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Environmental Toxicology and Chemistry, Vol. 22, No. 10, pp. 2375–2384, 2003

Printed in the USA
0730-7268/03 $12.00 + .00

ECOTOXICOLOGIC IMPACTS OF AGRICULTURAL DRAIN WATER IN THE SALINAS RIVER, CALIFORNIA, USA

BRIAN S. ANDERSON,*† JOHN W. HUNT,† BRYN M. PHILLIPS,† PATRICIA A. NICELY,† KRISTINE D. GILBERT,† VICTOR DE VLAMING,‡ VALERIE CONNOR,§ NANCY RICHARD,§ and RONALD S. TJEERDEMA†
†Department of Environmental Toxicology, University of California, Davis, California 95616, USA
‡Aquatic Toxicology Laboratory, VM:APC, 1 Shields Avenue, University of California, Davis, California 95616, USA
§Division of Water Quality, State Water Resources Control Board, 1001 I Street, Sacramento, California 95814, USA

(*Received* 11 *September* 2002; *Accepted* 6 *February* 2003)

Abstract—The Salinas River is the largest of the three rivers that drain into the Monterey Bay National Marine Sanctuary in central California (USA). Large areas of this watershed are cultivated year-round in row crops, and previous laboratory studies have demonstrated that acute toxicity of agricultural drain water to *Ceriodaphnia dubia* is caused by the organophosphate (OP) pesticides chlorpyrifos and diazinon. We investigated chemical contamination and toxicity in waters and sediments in the river downstream of an agricultural drain water input. Ecological impacts of drain water were investigated by using bioassessments of macroinvertebrate community structure. Toxicity identification evaluations were used to characterize chemicals responsible for toxicity. Salinas River water downstream of the agricultural drain was acutely toxic to the cladoceran *Ceriodaphnia dubia*, and toxicity to *C. dubia* was highly correlated with combined toxic units (TUs) of chlorpyrifos and diazinon. Laboratory tests were used to demonstrate that sediments in this system were acutely toxic to the amphipod *Hyalella azteca*, a resident invertebrate. Toxicity identification evaluations (TIEs) conducted on sediment pore water suggested that toxicity to amphipods was due in part to OP pesticides; concentrations of chlorpyrifos in pore water sometimes exceeded the 10-d mean lethal concentration (LC50) for *H. azteca*. Potentiation of toxicity with addition of the metabolic inhibitor piperonyl butoxide suggested that sediment toxicity also was due to other non–metabolically activated compounds. Macroinvertebrate community structure was highly impacted downstream of the agricultural drain input, and a number of macroinvertebrate community metrics were negatively correlated with combined TUs of chlorpyrifos and diazinon, as well as turbidity associated with the drain water. Some macroinvertebrate metrics were also correlated with bank vegetation cover. This study suggests that pesticide pollution is the likely cause of ecological damage in the Salinas River, and this factor may interact with other stressors associated with agricultural drain water to impact the macroinvertebrate community in the system.

Keywords—Pesticides Toxicity Macroinvertebrates Toxicity identification evaluations

INTRODUCTION

With a watershed area of more than 10,000 km^2, the Salinas River is the largest of the three coastal rivers flowing into the Monterey Bay National Marine Sanctuary in central California (USA). This river provides significant freshwater habitat in this semiarid region, and the lower river is a primary migration corridor for endangered steelhead trout (*Onchorhynchus mykiss*) [1]. Large areas in this watershed are cultivated year-round, primarily in row crops such as lettuce, strawberries, artichokes, and crucifer crops. Agriculture in the Salinas Valley accounts for 80% of the lettuce and a significant portion of the fresh vegetables produced in the United States. Current and past agricultural practices have included intensive use of pesticides, and previous studies have found pesticide residues in agricultural soils and in furrow runoff [2]. The California State Mussel Watch Program routinely has detected elevated bivalve tissue concentrations of pesticides in river and tributary samples, including chlorpyrifos and organochlorine pesticides [3,4]. Recent studies have shown that ambient water samples from the river and specific tributaries are toxic to various species in laboratory tests [5]. This study focused on drain water inputs from the most consistently toxic agricultural creek identified by Hunt et al. [5]. This creek originates as an ephemeral stream in the Gabilan Mountains on the eastern border of the Salinas Valley. Although the creek carries some natural water flow during the wettest winter months, headwater flow is underground above the study area. Flow in the lower portion of the creek is dominated by agricultural drain water.

In the current study, we investigated the impacts of this drain water in the Salinas River over an 18-month period. Salinas River water and sediment toxicity were characterized by 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 selected pesticide measures in both water and sediment matrices. Ecological impacts were assessed by characterizing macroinvertebrate community structure upstream and downstream of the drain-water input. Possible causes of toxicity and impacts on macroinvertebrate community structure were investigated by using a combination of toxicity identification evaluations (TIEs) and chemical analyses, dose–response information from the literature, and habitat and physical factor assessments. The results were combined in a weight-of-evidence evaluation of the impacts of agricultural drain water on the river ecosystem, and were used to investigate chemicals responsible for toxicity and ecological degradation.

MATERIALS AND METHODS

Sampling sites

The study was conducted at the confluence of an agricultural drain, approximately 50 river kilometers southeast of the

* To whom correspondence may be addressed (anderson@ucdavis.edu).

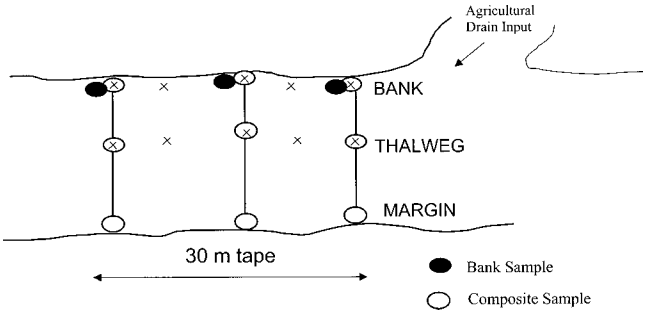

Fig. 1. Schematic diagram of Salinas River (CA, USA) sampling design. Solid circles indicate location of bank macroinvertebrate samples, open circles indicate locations of composite macroinvertebrate samples, × indicates locations of water and sediment samples for toxicity tests.

point where the river enters the Monterey Bay. A reference station was located approximately 30 m upstream of the drain confluence (station 1), and 3 additional stations were located approximately 1 (station 2), 100 (station 3), and 200 m (station 4) downstream of the input. The drain was sampled at the confluence with the river. Surveys were conducted in April, May, and September 2000, and May 2001 to account for temporal variability.

Each station comprised a 30-m reach of river bank marked with a transect tape along the bank. During each of the four sample periods, two randomly selected sets of replicate water samples were collected for *C. dubia* toxicity tests. The first set consisted of five separate samples of water collected midriver and parallel to the 30-m bank transect. The second set of water samples consisted of five separate samples of river water collected along the bank. The midriver samples were used to assess toxicity in the whole river as influenced by the drain. We assumed dilution of the incoming water would be less near the bank where the drain entered the river, so the bank water samples were presumed to represent worst-case conditions (Fig. 1). The five bank and midriver samples were tested separately.

Ceriodaphnia dubia *96-h survival tests*

Ceriodaphnia dubia 96-h toxicity tests were conducted with bank and midriver samples by using the U.S. Environmental Protection Agency (U.S. EPA) standard acute test protocol [6]. Each undiluted sample was tested with the five field replicates, each of which contained five neonatal *C. dubia* (<24 h old). Survival was monitored daily in each replicate of each sample. Water quality variables including conductivity, hardness, alkalinity, pH, dissolved oxygen, and ammonia were measured in a composite of each sample at the beginning and end of each test. Temperature was monitored continuously by placing a probe in an additional replicate in the controlled temperature room. Control water was 4:1 moderately hard water [6].

Hyalella azteca *toxicity test*

Toxicity of sediment samples from all stations was assessed in September 2000 by using the 10-d survival and growth toxicity test with *H. azteca*, an invertebrate that occurs in the Salinas River. Sediment was sampled with a 7.5-cm-diameter polycarbonate core tube. The top 2 cm of sediment was collected and composited in a polycarbonate tub before being aliquoted for toxicity tests and chemical analyses. Eight replicate test containers, each with ten 7- to 14-d-old amphipods, were tested by following U.S. EPA procedures [7]. The amphipods were exposed to 100 ml of sediment in 300-ml beakers, each of which contained 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, cerophyll, and trout chow) was added daily to each test container; the containers were not aerated. Surviving animals were dried (60°C) at the end of the test, and growth was measured as dry weight per individual amphipod relative to baseline organisms.

Hyalella azteca *toxicity identification evaluation*

To characterize and identify chemicals responsible for sediment toxicity to *H. azteca*, a TIE was conducted in October 2000. A preliminary test compared survival of amphipods in solid-phase exposures to survival of amphipods exposed to pore water. For this test, sediment samples were collected from stations 1 and 2 (the station nearest the drain input). Pore water was extracted via refrigerated centrifugation (2,500 *g* at 4°C), and 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 20-ml glass scintillation vials. Pore-water test solutions were renewed on day 5. Because the results of these experiments demonstrated comparable toxicity in solid-phase and pore-water exposures, the TIE was conducted with pore water. This allowed application of phase 2 C_8 column methanol elution treatments, which were not possible with a solid-phase TIE.

Because of severe toxicity, selected TIE treatments were conducted with pore water diluted to 10 and 50% by using the 4:1 moderately hard water used in the *C. dubia* tests. Toxicity identification evaluation procedures followed abbreviated phase 1 and 2 guidelines described by Mount and Anderson-Carnahan [8] and Durhan et al. [9]. A previous study indicated the primary chemicals of concern at this site were the metabolically activated organophosphate (OP) pesticides chlorpyrifos and diazinon [5], so the *Hyalella* TIE focused on treatments to mitigate toxicity due to these and other compounds [10]. In addition to assessing toxicity of 10 and 50% baseline samples, pore water was tested after treatment with a C_8 column and piperonyl butoxide (PBO). The PBO was added to samples to achieve a final concentration of 0.5 mg/L. Piperonyl butoxide is a metabolic inhibitor that prevents in vivo transformation of pesticides such as diazinon or chlorpyrifos into their toxic forms. The lowering of sample toxicity by the addition of PBO serves as evidence that toxicity is caused by metabolically activated pesticides. An increase in toxicity caused by the addition of PBO constitutes evidence that toxicity is caused by a nonmetabolically activated compound. Serial elutions of the C_8 column with 25 to 100% methanol treatments also were tested. To account for toxicity introduced by the TIE procedures, blanks consisting of control water subjected to each of the TIE treatments were included. Concentrations of chlorpyrifos and diazinon were measured in all treatment solutions of the 50% pore-water samples by using enzyme-linked immunosorbent assays (ELISAs, described below).

Benthic macroinvertebrate community characterization

Techniques for sampling streams with sand or mud bottoms were modeled after the California Department of Fish and Game Aquatic Bioassessment Laboratory procedures for wadeable streams [11], which were adapted from the U.S. EPA

Rapid Bioassessment Protocol for use in streams and rivers [12].

Samples were collected by placing a D-net (0.5-mm mesh) on the sandy river bottom or against the submerged vegetated bank substrate, then disturbing a 30 × 61-cm portion of substrate upstream of the net for 60 s. A bank and a composite sample were collected at each of three randomly selected locations per station. These were three of the five locations sampled for toxicity testing. The bank sample was collected along the drain-side bank and was presumed to be the most influenced by the drain. The composite sample was collected at the bank, thalweg, and margin (opposite bank; Fig. 1). All samples were fixed in the field in 95% ethanol. Samples were transferred to 70% ethanol after being transported to the laboratory. All benthic macroinvertebrates were identified to species or genus by following methods and quality assurance guidelines of the California Stream Bioassessment Protocol [11]. These data were used to calculate the following six metrics for each sample: the number of Ephemeroptera taxa (number of mayfly genera), richness (number of species), abundance (total number of organisms), number of daphnids, number of *Hyalella* sp., and the percentage of Chironomidae (percentage of midge larvae) [11,12]. Physical and habitat-quality assessments were conducted at each sampling station. Instream cover, epifaunal substrate, embeddedness, channel flow, channel alteration, sediment deposition, and riffle frequency were quantified on a scale from 1 (poor) to 20 (optimal) at each sample location during each survey. Bank vegetation, bank stability, and riparian zone cover also were quantified on a scale from 1 (poor) to 10 (optimal) at each sample location during each survey [11].

Chemical analyses

River samples from May and September 2000 and May 2001 were analyzed for OP, organochlorine, and carbamate pesticides; polycyclic aromatic hydrocarbons; polychlorinated biphenyls; and trace metals. Organochlorine compounds were measured by using U.S. EPA method 8080, with gas chromatography–electron capture with detection limits ranging from 0.3 to 5 ng/L. Organophosphate compounds were measured by using EPA method 8140/8141 and a nitrogen–phosphorus–specific detector (detection limits 0.04–33 μg/L; detection limit for chlorpyrifos = 0.05 μg/L; detection limit for diazinon = 0.04 μg/L). Carbamate compounds were measured by using U.S. EPA method 632 [*http://www.epa.gov/epaoswer/hazwaste/test8_series.htm#8_series*] with dual detection with ultraviolet visual mode and liquid chromatography–mass spectroscopy confirmation (detection limits 0.054–2.5 μg/L). Polychlorinated biphenyls were analyzed as arochlors by using U.S. EPA method 8080-polychlorinated biphenyls (detection limits 0.04–0.11 μg/L). Polycyclic aromatic hydrocarbons were analyzed by using U.S. EPA method 8310 with high-pressure liquid chromatography–ultraviolet detection (detection limits 0.05–1.0 μg/L). Selected water samples also were analyzed for trace metals (As, Ag, Cd, Cr, Cu, Hg, Mg, Ni, Pb, and Zn) by inductively coupled plasma–mass spectroscopy (ICP-MS) by using U.S. EPA method 200.7 (detection limits 0.33–4.1 μg/L). Selected sediment samples were analyzed for trace metals (As, Ag, Cd, Cr, Cu, Hg, Mg, Ni, Pb, and Zn) by ICP-MS by using U.S. EPA method 6010A. 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.

Ten percent of the water samples analyzed for trace organics also were analyzed at the California Department of Fish and Game's Trace Organics Analytical Laboratory at Rancho Cordova (CA, USA) to determine interlaboratory precision of the chemical analyses. Results of these comparative analyses are reported as the mean and range of the relative percent differences ((lab 1 − lab 2/mean) × 100) for all compounds measured.

ELISA tests

All samples were analyzed for chlorpyrifos and diazinon by using ELISAs following procedures recommended by Sullivan and Goh [13]. The ELISA readings were compared to a five-point standard curve by using standards provided by the manufacturer. After the analysis of every group of field samples, accuracy of the ELISA method was determined by measuring an external chlorpyrifos or diazinon standard. This standard also was spiked into uncontaminated river water taken upstream of the contaminant input to account for matrix effects. All standard measurements were within ± 20% of nominal. Precision of the ELISA method was determined with duplicate measures of one sample by calculating the coefficient of variance ((variance/mean) × 100). Coefficient of variation values always were less than 20%. A combined bottle-blank/process-blank was included during one sampling period, and 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.

Dissolved oxygen (mg/L), specific conductance (μS/cm), pH, temperature (°C), and turbidity were measured in situ by using Hydrolab Surveyor 4 and Datasonde 4x instruments (Hydrolab, Austin, TX, USA). These instruments were calibrated in the laboratory as per manufacturer's recommendations. Alkalinity (total as $CaCO_3$) and hardness (calcium as $CaCO_3$) were measured in field-collected samples in the laboratory. All samples were analyzed at room temperature <48 h after collection.

Statistical analyses

Statistically significant differences in toxicity among stations were investigated by using analysis of variance followed by Tukey's multiple comparison test [14]. Percentage survival data were transformed (arcsine) before analysis. Separate analyses were conducted for the bank samples and the midriver composite samples. Statistically significant differences in macroinvertebrate abundances among stations were investigated by the Kruskal–Wallis test [14]. Associations among survival of *C. dubia* in toxicity tests, combined toxic units (TUs) of diazinon and chlorpyrifos, and turbidity in water samples were quantified by Spearman rank correlations [14]. Variables correlated with the mean number of Ephemeroptera taxa, macroinvertebrate abundance, species richness, and percentage of Chironomidae in the samples also were investigated with Spearman rank correlations. The variables investigated for correlations were combined TUs, turbidity, the total habitat score from the physical habitat assessment, and the bank cover score from the physical habitat assessment.

RESULTS

Chemical analyses

In the comparison of the pesticide analyses between laboratories, the second laboratory detected both chlorpyrifos and diazinon at lower concentrations than either the first laboratory, which used gas chromatography–mass spectroscopy (GC-MS), or our laboratory, with ELISA. The relative percent difference between the first and second laboratory for diazinon ranged from 0 to 100%; the mean relative percent differences for diazinon and chlorpyrifos measures between the first and second analytical laboratory when using GC-MS was 51.3%. Although both laboratories reported similar method detection limits, the second laboratory detected lower concentrations of both pesticides in these samples. Twenty-seven of the 36 samples measured with ELISA kits also were measured with U.S. EPA analytical chemistry methods. The mean relative percent differences for chlorpyrifos and diazinon were 34.6 and 68.3%, respectively. In most cases, differences were caused by detectable concentrations of chlorpyrifos or diazinon when using ELISA where no chemical was detected when using GC-MS (data not shown). Results from the ELISA analyses were used to calculate combined TUs because the ELISA results were considered to be more accurate.

Ceriodaphnia dubia *toxicity tests*

Although all drain samples caused 100% mortality of *C. dubia*, toxicity in the Salinas River varied both spatially and temporally (Table 1). Although survival was consistently >90% in the bank and midriver samples collected upstream of the drain (station 1), no animals survived in samples collected from station 2 bank, immediately downstream from the input. Toxicity was observed in the bank sample from station 3 in September 2000 and again in May 2001; the bank sample from station 4 was toxic only once, in May 2001. Samples collected from bank stations were more toxic than samples collected midriver. The mid river samples collected at stations 2, 3, and 4 were toxic in May 2001 only (Table 1). Major water chemistry conditions were within tolerable ranges for *C. dubia*. Dissolved oxygen was always >8 mg/L; hardness ranged from 92 to 1,243 mg/L; and conductivity ranged from 70 to 2,040 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 [15]). Copper concentrations were <7 μg/L (LC50 = 200 μg/L [15]), and zinc concentrations were <49 μg/L (LC50 = 95 μg/L [15]). Various OP and organochlorine pesticides were detected in some drain and river samples. Concentrations of dieldrin, DDT, endosulfan, and endrin always were <1 μg/L (data not shown). Only diazinon, chlorpyrifos, or both were above acute toxicity thresholds for *C. dubia* (diazinon 96-h median lethal concentration [LC50] = 0.32 μg/L, chlorpyrifos 96-h LC50 = 0.053 μg/L [16]).

Toxicity to *C. dubia* in the Salinas River reflected concentrations of chlorpyrifos and diazinon. Chlorpyrifos and diazinon concentrations exceeded their respective 96-h LC50 values in April 2000 in the drain and station 2 bank sample, and these were the only samples during this sampling period that were toxic to *C. dubia* (Table 1). Concentrations of these pesticides were low in all other samples during this period and none of the other samples were toxic (Table 1). Patterns of chlorpyrifos concentrations were similar in May and September 2000, with elevated concentrations in the drain and station 2 bank samples only. Concentrations of both OP pesticides were greater in samples from all downstream bank and midriver samples in May 2001, coinciding with greater mortality of *C. dubia* during this sample period (Table 1).

Mortality of *C. dubia* in bank samples correlated strongly with combined TUs of chlorpyrifos and diazinon (one combined TU of diazinon and chlorpyrifos = 100/LC50 diazinon + 100/LC50 chlorpyrifos; Table 2). Turbidity and TUs of these pesticides correlated highly in these samples, and mortality of *C. dubia* in the bank samples also correlated significantly with turbidity. The correlation between combined TUs of chlorpyrifos and diazinon and mortality of *C. dubia* in the midriver samples also was significant (Table 3). Turbidity was lower in the midriver samples, and was not significantly correlated with either TUs or toxicity.

Table 1. Survival of *Ceriodaphnia dubia* and concentrations of major chemicals and turbidity detected in Salinas River (CA, USA) water. Concentrations of chlorpyrifos and diazinon are from enzyme-linked immunosorbent assay methods (see text). Turbidity is given as nephelometric turbidity units[a]

Sample	*C. dubia* (SD)	Turbidity	Chlorpyrifos (μg/L)	Diazinon (μg/L)
April 12, 2000				
Station 1 bank	93 (15)	16.2	ND	ND
Station 1 mid	95 (11)	16.9	ND	ND
Station 2 bank	0 (0)*	276.6	0.072	0.794
Station 2 mid	100 (0)	128.2	ND	ND
Station 3 bank	96 (9)	35.7	ND	0.083
Station 3 mid	100 (0)	35.1	ND	0.084
Station 4 bank	100 (0)	24.3	ND	0.048
Station 4 mid	100 (0)	21.8	ND	0.042
Drain	0 (0)*	NM	0.116	1.202
May 15, 2000				
Station 1 bank	100 (0)	28.4	ND	0.052
Station 1 mid	100 (0)	30.2	ND	0.046
Station 2 bank	0 (0)*	690.6	0.097	0.189
Station 2 mid	100 (0)	256.3	ND	0.050
Station 3 bank	96 (9)	237.0	ND	0.078
Station 3 mid	100 (0)	115.4	ND	0.051
Station 4 bank	100 (0)	104.1	ND	0.057
Station 4 mid	100 (0)	59.2	ND	0.048
Drain	0 (0)*	NM	0.052	ND
September 5, 2000				
Station 1 bank	100 (0)	20.4	ND	ND
Station 1 mid	100 (0)	24.1	ND	ND
Station 2 bank	0 (0)*	955.1	0.048	0.450
Station 2 mid	100 (0)	339.2	ND	ND
Station 3 bank	0 (0)*	206.0	ND	0.100
Station 3 mid	100 (0)	95.6	ND	ND
Station 4 bank	100 (0)	122.1	ND	ND
Station 4 mid	100 (0)	61.3	ND	ND
Drain	0 (0)*	NM	0.145	2.004
May 14, 2001				
Station 1 bank	96 (9)	61.8	ND	0.304
Station 1 mid	100 (0)	58.2	ND	0.352
Station 2 bank	0 (0)*	163.1	0.515	0.420
Station 2 mid	64 (43)	122.0	ND	0.417
Station 3 bank	0 (0)*	178.5	0.333	0.353
Station 3 mid	0 (0)*	175.2	0.337	0.421
Station 4 bank	0 (0)*	125.0	0.051	0.294
Station 4 mid	52 (48)	121.6	ND	0.310
Drain	0 (0)*	NM	2.524	0.302

[a] ND = not detected; NM = not measured.
* $p < 0.05$.

Table 2. Spearman rank correlation coefficients (rho) for selected factors correlated with survival of *Ceriodaphnia dubia* in laboratory toxicity tests with bank samples and with selected macroinvertebrate community metrics calculated with bank samples ($n = 16$)[a]

	Toxic units (rho)	Turbidity (rho)	Habitat (rho)	Bank cover (rho)
C. dubia survival	−0.829**	−0.701**	NA	NA
No. Ephemeroptera taxa	−0.784**	−0.683**	0.190	0.543*
Macroinvertebrate abundance	−0.150	−0.197	−0.024	−0.003
Species richness	−0.493*	−0.492*	0.405	0.371
% Chironomidae	−0.703**	−0.681**	−0.268	0.474*
Turbidity	0.726**			

[a] NA = not analyzed.
* $p < 0.05$, ** $p < 0.01$.

Hyalella azteca *toxicity in river sediments*

Sediments from the drain and from the river downstream of the drain input were toxic to *H. azteca* (Table 4). Amphipod growth and survival were not inhibited at the upstream reference station, but growth and survival were reduced in all of the downstream bank samples, and in the midriver sample collected at station 2. Conventional pore-water chemistry was suitable for this species: un-ionized ammonia ranged between 0.01 and 0.05 mg/L, hardness ranged between 114 and 163 mg/L, conductivity ranged between 700 and 715 μS/cm, and alkalinity ranged between 100 and 125 mg/L. Concentrations of chlorpyrifos in sediment pore water from the drain (0.057 μg/L), station 2 bank (0.084 μg/L), and station 3 bank (0.199 μg/L) samples were near or above the 10-d LC50 for *H. azteca* (chlorpyrifos 10-d LC50 = 0.086 μg/L [17]; Table 4). Diazinon was detected in pore-water samples from all stations downstream of the drain, but the concentrations of this pesticide were lower than this compound's 10-d LC50 (diazinon 10-d LC50 = 6.51 μg/L [17]; Table 4). Other organochlorine pesticides and several trace metals were detected in Salinas River sediment samples. Some of the organochlorine pesticides exceeded the consensus-based freshwater sediment threshold effect concentration (TEC) guidelines published by MacDonald et al. [18]. Total DDT concentrations exceeded the TEC value at station 2 bank and midriver, at stations 3 and 4 bank, and in the drain. The concentration of dieldrin exceeded the probable-effect concentration [18] at station 2 bank, and sediment concentrations of this pesticide exceeded the TEC at stations station 3 bank and in the drain. Concentrations of endrin exceeded the TEC at station 2 bank (Table 4). Of the seven metals for which guidelines have been calculated, only nickel exceeded the freshwater TEC guideline in our study (TEC = 22.7 mg/kg dry wt [18]). The highest nickel concentration was measured in sediment from the drain (45 mg/kg dry wt), lower than the nickel probable-effect concentration (48.6 mg/kg dry wt [18]).

Hyalella azteca *sediment TIE*

In May 2001, both the pore-water and solid-phase sediment samples from station 2 bank caused 100% mortality to *H. azteca* (Fig. 2A). Toxic pore water implies that exposure occurred via respiration, dermal uptake, or both. The TIEs were conducted with 10 and 50% pore-water concentrations. We noted 100% mortality in the 50% pore-water sample, and 50% mortality in the 10% pore-water concentration (Fig. 2B). Amphipod survival was >80% in all treatment blanks (data not shown). The concentration of chlorpyrifos in the 50% pore-water sample was 0.475 μg/L—a value five times greater than the 10-d *H. azteca* LC50 for chlorpyrifos. The C_8 column completely removed toxicity in the 10% pore-water sample, indicating toxicity due to nonpolar organic compounds. The C_8 column did not reduce toxicity of the 50% pore-water sample. The 80 and 85% methanol eluants of the C_8 column were relatively more toxic in both the 50 and 10% pore-water concentrations, and some chlorpyrifos and diazinon were recovered from these fractions. The addition of PBO did not mitigate toxicity of either the 10 or 50% pore-water samples (survival in the PBO blank was 80%). Amphipod mortality in 10% pore water increased when PBO was added, suggesting a potentiation effect. Because PBO inhibits a key metabolic pathway, previous studies have suggested that increased toxicity with the addition of PBO is an indicator of toxicity due to non–metabolically activated chemicals such as pyrethroid pesticides [19]. These results suggest sediment toxicity due to a combination of chemicals including the OP pesticide chlorpyrifos, and some other nonmetabolically activated compound.

Table 3. Spearman rank correlation coefficients (rho) for selected factors significantly correlated with survival of *Ceriodaphnia dubia* in laboratory toxicity tests with midriver samples or with selected macroinvertebrate community metrics calculated with composite data ($n = 16$)[a]

	Toxic units (rho)	Turbidity (rho)	Habitat (rho)
C. dubia survival	−0.499*	−0.197	NA
No. Ephemeroptera taxa	−0.690**	−0.462*	0.190
Macroinvertebrate abundance	0.014	−0.132	−0.024
Species richness	−0.358	0.108	0.698**
% Chironomidae	−0.258	−0.539*	−0.261
Turbidity	−0.299		

[a] NA = not analyzed.
* $p < 0.05$, ** $p < 0.01$.

Table 4. Results of September 2000 10-d sediment toxicity tests with *Hyalella azteca* (HA) and chemical analyses of sediment pore water (PW) and solid-phase sediment[a]

Station	HA % survival[a]	HA growth[a] (mg dry wt)	PW chlor-pyrifos (μg/L)	PW diazinon (μg/L)	TDDT[b] (μg/kg dry wt)	Dieldrin[c] (μg/kg dry wt)	Endrin[d] (μg/kg dry wt)	Sediment constitutents (% composition)			
								Coarse + medium	Fine	Silt + clay	TOC[e]
1 bank	93	0.2497	ND	ND	1.3	ND	ND	92	6	0	ND
1 mid	88	0.2290	ND	ND	1.3	ND	ND	85	12	0	ND
2 bank	16*	0.0846*	0.084	0.562	129.2	131.9	13.9	28	13	48	2.6
2 mid	73*	0.1140*	ND	0.067	8.8	1.3	1.3	78	5	4	1.0
3 bank	1*	0.0900*	0.199	0.133	52.5	3.8	5.0	51	15	28	4.4
3 mid	93	0.2015	ND	0.089	1.2	ND	ND	88	10	0	ND
4 bank	45*	0.1401*	ND	0.109	29.1	1.3	2.5	58	20	16	1.3
4 mid	90	0.2445	ND	0.054	1.3	ND	ND	85	14	1	ND
Drain	59*	0.1304*	0.057	0.488	79.2	9.7	4.2	13	11	62	1.9
Control	89	0.2429									

[a] TDDT = total DDT; TOC = total organic carbon; ND = not detected.
[b] Threshold effect concentration (TEC) = 5.28 μg/kg; probable-effect concentration (PEC) = 572 μg/kg.
[c] TEC = 1.9; PEC = 61.8 μg/kg.
[d] TEC = 2.2; PEC = 207 μg/kg [24].
[e] Total organic carbon.

Macroinvertebrate community structure

Macroinvertebrate community structure varied considerably in the Salinas River between station 1 and the downstream stations 2, 3, and 4. During all sample periods, macroinvertebrate abundances at the downstream stations reflected the gradient of exposure to the drain input. Abundances were lower at station 2 and increased at stations farther downstream (Table 5).

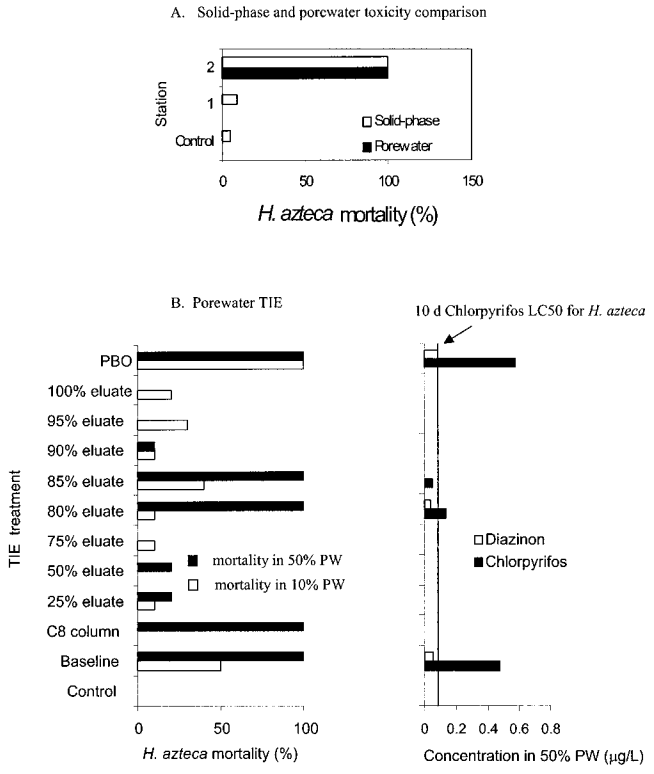

Fig. 2. (**A**) Results of 10-d *Hyalella azteca* solid-phase and porewater (PW) toxicity tests. (**B**) The lower left figure shows results of toxicity identification evaluation (TIE) conducted with 10% PW concentration and 50% PW concentration (PBO = piperonyl butoxide). The lower right figure shows concentrations of chlopryifos and diazinon measured in the 50% PW concentration after the TIE treatments (LC50 = 50% lethal concentration).

Although temporal and spatial variation were evident, abundances of macroinvertebrates were lower at all downstream stations, relative to station 1 (Table 5). For example, approximately 98% fewer macroinvertebrates were present in the station 2 bank sample than in the station 1 bank sample in April 2000. Macroinvertebrate abundances in the stations 3 and 4 bank samples were approximately 80% lower than those at station 1 in April 2000.

Species richness also was lower at some of the downstream stations relative to station 1, particularly the downstream bank samples collected in April, May, and September 2000 (Table 5). The reduction in species richness partly reflects the loss of Ephemeroptera taxa (mayflies) at the downstream stations during some of the sampling periods. Mayfly numbers were lower in the downstream samples during all of the sampling periods except May 2001, when few Ephemeroptera species were present in any of the samples. Differences in abundances of Ephemeroptera species between station 1 and stations 2, 3, and 4 were greatest in the April and September 2000 samples. In most cases for all sampling periods, fewer Ephemeroptera species were present in the downstream bank samples relative to the composite samples (Table 5). As with macroinvertebrate abundances, the decrease in Ephemeroptera was greatest at the downstream stations nearest the drain input. Impacts of the drain on macroinvertebrate species richness also reflect reductions in the percentage of Chironomidae (midges) at the downstream stations. As with Ephemeroptera taxa, impacts on the percentages of Chironomidae in these samples were more pronounced in the bank samples (Table 5).

Numbers of Daphniidae and *Hyalella* sp. in the macroinvertebrate samples were quantified separately to determine if the stations with greatest toxicity to *C. dubia* and *H. azteca* in the laboratory toxicity tests also had lower abundances of these organisms in field samples. In September 2000, relatively large numbers of *Hyalella* sp. were present at the station 1 bank, but considerably fewer individuals were present at all of the downstream bank stations (Table 5). Few individuals of *Hyalella* were collected during the other three sample periods.

Table 5. Summary of benthic macroinvertebrate community characterizations in bank and composite samples ($n = 3$). Station 1 is upstream of the agricultural drain, and stations 2, 3, and 4 are progressively farther downstream

	Bank samples				Composite samples			
	Station 1	Station 2	Station 3	Station 4	Station 1	Station 2	Station 3	Station 4
April 2000								
Mean no. Ephemeroptera taxa	4.7	0	0.7	1.3	3.7	1.3	1.7	2
Mean richness	14	2.7	9	7	12	6	10.3	9.3
Mean abundance	390	9*	78	79	302	35*	166	78
Mean no. daphnids per sample	0	0	1	0	0	0	0	0
Mean no. *Hyalella* per sample	0	0	0.3	0	0	0	0.7	0
Mean % Chironomidae per sample	38.6	4.3	45.4	74.1	38.4	16.3	58.3	80.5
May 2000								
Mean no. Ephemeroptera taxa	2.7	0.3	0.3	0	1.7	2	1	1
Mean richness	15.3	7.3	10.3	12.7	15	12.7	11	13.3
Mean abundance	7,747	1,394	1,471	2,051	3,892	1,950	2,160	2,260
Mean no. daphnids per sample	8	0	0.3	1.7	10.3	0.7	1.3	8.3
Mean no. *Hyalella* per sample	1.7	0	0	0	0.7	0	0	0.3
Mean % Chironomidae per sample	52	16	15	58	41	40	30	60
September 2000								
Mean no. Ephemeroptera taxa	3.3	0	0	0.7	3.7	1.3	3	0.7
Mean richness	18	10.3	10	14.3	22	21	19.3	17
Mean abundance	3,535	719	186*	1,650	3,434	2,084	2,106	7,166
Mean no. daphnids per sample	107	0	1	11	104.3	15.7	56	114
Mean no. *Hyalella* per sample	16.3	0	0.3	3	6.7	14	2.3	3
Mean % Chironomidae per sample	24	0	3	8	19	8	9	7
May 2001								
Mean no. Ephemeroptera taxa	0	0	0	0	0	0	0	0.3
Mean richness	10.3	12	12	12.3	11	12	14	11.7
Mean abundance	4,945	2,117	1,337	466	4,344	2,047	1,711	2,343
Mean no. daphnids per sample	122.7	0.7	1.7	2	33.7	3	0.3	1.3
Mean no. *Hyalella* per sample	0	0	0	0	0	0	0	0
Mean % Chironomidae per sample	5	2	3	6	8	2	6	4

* Significantly less than station 1 reference at $p < 0.05$.

In many cases, significantly fewer daphnids were present in the downstream samples relative to station 1. For example, lower numbers of Daphniidae were present in all of the downstream samples except the station 4 composite samples collected in May and September 2000. Fewer Daphniidae also were found in all of the downstream bank and composite samples collected in May 2001 (Table 5).

The number of Ephemeroptera taxa and the percentage of Chironomidae in the bank samples were positively correlated with each other and negatively correlated with the combined TUs of diazinon and chlorpyrifos in the river (Table 2). In addition, species richness was negatively correlated with combined OP TUs in the river. Because TUs of diazinon and chlorpyrifos were correlated with turbidity in the river, macroinvertebrate metrics that were negatively correlated with TUs also were negatively correlated with turbidity (Table 2). None of the macroinvertebrate metrics were significantly correlated with the habitat score calculated with the California Department of Fish and Game Rapid Bioassessment procedures. However, the number of Ephemeroptera taxa and the percentage of Chironomidae both were significantly correlated with river bank vegetation cover, a component of the habitat score (Table 2).

Similar relationships were found with the composite sample data. The number of TUs measured in the midriver samples correlated negatively with the number of Ephemeroptera taxa in the composite samples. The number of Ephemeroptera taxa and percentage of Chironomidae also correlated negatively with turbidity. Species richness in the composite samples correlated with habitat (Table 3). Because other physical and water-quality factors potentially affected by the drain input were similar between the stations, they probably did not affect macroinvertebrates at the downstream stations. Mean flows at stations 1, 2, 3, and 4 were 0.93, 0.90, 0.75, and 1.40 m/s, respectively. Mean conductivities were 614.9, 773.2, 657.1, and 629.7 μS/cm at stations 1, 2, 3, and 4, respectively. The Salinas River is less than 2 m deep in the study area, and wide daily fluctuations in water temperatures occurred during this study. However, little difference was found in the range of temperatures measured at the upstream and downstream stations. Temperatures ranged from 13.0 to 31.5°C, 13.7 to 25.4°C, 12.7 to 31.0°C, and 15.5 to 25.7°C, at stations 1, 2, 3, and 4, respectively.

DISCUSSION

This study demonstrated that sections of the Salinas River are significantly impacted by pesticides associated with agricultural drain water. River water downstream of the drain input was consistently toxic to *C. dubia* in laboratory tests. Mortality of *C. dubia* was significantly correlated with the combined TUs of chlorpyrifos and diazinon and turbidity in the bank samples. Turbidity was not significantly correlated with mortality of *C. dubia* in the composite samples. The negative correlation between survival of *C. dubia* in laboratory exposures and turbidity measured in bank samples is due to the co-occurrence of pesticides and particles in the field samples. Before testing in the laboratory, the samples were allowed to settle for 24 h. They were then decanted for testing. This effectively removed the majority of particles in the samples, negating their effect on *C. dubia*. In a previous study of this

drainage, Hunt et al. [5] used phase 1, 2, and 3 TIEs to demonstrate that toxicity to *C. dubia* was due to the combined effects of chlorpyrifos and diazinon. Our studies on the Salinas River corroborate results of other research conducted throughout California that show aquatic toxicity due to these two pesticides is prevalent in watersheds dominated by agricultural [10,20].

We have observed that drain water flow at this site increases throughout the day with the onset of irrigation, then gradually declines overnight. This pattern repeats itself daily (Bryn Phillips, Marine Pollution Studies Laboratory, Monterey, CA, USA, unpublished data). Favorable growing conditions allow farming in the Salinas Valley to proceed through most of the year. Thus, drain water discharge through this drainage creek is a potential source of chronic pollution in this system [5]. Our 96-h acute toxicity tests with *C. dubia* may have underestimated the magnitude and spatial extent of toxicity in this system. The 7-d LC50 for diazinon toxicity to *C. dubia* is 110 ng/L [21]; the 7-d LC50 for chlorpyrifos toxicity to this species is 20 ng/L [22]. Concentrations of both of these chemicals sometimes exceeded these values during the course of this study. Apparent underestimation of the spatial extent of toxicity in this study occurred in the May 2001 toxicity tests when complete mortality was observed at all stations downstream of the drain input.

Toxicity tests with *H. azteca* suggest that chemicals associated with the drainage input impact macroinvertebrates associated with sediments in this system. Sediments at all downstream stations were acutely toxic to the amphipods, so the downstream extent of sediment toxicity is not known (Table 4). 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. Chlorpyrifos concentrations in sediment reflect concentrations in the overlying water, and previous studies have shown that sediment concentrations decline rapidly with the decline of overlying water concentrations [23]. The presence of acutely lethal concentrations of chlorpyrifos in Salinas River sediment samples may be due to the consistency of the drain-water source. During the growing season, drain water containing diazinon and chlorpyrifos enters the river on a daily basis and these pesticides are transported to sediments through deposition of contaminated particles. We measured the greatest concentrations of pesticides in samples with the greatest percent silt, clay, and total organic carbon (Table 4). Concentrations of chlorpyrifos measured in our study are comparable to the highest concentration reported in California Central Valley suspended sediments (153 ng/L) [24].

The TIE results suggest that OP pesticides were not the only source of toxicity in the station 2 sediment sample. Increased toxicity of the 10% pore-water sample with the addition of PBO is consistent with the hypothesis that a non–metabolically activated chemical also was present in this sample (Fig. 2). If chlopyrifos was the only chemical responsible for toxicity, the PBO treatment should have increased survival of *C. dubia* by inhibiting chlorpyrifos metabolism to its toxic form by cytochrome P450. By inhibiting cytochrome P450, PBO also acts as a synergist in the presence of nonmetabolically activated pesticides such as pyrethroids [19]. The California Department of Pesticide Regulation report that pyrethroid pesticides are used widely as agricultural insecticides in the Salinas Valley area (D. Paradies, Central Coast Regional Water Quality Control Board, San Luis Obispo, CA, USA, personal communication). Amphipods are among the most sensitive taxa to synthetic pyrethroids [25]. Although the ELISA and TIE results indicate that chlorpyrifos in pore water was partially responsible for acute toxicity to *H. azteca*, examination of the TIE and pesticide use data suggests that future studies also should include consideration of non–metabolically activated pesticides such as pyrethroids. Sediments from station 2 also had the highest concentrations of organochlorine pesticides, and the dieldrin concentration at this station exceeded the probable-effect concentration [18]

Our results demonstrate impacts of agricultural drain water on macroinvertebrate communities in this section of the Salinas River. Because river flow, temperature, and conductivities were similar at the upstream and downstream stations, these factors are unlikely to account for impacts on macroinvertebrates at the downstream stations. Macroinvertebrate abundances, species richness, and the number of Ephemeroptera taxa often were lower at the downstream stations relative to the upstream reference station, particularly at the bank stations where drainwater effects tended to be more pronounced. These metrics correlated negatively with combined chlorpyrifos and diazinon TUs associated with the drain input, and with turbidity in the river. As discussed above, turbidity correlated with TUs. Although it is not possible to separate the negative effects of particles from the toxic effects of pesticides on resident macroinvertebrates by using these data, several lines of evidence suggest that OP pesticides played a role in ecological impacts in the river. In an extensive literature review, de Vlaming and Norberg-King [26] concluded that among the single-species toxicity tests used in ambient water assessments, tests with *C. dubia* were particularly reliable as predictors of instream biological responses. Examination of our results indicates that toxicity to *C. dubia* occurred in samples from stations where macroinvertebrate community structure also was impacted. In many cases, toxicity to *C. dubia* in laboratory toxicity tests occurred in samples from stations with lower Daphniidae abundances (Table 5). Results of previous TIEs suggested that toxicity to *C. dubia* was due to OP pesticides, not turbidity [5]. Toxicity to *Hyalella* also occurred at stations where macroinvertebrate community structure was most impacted (i.e., the downstream bank stations), and examination of TIE results suggests that sediment toxicity was due, in part, to elevated chlorpyrifos concentrations. The number of Ephemeroptera taxa in samples from the four river stations correlated negatively with combined TUs of diazinon and chlorpyrifos, and correlated less strongly with turbidity.

Although the relative effects of turbidity and pesticides to mayfly nymphs and other macroinvertebrates cannot be determined without further study, examination of dose–response data from the literature suggests that some of the species and genera found in the Salinas system are sensitive to pesticide concentrations within the range measured in this study. The 10-d LC50 for chlorpyrifos toxicity to the midge *Chironomus tentans* is 0.07 μg/L [17]; the 48-h LC50 to mayflies *Cloeon dipterum* and *Ephemerella* sp. are 0.37 μg/L and 0.40 μg/L, respectively [27,28]. The 48-h LC50 for chlorpyrifos toxicity to larvae of the blackfly *Simulium vittatum* is 0.43 μg/L [29]. The 10-d LC50 for chlorpyrifos toxicity to *H. azteca* is 0.086 μg/L [17]. *Hyalella azteca, S. vitattum, Chironomus* sp., and *Ephemerella* sp. are all species or genera that occurred at our reference station, and densities of all of these genera declined downstream of the drain input. Most of these values reflect

chlorpyrifos toxicity in short-term exposures. Furthermore, because toxicity of diazinon and chlorpyrifos is additive [16], inputs of drain water containing combinations of these chemicals would be more toxic than water containing either individually. Pesticides in this system likely influence macroinvertebrate community structure through behavioral or indirect mechanisms. These include sublethal influences on drift and predator-avoidance behavior [30,31].

Species richness, the number of Ephemeroptera taxa, and the percentage of Chironomidae were less strongly correlated with bank cover than with combined TUs and turbidity. The station with the lowest bank cover score was station 2 bank (data not shown), which also was the station with the highest TUs and turbidity, the greatest toxicity, and the most impacted macroinvertebrate community structure. In our study, lower bank cover was observed at station 2 in April and May 2000, but bank cover was similar at all stations in September 2000 and May 2001. The fact that declines in macroinvertebrate abundances occurred at the downstream stations even when bank vegetative cover was comparable to that of the reference station indicates that this factor was a less important determinant of insect community structure. Based on the weight of evidence in this study, pesticide pollution is the likely cause of ecological damage in the Salinas River. Future work will address several research directions based on the current work. Investigations of the causes of sediment toxicity to *H. azteca* will be conducted by using TIEs and chemical analyses that address toxicity due to non–metabolically activated compounds such as pyrethroids. More extensive surveys will be conducted to investigate the spatial extent of sediment toxicity associated with selected agricultural drains. Relative impacts of pesticides and physical factors (e.g., particles accounting for turbidity) on resident macroinvertebrates will be assessed by using laboratory dose–response experiments with mayfly and amphipod species.

Acknowledgement—We are grateful for all those who helped complete this study, including R. Kosaka, W. Piekarski, S. Huntley, K. Worcester, M. Adams, D. Paradies, J. Harrington, and P Ode. Access to river sampling stations was coordinated with the help of the Monterey County Farm Bureau and we gratefully acknowledge the cooperation of the Salinas Valley Growers and Kelly Huff. This manuscript was greatly improved by the comments of two anonymous reviewers. This study was funded by the California State Water Resources Control Board.

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