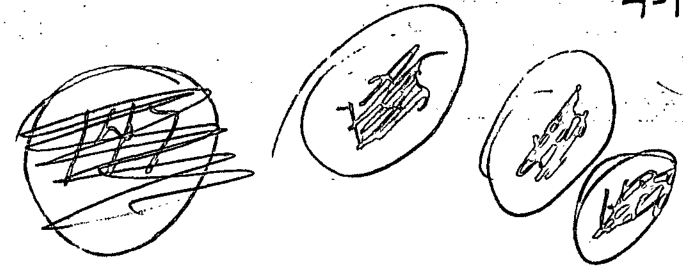
4-19



CAUSES OF AMBIENT TOXICITY IN THE CALLEGUAS CREEK WATERSHED OF SOUTHERN CALIFORNIA

135

B. S. ANDERSON[1]*, V. DE VLAMING[2], K. LARSEN[3], L. S. DEANOVIC[4], S. BIROSIK[5], D. J. SMITH[5], J. W. HUNT[1], B. M. PHILLIPS[1] and R. S. TJEERDEMA[1]

[1] *Department of Environmental Toxicology, University of California, Davis, California, U.S.A.;* [2] *Division of Water Quality, State Water Resources Control Board, Sacramento, California, U.S.A.;* [3] *Central Valley Regional Water Quality Board, Sacramento, California, U.S.A.;* [4] *Los Angeles Regional Water Quality Board, Los Angeles, California, U.S.A.;* [5] *Aquatic Toxicology Laboratory, VM:APC, University of California, Davis, California, U.S.A.*

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

(Received 6 February 2001; accepted 2 October, 2001)

Abstract. A combination of toxicity tests, chemical analyses, and Toxicity Identification Evaluations (TIEs) were used to investigate receiving water toxicity in the Calleguas Creek watershed of southern California. Studies were conducted from 1995 through 1999 at various sites to investigate causes of temporal variability of toxicity throughout this system. Causes of receiving water toxicity varied by site and species tested. Investigations in the lower watershed (Revolon Slough, Santa Clara Drain, Beardsley Wash) indicated that toxicity of samples to the cladoceran *Ceriodaphnia dubia* was due to elevated concentrations of the organophosphate pesticide chlorpyrifos, while causes of intermittent toxicity to fathead minnows (*Pimephales promelas*) and the alga *Selanastrum capricornutum* were less clear. Investigations at sites in the middle and upper reaches of the watershed (Arroyo Simi and Conejo Creek) indicated that the pesticide diazinon was the probable cause of receiving water toxicity to *Ceriodaphnia*. Elevated ammonia was the cause of toxicity to fathead minnows in the upper watershed sites. Results of these and previous studies suggest that biota are impacted by degraded stream quality from a variety of point and non-point pollution sources in the Calleguas Creek watershed. Water quality resource manager's efforts to identify contaminant inputs and implement source control will be improved with the findings of this study.

Keywords: ceriodaphnia, pesticides, TIE, toxicity

1. Introduction

The California State Water Resources Control Board and its nine Regional Boards are responsible for ensuring compliance with the federal Clean Water Act provisions as they apply to surface waters. As point source pollution has been reduced through improved monitoring and treatment, monitoring programs implemented by these agencies have emphasized water quality assessment of freshwater and marine ambient waters. Recent freshwater studies have incorporated chemical analyses, toxicity tests, Toxicity Identification Evaluations (TIEs), and in some cases, bioaccumulation and biological community studies to identify polluted receiving waters throughout the State (e.g., de Vlaming *et al.*, 2000).

Environmental Monitoring and Assessment **78:** 131–151, 2002.

In 1992, through funding from the State Water Resources Control Board, the Los Angeles (L.A.) Regional Water Quality Control Board began a cooperative water quality assessment of the Calleguas Creek watershed in southwestern California. Calleguas Creek and its major tributaries, Revolon Slough, Conejo Creek, Arroyo Conejo, Arroyo Santa Rosa, and Arroyo Simi, drain an area of 878 km^2 in southern Ventura County and a small portion of western Los Angeles County. This area is characterized by a southern Mediterranean climate with minimal summer rainfall. Annual rainfall averages 33 cm in the lower plains and up to 51 cm in the upper portions of this watershed; the majority of winter rain falls from November through March. Although there is some surface water flow from perennial springs, summer water sources in some portions of the watershed are reduced to treated effluent, agriculture return water, and urban runoff. A considerable number of reaches of the creeks and sloughs in this watershed have been channelized and/or lined for sedimentation and flood control purposes. Despite these localized modifications, the watershed as a whole provides significant freshwater habitat in this normally arid environment (CRWQCBLA, 1995). Land uses vary throughout the watershed. Urban developments are generally restricted to the city limits of Simi Valley, Moorpark, Thousand Oaks, and Camarillo. Although some residential development has occurred along the slopes of the watershed, most of the upland areas are still open space. Agricultural activities, primarily cultivation of orchard and row crops, are spread out along the valleys and on the coastal Oxnard Plain. The US Navy has a Naval Air Base on much of the area around Mugu Lagoon. Mugu Lagoon is the final receiving system where surface waters from the Calleguas Creek watershed enter the Pacific Ocean. The California State Ecological Preserve at Mugu Lagoon is one of the largest remaining estuarine systems in southern California and, as such, is of particular concern to regional regulatory agencies. Recent water quality assessments in this watershed have detected surface water toxicity, and this has been associated with a number of contaminants. Bailey *et al.* (1996a) found toxicity to *Ceriodaphnia dubia*, *Pimephales promelas*, and intermittent toxicity to *Selenastrum capricornutum* using samples from a number of stations located throughout Calleguas Creek and its tributaries. TIEs suggested that non-polar organic chemicals, and in some cases, divalent cations were responsible for toxicity of a number of samples to *C. dubia.* This investigation was limited by a lack of comprehensive chemical analyses with appropriate detection limits. These authors also reported a number of histopathological abnormalities and external lesions in feral fish collected at several Calleguas Creek sites. A number of reaches of Calleguas Creek and tributaries in the watershed have been included in California's Clean Water Act Section 303 (d) list of impaired water bodies due to surface water chemical contamination, sedimentation, and elevated contaminants in shellfish and fish tissue; these include Mugu Lagoon, Beardsley Wash, Calleguas Creek, and Revolon Slough (LARWQCB, 1995).

The current study was designed to succeed these previous studies by assessing spatial and temporal trends in surface water toxicity and chemical contamination

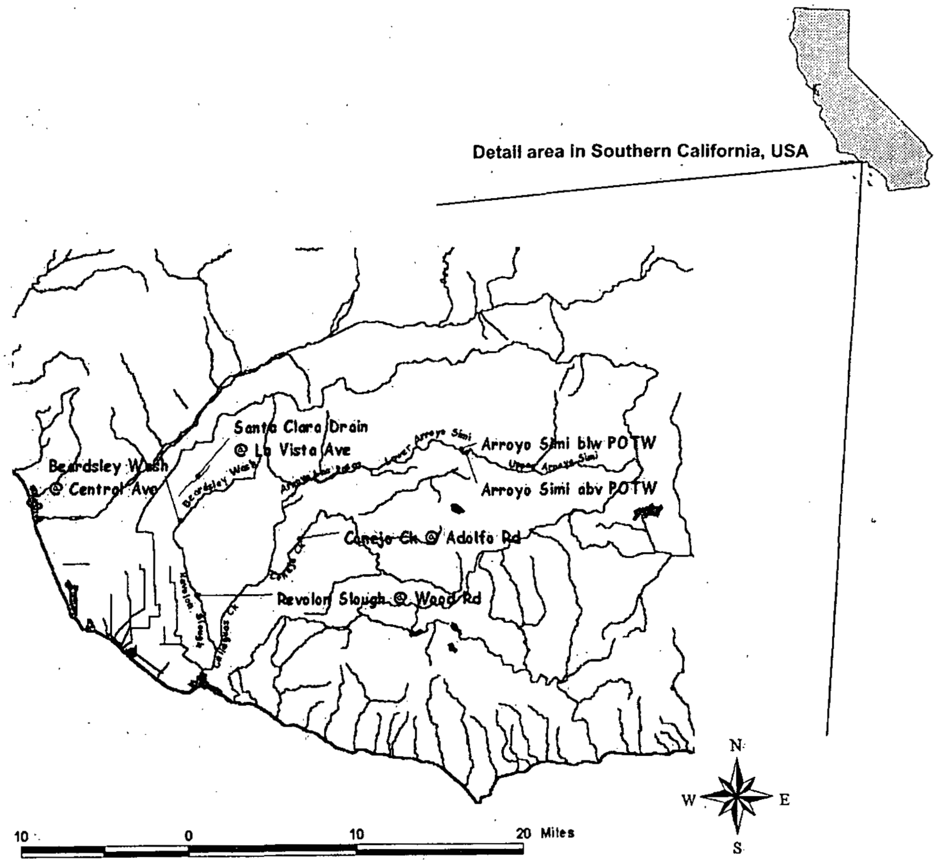


Figure 1. Map of study sites in the Calleguas Creek watershed of southern California.

throughout the Calleguas Creek watershed. This study proceeded in three phases from 1995 through 1999. In each phase, toxicity tests were combined with chemical analyses, and where appropriate, TIEs to characterize chemicals responsible for surface water toxicity.

2. Methods

2.1. Study Sites

Figure 1 shows the study sites in the Calleguas Creek Watershed. Revolon Slough and Beardsley Wash are located on the Oxnard Plain in the southwestern part of the system. The Revolon Slough site is at the uppermost extent of tidal influence. This is a sediment depositional area with rip-rap sides. Surface water sources at these sites are dominated by agricultural return waters, storm water runoff and some treated wastewater. The Beardsley Wash site is in a completely concreted portion of the waterway. Water quality at the Revolon Slough site was assessed monthly from February 1995 through January 1996 (defined as: dissolved oxygen, pH, hardness, conductivity, unionized ammonia, toxicity, selected trace organic and trace metal chemical compounds). Toxicity was assessed using *Ceriodaphnia dubia, Selenastrum capricornutum*, and *Pimelphales promelas* (US EPA, 1994). From October 1997 through July 1998 water quality was assessed approximately monthly at the Revolon Slough, Beardsley Wash, and Santa Clara Drain study sites (Figure 1). Toxicity at these sites was assessed using *C. dubia* because this was the most sensitive species in previous assessments. The Santa Clara drain study site is located in the northwestern part of the Calleguas Creek watershed and the surface waters at this site receive mostly agricultural return water with small amounts of urban runoff. This site has a natural bottom and rip-rap sides and experiences very low flows relative to the other sites. From November 1998 through June 1999 water quality was assessed monthly at three sites in the middle and upper watershed. Conejo Creek is located in the central part of the watershed and receives wastewater from two municipal treatment facilities. Two Arroyo Simi study sites were in the northeastern part of the watershed (Figure 1). The Lower Arroyo Simi study site was just below a municipal treatment facility and the Upper Arroyo Simi study site was just above this input. Toxicity at these sites was assessed using *C. dubia* and *P. promelas*.

2.2. Toxicity Studies

Methods for sampling, toxicity tests, TIEs, and analytical chemical analyses followed those described by Bailey *et al.* (1996a). Grab samples consisting of 100% river water were used for all toxicity tests. Samples were collected by L.A.W.Q. Regional Board staff in 2- and 4-liter amber glass bottles and shipped overnight on ice to the Aquatic Toxicology Laboratory at the University of California, Davis where they were stored in the dark at 4 °C. Testing was initiated on all samples within 48-h of collection. The toxicity test methods followed published Environmental Protection Agency guidelines for conducting short-term chronic toxicity tests with freshwater organisms (US EPA, 1994). The test species were fathead minnows (*P. promelas*), a cladoceran (*C. dubia*) and a green alga (*S. capricornutum*). Brief descriptions of the procedures follow.

For fathead minnow (*P. promelas*) tests, ten < 24-h post-hatch minnow larvae were exposed in 250 ml of sample water in each of 4 replicate 600 ml beakers. Test solutions were renewed daily (80%), and fish were fed *Artemia* nauplii 3 times daily. Samples with hardness exceeding 3000 μmhos were diluted to 3000 μmhos using glass-distilled water. Mortality was monitored daily and tests were concluded after 7 days. At termination, the fish were rinsed and dried to constant weight by replicate groups, and mean weight per surviving fish was determined. Test endpoints were survival and growth.

For *C. dubia* tests, ten <24-h neonates were exposed individually in 20 ml glass scintillation vials. Because stations in the lower watershed were heavily influenced by agricultural return water, and to a lesser extent, by tidal action from Mugu Lagoon, conductivities of some of the samples were beyond the range tolerated by *Ceriodaphnia*. To accommodate testing with *Ceriodaphnia*, samples with conductivity exceeding 2500 μmhos were diluted to 2500 μmhos using glass-distilled water. Samples for chemical analysis were not diluted. Test solutions were renewed and cultures fed a YCT and *Selenastrum* mix daily. After a 7-d exposure, survival and neonate production were quantified.

For *S. capricornutum* tests, algae were exposed to 100 ml of 4.5 μm-filtered sample in each of four replicate 250 ml Erlenmeyer flasks. Each flask was inoculated with 10,000 cells/ml and spiked with nutrients, then maintained on an orbital shaker (100 rpm) under constant illumination (400 ft. candles). The positions of the flasks on the shakers were changed 2 times daily to minimize position effects. All exposures were terminated after 96-h and cell numbers were counted with an electronic particle counter (Beckman Coulter Electronics, Fullerton, CA, USA).

Control procedures for the *P. promelas* and *C. dubia* tests were similar to those described above except that the test organisms were maintained in laboratory reference water (Sierra Springs™ water amended to EPA moderately hard – MHW) and diluted well water. MHW was prepared according to EPA moderately hard water standards by adding the specified four salts to spring water. Algal control water was prepared by adding EPA nutrient salts (w/o EDTA) to glass distilled water. The algal medium was filtered to 4.5 μm prior to use.

Dissolved oxygen and pH were measured at test initiation and after 24 hours; conductivity was measured prior to test initiation; hardness (as $CaC0_3$) and alkalinity (as $CaC0_3$) were measured within one week of sample collection. Total ammonia was measured if test organism mortality was greater than or equal to 30%. Monthly reference toxicant tests using NaCl were conducted on all three species as part of an overall laboratory quality assurance program.

In cases where the parametric statistic assumptions were met, mortality, growth, reproduction, and algal cell numbers in the sample waters were compared to performance in the respective control solutions using Dunnett's Test in the Analysis of Variance. In cases where the parametric statistical assumptions were not met, Kruskal-Wallis or the Wilcoxon Two-Sample Test was used to determine statistically significant differences between the sample and control responses.

2.3. Toxicity Identification Evaluations (TIEs)

Subsets of samples were treated with selected toxicity identification evaluation procedures to characterize the properties of the chemicals responsible for toxicity. TIE procedures were abbreviations of US EPA (1991) methods (Bailey *et al.*, 1996b). The treatments included filtration with 0.45 μm glass-fiber filters to remove particulates, solid-phase extraction with C8 columns, and treatment with the biochemical reagent piperonyl butoxide, PBO (Ankley *et al.*, 1991; Bailey *et al.*, 1996b), which inhibits the oxidation of phosphorothioate pesticides to the more toxic phosphate form (Fukuto, 1987). Reduction of toxicity in the presence of PBO suggests toxicity due to one or more metabolically activated organophosphate pesticides. PBO was added to the water samples at 200 μg/L and the exposures were conducted using procedures described above. Metals chelation with ethylenediaminetetraacetic acid (EDTA) was applied in cases where the other treatments did not eliminate toxicity. EDTA was added to the test samples at 12.5 to 50 mg/L. In fathead minnow tests where unionized ammonia was suspected to be the source of toxicity, sample pH was lowered using addition of hydrochloric acid (Mount and Anderson-Carnahan, 1988). In these cases pH was lowered from ambient levels to pH 6.5. One sample was subjected to Phase II TIE manipulations using U.S. EPA (1993) procedures. In this experiment, the C8 column was eluted with 25, 50, 70, 75, 80, 85, 90, 95, and 100% methanol:water solutions, and toxicity of each fraction was assessed using *C. dubia*. In all cases, the untreated sample was tested concurrently with the treated sample to verify that the sample retained toxicity.

2.4. Analytical Chemistry

Selected samples were analyzed for trace organic chemicals using gas chromatography-mass spectrometry following EPA procedures (EPA method 8140, 8080). Trace metal concentrations were analyzed using inductively coupled plasma- mass spectrometry (EPA methods 614, 632, 630) Chemical analyses were conducted by APPL Laboratories (Fresno, CA, USA). In addition, diazinon and chlorpyrifos concentrations were determined with enzyme-linked immunosorbent assays (ELISA – Millipore, Bedford, MA, USA). Each set of ELISA readings was accompanied with its own set of four calibration standards (30, 100, 250 and 500 ng/L for diazinon and 80, 180, 350, and 600 ng/L for chlorpyrifos). The specified minimum detection limits were 30 and 80 ng/L for diazinon and chlorpyrifos, respectively. All samples were run in duplicate. If the coefficient of variation for the duplicates differed by more than 10% the analysis was repeated. Results that were less than the specified minimum detection limits were included in the data set if the duplicate measurements did not differ by more than 10%. Quality control measures included testing of replicates within each analytical run, testing replicates in consecutive runs, testing a laboratory reference standard, and analyzing split samples with gas chromatography-mass spectrometry.

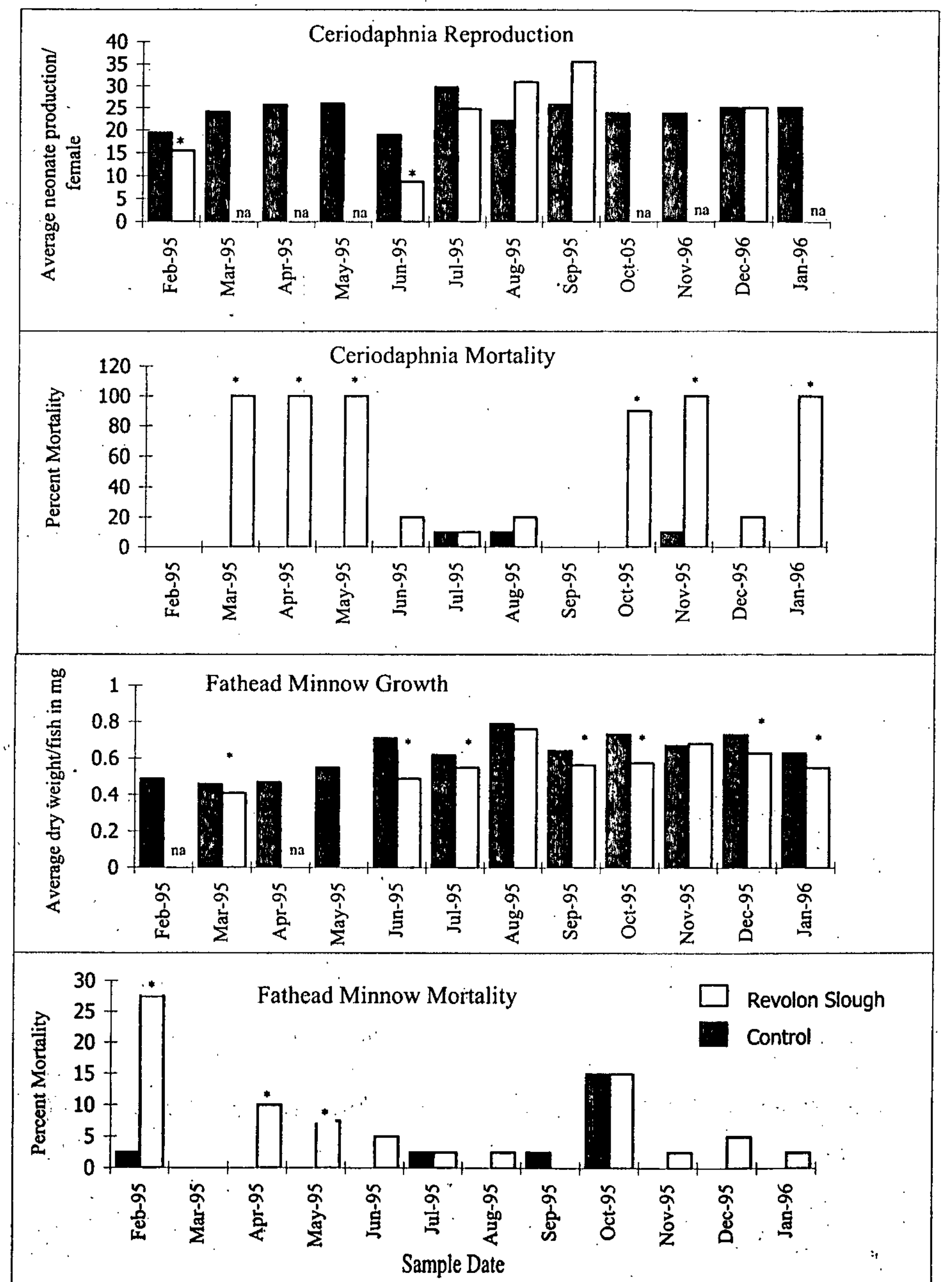


Figure 2. Temporal variability in *Ceriodaphnia dubia* reproduction (average numbers of neonates per female) and mortality, and *Pimephales promelas* growth and mortality at the Revolon Slough study site. Samples collected from March 1995 through January, 1996.

TABLE I

Summary of 95/96 TIEs with Ceriodaphnia using Revolon Slough samples

Sample date	Mortality (%)				Pesticide Concentrations (ug/L)		
	Untreated	PBO	C_8	Eluate.	Diazinon	Chlorpyrifos	Carbaryl
March 95	100	0	0	100	0.03	0.06[b]	0.18
May 95	100	0	0	100	nd	nd	0.78
Nov. 95	100	0	0	100	nd	0.11[a,c]	nd
Jan. 96	100	0	0	100	0.20[a]	0.09[a,c]	0.50

[a] = exceeds California Department of Fish and Game acute water quality criteria (diazinon = 0.08 μg/L; chlorpyrifos = 0.07 μg/L);
[b] = exceeds California Department of Fish and Game chronic water quality criteria (diazinon = 0.040 μg/L, chlorpyifos = 0.02 μg/L);
[c] = exceeds the 96-h LC50 for *C. dubia* (diazinon = 0.350-0.450 μg/L, chlorpyifos = 0.06-0.09 μg/L).

3. Results

3.1. Revolon Slough (1995 – 1996)

Intermittent toxicity was observed in Revolon Slough samples collected between February 1995 and January 1996. Toxicity assessments with the cladoceran *Ceriodaphnia dubia* indicated significant mortality in samples collected from March, April, May, October and November of 1995, and January of 1996 (Figure 2). Two of the samples from this site significantly reduced *C. dubia* reproduction without significantly increasing mortality (February and June 1995). Phase I TIEs were conducted on Revolon Slough samples collected in March, May, and November of 1995, and on the sample collected in January, 1996. In all cases, *C. dubia* mortality was eliminated by treating samples with solid-phase (C_8) extraction, and methanol elutions of the columns (eluates) were toxic to *C. dubia*, suggesting that non-polar organic compounds were responsible for toxicity (Table I). Toxicity of all samples was also eliminated by addition of piperonyl butoxide (PBO), indicating that toxicity to *C. dubia* was due to metabolically-activated pesticides. Concentrations of organophosphate pesticides were detected during March and November, 1995, and January, 1996 (Chlorpyrifos range = 0.06 – 0.11 μg/L; diazinon range = 0.03 – 0.20 μg/L). Concentrations of chlorpyrifos and/or diazinon exceeded acute water quality guidelines developed by the California Department of Fish and Game (for protection of aquatic biota) during two of these testing periods. The 96-h LC_{50} for chlorpyrifos toxicity to *C. dubia* was exceeded in November, 1995 and January, 1996. Carbaryl was sometimes detected in these samples but concentrations were below the reported LC_{50} value for this pesticide (11.6 μg/L; Oris *et al.*, 1991). Because it was sometimes necessary to dilute samples so that conductivity was within

TABLE III

Summary of 97/98 TIEs with *Ceriodaphnia* using selected samples from the Calleguas Creek watershed: Santa Clara Drain, Revolon Slough, Beardsley Wash

Site and Sample Date	Mortality (%)				Pesticide Concentration (ug/L)		
	Untreated	PBO	C8	Eluate	Diazinon	Chlorpyrifos	Carbaryl
Santa Clara Drain Oct. 97	100	0	5	100	nd	0.161[a]	nd
Santa Clara Drain Nov. 97	100*	5*	0	100	nd	0.328[a]	nd
Revolon Slough Nov. 97	92	10	0	100	<dl	0.177[a]	0.069
Santa Clara Drain Dec. 97	100	100**	5	100	nd	0.330[a]	nd
Beardsley Wash Dec. 97	100	30	10	100	<dl	0.149[a]	nd
Santa Clara Drain Jan. 98	100*	0*	0	100	nd	0.370[a]	nd
Beardsley Wash Jan. 98	100	0	0	100	<dl	0.149[a]	nd

* Sample diluted to 25%; ** Mortality delayed by PBO; nd = not detected; [a] = exceeds the 96-h LC50 for *C. dubia* (0.06 – 0.09 μg/L); < dl = concentration less than the detection limit.

3.2. Revolon Slough, Beardsley Wash, Santa Clara Drain 1997 – 1998

Less toxicity to *C. dubia* was detected in samples collected at the Revolon Slough site in 1997 and 1998 relative to the 1995–1996 sampling, although the reasons for this are not clear. There was a significant reduction in neonate production relative to the control in the October 1997 sample, and *C. dubia* mortality in the November 1997 sample was 90%. No mortality or reproductive effects were detected in the remaining samples from this site (Table II). Mortality in the Revolon Slough sample collected in November 1997 was reduced by treatment with the C_8 column. Mortality was 100% in the C_8 column eluate, and addition of PBO significantly reduced toxicity relative to the untreated sample (Table III). These results implicate a metabolically-activated pesticide as the cause of toxicity. The chlorpyrifos concentration in the sample was more than twice the 96-h LC_{50} value for *C. dubia* (chlorpyrifos concentration = 0.177 μg/L). As discussed above, toxicity may have been underestimated in some of the Revolon Slough samples because it was necessary to dilute 6 of the 8 samples collected during this time period to accommodate *Ceriodaphnia*. The mean dilution was 51% (range = 44 – 63%).

Significant mortality of *C. dubia* occurred in the Beardsley Wash samples collected in December 1997 and January 1998. No significant mortality or inhibition of reproduction occurred in any other samples collected from this site (Table II). TIE treatments conducted on two toxic samples suggested that chlorpyrifos was responsible for the observed toxicity (Table III). In both TIEs, toxicity was removed by the C_8 column, returned in the C_8 methanol eluate, and reduced with the addition of PBO. Chlorpyrifos concentrations in both samples were approximately twice the 96-h LC_{50} for this species (chlorpyrifos concentration = 0.149 μg/L).

TABLE II

Summary of *Ceriodaphnia dubia* toxicity tests conducted at Revolon Slough Beardsley Wash, and the Santa Clara Drain in 1997 and 1998

Sample	Revolon Slough		Beardsley Wash		Santa Clara Drain	
	Neonates/ female	Mortality	Neonates/ female	Mortality	Neonates/ female	Mortality
Oct. 97	15.1[a]	0.0	29.0	0	nc	100[a]
Nov. 97	nc	90[a]	18.7	11	nc	100[a]
Dec. 97	21.5	0.0	nc	100[a]	nc	100[a]
Jan. 98	27.6	0.0	nc	100[a]	nc	100[a]
Mar. 98	38.3	0.0	nt	nt	nt	nt
April. 98	24.3	0.0	27.4	0	24.3	0
May. 98	24.3	0.0	17.3	0	19.6	0
Jul. 98	16.0	0.0	20.8	0	29.3	0
Mean Control	23.0	2.5	23.0	2.5	23.0	2.5

[a] significantly different from the control response ($p < 0.05$); nc = not calculated due to significant mortality; nt = not tested.

the range tolerable to *Ceriodaphnia*, toxicity may have been greater in many of these samples. For example, 10 of 12 samples were diluted for these experiments, and the mean dilution was approximately 37% (range = 23 – 51% dilution).

Revolon Slough samples significantly increased mortality of fathead minnows in three samples collected in 1995 (Figure 2). A greater number of samples reduced fathead minnow growth (Figure 2). No TIEs were conducted on these samples.

Cell growth in *S. capricornutum* was significantly reduced in two months in 1995. Except for the April 1995 sampling period, cell growth was stimulated relative to the controls in all other samples from Revolon Slough (data not shown). Because there was measurable chlorine in the February 1995 sample (0.06 mg/L), a TIE was conducted using sodium thiosulfate. Toxicity was not reduced with this treament (control growth = 900,000 cells/ml; Revolon Slough sample growth = 105,000 cells/ml; Revolon Slough sample + $Na_2S_2O_3$ growth = 133, 000 cells/ml). Treatment of this sample with the C_8 SPE column also did not increase cell numbers, and toxicity tests conducted on the column eluate (column eluted with propanol and methanol) were not conclusive due to poor control performance. These results suggest that neither chlorine nor non-polar organic compounds were responsible for sample toxicity to the algae. The herbicide diuron was detected at a concentration sufficient to be toxic in March, 1995 (4.0 μg/L). The 96-h EC_{50} for diruon toxicity to *S. capricornutum* is 2.4 μg/L (US EPA, 1995), so it is possible this inhibited algal cell growth in the March 1995 Revolon Slough sample. No TIE was conducted on this sample.

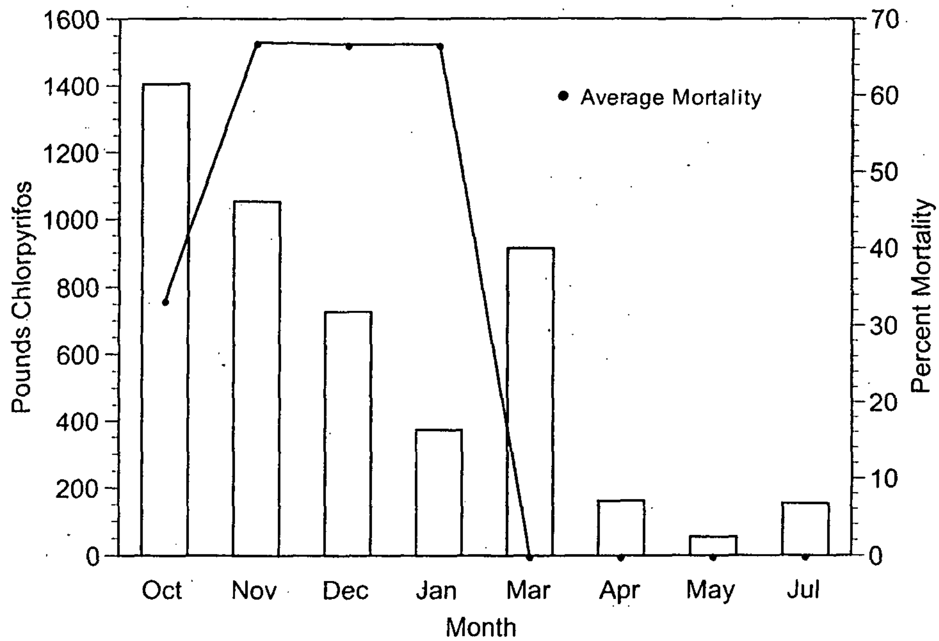


Figure 3. Relationship between monthly application of chlorpyrifos in Ventura County and average mortality of *Ceriodaphnia dubia* in samples collected from Revolon Slough, Santa Clara Drain, and Beardsley Wash in 1997 and 1998.

Significant *C. dubia* mortality occurred in four of the eight Santa Clara Drain samples tested in 1997 and 1998. There was 100% mortality in samples collected from October, 1997 through January, 1998. No significant mortality or reproductive effects were detected in any of the remaining samples (Table II). The TIE results were comparable to those for samples from the other sites analyzed during this period. TIEs conducted on Santa Clara Drain samples collected from October, 1997 through January, 1998, suggested chlorpyrifos was responsible for toxicity. In these TIEs, toxicity was removed by the C_8 column and returned in the C_8 methanol eluate. In two of the TIEs mortality was reduced with the addition of PBO to the sample, and in the third, mortality was delayed. Chlorpyrifos concentrations in all samples were approximately two to four times the 96-h LC_{50} for this species (Table III; chlorpyrifos range = 0.161 – 0.370 μg/L).

Because chlorpyrifos was identified as the primary chemical responsible for *C. dubia* mortality in the toxic Revolon Slough, Beardsley Wash, and Santa Clara Drain samples collected during this period, a pesticide use report database was used to explore the relationship between county-wide application of chlorpyrifos and the average mortality of *C. dubia* in these samples (e.g., the average toxicity observed in Revolon Slough, Beardsley Wash, and Santa Clara Drain samples). Figure 3 shows that except for the March 1998 samples, greater toxicity was observed in

TABLE IV

Summary of 98/99 TIEs with *Ceriodaphnia* using selected samples from Conejo Creek and Upstream and Downstream Arroyo Simi

	Mortality (%)				Pesticide Concentration (ug/L)		
Site and Sample Date	Untreated	PBO	C8	Eluate	Diazinon	Chlorpyrifos	Other
Conejo Cr. Dec. 98*	100	0	0	100	0.230[a]	0.05	nd
Arroyo Simi upstream Apr. 99	100	5	0	100	0.410[a,b]	nd	Prowl = 0.05
Arroyo Simi dnstream Apr. 99	95	0	0	100	0.400[a,b]	nd	Prowl = 0.06
Arroyo Simi upstream Jun. 99	100	0	0	100	0.430[a,b]	nd	Prowl = 0.33, Demeton- s = 0.57

* Phase II TIE indicated 100% mortality in the 75% and 80% methanol fractions only; [a] = exceeds California Department of Fish and Game acute water quality criteria (0.08 μg/L); [b] = exceeds the 96-h LC50 for *C. dubia* (0.350–0.450 μg/L); nd = not detected

months with greater application of chlorpyrifos in Ventura County. No toxicity was observed in April, May and July, 1998 when the lowest amount of pesticide were applied in Ventura County.

3.3. Upper Arroyo Simi, Lower Arroyo Simi, Conejo Creek 1998 – 1999

Toxicity at these sites was assessed monthly from November 1998 through June 1999 using *C. dubia* and *P. promelas*. Three of the 8 samples from the Upper Arroyo Simi site were toxic to *C. dubia*; the February 1999 sample significantly reduced neonate production, while 100% daphnid mortality was observed in samples from this site in April and June 1999 (Figure 4). TIEs conducted on the Upper Arroyo Simi samples in April and June 1999 indicated that toxicity in these samples was due to diazinon. Mortality was largely eliminated with the C_8 column treatment, returned in the C_8 column methanol eluates, and was significantly reduced or eliminated with the addition of PBO (Table IV). These results suggest that a metabolically-activated pesticide was responsible for toxicity. Diazinon concentrations in April and June 1999 exceed the 96-h LC_{50} for *C. dubia* (Table IV; diazinon range = 0.410 – 0.430 μg/L).

Five of the 8 samples from the Lower Arroyo Simi site were toxic to *C. dubia*. Three samples significantly reduced neonate production, while two additional samples significantly increased *C. dubia* mortality (Figure 4). Results of a TIE conducted on one Lower Arroyo Simi sample collected also suggested that diazinon was the cause of toxicity. As with previous samples, mortality was eliminated with the C_8 column treatment, returned in the C_8 column methanol eluate, and

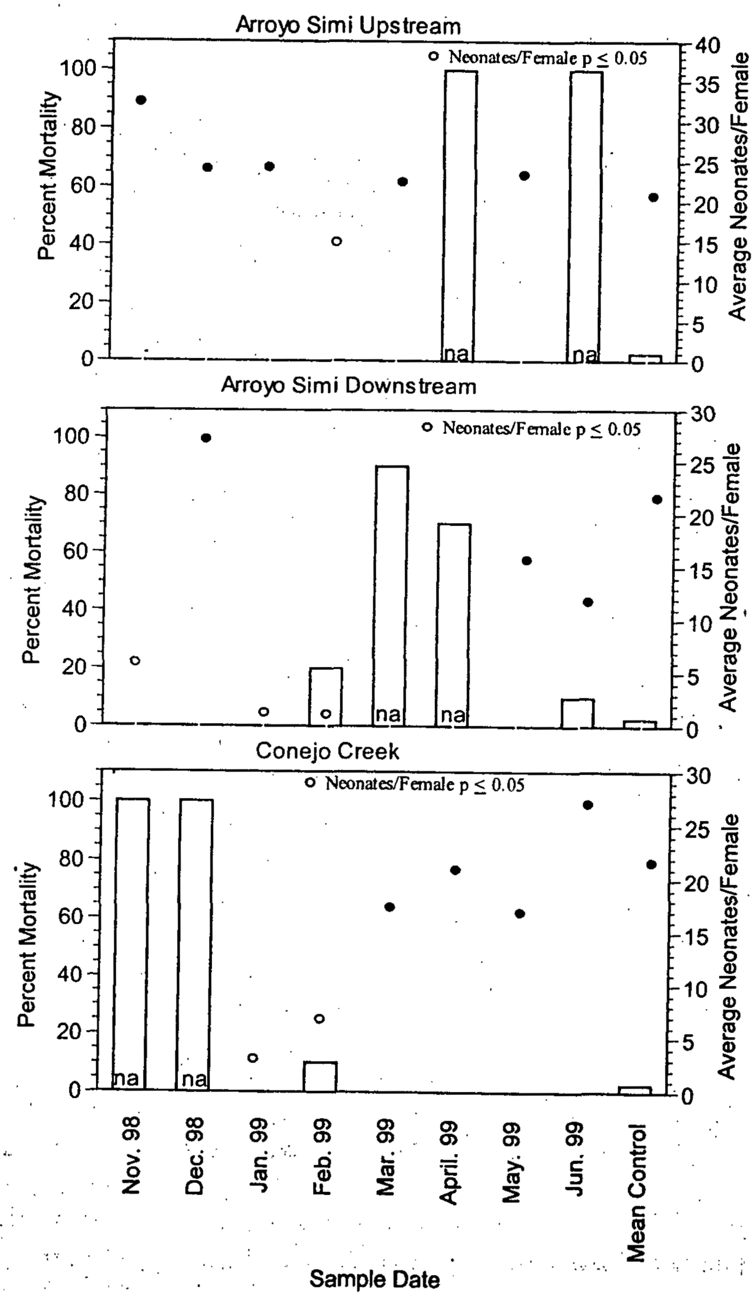


Figure 4. Ceriodaphnia dubia reproduction and mortality in samples collected from three sites in 1998 and 1999: Arroyo Simi Upstream of the POTW, Arroyo Simi Downstream of the POTW, and Conejo Creek. ° = average number of neonates per female significantly less than the control (@ p = 0.05), ● = average number of neonates per female not significantly different from the control (@ p = 0.05), □ = percent mortality.

TABLE V

Influence of pH adjustment on Fathead Minnow mortality in selected samples from Arroyo Simi (up and downstream) and Conejo Creek

Sample Date	Arroyo Simi Upstream			Arroyo Simi Downstream			Conejo Creek		
	NH_3^{**} mg/L	Mortality (ambient pH)	Mortality* adjusted	NH_3^{**} mg/L	Mortality (ambient pH)	Mortality* adjusted	NH_3^{**} mg/L	Mortality (ambient pH)	Mortality* adjusted
Nov. 98	0.79	12.5	nt	2.39	65.0	0.0	1.06	20.0	nt
Dec. 98	0.23	12.5	nt	0.26	0.0	nt	1.02	2.5	nt
Jan. 99	nd	22.5	nt	2.42	45.0	nt	1.34	30.0	nt
Feb. 99	0.01	15.0	nt	4.76	100.0	15.0	5.23	95.0	7.5
Mar. 99	nd	47.5	nt	2.55	75.0	5.0	2.77	77.5	7.5
April. 99	0.92	2.8	nt	0.76	7.5	nt	2.12	38.4	15.0
May. 99	nd	27.5	nt	2.49	82.5	10.0	1.87	2.5	nt
Jun. 99	0.16	5.0	nt	2.90	82.5	5.0	0.04	2.5	nt
Mean Control		2.8			2.8			2.8	

* pH was adjusted to 7.0 in the Nov. 1998 experiment, pH was adjusted to 6.5 in all subsequent experiments
** unionized ammonia; nt = not tested due to low initial mortality

eliminated with the addition of PBO (Table IV). The diazinon concentration in this sample exceeded the 96-h LC_{50} for *C. dubia* (diazinon = 0.400 – 0.430 μg/L).

Four samples from Conejo Creek were toxic to *C. dubia*. Two samples significantly inhibited neonate production but had no effect on survival; the other two caused significant mortality (Figure 4). No other samples from Conejo Creek were toxic to *Ceriodaphnia*. A TIE conducted on the Conejo Creek sample collected in December 1998 indicated that diazinon may have been responsible for the observed toxicity. Mortality was eliminated with the C_8 column treatment, returned in the C_8 column methanol eluates, and eliminated with the addition of PBO (Table IV). The diazinon concentration in the December 1998 Conejo Creek sample exceeded the California Department of Fish and Games 96-h acute water quality criteria for this chemical, but was less than the LC50 value (Table IV; diazinon = 0.230 μg/L; chlorpyrifos = 0.05 μg/L). A Phase II TIE conducted on this sample showed 100% mortality of *C. dubia* exposed to the 75 and 80% C_8 column methanol eluate fractions (no toxicity occurred in the other methanol fractions). Bailey *et al.* (1996b) found that diazinon elutes primarily in the 75 and 80% methanol fractions.

Significant mortality of larval fathead minnows occurred in 2 of 8 samples collected from the upper Arroyo Simi site, while 6 of 8 Lower Arroyo Simi samples were toxic. Conejo Creek samples collected in January through March 1999 were also toxic to fathead minnows. Toxicity was reduced in most cases by lowering sample pH (Table V). Samples from Lower Arroyo Simi and Conejo Creek having unionized ammonia concentrations above 1.25 mg/L were always toxic to fathead minnows; the LC50 for unionized ammonia is 0.6–1.0 mg/L (Markle *et al.*, 2000). In all cases where unionized ammonia in samples from these sites exceeded 1.25 mg/L, larval mortality was significantly reduced or eliminated by lowering sample pH (Table V).

4. Discussion

When used in combination with chemical analyses, TIEs, and other biological, physical and chemical information, toxicity tests originally developed for whole effluent monitoring are effective for identifying water quality impairments to aquatic life in ambient waters. These experiments indicate pervasive toxicity in water samples collected throughout the Calleguas Creek watershed. The apparent sources of toxicity in this system varied by site and species tested. It is likely that the majority of samples collected in the lower watershed (i.e., those collected in 1995 through 1997 in Revolon Slough) were more toxic than these results suggest because these samples were diluted by an average of 44% in order to accommodate the conductivity limitations of *Ceriodaphnia dubia* (samples for chemistry were not diluted).

Fifty percent of the Revolon Slough samples tested with *C. dubia* were toxic (Figure 1, Table II). Chemical analyses combined with results of the 5 Phase I TIEs conducted on these samples suggested that the organophosphate pesticide, chlorpyrifos was the cause of *Ceriodaphnia* mortality in the Revolon Slough samples. Diazinon and carbaryl were also often detected in Revolon Slough and though these pesticides were usually below their respective LC50s for *C. dubia*, they may have contributed toxicity to these samples. Toxicity of diazinon and chlorpyrifos has been shown to be approximately additive with this species (Bailey *et al.*, 1997). Eighty-three percent of the Revolon Slough samples were also toxic to fathead minnows (*P. promelas* – Figure 2) and two of the samples were toxic to the alga *Selenastrum capricornutum*. The causes of toxicity to these species were not clear. Concentrations of the herbicide diuron exceeded the toxicity threshold for *Selenastrum* in some cases. Twenty-five percent of the Beardsley Wash samples and fifty percent of the Santa Clara Drain samples were toxic to *Ceriodaphnia* (Table II), and chemical analyses and TIE results also indicated toxicity was apparently due to chlorpyrifos.

Sources of pesticides in the lower reaches of the Calleguas watershed include agriculture and urban inputs. The majority of the lower portion of the Revolon Slough is surrounded by cultivated fields including row crops and some citrus orchards. Runoff from these fields include direct tailwater, and tile-drain water from those fields nearer the coast (personal communication, S. Birosik). Additional pesticide sources in the Revolon Slough area include a storm drain from the City of Camarillo and a golf course. Land uses surrounding the Santa Clara Drain are also primarily agricultural while those around the Beardsley Wash area are a mixture of agriculture and some residential development. Bailey *et al.* (2000) found that both residential and agricultural sources contributed the majority of diazinon and chlorpyrifos measured in urban waterways in the central valley of California; while industrial inputs were less important. The relative contribution of pesticide inputs from these different land-use practices are of concern, but beyond the scope of the current study. However, there appears to be a relationship between the county-wide use of chlorpyrifos in Ventura County and average toxicity detected at these three study sites; in most cases greater toxicity occurred during months when agricultural application rates were higher (Figure 3).

Other studies have also suggested water quality impairment at the Revolon Slough study site. The California Department of Fish and Game conducted surveys of aquatic benthic macroinvertebrates throughout the watershed in 1998. Characterization of the Revolon Slough indicated a poor Index of Biological Integrity compared to a more pristine Northern California study area (Harrington *et al.*, 1998). These authors found few pollution sensitive indicator species, including only one Trichopteran (caddisfly) species and no Ephemeroptera (mayfly) or Plecoptera (stonefly) species. The benthic macroinvertebrate assemblage at this site was dominated by oligochaetes, dipteran larvae, and planarians, many species of which are considered to be more pollution tolerant (Harrington *et al.*, 1998). This survey was not designed to determine causes of impacts on aquatic insects, and other factors such as poor habitat quality and high sedimentation probably influenced insect numbers at this site. However, combined with the toxicological and chemical characterizations of the current study, this provides compelling evidence that water quality at Revolon Slough is degraded.

Apparent causes of toxicity to *C. dubia* in Conejo Creek samples differed from those in the lower watershed samples discussed above. Fifty percent of the Conejo Creek samples were toxic to *C. dubia*. Results of Phase I and Phase II TIEs conducted on the December 1998 sample from this site suggested that diazinon was the cause of toxicity. Agriculture inputs are limited in this part of the watershed and sources of diazinon are more likely associated with urban runoff and treated wastewater inputs. As part of local NPDES monitoring, two municipal wastewater treatment plants that discharge into Conejo Creek (the City of Thousand Oaks – Hill Canyon Plant, and the City of Camarillo Treatment Plant) are required to conduct routine chemical monitoring of their discharges and Creek receiving water. Analyses of receiving water concentrations in months during which this study was conducted indicated low concentrations of chlorpyrifos in both effluents and receiving waters; chlorpyrifos was detected in only one receiving sample. Diazinon was detected more often, twice in the effluent and more often in the receiving water; the concentration in the receiving water below the Camarillo discharge was as high as 140 ng/L in the month preceding the December sampling though this concentration is less than the LC_{50} for *C. dubia*.

Diazinon was also the likely cause of toxicity in the upper watershed samples. Sixty three percent of the Downstream Arroyo Simi samples, and 38% of the

Upstream Arroyo Simi samples were toxic to *Ceriodaphnia*. TIEs combined with chemical analyses conducted on subsets of these samples also implicated diazinon (Figure 4; Table IV). Sources of diazinon in the Upper Arroyo Simi sample are probably limited to urban runoff because there are minimal agricultural inputs in this part of the watershed (S. Birosik, personal communication). In addition to treated wastewater inputs, sources of diazinon in the Lower Arroyo Simi are dominated by residential and commercial inputs from the City of Simi Valley. No diazinon was measured in routine NPDES monitoring of the creek water upstream of the wastewater treatment plant during the months when toxicity was detected as part of this study. Approximately 100 ng/L of diazinon was measured in the creek water downstream of the Simi Valley treatment plant in November 1998, and December 1999, but creek water diazinon concentrations did not always correspond to those measured in the effluent. Elevated concentrations of diazinon (250 ng/L) were detected in the treatment plant effluent only in November 1998 and May 1999. Thus, diazinon is sometimes present in the effluent in concentrations that approach the range toxic to *C. dubia*, but effluent inputs do not always relate to the creek water toxicity measured as part of this study. This suggests that non-point pollution sources (e.g., residential and/or commercial runoff) are also contributing diazinon to this system.

Samples from the Upper Arroyo Simi, Conejo Creek, and especially Lower Arroyo Simi were often toxic to fathead minnows (*P. promelas*). It is not clear what caused fish mortality in the Upper Arroyo Simi Creek samples, but in the Lower Arroyo Simi samples, mortality was apparently due to elevated unionized ammonia. When sample pH was adjusted to 7.0 or lower, survival improved in all cases; a similar pattern was observed in the two Conejo Creek samples that were significantly toxic to minnows (Table V). Regional Board staff are currently working with the Simi Valley (Lower Simi) and Thousand Oaks (Conejo Creek) waste treatment facilities to reduce effluent ammonia concentrations to levels consistent with Basin Plan objectives for the Calleguas Creek watershed which must be achieved by June, 2002 (personal communication, S. Birosik, Los Angeles Regional Water Quality Control Board).

In addition to the toxicological information from the current study, previous investigations have found evidence of ecological impairment at several of the upper Calleguas watershed study sites. Benthic macroinvertebrate surveys conducted by Harrington *et al.* (1998) at the Conejo Creek, and the Upper and Lower Arroyo Simi stations suggest these sites have a poor biotic condition relative to more pristine sites. These authors found that the aquatic insects found in this system are indicative of a heavily sediment-impacted system, and that macroinvertebrate community structure is also apparently impacted by poor water quality. This is particularly evident at the Lower Arroyo Simi station, where there was decreased diversity and increased dominance of filter-feeding dipteran larvae (blackflies) relative to the Upper Arroyo Simi station, a common effect of waste treatment effluent. The effect of waste effluent on the benthic macroinvertebrate community was less evident at the Conejo Creek station (Harrington et al., 1998). In addition to toxicity studies, Bailey *et al.* (1996a) conducted histopathological analyses on fish collected from Conejo Creek, Calleguas Creek, and Upper and Lower Arroyo Simi Creek. Fish (*Pimephales promelas*) from Conejo Creek showed few obvious abnormalities, while those from lower Calleguas Creek (*P. promelas, Gambusia affinis*) displayed a number of tissue abnormalities associated with chemical exposure (e.g., hepatic necrosis, liver tumors, basophilic adenoma, hepatocyte vacuolation). Results from the California Toxic Substances Monitoring Program have indicated that fish sampled in the lower parts of the Calleguas watershed contain among the highest concentrations of arsenic, silver, DDT, and methoxychlor in California, and that fish from this watershed are also contaminated with high concentrations of a number of other organochlorine pesticides (Rasmussen *et al.,* 1995). While the studies discussed above were not designed to determine causes of insect community degradation or fish histopathological abnormalities, or the consequences of elevated tissue contaminant levels, when combined with available chemical, toxicity test and TIE information (present study and Bailey *et al.*, 1996a), they suggest chemical contaminants are impacting this system.

In a review of the application of toxicity tests in freshwater ambient monitoring in California, de Vlaming *et al.* (2000) found that since the first ambient studies conducted in 1985, these procedures consistently identified receiving water toxicity due to ammonia, pesticides, and herbicides in state waters. When combined with TIEs and chemical analyses, these tests were particularly useful for identifying causes of receiving water toxicity. Studies conducted in California's Central Valley, Imperial Valley, the Alamo River, the Sacramento-San Joaquin River Delta (see review by de Vlaming *et al.*, 2000), and in numerous urban creeks in the Sacramento Valley area (Bailey *et al.,* 2000), have identified toxic receiving waters. A majority of these studies have identified chemicals responsible for toxicity, and these results suggest toxicity due to contamination by organophosphate pesticides, ammonia, and in some cases, trace metals, and herbicides, is common in state waters.

In addition to pervasive water pollution, there is increasing evidence that sediment contamination is a significant problem in coastal watersheds of California (Fairey *et al.*, 1998, Anderson *et al.*, 1998, Hunt *et al.,* 2001). Results of a recent water quality monitoring program found significant mortality of the amphipod *Hyalella azteca* in 10-day exposures. Significant mortality of this species occurred in surficial sediment samples collected in November 1998 from lower Arroyo Simi, Conejo Creek, Revolon Slough, and lower Calleguas Creek (Walker, 2000). Relationships between amphipod mortality and sediment contamination were difficult to determine in this study because of a limited chemical analyte list and insufficient detection limits. For example, although some organochlorine pesticides (e.g., DDTs and chlordane) were elevated in many of these samples, PCBs and organophosphate pesticides were not measured in sediment samples in this study. In addition, Anderson *et al.* (1998) found sediments of Mugu Lagoon at the mouth of Calleguas Creek were contaminated by a number of organochlorine compounds, and these were sometimes associated with degraded benthic community structure and significant amphipod toxicity in sediment toxicity tests. These authors also found elevated concentrations of chlorpyrifos in some Mugu Lagoon sediments. Levels of chlorpyrifos comparable to the reported LC_{50} concentration have been measured in pore water of freshwater sediments toxic to the amphipod *Hyalella azteca* in another agriculturally-dominated receiving water (Anderson *et al.*, unpublished data). These results suggest that sediment contamination may be present in the Calleguas Creek system, and toxicity associated with contaminated sediments should be investigated in future investigations in the lower parts of the watershed.

5. Conclusions

The Water Quality Control Plan (Basin Plan) for the Los Angeles Region designates 21 beneficial uses for the Calleguas Creek watershed (CRWQCBLA 1994). In addition to ground water recharge and agriculture water supplies, this watershed provides significant freshwater habitat in this normally arid region of Southern California. In addition, this system supplies the majority of freshwater into the ecologically important wetland habitats of the Mugu Lagoon state ecological reserve. Because minimizing contaminant inputs is a primary objective of local and state regulatory agencies responsible for protecting this area, future sampling programs in this watershed will be designed to identify key inputs of pesticides and other chemicals responsible for water quality impairment by separating residential, commercial, and agricultural sources. For example, the current study found toxicity due to chlorpyrifos was more common in the lower watershed while toxicity due to diazinon and ammonia was more common in the upper watershed. Point and non-point source inputs differ in these two parts of the watershed, because agricultural sources are more prevalent in the lower watershed while the upper watershed is more heavily influenced by residential, commercial, and treated waste waters. In addition to upcoming Total Maximum Daily Load studies scheduled for the Calleguas Creek watersheds (California State Water Resources Control Board, 1998), future studies will be designed to investigate the relative contributions of contaminants by these different sources.

References

Anderson, B. S., Hunt, B. M., Phillips, S., Newman, J., Tjeerdema, R. S., Fairey, R., Oakden, J., Wilson, C. J., Kapahi, G., Stephenson, M. D., Puckett, Lyons, M. and Birosik, S.: 1998, 'Chemistry, Toxicity and Benthic Community Conditions in Selected Sediments of the Los Angeles Region', *Final Report*, State Water Resources Control Board, Sacramento California. pp 232.

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

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, USA', *Environ. Toxicol. Chem.* **19**, 82–87.

Bailey, H. C., Villalobos, A., Gottl, E., Brattin, L., Hanes, D. and Hinton, D. E.: 1996a, 'Toxicity Study of the Santa Clara River, San Gabriel-River, and Calleguas Creek', *Technical Report*, California Regional Water Quality Control Board, Los Angeles Region, Los Angeles, CA, 90013, pp 96–114.

Bailey, H. C., DiGiorgio, C., Kroll, K., Miller, J. L., Hinton, D. E. and Starrett, G.: 1996b, '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.

California Regional Water Quality Control Board, Los Angeles Region: 1994, Water Quality Control Plan- Los Angeles Region. Basin Plan for the Coastal Watersheds of Los Angeles and Ventura Counties.

California Regional Water Quality Control Board, Los Angeles Region: 1995, Calleguas Creek preliminary report: State of the Watershed. CRWQCB – Los Angeles, CA. 46 pp.

California State Water Resources Control Board: 1998, California 303 (d) list and TMDL priority schedule. Proposed study plan approved by U.S. EPA (May 12, 1999).

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.

U.S. Environmental Protection Agency: 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.

Fairey, R., Roberts, C., Jacobi, M., Lamerdin, S, Clark, R., Downing, J., Long, E., Hunt, J., Anderson, B, Newman, J., Stephenson, M. and Wilson, C. J.: 1998, 'Assessment of sediment toxicity and chemical concentrations in the San Diego Bay region, California', *Environ. Toxicol. Chem.* **17**(8), 1570–1581.

Fukuto, T. R.: 1987, 'Organophosphorous and Carbamate Esters: The Antichloinesterase Insecticides', in: J. Biggar and J. Seiber (eds), *Fate of Pesticides in the Environment, Publication 3320*, Agricultural Experimental Station, University of California, Davis, CA, USA, pp. 5–18.

Harrington, J. M.: 1998, 'Biological and Physical Habitat Assessment of California Water Bodies: Calleguas Creek Characterization Study – Benthic Macroinvertebrates', pp. 18.

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.

Markle, P. J., Gully, J. R., Baird, R. B., Nakada, K. M. and Bottomley, J. P.: 2000, 'Effects of several variables on whole effluent toxicity test performance and interpretation', *Environ. Toxocol. Chem.* **19**, 123–132.

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.

Oris, J. T., Winner, R. W. and Moore, M. V.: 1991, 'A four-day survival and reproduction test for *Ceriodaphnia dubia*', *Environ. Toxicol. Chem.* **10**, 217–224.

Rasmussen, D.: 1995, 'Toxic Substances Monitoring Program: 1992–1993 Draft Report', *Publication No. 95-1 WQ*, State Water Resources Control Board, California Environmental Protection Agency, Sacramento, CA, U.S.A.

U.S. Environmental Protection Agency: 1991, Phase I Toxicity Characterization Procedures, Second Edition. EPA/600/6-91/003.

U.S. Environmental Protection Agency: 1994, 'Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Water to Freshwater and Marine Organisms', 4th ed., *EPA 600/4-90/027. Technical Report*, Washington, DC.

U.S. Environmental Protection Agency: 1995, Office of Pesticide Programs, Environmental Effects Database (EEDB), Environmantal Fate and Effects Division, U.S. EPA, Washington, DC.

Walker, L.: 2000, *Calleguas Creek Characterization Study: Results of the Coordinated Water Quality Monitoring Program, Surface Water Element*. L. Walker and Associates, Thousand Oaks, CA 91360.