toxicity with the addition of PBO suggests toxicity caused by a pyrethroid pesticide. Organism response in enzyme and PBO treatment blanks was greater than 95% survival.

Interstitial water from the test sediment was analyzed for chlorpyrifos and diazinon, and solid-phase sediment was analyzed for organochlorines, organophosphates, carbamates, and several pyrethroids. The concentrations of the detected compounds are presented in Table 1. The interstitial water concentration of chlorpyrifos exceeded the 10-d LC50 for *H. azteca* by a factor of six (86 ng/L [16]), but no toxicity was observed in the initial interstitial water test. The concentration of total DDT in the sediment was 116.7 ng/g. This concentration was above the consensus-based freshwater sediment threshold effect concentration guideline (= 5.28 μg/kg), but well below the probable-effects concentration (= 572 μg/kg [18]). When normalized as total DDT per gram organic carbon, the concentration of μg total DDT/g organic carbon also was well below the mean *H. azteca* LC50 (371 μg/g organic carbon [30]). The concentrations of pyrethroids in the sediment were below the sediment LC50 values reported by Amweg et al. [17].

## DISCUSSION

The combination of TIE procedures presented in this study were designed to determine first whether organic chemicals caused toxicity in Orcutt Creek sediment and then to further resolve whether the cause of toxicity was due to organophosphate or pyrethroid pesticides. The PCC and Ambersorb amendments reduced toxicity, and the solvent eluate of Ambersorb was toxic when spiked into clean dilution water. These characterization experiments, therefore, implicated one or more organic contaminants as the cause of toxicity. Addition of PBO increased toxicity and addition of carboxylesterase reduced toxicity. Results of these treatments provided evidence that toxicity of Orcutt Creek sediment, at least in part, was due to pyrethroid pesticides.

Powdered coconut charcoal and Ambersorb perform the same function in a sediment TIE: Both reduce bioavailability of organic contaminants. Powdered coconut charcoal is considered especially effective because it has greater surface area for adsorption [14], but our results confirm the observation of other researchers that *H. azteca* do not tolerate higher concentrations of PCC. Ambersorb is effective at binding organic chemicals and is relatively nontoxic. An additional benefit of Ambersorb is that, unlike PCC, it can be separated from sediment and eluted with solvent. In solid-phase toxicity testing, this step is necessary before phase II (identification) procedures can be implemented. Several researchers have described the effectiveness of carbonaceous resins to reduce sediment toxicity due to organic chemicals [7,8,11,13], but we are aware

Table 1. Interstitial water and sediment chemistry results from Orcutt Creek (CA, USA) collected October 2003. LC50 indicates median lethal concentration; OC indicates organic carbon

| Chemical class | Analyte | Concn. | LC50 |
|---|---|---|---|
| Interstitial water | | | |
| Organophosphate | Chlorpyrifos | 588 ng/L | 86 ng/L[a] |
| | Diazinon | 600 ng/L | 6,510 ng/L[a] |
| Sediment | | | |
| Organophosphate | Chlorpyrifos | 17.4 ng/g | 399 ng/g[b] |
| | Diazinon | 1.21 ng/g | |
| Organochlorine | Dacthal | 17.2 ng/g | |
| | Hexachlorobenzene | 0.099 ng/g | |
| | Total DDT | 116.7 ng/g | |
| | Total DDT/g OC | 12.3 μg/g OC | 371 μg/g OC[c] |
| Pyrethroid | Esfenvalerate/fenvalerate | 0.56 ng/g | 41.8 ng/g[d] |
| | Permethrin | 1.54 ng/g | 201 ng/g[d] |

[a] Phipps et al. [16].
[b] Brown et al. [36].
[c] Nebeker et al. [30].
[d] Amweg et al. [17].

of only one published report that successfully has eluted Ambersorb with solvent as part of a sediment TIE process [31]. This presumably is due to the difficulty of separating the resin from sediment. For the treatments using Ambersorb, sediment was presieved through a 280-μm screen. This sediment retained its toxicity, and presieving facilitated isolation of the Ambersorb. Once it was eluted, clean dilution water spiked with this eluate was toxic to amphipods. Following published TIE procedures [3], the logical next step would have been to subject the Ambersorb eluate to high-performance liquid chromatography fractionation, conduct toxicity tests to identify toxic fraction(s), then analyze chemicals in those fractions. We attempted to do this with residual Ambersorb from this experiment. No toxicity was observed when the remaining Ambersorb was eluted (data not shown), so the additional phase II steps were not completed. Additions of PBO and carboxylesterase enzyme were used as alternative phase II TIE procedures.

Results of the carboxylesterase and PBO additions to sediment overlying water suggest pyrethroid pesticides caused toxicity of Orcutt Creek sediment. *H. azteca* are epibenthic amphipods closely associated with the sediment-water interface, and this species, therefore, is susceptible to contaminants fluxing into the overlying water. We have shown previously that treatments of the overlying water in sediment exposures reduce toxicity to epibenthic organisms [32]. In the current experiments, the addition of esterase reduced toxicity, and the addition of PBO increased toxicity. Toxicity also increased when overlying water was subjected to both treatments simultaneously.

The conclusion that pyrethroids were the cause of Orcutt Creek sediment toxicity was confounded by the fact that concentrations of pyrethroids measured in the TIE sediments were below LC50 values reported by Amweg et al. [17]. Because sediment was held two months before being analyzed, some degradation of contaminants may have occurred, but sediment remained significantly toxic up to six months after collection. Although measured concentrations of pyrethroids were low, only four pyrethroids were analyzed in these samples, and other pyrethroids might have been present in toxic concentrations. Previous concentrations of lambda-cyhalothrin and permethrin in sediments collected at this site were 43.3 and 23.1 ng/g, respectively [1]. This previous lambda-cyhalothrin concentration was well above the sediment LC50 value reported by Amweg et al. [17] (5.6 ng/g). Other pyrethroids that have been used in the counties adjacent to the Santa Maria River include cyfluthrin, cypermethrin, and deltamethrin (University of California Statewide Integrated Pest Management Program, www.ipm.ucdavis.edu/PUSE/puse1.html).

Previous studies have demonstrated that pyrethroids often occur in environmental samples in combination with toxic concentrations of other agricultural pesticides, such as organophosphates [33]. It is likely that the toxicity of pyrethroid pesticides in mixtures is additive ([34]; http://www.ecologyandsociety.org/vol9/iss6/art1), and the toxicity of pyrethroids and organophosphate pesticides in mixtures can be greater than additive [35]. Organophosphates that are cytochrome P450–metabolized inhibit esterase activity, and pyrethroids and organophosphates have complementary modes of action. Although the concentration of the organophosphate chlorpyrifos in the ORC sediment was well below the solid phase LC50 of 399 ng/g [36], it may have worked synergistically with low concentrations of pyrethroids to contribute to the observed toxicity.

## CONCLUSION

Ambient sediment toxicity due to pesticides is a continuing problem, particularly where intensive agriculture activities are conducted in areas adjacent to sensitive waterways. This study presents the results of sediment TIEs that incorporated a number of procedures, including the use of phase I TIE procedures with powdered coconut charcoal and the carbonaceous resin Ambersorb, and solvent elution of Ambersorb isolated from sediment. Additions of carboxylesterase enzyme and PBO, individually and in tandem, also were used to resolve toxicity caused by mixtures of pyrethroids and organophosphates. These techniques complement the evolving toolbox of procedures now available for sediment TIEs. When combined with results of chemical analyses of Orcutt Creek sediments and our previous TIE results from this study area, the results suggest that sediment toxicity was caused by pyrethroid pesticides, possibly in combination with the organophosphate pesticide chlorpyrifos. We anticipate that these results will be used to provide resource managers responsible for restoring water and sediment quality in the lower Santa Maria River with information necessary for identifying potential sources of these pesticides as part of the initial phase of total maximum daily loads in this water body.

*Acknowledgement*—The authors would like to thank the following people for assisting with this study: S. Miller, A. Gerke, and W. Piekarski of the University of California Davis Marine Pollution Studies Laboratory; M. Adams of the California Regional Water Quality Control Board, Central Coast Region; and D. Crane and A. Mekebri of the California Department of Fish and Game, Water Pollution Control Laboratory. This study was funded by the California State Water Resources Control Board.

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