

Attachment C.

Characterization of Stormwater Toxicity in Chollas Creek, San Diego

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Southern California Coastal Water Research Project

In collaboration with:

City of San Diego

Port of San Diego

Regional Water Quality Control Board, San Diego Region

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This report represents a collaborative effort among stakeholders in support of Total Maximum Daily Load (TMDL) development within the San Diego Region. The following groups assisted in the completion of this project:

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EXECUTIVE SUMMARY

Stormwater discharges from urban areas have been shown to be a large source of potential pollutants to coastal waterbodies. Chollas Creek, a tributary to San Diego Bay, contributes a variety of constituents during storm events. Moreover, samples of stormwater from Chollas Creek collected by the NPDES municipal stormwater co-permittees have elicited toxic responses using a freshwater organism (*Ceriodaphnia dubia*). The wet weather contributions and associated toxicity have led the Regional Water Quality Control Board (RWQCB), San Diego Region to add Chollas Creek to the State's list of impaired waterbodies, the 303(d) list. Pursuant to 303(d) legislation, the RWQCB is proceeding with a Total Maximum Daily Load (TMDL) to control toxicity in the Chollas Creek watershed.

This study was initiated by four concerned stakeholders in the TMDL process, each of which is an independent agency, but all of which are working collaboratively to cost-effectively address the technical issues surrounding the TMDL. The group designed two primary questions for research. The first question is: "What are the differences in toxic responses between freshwater and marine organisms to stormwater runoff?" The goal of this study objective is to evaluate potential impacts to either the freshwater or marine habitats that receive Chollas Creek discharges. The second question is: "What are the constituents responsible for toxicity in freshwater and marine organisms?" The goal of this study objective is to focus the TMDL on the constituent(s) of concern.

Three storm events were sampled from Chollas Creek between March 15 and April 6, 1999. Sampling techniques and chemical analyses were conducted using methods similar to those used in previously monitored events under the municipal stormwater NPDES permit. In addition, each sample was tested using one freshwater species (*Ceriodaphnia*, 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).

The results demonstrated that the toxic responses differed between freshwater and marine species. 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. In contrast, another marine species, *Mysidopsis*, exhibited no response to stormwater for any of the storms sampled. *Ceriodaphnia*, the freshwater species, exhibited intermediate toxic responses; two of three samples were toxic at high concentrations (100%) of stormwater. Moreover, the pattern of toxicity among storms was not consistent. 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 toxicity observed in the freshwater species *Ceriodaphnia*. The TIE manipulations that remove hydrophobic organic compounds (C18 column) or neutralized organophosphate pesticides (piperonyl butoxide) effectively removed toxicity. Moreover, concentrations of diazinon and chlorpyrifos, both organophosphate pesticides, were high enough in the stormwater samples to induce toxicity. Confirmation of diazinon as the likely toxic constituent was accomplished through the use of pH manipulations that degrade diazinon but not chlorpyrifos. The predicted toxicity of diazinon based upon measured concentrations in the study samples and responses of *Ceriodaphnia* from the peer-reviewed literature was sufficient to account for 90% of the observed toxicity in each of the storms measured. Chlorpyrifos was further discounted as a source of toxicity because *Mysidopsis*, a species that is known to be even more sensitive than *Ceriodaphnia* to this pesticide, exhibited no toxic response to the same stormwater sample.

Trace metals in stormwater runoff from Chollas Creek were responsible for toxicity observed to the sea urchin. The TIE manipulations that sequestered heavy metals (ethylenediaminetetraacetic acid, or EDTA) effectively removed toxicity. Moreover, concentrations of zinc, and to a lesser extent copper, were high enough in the stormwater samples to induce toxicity. Confirmation of zinc as the likely constituent was accomplished through the use of cation exchange columns that were used to reintroduce

the sequestered metals. The predicted toxicity of zinc and copper based upon measured concentrations in our samples and responses of the sea urchin from laboratory spiked seawater experiments was sufficient to account for between 55 and 95% of the observed toxicity, depending upon which storm was measured.

The representativeness of the three storms examined in this study were evaluated by comparing results to previously monitored events on this watershed. Tests of 11 stormwater samples for acute toxicity to *Ceriodaphnia* since 1993 show a similar or greater level of toxicity compared to the three storms measured in this study. Diazinon and chlorpyrifos concentrations have only been measured for two prior storm events and the concentrations were similar to the levels found in this study. No historical data exists for toxicity tests with any marine species, but dissolved trace metals have been measured previously. The dissolved concentrations of zinc during storms measured as part of this study were comparable to previously monitored events.

Three recommendations are given to increase confidence in the findings and target the next steps in the TMDL process. First, additional TIE testing is recommended to confirm toxicants and thus provide sufficient information to improve confidence in management actions. This study only sampled and analyzed three storms for comparing toxicity and identifying toxicants. Comparisons of the storm characteristics we sampled indicated that these storms were later in the storm season than most that have been monitored on this watershed to date. Other studies in southern California have indicated a potential for seasonal flushing (Bay *et al.* 1999, Schiff and Stevenson 1996). It is possible that other toxicants may be responsible for the toxicity found in early season storm events.

Second, a link needs to be established between in-channel measurements and impairments in the receiving water environment. While toxicity tests on discharges are predictors of receiving water impairments, the extrapolation from discharge toxicity to freshwater or marine beneficial use impairment needs to be confirmed. This is particularly true for freshwater habitats since this resource has not been well-documented and occurs upstream of the study site. In addition, receiving water measurements (either freshwater or marine) will establish the magnitude and extent of beneficial use

impairment necessary to set reduction goals, while at the same time identifying benchmarks for measuring success after the TMDL is implemented. Third, toxicological and chemical testing should be used jointly for source tracking. While we preliminarily identified the toxicants responsible for toxicity on the Chollas Creek watershed, these constituents can vary in their toxic levels due to natural and anthropogenic factors.

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INTRODUCTION

Stormwater runoff from urbanized watersheds has been a large source of pollutants to coastal waterbodies around the nation (US EPA 1995a). In southern California, runoff from urbanized watersheds has contributed substantial loadings of a variety of constituents to receiving water environments (Schiff 1997); it has also demonstrated aquatic toxicity to a range of species including larval fish, invertebrates such as molluscs, echinoderms, and crustaceans, as well as kelp (Bay *et al.* 1997). Urban runoff has been sampled and analyzed from several coastal watersheds within the county of San Diego since 1993, largely in support of the municipal stormwater National Pollutant Discharge Elimination System (NPDES) permit (WC 1998, WC 1997, WC 1996, KLI 1995, KLI 1994, Schiff and Stevenson 1996). Chollas Creek, a tributary to San Diego Bay, is one of these watersheds that have been monitored since the 1993/94 wet season. Over the past six seasons, samples of wet weather runoff from Chollas Creek have consistently exhibited chronic toxicity using the freshwater invertebrate *Ceriodaphnia dubia*. In addition, sediments collected at the mouth of the creek following the wet season were toxic to the marine amphipod *Eohaustorius estuarius*. Both of these species are native southern California fauna. Moreover, other studies have identified toxic sediments near the mouth of Chollas Creek (Fairey *et al.* 1998).

The toxicity observed on Chollas Creek has led the Regional Water Quality Control Board (RWQCB) to add this watershed to the State/Federal 303(d) list of impaired waterbodies. All waterbodies on the 303(d) list are subject to a total maximum daily load (TMDL). The TMDLs attempt to control water quality problems associated with multiple sources of constituents including non-point sources such as Chollas Creek. The objective of the TMDL approach is to address the cumulative loads that may be environmentally safe when taken individually, but can result in water quality problems when combined together.

The TMDLs focus on improving beneficial use impairments in receiving waters. Two receiving water environments are of concern for Chollas Creek. The first is freshwater habitat within the watershed, particularly ephemeral pools that exist following the wet weather season; the second is the marine/estuarine habitat that exists within San Diego Bay. The delineation of freshwater habitat has not been well documented and historical data has been collected near the mouth of the creek, downstream of any existing habitat. The marine/estuarine habitat and its beneficial uses within the Bay have been described in detail (IWQP 1998).

The RWQCB needs to obtain additional data in order to complete their TMDL. They must identify what constituents are responsible for the observed toxicity before they can define the problem, set numeric targets, assess sources, conduct linkage analysis, or assign load allocations. Without knowing what constituent(s) are eliciting the toxic responses, the TMDL process cannot move forward.

This study addresses two questions of environmental concern to further the TMDL process for Chollas Creek. The first question is: “What are the differences in toxic responses between freshwater and marine organisms to stormwater runoff?” The goal of this study objective is to evaluate potential impacts to either the freshwater or marine habitats that receive Chollas Creek discharges. The second question is: “What are the constituents responsible for toxicity in freshwater and marine organisms?” The goal of this study objective is to focus the TMDL on the constituent(s) of concern.

The RWQCB has assembled a group of stakeholders to address these two study questions. These stakeholders include the City of San Diego, the Port of San Diego (Port District), and the Southern California Coastal Water Research Project (SCCWRP). The City of San Diego maintains jurisdiction over the majority (91%) of the stormwater conveyance systems in the watershed. The Port District maintains much of the Bay waterfront. The SCCWRP, a non-regulated public agency, has been tasked by each of the RWQCBs in southern California to assist RWQCB/stakeholder groups with technical expertise to enhance TMDLs.

This document is divided into eight sections. The first section describes the methods used in the study. The second section addresses the hydrologic and chemical results. The third section compares the relative toxicity of freshwater and marine organisms. The fourth section identifies the principal toxicants to the marine species. The fifth section identifies the principal toxicants to the freshwater species. The sixth section discusses the interpretation and limitations of the results. The seventh and eighth sections summarize the major conclusions and recommendations of the study.

MATERIALS AND METHODS

Watershed Characteristics and Sampling Strategy

Chollas Creek is a highly developed watershed 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%) (WC 1998). Much of the mainstem of Chollas Creek is an earthen, unlined open channel. The confluence of the North Fork and South Fork sub-watersheds are 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. The sampling site captured approximately 57% of the entire watershed. Land use characteristics in the North Fork are in similar proportions to the entire watershed.

Stormwater samples were collected using a flow-weighted composite strategy identical to the current NPDES stormwater monitoring program (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 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 (Table 1). All methods include approved EPA methods (1983) or Standard Methods (APHA 1998).

Specialized chemical analyses were conducted on selected toxicological samples. These analyses were used to assist in the characterization and confirmation of specific compounds that were responsible for stormwater toxicity. These constituents included diazinon, chlorpyrifos, and dissolved trace metals.

Diazinon and chlorpyrifos were measured in bulk stormwater and in toxicity samples that had been manipulated in the laboratory. These organophosphate pesticides were measured using enzyme linked immunosorbant assay (ELISA) methods. This methodology uses biochemical techniques to measure low-level quantities of these difficult-to-detect compounds. Briefly, enzyme conjugates (antibodies) specific for each target analyte are allowed to incubate with each sample. The antibody-antigen complex is concentrated by washing off excess solution and a chromogen-peroxide solution is added. The chromogen-peroxide solution produces color intensities proportional to the quantity of antigen-antibody concentration present. Color intensity is measured using a microwell-reader at specific light wavelengths.

Dissolved metals were measured in bulk stormwater and in toxicity samples that had been manipulated in the laboratory. Standard EPA methods (EPA 200.8) were used for trace metal analysis, but samples were centrifuged (3,000 g for 30 min) to remove the particulate phase.

Baseline Toxicity

The three test species used in this study were chosen to satisfy four criteria: 1) sensitivity to stormwater toxicants, 2) comparability with prior data, and 3) representativeness of local fauna or key animal groups, and 4) suitability for TIE methods. The freshwater water flea, *Ceriodaphnia*, has been shown to be sensitive to stormwater toxicity in many

studies throughout California, prior toxicity data exists for Chollas Creek, and this species represents a taxonomic group (crustacean) that is an important member of freshwater aquatic communities. The two marine test species, the mysid (*Mysidopsis bahia*) and the purple sea urchin (*Strongylocentrotus purpuratus*) also represent ecologically important marine animal groups (crustaceans and echinoderms) that are likely to be exposed to stormwater plumes in marine surface waters. The sea urchin and water flea are native to southern California. An east coast species of mysid was selected for this study because the availability of west coast species is unpredictable during winter storms.

Sample Handling

Upon receipt in the laboratory, stormwater samples were stored at 4° C in the dark until used in toxicity testing. In all but one case, toxicity testing commenced within 48 h of sample collection. For the *Ceriodaphnia* test of storm 2, testing began 5 d after sampling.

The relative toxicity of each sample was evaluated using three test methods, incorporating one freshwater and two marine invertebrate species. For these baseline tests, water samples were tested whole (no filtration or removal of particulates) and diluted with laboratory water to produce a concentration series using procedures specific to each test method.

Daphnid 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* (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. This feeding regime differed from standard EPA methods in frequency (day 2 only), but not in the total amount of food added. This deviation in procedure was employed in order to ensure acceptable survival in control treatments. The modified feeding schedule may have influenced the test results, by affecting animal health or bioavailability of contaminants (due to binding on food particles). This effect was considered to be small, since the same amount of food was added during the test and reference toxicant results showed no difference in response between tests using the EPA and modified feeding methods (unpublished Ogden data).

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 (lab 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 desired ranges and did not create unintended stress on the test organisms. In addition, a reference toxicant test was included with each stormwater test series in order to document intra-laboratory variability. Each reference toxicant test consisted of a concentration series of copper with four replicates tested per concentration. The median effective concentration (LC50) was calculated from the data and compared to control limits based upon the cumulative mean and two standard deviations of recent experiments.

Mysid Survival Test

The Chollas Creek samples from each storm were assessed for toxicity using an acute exposure test using the marine mysid, *Mysidopsis bahia* (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 μ m 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 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 was included with each stormwater test series in order to document intra-laboratory variability. Each reference toxicant test consisted of a concentration series of copper with three replicates tested per concentration. The median lethal concentration (LC50) was calculated from the data and compared to control limits based upon the cumulative mean and two standard deviations from recent experiments.

Sea Urchin Fertilization

All samples of stormwater were evaluated for toxicity using the purple sea urchin fertilization test (EPA 1995b). The test consisted of a 20-min exposure of sperm to the samples. Eggs were then added and given 20 min 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 desired ranges and did not create unintended stress on the test organisms. In addition, a reference toxicant test was included with each stormwater test series in order to document intra-laboratory variability. Each reference toxicant test consisted of a concentration series of copper with five replicates tested per concentration. The median effective concentration (EC50) was estimated from the data and compared to control limits based upon the cumulative mean and two standard deviations of recent experiments.

Toxicity Identification Evaluations

Toxicity identification evaluations (TIE) were conducted on each stormwater sample in order to determine which constituents were most likely to cause the observed toxic responses. The complete TIE process is generally broken into three phases. Phase I is used to characterize the toxicants present. Phase I TIE treatments are designed to

selectively remove or neutralize classes of compounds (e.g., metals, nonpolar organics) and thus the toxicity that may be associated with them. Phase II testing is designed to more specifically identify what chemicals in the sample are causing toxicity. Phase II analysis involves a variety of techniques, including fractionation and chemical analysis. Phase III studies are intended to confirm that the identified constituents are indeed responsible for the observed toxicity. Confirmation procedures often include statistical comparisons of observed and predicted toxicity in addition to experiments using samples spiked with the suspected toxicants.

The TIE studies for the Chollas Creek samples emphasized Phase I and II testing. Limited confirmation (Phase III) studies were conducted due to the short time frame of this study.

Toxicant Characterization (Phase I TIE)

A modified Phase I TIE, using methods described by the EPA (1991 and 1996), was conducted on each of the three stormwater samples to characterize 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 was tested as recommended by EPA guidance.

The salinity of each water sample was adjusted as appropriate for each species before the application of the treatments. The specific TIE manipulations conducted varied by species because of differences in organism physiology (Table 2). 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 (Figure 1). A control sample (lab 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 1h 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 organophosphorus 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 pH's 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 (200-1,000 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.

Toxicant Identification and Confirmation (Phase 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 3).

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

The C18 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 on their polarity. Methanol concentrations ranging from 0 to 100% methanol were sequentially passed through the column to remove compounds of different polarity. The eluates represented a 200x (SS1) or 500x (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 2x or 5x 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 greater 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: laboratory dilution water and water containing 1.5% methanol (the highest concentration used in the experiment).

Prior data from Chollas Creek and other locations indicated that the organophosphorus pesticides diazinon and chlorpyrifos were probably present in the stormwater samples. 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 organophosphorus 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 as well as 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 back to the initial pH for toxicity testing.

Sea Urchin . The Phase II TIE tests with sea urchin sperm focused upon trace metals. The approach was to determine if 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 (SS1) or 2.0 N HCl (SS2 and SS3). The resulting eluate was approximately 20x more concentrated than the original stormwater sample. The eluate was then diluted with seawater to 1.5x the original stormwater concentration and tested for toxicity. Additional dilutions (50% and 25%) of the 1.5x sample were also tested. Two blanks were tested for quality control purposes: a column blank containing deionized water passed through the column and 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 obtained from SCCWRP research.

Statistical Analysis

The sea urchin toxicity tests were normalized to the control response in order to facilitate comparisons of toxicity between 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 TUa) were calculated to describe the magnitude of stormwater toxicity. The NOEC (No Observed Effects Concentration) is the highest test concentration not producing a significant toxic response and the LOEC (Lowest Observed Effects Concentration) 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 EC50 (Effects Concentration 50%) or LC50 (Lethal Concentration 50%) are the concentrations of stormwater producing a 50% reduction in fertilization or survival, respectively. The EC50 and LC50 were calculated using either probit or trimmed Spearman-Kärber methods. These statistics were calculated using State- or U.S. EPA-approved software (ToxCalc or ToxStat).

Acute toxic units (TUa), an alternate expression of the stormwater EC or LC50, were calculated as $100/\text{EC}_{50}$ or $100/\text{LC}_{50}$. Toxic units (TUa) 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 EC or LC50, toxic units 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.

Table 1. Constituents, reporting limits, and analytical methods for bulk stormwater samples.

Constituent	Units	Reporting Limit	Method ^a
Total Suspended Solids	mg/l	5	EPA 160.2
Total Dissolved Solids	mg/l	10	EPA 160.1
Turbidity	ntu	0.05	EPA 180.1
Hardness	mg/l	3	SM2340B
Surfactants (MBAS)	mg/l	0.05	EPA 425.1
Biological Oxygen Demand	mg/l	5	EPA 405.1
Oil and Grease	mg/l	1	EPA 413.1
Total Coliform	cfu/100 ml	2	SM9221B
Fecal Coliform	cfu/100 ml	2	SM9221E
Fecal Streptococci	cfu/100 ml	2	SM9230B
Ammonia	mg/l	0.1	EPA 350.1
Nitrate-Nitrogen	mg/l	0.2	EPA 300.0
Nitrite-Nitrogen	mg/l	0.1	EPA 354.1
Kjedahl Nitrogen	mg/l	0.1	EPA 351.2
Total Phosphorous	mg/l	0.05	EPA 365.2
Dissolved Phosphorous	mg/l	0.05	EPA 365.2
Arsenic	ug/l	5	EPA 200.8
Cadmium	ug/l	2	EPA 200.8
Chromium	ug/l	20	EPA 200.8
Copper	ug/l	10	EPA 200.8
Lead	ug/l	10	EPA 200.8
Nickel	ug/l	20	EPA 200.8
Zinc	ug/l	10	EPA 200.8

^a EPA Methods (1983), Standard Methods (APHA 1998)

Table 2. Summary of Phase I TIE treatments performed on samples of Chollas Creek stormwater.

Treatment	Purpose	Daphnid	Sea urchin	Mysid
Centrifugation	Remove particles	1540 x g	3000 x g	3000 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 organophosphorus pesticides	50 µg/l	nt ^a	100 µg/l
Graduated pH	Identifies if toxicity is pH dependent	✓	nt	nt

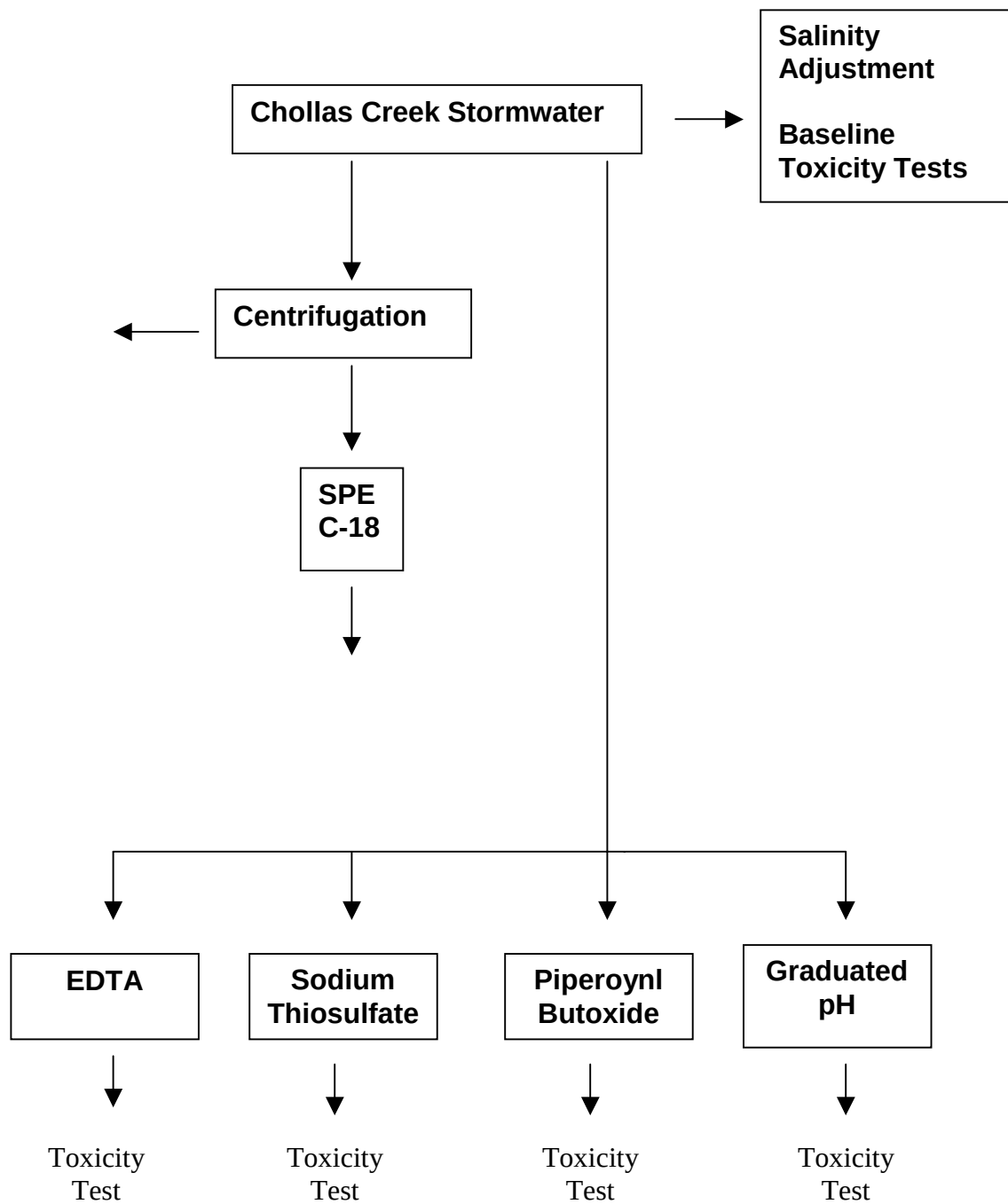
^a Treatment not tested with this species.

Table 3. 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	Separated and identifies chemicals removed by columns	✓	✓
pH adjustment	Alters toxicant characteristics and /or degradation	✓	nt

^a Treatment not tested with this species.

Figure 1. Schematic of phase I TIE testing. Salinity adjustments were made for marine species only.



STORMWATER RESULTS

Three storms were sampled between March 15 and April 6, 1999, for this study (Table 4). 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, 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. A second difference between the storms sampled for this study and those previously monitored is that the more recent storms were sampled later in the season than most. 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.

Event mean concentrations (EMCs) for stormwater constituents were not consistently higher from any single storm event sampled as part of this study (Table 5). 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.

Storms sampled during our study were not distinctly different in constituent concentrations relative to other storms monitored in this watershed (Table 5). Most trace metals were near or below the median EMC sampled between the 1993/94 and 1997/98 wet seasons. For example, zinc 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. 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.

Comparison of trace metal concentrations to water quality criteria provides another context for evaluating stormwater results (Figure 2). No water quality criteria have been established by the State for stormwater discharges. However, the U.S. EPA has proposed the California Toxics Rule (40CFR Part 131, Aug.5,1997), which establishes water quality standards based upon hardness (EPA 1994) for NPDES discharges to freshwater or estuarine (saltwater) receiving waters. Concentrations of copper and zinc, constituents that we have chosen as examples, have routinely exceeded these water quality thresholds over the past six years from this watershed. For instance, 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 criteria during two of three storms sampled during this study. However, storm 3 was the lowest observed over the NPDES monitoring history on Cholls Creek. The storms that were above water quality thresholds from this study exceeded the thresholds by a smaller magnitude than has been typically measured (Figure 2). Copper has exceeded water quality thresholds by as much as a factor of five while our storms exceeded by a maximum factor of three. Zinc has exceeded water quality criteria by as much as a factor of four while our storms exceeded only by a maximum factor of two.

Table 4. Precipitation results for monitored storms from this study (Nos. SS1 – SS3) and from all storms monitored at the study site between 1993 and 1998.

Storm No.	Storm Date	Rainfall Quantity (inches)	Rainfall Intensity (in/hr)
1 (SS1)	15-Mar-99	0.24	0.01
2 (SS2)	25-Mar-99	0.59	0.12
3 (SS3)	6-Apr-99	0.63	0.05
All Storms 1993-1998	N	15	15
	min	0.18	0.02
	median	0.32	0.04
	max	1.37	0.11
	average (sd)	0.56 (0.43)	0.05 (0.03)

Table 5. Stormwater event mean concentrations (EMC) during this study compared to the minimum, median, maximum, and average (standard deviation) EMC for previously monitored storms at the sampling location on Chollas Creek between 1993/94 and 1997/98 (N=15).

		Storm 1	Storm2	Storm 3	Previously Monitored Events			
		15-Mar-99	25-Mar-99	5-Apr-99	Min	Median	Max	Average (SD)
Total Susp. Solids	mg/l	159	150	44	75	330	1200	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	5600	160000	240000	160000 (89000)
Fecal Coliform	cfu/100 ml	>1,600	140,000	no data	3000	17000	240000	42000 (67000)
Fecal Streptococci	cfu/100 ml	240	300,000	no data	110	16000	>160000	42000 (55000)
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)
Diss. 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)

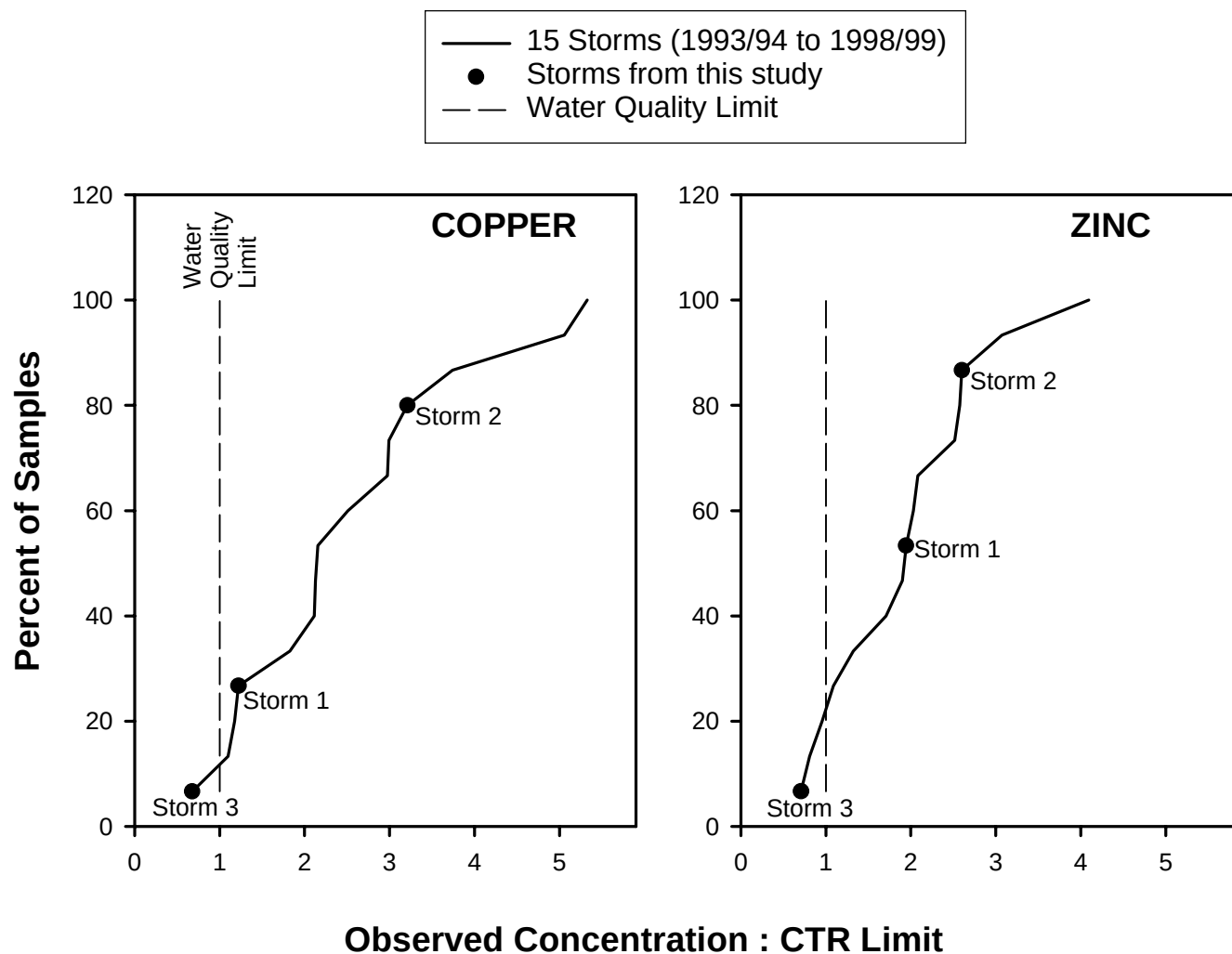


Figure 2. Cumulative distribution functions (CDFs) of copper and zinc concentrations in Chollas Creek stormwater relative to proposed water quality standards from the California Toxics Rule (CTR). The CDFs were generated by dividing the observed concentration by the water quality standard; a value of 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.

TOXICITY RESULTS

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 12-25% (Table 6). Toxicity to sea urchins was present in each of the three samples tested. The EC50 for reduced fertilization was 18-47%, which represented 2.1-5.5 TUa. 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 6). 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 (75-79%) and lower TUa values (1.3 TUa). A similar level of toxicity was present in both storms. No toxicity was detected for the third stormwater sample.

The differential sensitivity of each test species to Chollas Creek stormwater is illustrated by their dose-response patterns (Figure 3). No dose-response pattern was evident for the mysid, as survival was not significantly reduced by exposure to stormwater. Survival of *Ceriodaphnia* showed a steep dose-response pattern in the first two samples; no toxicity (100% survival) was

measured at a concentration of 50% stormwater while 15-25% survival resulted from exposure to 100% stormwater. The sea urchin fertilization dose-response pattern was more gradual, with 1-2 concentrations producing intermediate responses between the maximum and no-effect levels. Sea urchin fertilization was affected at lower stormwater concentrations than *Ceriodaphnia* survival, a reflection of the greater sensitivity of the sea urchin test to these samples.

Table 6. Summary of toxicity test results for Chollas Creek stormwater samples. 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; TU, toxic units; nd, no toxicity detected.

Sample	Sea Urchin Fertilization				Daphnid Survival				Mysid Survival			
	EC50 (%)	NOEC (%)	LOEC (%)	TU	LC50 (%)	NOEC (%)	LOEC (%)	TU	EC50 (%)	NOEC (%)	LOEC (%)	TU
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

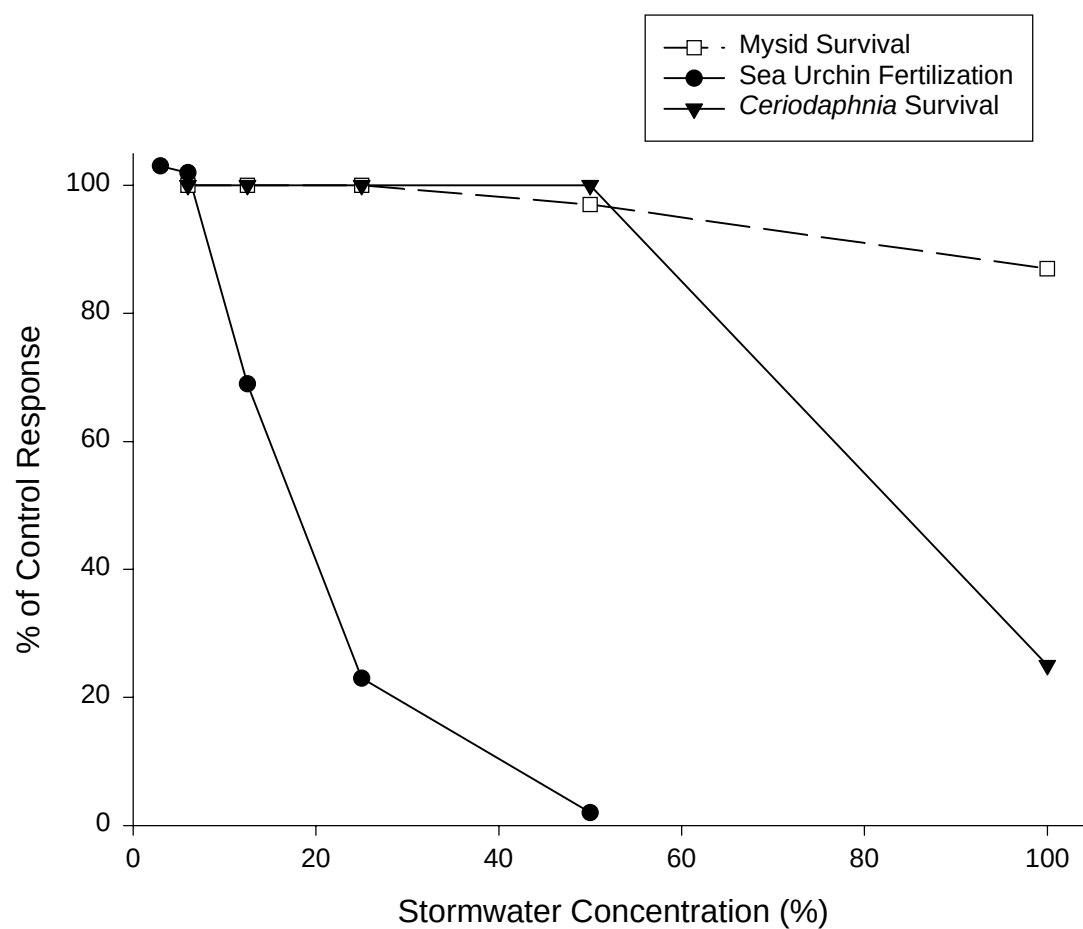


Figure 3. Comparative response of the three toxicity test methods to Chollas Creek stormwater sample SS2.

IDENTIFICATION OF TOXICANTS TO SEA URCHINS

Toxicant Characterization (Phase I)

Four treatments were applied to each of the three Chollas Creek stormwater samples in order to characterize the toxicants affecting sea urchin fertilization. A similar pattern of response to the treatments was obtained for each sample, suggesting that similar toxicants were present in each sample (Figure 4). Complete removal of the toxicity, as indicated by the large increase in fertilization relative to the baseline value, was produced only by 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 the treatments produced interferences that influenced the toxicity responses.

Toxicant Identification (Phase II)

Subsequent toxicant identification steps (Phase II) were directed towards determining whether toxic concentrations of cationic trace metals were present. Two types of analyses were conducted. First, the samples were extracted with cation exchange columns to determine whether the toxic materials could be separated from the stormwater sample for subsequent

analysis. The second procedure consisted of trace metal measurements of stormwater and materials eluted from the cation exchange columns.

Extraction of the stormwater samples with the cation exchange column eliminated toxicity for all three samples (Figure 5). Elution of the columns with acid was able to recover 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 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. Arsenic, chromium (total), copper, lead, nickel, selenium, and zinc were detected in the fractions (Table 7). Concentrations were low ($<5 \mu\text{g/L}$) 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 4. Most of the copper and zinc removed by the column was recovered in the acid eluate (Table 7).

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 $\mu\text{g/L}$ in the samples demonstrating toxicity. All of these concentrations were above the concentration of zinc found to be strongly toxic in prior SCCWRP experiments (EC_{50} of 29 $\mu\text{g/L}$). Copper concentrations, though elevated in the toxic samples, were less than 10-43% of the EC_{50} of 30 $\mu\text{g/L}$.

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 TU_a for the stormwater samples. Comparisons were made between the observed TU_a for the centrifuged stormwater sample used for the cation exchange treatments and the predicted TU_a . Between 55 and 95% of the total (observed) toxicity was accounted for by zinc and copper depending upon the storm evaluated

(Figure 6). Of the two metals, zinc accounted for the majority of the predicted toxicity due to its higher concentration in each stormwater sample (Table 7).

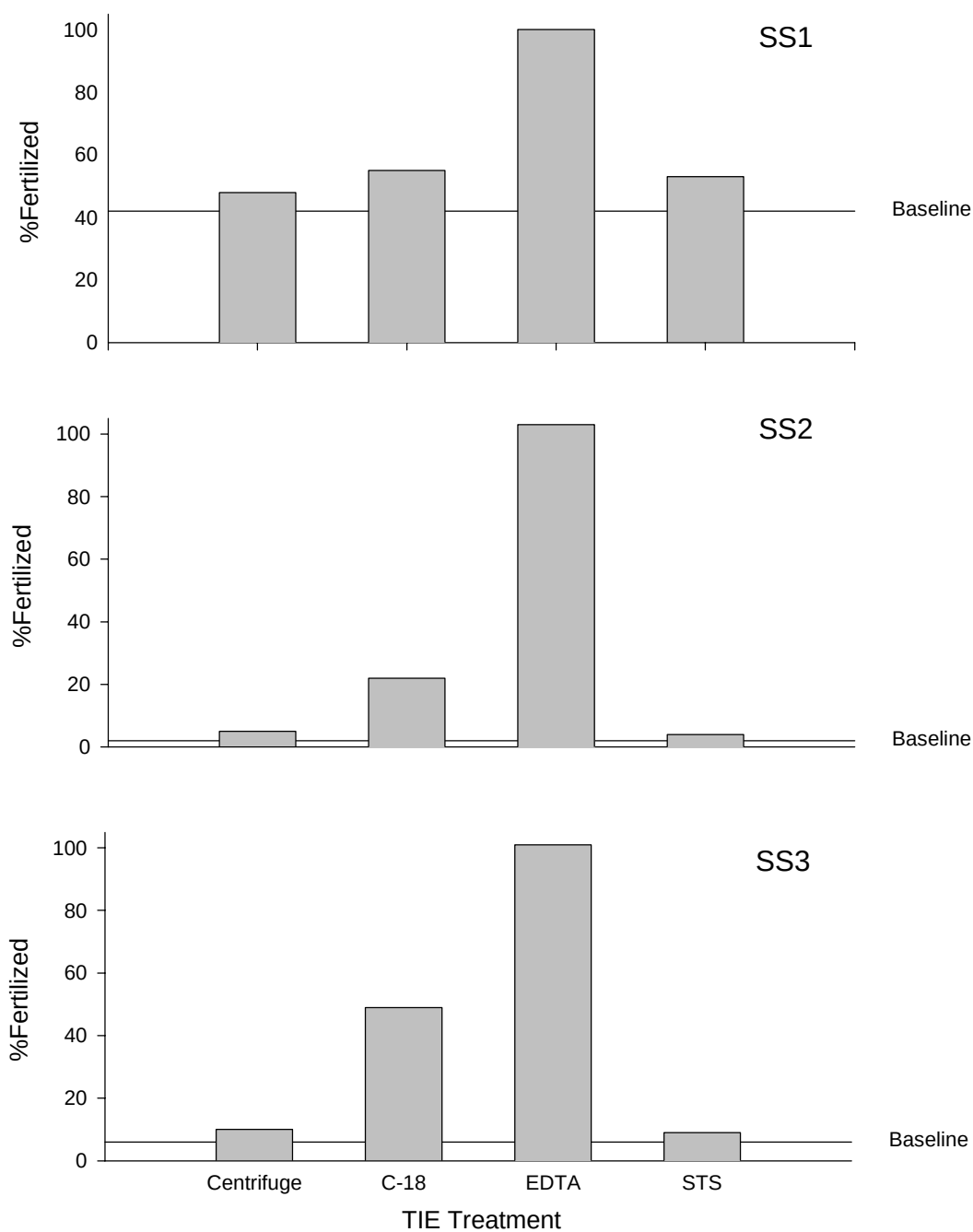


Figure 4. Summary of toxicity characterization results for Chollas Creek stormwater samples tested with the sea urchin fertilization test. Values shown are survival following treatment of a sample containing 50% stormwater.

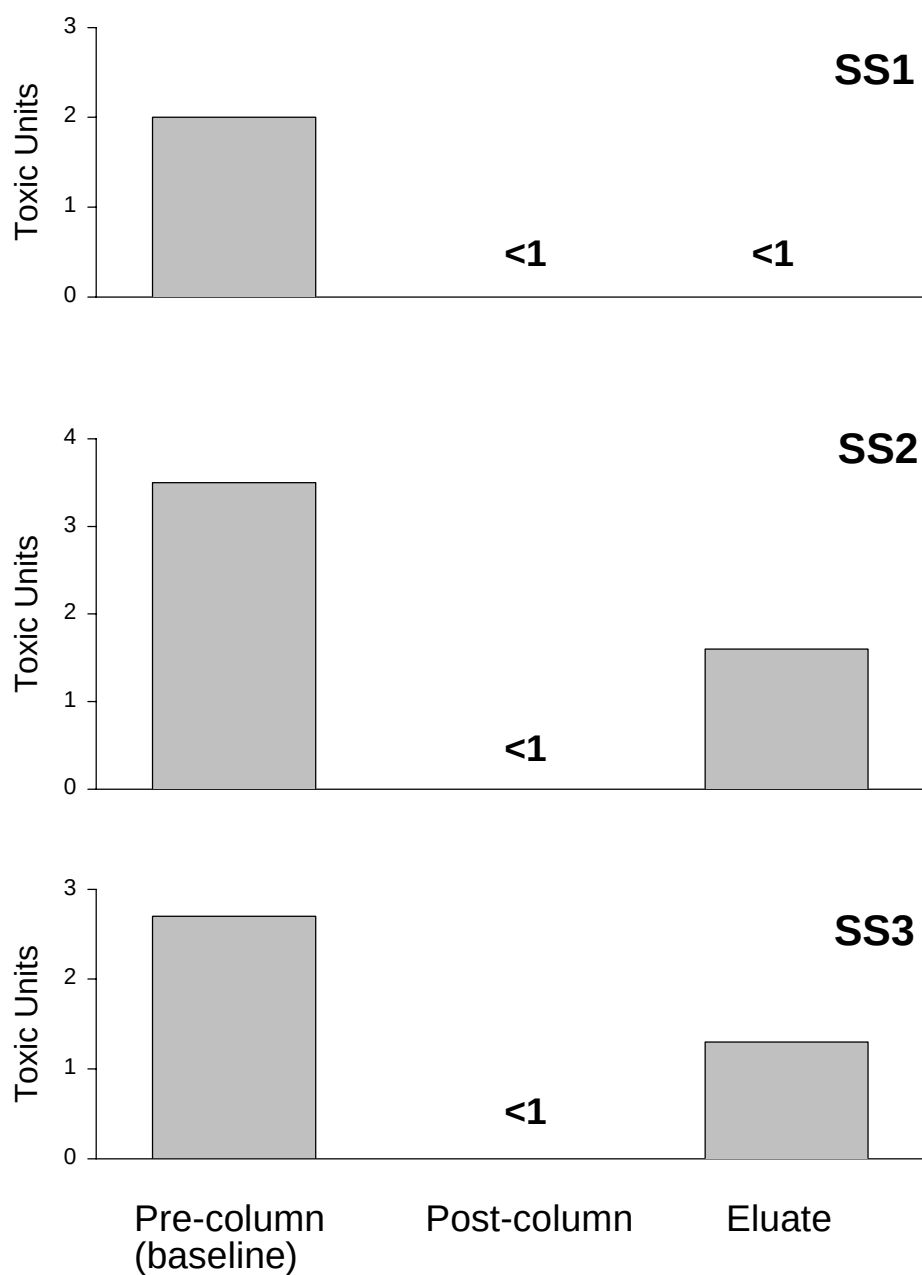


Figure 5. Recovery of toxicity in eluates from cation exchange columns used to treat Chollas Creek stormwater. No toxicity was recovered from sample SS1.

Table 7. Concentrations of metals in cation exchange column fractions. Shaded values indicate constituents present at concentrations near or above levels highly toxic to sea urchin sperm.

	EC50	SS1			SS2			SS3		
		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 (TU)		2.0	<1	<0.7	3.5	<1	1.6	2.7	<1	1.3

^a Pre-column stormwater sample (centrifuged to remove particles).

^b Post-column sample.

^c Acid eluate of cation exchange column.

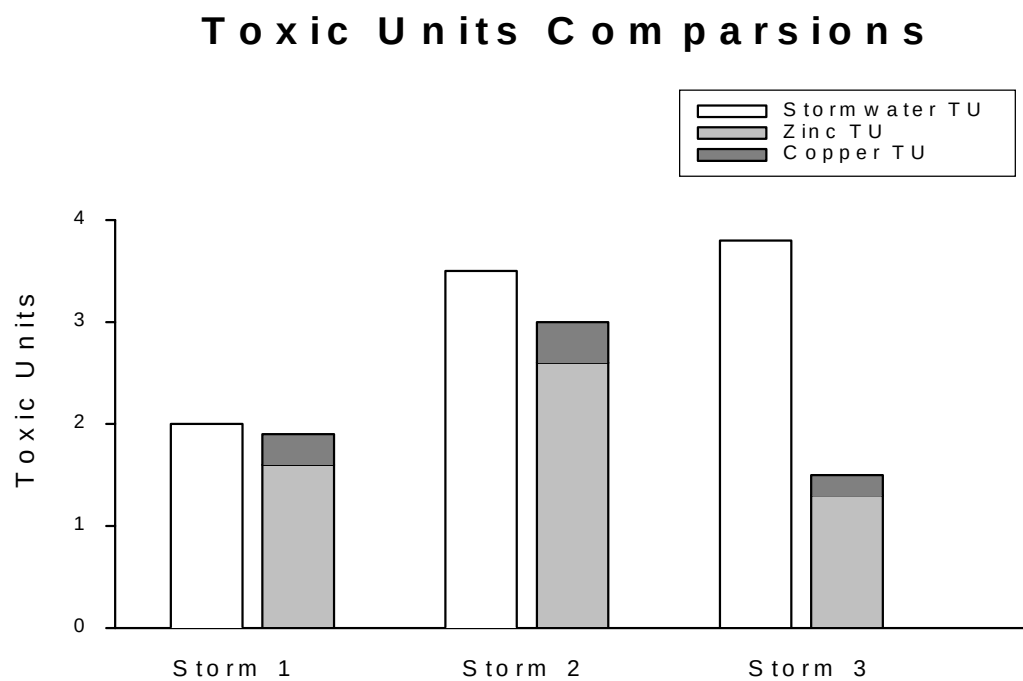


Figure 6. Comparison of observed and predicted toxicity of Chollas Creek stormwater samples to sea urchin sperm.

IDENTIFICATION OF TOXICANTS TO CERIODAPHNIA

Toxicant Characterization (Phase I)

The characterization of Chollas Creek stormwater toxicity to *Ceriodaphnia* consisted of six treatments. Control survival in each of the TIE treatments was high with one exception; the control samples for the sodium thiosulfate (STS) treatment showed a partial reduction in survival. The concentration of STS added was reduced by 50% in subsequent tests and no further evidence of control toxicity was detected.

Two treatments, solid phase extraction (SPE) with a C-18 column and addition of piperonyl butoxide (PBO), had a similar beneficial effect on the toxicity of samples SS1 and SS2 (Figure 7). 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 organophosphorus pesticides.

The addition of STS increased the toxicity of Chollas Creek stormwater. This effect was observed for each sample, including SS3, which was not toxic prior to treatment (data not shown).

Variable responses of *Ceriodaphnia* to the other characterization treatments were observed between samples. For sample SS1, survival was partially increased by centrifugation and manipulation of pH (Figure 7). An increase in toxicity was observed following the addition of EDTA. Centrifugation and adjustment to a pH of 6 resulted in increased toxicity for sample SS2. The toxicity of sample SS2 was partially reduced by the addition of EDTA and the adjustment to a pH of 8.

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.

Toxicant Identification (Phase II)

Subsequent TIE experiments were conducted in order to determine whether organophosphorus pesticides, the major toxicant class indicated by the characterization tests, were present in significant amounts in the samples. Three types of tests were conducted: (1) elution of the C-18 columns to determine if the toxicity could be recovered, (2) analysis of samples for pesticides, and (3) a pH adjustment test to examine toxicant stability.

A graduated concentration series of methanol was used to sequentially elute compounds from the C-18 SPE columns. Toxicity (reduced survival) was present in some of the eluates for all three storm samples, indicating that the toxicants were probably nonpolar organic compounds (Table 8). Toxicity was consistently recovered in the fractions containing 80-90% 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 75-90% methanol (Bailey *et al.* 1996).

Interestingly, toxicity was recovered from the SS3 C-18 sample, even though the original sample was nontoxic (Table 8). This result was due to the fact that the eluates were tested at 5x 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 organophosphorus pesticides diazinon and chlorpyrifos using an ELISA. Either one or both pesticides were present at potentially toxic concentrations in every sample analyzed (Table 9). 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 using EPA method 507/508. The purpose of this analysis was to determine if additional organic compounds were present in the toxic eluate samples at significant concentrations. 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 on published LC50 data (Table 9).

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 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 >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. Toxicity was greatly reduced by maintaining the sample at a low pH (approximately 3) for 5h (Figure 8). The sample was adjusted to a pH of 7 before toxicity measurement. Adjustment to a basic pH (approximately 10) had little effect on toxicity. These results indicate that the toxicants were either degraded or rendered biologically unavailable by storage under acidic conditions. Time constraints prevented use of the pH adjustment test with sample SS2.

The change in toxicity observed in the pH adjustment experiment was similar to results reported by Bailey *et al.* (1996), who studied the stability of diazinon under acid and alkaline storage conditions. Up to 85% of the diazinon in an aqueous solution was found to degrade during storage at pH 3 for 6 h, while alkaline conditions had no effect. Chlorpyrifos was unaffected by acid or alkaline storage conditions. These results suggest that diazinon was the principal toxicant in sample SS1.

Toxicant Confirmation (Phase III)

The ELISA measurements were used to calculate the TUa associated with diazinon and chlorpyrifos in the stormwater samples. There were 1.23 and 1.16 TUa of diazinon present in samples SS1 and SS2, respectively. Chlorpyrifos concentrations were higher (1.33 and 1.67 TUa), a reflection of 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 (Figure 9).

These pesticides have been shown to act in an additive manner (Bailey *et al.* 1997), so it is appropriate to use the sum of the TUa to predict the total toxicity of the sample. Comparisons of the summed TUa indicated that the concentrations of these pesticides were more than sufficient to cause all of the observed toxicity in samples SS1 and SS2 (Figure 9).

Table 8. Effect of extraction and elution of Chollas Creek stormwater samples using C-18 columns on toxicity (C. dubia survival). The pre- and post C-18 samples were tested at 100% concentration, while the methanol eluates were tested at 2x (SS1) or 5x (SS2 and SS3) the original concentration.

	Survival (%)		
	SS1	SS2	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

Table 9. Concentration and toxicity of organophosphorus and organochlorine pesticides in Chollas Creek stormwater samples. Shaded values indicate constituents present at concentrations near or above levels highly toxic to daphnids.

Sample	ELISA ^a analysis (µg/L)		GC/NPD/ECD ^b analysis (µg/L)				
	Diazinon	Chlorpyrifos	Diazinon	Chlorpyrifos	beta-BHC	<i>p,p'</i> -DDE	Metribuzin
SS1	0.54	0.08	na ^c	na	na	na	na
SS2	0.51	0.10	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	0.10	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 Immunosorbant Assays conducted by Aqua-Science Laboratories.

^c Gas Chromatography analyses using EPA method 507/508 conducted by Babcock Laboratories. 32 other pesticides were included in the analysis but were not detected in the samples.

^c Sample not analysed for this constituent.

^d 96 hour LC50 for *Ceriodaphnia* (Bailey *et al.* 1996).

^e 96 hour LC50 for *Daphnia* sp. (Johnson and Finley 1980).

^f 48 hour DDT LC50 for daphnids (Johnson and Finley 1980), acute toxicity of DDE likely to be lower.

^g 48 hour LC50 for *Daphnia magna* (Weed Science Society of America 1994).

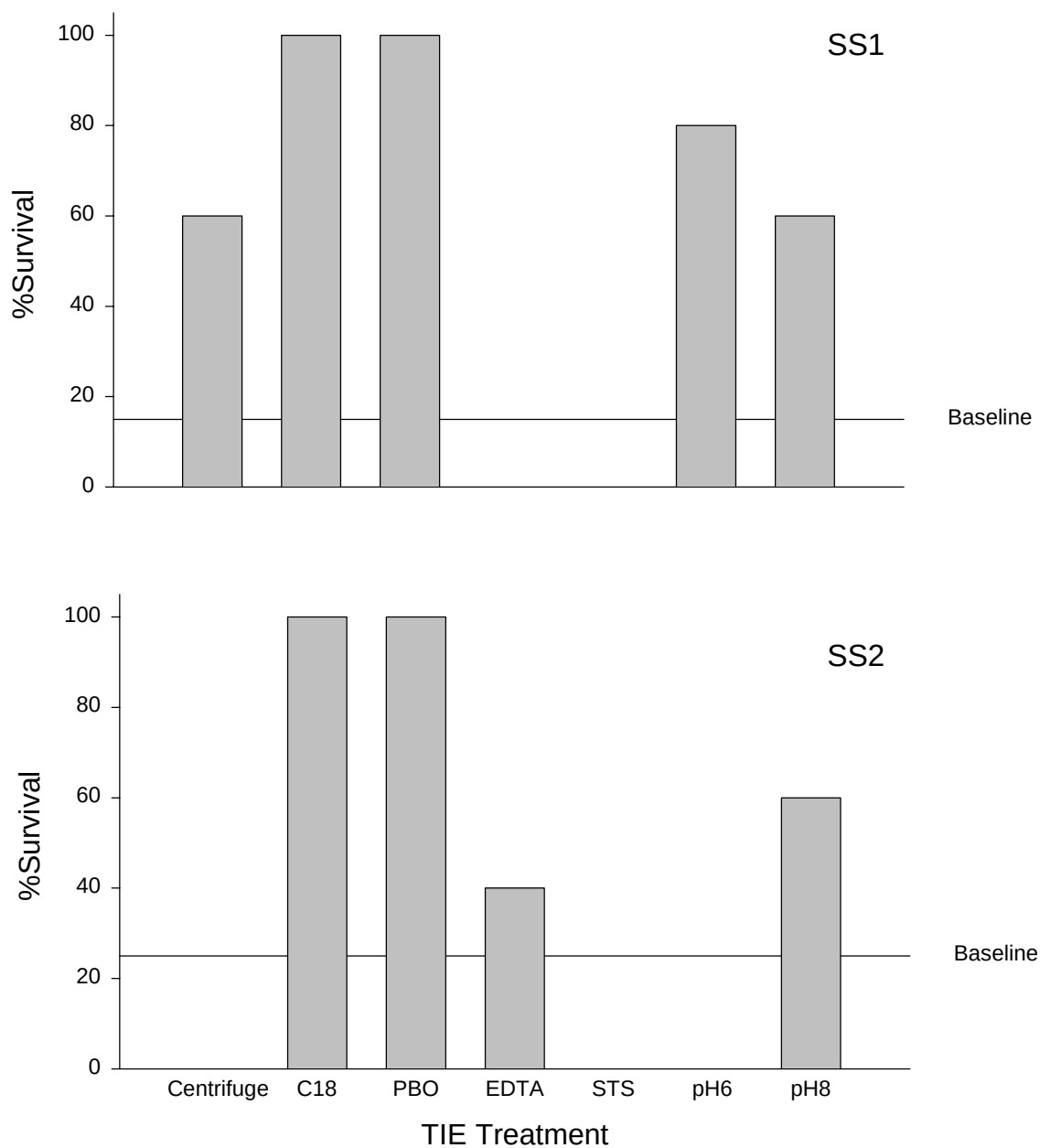


Figure 7. Summary of toxicity characterization results for Chollas Creek stormwater samples tested with the *Ceriodaphnia* survival test. Values shown are survival following treatment of a sample containing 100% stormwater.

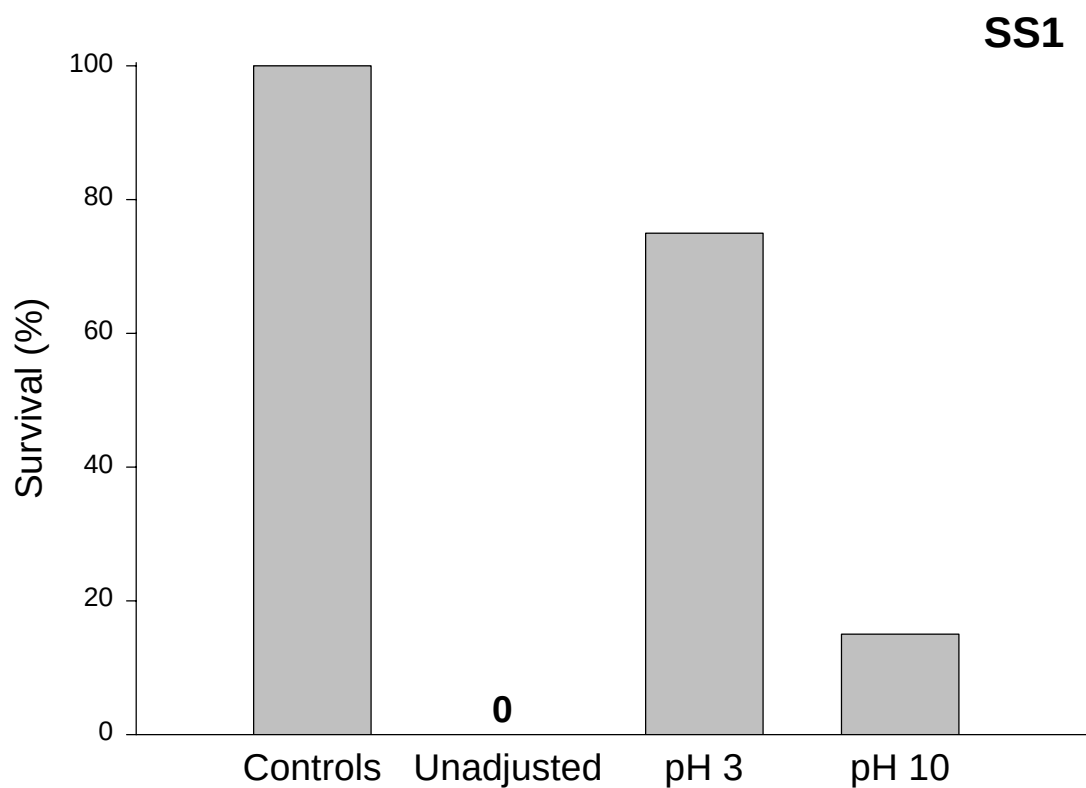


Figure 8. Survival of *Ceriodaphnia* following a 96 hour exposure to pH-adjusted Chollas Creek stormwater (100%) from storm 1. Samples were maintained at the indicated pH for 6 hours before adjustment back to the initial pH (approximately 7-8).

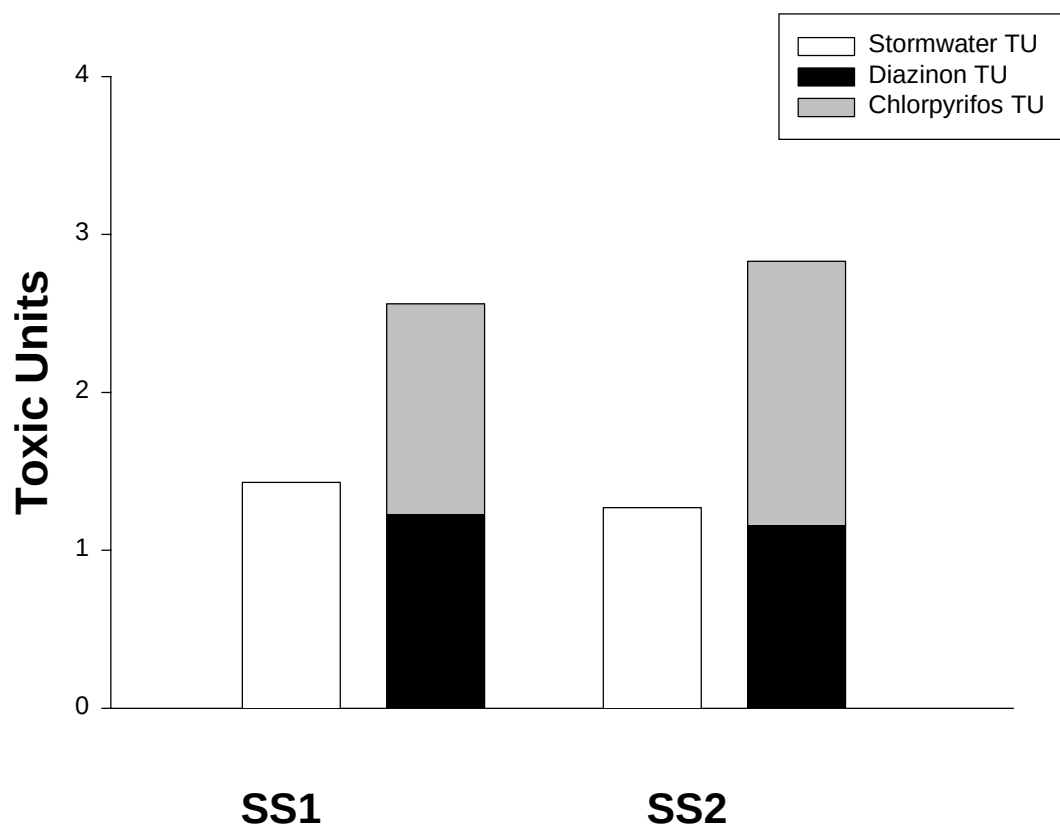


Figure 9. Summary of observed and predicted toxicity of Chollas Creek stormwater samples to *Ceriodaphnia*.

DISCUSSION

Key findings from this study showed that a different pattern of response was obtained for each species tested. The sea urchin fertilization test showed the highest toxic response to each sample and indicated that zinc was the probable cause of toxicity. The *Ceriodaphnia* survival test showed a lesser response to stormwater samples and indicated that a different toxicant, the organophosphate pesticide diazinon, was the principal cause of toxicity. A third species, the marine crustacean *Mysidopsis*, showed high survival (e.g. no toxicity) after exposure to stormwater samples.

The results represent the first application of toxicity identification procedures for stormwater from the Chollas Creek watershed and are based on a very limited set of data. Only three storms that occurred at the end of the 1998/99 wet season were analyzed. Since TMDLs developed for Chollas Creek are expected to apply to all wet weather discharges, at a minimum, it is important to determine whether the results of this study are representative of other storm events. This question can be addressed by comparing the results among the three storms and with data from previous seasons.

Confidence in the comparative toxicity and TIE results is bolstered by the consistency of the results obtained. The relative responses of the three species were similar for all three stormwater samples, indicating that the results were reproducible and that similar toxicants were present in each sample.

The confidence of the toxicant identifications is further supported by a comparison of the sensitivity of each species to selected stormwater constituents (Table 10). Laboratory studies have shown that sea urchin sperm are approximately ten times more sensitive to zinc than the other two test species (*Ceriodaphnia* and *Mysidopsis*). Similarly, *Ceriodaphnia* is the most sensitive of the three species to diazinon. Thus, the different TIE results obtained for each species are consistent with independent data regarding the toxicity of these constituents.

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 San Diego Bay and surrounding watersheds. Other species that are susceptible to stormwater exposures may respond differently to runoff discharges. Additional tests with local species, including mussel larvae and resident species of phytoplankton, zooplankton, and sediment-dwelling animals could provide assurance that the toxicity test results in this study adequately reflect the responses of other species in the Bay and surrounding watersheds.

Some uncertainty exists regarding the relative contributions of diazinon and chlorpyrifos to the toxicity of Chollas Creek stormwater. Chemical analyses indicated that both of these pesticides were present in sufficient quantities to account for most of the toxicity to *Ceriodaphnia* (Table 10). In addition, the total predicted toxicity due to these pesticides is approximately double the observed toxicity (Figure 9), 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 15-90% in water samples (Miller *et al.* 1997).

The relative influence of diazinon and chlorpyrifos is difficult to distinguish because both pesticides have similar responses to many TIE procedures. Two pieces 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 (Figure 8). This pattern is characteristic of diazinon, but not of chlorpyrifos (Bailey *et al.* 1996). 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 (Table 10), but only *Ceriodaphnia* showed a response to the stormwater samples. If chlorpyrifos was causing a significant amount of toxicity, then the survival of *Mysidopsis* should have been reduced in the tests.

The identification of zinc and diazinon as important toxicants is also supported by the findings of other stormwater toxicity investigations in California. 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). Diazinon and trace metals are commonly found constituents in surface runoff around the country (US EPA 1995a).

The representativeness of the three storms examined in this study were evaluated by comparing results to previously monitored events in this watershed. Although the data are limited, similar concentrations of toxicants and similar toxic responses were observed (Table 11). Tests of 11 stormwater samples for acute and chronic toxicity to *Ceriodaphnia* since 1993 show a similar or greater level of toxicity compared to the three storms measured in this study. Moreover, these prior toxicity results show two characteristics that are consistent with organophosphate pesticide toxicity: (1) a steep dose-response relationship; and (2) little difference between the stormwater concentrations causing lethal and sublethal (reproduction) effects. Diazinon and chlorpyrifos concentrations have only been measured for two prior storm events and the concentrations were similar to the levels found in the present study. Cumulatively, these findings indicate that the organophosphorus pesticides identified in this study are likely to be significant toxicants for other storm events in the watershed.

Seasonal variability in stormwater toxicity is a potentially important factor influencing the representativeness of the Chollas Creek toxicity results. Prior monitoring data for Chollas Creek show that stormwater toxicity varies approximately two-fold within a season and that early storms often contain the highest toxicity (Figure 10). Similar seasonal patterns in stormwater toxicity have been reported for Tecolote Creek in San Diego County (Schiff and Stevenson 1996) and for Ballona Creek in Los Angeles County (Bay *et al.* 1999). Seasonal variations in toxicity may be due to several factors, such as

changes in toxicant concentrations or the presence of different types of toxicants. Variability in toxicant concentrations can affect the development of TMDLs by altering the extent of water body impairment present, while variability in the cause of toxicity may necessitate the control of additional constituents.

It is difficult to gauge the representativeness of the marine toxicity data since there are no prior monitoring data for sea urchins or other marine species at Chollas Creek. Review of total and dissolved zinc measurements in Chollas Creek stormwater for other storms (Tables 5 and 11) indicate that the concentrations of zinc measured for the March and April, 1999, storms were typical of prior events. It is a reasonable assumption, therefore, that zinc will be an important toxicant for other storm events.

The TIE results obtained in this study appear to be reliable and accurate for the storms investigated. The identification of diazinon and zinc as principal toxicants is consistent with the results of studies in other locations; prior monitoring data indicate that these constituents are often present in stormwater discharges from the Chollas Creek watershed. However, additional TIE studies are needed in order to determine whether other significant toxicants are present. The concentration of individual constituents in Chollas Creek stormwater can vary by more than an order of magnitude between storms (Table 5); therefore it is possible that other constituents (e.g., copper and chlorpyrifos) may play a significant role in other samples. The relative toxicity to freshwater and marine species may also differ for other storm events. The presence of additional toxicants, and their relative importance, can only be determined through additional TIE studies.

Table 10. Sensitivity of toxicity test species to selected constituents of concern. Shaded areas indicate cases where constituents are present at potentially toxic concentrations to the test species.

		LC50 (96 hr)		EC50 (40 min)	Measured Range
		<i>Ceriodaphnia</i> Survival	Mysid Survival	Sea Urchin Fertilization	Storms 1-3
Copper	µg/l	25	267	30	7-13
Zinc	µg/l	208	400	29	37-75
Diazinon	µg/l	0.44	4.5	>1,000	0.32-0.54
Chlorpyrifos	µg/l	0.06	0.04		0.08-0.11

Table 11. Stormwater event mean concentrations (EMC) during this study compared to the minimum, maximum, and median EMC for previously monitored storms at the sampling location on Chollas Creek between 1993/94 and 1997/98. Toxicity data are for *Ceriodaphnia dubia*.

		Storm 1	Storm2	Storm 3	Previously Monitored Events					
		15-Mar-99	25-Mar-99	5-Apr-99	N	Min	Median	Mean	SD.	Max
96 hr LC50	%	75	79	>100	11	35	55	52.8	18.1	82
TUa		1.3	1.3	<1.0	11	1.2	1.8	2.1	0.7	2.8
7 d NOEC (Survival)	%				11	12	25	30.3	11.1	50
7 d NOEC (Reproduction)	%				11	12	30	35.4	13.8	50
Diazinon	µg/l	0.54	0.51	0.32	2	0.46				0.46
Chlorpyrifos	µg/l	0.08	0.10	0.11	1		0.1			
Zinc (dissolved)	mg/l	45	75	37	4	25	60	71.5	51.8	141

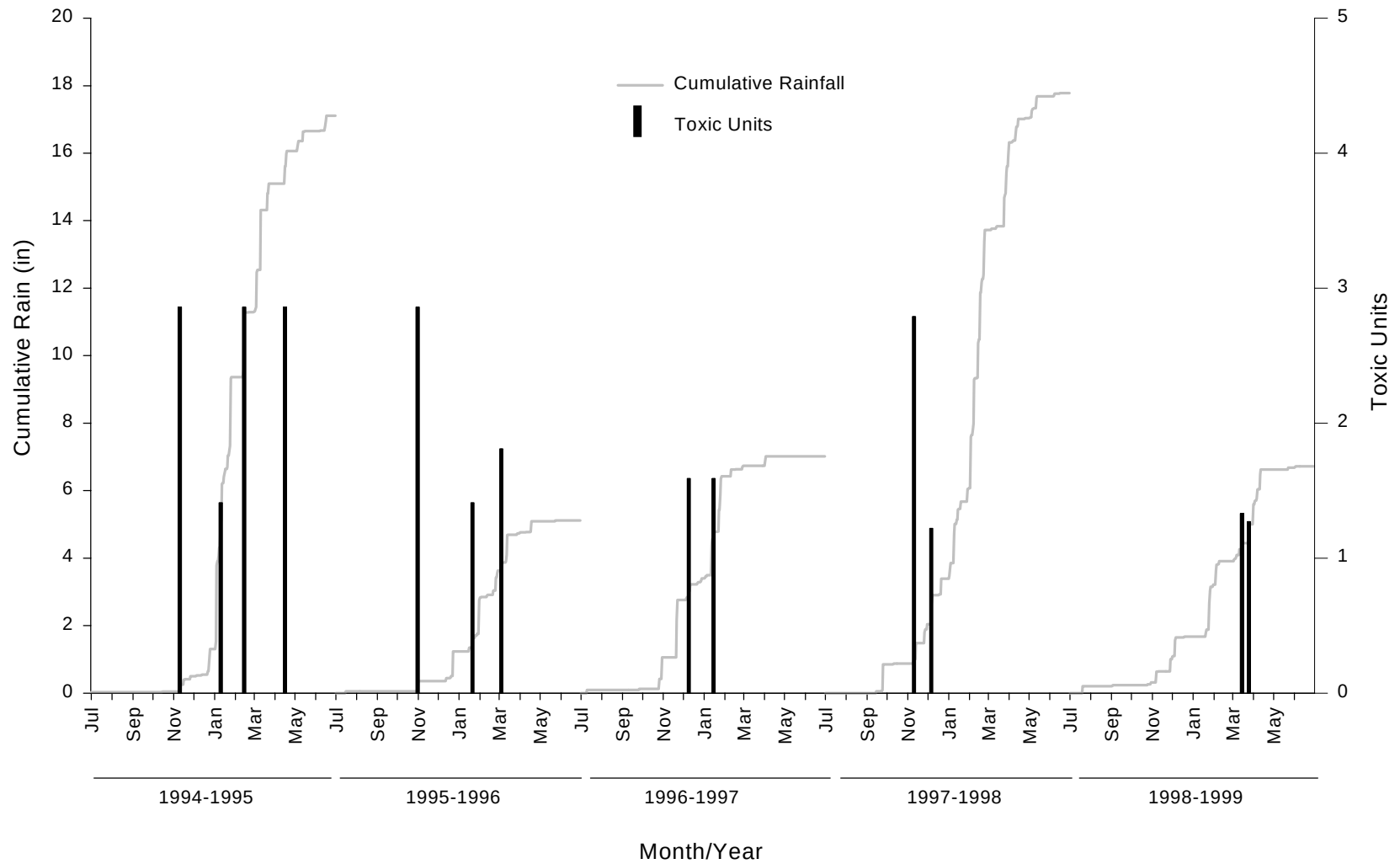


Figure 10. Variation in toxicity of Chollas Creek stormwater to *Ceriodaphnia* relative to seasonal rainfall pattern. Rainfall data for Lindberg field are shown. Toxicity data (TUa based on 96 hr LC50) were obtained from previous stormwater monitoring program reports.

CONCLUSIONS

- *The toxic responses differed between freshwater and marine species after exposure to stormwater.*

The results of this study demonstrated that no two species responded similarly after exposure to stormwater from Chollas Creek. *Strongylocentrotus* (sea urchin), a marine species, was extremely sensitive to stormwater, exhibiting responses during every storm at concentrations as low as 12% stormwater. In contrast, another marine species, *Mysidopsis* (mysid), exhibited no response to stormwater for any of the storms sampled. *Ceriodaphnia* (water flea), the freshwater species, exhibited intermediate toxic responses; two of three samples were toxic at 100% stormwater concentrations. Moreover, the pattern of toxicity among storms was not consistent. 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 toxicity observed in the freshwater species Ceriodaphnia dubia. Diazinon was the most likely constituent causing the toxicity.*

The TIEs characterized toxicity during each storm as organophosphate pesticides. The TIE manipulations that remove hydrophobic organic compounds (C18 column) or neutralized organophosphate pesticides (piperonyl butoxide) both effectively removed toxicity. Moreover, concentrations of diazinon and chlorpyrifos, both organophosphate pesticides, were high enough in the stormwater samples to induce toxicity. Confirmation of diazinon as the likely constituent was accomplished through the use of pH manipulations that degrade diazinon but not chlorpyrifos. The predicted toxicity of diazinon based upon measured concentrations in our samples and responses of *Ceriodaphnia* from the peer-reviewed literature was sufficient to account for 90% of the observed toxicity in each of the storms measured.

Chlorpyrifos was further discounted because *Mysidopsis*, a species that is known to be even more sensitive than *Ceriodaphnia* to this pesticide, exhibited no toxic response to the same stormwater sample.

- *Trace metals in stormwater runoff from Chollas Creek were responsible for toxicity observed in the marine species Strongylocentrotus purpuratus. Zinc was the most likely constituent causing toxicity.*

The TIEs characterized toxicity during each storm as trace metals. The TIE manipulations that sequestered heavy metals (EDTA) effectively removed toxicity. Moreover, concentrations of zinc, and to a lesser extent copper, were high enough in the stormwater samples to induce toxicity. Confirmation of zinc as the likely constituent was accomplished through the use of cation exchange columns that can be used to reintroduce the sequestered metals. The predicted toxicity of zinc and copper based upon measured concentrations in our samples and responses of *Strongylocentrotus* (sea urchin) from laboratory-spiked seawater experiments was sufficient to account for between 55 and 95% of the observed toxicity, depending upon which storm was measured.

RECOMMENDATIONS

- *Additional TIE testing would confirm toxicants and improve confidence in management actions.*

This study only sampled and analyzed three storms for comparing toxicity and identifying toxicants. Comparisons of the storm characteristics we observed indicated that these storms were later in the storm season than most that have been monitored on this watershed to date. Other studies in southern California have indicated a potential for seasonal flushing (Bay *et al.* 1999, Schiff and Stevenson 1996). It is possible that other toxicants may be responsible for the toxicity found in early season storm events. Understanding and confirming the magnitude and variability of runoff toxicity will greatly assist stakeholders in evaluating best management practices to reduce toxicity in the most cost-efficient manner.

- *A link needs to be established between in-channel measurements and effects in the receiving water environment.*

All of the samples examined in this study were taken from the current NPDES monitoring station on the North Fork of Chollas Creek. In order to assume impairments of beneficial uses in the receiving waters, one needs to extrapolate these samples either upstream to the freshwater habitat or downstream to the marine habitat in San Diego Bay. At this point in time, no receiving water studies have been conducted to demonstrate that these extrapolations are correct. Making the connection between in-channel sampling and impacts to the receiving water environment should be an imperative goal to further the TMDL. This information not only provides the rationale and justification for implementation, but it provides an assessment of the magnitude and extent of beneficial use impairment necessary to set

target reduction goals. Furthermore, as implementation of the TMDL unfolds, receiving water monitoring provides the benchmark for measuring success in improving water quality.

- *Toxicological and chemical testing should be used jointly for source tracking.*

Among the initial steps in the TMDL is identifying sources within the watershed for assigning load allocations. Since the TMDL on Chollas Creek is a toxicity TMDL, both toxicity and chemical testing are appropriate for source tracking. Although this study identified a small handful of constituents that play a role in the observed toxicity, these toxicants may not be the primary toxicants in other regions of the watershed. Furthermore, these constituents may vary in their toxic levels due to changes in bioavailability that are not chemically measured, such as inhibition/degradation, sorption/desorption, ionic state, or antagonistic/synergistic effects from additional constituents that commingle in stormwater runoff.

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