Characterization of stormwater toxicants from an urban watershed to freshwater and marine organisms

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ABSTRACT

Numerous studies have shown that urban stormwater discharges in southern California are a large source of pollutants to coastal waterbodies, but their biological effects have been studied rarely. This study addressed two primary questions: (1) How do the toxic responses to urban stormwater runoff differ between freshwater and marine organisms? and (2) Which constituents are responsible for stormwater toxicity in freshwater and marine organisms?

Samples were collected from Chollas Creek, a highly urbanized watershed in San Diego, California, that discharges directly to San Diego Bay. Stormwater samples were tested using one freshwater species (*Ceriodaphnia dubio*, water flea) and two marine species (*Strongylocentrotus purpuratus*, purple sea urchin; and *Mysidopsis bahia*, mysid shrimp). Toxicity identification evaluations (TIEs) were conducted on each species to determine the toxic constituent(s).

No two species responded similarly after exposure to stormwater from Chollas Creek. The sea urchin was extremely sensitive to stormwater, exhibiting responses during every storm at concentrations as low as 12% stormwater. The other marine species, *Mysidopsis*, exhibited no response to stormwater for any of the storms sampled. *Ceriodaphnia* exhibited intermediate toxic responses; two of three samples were toxic at relatively high concentrations (100%) of stormwater. The pattern of toxicity was also inconsistent; no single storm was the most toxic to both the marine and freshwater species.

Organophosphate pesticides in stormwater runoff from Chollas Creek were responsible for the toxicity observed in the freshwater species *Ceriodaphnia*. Concentrations of diazinon and chlorpyrifos, both organophosphate pesticides, were found in the stormwater samples in sufficient amounts to induce toxicity. Trace metals in stormwater runoff from Chollas Creek were responsible for the toxicity observed to the sea urchin. Concentrations of zinc, and to a lesser extent copper, were of sufficient quantity in the stormwater samples to induce toxicity.

INTRODUCTION

Stormwater runoff from urbanized watersheds has been receiving increasing attention from the public and scientific community in recent years because it is perceived to be a large source of pollutants to coastal waterbodies around the nation (U.S. EPA 1995a). In southern California, the runoff from urbanized watersheds has contributed substantial loadings of a variety of constituents to receiving water environments (Schiff 1997). For example, the cumulative loads of lead and zinc from all of the urbanized watersheds in the Southern California Bight to the coastal oceans were estimated to be 39 and 316 metric tons during the 1994/95 water years, respectively. These inputs represent over half of the combined mass emissions from all sources, which include traditional point sources such as publicly owned treatment works, industrial facilities, and power generating stations (Schiff *et al.* in press).

Although mass emissions from urban runoff in southern California have been determined to be large, little is known about the extent that stormwater inputs affect biota. Some studies have analyzed the effect of stormwater discharges on freshwater organisms including algae, invertebrates, and fish (Katznelson *et al.* 1995; Field and Pitt 1990; Pitt *et al.* 1990, 1995). However, studies that have examined the effects of stormwater discharges on marine species are rare (Bay *et al.* 1997). Southern California has very little freshwater habitat because of its semi-arid climate; therefore, the ocean environment has generated the most concern from the public. The coastal environment of the SCB has been estimated to generate as much as $9 billion

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per year in revenues from recreational uses.

This study addressed two questions of environmental concern to further assess the impacts of stormwater discharges on aquatic organisms. The first question was: "How do the toxic responses to stormwater runoff differ between freshwater and marine organisms?" The goal of this study objective was to evaluate potential impacts to either the freshwater or marine habitats that receive runoff discharges. The second question was: "Which constituents are responsible for toxicity in freshwater and marine organisms?" The goal of this study objective was to identify the constituent(s) of concern in stormwater discharges and to determine whether the constituents are similar between the two habitats.

METHODS

Study Site and Sampling Strategy

This study focused on Chollas Creek, a highly urbanized watershed in San Diego, California, that drains 16,273 acres to San Diego Bay. The predominant land uses are residential (67%), commercial/industrial (12%), roadways (4%), and open space (16%) (Schiff and Stevenson 1996). The watershed is comprised of two main tributaries, the North Fork and South Fork subwatersheds, whose confluence is located below the tidal prism to San Diego Bay. Tidal influence inhibits flow measurements and accurate sampling; therefore, the sampling site was located on the North Fork subwatershed. The sampling site captured approximately 57% of the entire watershed. Land use characteristics in the North Fork subwatershed are similar in proportion to the entire watershed.

Three storm events were sampled during the 1998/99 wet season using a flow-weighted composite strategy (WC 1998). Automated sampling equipment, triggered by increases in storm flow, was used to collect the composite samples. Flow was determined using pressure transducers to measure stage and calculate cross-sectional wetted surface area while Doppler motion sensors were used to measure velocity. The sampler electronically logged flow and sampling intervals. In addition, grab samples were taken for individual chemical analysis not amenable to compositing due to contamination or holding time constraints. Flow-weighted composite samples were split in the laboratory for chemical and toxicological analysis.

Chemical Analysis

The target analytes and laboratory methods for stormwater constituents were similar to the current National Pollutant Discharge Elimination System (NPDES) stormwater monitoring program and have been described in detail by others (WC 1998). These analytes include general constituents, microbiological indicators, nutrients, and trace metals. All analytical methods utilized approved EPA methods (1983a) or Standard Methods (APHA 1998).

Analysis of organophosphate pesticide and dissolved trace metals was conducted to assist in the characterization and confirmation of specific compounds that were responsible for stormwater toxicity. The organophosphate pesticides diazinon and chlorpyrifos were measured in bulk stormwater and in toxicity samples using enzyme-linked immunosorbent assay (ELISA) methods. Dissolved metals were measured in bulk stormwater and in toxicity samples using standard EPA methods (EPA 200.8); dissolved fractions were obtained using centrifugation (3,000 g for 30 min) and analyzing the supernatent.

Baseline Toxicity

One freshwater and two marine species were used in this study. The freshwater species included the water flea *Ceriodaphnia dubia*. The marine species included the mysid *Mysidopsis bahia* and the purple sea urchin *Strongylocentrotus purpuratus.*

Ceriodaphnia Survival Test

Each of the three stormwater samples was tested for baseline toxicity using an acute exposure test with the freshwater daphnid (water flea) *Ceriodaphnia dubia* (U.S. EPA 1993a). The test procedure consisted of exposing less than 24-h-old daphnids to the samples for 96 h. Five animals were added to each 30 mL glass scintillation vial containing 10 mL of test material. A single 50% renewal of test solutions was performed at 48 h. At the end of the test, the animals were evaluated for survival. The *Ceriodaphnia* were fed a half ration of food (mixture of yeast, Cerophyll®, trout chow [YCT], and *Selenastrum* algae) on days 2 and 3 of the exposure.

Stormwater samples were diluted with laboratory water to concentrations ranging from 100 to 6% runoff. This dilution water consisted of eight parts Nanopure water and two parts Perrier water (8:2 vol:vol). Four replicates of each concentration were tested.

Negative controls (laboratory water) were included in each test series for quality control purposes. Water quality parameters (temperature, dissolved oxygen, pH, and conductivity) were measured on the test samples to ensure that the experimental conditions were within the desired ranges and did not create unintended stress on the test organisms. In addition, a reference toxicant test (copper) was included with each stormwater test series in order to document intra-laboratory variability.

Mysid Survival Test

The Chollas Creek samples from each storm were assessed for toxicity using an acute exposure test with the marine mysid *Mysidopsis bahia* (U.S. EPA 1993a). The procedure consisted of a 96-h exposure of 3-d-old juvenile mysids to the stormwater samples, with 10 animals in each test chamber. A single 75% renewal of test solution was performed at 48 h. At the end of the test, the animals were evaluated for survival. The exposure was conducted in 250 mL glass beakers with 200 mL of test solution in each beaker. The mysids were fed brine shrimp nauplii daily during the exposure.

Before testing, the stormwater samples were adjusted to a salinity of 30 g/kg by adding a sea salt mixture (Forty Fathoms Bioassay Laboratory Formula). Stormwater samples were mixed with sea salts and diluted with seawater to produce concentrations ranging from 100 to 6% runoff. Three replicates of each concentration were tested.

Negative control samples (0.45 µm and activated carbon filtered natural seawater from Redondo Beach diluted to 30 g/kg with distilled water) and sea salt control samples (distilled water mixed with sea salts) were included in each test series for quality control purposes. Water quality parameters (temperature, dissolved oxygen, pH, ammonia, and salinity) were measured in the test samples to ensure that the experimental conditions were within the desired ranges and did not create unintended stress on the test organisms. In addition, a reference toxicant test (copper) was included with each stormwater test series in order to document intra-laboratory variability.

Sea Urchin Fertilization

All samples of stormwater were evaluated for toxicity using the purple sea urchin fertilization test (U.S. EPA 1995b). The test consisted of a 20-min exposure of sperm to the samples. Eggs were then added and 20 min was given for fertilization to occur. The eggs were then preserved and examined later with a microscope to assess the percentage of successful fertilization. Toxic effects are expressed as a reduction in fertilization percentage. Purple sea urchins (*Strongylocentrotus purpuratus*) used in the tests were collected from the intertidal zone in northern Santa Monica Bay. The tests were conducted in glass shell vials containing 10 mL of solution at a temperature of 15° C.

The stormwater samples were adjusted to a salinity of 34 g/kg. Previous experience has shown that many sea salt mixes are toxic to sea urchin sperm. Therefore, the salinity for the urchin test was adjusted by the addition of hypersaline brine. The brine was prepared by freezing and partially thawing seawater. Since the addition of brine dilutes the sample, the highest stormwater concentration that could be tested for the sperm cell test was 50%. The adjusted samples were diluted with seawater to produce test concentrations ranging from 50 to 3%. Five replicates of each concentration were tested.

Seawater control samples (0.45 µm and activated carbon filtered natural seawater from Redondo Beach) and brine control samples (50% distilled water and 50% brine) were included in each test series for quality control purposes. Water quality parameters (temperature, dissolved oxygen, pH, ammonia, and salinity) were measured on the test samples to ensure that the experimental conditions were within the desired ranges and did not create unintended stress on the test organisms. In addition, a reference toxicant test (copper) was included with each stormwater test series in order to document intra-laboratory variability.

Toxicant Characterization (Phase I TIE)

A modified Phase I TIE, using methods described by the U.S. EPA (1991 and 1996), was conducted on each of the three stormwater samples to characterize the toxicants present. Phase I testing was performed using all three test species. These tests were conducted simultaneously with the baseline testing to minimize holding time and any possible associated change in toxicity. Test conditions were the same as for the baseline test, except that a reduced number of replicates were tested, as recommended by EPA guidance.

The salinity of each water sample was adjusted as appropriate for each species before application of the treatments. The specific TIE manipulations conducted varied by species because of differences in organism physiology (Table 1). A core group of four treatments was applied to each sample. These treatments were particle removal, trace metal chelation, nonpolar organic extraction, and chemical reduction. Additional treatments were applied to the daphnid and mysid tests to examine the effects of metabolic activation and pH variation on toxicity.

All treatments that involved the addition of a chemical agent were performed on otherwise unmodified samples. A control sample (laboratory dilution water) was included with each type of treatment to verify that the manipulation itself was not causing toxicity. The toxicity methods used to evaluate the effectiveness of the TIE treatments were the same as those used to measure baseline toxicity, except that only the three highest concentrations were tested and fewer replicates were used.

Ethylenediaminetetraacetic acid (EDTA), a chelator of metals, was added to the test samples. Sodium thiosulfate (STS), a treatment that reduces oxidants such as chlorine and also decreases the toxicity of some metals, was added to separate portions of each sample. The EDTA and STS treatments were given at least 1 h prior to the addition of the test organisms to allow interaction with the sample.

For the *Ceriodaphnia* and mysid tests, piperonyl butoxide (PBO) was added to an aliquot of sample. The PBO is an inhibitor of organophosphate pesticide metabolism, thus blocking the toxicity of these compounds.

For the *Ceriodaphnia* test only, samples were tested at different pH levels (graduated pH test), which may affect the solubility, stability, volatility, polarity, and speciation of some compounds. Samples were adjusted to pHs of 7 and 9 with dilute solutions of HCl or NaOH. A stable pH was not obtained with this method; the pH of the samples drifted towards the original value during the 48-h interval between water changes.

Samples were centrifuged for 30 min to remove particle-borne contaminants and prevent clogging of the C-18 and cation exchange columns. A portion of the centrifuged sample (200 to 1,000 m/L) was passed through a 6 mL Varian Mega Bond Elut or 5 mL Baker C-18 solid phase extraction column in order to remove nonpolar organic compounds. The filtrate was retained for toxicity testing. The C-18 columns were placed in a sealed container and stored under refrigeration for later elution during Phase II testing.

TABLE 1. Summary of Phase I TIE treatments performed on samples of Chollas Creek stormwater.

Treatment	Purpose	Daphnid	Sea Urchin	Mysid
Centrifugation	Removes particles	1,540 x g	3,000 x g	3,000 x g
EDTA	Complexes trace metals	200 mg/L	60 mg/L	60 mg/L
Sodium Thiosulfate	Neutralizes oxidants and complexes some metals	400 or 200 mg/L	50 mg/L	50 mg/L
C-18 SPE	Removes nonpolar organics	✓	✓	✓
Piperonyl Butoxide	Blocks metabolism of organophosphate pesticides	50 mg/L	nt[a]	100 mg/L
Graduated pH	Identifies whether toxicity is pH dependent	✓	nt	nt

[a]Treatment not tested with this species.

Toxicant Identification and Confirmation (Phases II and III TIE)

Due to the lack of toxicity observed during baseline testing of the mysids, Phase II procedures were carried out only for the *Ceriodaphnia* and sea urchin tests. The Phase I testing indicated that these two species were responding to different types of toxicants; therefore, different methods were used for each test species (Table 2).

Ceriodaphnia

Based upon the Phase I results, the Phase II testing was focused on identifying whether organic compounds, especially organophosphate pesticides, were present in toxicologically significant amounts. The experimental procedures included the fractionation and analysis of materials retained by the C-18 SPE column (U.S. EPA 1993b) and measurement of toxicant stability following pH adjustment (U.S. EPA 1991).

The C-18 SPE columns used to treat the stormwater samples during Phase I were eluted with a series of methanol/water concentrations to fractionate the organic compounds responsible for the observed toxicity based upon their polarity. Methanol concentrations ranging from 0 to 100% were sequentially passed through the column to remove compounds of different polarity. The eluates represented a 200 times (Sample SS1) or 500 times (Samples SS2 and SS3) concentration of the original stormwater sample. Each eluate was diluted 100-fold with laboratory water to produce a maximum test concentration of 2 times or 5 times the original sample and tested for toxicity. Two additional dilutions (50 and 25% of the maximum test concentration) were also tested. Toxicity tests were conducted at concentrations higher than the original sample in order to compensate for the potential loss of toxicity resulting from the elution/fractionation process. Two controls were tested with the extracts: (1) laboratory dilution water and (2) water containing 1.5% methanol (the highest concentration used in the experiment).

Prior data from Chollas Creek and other locations indicated that the organophosphate pesticides diazinon and chlorpyrifos were probably present in the stormwater samples.

TABLE 2. Summary of Phase II TIE treatments performed on samples of Chollas Creek stormwater.

Treatment	Purpose	Ceriodaphnia	Sea Urchin
C-18 SPE Elution	Separates possible organic toxicants by polarity	✔	nt[a]
Cation Exchange Extraction/Elution	Verifies that metals removed by cation exchange can be recovered from column	nt	✔
Chemical Analysis of Column Eluates and Post Column Samples	Separates and identifies chemicals removed by columns	✔	✔
pH Adjustment	Alters toxicant characteristics and/or degradation	✔	nt

[a]Treatment not tested with this species.

Samples of stormwater and selected toxic methanol eluates were analyzed for these two pesticides using an ELISA technique. Methanol eluates were also analyzed for organochlorine and organophosphate pesticides using gas chromatography (GC) (EPA Method 507/508). The values measured were then compared to levels reported in the literature to be toxic to *Ceriodaphnia* or related species.

A pH adjustment test was also conducted to examine the stability of the toxicants in Sample SS1. A sample of stormwater from the first storm event and a sample of laboratory dilution water were adjusted to pH 3 and pH 10 with HCl and NaOH, respectively. The samples were maintained at those pH levels for 5 h at 25° C. The pH was then readjusted to the initial pH for toxicity testing.

Sea Urchin

The Phase II TIE tests with sea urchin sperm focused on trace metals. The approach was to determine whether the toxicity: (1) was removed from stormwater by a cation exchange column and (2) could be recovered by elution of the column with acid. Chemical analysis of the sample before and after application to the column, as well as the eluate, was performed to determine which metals were present in significant amounts.

A sample of centrifuged stormwater was passed through a Supelco LC-WCX 3 mL cation exchange column to remove cationic trace metals. The filtrate passing through the column was retained for toxicity testing. The cation exchange columns were then eluted with 0.7 (Sample SS1) or 2.0 N HCl (Samples SS2 and SS3). The resulting eluate was approximately 20 times more concentrated than the original stormwater sample. The eluate was then diluted with seawater to 1.5 times the original stormwater concentration and tested for toxicity. Additional dilutions (50 and 25%) of the 1.5 times sample were also tested. Two blanks were tested for quality control purposes: (1) a column blank containing deionized water passed through the column and (2) an eluate blank containing the acid eluate from a deionized water-rinsed column.

Stormwater and eluate samples were analyzed for trace metals using a high-resolution inductively coupled plasma mass spectrometer (ICP/MS). Identification of metals present in toxicologically significant amounts was accomplished by comparing the analytical data to EC50 values for the metals (Southern California Coastal Water Research Project, unpublished data).

Statistical Analysis

The sea urchin toxicity tests were normalized to the control response in order to facilitate comparisons of toxicity among experiments. Normalization was accomplished by expressing the individual replicate values as a percentage of the control value.

Four statistical parameters (NOEC, LOEC, EC50 or LC50, and TUs) were calculated to describe the magnitude of stormwater toxicity. The no observed effects concentration (NOEC) is the highest test concentration not producing a significant toxic response and the lowest observed effects concentration (LOEC) is the lowest test concentration producing a significant toxic response. The NOEC and LOEC were determined by testing the response at each concentration for a statistically significant difference from the control. The data were first arcsine transformed, and then tested for homogeneity of variance and normal distribution. Data that met these criteria were then tested using one-way analysis of variance (ANOVA) and Dunnett's test to identify differences relative to the control value. Data that did not pass the test for homogeneity of variance and/or normal distribution were analyzed by the non-parametric Steel's Many-One Rank test.

The effects concentration 50% (EC50) or lethal concentration 50% (LC50) are the concentrations of stormwater producing a 50% reduction in fertilization or survival, respectively. The LC50 and EC50 were calculated using either probit or trimmed Spearman-Karber methods, respectively. The toxic units (TUs), an alternate expression of the stormwater EC50 or LC50, were calculated as 100/

EC50 or 100/LC50. The TUs were also calculated for individual chemical constituents in stormwater as the concentration in the sample divided by the EC50 or LC50 of the single chemical (obtained from the literature). Unlike the EC50 or LC50, TUs are directly proportional to the magnitude of toxicity and can be used to estimate the fraction of toxicity associated with specific chemicals or removed by procedures during the TIE confirmation phase.

The final data category analysis compared the data collected during this study to data collected during previous storms monitored in this watershed, dating back as far as 1993. Most data were collected as part of the municipal stormwater NPDES permit requirements (WC 1998). Different indicators were compared including storm size (rainfall), water quality (constituent concentrations), and toxicity. The comparisons were used to assess the representativeness of the selected events. Therefore, the minimum, maximum, median, and average of the historical data is provided.

RESULTS

The three storms sampled for this study were of a moderate size for this region (Table 3). Rainfall quantities ranged from 0.24 to 0.63 inch. The third storm produced the largest rainfall, but the second storm produced the highest rainfall intensities. These storms were similar in size to many of the storm events monitored in this watershed since the 1993/94 wet season. However, two differences were noted. First, the second storm produced the highest rainfall intensities that have been monitored on the watershed to date. Higher rainfall intensities have the potential to generate larger flows, which mobilize particles and their associated pollutants. Second, the storms studied herein were sampled later in the season than those previously monitored. Of the 15 events sampled between 1993/94 and 1997/98, 12 were sampled before March and only 2 were sampled later than April 6.

Storms sampled during the present study were not distinctly different in constituent concentrations relative to other storms monitored previously in this watershed. Event mean concentrations (EMCs) for stormwater constituents from any single storm event sampled as part of this study were not consistently higher than those for previous storm events (Table 4).

Trace metal concentrations were highest for three of seven trace metals during the first storm. However, the second storm generated the highest concentrations for three of five nutrient constituents. Although the third storm was larger than the first two events, it did not generate the highest concentrations for most constituents. All three storms generated relatively high bacteriological measurements.

Most trace metals were near or below the median EMC sampled between the 1993/94 and 1997/98 wet seasons. For example, the zinc concentrations measured during our three storm events ranged from 90 to 220 µg/L while the range of EMCs over the past six years has ranged from 11 to 560 µg/L. Except for nickel and chromium, no sample during this study exceeded the six-year maximum in this watershed. Most storms in this watershed are characterized by having large bacterial densities for each of the microbiological indicators.

The comparison of trace metal concentrations to water quality criteria provides another context for evaluating stormwater results (Figure 1). No water quality criteria have been established by the State of California for stormwater discharges. However, the U.S. EPA has proposed the California Toxics Rule (40CFR Part 131, August 5, 1997), which establishes water quality standards based upon hardness (U.S. EPA 1994) for NPDES discharges to freshwater or estuarine (bays and estuaries) receiving waters. Concentrations of copper and zinc, the constituents chosen as examples in this study, have routinely exceeded these water quality thresholds over the past six years from this watershed. For example, 11 of 15 storm EMCs have exceeded the water quality thresholds for copper and 11 of 15 storm EMCs have exceeded the water quality thresholds for zinc. Copper and zinc exceeded water quality thresholds during two of three storms sampled during this study. However, Storm 3 had the lowest concentrations of copper and zinc observed over the NPDES monitoring history on Chollas Creek. The storms that were

TABLE 3. Precipitation results for monitored storms from this study (Samples SS1 – SS3) and from all storms monitored at the study site between the 1993/94 and 1998/99 wet seasons.

Storm Number	Storm Date	Rainfall Quantity (inches)	Rainfall Intensity (inches per hour)
1 (Sample SS1)	15-Mar-99	0.24	0.01
2 (Sample SS2)	25-Mar-99	0.59	0.12
3 (Sample SS3)	6-Apr-99	0.63	0.05
All Storms 1993-1998	N	15	15
	Minimum	0.18	0.02
	Median	0.32	0.04
	Maximum	1.37	0.11
	Average (standard deviation)	0.56 (0.43)	0.05 (0.03)

TABLE 4. Stormwater event mean concentrations (EMCs) during this study compared to the minimum, median, maximum, and average (standard deviation [SD]) EMCs for previously monitored storms at the sampling location on Chollas Creek between 1993/94 and 1997/98 (N=15).

		Storm 1	Storm 2	Storm 3	Previously Monitored Events			
		15-Mar-99	25-Mar-99	5-Apr-99	Minimum	Maximum	Median	Average (SD)
Total Suspended Solids	mg/L	159	150	44	75	330	1,200	416 (314)
Total Dissolved Solids	mg/L	222	150	300	39	250	460	217 (98)
Turbidity	ntu	21	110	31	18.4	54.2	290	70.6 (64.7)
Hardness	mg/L	90.8	68	110	39	91	150	90 (30)
Surfactants (MBAS)	mg/L	0.7	0.27	0.25	0.07	0.23	1	0.34 (0.31)
BOD	mg/L	11	22	15	< 3	17	49	22 (13)
Oil and Grease	mg/L	0.95	14	5	< 0.5	2.2	6.9	2.8 (1.8)
Total Coliform	cfu/100 mL	>2,400,000	1,100,000	no data	56,000	160,000	240,000	160,000 (89,000)
Fecal Coliform	cfu/100 mL	>16,000	140,000	no data	3,000	17,000	240,000	42,000 (67,000)
Fecal Streptococci	cfu/100 mL	240	300,000	no data	110	16,000	>160,000	42,000 (55,000)
Ammonia	mg/L	1.06	0.5	< 0.1	< 0.2	0.67	10	1.5 (2.7)
Nitrate-Nitrogen	mg/L	0.44	0.7	0.4	0.7	1.3	2.7	1.6 (2.7)
Nitrite-Nitrogen	mg/L	0.14	< 0.1	< 0.1	< 0.05	< 0.05	< 0.05	< 0.05 (0.0)
Kjedahl Nitrogen	mg/L	3.61	2.4	1	<1.0	3.1	15	4.1 (3.8)
Total Phosphorous	mg/L	0.17	0.62	0.33	< 0.1	0.65	2.2	0.74 (0.49)
Dissolved Phosphorous	mg/L	0.22	0.42	0.2	< 0.1	0.4	1.41	0.43 (0.32)
Arsenic	ug/L	3	< 5	< 5	2	5.9	11	5.9 (2.8)
Cadmium	ug/L	< 0.3	< 2	< 2	0.3	0.8	2.5	1.1 (0.7)
Chromium	ug/L	35	< 20	< 20	3.4	6.7	11	7.2 (2.8)
Copper	ug/L	15	30	10	10	29	85	33 (19)
Lead	ug/L	82	30	< 10	3	64	140	70 (48)
Nickel	ug/L	16	< 20	< 20	5.6	11	14	10 (2.8)
Zinc	ug/L	210	220	90	11	185	560	215 (141)

above water quality thresholds in this study exceeded the thresholds by a smaller magnitude than typically has been measured (Figure 1). Copper has exceeded water quality thresholds by as much as a factor of five, while the storms in this study exceeded water quality thresholds by a maximum factor of three. Zinc has exceeded water quality criteria by as much as a factor of four, while storms in the present study exceeded water quality thresholds by a maximum factor of only two.

Comparative Toxicity

Each of the three test species responded differently to the Chollas Creek stormwater samples. Toxicity was detected with the freshwater test (*Ceriodaphnia dubia* 96-h survival test) and one of the marine tests (purple sea urchin [*Strongylocentrotus purpuratus*] fertilization test). No toxicity was detected with the third test species, a marine mysid (*Mysidopsis bahia* 96-h survival). Mysid survival was unaffected by exposure to any of the three Chollas Creek stormwater samples tested.

The greatest sensitivity to stormwater was observed for the purple sea urchin. Inhibition of fertilization of sea urchin eggs was produced following a 40-min exposure to stormwater concentrations of 12 to 25% (Table 5). Toxicity to sea urchins was present in each of the three samples tested. The EC50 for reduced fertilization was 18 to 47%, which represented 2.2 to 5.5 TUs. The sample from Storm 2 was most toxic.

Chollas Creek stormwater was also acutely toxic to the daphnid *Ceriodaphnia dubia*. Toxicity was detected in the first two storms, where 96-h survival was reduced by exposure to undiluted stormwater (Table 5). Mortality was only observed in the 100% stormwater treatment. Daily observations indicated a similar pattern of toxic response for Samples SS1 and SS2. All mortality occurred between 48 and 96 h of exposure and any surviving *Ceriodaphnia* in the 100% concentration were immobilized, displaying only slight twitching movements. *Ceriodaphnia* survival was less sensitive than the sea urchin fertilization test, as indicated by the higher LC50 values (75 to 79%) and lower TU values (1.3 TU). A similar level of toxicity was present in both storms. No toxicity was detected for the third stormwater sample.

The toxicity observed with *Ceriodaphnia* during this study was similar to previous storms monitored in this watershed (Table 5). Tests of 11 stormwater samples for acute toxicity to *Ceriodaphnia* since 1993 show a similar or higher level of toxicity compared to the three storms measured in this study. Only one other storm in this watershed had been tested with a marine species previously; the toxicity test was similar to the one used herein. Like the freshwater tests, the results were consistent with those measured in this study.

Identification of Toxicants to Sea Urchins

Toxicant Characterization (Phase I)

A similar pattern of response to each of the treatments was obtained for each sample, suggesting that similar toxicants were present in each sample (Figure 2). Complete removal of the toxicity, as indicated by the large increase in fertilization relative to the baseline value, was produced only by the addition of EDTA. This treatment is effective when cationic trace metals (e.g., cadmium, copper, lead, and zinc) are important toxicants.

The remaining characterization treatments were variable in their effectiveness for removing toxicity. Extraction of the sample with a column containing C-18 media was partially effective, usually removing less than 50% of the toxicity present. Extraction with C-18 is effective with some non-polar organic compounds, but this treatment may also reduce toxicity caused by some trace metals. Two other treatments, removal of particles by centrifugation and addition of STS, were not effective. Fertilization measured after the application of these two treatments remained similar to the baseline value.

The fertilization percentage was high in control samples (laboratory seawater or deionized water) that received each of the characterization treatments. These results indicated that none of

FIGURE 1. Cumulative distribution functions (CDFs) of copper and zinc concentrations in Chollas Creek stormwater relative to proposed water quality standards from the California Toxics Rule (CTR). The CDFs were generated by dividing the observed concentration by the water quality standard; a value of 1 occurs when the concentration equals the standard. The CDFs for marine and freshwater standards were similar. The samples collected during this study are indicated.

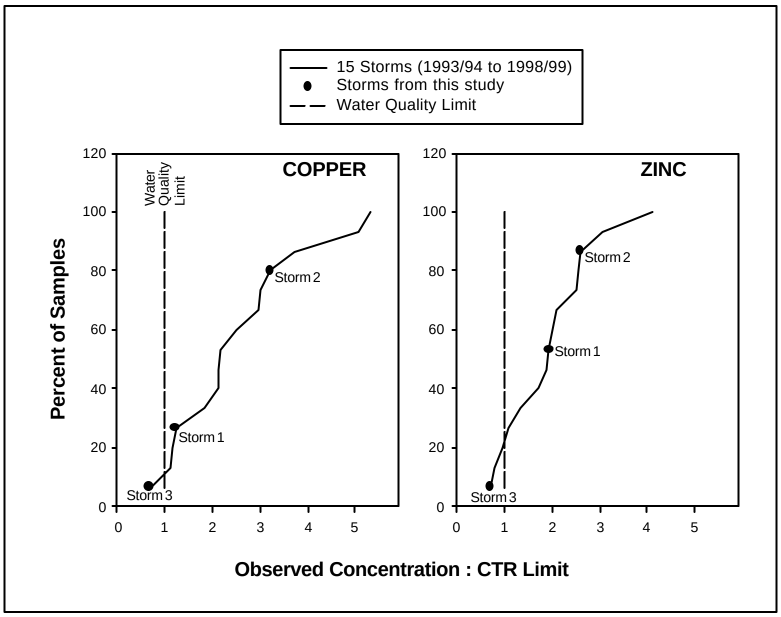


TABLE 5. Summary of toxicity test results for Chollas Creek stormwater samples during the three storms sampled as part of this study and from storms sampled previously from this watershed. Abbreviations: EC50, concentration producing a 50% effect (reduced fertilization); NOEC, highest concentration not producing a significant effect; LOEC, lowest concentration producing a significant effect; %, percent of stormwater eliciting response; TUs, toxic units; nd, no toxicity detected.

					Daphnid Survival				Mysid Survival			
	EC50	NOEC	LOEC	TUs	LC50	NOEC	LOEC	TUs	EC50	NOEC	LOEC	TUs
Sample	(%)	(%)	(%)		(%)	(%)	(%)		(%)	(%)	(%)	
SS1	46.6	12	25	2.2	75	50	100	1.3	nd	nd	nd	nd
SS2	18.2	6	12.5	5.5	79	50	100	1.3	nd	nd	nd	nd
SS3	27.7	6	12.5	3.6	nd	nd	nd	nd	nd	nd	nd	nd
All Storms 1993-1998												
Number	1	1	1	1	11	11	11	11				
Minimum	-	-	-	-	35	12	25	1.2				
Median	36.2	12	25	2.8	55	25	50	2.7				
Maximum	-	-	-	-	82	50	>100	1.8				
Average (sd)	-	-	-	-	52.8	30.3		2.1				

the treatments produced interferences that influenced the toxicity responses.

Toxicant Identification (Phase II)

Extraction of the stormwater samples with the cation exchange column eliminated toxicity for all three samples (Table 6). Elution of the columns with acid recovered toxicity from two of the columns; approximately 47% of the toxicity was recovered for Samples SS2 and SS3. The reason for the lack of recovery of toxicity from the Sample SS1 cation exchange column is not known.

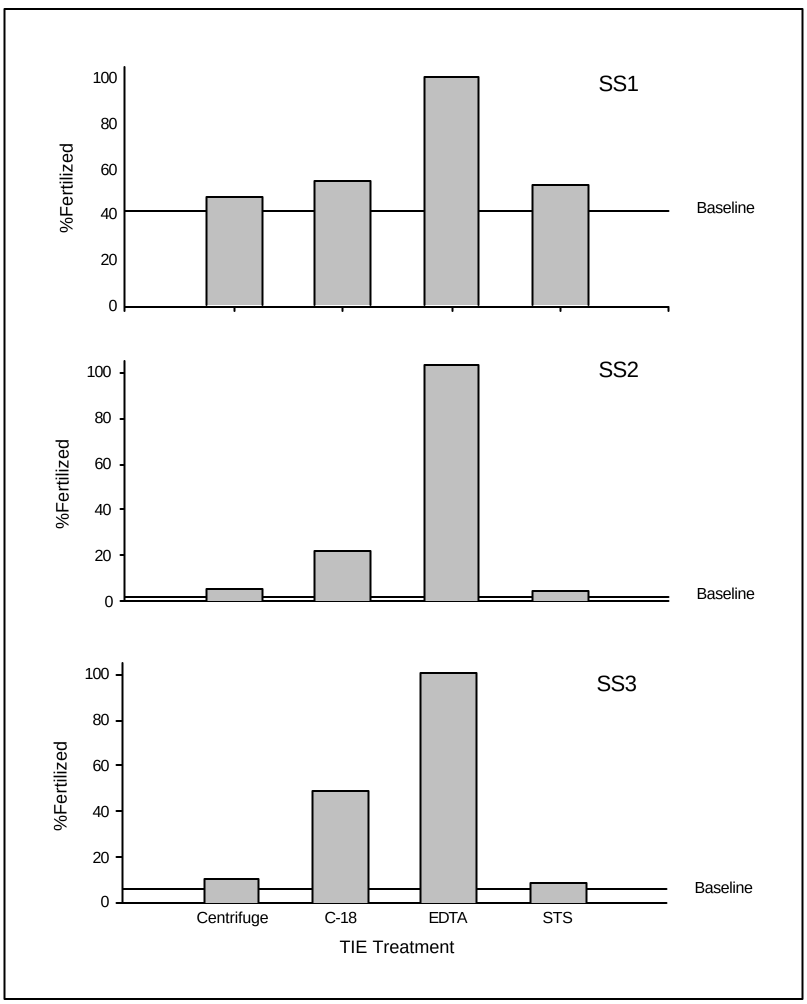
Samples of the centrifuged stormwater, column filtrate, and acid eluate from the column were analyzed by ICP/MS for trace metals (Table 6). Arsenic, chromium (total), copper, lead, nickel, selenium, and zinc were detected in the fractions. Concentrations were low (<5 µg/L) for all metals except copper and zinc. Comparison of the pre- and post-column fractions indicated that copper and zinc were the only metals that were removed by the cation exchange column, and thus could potentially account for the toxicity changes observed in Figure 2. Most of the copper and zinc removed by the column was recovered in the acid eluate (Table 6).

Examination of the metals data indicated that zinc and possibly copper were present at concentrations likely to be toxic to sea urchin sperm. Concentrations of zinc ranged from 32 to 75 µg/L in the samples demonstrating toxicity. All of these concentrations were above the concentration of zinc found to be strongly toxic in prior SCCWRP experiments (EC50 of 29 µg/L). Copper concentrations, although elevated in the toxic samples, were less than 10 to 43% of the EC50 of 30 µg/L.

Toxicant Confirmation (Phase III)

The role of zinc and copper in the toxicity of Chollas Creek stormwater to sea urchins was confirmed by a comparison of observed and predicted TUs for the stormwater samples. Comparisons were made between the observed TUs and the predicted TUs for the centrifuged stormwater sample used for the cation exchange treatments. Between 55 and 95% of the total (observed) toxicity was accounted for by zinc and copper, depending upon the storm evaluated (Figure 3). Of the two metals, zinc accounted for the majority of the predicted toxicity due to its higher concentration in each stormwater sample (Table 6).

FIGURE 2. Summary of toxicity characterization results for Chollas Creek stormwater samples tested with the sea urchin fertilization test. Values shown are survival following treatment of a sample containing 50% stormwater.



Identification of Toxicants to Ceriodaphnia

Toxicant Characterization (Phase I)

Two Phase I TIE treatments, solid phase extraction (SPE) with a C-18 column and the addition of PBO, had a similar beneficial effect on the toxicity of Samples SS1 and SS2 (Figure 4). These two treatments eliminated all toxicity in both samples. The SPE using C-18 is effective when nonpolar toxicants are present and PBO blocks the action of organophosphate pesticides.

Variable responses of *Ceriodaphnia* to the other characterization treatments were observed between samples (Figure 4). For Sample SS1, survival was partially increased by centrifugation and manipulation of pH. In contrast, centrifugation and adjustment of pH to 6 decreased survival in Sample SS2. In reverse order, the addition of EDTA increased survival in Sample SS1, but decreased survival in Sample SS2. An increase in toxicity was observed following the addition of STS to both samples.

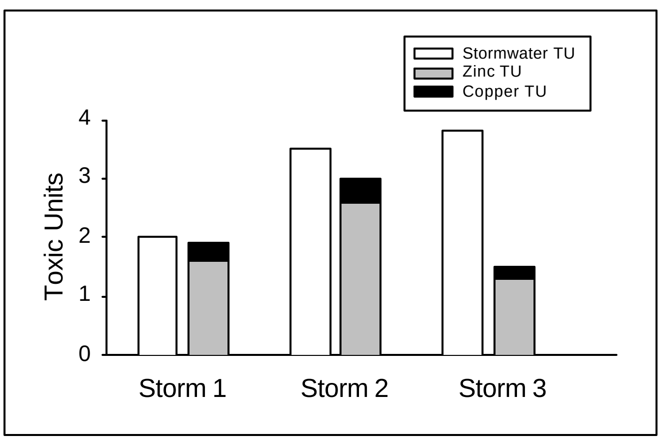
TABLE 6. Concentrations of metals in cation exchange column fractions. Bolded values indicate constituents present at concentrations near or above levels highly toxic to sea urchin sperm.

		Sample SS1			Sample SS2			Sample SS3		
	EC50	Pre[a]	Post[b]	Eluate[c]	Pre	Post	Eluate	Pre	Post	Eluate
Arsenic (µg/L)		2	2	<1	2	2	<1	2	2	<1
Cadmium (µg/L)	11,500	<1	<1	<1	<1	<1	<1	<1	<1	<1
Chromium (µg/L)		<1	2	0	2	1	1	4	3	1
Copper (µg/L)	30	10	7	5	13	7	6	7	5	3
Lead (µg/L)	>4,000	1	3	1	2	1	1	1	6	1
Nickel (µg/L)		3	2	9	4	2	3	3	2	3
Selenium (µg/L)		1	<1	<1	<1	1	<1	1	1	<1
Zinc (µg/L)	29	**45**	9	**40**	**75**	7	**64**	**37**	10	**32**
Toxicity (TUs)		2	<1	<0.7	3.5	<1	1.6	2.7	<1	1.3

The cause of the variable results obtained for the EDTA, centrifugation, and pH treatments is not known, but may reflect differences in stormwater sample composition. Part of the variability may be due to the use of only two replicates in the characterization tests, which would tend to increase the variability of the results. For some experiments, survival varied by 40% between replicate test chambers.

The variability between treatments was not attributable to the effect of the treatment alone. Control survival in each of the TIE treatments was high with one exception; the control samples for the STS treatment showed a partial reduction in survival. The concentration of STS added was reduced by 50% in subsequent tests and no additional evidence of control toxicity was detected.

FIGURE 3. Comparison of observed and predicted toxicity of Chollas Creek stormwater samples to sea urchin sperm.



Toxicant Identification (Phase II)

Toxicity was present in C-18 eluates for all three storm samples, indicating that the toxicants were non-polar organic compounds (Table 7). Toxicity was consistently recovered in the fractions containing 80 to 90% methanol, with the 85% fraction yielding the most consistent results. This pattern is consistent with the elution pattern shown for diazinon and chlorpyrifos, which elute in fractions containing 75 to 90% methanol (Bailey *et al.* 1996).

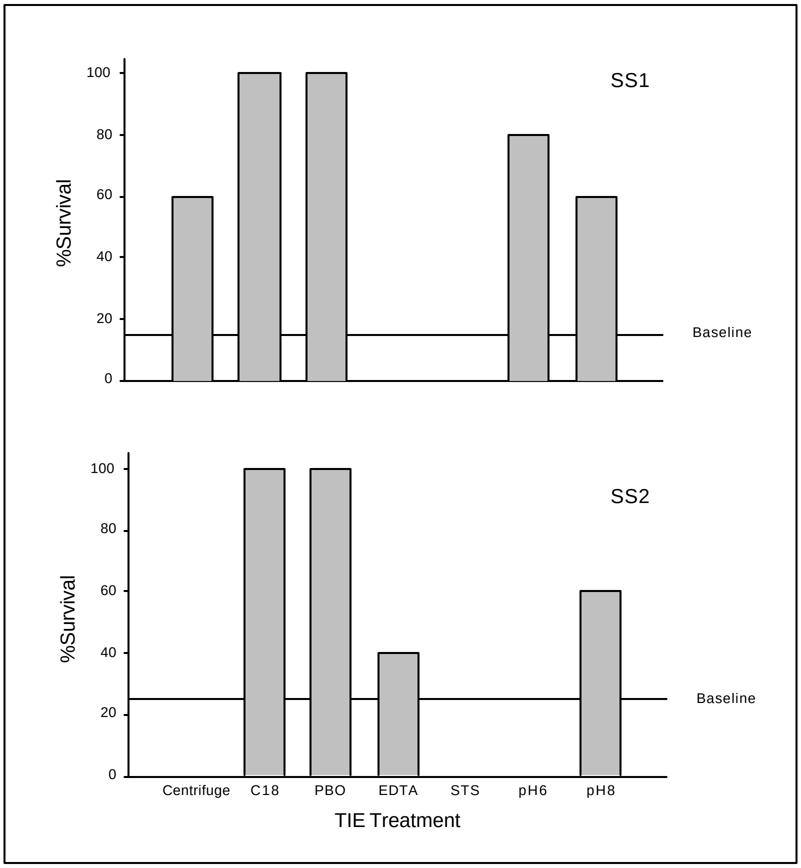
Interestingly, toxicity was recovered from the SS3 C-18 sample, even though the original sample was nontoxic (Table 7). This result was due to the fact that the eluates were tested at 5 times the original sample concentration. This finding indicates that similar toxicants were present in all three stormwater samples.

Chollas Creek stormwater samples and toxic methanol eluates of the C-18 columns were analyzed for pesticides using two techniques. All samples were measured for the organophosphate pesticides diazinon and chlorpyrifos using an ELISA. Either one or both pesticides were present at potentially toxic concentrations in every sample analyzed (Table 8). Concentrations of diazinon in the stormwater and eluate ranged from 0.32 to 1.63 µg/L, with all but one sample above the 96-h LC50 of 0.44 µg/L for *Ceriodaphnia* reported by Bailey *et al.* (1996). Chlorpyrifos concentrations in the stormwater and eluate ranged from 0.04 to 0.17 µg/L, with most above the LC50 of 0.06 µg/L (Bailey *et al.* 1996).

Selected eluate samples were also analyzed by GC for 37 organochlorine and organophosphate compounds to assess whether other potential toxicants were present at significant concentrations (Table 8). These analyses detected diazinon and three other compounds (beta-BHC, *p,p'*-DDE, and Metribuzin). Concentrations of these additional detected compounds were well below levels likely to be toxic to *Ceriodaphnia*, based upon published LC50 data.

The GC analyses reported substantially lower concentrations for diazinon and chlorpyrifos than did ELISA measurements on the same samples. The cause for this discrepancy is unknown, but may be related to the variability associated with sample handling, as only small volumes of eluate were available for GC analysis. Quality assurance data provided by the analytical laboratory demonstrated that

FIGURE 4. Summary of toxicity characterization results for Chollas Creek stormwater samples tested with the *Ceriodaphnia* survival test. Values shown are survival rates following treatment of a sample containing 100% stormwater.



>75% recovery of the analytes was attained from spiked samples analyzed by GC.

A pH adjustment test was conducted once, on Sample SS1, to discriminate between diazinon and chlorpyrifos as the likely toxicants. Survival increased to 100% after maintaining the sample at low pH (ca. 3) for 5 h, then readjusting to a pH of 7 before retesting. Adjustment to a basic pH (ca. 10) had little effect on toxicity. These results indicate that the toxicants either were degraded or rendered biologically unavailable by storage under acidic conditions. These results also suggest that diazinon was the principal toxicant in Sample SS1 because diazinon is known to degrade under acidic conditions while chlorpyrifos is not (Bailey *et al.* 1996). Both diazinon and chlorpyrifos are stable under basic conditions.

Toxicant Confirmation (Phase III).

The ELISA measurements were used to calculate the TUs associated with diazinon and chlorpyrifos in the stormwater samples (Figure 5). There was 1.23 and 1.16 TU of diazinon present in Samples SS1 and SS2, respectively. Chlorpyrifos concentrations were lower, but predicted TUs were higher (1.33 and 1.67 TU, respectively), reflecting the higher toxicity of this pesticide in laboratory tests. The toxicity associated with each pesticide was similar to the total toxicity measured in each stormwater sample.

TABLE 7. Effect of extraction and elution of Chollas Creek stormwater samples using C-18 columns on toxicity (Ceriodaphnia dubia survival). The pre- and post-C-18 samples were tested at 100% concentration, while the methanol eluates were tested at 2x (Sample SS1) or 5x (Samples SS2 and SS3) the original concentration.

	Survival (Percent)		
	Sample SS1	Sample SS2	Sample SS3
Stormwater (pre-C18)	15	25	100
Post-C-18	100	100	100
Sequential Methanol Elution			
25%	100	100	100
50%	100	100	100
70%	100	100	100
75%	100	100	100
80%	80	0	100
85%	0	0	0
90%	0	100	100
95%	90	100	100
100%	90	100	90

DISCUSSION

A different pattern of response for marine and freshwater species was obtained after exposure to urban runoff. The sea urchin was the most sensitive species tested (i.e., greatest toxic response) while the other marine species, *Mysidopsis*, was the least sensitive species tested (i.e., no toxicity). The freshwater species *Ceriodaphnia* had an intermediate response; two of the samples were toxic at 100% stormwater concentrations. Few evaluations have been conducted on the sensitivity of other marine species to urban runoff discharges, particularly on species found in the western coastal regions of the United States. In southern California, Bay *et al*. (1996) compared the sensitivity of the sea urchin to a marine plant (giant kelp, *Macrocystis pyrifera*) and a mollusc (abalone, *Haliotis refuscens*) to dry weather runoff from an urbanized watershed in southern California. These investigators found that the sea urchin was among the most sensitive of these various taxonomic groups, with NOECs occurring at levels as low as 6% runoff.

TABLE 8. Concentration and toxicity of organophosphate and organochlorine pesticides in Chollas Creek stormwater samples. Bolded values indicate constituents present at concentrations near or above levels highly toxic to daphnids.

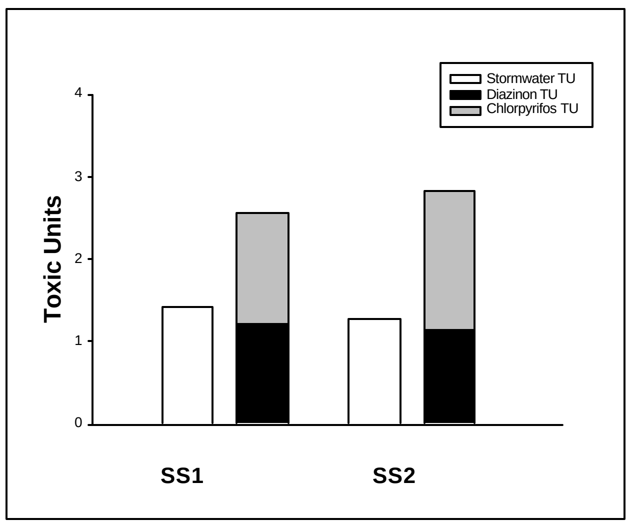
	ELISA[a] Analysis (mg/L)		GC/NPD/ECD[b] Analysis (mg/L)				
Sample	Diazinon	Chlorpyrifos	Diazinon	Chlorpyrifos	beta-BHC	*p,p'*-DDE	Metribuzin
SS1	**0.54**	**0.08**	na[c]	na	na	na	na
SS2	0.51	**0.1**	na	na	na	na	na
SS3	0.32	**0.11**	na	na	na	na	na
C-18 Methanol Eluates							
SS1: 85+90%	**0.45**	**0.05**	0.12	<0.07	<0.007	0.06	0.01
SS2: 80%	**0.51**	**0.17**	0.04	<0.02	0.006	<0.002	<0.02
SS2: 85%	**1.63**	**0.1**	0.19	<0.02	0.002	<0.002	<0.02
SS3: 85%	**0.75**	0.04	na	na	na	na	na
LC50	0.44[d]	0.06[d]	0.44	0.06	460[e]	4.7[f]	4,500[g]

[a]Enzyme-Linked Immunosorbent Assays.
[c]Gas chromatography analyses using EPA Method 507/508. Thirty-two other pesticides were included in the analysis but were not detected in the samples.
[c]Sample not analyzed for this constituent.
[d]96-h LC50 for *Ceriodaphnia* (Bailey *et al*. 1996).
[e]96-h LC50 for *Daphnia sp.* (Johnson and Finley 1980).
[f]48-h DDT LC50 for daphnids (Johnson and Finley 1980), acute toxicity of DDE likely to be lower.
[g]48-h LC50 for Daphnia magna (Weed Science Society of America 1994).

A second key finding of this study was the difference in toxicants identified among the marine and freshwater species. Trace metals, most likely zinc, were responsible for the toxicity observed in the marine species TIEs with the sea urchin. However, the freshwater species was not sensitive to trace metals. Organophosphate pesticides, most likely diazinon, was responsible for the toxicity in TIEs with *Ceriodaphnia*. This finding is not altogether surprising as sea urchins are an order of magnitude more sensitive to zinc than *Ceriodaphnia*, while *Ceriodaphnia* are four orders of magnitude more sensitive to diazinon than sea urchins.

Some uncertainty exists regarding the relative contributions of diazinon and chlorpyrifos to the freshwater toxicity of Chollas Creek runoff. Chemical analyses indicated that both of these pesticides were present in sufficient quantities to account for most of the toxicity to *Ceriodaphnia* (Table 8). In addition, the total predicted toxicity due to these pesticides is approximately double the observed toxicity (Figure 5), indicating that some fraction of one or both of these pesticides is not in a biologically available form. These results are consistent with recent studies indicating that the bioavailability of diazinon and chlorpyrifos can range from 15 to 90% in water samples (Miller *et al.* 1997).

FIGURE 5. Summary of observed and predicted toxicity of Chollas Creek stormwater samples to *Ceriodaphnia.*



The relative influence of diazinon and chlorpyrifos is difficult to distinguish because both pesticides have similar responses to many of the TIE procedures. Two findings of evidence indicate that diazinon was probably the major cause of toxicity to *Ceriodaphnia* in this study. First, the pH adjustment test results indicated that most of the toxicity to *Ceriodaphnia* was eliminated by storage under acidic conditions. This pattern is characteristic of diazinon, but not of chlorpyrifos (Bailey *et al.* 1997). Second, differences in the response of *Ceriodaphnia* and *Mysidopsis* to the stormwater samples also support the conclusion that diazinon is the most probable cause of toxicity. Both species have a similar sensitivity to chlorpyrifos, but *Ceriodaphnia* is an order of magnitude more sensitive to diazinon (Table 8). During this study, only *Ceriodaphnia* showed a response to the stormwater samples; however, if chlorpyrifos were causing a significant amount of toxicity, then the survival of *Mysidopsis* would have been reduced in the tests.

The identification of zinc and diazinon as important toxicants is not uncharacteristic of the findings of large urban watersheds. Both zinc and other trace metals are commonly found in runoff from urbanized watersheds in southern California (Schiff 1997) and around the country (U.S. EPA 1983b). Organophosphate pesticides such as diazinon and chlorpyrifos are also widespread in runoff (Bailey *et al.* 1999). Diazinon has been identified as the probable toxicant in studies of stormwater from the San Francisco Bay region (Katznelson and Mumley 1997) as well as in stormwater studies in Los Angeles and Orange counties (Lee *et al.* 1999). Metals, primarily copper and zinc, have been identified as significant toxicants in stormwater samples from Los Angeles County (Bay et al. 1997) and the San Francisco Bay area (Cooke and Lee 1995).

While the species we tested have been shown to be sensitive measures of toxicity, the use of only three test species may not be sufficient to represent the diversity of aquatic life in marine receiving waters and surrounding watersheds. Other species that are susceptible to stormwater exposures may respond differently to runoff discharges. In particular, water column residents such as plankton are important candidates due to their potential exposure to stormwater plumes. Moreover, toxicity tests are used as surrogates for impairments that might potentially occur in receiving waters. When toxicity is observed, receiving water environments should be examined to assess whether these impairments are actually occurring.

LITERATURE CITED

American Public Health Association (APHA). 1998. Standard Methods for the Examination of Water and Wastewater. American Public Health Association. Philadelphia, PA.

Bailey, H., L. Deanovic, E. Reyes, T. Kimball, K. Larson, K. Cortwright, V. Conner and D. Hinton. 1999. Diazinon and chlorpyrifos in urban waterways in Northern California, USA. *Environmental Toxicology and Chemistry* 19:82-87.

Bailey, H.C., C. DiGiorgio, K. Kroll, J.L. Miller, D.E. Hinton and G. Starrett. 1996. Development of procedures for identifying pesticide toxicity in ambient water: Carbofuran, diazinon, chlopyrifos. *Environmental Toxicology and Chemistry* 15:837-845.

Bailey, H.C., J.L. Miller, M.J. Miller, L.C. Wiborg, L. Deanovic and T. Shed. 1997. Joint acute toxicity of diazinon and chlorpyrifos to *Ceriodaphnia dubia*. *Environmental Toxicology and Chemistry* 16:2304-2308.

Bay S., D. Greenstein, S. Lau, M. Stenstrom and C. Kelley. 1996. Toxicity of dry weather flow from the Santa Monita Bay watershed. *Southern California Academy of Sciences* 95:33-45.

Bay, S., D. Greenstein, A. Jirik and A. Zellers. 1997. Toxicity of stormwater from Ballona and Malibu creeks. pp. 96-104 *in*: S. Weisberg, C. Francisco, and D. Hallock (eds.), Southern California Coastal Water Research Project Annual Report 1995-1996. Westminster, CA.

Bay, S., B.H. Jones, and K. Schiff. 1999. Study of the Impact of Stormwater Discharge on the Beneficial Uses of Santa Monica Bay. Prepared for Los Angeles County Department of Public Works. Los Angeles, CA.

Cooke, T.D., and C.C. Lee. 1995. Toxicity identification evaluations (TIE) in San Francisco Bay area urban storm water runoff. Presented at 16th Annual Meeting of the Society of Environmental Toxicology and Chemistry (SETAC). Vancouver, British Columbia.

Field, R., and R. Pitt. 1990. Urban storm-induced discharge impacts. *Water Environment and Technology* 2:64-67.

Katznelson, R., W. Jeweel, and S. Anderson. 1995. Spatial and temporal variations in toxicity in an urban runoff treatment marsh. *Environmental Toxicology and Chemistry* 14:471-482.

Katznelson, R., and T. Mumley. 1997. Diazinon in Surface Waters in the San Francisco Bay Area: Occurrence and Potential Impact. Prepared for California State Water Resources Control Board, Alameda County Flood Control and Water Conservation District, and Alameda Countywide Clean Water Program. Alameda, CA.

Johnson, W.W., and M.T. Finley. 1980. Handbook of Acute Toxicity of Chemicals to Fish and Aquatic Invertebrates. Resource Publication 137. U.S. Department of the Interior, Fish and Wildlife Service. Washington, DC.

Lee, G.F., S. Taylor, and D. Neiter. 1999. Review of Existing Water Quality Characteristics of Upper Newport Bay, Orange County, California, and its Watershed and Results of Aquatic Life Toxicity

Studies Conducted during 1997-98 in the Upper Newport Bay Watershed (draft report). Prepared for California State Water Resources Control Board, Santa Ana Regional Water Quality Control Board, and Orange County Public Facilities and Resources Department.

Miller, J., M. Miller, C. Foe and V. DeVlaming. 1997. Selective removal of diazinon and chlorpyrifos from aqueous matrices using antibody-mediated procedures. Presented at 18th Annual Meeting of the Society of Environmental Toxicology and Chemistry (SETAC). San Francisco, CA.

Pitt, R. 1995. Biological effects of urban runoff discharges. pp. 127-162 *in:* E. Herricks (ed.), Assessment of Urban Runoff Impacts in Receiving Waters. Lewis Publishers. Boca Raton, FL.

Pitt, R., R. Field, M. Lalor and M. Brown. 1990. Urban stormwater toxic pollutants: Assessment sources and treatability. *Water Environment Research* 67:260-275.

Pitt, R., R. Field, M. Lalor and M. Brown. 1995. Urban stormwater toxic pollutants: Assessment sources and treatability. *Water Environment Research* 67:260-275.

Schiff, K., S.M. Bay, M.J. Allen and E. Zeng. 2000. Southern California. pp. 385-404 *in:* C. Sheppard (ed.), Seas at the Millennium. Elsevier Press. Oxford, UK.

Schiff, K. 1997. Review of existing stormwater monitoring programs for estimating bight-wide mass emissions from urban runoff. pp. 44-55 *in*: S. Weisberg, C. Francisco, and D. Hallock (eds.), Southern California Coastal Water Research Project Annual Report 1995-96. Westminster, CA.

Schiff, K. and M. Stevenson. 1996. San Diego Regional Stormwater Monitoring Program: Contaminant inputs to wetlands and bays. *Bulletin of the Southern California Academy of Sciences* 95:7-16.

U.S. Environmental Protection Agency (U.S. EPA). 1983a. Chemical Methods for the Examination of Water and Wastes. EPA-600/4-79-020. U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory. Cincinnati, OH.

U.S. Environmental Protection Agency (U.S. EPA). 1983b. Results of the Nationwide Urban Runoff Study. PB84-185545. U.S. Environmental Protection Agency, Water Planning Division. Washington, DC.

U.S. Environmental Protection Agency (U.S. EPA). 1991. Methods for Aquatic Toxicity Identification Evaluation: Phase I Toxicity Characterization Procedures. Second Edition. EPA/600/6-91/003. U.S. Environmental Protection Agency, Environmental Research Laboratory. Duluth, MN.

U.S. Environmental Protection Agency (U.S. EPA). 1993a. Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms. Fourth Edition. EPA/600/4-90/027F. U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory. Cincinnati, OH.

U.S. Environmental Protection Agency (U.S. EPA). 1993b. Methods for Aquatic Toxicity Identification Evaluations: Phase II Toxicity Identification Procedures for Samples Exhibiting Acute and Chronic Toxicity. Second Edition. EPA/600/R-92/080. U.S. Environmental Protection Agency, Environmental Research Laboratory. Duluth, MN.

U.S. Environmental Protection Agency (U.S. EPA). 1994. Water Quality Standards Handbook. Second Edition. EPA/823/B-94/005a. U.S. Environmental Protection Agency, Office of Water. Washington, DC.

U.S. Environmental Protection Agency (U.S. EPA). 1995a. National Water Quality Inventory: 1994 Report to Congress. EPA/841/R-95/005. U.S. Environmental Protection Agency. Washington, DC.

U.S. Environmental Protection Agency (U.S. EPA). 1995b. Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to West Coast Marine and Estuarine Organisms. First Edition. EPA/600/R-95/136. U.S. Environmental Protection Agency, National Exposure Research Laboratory. Cincinnati, OH.

U.S. Environmental Protection Agency (U.S. EPA). 1996. Marine Toxicity Identification Evaluation (TIE): Phase I Guidance Document. EPA/600/R-96/054. U.S. Environmental Protection Agency, National Health and Environmental Effects Research Laboratory. Narragansett, RI.

Weed Science Society of America. 1994. Herbicide Handbook. Seventh Edition. Champaign, IL.

Woodward-Clyde (WC). 1998. 1997-1998 City of San Diego and Co-Permittee NPDES Stormwater Monitoring Program Report. Prepared for the City of San Diego Engineering and Development Department, San Diego, CA. Woodward-Clyde International-Americas. San Diego, CA.

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