SETAC PRESS

Environmental Toxicology and Chemistry, Vol. 25, No. 4, pp. 1160–1170, 2006

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

Hazard/Risk Assessment

EVIDENCE OF PESTICIDE IMPACTS IN THE SANTA MARIA RIVER WATERSHED, CALIFORNIA, USA

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(*Received* 5 *April* 2005; *Accepted* 29 *August* 2005)

Abstract—The Santa Maria River provides significant freshwater and coastal habitat in a semiarid region of central California, USA. We conducted a water and sediment quality assessment consisting of chemical analyses, toxicity tests, toxicity identification evaluations, and macroinvertebrate bioassessments of samples from six stations collected during four surveys conducted between July 2002 and May 2003. Santa Maria River water samples collected downstream of Orcutt Creek (Santa Maria, Santa Barbara County, CA, USA), which conveys agriculture drain water, were acutely toxic to cladocera (*Ceriodaphnia dubia*), as were samples from Orcutt Creek. Toxicity identification evaluations (TIEs) suggested that toxicity to *C. dubia* in Orcutt Creek and the Santa Maria River was due to chlorpyrifos. Sediments from these two stations also were acutely toxic to the amphipod *Hyalella azteca*, a resident invertebrate. The TIEs conducted on sediment suggested that toxicity to amphipods, in part, was due to organophosphate pesticides. Concentrations of chlorpyrifos in pore water sometimes exceeded the 10-d median lethal concentration for *H. azteca*. Additional TIE and chemical evidence suggested sediment toxicity also partly could be due to pyrethroid pesticides. Relative to an upstream reference station, macroinvertebrate community structure was impacted in Orcutt Creek and in the Santa Maria River downstream of the Creek input. This study suggests that pesticide pollution likely is the cause of ecological damage in the Santa Maria River.

Keywords—Pesticides Toxicity Macroinvertebrates Toxicity identification evaluations

INTRODUCTION

The Santa Maria River provides significant freshwater and coastal habitat in a semi-arid region of central California, USA. The lower river is a primary migration corridor for endangered steelhead trout (*Onchorhynchus mykiss*), and the Santa Maria River estuary provides an important aquatic habitat for numerous marine and estuarine fish species. Mudflat habitats in the estuary are important foraging areas for numerous sensitive shorebird species ([1]; http://www.dunescollaborative.org/SMRE_A.pdf). Large areas in this watershed are cultivated year-round, primarily in row crops such as lettuce and strawberries and in crucifer crops. Current and past agricultural practices have included intensive use of pesticides, and previous studies have found pesticide residues in sediments of the Santa Maria River estuary ([2]; http://www.swrcb.ca.gov/bptcp.reports.html).

In the current study, we investigated the impacts of agriculture drain water at six stations in the Santa Maria River watershed over 12 months. Water and sediment toxicity were characterized using the cladoceran *Ceriodaphnia dubia* and the amphipod *Hyalella azteca*, respectively. The results of these tests were compared to physical and water-quality analyses, as well as to concentrations of selected pesticides in both water and sediment. Ecological impacts at selected sites in the lower watershed were assessed by characterizing macroinvertebrate community structure and in-stream habitat. Possible causes of toxicity and impacts on macroinvertebrate community structure were investigated using a combination of toxicity identification evaluations (TIEs) and chemical analyses, dose-response information from the literature, and physical and habitat assessments. The results were combined in a weight-of-evidence evaluation of the impacts of agriculture drain water on the river ecosystem and were used to investigate chemicals responsible for toxicity and ecological degradation.

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MATERIALS AND METHODS

Sampling sites

Like most coastal rivers in central and southern California, the Santa Maria River heavily is influenced by winter rainfall occurring primarily from November through March. The upper reaches of the river may flow year round, and the middle reaches, extending from the confluence of the Sisquoc River to the Highway 1 crossing, flow underground much of the year (Fig. 1). The river resurfaces and flows year-round from the Highway 1 crossing to the Santa Maria River estuary. This study was conducted at six stations, one in the upper Santa Maria River watershed and five in the lower watershed (Fig. 1). A watershed reference station was located approximately 55 km upstream of the Santa Maria River estuary near the lower end of the Sisquoc River (station SIV = Sisquoc River). This river is a primary tributary of the Santa Maria River and minimally is influenced by agriculture at this location. The remaining five stations were located in the lower watershed in areas surrounded by intensive agriculture. Large areas in this watershed are cultivated year-round, primarily in row crops such as lettuce and strawberries and in crucifer crops. The Main Street Ditch (station MSD) is a storm water and agriculture drain water canal located near the city of Santa

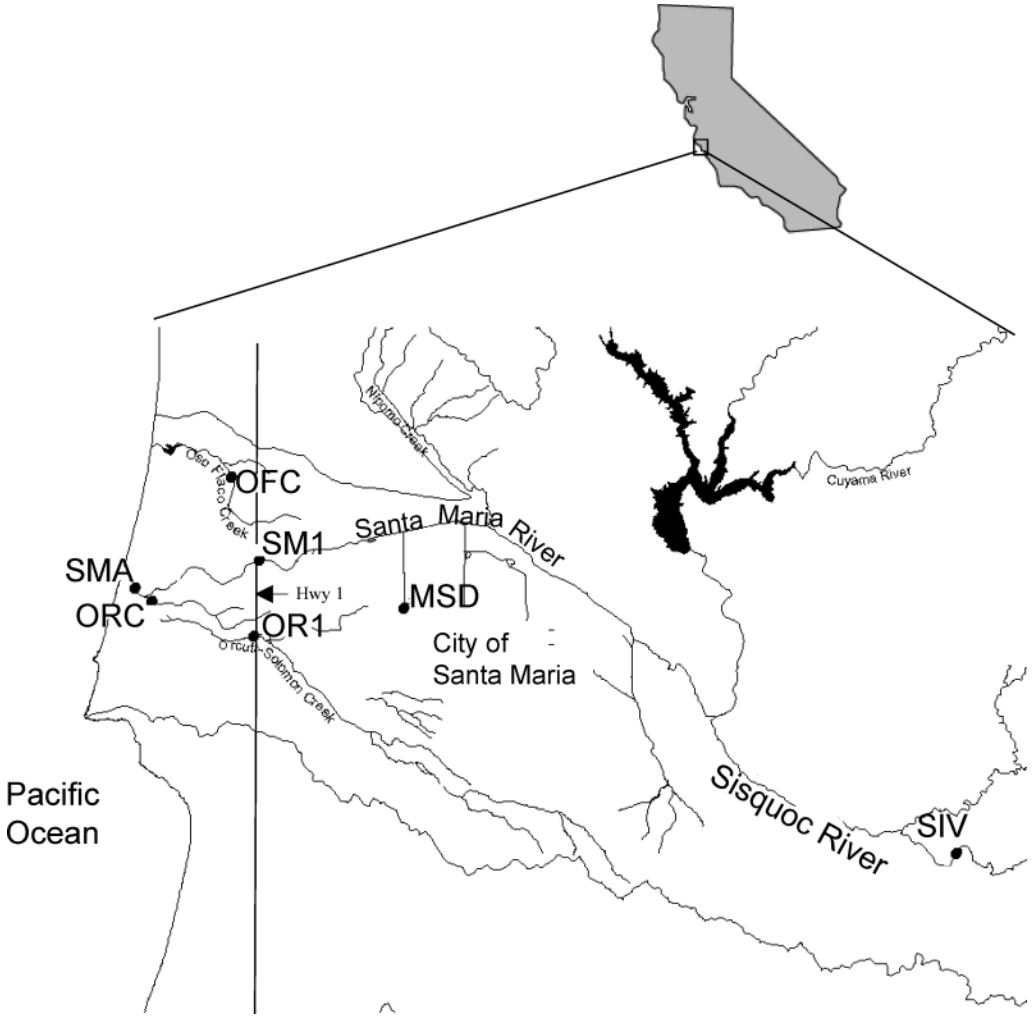

Fig. 1. Map of the Santa Maria River watershed (Santa Maria, CA, USA) showing sampling stations. SIV = Sisqouc River; MSD = Main St. Ditch; SM1 = Santa Maria River at Highway 1; OR1 = Orcutt Creek at Highway 1; ORC = Orcutt Creek at Santa Maria River; SMA = lower Santa Maria River; OFC = Oso Flaco Creek. All stations are located in the vicinity of Santa Maria, CA, USA. (scale: 1.3 cm = 1 km)

Maria and flows directly into the river approximately 13 km upstream of the Highway 1 bridge. The river channel usually is dry where flow from station MSD enters. The Santa Maria River at Highway 1 (SM1) was selected as a reference station for the lower watershed. Resurfacing Santa Maria River flow combined with limited drain water inputs primarily influence this station, which is approximately 7 km from the estuary. Two stations were located on Orcutt Creek, a main tributary to the lower river and its estuary. Station OR1 was located in the middle reaches of Orcutt Creek at the Highway 1 crossing. This station is approximately 7 km from the lower Orcutt Creek station (Fig. 1). Station ORC (Orcutt Creek at the Santa Maria River) was located on lower Orcutt Creek 0.5 km above its confluence with the river. Both stations largely are fed by agriculture drain water. Station SMA (lower Santa Maria River) was located in the Santa Maria River 1 km downstream of Orcutt Creek and 1 km from the estuary. During the dry season, 90% of the flow at this station comes from Orcutt Creek. The final station was located on Oso Flaco Creek (station OFC), which is not part of the Santa Maria River watershed. This creek conveys agriculture drain water to Oso Flaco Lake, a coastal freshwater lake that drains to the Pacific Ocean north of the Santa Maria River estuary. This station was included in the current study due to concerns about the influence of Oso Flaco Creek on the water quality of Oso Flaco Lake and the ecological importance of this coastal water body.

Surveys were conducted in July and September 2002, and March and May 2003 to account for seasonal variability. Water samples for toxicity testing and chemical analyses were collected by hand mid-channel, using 2-L amber glass bottles. Water samples were shipped on ice and then refrigerated at 4°C before analyses. Sediment sampling is described below (this section).

Ceriodaphnia dubia *7-d survival and reproduction tests*

Ceriodaphnia dubia 7-d tests were used to assess toxicity of water samples from all stations using U.S. Environmental Protection Agency (U.S. EPA) procedures [3]. Each undiluted grab sample was tested using 10 replicates, each containing one *C. dubia* neonate (<24-h-old). Tests were initiated within 48 h of sample collection. Daphnid survival and neonate production was monitored daily in each replicate of each sample.

Phase I TIEs with *C. dubia* were conducted on stations SMA and ORC water samples collected on May 28, 2003. The TIE procedures followed those developed by Mount and Anderson-Carnahan [4] and Durhan et al. [5]. The following is a brief description of the procedures. Baseline toxicity was confirmed on unmanipulated samples using five concentrations

chosen to bracket the effect concentration of the initial (original) sample. Centrifugation was used to determine whether toxicants were associated with particles. Samples were subjected to 2,500 rpm at 4°C in a refrigerated centrifuge for 30 min, decanted, and then tested for toxicity. The centrifugation step also was used as a pretreatment step for the solid-phase extraction column treatments discussed below. An aeration step consisted of vigorous bubbling for 30 min. Aeration was used to determine if toxicity was due to volatile compounds or surfactants. Ethylenediaminetetraacidic acid was added to the sample to investigate toxicity due to divalent metals. Samples were subjected to two pH shifts, pH 3 and pH 11, through addition of hydrochloric acid (1 normal) or sodium hydroxide, respectively. Sample pH was shifted and held for 3 h and then returned to the initial pH (8.0). Changes in pH can affect solubility, polarity, volatility, stability, and speciation of a compound, thereby affecting its bioavailability and toxicity. Shifting of pH is designed to determine how much sample toxicity can be attributed to volatile, sublateable, or oxidizable compounds. Samples were subjected to solid-phase extraction using a bond elute 500 mg C18 column (Varian, Lake Forest, CA, USA). The C18 column removes nonpolar organic compounds from the sample. In the manipulation, reverse-phase liquid chromatography was applied to extract nonionic organic toxicants from the aqueous sample. The column then was eluted with 100% methanol, and the resulting eluate was tested to determine if substances removed by the column were returned to control water at toxic concentrations. Piperonyl butoxide (PBO) was added to assess toxicity due to pesticides. Piperonyl butoxide is a metabolic inhibitor that prevents in vivo transformation of pesticides such as diazinon or chlorpyrifos into their toxic forms. If toxicity declines when PBO is added, it is presumed that toxicity was due to metabolically activated pesticides. An increase in toxicity caused by the addition of PBO is evidence that toxicity is due to a nonmetabolically activated compound such as a pyrethroid pesticide. Toxic units (TUs) were calculated for each treatment and were used for comparison among treatments to determine which were most effective at reducing toxicity (1 TU = 100/sample median lethal concentration [LC50]). Baseline toxicity was compared to toxicity of sample subjected to the following TIE manipulations. Treatment blanks for all of the TIE treatments listed above were used to verify tolerance of *Ceriodaphnia* to the TIE manipulations. Blanks consisted of control water subjected to the same manipulations as the samples. Exposures with *Ceriodaphnia* were conducted in 50-ml beakers (3 replicates) containing 15 ml of sample. All exposures in the TIEs were conducted for 96 h, following U.S. EPA procedures [6].

Hyalella azteca *toxicity test*

Sediment toxicity was assessed at each station in June 2002 and May 2003, using the 10-d survival and growth toxicity test with *H. azteca*, a genus that occurs in the Santa Maria River watershed. Sediment was sampled with a polycarbonate core tube (7.5-cm diameter). The top 5 cm of sediment was collected and composited in a polycarbonate tub, and aliquots of the sediment were used for toxicity tests and chemical analyses. Eight replicate test containers, each containing ten 7- to 14-d-old amphipods, were used for the test [7]. Amphipods were exposed to 100 ml of sediment in 300-ml beakers, with each beaker containing 175 ml of overlying water. The test temperature was 23°C. The overlying water was renewed twice daily, and 1.0 ml of food (Yeast, Cerophyl, and Trout Chow; Aquatic Biosystems, Fort Collins, CO, USA) was added daily to each test container. The containers were not aerated. Surviving animals were dried at 60°C at the end of the test, and growth was measured as dry weight per individual amphipod relative to baseline organisms.

Hyalella azteca *TIE*

Chemicals responsible for sediment toxicity to *H. azteca* were characterized with phase I TIEs using samples from station SMA (sample collected and TIE conducted in June 2003), and station ORC (sample collected in October 2003, TIE conducted January 2004). A preliminary test compared survival of amphipods in solid-phase exposures to survival of amphipods exposed to pore water extracted via refrigerated centrifugation (2,500 *g* at 4°C). Side-by-side pore water and solid-phase sediment tests were conducted for 10 d. For the pore water test, individual amphipods were exposed to 15 ml of pore water in ten 20-ml glass scintillation vials. Pore water test solutions were renewed on day five. Because the results of these experiments demonstrated comparable toxicity in the solid-phase and pore water exposures on the SMA sample, this TIE was conducted using pore water.

For the pore water TIE, amphipods were exposed to the TIE treatments for 96 h. To facilitate interpretation of the results, selected TIE treatments on SMA sediment were conducted using pore water concentrations of 100, 50, and 10%. Pore water was diluted using laboratory well water. The TIE procedures followed abbreviated phase I described by Mount and Anderson-Carnahan [4] and Durhan et al. [5]. Because our previous results indicated the primary chemical of concern at this site was the metabolically activated organophosphate pesticide chlorpyrifos, the *Hyalella* TIE focused on treatments to mitigate toxicity due to this and other pesticides. In addition to assessing toxicity of baseline samples (100–10% concentrations), pore water was tested using all treatments and blanks discussed above for the *Ceriodaphnia* tests. Addition of a carboxyl-esterase enzyme was included to investigate toxicity due to pyrethroid pesticides [8]. Liquid enzyme was obtained from J. Miller (AquaScience, Davis, CA, USA). Buffered, secondary stock was added to give 0.1 units/ml or about 40× enzyme activity. Concentrations of chlorpyrifos and diazinon were measured in all treatment solutions of the 100% pore water samples using enzyme-linked immunosorbent assays (ELISAs, described below).

Because the ORC sample pore water was not toxic, the TIE of this sample was conducted using solid-phase procedures. The solid-phase treatments included addition of the carbonaceous resin, Ambersorb 563(Rohm and Haas, Philadelphia, PA, USA), which preferentially binds nonpolar organic compounds in sediments [9]. A 5% wet weight volume of Ambersorb 563 was mixed with ORC sediment on a rolling mill for 24 h and then distributed to the test containers. Test procedures followed the 10-d *Hyalella* method described above. To account for the 5% dilution of ORC sediment, a treatment blank was included that consisted of 5% wet weight addition of formulated sediment to the ORC sediment.

Benthic macroinvertebrate community characterization

The macroinvertebrate bioassessments were modeled after the California Department of Fish and Game Aquatic Bioassessment Laboratory procedures for wadeable streams with sand or mud bottoms ([10]; http://www.dfg.ca.gov/cabw/

csbp_2003.pdf), which were adapted from the U.S. EPA Rapid Bioassessment Protocol for use in streams and rivers [11].

Samples were collected in May 2003 by placing a D-shaped net (0.5-mm mesh) on the sandy river bottom or against the submerged vegetated bank substrate and then disturbing a 30- × 60-cm portion of substrate upstream of the net for 60 s. A composite of a bank, midriver (thalweg), and opposite bank sample was collected at each of three randomly selected locations per station. All samples were preserved in the field in 95% ethanol. Samples were transferred to 70% ethanol after being transported to the laboratory. Benthic macroinvertebrates were identified to species or genus using methods and quality assurance guidelines of the California Stream Bioassessment Protocol [10]. These data were used to calculate the following metrics for each sample: The ephemeroptera, plecoptera, trichoptera (EPT) index (= number of mayfly [ephemeroptera], stonefly [plecoptera], and caddisfly [trichoptera] genera), taxa richness (= number of species), abundance (= total number of organisms), number of *Hyalella* sp., and the number of chironomidae individuals [10,11]. Physical and habitat-quality data were collected at each sampling station. Instream cover, epifaunal substrate, embeddedness, channel flow, channel alteration, sediment deposition, and riffle frequency were quantified on a scale from one (poor) to 20 (optimal) at each sampling location during each survey. Bank vegetation, bank stability, and riparian zone cover also were quantified on a scale from one (poor) to 10 (optimal) at each sampling location during each survey [10].

Chemical analyses

Water samples from September 2002 and May 2003 were analyzed for organophosphate, organochlorine, and pyrethroid pesticides, polychlorinated biphenyls, and trace metals. Organochlorine compounds were measured using U.S. EPA method 8080 [12], using gas chromatograph/electron capture with detection limits ranging from 0.3 to 5 ng/L. Organophosphate compounds were measured using U.S. EPA method 8140/8141 [13] and a nitrogen-phosphorus–specific detector (detection limits ranging from 0.04–33 μg/L; detection limit chlorpyrifos = 0.05 μg/L; detection limit diazinon = 0.04 μg/L). The polychlorinated biphenyls were analyzed as arochlors using U.S. EPA method 8080–polychlorinated biphenyls [12] (detection limits 0.04–0.11 μg/L). Selected water samples also were analyzed for trace metals (As, Ag, Cd, Cr, Cu, Hg, Mg, Ni, Pb, Zn) by inductively coupled plasma mass spectroscopy using U.S. EPA method 200.7 [14] (detection limits 0.33–4.1 μg/L). Sediment samples from June 2002 and May 2003 also were analyzed for organophosphate and organochlorine pesticides using the procedures described above. Pyrethroid pesticide concentrations in sediment were analyzed in the June 2002 sediment samples. Selected sediment samples were analyzed for trace metals (As, Ag, Cd, Cr, Cu, Hg, Mg, Ni, Pb, Zn) by inductively coupled plasma mass spectroscopy using U.S. EPA method 6010A [15]. Standard quality-assurance procedures, including measurement of standard reference materials and quantification of surrogate recoveries and matrix spikes, were used in all analyses. All chemical analyses met prescribed quality-assurance guidelines.

ELISA tests

All water samples and selected sediment pore water samples were analyzed for chlorpyrifos and diazinon using the following ELISA procedures recommended by Sullivan and Goh [16]. The ELISA readings were compared to a five-point standard curve prepared using standards provided by the manufacturer. After the analysis of every group of field samples, accuracy of the ELISA method was determined by measuring external chlorpyrifos or diazinon standard. To account for matrix effects, this standard also was spiked into a river water sample collected upstream of the contaminant input. All standard measurements were within ±20% of nominal. Precision of the ELISA method was determined with duplicate measures of one sample per batch by calculating the coefficient of variation ([CV] = [variance/mean] × 100). The CVs always were less than 20. A combined bottle-blank/process-blank was included during one sampling period; this indicated no contamination. Samples were tested at full strength unless initial readings indicated that the chemical was at concentrations above the range of the test kits. In such cases, samples were diluted to known concentrations before definitive analysis. The lowest detectable dose was 30 ng/L for diazinon and 50 ng/L for chlorpyrifos.

Water quality

Dissolved oxygen (mg/L), specific conductance (μS/cm, where S = siemens), pH, temperature (°C), and turbidity (nephelometric turbidity unit [NTU]) were measured in situ using a Hach Sension 156 and Hach 2100 P portable turbidimeter (Loveland, CO, USA). These instruments were calibrated in the laboratory per manufacturer's recommendations. Alkalinity (total as $CaCO_3$) and hardness (calcium as $CaCO_3$) were measured in field-collected samples in the laboratory. Nitrate and phosphate concentrations were measured on a Hach 2010 spectrophotometer.

Statistical analyses

Statistical differences in survival and reproduction of daphnids and survival and growth of amphipods in toxicity tests were determined by comparing sample results to controls using separate variance *t* tests (at $\alpha = 0.05$). Statistically significant differences in macroinvertebrate abundances among stations were determined using analysis of variance followed by Tukey's multiple comparison test [17].

RESULTS

C. dubia *toxicity tests*

Water toxicity to *C. dubia* in the Santa Maria River watershed varied spatially and temporally (Table 1). Survival consistently was >90% in station SIV, and survival was 100% in all MSD samples. Only one sample from station SM1 was toxic (September 2002). Samples from station OR1 were toxic in July 2002, but not in March or May 2003. Survival in samples from all stations was greater than 90% in March 2003. In part, this may have been due to dilution from rainfall or reduction of irrigation drain water in the system. Heavy rain (3.63 cm) fell in the Santa Maria watershed in the 48 h preceding this sampling event. Survival in stations ORC and SMA was identical in this study. Survival was 100% at both stations in July 2002 and March 2003, and 0% survival was observed in samples from these stations in September 2002 and May 2003. The only toxic sample from station OFC was the one collected in May 2003 (Table 1). Major conventional water-chemistry variables were within tolerable ranges for *C. dubia*. Dissolved oxygen always was >7 mg/L; hardness ranged from 107 to 866 mg/L, and conductivity ranged from 544 to 2,960

Table 1. *Ceriodaphnia dubia* survival, enzyme-linked immunosorbent assay (ELISA) concentrations of chlopyrifos, combined toxic units (TUs) of chlorpyrifos and diazinon, and nitrate (NO_3) concentrations in water samples from the Santa Maria River watershed. All sample locations in Santa Maria (CA, USA); * indicates significantly different from the control at $p < 0.05$

Station name[a]	Sample date	*C. dubia* survival (SD)[b]	*C. dubia* reproduction (SD)	Chlorpyrifos ELISA (μg/L)	TUs	NO_3 mg/L
SIV	July 2002	100	31 (11)	ND[c]	0	0.4
MSD		100	36 (6)	ND	0	8.6
SM1		100	29 (4)	ND	0	69.2
OR1		0*	NA[d]	0.811	15.3	200.0
ORC		100	25 (4)	0.092	1.7	119.7
SMA		100	27 (6)	0.112	2.1	109.1
OFC		80	21 (8)	0.084	1.6	72.9
Control		90 (32)	23 (6)			
SIV	September 2002	90	26 (3)	0.063	1.2	0.6
MSD		100	25 (6)	0.08	1.6	4.6
SM1		0*	NA	0.083	1.7	167.0
OR1		100	17* (7)	0.09	2.4	288.0
ORC		0*	NA	0.344	6.6	108.2
SMA		0*	NA	0.394	7.6	82.8
OFC		100	18* (7)	0.071	1.5	144.0
Control		100	28 (3)			
SIV	March 2003	90	27 (6)	ND	0.2	6.0
MSD		100	30 (5)	0.119	2.7	14.4
SM1		90	20 (4)	0.048	0.9	46.2
OR1		100	29 (3)	0.159	3.1	112.1
ORC		100	27 (3)	0.149	2.8	74.8
SMA		100	25 (5)	0.15	2.8	79.2
OFC		100	22 (3)	0.052	1.3	184.6
Control		100	24 (5)			
SIV	May 2003	100	15* (7)	ND	0	5.2
MSD		100	7* (2)	0.088	1.7	78.8
SM1	100	14* (6)	0.07	1.3	83.3	
OR1	100	15* (5)	0.133	2.5	208.8	
ORC	0*	NA	0.489	9.2	118.5	
SMA	0*	NA	0.514	9.7	116.2	
OFC	30* (48)	11* (9)	0.087	1.6	149.0	
Control	100	26 (2)				

[a] SIV = Sisquoc River; MSD = Main St. Ditch; SM1 = Santa Maria River at Hwy 1; OR1 = Orcutt Creek at Hwy 1; ORC = Orcutt Creek at River; SMA = lower Santa Maria River; OFC = Oso Flaco Creek.
[b] SD = standard deviation.
[c] ND = not detected.
[d] NA = not applicable.

μS/cm, except that one water sample collected from station OR1 in September 2002 had a conductivity of 3,350 μS/cm.

None of the measured metals exceeded published toxicity thresholds for *C. dubia*. Concentrations of cadmium were <1 μg/L (LC50 = 120 μg/L [18]). Copper concentrations were <9 μg/L (LC50 = 200 μg/L [18]), and zinc concentrations were <25 μg/L (LC50 = 95 μg/L [18]). Several organophosphate and organochlorine pesticides were detected in these samples. When detected, concentrations of dieldrin, dichlorodiphenyltrichloroethane (DDT), endosulfan, and endrin were less than 0.1 μg/L, and parathion and malathion in water always were <1.0 μg/L (analyses conducted on September 2002 and May 2003 water samples; not shown).

Only chlorpyrifos was above the acute toxicity threshold for *C. dubia*, and toxicity to *C. dubia* was observed in samples with the highest concentrations of chlorpyrifos. Combined TUs of chlorpyrifos and diazinon were calculated for all water samples (one combined TU of diazinon and chlorpyrifos = sample concentration diazinon/*C. dubia* LC50 diazinon + sample concentration chlorpyrifos/*C. dubia* LC50 chlorpyrifos; chlorpyrifos and diazinon 96-h LC50 = 0.053 and 0.32 μg/L, respectively [19]). In all but one case (OR1, Sept. 2002, [diazinon] = 0.232 μg/L), the combined TUs were dominated by concentrations of chlorpyrifos. The *C. dubia* mortality in these samples significantly was correlated with combined TUs (Spearman-Rank correlation coefficient [rho] = 0.415; significant at $p < 0.05$).

In some cases, *C. dubia* reproduction was inhibited in samples where no impacts on survival were observed. For example, although survival was 100%, reproduction was reduced significantly in OR1 and OFC in September 2002. Relative to control reproduction, all of the samples significantly reduced reproduction in May 2003.

C. dubia *TIEs*

Phase I TIEs were conducted on the Orcutt Creek (ORC) and Santa Maria River (SMA) water samples collected in May 2003, and results of both TIEs were similar. The *C. dubia* survival increased from 0% in the ORC baseline sample to 87% after treatment with C18 solid-phase extraction (Table 2). The increase in survival coincided with a reduction in chlorpyrifos concentration from 7.6 TUs to nondetectable after the C18 treatment (Table 2). Methanol elution recovered only a fraction of the chlorpyrifos from the C18 column, and no toxicity was observed in the eluate (Table 2). Centrifugation also reduced toxicity in the 50 and 100% samples, and this coin-

Table 2. Results of phase 1 toxicity identification evaluations (TIEs) conducted on water samples from Orcutt Creek (ORC) and the Santa Maria River (SMA), in Santa Maria (CA, USA)

		Mean % survival with each % sample				Chloropyrifos	
	Toxic units	0%	5%	50%	100%	Concn. (μg/L)	Toxic units
ORC Treatments							
Baseline	2.6	100	100	47	0	0.410	7.6
EDTA[a]	2.6	87	100	45	0	05.55	10.3
STS[b]	3.5	93	100	20	0	0.598	11.1
Aeration	1.7	100	100	87	0	0.560	10.4
Centrifuge	<1	100	100	100	87	0.213	3.9
pH 3 shift	4.1	87	100	7	0	0.425	7.9
pH 11 shift	4.5	100	100	0	0	0.546	10.1
C18 Column	<1	100	94	100	87	ND[c]	ND
C18 eluate	<1	100	100	100	100	0.148	2.7
PBO[d]	<1	100	100	100	87	0.635	11.8
SMA treatments							
Baseline	4.5	93	93	0	0	0.422	7.8
EDTA	5.7	87	67	0	0	0.420	7.8
STS	4.5	100	100	0	0	0.484	9.0
Aeration	4.8	100	87	0	0	0.431	8.0
Centrifuge	1.3	100	100	93	20	0.349	6.5
pH 3 shift	4.5	94	100	0	0	0.524	9.7
pH 11 shift	4.5	93	93	0	0	0.683	12.6
C18 Column	<1	100	100	92	93	0.076	1.4
C18 eluate	<1	93	100	100	93	0.204	3.8
PBO	<1	93	100	93	80	0.697	12.9

[a] EDTA = ethylenediaminetetraacetic acid.
[b] STS = sodium thiosulfate.
[c] ND = not detected.
[d] PBO = piperonyl butoxide.

cided with a reduction in chlorpyrifos. Addition of the metabolic inhibitor PBO increased survival to 87% in the 100% sample, indicating that toxicity was caused by a metabolically activated compound. Similar results were observed with the SMA sample. The *C. dubia* survival increased from 0% in SMA baseline samples to 93% after treatment with C18 solid-phase extraction (Table 2). The increase in survival coincided with a reduction in chlorpyrifos concentration from 7.8 to 1.4 TUs after the C18 treatment (Table 2). Methanol elution of chlorpyrifos from the C18 column again was incomplete, and no toxicity was observed in the eluate (Table 2). Addition of PBO to the SMA sample also increased survival to 93%, even though the concentration of chlorpyrifos in this treatment was more than sufficient to cause *Ceriodaphnia* mortality. Combined with information on pesticide concentrations, the TIE results for both samples suggest mortality was caused by chlorpyrifos.

Nutrients

As has been documented in agricultural areas throughout California, we detected elevated nutrient concentrations at all stations in the lower Santa Maria watershed. Nitrates in particular exceeded the California Regional Water Quality Control Board's Basin Plan objective (45 mg/L as nitrate [20]; http://www.waterboards.ca.gov/centralcoast/basinplan/index/html) in the majority of samples, except those collected at stations SIV and MSD (Table 1). The highest concentrations of nitrates were measured in stations OR1 and ORC, station SMA, and station OFC, with nitrates ranging between 72.9 and 288 mg/L at these four stations.

H. azteca *toxicity in watershed sediments*

Sediments from many of the stations were toxic to *H. azteca* (Table 3). In June 2002, there was no inhibition of amphipod growth or survival in sediment from station SIV, and there was no inhibition of survival in samples from SM1 or OR1. The remaining samples significantly inhibited amphipod survival, especially the sample from station MSD and the samples from stations ORC and SMA, which were the stations located lowest in the watershed. Significant reductions in amphipod growth were measured in all samples except SIV and ORC (Table 3). In May 2003, all amphipods died in samples from SMA and ORC, and slight reductions in survival were observed in samples from MSD and SM1. Significant reductions in amphipod growth were measured in samples from MSD, SM1, and OR1 in May 2003.

Major water-quality properties of the sediment overlying water were suitable for this species: Unionized ammonia ranged between 0.006 and 0.16 mg/L, hardness ranged from 84 to 139 mg/L, conductivity ranged from 736 to 989 μS/cm, and alkalinity ranged from 114 to 160 mg/L.

In June 2002, concentrations of chlorpyrifos in sediment pore water from stations ORC (0.231 μg/L) and SMA (0.127μg/L) were above the 10-d LC50 for *H. azteca* (chlorpyrifos 10-d LC50 = 0.086 μg/L; [21]; Table 3). The chlorpyrifos concentration in pore water from MSD sediment was 5.81 μg/L. This represents approximately 67 TUs chlorpyrifos. Diazinon was detected only in pore water from sediment collected from station MSD and the concentration (0.069 μg/L) was considerably lower than the 10-d LC50 for this pesticide (diazinon 10-d LC50 = 6.51 μg/L; [21]; Table 3). Pore water concentrations of chlorpyrifos also exceeded the LC50 in sam-

Table 3. Results of June 2002 and May 2003 *Hyalella azteca* (HA) 10-d sediment toxicity tests and chemical analyses of sediment pore water (PW) and solid-phase sediment. Values with an asterisk (*) are significantly different from the control value at $p < 0.05$. Values with double asterisk (**) exceed 10-d median lethal concentration (LC50) for chlorpyrifos toxicity to *H. azteca* (0.086 μg/L). Values with triple asterisk (***) are greater than the LC50 for L-cyhalothrin (LC50 = 5.2–6.4 μg/kg dry wt; Amweg et al. [23])

Station[a]	HA survival (%)	HA growth (mg dry wt)	PW chlorpyrifos μg/L	Permethrin μg/kg dry wt			Sediment constituents (% composition)		
				L-cyhalothrin		Esfenvalerate	Fines	Silt + Clay	TOC[b]
June 2002									
SIV	98 (7)	0.327	ND[c]	NA[d]	NA	NA	NA	NA	0.59
MSD	0*	—	5.81**	59.4***	107.0	32.6	68.5	31.3	ND
SM1	83 (34)	0.183*	ND	ND	ND	ND	61.9	36.6	1.90
OR1	93 (7)	0.213*	ND	ND	ND	ND	26.6	72.7	1.10
ORC	6 (5)*	0.363	0.231**	43.1***	23.1	ND	29.7	69.8	1.30
SMA	6 (12)*	0.060*	0.127**	18.5***	ND	ND	27.4	72.2	2.10
OFC	71 (23)*	0.129*	ND	ND	ND	ND	31.9	67.6	1.10
Control	93 (12)	0.307							
May 2003									
SIV	98 (5)	0.371	ND	NA	NA	NA	NA	NA	NA
MSD	78 (10)*	0.208*	0.043	NA	NA	NA	NA	NA	NA
SM1	74 (37)*	0.241*	ND	NA	NA	NA	NA	NA	NA
OR1	88 (18)	0.215*	ND	NA	NA	NA	NA	NA	NA
ORC	0*	—	0.124**	NA	NA	NA	27.4	71.0	0.95
SMA	0*	—	0.281**	NA	NA	NA	28.0	68.6	1.10
OFC	NA	NA	ND	NA	NA	NA	NA	NA	NA
Control	100	0.324							

[a] See Table 1 for definitions of station abbreviations.
[b] TOC = total organic carbon.
[c] ND = not detected.
[d] NA = not analyzed.

ples from ORC and SMA in May 2003, but no diazinon was detected in these samples. Because *H. azteca* primarily is an epibenthic species, measures of pore water concentrations of these pesticides probably overestimated the concentrations to which this species is exposed. Pesticide concentrations were not measured in the water overlying the sediment in these experiments.

Other pesticides and trace metals were detected in Santa Maria watershed sediment samples. Total DDT concentrations at ORC and SMA were 116.7 and 116.4 μg/kg dry weight, respectively, in May 2003. These concentrations exceeded the threshold effect concentration (5.28 μg/kg dry wt), but were considerably lower than the probable effects concentration (572 μg/kg; [22]). All organochlorine pesticides measured in sediments from all these stations were less than 10.0 μg/kg in June 2002, except at station MSD, when total DDT was 32 μg/kg dry weight. Concentrations of the pyrethroid pesticides lambda-cyhalothrin and esfenvalerate in the MSD sediment were 59.4 and 32.6 μg/kg dry weight, respectively. These concentrations exceeded the respective 10-d LC50s for these pyrethroids reported by Amweg et al. [23] (Table 3). The concentrations of lambda-cyhalothrin in the ORC and SMA sediments were 43.1 and 18.5 μg/kg dry weight, respectively. These concentrations also exceeded the 10-d LC50 Amweg et al. [23] reported for this pyrethroid (Table 3). Of the trace metals, only nickel in sediment from OR1 (34 mg/kg dry wt) exceeded the threshold effect concentration (22.7 mg/kg dry wt; probable effects concentration = 48.6 mg/kg dry wt, [22]).

Hyalella azteca *sediment TIE*

Amphipod survival was 0% in the 100% pore water sample from station SMA and 20% in the 50% pore water concentration after 96 h (Table 4). Amphipod survival was equal to or greater than 80% in all treatment blanks, except for the pH 11 shift (survival = 55%). The concentration of chlorpyrifos in the 100% pore water sample was 0.589 μg/L (6.8 TUs). The C18 column treatment increased survival from 0 to 87% in 100% pore water and reduced chlorpyrifos TUs from 6.8 to 0.7 (Table 4). This suggests that toxicity partly was due to chlorpyrifos. No toxicity was observed in C18 column eluate, but this may have been due to insufficient recovery. The addition of PBO did not mitigate toxicity of either the 100 or 50% pore water samples (survival in the PBO blank was 85%). A slight increase in mortality with PBO addition to the 10 and 50% pore water samples suggests some potentiation of toxicity, and this potentiation also was evident at 48 h (data not shown). Because PBO inhibits a key metabolic pathway, previous studies have suggested that increased toxicity with the addition of PBO indicates toxicity due to pyrethroid pesticides [24]. As discussed above, the solid-phase concentration of the pyrethroid pesticide lambda-cyhalothrin in this sample exceeded the 10-d LC50 for *H. azteca* [23]. Addition of the carboxylesterase enzyme, however, did not reduce toxicity of the pore water, as would be expected if toxicity solely were due to the actions of a pyrethroid pesticide. Survival also was lower at the 15°C exposure temperature, relative to 23°C, especially in the 10% pore water concentration. Increased toxicity at lower temperatures is a characteristic of type I pyrethroid pesticides [25]. The TIE results suggest sediment toxicity due to a combination of chemicals, including the organophosphate pesticide chlorpyrifos and, possibly, a pyrethroid pesticide.

Because toxicity was not observed in pore water extracted from ORC sediment collected in October 2003, the TIE of this sample consisted of the addition of Ambersorb 563 to reduce bioavailability of organic chemicals. Survival of *H. azteca* was 28% in the baseline ORC sediment (Fig. 2). Survival was 84%

Table 4. Results of 96-h phase 1 toxicity identification evaluation (TIF) using sediment pore water from lower Santa Maria River (Santa Maria, CA, USA). See text for descriptions of TIE treatments

Treatments[a]	LC50[b]	Toxic units	% Pore water				Chlorpyrifos concn. (μg/L)	Chlorpyrifos toxic units LC50 = 0.086
			0% Mean survival	10% Mean survival	50% Mean survival	100% Mean survival		
Baseline 23°C	28.9	3.5	85	95	20	0	0.589	6.8
Baseline 15°C			85	60	20	0		
Aeration	27.1	3.7	90	93	13	7		
pH 3	5.0	20.0	80	0	0	0		
pH 11			55	0	69	0		
C18 column	>100	<1	80	87	87	87	0.059	0.7
C18 eluate	>100	<1	80	80	93	93	0.061	0.7
Enzyme	30.1	3.3	87	100	27	0	0.621	7.2
PBO[c]	23.9	4.2	85	87	7	0	0.660	7.7

[a] All treatments except baseline 15°C were conducted at 23°C.
[b] LC50 = median lethal concentration.
[c] PBO = Piperonyl butoxide.

with the addition of Ambersorb. To account for possible dilution of toxicity with the addition of 5% volume of Ambersorb, a 5% volume of formulated sediment was added to the ORC sample. Survival of *Hyalella* was 30% in this treatment (ORC + formulated; Fig. 2), indicating negligible dilution due to the Ambersorb. No toxicity was observed in the Ambersorb treatment blank (5% Ambersorb + formulated; Fig. 2). The ORC sediment toxicity reduction with the addition of Ambersorb implicates a nonpolar organic compound. Concentrations of chlorpyrifos and diazinon measured in ORC pore water when the sample was collected in October 2003 were 0.59 and 0.60 μg/L, respectively. The chlorpyrifos concentration was not measured in pore water as part of the solid-phase TIE, so it is not possible to determine whether there was a decline of this pesticide in the sample in the four-month holding period preceding the solid-phase TIE. The previous analyses of ORC sediments discussed above showed that concentrations of chlorpyrifos and lambda cyhalothrin exceeded the 10-d LC50 for *Hyalella*.

Macroinvertebrate community structure

Macroinvertebrate community structure in stations ORC and SMA was quite different from that in the river upstream at station SM1. Generally low taxa richness was at all three stations, and the EPT index was 0 in all samples because no mayflies (Ephemeroptera), stoneflies (Plecoptera), or caddisflies (Tricoptera) were observed (Table 5). Macroinvertebrate abundances were lower at ORC and SMA relative to station SM1 (analysis of variance $p = 0.008$; Table 5). The numbers of amphipods (*Hyalella*) in ORC (198) and SMA (41) were considerably lower than those at station SM1 (464; Table 5). Similarly, there were lower numbers of chironomids at stations ORC and SMA relative to station SM1.

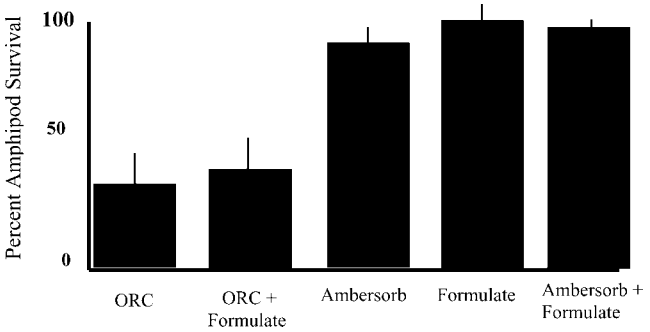

Fig. 2. Results of solid-phase toxicity identification evaluation (TIE) of Orcutt Creek (Santa Maria, CA, USA) sediment. ORC = Orcutt Creek; error bars are 1 standard deviation.

The composite habitat scores were similar at all three stations, and there was little difference in bank cover scores (data not shown). Mean water conductivities were similar: 1,882, 2,560, and 2,420 (μS/cm) at stations SM1, SMA, and ORC, respectively. Total suspended solids were lower at SM1 than at SMA or ORC with values of 8.4, 3,290, and 2,190 (mg/L) and turbidities of 1.64, >1,000, and >1,000 (NTUs) at stations SM1, SMA, and ORC, respectively.

DISCUSSION

This study demonstrates that the lower Santa Maria River and some of its tributaries are impacted by pesticides and nutrients associated with agricultural drain water. Intermittent water toxicity to *C. dubia* was observed and, in most cases, high mortality of daphnids occurred in samples with the highest concentrations of chlorpyrifos (Table 1). The TIE evidence suggests toxicity of water samples from Orcutt Creek (ORC) and the lower Santa Maria River (SMA) was due to chlorpyrifos. Toxicity was removed in both cases when samples were subjected to solid-phase extraction and when the metabolic inhibitor PBO was added to test solutions. These results suggest toxicity due to a metabolically activated nonpolar organic compound (Table 2). Chlorpyrifos concentrations were sufficient to account for toxicity in both the ORC and SMA

Table 5. Macroinvertebrate bioassessment results at three stations in the lower Santa Maria River watershed. See text for description of methods. An asterisk indicates significantly different from SM1[a] at $p = 0.008$

	Station[a]		
	SM1 (Upstream)	ORC (Tributary)	SMA (Downstream)
Taxa richness	18	18	23
EPT[b] index	0	0	0
Abundance	3,773	498*	214*
Number *Hyalella*	464	198	41
Number *Chironomini*	9	3	1

[a] See Table 1 for definitions of station abbreviations. All stations are in Santa Maria (CA, USA).
[b] EPT = Ephemeroptera, Plecoptera, Trichoptera.

water samples subjected to TIEs. The lack of methanol eluate toxicity in the TIEs apparently was due to poor recovery of chlorpyrifos from C18 resin. Despite this, the weight of evidence in our data suggests toxicity of water samples in the Santa Maria watershed was due to chlorpyrifos.

Results of toxicity tests and TIEs with *H. azteca* indicate that chemicals associated with agriculture drain water in the Santa Maria watershed impact benthic macroinvertebrates in this system. The concentration of chlorpyrifos in pore water extracted from station MSD sediment collected in June 2002 was 5.81 μg/L. This is equal to 67.5 TUs of chlorpyrifos, based on a 10-d LC50 of 0.086 μg/L for *H. azteca* [21]. Consistent sediment toxicity also was measured at two of the same stations where water column toxicity was observed, ORC and SMA, and concentrations of chlorpyrifos exceeded the 10-d LC50 for *H. azteca* in the ORC and SMA sediment pore water samples (Table 3). Results of the sediment pore water TIE, plus the fact that chlorpyrifos concentrations in pore water exceeded the 10-d LC50 value for this species, indicate that chlorpyrifos contributed to amphipod mortality in the SMA sediment.

The TIE results suggest that chlorpyrifos was not the only likely source of toxicity in SMA pore water. Increased toxicity of the 10 and 50% pore water sample with the addition of PBO is consistent with the hypothesis that a pyrethroid pesticide also was present in this sample (Table 4). If chlorpyrifos was the only chemical responsible for toxicity, the PBO treatment should have reduced *H. azteca* mortality by inhibiting cytochrome P450 metabolism of chlorpyrifos to its toxic form. However, by inhibiting cytochrome P450, PBO also acts as a synergist in the presence of pyrethroids [24]. Concentration of the pyrethoid lambda-cyhalothrin exceeded the LC50 for *Hyalella* in the solid-phase SMA sample collected in June 2002 (Table 3). We also observed lower survival when the test temperature was lowered from 23 to 15°C (Table 4). Increased toxicity at lower temperature is a characteristic of type I pyrethroid pesticides (e.g., permethrin [25]). Amphipods are among the most-sensitive taxa to synthetic pyrethroids [26]. We observed minimal reduction of toxicity with the addition of the carboxylesterase enzyme to this sample, which might be due to the presence of both pyrethroid and organophosphate pesticides in this sample. Future TIEs of sediments containing mixtures of pesticides will emphasize treatments to reduce toxicity of compounds with dissimilar modes of action and will include combinations of the esterase enzyme with PBO additions to mitigate simultaneously toxicity of mixtures that include organophosphate and pyrethroid pesticides.

Increased amphipod survival in the ORC sediment with the addition of Ambersorb 563 also suggests toxicity was due to an organic chemical, and chemical analyses of samples from this station have shown mixtures of organophosphate, pyrethroid, and organochlorine pesticides.

Declines in macroinvertebrate densities in the lower sections of the Santa Maria River watershed are consistent with our observations of high pesticide concentrations and water and sediment toxicity at these same stations. No EPT taxa were observed in these samples, and there were low species diversities at all stations sampled. This implies that conditions at all three stations were inadequate to support rich macroinvertebrate communities. Despite this, we observed lower numbers of macroinvertebrates in Orcutt Creek and the lower Santa Maria River relative to the upper river station (SM1). Lower numbers of amphipods *Hyalella* also were at ORC and SMA, relative to station SM1. Combined with chemistry, water and sediment toxicity tests, and TIE results showing greatest toxicity to *Ceriodaphnia* and *Hyalella* in samples from lower watershed stations having the highest pesticide contamination, these results suggest impacts on benthic macroinvertebrates at ORC and SMA partly are due to pesticides associated with agriculture drain water. Because *Hyalella* is a resident genus in this system, laboratory tests with this amphipod provide a powerful investigative tool to determine causes of macroinvertebrate impacts, particularly when amphipod declines are observed in bioassessments from the same stations.

Macroinvertebrate densities also might respond to other stressors such as habitat degradation and extremes in temperature, flow, and conventional water quality parameters such as low dissolved oxygen or high conductivities, or suspended solids. We did not observe any obvious differences in habitat between the upper river SM1 station and the lower river stations (ORC and SMA), and the habitat scores were similar between these stations. We measured much lower concentrations of both suspended solids and turbidities at station SM1, relative to SMA and ORC. In previous studies, we have found a number of macroinvertebrate metrics to be correlated negatively with turbidity [27]. Recent dose–response experiments at our laboratory with the baetid mayfly *Procloeon* sp., the amphipod *H. azteca*, and the midge *Chironomous tentans* have shown that all three of these species are insensitive to suspended particles (measured as turbidity) at nominal concentrations as high as 1,000 NTUs [28], indicating it is unlikely suspended particles alone influenced densities of amphipods, chironomids, or mayflies in the Santa Maria River.

Our current results are similar to those documented on the Salinas River (Monterey County, CA, USA), another coastal river influenced by agriculture drain water [27,29,30]. In sections of the Salinas River, declines in macroinvertebrate densities were observed at stations downstream of agricultural drains where the greatest pesticide contamination and toxicity were observed. As in the Salinas River, multiple lines of evidence suggest that organophosphate pesticides played a role in ecological impacts in the Santa Maria River. These results corroborate other research conducted throughout California, which show that aquatic toxicity due organophosphate pesticides is prevalent in watersheds dominated by agriculture [27,29,31,32]. Increasing evidence supports that pyrethroid pesticides also may be contributing to increased toxicity and associated ecological impacts in watersheds influenced by agriculture [23,28,33,34]. The Santa Maria River divides two counties, San Luis Obispo County to the north, and Santa Barbara County to the south. Pesticide use data for San Luis Obsipo County showed that, after malathion, chlorpyrifos (6,922 gross kg applied) and diazinon (4,008 gross kg applied) were second and third most commonly used organophosphorous pesticides in this county in 2003. Permethrin (3,921 gross kg applied) was the most commonly used pyrethroid pesticide in San Luis Obispo County in 2003. Pesticide use data for Santa Barbara County showed that, after malathion, chlorpyrifos (12,750 gross kg applied) was the second most commonly used organophosphorous pesticide in this county in 2003. Permethrin (1,965 gross kg applied) was the most commonly used pyrethroid pesticide in Santa Barbara County 2003 (http://www.cdpr.ca.gov/docs/pur/purmain/htm).

Based on the weight-of-evidence, pesticide pollution likely is causing ecological damage in the lower Santa Maria River watershed. Previous studies have shown that pesticide pollu-

tion extends into the Santa Maria estuary. Results of a statewide monitoring program investigating toxicity associated with sediment contamination in California's bays and estuaries found that total DDT in Santa Maria River estuary sediments was among the highest measured statewide, and these sediments were highly toxic to the amphipod *Eohaustorius estuarius* [2]. A more recent survey of organochlorine pesticide contamination in sand crab tissues (*Emerita analoga*) found total DDT concentrations as high as 556 ng/g dry weight in crabs collected on the beach adjacent to the mouth of the estuary. Total sand crab tissue DDT concentrations at this site were the highest measured on the central coast of California ([35]; http:www.swrcb2.swrcb.ca.gov/swamp/docs/sandcrab.pdf). Given the proximity of our current study stations to the estuary and the likelihood that high concentrations of current-use pesticides also are reaching this ecologically important habitat, we recommend that monitoring of pesticides and their effects in water and sediment be extended to the estuary. Awareness is growing of the importance of monitoring nonpoint pollution runoff from agriculture in the central California coast, and agriculture-monitoring programs now are being implemented in this coastal area. These are comprehensive monitoring programs developed through the cooperation of local agriculture interests and under the jurisdiction of the respective California Regional Water Quality Control Boards. It is anticipated that, as this work proceeds, on-farm practices designed to reduce pesticide and nutrient runoff will be implemented.

Acknowledgement—We are grateful for all those who helped complete this study and we thank the following people: A. Gerke, P. Nicely, D. Crane, A. Mekebri, and K. Goding. This study was funded by the California State Water Resources Control Board. Additional funding for chemical analyses was provided by the Central Coast Regional Water Quality Control Board.

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