AMBIENT TOXICITY DUE TO CHLORPYRIFOS AND DIAZINON IN A CENTRAL CALIFORNIA COASTAL WATERSHED

JOHN W. HUNT[1]*, BRIAN S. ANDERSON[1], BRYN M. PHILLIPS[1], PATRICIA N. NICELY[1], RON S. TJEERDEMA[1], H. MAX PUCKETT[2], MARK STEPHENSON[2], KAREN WORCESTER[3] and VICTOR DE VLAMING[4]

[1] *Department of Environmental Toxicology, University of California, Davis Marine Pollution Studies Laboratory, Monterey, California, U.S.A.;* [2] *California Department of Fish and Game, Marine Pollution Studies Laboratory, Granite Canyon, U.S.A.;* [3] *California Regional Water Quality Control Board, Central Coast Region, San Luis Obispo, California, U.S.A.;* [4] *California State Water Resources Control Board, Sacramento, California, U.S.A.*

(author for correspondence, e-mail: jwhunt@ucdavis.edu)*

(Received 20 March 2001; accepted 15 April 2002)

Abstract. The Salinas River watershed along the central coast of California, U.S.A., supports rapidly growing urban areas and intensive agricultural operations. The river drains to an estuarine National Wildlife Refuge and a National Marine Sanctuary. The occurrence, spatial patterns, sources and causes of aquatic toxicity in the watershed were investigated by sampling four sites in the main river and four sites in representative tributaries during 15 surveys between September 1998 and January 2000. In 96 hr toxicity tests, significant *Ceriodaphnia dubia* mortality was observed in 11% of the main river samples, 87% of the samples from a channel draining an urban/agricultural watershed, 13% of the samples from channels conveying agricultural tile drain runoff, and in 100% of the samples from a channel conveying agricultural surface furrow runoff. In six of nine toxicity identification evaluations (TIEs), the organophosphate pesticides diazinon and/or chlorpyrifos were implicated as causes of observed toxicity, and these compounds were the most probable causes of toxicity in two of the other three TIEs. Every sample collected in the watershed that exhibited greater than 50% *C. dubia* mortality (n = 31) had sufficient diazinon and/or chlorpyrifos concentrations to account for the observed effects. Results are interpreted with respect to potential effects on other ecologically important species.

Keywords: agriculture, *Ceriodaphnia dubia*, organophosphate, Salinas River, toxicity, watershed

1. Introduction

The State of California is the nation's fastest growing and most populous state, with over 12 000 km^2 of developed urban area. The state also has 112 500 km^2 in agricultural production and supplies more than 50% of the fruits, nuts, and vegetables grown in the U.S.A. (CFBF, 2000). Human activity over the past 150 yr has resulted in the release of metals, hydrocarbons and industrial compounds into the state's environment (Ehrlich *et al.*, 1977); and industrial, residential, and agricultural pest control operations in California apply over 86 000 metric tons of pesticides each year (CDPR, 2000). Rain and irrigation water draining from urban and agricul-

Environmental Monitoring and Assessment **82:** 83–112, 2003.

tural surfaces transport pesticides and other contaminants into surface waters and aquatic habitats (De Vlaming *et al*., 2000). Previous studies have identified numerous drainage channels, rivers, and estuaries where samples of water and sediment were both toxic to test organisms and contained contaminants at concentrations capable of inducing the observed toxicity (Norberg-King *et al*., 1991; De Vlaming *et al*., 2000; Hunt *et al*., 2001). Toxicity has been observed in waterways receiving both urban and agricultural runoff (De Vlaming *et al*., 2000; Bailey *et al*., 2000; Hansen and Associates, 1994). Toxicity identification evaluations (TIEs) employed in many of these studies have determined that toxicity to *Ceriodaphnia dubia*, a commonly used test organism, has been caused primarily by two organophosphate (OP) pesticides: chlorpyrifos and diazinon (De Vlaming *et al*., 2000).

While the Sacramento, San Joaquin, and Imperial Valleys are relatively well studied areas of California with respect to transport and toxicity of the two OP insecticides (De Vlaming *et al*., 2000; Tierney *et al*., 1997), fewer reports are available describing the fate and effects of non-point source contaminants in California's coastal areas. Coastal regions of California are subject to rapid urbanization, and the milder coastal climate supports year-round intensive cultivation of many high-value crops. A previous investigation of one California coastal watershed documented toxicity to resident mysid crustaceans in most agricultural drain samples, 25% of wetland samples, and 11% of samples from the main stem of the Pajaro River (Hunt *et al*., 1999). In that study, organic compounds were implicated as causes of toxicity in TIEs, and both organochlorine and OP pesticides were measured at potentially toxic concentrations.

This article describes an assessment of the occurrence and effects of non-point source contaminants in the Salinas River, along the central coast of California. The Salinas River watershed covers 11 500 km^2, with approximately 100 km^2 of urban area and 950 km^2 of irrigated agricultural land. Rainfall is variable and highly seasonal, and river flow varies from a monthly average of 0.57 $m^3\ s^{-1}$ in August to 45 $m^3\ s^{-1}$ in February. The valley is known as the nation's salad bowl, and is the most productive vegetable producing region in the U.S.A. (CFBF, 2000). Lettuce, broccoli, strawberries, nursery crops and grapes are among the major crops grown year-round on irrigated fields. This intensive agricultural activity and rapid urban growth occur adjacent to recognized critical aquatic habitats, as the river drains to the Salinas River National Wildlife Refuge estuarine area and the Monterey Bay National Marine Sanctuary. The river is a migration corridor for threatened salmonids, such as the steelhead *Oncorhynchus mykiss* (Busby *et al*., 1997), and provides habitat for a diversity of waterfowl, mammal, and amphibian species. Risk of pesticide and sedimentation impacts have caused the Salinas River to be placed on the federal Clean Water Act 303(d) list of impaired water bodies.

Because the Salinas River and tributaries function both to support wildlife and to drain surface water from urban and agricultural areas, and because of previous patterns of aquatic toxicity identified elsewhere in California, a watershed-based assessment was conducted to investigate the potential occurrence of ambient tox-

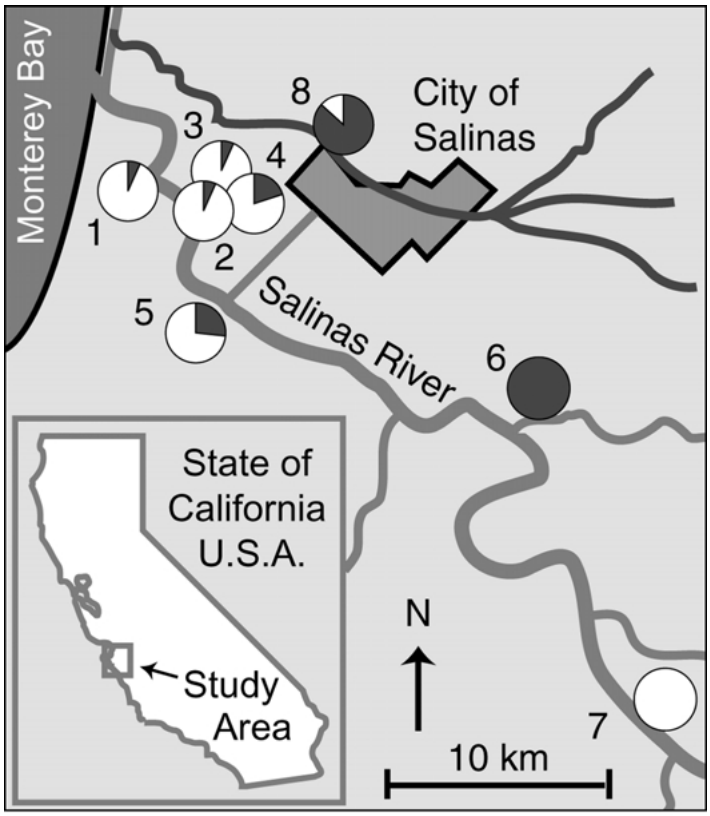

Figure 1. Map of the lower Salinas River watershed area, showing the location of sampling sites. Site numbers correspond to those used in the text and tables. The number of samples exhibiting significant *C. dubia* mortality in 15 surveys is indicated by the size of the dark sectors in pie graphs at each site.

icity. This assessment was planned with input from the local farm community, water management agencies, and urban storm water managers, who provided information on land use and hydrology in the watershed. Four study sites were selected in the main stem of the river, and four additional sites were located in modified tributary creeks that convey runoff from surface furrows, subsurface tile drains, urban areas, and a combined urban/agricultural subwatershed.

This study was designed as the first phase of an ecotoxicological assessment in this watershed. The two major objectives were to screen river reaches and tributaries to locate general sources of toxic runoff in the watershed, and to use TIEs to determine constituents responsible for observed toxicity. After source areas and causative agents were identified in this first phase, a second phase has been initiated to assess ecological impacts to resident invertebrate communities in main river aquatic habitats.

2. Materials and Methods

2.1. Study sites

Eight study sites were selected (Figure 1). Site 1 was located approximately 6 km upstream of the point where the river enters Monterey Bay. This site was at the upper end of the coastal lagoon, where seawater influence was sufficiently low to allow year-round testing with freshwater organisms. Site 2 was located 1 km further upstream and on the opposite side of the river from Site 1, approximately 30 m downstream from a modified channel discharging tile drain runoff. (A *tile drain* is a system of perforated drain pipes buried approximately 1 m beneath the soil surface to allow water percolating through the soil to flow to collection sumps, from which it is pumped into open drainage channels). Sites 3 and 4 were branches of the same channel that drained to the river just upstream of Site 2. Each channel branch received tile drain runoff from agricultural fields. Site 5 was located in the main stem of the river 6.5 km upstream from Site 2, and was approximately 30 m downstream of an urban stormwater discharge pipe. Site 6 was in a modified creek channel that drained agricultural furrow runoff into the main river 14 km upstream of Site 5. Site 7 was in the main stem of the river 11 km upstream of Site 6. Site 8 was in a modified creek that drained urban and agricultural areas. This channel emptied into the Moss Landing Harbor via the Tembladero Slough and Old Salinas River channel, which is connected with the main Salinas River only during times of extremely high flow.

2.2. Sample handling

Each site was sampled 15 times from 23 September 1998 to 20 January 2000. Ten of these surveys were scheduled at regular 7 week intervals throughout the study period, with five surveys conducted at selected times during the fall and early winter seasons to coincide with rainfall events. Samples were collected in pre-cleaned 1 L amber glass bottles attached to the end of a plastic pole. At Sites 2 and 5, which were in the main stem of the river approximately 30 m downstream of agricultural and urban drains, respectively, samples were collected along the edge of the plume where tributary and river water mixed, approximately 2 m from the bank. Bottles were immersed just below the water surface to avoid entrainment of the surface microlayer. Samples were transported on ice and stored in the dark at $4\pm3^{\circ}$C. Samples were settled overnight, decanted, and split into aliquots for toxicity testing and chemical analysis.

2.3. Toxicity testing

Each sample was tested for toxicity using 96 hr *Ceriodaphnia dubia* survival tests (USEPA, 1993). All samples were tested within 48 hr of collection, using <24 hr old *C. dubia* neonates. Mortality was recorded daily, and sample water was changed

once at 48 hr. For each sample, ammonia was measured at the beginning of the test; conductivity, hardness, dissolved oxygen, and pH were measured at the beginning and end of the test and at the water change; and temperature was recorded continuously. Two control solutions were prepared for each test, using the protocol-specified mixture of distilled water and commercial mineral water, with filtered seawater added to adjust conductivity to match the lowest and highest values for the set of samples being tested. Performance in the high and low conductivity controls averaged 98% survival, with a minimum of 88%. Reference toxicant tests using copper chloride were conducted concurrently with each set of sample tests. The mean Cu LC50 was 16.1 μg L^{-1} $\pm$29% (CV), and all LC50 values fell within control limits of $\pm$2 standard deviations of the mean. A field duplicate sample was collected from one site during each survey, selected on a rotating basis. Of 15 sets of duplicates, the average relative percent difference in *C. dubia* survival was 2%, with a maximum difference of 9%.

Significant sample toxicity was determined by comparison to a threshold of 75% of control survival. This value was determined using the *detectable difference* method of Thursby *et al*. (1997). To derive this threshold value, each sample was compared to its respective laboratory control using a *t*-test (alpha = 0.05), and the minimum significant difference (MSD) for each *t*-test (n = 114) was determined. These MSD values were ranked from lowest to highest, and the 90th percentile MSD value was determined to be 25% of control survival. This value represented the minimum difference between sample and control that could be detected as statistically significant in 90% of the sample comparisons. This is equivalent to setting the level of statistical power at 0.90 for all comparisons (Thursby *et al*., 1997). In practice, no sample identified as toxic in this study had *C. dubia* survival greater than 52%.

2.4. Chemical analyses

All samples from eight of the surveys conducted between 10/28/1998 and 1/20/2000 were analyzed for organochlorine and OP pesticides, and samples from seven of those surveys were also analyzed for PCBs and carbamate pesticides. Samples from 13 surveys were analyzed for diazinon and chlorpyrifos using enzyme-linked immunosorbant assays (ELISA). Trace metals were analyzed in all samples from four surveys, and polynuclear aromatic hydrocarbons (PAHs) were measured at all sites once. Volatile organic compounds and PAHs were measured once each in specific samples as part of toxicity identification evaluations. The concentration of total suspended solids, as the mass of particles retained on a 0.45 μm glass fiber filter, was quantified in all samples from 13 surveys.

Organochlorine, OP, and carbamate pesticides were measured using EPA gas chromatography/mass spectroscopy methods 8080, 8140, and 632, respectively. The screen included 26 organochlorine compounds (including seven PCB aroclors), with minimum detection limits ranging from 0.3 to 5 ng L^{-1}. Twenty OP

pesticides were included in the analysis, with minimum detection limits of 0.05 μg L^{-1} for chlorpyrifos, 0.04 μg L^{-1} for diazinon, and ranging from 0.04 to 0.33 μg L^{-1} for all other OPs. Minimum detection limits for the 15 carbamate pesticides ranged from 0.054 to 2.52 μg L^{-1}. The mean coefficient of variation for GCMS analyses of two sets of field duplicates was 23% for chlorpyrifos, 104% for diazinon, and 17% for all other chemicals detected. The mean coefficient of variation between results of split samples analyzed by both GCMS and ELISA was 35% for chlorpyrifos (n = 53) and 67% for diazinon (n = 54).

Enzyme-linked immunosorbent assays (ELISAs) for the pesticides diazinon and chlorpyrifos were conducted according to manufacturer's specifications (Beacon Analytical, Bedford, Ma). Two replicates of each sample were analyzed, and all duplicate measurements had coefficients of variation less than 15%. Measurements were compared to a five point standard curve. The lowest detectable dose was calculated from analysis of laboratory standards, according to the manufacturer's methodology, as the amount of the chemical of interest required to achieve a ratio of 85% between the mean absorbence of the standard and the mean absorbence of a negative control (Sullivan and Goh, 2000). Absorbence is inversely proportional to concentration. The lowest detectable dose was 0.03 μg L^{-1} for both diazinon and chlorpyrifos. The mean coefficient of variation for replicate measurements was 11.3% (n = 7 replicates per standard). The mean coefficient of variation for ELISA analyses of six sets of field duplicates was 7% for chlorpyrifos and 37% for diazinon.

Samples from one survey (2/1/1999) were analyzed for 16 PAH compounds using EPA Method 8310. Minimum detection limits ranged from 0.05 to 1.0 μg L^{-1}. Trace metals were analyzed using an Elan 6000 inductively-coupled plasma mass spectrometer (Perkin Elmer Corp., Norwalk, CT, U.S.A.). Eleven elements (Ag, Al, As, Cd, Cr, Cu, Mn, Ni, Pb, Se, and Zn) were measured with detection limits of 0.02 μg L^{-1}. Samples, blanks, matrix modifiers, and standards were prepared with ASTM Type II water and ultra clean chemicals, using clean techniques inside a positive pressure filtered-air laboratory.

2.5. Toxicity identification evaluations

Ten TIEs were conducted, including Phase I, II, and III TIEs, depending on specific objectives. All TIE treatments were tested with the 96 hr *C. dubia* mortality test, as described above. Phase I TIEs were designed to characterize the classes of compounds associated with sample toxicity (Mount and Anderson-Carnahan, 1988). All treatments, including an unmanipulated baseline sample, were tested in a series of dilutions that included a control and three to five concentrations, selected on the basis of original sample toxicity. All Phase I TIEs, with the exception of the filtration TIE described in the next paragraph, included the following treatments: adjustment of sample pH to selectively degrade potentially toxic compounds, removal of particulates by filtration (0.45 μm glass fiber) or centrifugation (see below), che-

lation of divalent cations (including many trace metals) with EDTA, neutralization of oxidants (such as chlorine) with sodium thiosulfate (STS), aeration to remove volatiles, solid-phase extraction (SPE) of non-polar organic compounds on C-8 columns, subsequent elution and add back (1 ×) of SPE-extracted compounds (to identify organic compound toxicity), and treatment with piperonyl butoxide (PBO) to block the metabolic activation of acetylcholinesterase-inhibiting OP pesticides (Ankley *et al*., 1991). Phase I TIEs were conducted on one sample each from Sites 3 and 6, and on two samples each from Sites 5 and 8 (Figure 1).

Phase I results indicated that filtration reduced toxicity in one sample from Site 6, and so a modified TIE was conducted to investigate sample constituents affected by filtration. This sample was split into aliquots for filtration, centrifugation, and solid-phase organic extraction, as well as for sequential combinations of these treatments. Centrifugation was at 4 °C and 2500 × *g* for 20 min. Filters and SPE columns were subsequently eluted with 100% methanol to recover any toxicity associated with adsorbed organic compounds.

Phase II TIEs were designed to identify compounds responsible for toxicity by isolating organic compounds in methanol fractions of decreasing polarity (Durhan *et al*., 1993). Samples were extracted on C-8 SPE columns, then sequentially eluted with increasing concentrations of methanol in water. Each methanol fraction was intended to recover a suite of compounds at a given polarity, along with any associated toxicity. The unmanipulated baseline sample and all sample treatments were tested at three concentrations and a control. Phase II TIEs were conducted on samples from Sites 6 and 8 (Figure 1).

A Phase III TIE (Mount and Norberg-King, 1993) was conducted on a sample from Site 6 to confirm the characterization and identification of compounds responsible for toxicity. The original sample was split into two aliquots: the untreated baseline and an aliquot to be treated by solid-phase extraction. The SPE column rinsate, from which organic compounds were removed, was then spiked with technical grade chlorpyrifos and diazinon to match the concentrations in the original sample. The SPE column was eluted with 100% methanol to recover compounds from the original sample that had been retained on the column, which were then added back (1×) to clean water for testing. The three solutions, untreated baseline, SPE eluate, and spiked rinsate, were then each diluted to six concentrations ranging from 1.5 to 50%. These were all tested with the 96 hr *C. dubia* mortality test, and diazinon and chlorpyrifos were measured by ELISA in each concentration.

All TIEs employed dilution series of each sample and treatment, allowing the calculation of toxic units (TU) to assist in sample characterization. Toxic units were calculated as 100 divided by the sample LC50. The toxic units of a specific chemical were calculated as the measured concentration in a sample divided by the compound's *C. dubia* LC50. Joint toxic units for diazinon plus chlorpyrifos, which are known to exhibit additive toxicity (Bailey *et al*., 1997), were calculated as the sum of the TUs for each pesticide.

3. Results

3.1. Spatial patterns of toxicity

Intermittent toxicity was observed in the main stem of the Salinas River. The furthest downstream sites, Sites 1 and 2, each had one sample out of 15 that exhibited significant *C. dubia* mortality. Site 5, below the urban storm drain, had 4 toxic samples out of 15 collected; and the furthest upstream site (Site 7) had no toxic samples (Figure 1). Of the samples collected from tributaries, Sites 3 and 4, in tile drain channels, had 1 and 3 toxic samples out of 15, respectively. At Site 6, the channel draining furrow runoff, all samples produced 100% *C. dubia* mortality. At Site 8, located in a combined urban/agricultural drainage channel, there were 13 toxic samples out of 15. There were no obvious or statistically significant temporal trends in toxicity, despite a sampling schedule that included 10 surveys at regular intervals and 5 targeting early season rain events (Table I).

3.2. Causes of toxicity at test sites

The furthest downstream site, at the upper end of the coastal lagoon, produced one toxic sample, with 0% *C. dubia* survival. Of the seven chemical compounds detected in this sample, only chlorpyrifos was measured at concentrations toxic to *C. dubia*, with a concentration more than three times the LC50 value (Table II; LC50 values given in Table III). No TIEs were conducted on samples from this site. Rainfall in the days prior to sampling produced runoff throughout the watershed (Table I), and river flow was at about twice the yearly average.

Site 2 produced only one sample with significantly reduced *C. dubia* survival (28%), and this sample was collected on the same date as the toxic sample from Site 1, located 1 km further downstream. As with Site 1, chlorpyrifos was the only chemical of eight detected that was measured at concentrations above the *C. dubia* LC50 (Table II). This concentration (0.079 μg L^{-1}) was measured by ELISA; chlorpyrifos was not detected by the GCMS scan. No TIEs were conducted on samples from this site. Samples from Sites 3 and 4, in the tile drain tributary that flows into the main river just above Site 2, had no significant toxicity at the time the toxic Site 2 sample was collected, indicating a further upstream source for this toxic runoff.

One toxic sample was collected from Site 3. *C. dubia* survival was 0%, and of 4 compounds detected in the sample, only diazinon was above the *C. dubia* LC50 (Table II). Chlorpyrifos was not detected by ELISA or GCMS, but both measured diazinon at concentrations above the LC50: 0.436 μg L^{-1} for ELISA, 0.570 μg L^{-1} for GCMS. There was no rainfall in the week prior to collection of this sample; summer runoff was dominated by tile drainage from agricultural irrigation. A Phase I TIE was conducted, providing three lines of evidence implicating diazinon. Toxicity was eliminated by SPE removal of non-polar organic compounds. Toxicity was not recovered in the eluate, as incomplete recovery of

TABLE I

Survival of *Ceriodaphnia dubia* in 96 hr exposures to samples from the study sites

Date	Rainfall	Site number															
	(cm)[r]	1		2		3		4		5		6		7		8	
		Mean	sd	Mean	sd	Mean	sd	Mean	sd	Mean	sd	Mean	sd	Mean	sd	Mean	sd
09/21/1998	0.0	100	0	96	9	96	9	100	0	0[a]	0	0[a]	0	100	0	0[a]	0
10/26/1998[c]	1.3	100	0	100	0	100	0	100	0	100	0	0[a]	0	–[b]	–	0[a]	0
11/30/1998[c]	1.6	100	0	100	0	100	0	100	0	40[a]	40	0[a]	0	100	0	100	0
12/14/1998	0.2	100	0	100	0	100	0	92	11	88	11	0[a]	0	100	0	0[a]	0
02/01/1999[c]	2.2	0[a]	0	28[a]	30	100	0	96	9	88	18	0[a]	0	100	0	36[a]	26
03/22/1999	0.4	100	0	100	0	100	0	16[a]	17	100	0	0[a]	0	100	0	100	0
05/10/1999[c]	0.0	100	0	100	0	100	0	100	0	92	11	0[a]	0	–[b]	–	0[a]	0
07/12/1999	0.0	100	0	100	0	0[a]	0	100	0	100	0	0[a]	0	100	0	0[a]	0
08/16/1999[c]	0.0	100	0	100	0	100	0	96	9	100	0	0[a]	0	100	0	0[a]	0
09/23/1999	0.0	100	0	100	0	97	7	52[a]	30	97	7	0[a]	0	–[b]	–	0[a]	0
10/04/1999	0.0	100	0	100	0	100	0	96	9	92	18	0[a]	0	–[b]	–	0[a]	0
11/02/1999[c]	0.0	96	9	96	9	88	27	100	0	100	0	0[a]	0	–[b]	–	20[a]	14
11/08/1999[c]	1.4	100	0	100	0	100	0	100	0	28[a]	39	0[a]	0	–[b]	–	0[a]	0
11/30/1999	0.4	96	9	96	9	96	9	96	9	92	11	0[a]	0	100	0	0[a]	0
01/18/2000[c]	3.1	100	0	97	7	100	0	0[a]	0	20[a]	35	0[a]	0	100	0	0[a]	0

[r] Rainfall during the 48 hr period prior to sample collection.

[a] *C. dubia* survival was significantly lower than in test controls.

[b] Sampled not collected because this reach of the river was dry.

[c] Chemical analysis for pestici des and PCBs was conducted on samples from these surveys (no carbamates or PCBs on samples from 11/30/1998).

TABLE II

Chemicals detected in samples exhibiting toxicity to *Ceriodaphnia dubia*[c]. Samples from each site were tested for toxicity during 15 separate surveys. All concentrations in μg L^{-1}

Chemicals detected	Concentration	Chemicals detected	Concentration
Site 1 Number of toxic samples: 1		Date: 02/01/1999	*C. dubia* Survival: 0%
Chlorpyrifos[b]	0.18[a]	Dieldrin	0.008
Diazinon	0.076	Endrin	0.006
4,4′-DDE	0.017	Gamma-BHC	0.005
4,4′-DDT	0.018		
Site 2 Number of toxic samples: 1		Date: 02/01/1999	*C. dubia* Survival: 28%
Chlorpyrifos	0.079[a]	Dieldrin	0.026
Diazinon	0.083	Endrin	0.006
4,4′-DDE	0.02	Gamma-BHC	0.003
4,4′-DDD	0.006	4,4′-DDT	0.014
Site 3 Number of toxic samples: 1		Date: 07/12/1999	*C. dubia* Survival: 0%
TIE conducted (see Table IV)		4,4′-DDD	0.006
Diazinon	0.44[a]	4,4′-DDE	0.017
Dieldrin	0.013		
Site 4 Number of toxic samples: 3			
Date: 03/22/1999	*C. dubia* Survival: 16%		
Diazinon	0.493[a]	Chlorpyrifos	0.032
Date: 09/23/1999	*C. dubia* Survival: 52%		
Diazinon	0.285		
Date: 01/18/2000	*C. dubia* Survival: 0%		
Chlorpyrifos	0.057[a]	4,4′-DDE	0.026
Diazinon	0.14	Dieldrin	0.031
Diuron	13	Endosulfan I	0.24
Site 5 Number of toxic samples: 4			
Date: 09/21/1998	*C. dubia* Survival: 0%		
No chemical measurements this survey			
Date: 11/30/1998	*C. dubia* Survival: 40%		
Chlorpyrifos	0.070[a]	Diuron	0.6
Diazinon	0.224	4,4′-DDE	0.032
Zinc	58.7		

[a] Chemical concentration above LC50 for *C. dubia* or related organism (see legend in Table III for LC50 values and references); [b] All chlorpyrifos and diazinon values in this table were measured by ELISA; [c] Metals are only listed if concentrations are greater than one tenth the LC50; [d] Mean values for chemical analyses of 8 of 15 toxic samples from Sites 6, and 7 of 13 toxic samples from Site 8.

TABLE II
(continued)

Chemicals detected	Concentration	Chemicals detected	Concentration
Site 5 (continued)	Number of toxic samples: 4		
Date: 11/08/1999	*C. dubia* Survival: 28%		
TIE conducted (see Table IV)		Chlorpyrifos	0.116[a]
Carbofuran	12[a]	Diazinon	0.376[a]
Date: 01/18/2000	*C. dubia* Survival: 20%		
TIE conducted (see Table IV)		Diuron	10
Chlorpyrifos	0.056[a]	Endosulfan I	0.12
Diazinon	0.055	Zinc	22.7
Chemicals detected	Mean concentration[d]	Chemicals detected	Mean concentration[d]
Site 6	Number of toxic samples: 15	All	*C. dubia* Survival: 0%
TIEs on 4 Samples (see Table IV)		Beta-BHC	0.033
Chlorpyrifos	0.390[a]	Delta-BHC	0.016
Diazinon	1.174[a]	Gamma-BHC	0.351
Fensulfothion	0.110	4,4′-DDD	0.032
Parathion methyl	0.130	4,4′-DDE	0.060
Tokuthion	0.180	4,4′-DDT	0.134[a]
Diuron	0.390	Dieldrin	0.044
Fenuron	1.940	Endrin	0.041
Chromium	30.7	Endosulfan I	0.053
Copper	31.2	Endosulfan sulfate	0.038
Zinc	99.4		
Site 7	Number of toxic samples: 0	(No toxic samples)	
Site 8	Number of toxic samples: 13	Mean	*C. dubia* Survival: 4.3%
TIEs on 3 samples (see Table IV)			
Chlorpyrifos	0.077[a]	Carbofuran	27[a]
Diazinon	0.46[a]	Beta-BHC	0.050
Disulfoton	0.22	Gamma-BHC	0.015
Diuron	11	4,4′-DDD	0.012
Fenuron	8.6	4,4′-DDE	0.019
Methomyl	3.6	4,4′-DDT	0.021
Monuron	0.15	Dieldrin	0.026
Chromium	5.7	Endrin	0.010
Zinc	24.3	Endosulfan II	0.011

[a] Chemical concentration above LC50 for *C. dubia* or related organism (see legend in Table III for LC50 values and references); [b] All chlorpyrifos and diazinon values in this table were measured by ELISA; [c] Metals are only listed if concentrations are greater than one tenth the LC50; [d] Mean values for chemical analyses of 8 of 15 toxic samples from Sites 6, and 7 of 13 toxic samples from Site 8.

TABLE III

Listing of all chemicals that were detected in samples from the study area, with concentrations compared to literature LC50 values (all concentrations in μg L^{-1}). Survival of *Ceriodaphnia dubia* and combined toxic units of chlorpyrifos and diazinon are given for each sample listed

Chemical	Sample with highest concentration		Highest concentration measured	Literature		Mean (sd) of all detected concentrations	n	*C. dubia* survival in sample	Diazinon and chlorpyrifos combined TUs
	Site	Date		LC50	Reference				
Aldrin	4	26/10/1998	0.010	4300	7	0.005 (0.005)	3	100	nd
Delta-BHC	6	26/10/1998	0.016	na		0.009 (0.010)	2	0	10
Beta-BHC	8	26/10/1998	0.050	na		0.037 (0.015)	4	0	1
Disulfoton	8	26/10/1998	0.22	52	2	0.22 (0)	1	0	1
Copper[a]	6	30/11/1998	101	200	11	11.8 (28.1)	all	0	33
Zinc	6	30/11/1998	321*	95	11	37.1 (78.2)	all	0	33
Chromium	6	30/11/1998	114*	45	4	12.1 (28.1)	all	0	33
4,4'-DDD	6	30/11/1998	0.055	0.170	8	0.015 (0.014)	26	0	33
4,4'-DDE	6	30/11/1998	0.38	1.4	8	0.033 (0.065)	50	0	33
4,4'-DDT	6	30/11/1998	0.56*	0.07	8	0.068 (0.146)	21	0	33
Dieldrin	6	30/11/1998	0.095	7.6	8	0.026 (0.020)	49	0	33
Endrin	6	30/11/1998	0.14*	0.051	10	0.029 (0.044)	14	0	33
Diuron	8	30/11/1998	18	160	2	3.8 (5.7)	13	100	nd
Chlorpyrifos	6	12/14/1998	3.2*	0.053	1	0.71 (0.90)[a]	52	0	60
Parathion methyl	6	01/02/1999	0.13	2.6	3	0.13 (0)	1	0	39
Prothiophos	6	01/02/1999	0.18	na		0.18 (0)	1	0	39
Gamma-BHC	6	01/02/1999	0.66	5.1	5	0.14 (0.23)	13	0	39
Endosulfan II	8	01/02/1999	0.011	na		0.011 (0)	1	36	3
Endosulfan sulfate	6	22/03/1999	0.054	na		0.038 (0.023)	2	0	33

TABLE III
(continued)

Chemical	Sample with highest concentration		Highest concentration measured	Literature		Mean (sd) of all detected concentrations	n	*C. dubia* survival in sample	Diazinon and chlorpyrifos combined TUs
	Site	Date		LC50	Reference				
Fensulfothion	6	10/05/1999	0.11	10	2	0.11 (0)	1	0	3
Diazinon	6	08/16/1999	5.2*	0.32	1	0.87 (1.5)*	75	0	21
Fenuron	8	16/08/1999	12	na		3.5 (3.3)	13	0	18
Unionized ammonia	6	23/09/1999	624	1350	4	0.064 (0.11)	82	0 [b]	2.31 [b]
Chloropropham	3	02/11/1999	2.3	na		2.3 (0)	1	100	nd
Methomyl	8	02/11/1999	3.6	47	5	3.6 (0)	1	20	2
Monuron	8	02/11/1999	0.15	na		0.15 (0)	1	20	2
Carbofuran	4	08/11/1999	40*	0.23	4	28.3 (12.1)*	4	100	nd
Endosulfan I	4	18/01/2000	0.24	52.9	9	0.10 (0.082)	10	0	nd
Propham	6	18/01/2000	10	19000	6	10 (0)	1	0	30

* Asterisks indicate that a measured concentration exceeds a known LC50 value.

[a] All other metals concentrations were at least an order of magnitude below C. dubia LC50 values. Mean (sd) values for eight metals not listed above were: Ag 0.04 (0.04); Al 5550 (14 050); As 4.02 (2.63); Cd 0.43 (0.64); Mn 205 (423); Ni 10.39 (12.13); Pb 5.40 (13.07); Se 3.49 (3.68). Each of the metals was above detection limits in all samples analyzed.

[b] ELISA measurements; GCMS data not available. All others measured by GCMS.

References: 1: *Ceriodaphnia dubia*, Bailey *et al*., 1997; 2: *Gammarus fasciatus*, Johnson and Finley, 1980; 3: *Ceriodaphnia dubia*, Norberg-King *et al*., 1991; 4: *Ceriodaphnia dubia*, Bailey *et al*., 1996; 5: *Gammarus italicus*, Pantani *et al*., 1997; 6: *Gammarus fasciatus*, Sanders, 1970; 7: *Gammarus fasciatus*, Sanders, 1972; 8: *Hyalella azteca*, Phipps *et al*., 1995; 9: *Daphnia magna*, Schoettger, 1970; 10: *Gammarus fasciatus*, Trnkova, 1977; 11: *Ceriodaphnia dubia*, Schubauer-Berrigan *et al*., 1993.

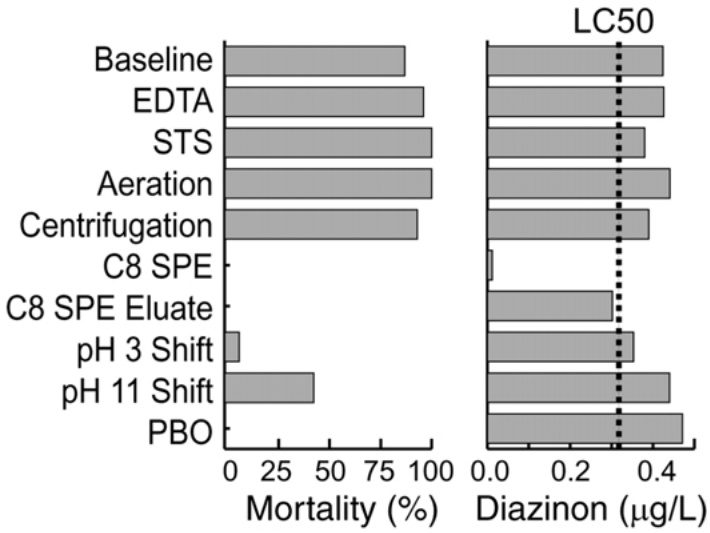

Figure 2. Phase I toxicity identification evaluation results of a sample from Site 3 (7/21/1999). Treatments listed on the left axis are described in the text. EDTA = ethylenediaminetetraacetic acid, STS = sodium thiosulfate, C8 SPE = solid-phase extraction on a C-8 column, and PBO = piperonyl butoxide.

diazinon resulted in an eluate concentration below the LC50 value (Figure 2). Toxicity was reduced by acidifying the sample to pH 3 for 3 hr and then restoring original pH for testing. This result was consistent with the hydrolytic degradation of diazinon at low pH. (Bailey *et al*., 1996, observed a 45% reduction in diazinon recovery following 3 hr pH 3 treatment). The third line of evidence was the elimination of toxicity by addition of PBO. Diazinon was present in the PBO treatment at the original baseline concentration, but toxicity was eliminated in the presence of PBO, which blocks metabolic activation of this OP insecticide (Table IV).

The three toxic samples from Site 4 were collected on dates other than those on which toxic samples were collected from Site 3 (the adjacent tile drain) or Site 2 (in the main river just downstream (Table I). Only ELISA measurements were available in the first 2 of 3 toxic samples from this site. In the first sample (*C. dubia* survival 16%), diazinon was measured above the LC50 (Table II). In the second toxic sample (*C. dubia* survival 52%), neither diazinon nor chlorpyrifos were above the LC50. In the third toxic sample, collected after 48 hr rainfall of 3.1 cm, *C. dubia* survival was 0%. Chlorpyrifos was measured by ELISA at about the LC50, but was not detected by GCMS. None of the 5 other chemicals detected were near known toxic concentrations (Table II). No TIEs were conducted on samples from this site.

Four samples collected in the main stem of the river downstream of the urban storm drain at Site 5 were toxic to *C. dubia* (Figure 1, Table I). The first toxic sample was not analyzed chemically. In the second toxic sample, collected after

TABLE IV
Summary of toxicity identification evaluation (TIE) results

Site #	Date	Land use	Baseline sample toxicity (TUs[a])	Effective TIE treatments	Chemicals detected above LC50	Conclusion: Toxicity due to:
5	11/30/1999	Urban	1.3	Phase I: SPE[b], PBO[c]	Chlorpyrifos,[d] Diazinon,[d] Carbofuran	Chlorpyrifos, Diazinon
5	01/18/2000	Urban	1.1	Phase I: All except PBO	None	Unknown [e]
8	05/10/1999	Urban/Ag	1.4	Phase I: SPE, PBO	Diazinon	Diazinon
8	01/18/2000	Urban/Ag	1.3	Phase I: SPE, (PBO)	Chlorpyrifos[d]	Unknown organic (OP)[f]
3	07/12/1999	Ag (tile-drain)	1.3	Phase I: SPE, pH, PBO	Diazinon	Diazinon
6	12/14/1998	Ag (furrow)	>16	Phase I: SPE, filtration	Chlorpyrifos, Diazinon[d]	Organic compound
6	02/01/1999	Ag (furrow)	>16	Partial: SPE, filtration[g]	Chlorpyrifos, Diazinon	Chlorpyrifos, Diazinon
6	03/22/1999	Ag (furrow)	>20	Phase II fractionation	Chlorpyrifos	Chlorpyrifos
6	01/18/2000	Ag (furrow)	20	Phase III confirmation	Chlorpyrifos, Diazinon	Chlorpyrifos, Diazinon

[a] Toxic units are 100 divided by the sample LC50, calculated from responses in a series of sample dilutions.
[b] SPE = solid-phase extraction on C-8 columns.
[c] PBO = piperonyl butoxide, which blocks the metabolic activation of chloinesterase-inhibiting organophosphate pesticides.
[d] Concentrations measured by enzyme-linked immunosorbent assays (ELISA) only; all others measured by both ELISA and GCMS.
[e] Sample was analyzed for volatile organics, but none were detected, possibly because of loss during sample storage and handling.
[f] Conclusions uncertain because PBO only partially reduced toxicity.
[g] This TIE employed only filtration, centrifugation and SPE treatments, with subsequent elution of columns and filters. Each Phase I TIE used all Phase I treatments as described in the Methods Section.
Phases II and III treatments are also described in the Methods Section.

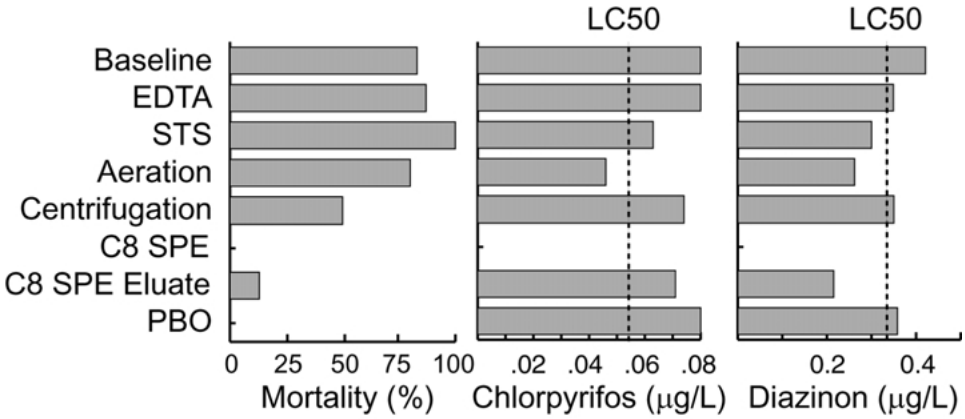

Figure 3. Phase I toxicity identification evaluation results of a sample from Site 5 (11/30/1999). Treatments listed on the left axis are described in the text, and abbreviations are as in Figure 2.

1.6 cm of rainfall, chlorpyrifos was the only chemical measured above the LC50. A TIE was conducted on the third toxic sample from this site, which was collected after rainfall of 1.4 cm. Both chlorpyrifos and diazinon were measured above LC50 values, and toxicity was eliminated by solid-phase extraction of organic compounds and by treatment with PBO (Figure 3). Incomplete recovery of toxicity in the SPE column eluate was coincident with incomplete recovery of diazinon. Centrifugation slightly reduced both toxicity and diazinon. Carbofuran was measured at well above the LC50 value (Tables II and III). However, carbofuran was measured at concentrations more than three times higher in another sample that exhibited no toxicity (Table III). Examination of laboratory blanks and other quality assurance data did not indicate carbofuran contamination during chemical analysis, and it is not clear why the elevated carbofuran concentrations measured during this survey did not correspond predictably with toxicity test results. It does not appear that carbofuran was related to toxicity in this sample, since carbofuran toxicity is not affected by PBO, and PBO eliminated toxicity in the TIE.

A TIE was also conducted on the fourth toxic sample from Site 5, which was collected 10 weeks after the sample described above, and following 3.1 cm of rainfall. Chlorpyrifos was measured at approximately the LC50, with no other chemicals measured at toxic concentrations (Table II). However, PBO did not reduce sample toxicity, while all of the other TIE treatments did, indicating that toxicity was not caused by OP pesticides. TIE results suggested that mixtures of constituents, possibly including volatile compounds, were responsible for toxicity. In a subsequent chemical analysis for volatile organics, none were detected; and the cause of toxicity in this sample remains unknown.

Site 8, in the channel draining both urban and agricultural areas, produced 13 toxic samples in 15 surveys. Chlorpyrifos was measured above the LC50 in eight of the toxic samples, diazinon was measured above the LC50 in nine of the toxic

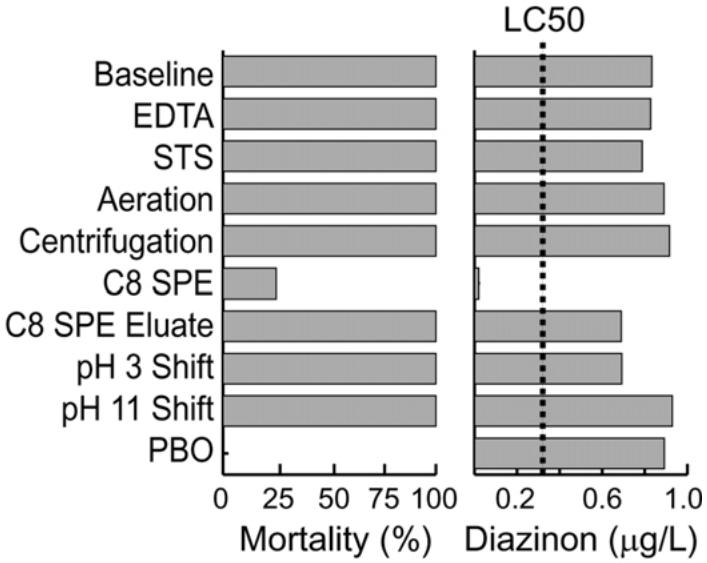

Figure 4. Phase I toxicity identification evaluation results of a sample from Site 8 (5/19/1999). Treatments listed on the left axis are described in the text, and abbreviations are as in Figure 2.

samples, and both were above LC50 values in four. Neither was above the LC50 in one of the non-toxic samples, and diazinon was measured at just above the LC50 in the other.

A Phase I TIE was conducted on a sample from this site (Figure 4). Diazinon was measured at more than twice the LC50 in the baseline sample, toxicity and diazinon were reduced by solid-phase extraction of organic compounds, both were recovered in the SPE column eluate, and toxicity was eliminated by PBO. Each of these results provided evidence that diazinon was the likely cause of toxicity. Sample treatment at pH 3 decreased the diazinon concentration, but it was still well above the LC50, and there was no decrease in toxicity.

A Phase II TIE was conducted on a second sample from Site 8, collected seven months after the Phase I TIE described above. Chlorpyrifos was measured above the LC50 in the original test of this sample, but not in the subsequent TIE tests. PBO treatment reduced *C. dubia* mortality from 100 to 60%, and solid-phase organic extraction removed toxicity completely. However, toxicity was only recovered in the full strength methanol eluate of the SPE column, and was not recovered in any of the sequential methanol fractions that would help identify toxic constituents. PAH compounds were considered because of possible influence from urban runoff, but none were detected in chemical analyses. The results indicated toxicity due to a mixture of organic constituents at moderately toxic concentrations, possibly including chlorpyrifos.

At Site 6, a modified creek channel collecting agricultural furrow runoff, samples from every survey produced 100% *C. dubia* mortality. Chlorpyrifos was measured

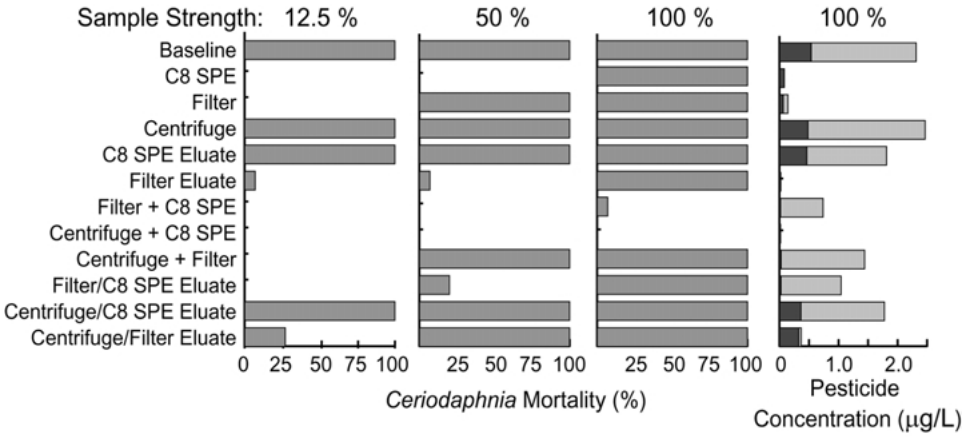

Figure 5. Evaluation of filtration, centrifugation, and C-8 column solid-phase extraction (Column) treatments on sample toxicity and pesticide concentrations at Site 6 (2/3/1999). The order in which treatments are listed is the order in which samples were treated in sequential manipulations; thus Centrifuge + Filter indicates sample was first centrifuged then filtered. In the filter/column eluate, the column was eluted. Dark bars in the graph at right represent chlorpyrifos concentrations, and lighter bars represent diazinon concentrations.

at more than double the LC50 concentration in every sample. Diazinon exceeded the LC50 in 10 of 15 surveys. Numerous other chemicals were measured at elevated concentrations in samples from this site; in one sample, eight chemicals were at their highest concentrations, four of them above LC50 values (Table III).

Four TIEs were conducted on samples from this site: a Phase I TIE, an evaluation of filtration treatments (Figure 5), a Phase II TIE (Figure 6), and a Phase III TIE (Figure 7). All of the TIE samples were highly toxic, each with more than 16 toxic units (Table III). The Phase I TIE resulted in toxicity reduction by solid-phase extraction, with the toxicity recovered in the SPE column eluate. Filtration reduced toxicity by nearly as much as the SPE column in dilutions up to 100% sample. This indicated toxicity due to organic compounds, possibly associated with suspended particles.

In a modified TIE to evaluate filtration effects, a second sample was treated with filtration, centrifugation, and solid-phase extraction, as well as combinations of these treatments and methanol elutions of filters and columns (Figure 5). The SPE column removed toxicity at sample concentrations up to 50%, the glass fiber filter removed toxicity in sample concentrations up to 25%, and centrifugation did not remove toxicity even at 12.5%, the lowest concentration tested (Figure 5; 25% dilution not shown). Filters and SPE columns also strongly reduced OP pesticide concentrations, while centrifugation did not. Filters reduced toxicity even when the sample was centrifuged to remove particles prior to filtration. Methanol eluates of SPE columns were highly toxic, and methanol eluates of filters, even those that filtered samples after centrifugation, recovered some of the original toxicity.

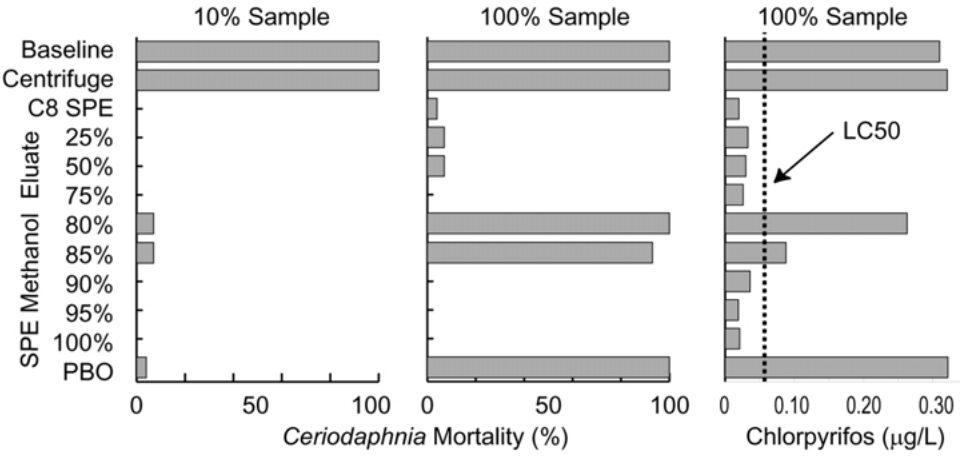

Figure 6. Results of a Phase II toxicity identification evaluation of a sample from Site 6 (3/24/1999). C8 SPE = solid-phase extraction on a C-8 column, PBO = piperonyl butoxide, and percentage values on the left axis represent concentrations of methanol in water that were sequentially pumped through the C-8 column after the column was used to extract the original sample. The diazinon concentration was at about half the LC50 value in the sample baseline.

These results indicated that filters might reduce toxicity primarily by adsorbing pesticides from solution rather than by removing particulates or particle-associated compounds. Consequently, centrifugation, rather than filtration, was used to remove particles in all subsequent TIEs, including all reported here except the above Phase I TIE at Site 6.

In a Phase II TIE of a Site 6 sample, toxicity was reduced after PBO treatment in the 10% sample, but the blocking capacity of PBO was apparently overwhelmed in 100% sample, which had more than 20 toxic units (Figure 6, Table III). Both toxicity and chlorpyrifos were recovered in the 80 and 85% methanol fractions, a result identical to that first described by Bailey *et al*. (1996).

To confirm the identification of OP pesticides as the toxic agents, a Phase III TIE was conducted. Original baseline sample, SPE column eluate, and SPE column rinsate spiked with technical grade diazinon and chlorpyrifos were each tested at 6 concentrations and a control (Figure 7). Because the toxicity of chlorpyrifos and diazinon has previously been demonstrated to be additive (Bailey *et al*., 1997), Figure 7 shows toxic units for the combination of these two compounds. Toxicity and toxic units of diazinon and chlorpyrifos in the SPE column eluate were about half that in the original sample, indicating incomplete recovery, as has been previously observed (Bailey *et al*., 1996). The rinsate, stripped of organic compounds by SPE, was then spiked with technical grade diazinon and chlorpyrifos. Their measured concentrations matched those of the original sample, and with diazinon and chlorpyrifos as the only organic compounds present in the matrix, the sample

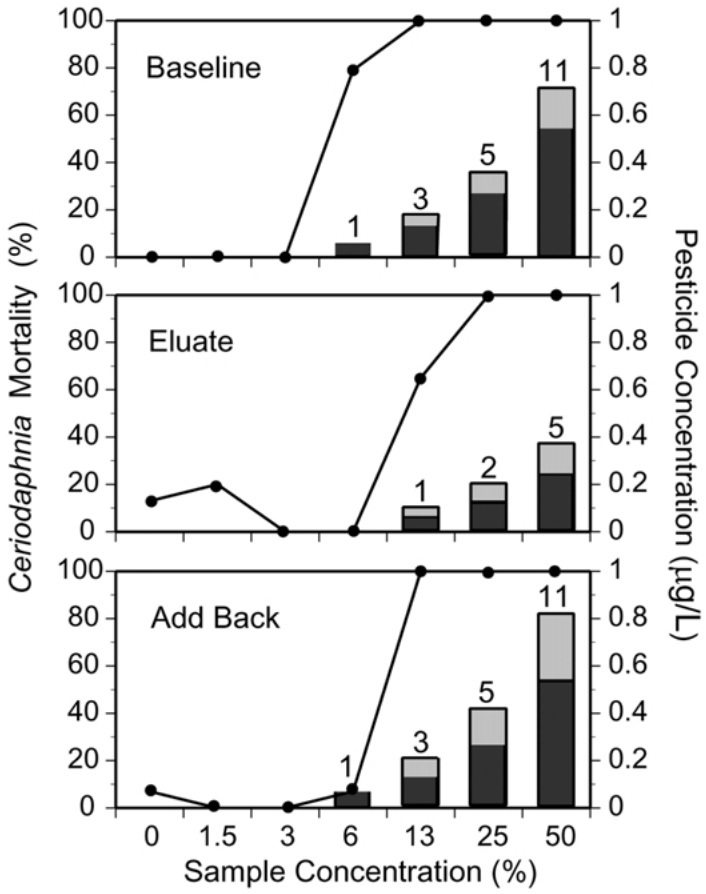

Figure 7. Results of a Phase III toxicity identification evaluation of a sample from Site 6 (2/1/2000). Each of the three treatments was diluted to 6 concentrations and a control. Dark bars represent chlorpyrifos concentrations, and lighter bars represent diazinon concentrations. Numbers above the bars are joint toxic units for the additive toxicity of these two compounds (see text). Curves indicate *C. dubia* mortality at each treatment concentration. Baseline is untreated sample, eluate was produced by extracting the sample on a C-8 column, recovering the extracted compounds in a methanol eluate, and adding the eluate to a volume of clean water equal to the volume of the extracted sample. Add Back is a solution prepared by adding reagent grade chlorpyrifos and diazinon to the sample rinsate after extraction of organic compounds on the C-8 column.

was nearly as toxic as the original, with the only difference occurring at the 6% sample concentration.

At Site 7, in the main stem of the river upstream from all the other sites, the river was dry during six surveys. There were no toxic samples in any of the other nine surveys. Diazinon was detected twice, and chlorpyrifos once, both at concentrations below their LC50 values. DDE and dieldrin were detected in one sample

from this site, both at concentrations three orders of magnitude below their *C. dubia* LC50s.

3.3. Possible alternative causes of toxicity

Of 61 samples analyzed for at least 78 compounds, only 5 samples had concentrations of compounds other than diazinon or chlorpyrifos above published LC50 values for either *C. dubia* or *Hyalella azteca* (the commonly tested resident freshwater amphipod which was used to screen chemicals for which no *C. dubia* data were available). Four samples exceeded *C. dubia* LC50 values for carbofuran, and one sample exceeded the *C. dubia* LC50 values for both DDT and zinc. The sample with the elevated DDT and zinc concentrations had sufficient amounts of fine particulates to cloud the water even after overnight settling and decanting, something not noticed in other samples from this study (personal observation). The two samples with the highest carbofuran levels were both non-toxic, and toxicity in the other two samples with elevated carbofuran coincided with high concentrations of diazinon and chlorpyrifos (Table III).

Unionized ammonia was never measured at more than 46% of the LC50 (Table III). Total suspended solids did not correlate significantly with toxicity, though the four samples with the highest TSS concentrations (680 to 1190 mg L^{-1}) were significantly toxic. Each of these were from Site 6 and contained more than 4 TU of chlorpyrifos.

3.4. Chlorpyrifos, diazinon, and toxicity in the watershed

Diazinon was detected in 63% of the watershed samples analyzed by ELISA, and was detected in 52% of the samples from the main stem of the Salinas River (Table V). The highest diazinon concentration measured in the watershed was 5.8 μg L^{-1} (18 toxic units) at Site 6, and the highest concentration measured in the main stem of the river was just above the LC50 at 0.38 μg L^{-1} (1.2 TU) at Site 5, below an urban storm drain. The diazinon water quality criterion for the protection of freshwater aquatic life, 0.080 μg L^{-1} (Menconi and Cox, 1994; International Joint Commission Canada and United States, 1987), was exceeded in 15% of the main stem river samples (Table V).

Chlorpyrifos was detected in 44% of the samples analyzed by ELISA, and was detected in 27% of the samples from the main stem of the river (Table V). The highest chlorpyrifos concentration measured in the watershed was 3.2 μg L^{-1} (60 TU) at Site 6, and the highest concentration measured in the main stem of the river was 0.20 μg L^{-1} (3.8 TU) at Site 1, near the wildlife refuge. The USEPA (1999) criteria maximum concentration of 0.083 μg L^{-1} was exceeded in 8% of the main stem river samples, and measured chlorpyrifos concentrations were above the *C. dubia* LC50 in 16% of the main stem river samples (Table V).

Because diazinon and chlorpyrifos are both acetylcholinesterase-inhibiting OP pesticides that exhibit additive toxicity when present together (Bailey *et al.*, 1997),

TABLE V
Incidence of diazinon and chlorpyrifos in samples from the lower Salinas River and tributaries

	Diazinon		Chlorpyrifos	
	Watershed	Main stem river	Watershed	Main stem river
Number of samples analyzed	121	54	113	51
Percentage of samples with compound detected[a]	63%	52%	44%	27%
Percentage of samples measured above *C. dubia* LC50[b]	17%	2%	30%	16%
Percentage of samples measured above 10th centile acute[c]	10%	0%	17%	4%
Percentage of samples measured above WQC[d]	36%	15%	19%	8%

[a] Lowest detectable dose = 0.03 μg L^{-1} for both diazinon and chlorpyrifos enzyme-linked immunosorbant assays (ELISAs).
[b] *C. dubia* LC50 values are 0.32 μg L^{-1} for diazinon and 0.053 μg L^{-1} for chlorpyrifos (Bailey *et al*., 1997).
[c] The 10th centile acute values from previous risk assessments are 0.483 μg L^{-1} for diazinon (Tierney *et al*., 1997) and 0.102 μg L^{-1} for chlorpyrifos (Geisy *et al*., 1999).
[d] WQC = water quality criteria are 0.080 μg L^{-1} for diazinon (Menconi and Cox, 1994; IJCCUS, 1987) and 0.083 μg L^{-1} for chlorpyrifos (CMC; USEPA, 1999).

their combined concentrations can be presented as joint toxic units (Figure 8). Thus, a sample with one joint toxic unit (dashed line) should produce 50% *C. dubia* survival, depending on relative bioavailability and the presence of other compounds in toxic concentrations. Mean *C. dubia* survival in all samples having less than 1 joint TU was 97±8% (n = 80), and mean *C. dubia* survival in all samples having more than 1 joint TU was 34 ± 43% (n = 47). All samples collected in the watershed exhibiting survival of 50% or less had more than 1 joint TU of diazinon plus chlorpyrifos (Figure 8).

4. Discussion

This study identified patterns, sources, and causes of ambient toxicity in this urban and agricultural watershed. The toxicity tests using *Ceriodaphnia dubia* were able to resolve differences in biologically active contaminant loadings in a variety of tributaries draining different land use areas. The toxicity tests and chemical analyses clearly identified the OP pesticides chlorpyrifos and diazinon as pervasive in both urban and agricultural drains, and toxicity identification evaluations linked these compounds to toxicity in samples from a variety of sources.

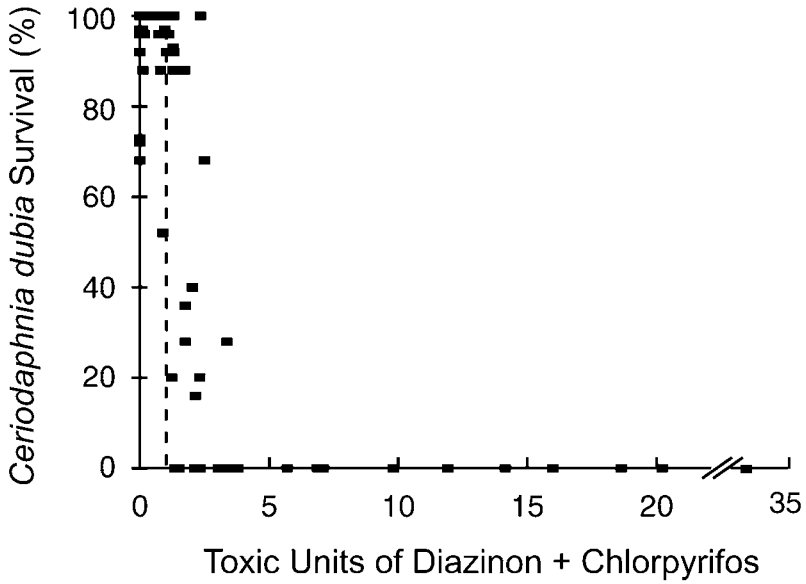

Figure 8. Relationship between toxicity and concentrations of diazinon and chlorpyrifos in samples from throughout the watershed. Concentrations of these additively toxic compounds are represented by joint toxic units (see text).

4.1. Characterization of runoff from different land uses

During this study, no toxic samples were collected from Site 7 at the furthest upstream point in the main stem of the river, above the sampled tributaries. That result was consistent with a previous study that sampled only that one site in the watershed 27 times over a one year period, while observing no significant mortality in 96 hr *C. dubia* or fathead minnow tests (Ganapathy *et al.*, 1997). In the present study, the river reach at Site 7 was dry in 6 out of 15 surveys, because the sandy substrate upstream allows low river flows to go underground. Upstream inputs that might contain anthropogenic chemicals would tend to be diluted during higher flows that reach the site. Eleven km downstream, the tributary on which Site 6 was located enters the main stem of the river. This highly modified creek channel primarily receives surface furrow runoff from intensive year-round irrigated agriculture, as headwater flows from the adjacent Gavilan mountains go underground above the study area during most of the year. Water from this channel produced 100% mortality in every survey, and had the highest concentrations of diazinon and chlorpyrifos, as well as the highest concentrations of 17 out of the other 29 compounds detected in this study (Table III). Samples from tributaries conveying tile drain runoff (Sites 3 and 4) were toxic far less frequently and had lower contaminant concentrations, despite similar cropping patterns, irrigation, and pesticide application rates. It is reasonable to assume that significant quantities of agricultural chemicals are removed from runoff water during percolation through

soil to subsurface tile drains before runoff is conveyed to the river during low to moderate flow regimes.

The mixture of urban and agricultural runoff flowing in the drainage channel at Site 8 was toxic to *C. dubia* in 13 of 15 surveys. This channel conveys runoff from two-thirds of the City of Salinas, as well as from extensive agricultural areas upstream. The fact that diazinon and chlorpyrifos were identified as causes of toxicity here does not clarify the land use practices responsible for contaminant loadings, since both compounds are used widely in agricultural, urban and residential settings. Hoffman *et al*. (2000) found that insecticide use was similar in urban and agricultural areas, and that diazinon and chlorpyrifos were among the four most frequently detected insecticides in urban streams. Other studies have indicated that within urban areas, runoff from residential areas may convey greater pesticide loadings than runoff from industrial areas (Bailey *et al*., 2000). Previous studies of this subwatershed have observed significant sediment toxicity at Site 8 and elsewhere downstream into the Moss Landing Harbor (Downing *et al*., 1998). While the sources and causes of that sediment toxicity were not determined, its occurrence serves as a reminder that the present study evaluated only the water matrix, and that biological resources in the Salinas River watershed may also be affected by contaminants associated with sediments. The commonly tested freshwater benthic amphipod *Hyalella azteca*, for example, has a chlorpyrifos 10 d LC50 value of 0.086 μg L^{-1} (Phipps *et al*., 1995), only slightly higher than that of the free-swimming *C. dubia*.

Site 5, in the main stem of the river just downstream of a culvert draining exclusively urban runoff from the other one-third of the City of Salinas, produced four toxic samples in 15 surveys. Three of those toxic samples were collected after rain events. Chlorpyrifos and diazinon were identified as causes of toxicity at this site in one TIE, but the cause of toxicity was not resolved in another sample from this site, collected after 3.1 cm of rainfall, 10 weeks after collection of the first TIE sample. The two furthest downstream sites (1 and 2) each produced toxic samples only once, both during the same February survey following 2.2 cm of rainfall. Chlorpyrifos was measured at concentrations above the LC50 value in both samples.

4.2. Causes of ambient toxicity

Diazinon and/or chlorpyrifos were specifically implicated as causes of *C. dubia* mortality in 6 of 9 TIEs, and one or both were the most probable cause of toxicity in two other Phase I TIE characterizations (Table IV). Diazinon and/or chlorpyrifos were identified in TIE samples from a variety of sources, including runoff from surface furrows, tile drains, urban surfaces, and in the main stem of the river downstream of an urban storm drain. Only one TIE, the second at Site 5, indicated the cause of toxicity might be other than diazinon or chlorpyrifos.

Thirty-one chemicals were detected in samples from throughout the watershed, including organochlorine, OP, and carbamate pesticides, as well as trace metals. No PCBs were detected in 56 samples for which they were analyzed, and no PAHs were detected at any site in one survey. Of the detected chemicals, only chlorpyrifos, diazinon, carbofuran, DDT, endrin, zinc and chromium were found above available LC50 values for daphnids or amphipods (Table III). The two samples with the highest carbofuran concentrations were not toxic, and the potentially toxic concentrations of the other four chemicals were all from the same sample, which also contained more than 30 toxic units of chlorpyrifos and diazinon (Table III). Toxicity caused by these two OP insecticides has been documented in a number of watersheds throughout California (De Vlaming *et al*., 2000).

4.3. Ecological implications

The *C. dubia* toxicity test is a useful tool for screening ambient waters to detect loadings of numerous chemicals. It is logistically simple, widely documented, of short duration, and highly sensitive to a variety of chemicals, including OP and other insecticides. Tierney *et al*. (1997) found *C. dubia* to be the second most sensitive of 63 test organisms for which acute diazinon toxicity data were available, and the test has been used successfully to identify contaminated ambient waters in numerous studies (De Vlaming *et al*., 2000). Previous reviews have shown high levels of concordance between toxicity to *C. dubia* and in-stream ecological impacts (Eagleson *et al*., 1990; Waller *et al*., 1996; De Vlaming and Norberg King, 1999). Other reviewers have had difficulty finding data to validate the test in systems dominated by OP pesticides (Tierney *et al*., 1997), but a number of studies have concluded that tests with daphnids are useful qualitative predictors of field effects on resident species exposed to chlorpyrifos (e.g., Van Wijngaarden *et al*., 1996; Van der Hoeven and Gerritsen, 1997).

Ecological risk assessments for diazinon and chlorpyrifos have used available acute effects concentrations (48 hr LC50s) to estimate 10th centile values for toxicity to all aquatic organisms (Geisy *et al*., 1999; Tierney *et al*., 1997). Concentrations above the 10th centile values are expected to be acutely toxic to at least 10% of aquatic species. The 10th centile value for diazinon was exceeded in 10% of the watershed samples, but in none of the main stem river samples. The 10th centile value for chlorpyrifos was exceeded in 17% of watershed samples and in 4% of main stem river samples (Table V). While these values indicate a potential for ecological impacts, they consider only 48 hr mortality and not chronic effects. If resident organisms were subjected to longer pesticide exposures, lethal or sublethal effects would likely occur at lower concentrations, putting a greater number of species at risk.

The in-stream concentrations of OP pesticides measured during this study suggest that these relatively short-lived chemicals are being continually applied and transported into the river system. In 1998, the first year of this study, 26 538 kg

of chlorpyrifos and 26 941 kg of diazinon (active ingredients) were applied to farmland in the Salinas River watershed. Residential and industrial uses are not known, but may be similar on a mass per land area basis (Hoffman *et al*., 2000). At Site 6, the channel receiving year-round furrow runoff from irrigated fields, the *average* concentrations (measured by GCMS; n = 7), were 1.49 μg L^{-1} chlorpyrifos (28 TU) and a similar 1.48 μg L^{-1} diazinon (4.6 TU). When seasonal river flow is low relative to inputs from tributary drains, the likelihood of impacts to a larger portion of the aquatic community increases.

Under these exposure regimes, effects may not be limited to aquatic invertebrates. The Salinas River is utilized by resident and migratory waterfowl, including endangered species, that are known to ingest OP pesticides and other contaminants during preening. Available data suggest that some birds, including juvenile waterfowl species, have LD50 values for diazinon that are 100 fold lower than those for mammals (Larkin and Tjeerdema, 2000). We do not know what pesticide levels these animals might ingest while preening wet feathers in a river system that consistently receives contaminated runoff.

The river system is also used as a migration corridor for threatened salmonids, including steelhead *Oncorhynchus mykiss* (Busby *et al*., 1997). While acute mortality in fish occurs at much higher OP concentrations, recent evidence suggests that diazinon affects sensitive salmonid olfactory organs at concentrations as low as 0.3 μg L^{-1} (Scholz and Collier, 2000; Scholz *et al*., 1999), a concentration comparable to the *C. dubia* LC50 (Bailey *et al*., 1997). Diazinon concentrations observed in the present study would be sufficient to disrupt nervous system function, reproductive physiology, and predator avoidance behaviors related to survival of exposed salmonids (Scholz and Collier, 2000).

Additional recent studies have shown that amphibians from populations downwind of intensive agricultural areas in California had tissue concentrations of diazinon and chlorpyrifos as high as 190 ppb wet weight, and a majority of individuals in the exposed populations exhibited acetylcholinesterase inhibition responses indicative of nervous system impacts (Sparling *et al*., 2001). Thus, while the *C. dubia* test serves as a useful screening tool, it also indicates the presence of bioavailable contaminants at concentrations that might adversely impact key migratory fish and other ecologically important species in this and other urban/agricultural watersheds.

5. Conclusions

This watershed-based assessment has identified urban runoff from residential areas and furrow runoff from irrigated fields as primary sources of chlorpyrifos and diazinon to the Salinas River. These pesticides were frequently found at concentrations causing *C. dubia* mortality in laboratory tests and TIEs, and have the potential to cause adverse effects in other ecologically important organisms. The results of

this study have led to current investigations of resident aquatic invertebrate populations, but additional studies may be warranted to investigate the potential impacts of these compounds on fish, amphibian, and bird species that utilize the river and its estuarine wildlife refuge.

Acknowledgements

We would like to thank Thelma Puckett and the members and growers of the Monterey County Farm Bureau, Kathleen Thomasberg and Russ Yoshimaru of the Monterey County Water Resources Agency, Tom Lockhart of the Natural Resources Conservation Service, Ron Cole of the City of Salinas Public Works Department, and Alison Jones of the Central Coast Regional Water Quality Control Board for providing information on regional land use and hydrology, as well as assistance in site selection and experimental design. Witold Piekarski of the California Department of Fish and Game assisted with field studies. Trace metal analyses were conducted by Jon Goetzl, California Department of Fish and Game Trace Metal Analytical Facility at Moss Landing, and organic GCMS analyses were conducted by BC Laboratories, Inc. Funding was provided by the State Water Resources Control Board, Division of Water Quality (Contract # 7-093-250-0).

References

Ankley, G. T., Dierkes, J. R., Jensen, D. A. and Peterson, G. S.: 1991, 'Piperonyl butoxide as a tool in aquatic toxicological research with OP insecticides', *Ecotoxicol. Environ. Saf.* **21**, 266–274.

Bailey, H. C., DiGiorgio, C., Kroll, K., Miller, J. L., Hinton, D. E. and Starrett, G.: 1996, 'Development of procedures for identifying pesticide toxicity in ambient waters: Carbofuran, diazinon, chlorpyrifos', *Environ. Toxicol. Chem.* **15**, 837–845.

Bailey, H. C., Miller, J. L., Miller, M. J., Wiborg, L. C., Deanovic, L. and Shed, T.: 1997, 'Joint acute toxicity of diazinon and chlorpyrifos to *Ceriodaphnia dubia*', *Environ. Toxicol. Chem.* **16**, 2304–2308.

Bailey, H. C., Deanovic, L. A., Reyes, E., Kimball, T., Larson, K., Cortright, K., Connor, V. and Hinton, D. E.: 2000, 'Diazinon and chlorpyrifos in urban waterways in northern California, U.S.A.', *Environ. Toxicol. Chem.* **19**, 82–87.

Busby, P. J., Wainwright, T. C., Bryant, G. J., Lierheimer, L. J., Waples, R. S., Waknitz, F. W. and Lagomarsino, I. V.: 1997, 'Status Review of West Coast Steelhead from Washington, Idaho, Oregon, and California', NOAA-NMFS, Technical Memorandom MNFS-NWFSC-27. National Oceanic and Atmospheric Administration, Springfield, VA, U.S.A.

CFBF: 2000, California Farm Bureau Federation Website: http://www.cfbf.com.

CDPR: 2000, California Department of Pesticide Regulation Website: http://www.cdpr.ca.gov.

De Vlaming, V. and Norberg-King, T. J.: 1999, 'A Review of Single Species Toxicity Tests: Are the Tests Reliable Predictors of Aquatic Ecosystem Community Responses?' EPA 600/R-97/11, *Technical Report*, U.S. Environmental Protection Agency, Duluth, MN, U.S.A.

De Vlaming, V., Connor, V., DiGiorgio, C., Bailey, H. C., Deanovic, L. A. and Hinton, D. E.: 2000, 'Application of whole effluent toxicity test procedures to ambient water quality assessment', *Environ. Toxicol. Chem.* **19**, 42–62.

Downing, J., Jacobi, M., Fairey, R. F., Roberts, C., Clark, R., Anderson, B. S., Hunt, J. W., Tjeerdema, R. S., Oakden, J., Wilson, C. J., Kapahi, G., Stephenson, M. D., Puckett, H. M.: 1998, 'Chemical and Biological Measures of Sediment Quality in the Central Coast Region', New series No. 5, *Technical Report*, State Water Resources Control Board, Sacramento, CA, U.S.A.

Durhan, E. J., Norberg-King, T. J. and Burkhard, L. P.: 1993, 'Methods for Aquatic Toxicity Identification Evaluations; Phase II Toxicity Identification Procedures for Samples Exhibiting Acute and Chronic Toxicity', EPA/600/R-92/080, *Technical Report*, U.S. Environmental Protection Agency, Duluth, MN, U.S.A.

Eagleson, K. W., Lenat, D. L., Ausley, L. W. and Winborne, F. B.: 1990, 'Comparison of measured instream biological responses with responses predicted using the *Ceriodaphnia dubia* chronic toxicity test', *Environ. Toxicol. Chem.* **9**, 1019–1028.

Ehrlich, P. R., Ehrlich, A. H. and Holdren, J. P.: 1977, *Ecoscience*, W. H. Freeman and Co., 1051 pp.

Ganapathy, C., Nordmark, C., Bennett, K., Bradley, A., Feng, H., Hernandez, J. and White, J.: 1997, 'Temporal Distribution of Insetcicide Residues in Four California Rivers', DPR EH97-06, *Technical Report*, California Department of Pesticide Regulation, Sacramento, CA, U.S.A.

Giesy, J. P., Solomon, K. R., Coates, J. R., Dixon, K. R., Giddings, J. M. and Kenaga, E. E.: 1999, 'Chlorpyrifos: Ecological risk assessment in north american aquatic environments', *Rev. Environ. Contam. Toxicol.* **160**, 1–129.

Hansen and Associates: 1994, 'Identification and Control of Toxicity in Storm Water Discharges to Urban Creeks', *Final Report*, Alameda County Urban Runoff Clean Water Program, Hayward, CA, U.S.A.

Hoffman, R. S., Capel, P. D. and Larson, S. J.: 2000, 'Comparison of pesticides in eight urban streams', *Environ. Toxicol. Chem.* **19**, 2249–2258.

Hunt, J. W., Anderson, B. S., Phillips, B. M., Tjeerdema, R. S., Puckett, H. M. and De Vlaming. V.: 1999, 'Patterns of aquatic toxicity in an agriculturally dominated coastal watershed in California', *Agric. Ecosyst. Environ.* **75**, 75–91.

Hunt, J. W., Anderson, B. S., Phillips, B. M., Tjeerdema, R. S., Taberski, K. M., Wilson, C. J., Puckett, H. M., Stephenson, M., Fairey, R. and Oakden, J.: 2001, 'A large-scale categorization of sites in San Francisco Bay based on the sediment quality triad, toxicity identification evaluations, and gradient studies', *Environ. Toxicol. Chem.* **20**, 1252–1265.

International Joint Commission Canada and United States: 1987, 'Revised Great Lakes Water Quality Agreement of 1978 – Agreement with Annexes and Terms of Reference, Between the United States and Canada Signed at Ottawa November 22, 1978 and Phosphorus Load Reduction Supplement Signed October 16, 1983 as Amended by Protocol Signed November 18, 1987', International Joint Commission Canada and United States, Windsor, ON, Canada.

Johnson, W. W. and Finley, M. T.: 1980, 'Handbook of Acute Toxicity of Chemicals to Fish and Aquatic Invertebrates', Resour. Publ. 137, Fish Wildl. Serv., U.S.D.I. Washington DC, U.S.A., 98 pp.

Larkin, D. J. and Tjeerdema, R. S.: 2000, 'Fate and effects of diazinon', *Rev. Environ. Contam. Toxicol.* **166**, 49–82.

Menconi, M. and Cox, C.: 1994, 'Hazard Assessment of the Insecticide Diazinon to Aquatic Organisms in the Sacramento/San Joaquin River System', *Administrative Report 94-2*, California Department of Fish and Game, Sacramento, CA, U.S.A.

Mount, D. I. and Anderson-Carnahan, L.: 1988, 'Methods for Aquatic Toxicity Identification Evaluations: Phase I Toxicity Characterization Procedures', EPA/600/3-88/034, *Technical Report*, U.S. Environmental Protection Agency, Duluth, MN.

Mount, D. I. and Norberg-King, T. J.: 1993, 'Methods for Aquatic Toxicity Identification Evaluations; Phase III Toxicity Confirmation Procedures for Samples Exhibiting Acute and Chronic Toxicity', EPA/600/R-92/081, *Technical Report*, U.S. Environmental Protection Agency, Duluth, MN.

Norberg-King, T. J., Durhan, E. J., Ankley, G. T. and Robert, E.: 1991, 'Application of toxicity identification evaluation procedures to the ambient waters of the Colusa Basin drain, California', *Environ. Toxicol. Chem.* **10**, 891–900.

Pantani, C., Pannunzio, G., DeChristofaro, M., Novelli, A. A. and Salvatori, M.: 1997, 'Comparative acute toxicity of some pesticides, metals, and surfactants to *Gammarus italicus* and *Echinogammarus tibaldi*', *Bull. Environ. Contam. Toxicol.* **59**, 963–967.

Phipps, G. L., Mattson, V. R. and Ankley, G. T.: 1995, 'The relative sensitivity of three benthic test species to 10 chemicals', *Arch. Environ. Toxicol. Chem.* **28**, 281–286.

Sanders, H. O.: 1970, 'Toxicities of some herbicides to six species of freshwater crustaceans', *J. Water Pollut. Control Fed.* **24**, 1544–1550.

Sanders, H. O.: 1972, 'Toxicity of Some Insecticides to Four Species of Malacostracan Crustaceans', Tech. Pap., No. 66, Bur. Sports Fish. Wildl., Fish Wildl. Serv., U.S.D.I., Washington, DC, U.S.A. 19 p.

Schoettger, R. A.: 1970, 'Toxicology of Thiodan in Several Fish and Aquatic Invertebrates', Invest. Fish Control, No. 35, Fish Wildl. Serv., Bur. Sport Fish. Wildl., U.S.D.I., Washington, D.C., 31 p.

Scholz, N. L., Truelove, N. K., French, B. L., Berejikian, B. A., Quinn, T. P., Casillas, E. and Collier, T. K.: 1999, 'Diazinon disrupts antiprenditor and homing behaviors in Chinook Salmon (*Oncorhynchus tshawytscha*)'. *Can. J. Fish. Aquat. Sci.* **57**, 1911–1918.

Scholz, N. L. and Collier, T. K.: 2000, 'Endangered salmon and probabilistic risk assessment for organophosphate pesticides', SETAC *Globe* **1**(6), 32–33. (Correction of units from '0.3 to 1.0 mg L^{-1}' to '0.3 to 1.0 μg L^{-1}' – personal communication: N. L. Scholz, National Marine Fisheries Service, Seattle, WA, U.S.A.).

Schubauer-Berigan,, M. K., Dierkes, J. R., Monson, P. D. and Ankley, G. T.: 1993, 'pH-dependent toxicity of Cd, Cu, Ni, Pb, and Zn, to *Ceriodaphnia dubia, Pimephales promelas, Hyalella azteca*, and *Lumbriculus variegatus*', *Environ. Toxicol. Chem.* **12**, 1261–1266.

Sparling, D. W., Fellers, G. M. and McConnell, L. L.: 2001, 'Pesticides and amphibian population declines in California, U.S.A.', *Environ. Toxicol. Chem.* **20**, 1591–1595.

Sullivan, J. J. and Goh, K. S.: 2000, 'Evaluation and validation of a commercial ELISA for diazinon in surface waters', *J. Agric. Food. Chem.* **48**, 4071–4078.

Thursby, G. B., Heltshe, J. and Scott, K. J.: 1997, 'Revised approach to toxicity test acceptability criteria using a statistical performance assessment', *Environ. Toxicol. Chem.* **16**, 1322–1329.

Tierney, D., Giddings, J., Hall, L., Solomon, K., Adams, W., Vogel, D., Davis, L. and Smith, R. D.: 1997, 'An Ecological Risk Assessment of Diazinon in the Sacramento and San Joaquin River Basins', *Technical Report 11/97*, Novartis Crop Protection, Greensboro NC, U.S.A.

Trnkova, J.: 1977, 'Toxic Effect of Endrin on Aquatic Invertebrates', *Prirodoved. Pr. Ustavu Cesk. Akad. Ved. Brne* **11**(3), 1–30 (GER) (ENG ABS) (via EPA ECOTOX Internet Database).

U.S. Environmental Protection Agency: 1999, 'National Recommended Water Quality Criteria – Correction', EPA 822-Z-99-001, Washington, DC.

U.S. Environmental Protection Agency: 1993, 'Methods for Measuring Acute Toxicity of Effluents and Receiving Water to Freshwater and Marine Organisms', 4th ed., EPA 600/4-91/002, *Technical Report*, Washington, DC.

Van der Hoeven, N. and Gerritsen, A. A. M.: 1997, 'Effects of chlorpyrifos on individuals and populations of *Daphnia pulex* in the laboratory and field', *Environ. Toxicol. Chem.* **16**, 2438–2447.

Van Wijngaarden, R. P. A., Van den Brink, P. J., Crum, S. J. H., Oude Voshaar, J. H., Brock, T. C. M. and Leeuwangh, P.: 1996, 'Effects of the insecticide Dursban 4E (Active ingredient chlorpyrifos) in outdoor experimental ditches: I. Comparison of short-term toxicity between the laboratory and the field', *Environ. Toxicol. Chem.* **15**, 1133–1142.

Waller, W. T., Ammann, L. P., Birge, W. J., Dickson, K. L., Dorn, P. B., LeBlanc, N. E., Mount, D. I., Parkhurst, B. R., Preston, H. R., Schimmel, S. C., Spacie, A. and Thursby, G. B.: 1996, 'Predicting Instream Effects from WET Tests', in D. R. Grothe, K. L. Dickson and D. K. Reed-Judkins

(eds), *Whole Effluent Toxicity Testing: An Evaluation of Methods and Prediction of Receiving System Impacts*, Society of Environmental Toxicology and Chemistry, Pensacola, FL, U.S.A., pp. 271–286.