

A topographic map of a coastal region, likely in California, showing a network of creeks and rivers. The map uses a color gradient from green (low elevation) to red (high elevation) to indicate terrain. A prominent creek flows from the interior towards the coast. The coastline is visible on the left side, and a bay or estuary is shown at the bottom left. The text is centered over the map.

## **APPENDIX I**

# **Toxicity Identification Evaluation Report for Chollas Creek**

# Toxicity Identification Evaluation (TIE) of County of San Diego and Copermittees Chollas Creek Storm Water Sample

Prepared For:

County of San Diego and Copermittees

August 2007



**Toxicity Identification Evaluation (TIE) of  
County of San Diego and Copermittees  
Chollas Creek Storm Water Sample**

**Prepared For:**

**County of San Diego and Copermittees**

**Prepared By:**

**Weston Solutions, Inc.  
2433 Impala Drive  
Carlsbad, California 92010**

**August 2007**

## TABLE OF CONTENTS

EXECUTIVE SUMMARY .....	v
1. INTRODUCTION.....	1
1.1 Background and History .....	1
1.2 Toxicity Identification Evaluation (TIE) Testing.....	2
1.3 Initial Toxicity Testing Summary For Chollas Creek Storm water .....	4
2. MATERIALS AND METHODS .....	5
2.1 Test Procedures .....	5
2.1.1 Toxicity Test Using <i>Hyalella azteca</i> .....	5
2.2 Test Solution Preparation .....	5
2.3 Water Quality.....	5
2.4 Sample Receipt .....	5
2.5 Phase I TIE Methods .....	6
2.5.1 Protocol Modifications.....	6
2.5.2 Baseline Tests .....	6
2.5.3 Ethylenediaminetetraacetic Acid (EDTA) Tests.....	6
2.5.4 Sodium Thiosulfate (STS) Tests.....	6
2.5.5 Aeration Tests.....	7
2.5.6 Filtration Tests .....	7
2.5.7 C <sub>18</sub> Solid Phase Extraction (SPE) and Methanol Add-Back Tests.....	7
2.5.8 Graduated pH Tests .....	8
2.5.9 Piperonyl Butoxide (PBO) Tests.....	8
2.5.10 Carboxyl Esterase Tests.....	8
2.6 Statistical Analysis.....	9
3. RESULTS.....	10
3.1.1 Results of Phase I TIE Performed on the October 14, 2006 Sample .....	10
3.1.2 Summary of TIE Performed on the October 14, 2006 Storm water Sample .....	12
3.1.3 Results of Phase I TIE Performed on December 10, 2006 Sample .....	12
3.1.4 Summary of TIE performed on December 10, 2006 Sample.....	14
3.1.5 Results of Phase I TIE Performed on February 19, 2007 Sample.....	15
3.1.6 Summary of TIE performed on February 19, 2007 Sample.....	15
3.2 Chemical Analyses of Chollas Creek Storm water .....	16
4. DISCUSSION .....	19
5. REFERENCES.....	22

## LIST OF TABLES

Table 1. Triad Definitions for San Diego Storm Water Monitoring Program. ....	1
Table 2. Toxicity Identification Evaluation Manipulations.....	3
Table 3. Percent Survival of <i>Hyaella azteca</i> in TIE Tests performed on the Chollas Creek Storm water Sample Collected on October 14, 2006.....	10
Table 4. Percent Survival of <i>Hyaella azteca</i> in TIE Tests performed on the Chollas Creek Storm water Sample Collected on December 10, 2006 .....	13
Table 5. Percent Survival of <i>Hyaella azteca</i> in TIE Tests performed on the Chollas Creek Storm water Sample Collected on February 19, 2007 .....	15
Table 6. Chemical Analyses of Chollas Creek Storm water Samples Collected During the 2006-2007 Monitoring Season.....	17

## ACRONYMS AND ABBREVIATIONS

BSA	Bovine serum albumin
COC	Constituent of concern
CDPR	California Department of Pesticide Regulation
DO	Dissolved oxygen
EC <sub>p</sub>	Estimated concentration causing an effect on p% of the population
EDTA	Ethylenediaminetetraacetic acid
GC-MS	Gas chromatography – mass spectrometry
HCl	Hydrochloric acid
IC	Inhibitory concentration
K <sub>ow</sub>	Octanol – Water Partition Coefficient
LC	Lethal concentration
LC <sub>50</sub>	Median Lethal Concentration
MEC	MEC Analytical, Inc.
MLS	Mass loading stations
NaOH	Sodium hydroxide
NICI	Negative Ion Chemical Ionization
NOEC	No Observable Effect Concentration
NPDES	National Pollutant Discharge Elimination System
PBO	Piperonyl butoxide
pH	Hydrogen ion concentration
STS	Sodium Thiosulfate
SPE	Solid Phase Extraction
TIE	Toxicity Identification Evaluation
TRE	Toxicity Reduction Evaluation
TSS	Total Suspended Solids
USEPA	United States Environmental Protection Agency
Weston	Weston Solutions, Inc.

## UNITS OF MEASURE

°C	degree(s) Celsius
>	greater than
<	less than
µg/L	microgram(s) per liter
µm	micron(s)
g	gram
L	liter
M	moles
mg/L	milligram(s) per liter
mL	milliliter(s)
mm	millimeter(s)
N	Normality
ng/L	nanogram(s) per liter
ppt	parts per thousand
%	percent

## EXECUTIVE SUMMARY

As part of the San Diego municipal storm water monitoring permit (National Pollutant Discharge Elimination System [NPDES] Order 2001-01) for the San Diego, California region, storm water runoff from Chollas Creek is evaluated during the wet weather season for chemical constituents, toxicity to test organisms, and health of the benthic community. A decision matrix based on these three lines of evidence is then used to determine whether Toxicity Identification Evaluations (TIEs) will be initiated on Chollas Creek samples to identify the causative agents of toxicity in storm water samples collected during major storm events. The decision to initiate a TIE is based on the detection of high frequency contaminants of concern, persistent toxicity, and evidence of an impaired benthic community. In the 2005-2006 storm water monitoring period for the San Diego County Municipal Storm water Copermittees, high frequency constituents of concern (COCs), including turbidity, total and dissolved copper, and total zinc were measured in Chollas Creek storm water samples. Moreover, using the negative ion chemical ionization (NICI) mode on the gas chromatography–mass spectrometry (GC-MS), analytical results demonstrated the presence of pyrethroids including bifenthrin and permethrin, measured at concentrations in Chollas Creek storm water samples that exceeded the median lethal concentrations (LC<sub>50</sub>) for *H. azteca*. Persistent toxicity to *Hyalella azteca* was found because more than 50% of the toxicity tests conducted to date with *H. azteca* had a no observable effect concentration (NOEC) of less than 100%. As a consequence, TIEs were conducted which provided strong evidence that pyrethroids were the causative agent of toxicity in Chollas Creek storm water samples; the causative agent(s) of toxicity shared all of the physicochemical properties of pyrethroids, and lacked properties that characterize other classes of chemicals. In addition to toxicity and chemistry results, the benthic community was rated as poor and was determined to be impacted. Based on these findings, it was determined that TIEs would be performed on Chollas Creek storm water samples collected during the 2006-2007 monitoring period if toxicity was observed in standard toxicity tests.

During the 2006-2007 monitoring period, samples were collected from three major storm events and analyzed for toxicity in tests using *Ceriodaphnia dubia*, *H. azteca*, and *Selenastrum capricornutum*. Toxicity tests with the amphipod *H. azteca* showed significant toxicity during initial tests performed on samples collected October 14 and December 10, 2006, and February 19, 2007. As a result, separate TIEs were conducted on storm water samples from each of these three sampling events in an attempt to establish the potential cause or causes of toxicity.

Storm water toxicity tests and TIEs using the freshwater amphipod *H. azteca* were performed according to a modified version of the United States Environmental Protection Agency (USEPA) protocol for testing sediment-associated contaminants with freshwater invertebrates (EPA/600/R-99/064). TIEs were conducted according to guidelines for characterizing chronically toxic effluents (USEPA, 1991, 1992, 1993a, and 1993b). Phase I TIEs included the following battery of tests to help establish potential causative agents of toxicity in storm water samples:

- *Baseline tests* were performed to benchmark toxicity of the unmanipulated storm water samples run concurrently with the TIE tests for comparative purposes.
- *Filtration tests* were performed to establish whether the chemicals causing toxicity were particulate bound or freely dissolved.
- *Aeration tests* were performed to determine whether volatile chemicals and/or surfactants were potential causative agents of toxicity.
- *Graduated pH tests* were performed to determine if the causative agents showed pH-dependent changes in toxicity such as ammonia and aluminum.

- *Ethylenediaminetetraacetic Acid (EDTA) tests* were performed to determine whether metals were a potential contributor to the toxicity of the sample.
- *Sodium Thiosulfate (STS) tests* were performed to determine whether oxidative chemicals were contributing to toxicity of the sample.
- *Solid Phase Extraction (SPE) Tests* (followed by methanol elution) were performed to evaluate whether non-polar organics could be contributing to toxicity of the sample.
- *Piperonyl butoxide (PBO) tests* were performed to determine whether organophosphate pesticides or pyrethroids could be potential contributors to toxicity.
- *Carboxyl esterase tests* were performed to help determine potential contribution of pyrethroids to toxicity.

Results of TIE tests conducted on storm water samples collected from Chollas Creek during the 2006-2007 monitoring season provided strong evidence that pyrethroids were the causative agents of toxicity. TIE tests including the PBO and the carboxyl esterase tests, indicated that pyrethroids were a likely causative agent. PBO treatments led to increased toxicity in the storm water samples, indicative of pyrethroids because PBO is known to potentiate pyrethroid toxicity by interfering with key metabolic pathways (e.g., the P-450 mixed function oxygenase system) important in the inactivation of pyrethroid compounds. Conversely, carboxyl esterase, a known antagonist of pyrethroid toxicity, reduced toxicity in the storm water samples, also indicating pyrethroids as the causative agents. The carboxyl esterase enzyme has a strong affinity for pyrethroids and readily metabolizes pyrethroids to less toxic forms. Results of filtration tests also provided evidence of pyrethroids. In the filtration test, toxicity was reduced in filtered storm water samples, suggesting that the causative agents were bound to particulates. Pyrethroids are insoluble in water and have high adsorption coefficients, indicating their tendency to adsorb to particulates. Finally, all other TIE manipulations indicated that the causative agents of toxicity did not share similar physicochemical properties to those of metals, oxidative chemicals, pH-sensitive chemicals, or volatile chemicals/surfactants, as demonstrated by the lack of toxicity reduction in the EDTA, STS, graduated pH, and aeration tests, respectively.

Chemical analyses of Chollas Creek storm water samples indicated that pyrethroids were present. Pyrethroids including bifenthrin were measured in Chollas Creek storm water samples at levels that exceeded the aqueous 96-hour LC<sub>50</sub> for *H. azteca*. These results indicate that bifenthrin was a likely contributor to the toxicity observed in the Chollas Creek storm water samples. A number of other pyrethroids were detected in storm water samples including cyfluthrin, cypermethrin, esfenvalerate, fenvalerate, lambda cyhalothrin, and prallethrin. While the aqueous LC<sub>50</sub>s for *H. azteca* exposed to these pyrethroids are currently unknown, concentrations of cyfluthrin, cypermethrin, L-cyhalothrin, and prallethrin were comparable to those of bifenthrin, suggesting that these pyrethroids also may have contributed to the toxicity observed in the Chollas Creek samples. Other chemicals including metals were detected in the Chollas Creek storm water samples; however, the detected concentrations were below known effect levels for the test species and therefore were unlikely to have contributed to the observed toxicity.

Results of the TIE manipulations taken in concert with results of chemical analyses indicate that pyrethroids are the likely primary cause of toxicity in Chollas Creek storm water samples. It is interesting to note that this finding is consistent with recent changes in residential insecticide formulations where pyrethroids (e.g., bifenthrin) have replaced traditional organophosphate pesticides (e.g., diazinon and chlorpyrifos).

## 1. INTRODUCTION

### 1.1 BACKGROUND AND HISTORY

The San Diego municipal storm water monitoring permit (National Pollutant Discharge Elimination System [NPDES] Order 2001-01) for the San Diego, California region requires monitoring of storm water runoff at ten mass loading stations (MLS) during the wet weather season within San Diego’s watersheds. The ten MLS evaluated in this program include: San Luis Rey River, Aqua Hedionda Creek, Escondido Creek, San Dieguito River, Peñasquitos Creek, Tecolote Creek, San Diego River, Chollas Creek, Sweetwater River, and the Tijuana River. The determination of when to perform a Toxicity Identification Evaluation (TIE) at a MLS is identified by following a triad decision matrix method found in the Watershed Data Assessment Framework (MEC Analytical, Inc. [MEC]-Weston Solutions, Inc. [Weston], 2004). The triad decision matrix assesses data collected from the program including water chemistry and toxicity results from the mass loading stations, and results from benthic community structure analysis from rapid stream bioassessment. Definitions that are utilized to determine when a TIE is to be performed are listed below in Table 1.

**Table 1. Triad Definitions for San Diego Storm Water Monitoring Program.**

Triad Component	Definition
Persistent Exceedance of Water Quality Objectives	A constituent of concern with a high frequency of occurrence <sup>1</sup> based on wet and dry weather data exceedances compared to established list of benchmarks or trigger levels
Evidence of Persistent Toxicity	More than 50% of the toxicity tests for any given species have a NOEC of less than 100%.
Indication of Benthic Alteration	IBI score indicates a substantially degraded community (very poor)

Source: Watershed Data Assessment Framework (MEC-Weston, 2004)

Samples from three storm events are analyzed for toxicity using *Ceriodaphnia dubia* (7-day Survival and Reproduction), *Hyalella azteca* (4-day Survival), and *Selenastrum capricornutum* (4-day Growth). When findings from these toxicity tests at the MLS indicate the presence of persistent toxicity, a TIE may be conducted to determine the potential cause or causes of toxicity.

As listed in Table 1 above, toxicity test results are reported as the no observable effects concentration (NOEC). The NOEC is the lowest concentration at which there is no statistical difference from the control. Therefore, a concentration of less than 100% is considered to have some degree of toxic effect. Persistent toxicity is evident when more than 50% of the toxicity tests conducted to date for any given species at a specific site have a NOEC of less than 100%. The results of this determination are then combined with the high frequency constituents of

<sup>1</sup> A more detailed definition of high frequency occurrence of constituents may be found in Section 3 of the *San Diego County Municipal Copermittees 2006-2007 Urban Runoff Monitoring Final Report*

concern (chemistry data) and benthic data in the Triad Decision Matrix to determine the actions to be taken.

Results from chemistry, toxicity and relative benthic community health were assessed together using the triad approach to determine what short and/or long term actions are appropriate in a watershed. This approach examines persistence of toxicity using several indicators to provide an indication of an ecological concern. When persistence is found, this triggers the initiation of short term actions such as a TIE to identify the constituents of concern (COCs) in the watershed that may be responsible for storm water toxicity and/or benthic community degradation.

In the 2005-2006 storm water monitoring period for the San Diego County Municipal Storm water Copermitttees, high frequency constituents of concern (COCs), including turbidity, total and dissolved copper, and total zinc were measured in Chollas Creek storm water samples. Moreover, using the negative ion chemical ionization (NICI) mode on the gas chromatography–mass spectrometry (GC-MS), analytical results demonstrated the presence of pyrethroids including bifenthrin and permethrin, measured at concentrations in Chollas Creek storm water samples that exceeded the median lethal concentrations (LC50) for *H. azteca*. Persistent toxicity to *Hyalella azteca* was found because more than 50% of the toxicity tests conducted to date with *H. azteca* had a no observable effect concentration (NOEC) of less than 100%. As a consequence, TIEs were conducted which provided strong evidence that pyrethroids were the causative agent of toxicity in Chollas Creek storm water samples; the causative agent(s) of toxicity shared all of the physicochemical properties of pyrethroids, and lacked properties that characterize other classes of chemicals. In addition to toxicity and chemistry results, the benthic community was rated as poor and was determined to be impacted. Based on these findings, it was determined that TIEs would be performed on Chollas Creek storm water samples collected during the 2006-2007 monitoring period if toxicity was observed in standard toxicity tests.

## 1.2 TOXICITY IDENTIFICATION EVALUATION (TIE) TESTING

The United States Environmental Protection Agency (USEPA) has issued TIE testing guidelines for characterizing chronically toxic effluents (USEPA, 1991, 1992, 1993a, and 1993b). These guidelines are often effective for effluents that have similar toxic constituents to those identified in the model effluents used to develop the TIE guidelines. A Toxicity Reduction Evaluation (TRE) is an evaluation which involves the identification of toxicants, location of the source, and treatment of the causative agents to a less toxic form; the ultimate goal of a TRE is to reduce toxicity associated with contaminated water (or sediment). Thus, TIEs are important tools used in a TRE to initially help with the identification of toxicants, such that the source of the toxicant can be determined.

The TIE typically consists of three test phases. Phase I of a TIE involves procedures designed to provide information for identifying the class of the toxic constituents within an effluent<sup>2</sup> sample based on their chemical characteristics (e.g., volatility, ionization state, degree of adsorption to particulates, polarity, oxidative state, pH sensitivity, and interaction with synergistic and antagonistic compounds). These classification characteristics are examined by comparing the results of tests conducted on raw effluent samples to effluent samples that have been physically or chemically manipulated. Phase I testing involves manipulating the sample at the effluent's initial pH using the manipulations shown in Table 2 below.

---

<sup>2</sup> The USEPA protocol is designed for performing TIEs on effluent samples, however, modifications have been made for performing TIEs on storm water samples.

**Table 2. Toxicity Identification Evaluation Manipulations**

Physical and Chemical Manipulations (Tests) on Storm water Samples	Purpose of Test
Filtration	Detects filterable compounds (e.g., TSS related)
Aeration	Detects volatile, oxidizable, sublutable, or spargeable compounds
Graduated pH Adjustment	Detects pH dependent chemicals (e.g., ammonia and sulfides)
Ethylenediaminetetraacetic Acid (EDTA) Addition	Detects cationic metals (e.g., cadmium)
Sodium Thiosulfate (STS) Addition	Detects oxidative compounds (e.g., chlorine)
Solid Phase Extraction (SPE) over C <sub>18</sub> Column, followed by Methanol Elution	Detects non-polar organics and some surfactants
Piperonyl Butoxide (PBO) Addition	Detects organophosphate pesticides and pyrethroids
Carboxyl esterase Addition	Detects pyrethroids

The goal of Phase II TIE testing is to identify the toxicants in the sample, and Phase III methods are used to confirm that the suspected toxicants are the true cause of toxicity in the effluent samples (USEPA, 1993a and 1993b). It should be noted that the boundaries between Phases I, II, and III are not distinct and there may be cases where it is appropriate for their respective procedures to overlap because confirmation information can be obtained during Phases I and II.

TIEs are initiated when standard toxicity testing demonstrates toxicity in an effluent or storm water sample that has previously been toxic to test organisms. Standard toxicity test methods sometimes rely on sublethal endpoints, such as *C. dubia* reproduction as indicators of chronic toxicity, and require substantially more time and resources to evaluate than methods that rely exclusively on a mortality endpoint. In addition, the USEPA guidelines do not provide specific guideline for test procedures using each toxicity test species, given the large number of test organisms. Therefore, the USEPA's TIE documents are used as guidance for conducting TIEs because it may not be possible or cost-effective to strictly adhere to these protocols. In addition, the USEPA protocols were initially designed for TIEs on whole effluent samples, and not the more variable, temporally distinct, and less predictable storm water samples. Specifically, in contrast to effluent samples, chemical constituents found in storm water may vary extensively between storm events due to seasonal chemical applications associated with non-point source pollution (i.e., pesticide applications associated with seasonal crops), duration and extent of rainfall, and other water quality measures such as total suspended solids (TSS) and temperature. Thus, modifications for efficiently conducting TIEs on storm water samples using specific test organisms and for specific site conditions may sometimes include the following: changes in test volumes, test duration, replicate number, number of test concentrations, and reduction in frequency of test solution renewal.

Phase I test procedures are designed to identify obvious alterations in effluent toxicity, which may be achieved using modified chronic test methods.

### **1.3 INITIAL TOXICITY TESTING SUMMARY FOR CHOLLAS CREEK STORM WATER**

The initial toxicity test from the October 14, 2006 storm event demonstrated that Chollas Creek storm water caused significant toxicity. Survival in the 6.25, 12.5, 25, 50, and 100 percent sample concentrations was 85.0, 72.5, 60.0, 37.5, and 5.0%, respectively. The LC<sub>50</sub> at 96 hours was estimated to be 26.7% of the storm water sample, while the NOEC was 6.25%. Weston initiated Phase I TIE testing on October 24, 2006 with this sample utilizing the manipulations listed above.

The initial toxicity test from the December 10, 2006 storm event also demonstrated that storm water from Chollas Creek caused significant toxicity. Survival in the 6.25, 12.5, 25, 50, and 100 percent sample concentrations was 70.0, 85.0, 85.0, 47.5, and 17.5%, respectively. The LC<sub>50</sub> at 96 hours was estimated to be 56.3% of the storm water sample, while the NOEC was 25%. Weston initiated Phase I TIE testing on December 21, 2006 with this sample utilizing the manipulations listed above.

Similar to previous storm water events, initial toxicity tests from the February 19, 2007 storm event also demonstrated that storm water from Chollas Creek caused significant toxicity. Survival in the 6.25, 12.5, 25, 50, and 100 percent sample concentrations was 92.5, 92.5, 82.5, 62.5, and 10.0%, respectively. The LC<sub>50</sub> at 96 hours was estimated to be 54.1% of the storm water sample, while the NOEC was 25%. Weston initiated Phase I TIE testing on March 5, 2007 with this sample. Due to limitations on sample volume, only a limited number of TIE manipulations were tested (Baseline, Filtration, Piperonyl butoxide (PBO) 0.025 mg/L, and Carboxyl esterase).

## 2. MATERIALS AND METHODS

### 2.1 TEST PROCEDURES

Bioassay methods for the species *H. azteca* test are based on those in the USEPA guidance manual, "Methods for Measuring the Toxicity and Bioaccumulation of Sediment-associated Contaminants with Freshwater Invertebrates" (USEPA, 2000).

#### 2.1.1 Toxicity Test Using *Hyalella azteca*

Storm water tests for acute toxicity using the freshwater amphipod *H. azteca* were performed according to a modified version of the USEPA protocol for testing sediment-associated contaminants with freshwater invertebrates (EPA/600/R-99/064). This protocol provides test methods for measuring acute and chronic toxicity in *H. azteca* exposed to freshwater sediments, as well as a test method for conducting a water-only acute reference toxicant test. The reference toxicant test protocol was modified to conduct the toxicity testing on samples collected from the MLS. The test solution was prepared and 250-mL aliquots were placed into four replicate test chambers. Clean sand was placed as a thin "monolayer" in the bottom of the test chamber. Temperature, pH, dissolved oxygen (DO), and salinity were measured at test initiation prior to adding organisms. Ten organisms per replicate were added. Tests were run at  $23 \pm 1^\circ\text{C}$  under a 16 hour light: 8 hour dark photoperiod. Water quality was performed daily on a surrogate chamber. The animals were exposed for 4 days, and fed on day 0 and 2. At the end of the test, the survivors were removed from the sand and counted. Prior to analysis of the data, test acceptability was determined by evaluating the response of the control organisms. The test was considered invalid if survival of control animals was less than 90%. A reference toxicant test was conducted using copper sulfate with concentrations of 62.5, 125, 250, 500, and 1000  $\mu\text{g Cu}^{2+}/\text{L}$  to establish the sensitivity of test organisms used in the evaluation of the Chollas Creek storm water.

### 2.2 TEST SOLUTION PREPARATION

Control and dilution water for the *H. azteca* tests was Evian™ mineral water that was diluted with deionized water to achieve a moderate hardness (80-100 mg/L as  $\text{CaCO}_3$ ). This water source has been used successfully on numerous similar bioassay testing programs conducted by Weston and others. Extensive testing with a variety of species and biannual chemical analysis of this water type has shown that this water source provides for good survival in laboratory controls with little to no measurable levels of contaminants.

### 2.3 WATER QUALITY

Water quality was monitored daily as appropriate for each test, and data were recorded on data sheets. DO and temperature was measured using Orion™ Model 830A oxygen meters and probes; pH was measured using Orion™ Model 230A pH meters and probes. Conductivity was measured with Orion™ Models 142 conductivity/salinity meters. Ammonia was analyzed using an Orion™ 720A digital ion analyzer with a three-point calibration curve (1, 10, and 100 mg/L). Hardness and alkalinity were measured utilizing LaMotte™ titration kits.

### 2.4 SAMPLE RECEIPT

The storm water samples were composited at the laboratory and stored at  $4^\circ\text{C}$ . A chain-of-custody was completed for all samples received. Before samples were used in the tests, initial

water quality measurements were taken. These measurements included temperature, total chlorine, total ammonia, pH, DO, salinity, hardness, and alkalinity.

## **2.5 PHASE I TIE METHODS**

### **2.5.1 Protocol Modifications**

Measures to conserve time and resources required to conduct TIE testing have been developed and approved by the USEPA (USEPA, 1992). This study incorporated modifications which allowed for the reduction in number of test concentrations, organisms per replicate, and volumes for all TIE tests conducted, relative to test conditions used in standard toxicity tests. The test concentrations used for the TIE tests performed in October included the 50 and 100 percent sample concentrations for baseline, PBO, carboxyl esterase, and bovine serum albumin (BSA) treatments. The remaining treatments were only tested with the 100 percent sample concentration due to limited volume of storm water sample. In December, only the 75 percent sample concentration was tested for all treatments except baseline. The baseline treatment was tested at concentrations of 50, 75, and 100 percent. In February the test concentrations included 75 and 100 percent sample concentrations for all treatments. The differences in sample concentrations between TIE tests were determined by volume of sample and toxicity observed in the initial test. For the *H. azteca* toxicity test, five replicates with five organisms per replicate in a volume of 50 mL were used. Treatment blanks were created for each TIE test to determine the effects of the manipulation on laboratory dilution water. The results of these blanks were used to determine if any changes in toxicity of the control (dilution water) were impacted by the chemical or physical manipulation of the sample.

### **2.5.2 Baseline Tests**

The baseline test assesses the toxicity of the unmanipulated sample run concurrently with the TIE tests. This test confirms the presence of toxicity in the storm water sample, and benchmarks the toxicity for comparison to toxicity in TIE treatments.

### **2.5.3 Ethylenediaminetetraacetic Acid (EDTA) Tests**

EDTA tests were performed to determine whether metals were the causative agent of toxicity in the Chollas Creek storm water sample. Specifically, EDTA binds certain ionic metals, making them biologically unavailable to the test organisms. A reduction in the toxic response of a sample treated with EDTA may indicate the presence of divalent (metal) cation toxicity.

EDTA tests were conducted with a concentration of 3.0 mg/L. First, a stock solution of 2.5 g/L of EDTA was prepared. EDTA treatments were prepared by diluting the EDTA stock solution to a final concentration of 3.0 mg/L in storm water. The EDTA was allowed to react with the sample for a minimum of 2 hours prior to the addition of the test organisms. Prior to test initiation, the pH was adjusted to the initial pH of the storm water sample using 0.12 and 2.0 N hydrochloric acid (HCl) and 0.5 M sodium hydroxide (NaOH). EDTA treatment blanks for all TIEs were prepared identically to the storm water samples described above, using dilution water instead of storm water.

### **2.5.4 Sodium Thiosulfate (STS) Tests**

STS tests were performed to determine whether oxidative chemicals were the causative agent of toxicity in the Chollas Creek storm water sample. Specifically, the addition of STS removes

the effects of oxidative compounds including compounds such as chlorine, bromine, and ozone. This treatment also has success in removing some cationic metals.

STS tests were conducted with a concentration of 10 mg/L. First, a stock solution of 2.5 g STS/L was prepared. STS treatments were prepared by diluting the STS stock solution to a final concentration of 10 mg/L in storm water. STS treatment blanks for all TIEs were prepared identically to the storm water samples described above, using dilution water instead of storm water.

### 2.5.5 Aeration Tests

Aeration tests were performed to determine whether volatile chemicals and surfactants were the causative agent of toxicity in the Chollas Creek storm water sample. Aeration helps reduce concentrations of volatile chemicals and surfactants.

The storm water sample (300 ml) was aerated with filtered air at a medium to high rate (500 mL air/min) for a minimum of one hour at test temperature. After one hour the sample was collected by siphoning from the middle of the beaker to avoid surfactants on the side of the beaker. For the December 21, 2006 TIE, this aerated sample was then used to prepare the 75 percent dilution. Aeration treatment blanks for all TIEs were prepared identically to the storm water samples described above, using dilution water instead of storm water.

### 2.5.6 Filtration Tests

Filtration tests were performed to determine whether the chemicals causing toxicity were bound to particulate matter in the Chollas Creek storm water sample. A reduction in toxicity following filtration indicates that chemicals are particulate bound.

The storm water sample manipulation was prepared by filtering storm water (1.35 L) through a preconditioned 0.45  $\mu\text{m}$  glass fiber filter using a vacuum pump. Approximately 300 mL of the filtrate was used in the filtration test. The remaining filtrate (~1.0 L) was reserved for the C<sub>18</sub> solid phase extraction (SPE) described below. For the March 5, 2007 TIE, the SPE treatment was not performed. Therefore a reduced volume (350 mL) of sample was filtered. The filtration treatment blanks for all TIEs were prepared identically to the storm water sample filtration described above, using dilution water instead of storm water.

### 2.5.7 C<sub>18</sub> Solid Phase Extraction (SPE) and Methanol Add-Back Tests

SPE followed by methanol elution tests were performed to evaluate whether non-polar organics were the causative agents of toxicity. Specifically, non-polar organics are initially retained on the C<sub>18</sub> column and may be eluted with methanol. In subsequent toxicity tests, toxicity associated with the methanol extract is indicative of non-polar organics.

The storm water sample manipulation was prepared by passing 1.0 L of filtered storm water sample through a pre-conditioned 1 g C<sub>18</sub> SPE column at a rate of 5 mL per minute using a peristaltic pump. The SPE C<sub>18</sub> treatment blanks for all TIEs were prepared identically to the storm water sample extractions described above, using dilution water instead of storm water.

The SPE C<sub>18</sub> column was then eluted with 100% reagent grade methanol to extract any contaminants bound to the column. To elute the column, a total of 10 mL of 100% methanol was passed through the 1 g column in three aliquots, and collected into a test tube. The methanol

elution sample was concentrated using nitrogen gas to reduce the sample volume such that toxicity associated with methanol would be reduced. Specifically, the methanol elution sample was placed on a warming plate, and nitrogen gas was blown into the headspace of the vial until the volume was reduced to approximately 2.0 – 2.5 mL. The exact concentration was determined and the methanol elution was added to clean control water at a concentration of 1.5 times (October TIE) and 2.0 times (December TIE) the concentration of the potential contaminants in the original storm water sample.

### 2.5.8 Graduated pH Tests

Graduated pH tests are performed to see if the causative agents are pH-sensitive chemicals such as ammonia and aluminum. If changes in pH increase or decrease toxicity, this indicates that pH sensitive chemicals are present.

Aliquots of storm water (300 mL) and dilution water (300 mL) were elevated to test temperature and then pH was adjusted. For the October TIE, pH was adjusted to 8.5 because the initial pH of sample CC was 7.2. For the December TIE, pH was adjusted to 6.8 because the initial pH of sample CC was 8.2. The pH was adjusted to the target using 4 N HCl and 1.5 M NaOH. Treatment blanks for all TIEs were prepared identically to the storm water samples described above, using dilution water instead of storm water.

### 2.5.9 Piperonyl Butoxide (PBO) Tests

PBO tests are performed to identify whether the causative agents are organophosphate pesticides or pyrethroids. Specifically, PBO blocks specific cytochrome P450 enzymes that are involved in metabolizing chemicals such as organophosphates to more toxic metabolites and chemicals such as pyrethroids to less toxic metabolites. Thus, if results from this test demonstrate increased toxicity in the storm water sample, this is indicative of chemicals (e.g. pyrethroids) that are metabolized to less toxic forms by cytochrome P450 enzymes. In contrast, if the results demonstrate decreased toxicity in the storm water samples, this is indicative of chemicals (e.g. malathion, organophosphates) that are metabolized to more toxic forms by cytochrome P450 enzymes.

PBO tests were conducted with a concentration of 0.025 mg/L. First, a stock solution of 25 mg PBO/L was prepared. PBO treatments were prepared by diluting the PBO stock solution to a final concentration of 0.025 mg/L in storm water. PBO treatment blanks for all TIEs were prepared identically to the storm water samples described above, using dilution water instead of storm water.

### 2.5.10 Carboxyl Esterase Tests

Carboxyl esterase treatments are performed to remove toxicity in samples that may contain pyrethroids. Carboxyl esterase is an enzyme that degrades type I and type II pyrethroids. Thus, recent studies have been performed in which carboxyl esterase has been used to reduce pyrethroid-associated toxicity to test organisms such as *H. azteca* (Wheelock et al., 2004). The carboxyl esterase test method used was provided to Weston Solutions by Bryn Phillips (personal communication, Granite Canyon Laboratory). In this method, the protein BSA is used as a control for the esterase. Specifically, if toxicity is reduced in both the BSA treatment and the carboxyl esterase treatment, this indicates that both BSA and esterase are adsorbing the chemicals in the water samples. In contrast, if toxicity is only reduced in the carboxyl esterase treatment, this indicates that the chemicals in the water sample have been enzymatically altered

by the carboxyl esterase, and that these chemicals may be pyrethroids due to the affinity of carboxyl esterase for these compounds.

Carboxyl esterase and BSA tests were conducted with a concentration of 0.52  $\mu\text{g/mL}$  (or 1.25 Units/ml). A stock solution of 2 mg carboxyl esterase/mL was prepared. The carboxyl esterase treatments were prepared by diluting the carboxyl esterase stock solution to a final concentration of 0.52  $\mu\text{g/mL}$  in storm water. A stock solution of 2 mg BSA/mL was also prepared. The BSA treatments were prepared by diluting the BSA stock solution to a final concentration of 0.52  $\mu\text{g/mL}$  in storm water. Carboxyl esterase and BSA treatment blanks for all TIEs were prepared identically to the storm water samples described above, using dilution water instead of storm water.

## 2.6 STATISTICAL ANALYSIS

At the conclusion of all tests, test species data were evaluated statistically using ToxCalc™ to determine the estimated concentration that causes any effect (ECp), either lethal concentration (LC) or inhibitory concentration (sublethal; IC), on p% of the test population, and NOEC values. ToxCalc™ is a comprehensive statistical application that follows standard guidelines for acute and chronic toxicity data analysis.

Statistical effects can be measured by the ECp. The LC<sub>50</sub> or LC<sub>25</sub> is the point estimate of the concentration at which a lethal effect is observed in 50% or 25% of the test organisms. ECp values include 95% confidence limits where available. The NOEC is the highest tested concentration at which mortality and other sublethal measured effects are not significantly different from the those in the control treatment. All statistics were run against treatment blanks to mitigate for any artifactual effect that the treatment had upon the toxicity.

### 3. RESULTS

#### 3.1.1 Results of Phase I TIE Performed on the October 14, 2006 Sample

Results for the TIE performed on the October 14, 2006 sample are summarized in Table 3.

**Table 3. Percent Survival of *Hyalella azteca* in TIE Tests performed on the Chollas Creek Storm water Sample Collected on October 14, 2006.**

Test	Storm water Dilution			NOEC	LC <sub>50</sub>
	Control (Blank) – Dilution Water	50% Storm water	100% Storm water (2X <sup>1</sup> )		
Baseline	92	8	0	<50	27.4
EDTA – 3.0 mg/L	96		0		
STS - 10 mg/L	60		4		
Aeration	96		0		
Filtration	92		68		
Solid Phase Extraction (C <sub>18</sub> )	88		88		
Methanol Elution	80		20		
pH 8.5	92		0		
PBO - 0.025 mg/L	68	0	0	<50	25.0
Carboxyl esterase	88	32	8	<50	39.3
BSA (esterase control)	80	0	0	<50	25.0

<sup>1</sup> 2X concentration is associated with Methanol Elution manipulation only.

In the Baseline test, toxicity of *H. azteca* exposed to the 100 percent sample concentration (0% survival) was very similar to survival in the initial toxicity test (5% survival) started on October 14, 2006. The NOEC was less than 50%, and the LC<sub>50</sub> was greater than 27.4%. Survival in the dilution water control was 92%.

The 3.0 mg/L EDTA manipulation did not reduce toxicity in the 100 percent sample concentration (0% survival) relative to toxicity in the unmanipulated Baseline test (0% survival in the 100 percent sample concentration). Survival in the 3.0 mg/L EDTA treatment blank (dilution water) was 96%. These results indicate that ionic metals were not the causative agent of toxicity in the storm water sample.

The 10 mg/L STS manipulation also did not reduce toxicity in the 100 percent sample concentration (4% survival) relative to toxicity in the unmanipulated Baseline test (0% survival in the 100 percent sample concentration). Survival in the 10 mg/L STS treatment blank was 60%. These results indicate that the causative agent was not an oxidative compound such as chlorine, bromine, or ozone.

The Aeration treatment also did not reduce toxicity in the 100 percent sample concentration (0% survival) relative to toxicity in the unmanipulated Baseline test (0% survival in the 100 percent sample concentration). Survival in the Aeration treatment blank was 96%. These results indicate that volatile chemicals and/or surfactants were not the causative agent of toxicity in the storm water sample.

The Filtration manipulation using a 0.45  $\mu\text{m}$  Glass Fiber Filter reduced toxicity in the 100 percent sample concentration (68% survival) relative to toxicity in the unmanipulated Baseline test (0% survival in the 100 percent sample concentration). Survival in the Filtration treatment blank was 92%. These results indicate that the causative agent may be associated with particulates in the storm water sample.

The SPE procedure reduced toxicity in the 100 percent sample concentration (88% survival) relative to toxicity in the unmanipulated Baseline test (0% survival in the 100 percent sample concentration). Survival in the SPE  $\text{C}_{18}$  treatment blank was 88%. Since this sample is pre-filtered prior to the  $\text{C}_{18}$  manipulation, the reduction in toxicity was most likely due to pre-filtration of the sample prior to  $\text{C}_{18}$  elution. However, the slight increase in survival between the filtration and SPE may be associated with additional removal of fine particulates from the sample during the extraction process. Alternatively, a small fraction of the causative agent may be found in the dissolved phase, indicating that the causative agent of toxicity is likely an organic compound.

The methanol add-back manipulation resulted in 20% survival in the 2X methanol add-back concentration. Survival in the Methanol treatment blank was 80%. Because toxicity was present in the diluted methanol extract, this indicates that a fraction of the causative agent was associated with either the fine particles or the dissolved fraction of the sample that was extracted over the  $\text{C}_{18}$  column and then eluted via methanol.

The pH 8.5 manipulation did not reduce toxicity in the 100 percent sample concentration (0% survival) relative to toxicity in the unmanipulated Baseline test (0% survival in the 100 percent sample concentration). Survival in the pH 8.5 treatment blank was 92%. These results indicate that the causative agent was likely not a pH sensitive chemical such as ammonia.

The 0.025 mg/L PBO treatment had similar toxicity in the 100 percent sample concentration (0% survival) relative to toxicity in the unmanipulated Baseline test (0% survival in the 100 percent sample concentration). However, toxicity slightly increased in the 50 percent sample concentration (0% survival) relative to toxicity in the unmanipulated Baseline test (8% survival in the 50 percent sample concentration). The NOEC was less than 50% in both tests. The  $\text{LC}_{50}$  was 27.4% and 25.0% in the Baseline and PBO 0.025 mg/L tests, respectively. Survival in the 0.025 mg/L PBO treatment blank was 68%. The increase of toxicity in this manipulation suggests that pyrethroids may be a causative agent in this storm water sample. Specifically, it is well-known that sublethal concentrations of PBO potentiate the toxicity of pyrethroids (Budavari, 1989).

The carboxyl esterase test reduced toxicity in the 50 and 100 percent sample concentrations (32% and 8% survival, respectively), relative to toxicity in the unmanipulated Baseline test (8% and 0% survival in the 50 and 100 percent sample concentrations, respectively). Survival in the carboxyl esterase treatment blank was 88.0%. In contrast, the BSA treatment did not reduce toxicity in the 50 and 100 percent sample concentration (0% survival in both concentrations) relative to toxicity in the unmanipulated Baseline test (8% and 0% survival in the 50 and 100 percent sample concentrations, respectively). Survival in the BSA treatment blank was 80%. These data indicate that the chemical(s) responsible for the observed toxicity were not just adsorbing to binding sites on proteins (BSA or carboxyl esterase), but were enzymatically altered by the carboxyl esterase. This provides further evidence that the causative agents may be pyrethroids.

A copper sulfate reference toxicant test was conducted at nominal concentrations of 62.5, 125, 250, 500 and 1000  $\mu\text{g Cu}^{2+}/\text{L}$ . The calculated 96-hour  $\text{LC}_{50}$  (158  $\mu\text{g Cu}^{2+}/\text{L}$ ) was within two

standard deviations of the laboratory mean (309  $\mu\text{g Cu}^{2+}/\text{L}$ ) at the time of testing. This indicates that the sensitivity of *H. azteca* used in this evaluation fell within the normal range.

### 3.1.2 Summary of TIE Performed on the October 14, 2006 Storm water Sample

The results from the Phase I TIE performed on the Chollas Creek storm water sample collected in October indicated that pyrethroids may be the cause of the toxicity observed in the initial toxicity tests. The lack of toxicity reduction in the EDTA, STS, graduated pH, and aeration treatments indicates that the causative agent was likely not a metal, an oxidative chemical, a pH-sensitive chemical, or a volatile chemical or surfactant, respectively. However, the reduction in toxicity of *H. azteca* following filtration of the storm water sample indicates that the causative agent was highly bound to particulates in the sample. Pyrethroids have physicochemical properties that match the results of the present TIE; pyrethroids are insoluble in water but soluble in solvents (have high  $K_{ow}$ s), have low vapor pressures (indicating low volatility), and have high adsorption coefficients, indicating their tendency to adsorb to particulates (Kidd and James, 1991). In addition, toxicity was increased in the diluted PBO-treated storm water samples, as compared to toxicity in the baseline test (untreated diluted storm water). These results also indicate pyrethroids as the causative agents because pyrethroid toxicity is potentiated by PBO (Budavari, 1989). The carboxyl esterase treatment caused a reduction in toxicity in the undiluted storm water sample, whereas toxicity was not removed in the BSA treatment, used as a control for the carboxyl esterase. These results indicate that the chemical(s) causing toxicity in the storm water sample was enzymatically degraded, and not just adsorbed to binding sites on these proteins (BSA and esterase). These data further support the idea that pyrethroids were the causative agent in the Chollas Creek storm water sample because carboxyl esterase is an enzyme that metabolizes pyrethroids to less toxic forms.

### 3.1.3 Results of Phase I TIE Performed on December 10, 2006 Sample

Results for the TIE performed on the December 10, 2006 sample are summarized in Table 4.

In the Baseline test, toxicity of *H. azteca* exposed to the 100 percent sample concentration (56% survival) was lower than in the initial toxicity test (17.5% survival) started on December 10, 2006. The NOEC was 50%, and the  $LC_{50}$  was greater than 100%. Survival in the dilution water control was 88%.

The 3.0 mg/L EDTA manipulation did not reduce toxicity in the 75 percent sample concentration (44% survival) relative to toxicity in the unmanipulated Baseline test (48% survival in the 75 percent sample concentration). Survival in the 3.0 mg/L EDTA treatment blank was 100%. These results indicated that metals were likely not responsible for the observed toxicity.

The 10 mg/L STS manipulation led to slightly reduced toxicity in the 75 percent sample concentration (60% survival) by only 12%, relative to toxicity in the unmanipulated Baseline test (48% survival in the 75 percent sample concentration). Survival in the 10 mg/L STS treatment blank was 92%. This slight reduction in toxicity was likely due to variability in the test procedures and not due to an oxidative compound such as chlorine, bromine, or ozone contributing to toxicity in the storm water sample.

**Table 4. Percent Survival of *Hyalella azteca* in TIE Tests performed on the Chollas Creek Storm water Sample Collected on December 10, 2006**

Test	Storm water Dilution				NOEC	LC <sub>50</sub>
	Control (Blank) – Dilution Water	50% Storm water	75% Storm water	100% Storm water (1.5X <sup>1</sup> )		
Baseline	88	64	48	56	50	>100
EDTA – 3.0 mg/L	100		44			
STS - 10 mg/L	92		60			
Aeration	96		76			
Filtration	88		92			
Solid Phase Extraction (C <sub>18</sub> )	96		100			
Methanol Elution	100		72			
pH 6.8	96		60			
PBO - 0.025 mg/L	100		24			
Carboxyl esterase	92		84			
BSA (esterase control)	92		32			

<sup>1</sup> 1.5X concentration is associated with Methanol Elution of C<sub>18</sub> column only.

The Aeration treatment reduced toxicity in the 75 percent sample concentration (76% survival), by 28%, relative to toxicity in the unmanipulated Baseline test (48% survival in the 75 percent sample concentration). Survival in the Aeration treatment blank was 96%. These results indicate that volatile chemicals and/or surfactants may have slightly contributed to toxicity in the storm water sample.

The Filtration manipulation significantly reduced toxicity to organisms in the 75 percent sample concentration (92% survival) relative to toxicity in the unmanipulated Baseline test (48% survival in the 75 percent sample concentration). Survival in the Filtration treatment blank was 88%. These results indicate that the causative agent is likely associated with particulates in the storm water sample.

In the SPE C<sub>18</sub> manipulation, no toxicity was observed in the 75 percent sample concentration (100% survival). Because this sample was filtered prior to the C<sub>18</sub> extraction, it is likely that the lack of toxicity observed was primarily due to removal of particulates by filtration. However, the slight increase in survival between the filtration and SPE may be associated with additional removal of fine particulates from the sample during the extraction process. Alternatively, a small fraction of the causative agent may be found in the dissolved phase, indicating that the causative agent of toxicity is likely an organic compound. Specifically, there was slightly higher survival in the 75 percent sample concentration from the SPE C<sub>18</sub> manipulation (100% survival) relative to the 75 percent sample concentration from the filtration manipulation (92% survival). Survival in the SPE C<sub>18</sub> treatment blank was 96%.

The methanol add-back manipulation resulted in 72% survival at the 1.5X methanol add-back concentration. Because this level of survival was greater to that found in the 75 percent sample concentration from the Baseline test (48% survival), this indicates that there was no elution of a toxic chemical off of the C<sub>18</sub> column. Survival in the Methanol treatment blank was 100%. Results of the SPE C<sub>18</sub> manipulation together with methanol extraction indicate that the causative agent was likely not a non-polar organic compound.

The pH 6.8 manipulation led to slightly reduced toxicity in the 75 percent sample concentration (60% survival) by only 12%, relative to toxicity in the unmanipulated Baseline test (48% survival in the 75 percent sample concentration). Survival in the pH 6.8 treatment blank was 96%. This slight reduction in toxicity was likely due to variability in the test procedures likely not due a pH sensitive chemical such as ammonia.

The 0.025 mg/L PBO treatment led to increased toxicity in the 75 percent sample concentration (24% survival) relative to toxicity in the unmanipulated Baseline test (48% survival in the 75 percent sample concentration). Survival in the 0.025 mg/L treatment blank was 100%. The increase of toxicity in these manipulations suggests that pyrethroids may be a causative agent in this storm water sample. Specifically, it is well-known that sublethal concentrations of PBO potentiate the toxicity of pyrethroids (Budavari, 1989).

The carboxyl esterase treatment reduced toxicity in the 75 percent sample concentration (84% survival), relative to toxicity in the unmanipulated Baseline test (48% survival in the 75 percent sample concentration). Survival in the carboxyl esterase treatment blank was 92%. In contrast, the BSA treatment did not reduce toxicity in the 75 percent sample concentration (32% survival), relative to toxicity in the unmanipulated Baseline test (48% survival in the 75 percent sample concentration). Survival in the BSA treatment blank was 92%. These data indicate that the chemical(s) responsible for the observed toxicity were not just adsorbing to binding sites on proteins (BSA or carboxyl esterase), but were enzymatically altered by the carboxyl esterase.

A copper sulfate reference toxicant test was conducted at nominal concentrations of 62.5, 125, 250, 500 and 1000  $\mu\text{g Cu}^{2+}/\text{L}$ . The calculated 96-hour  $\text{LC}_{50}$  (166  $\mu\text{g Cu}^{2+}/\text{L}$ ) was within two standard deviations of the laboratory mean (250  $\mu\text{g Cu}^{2+}/\text{L}$ ) at the time of testing. This indicates that the sensitivity of *H. azteca* used in this evaluation fell within the normal range.

### 3.1.4 Summary of TIE performed on December 10, 2006 Sample

Results from the TIE performed on the Chollas Creek storm water sample collected in December provided additional evidence that pyrethroids were likely responsible for the observed toxicity. In accordance with the October TIE, there was a lack of toxicity reduction in the EDTA treatment, which indicates that the causative agent was likely not a metal. There was a slight toxicity reduction in the STS, graduated pH, and aeration treatments, which indicates that an oxidative chemical, a pH-sensitive chemical, or a volatile chemical or surfactant, respectively, may have slightly contributed to toxicity. However, there is little evidence that any of these chemicals were a major cause of toxicity. The reduction in toxicity of *H. azteca* following filtration of the storm water sample indicates that the causative agent was highly bound to particulates in the sample. These results support the evidence provided by the October TIE which indicate that the causative agents have similar physicochemical properties to those of pyrethroids. Also similar to results of the TIEs performed in October, the PBO treatment led to increased toxicity in the diluted storm water sample. The repeated potentiation of toxicity using PBO in all recent TIEs provides strong evidence that pyrethroids are the causative agents, because pyrethroid toxicity is well-known to increase following the addition of PBO (Budavari, 1989). The carboxyl esterase treatment caused a reduction in toxicity in the undiluted storm water sample, whereas toxicity was not removed in the BSA treatment, used as a control for the carboxyl esterase. These results indicate that the chemical(s) causing toxicity in the storm water sample was enzymatically degraded, and not just adsorbed to binding sites on these proteins (BSA and esterase). These data further support the idea that pyrethroids were the causative

agent in the Chollas Creek storm water sample because carboxyl esterase is an enzyme that metabolizes pyrethroids to less toxic forms.

### 3.1.5 Results of Phase I TIE Performed on February 19, 2007 Sample

Results for the TIE performed on the February 19, 2007 sample are summarized in Table 5.

**Table 5. Percent Survival of *Hyalella azteca* in TIE Tests performed on the Chollas Creek Storm water Sample Collected on February 19, 2007**

Test	Storm water Dilution			NOEC	LC <sub>50</sub>
	Control (Blank) – Dilution Water	75% Storm water	100% Storm water		
Baseline	100	96	100	100	>100
Filtration	100	100	100	100	>100
PBO - 0.025 mg/L	100	92	44	75	96.9
Carboxyl esterase	100	96	96	100	>100

In the Baseline test, toxicity of *H. azteca* exposed to the 100 percent sample concentration (100% survival) significantly degraded compared to the initial toxicity test (10% survival) started on February 20, 2007. The NOEC was 100%, and the LC<sub>50</sub> was greater than 100%. Survival in the dilution water control was 100%.

Toxicity in the 100 percent Filtration manipulation (100% survival) was similar to toxicity in the unmanipulated Baseline test (100% survival in the 100 percent sample concentration). Survival in the Filtration treatment blank was 100%.

The 0.025 mg/L PBO test led to increased toxicity in the 100 percent sample concentration (44% survival) relative to toxicity in the unmanipulated Baseline test (100% survival in the 100 percent sample concentration). Survival in the 0.025 mg/L PBO test blank was 100%. The increase of toxicity in the 0.025 mg/L PBO manipulation suggests that pyrethroids may be a causative agent in this storm water sample.

Toxicity in the 100 percent carboxyl esterase manipulation (96%) was similar to toxicity in the unmanipulated Baseline test (100% survival in the 100 percent sample concentration). Survival in the carboxyl esterase treatment blank was 100%.

A copper sulfate reference toxicant test was conducted at nominal concentrations of 62.5, 125, 250, 500 and 1000 µg Cu<sup>2+</sup>/L. The calculated 96-hour LC<sub>50</sub> (139 µg Cu<sup>2+</sup>/L) was within two standard deviations of the laboratory mean (236 µg Cu<sup>2+</sup>/L) at the time of testing. This indicates that the sensitivity of *H. azteca* used in this evaluation fell within the normal range.

### 3.1.6 Summary of TIE performed on February 19, 2007 Sample

Results from the TIE performed on the Chollas Creek storm water sample collected in February re-confirmed that pyrethroids were likely responsible for the observed toxicity. Because of limited volume of storm water collected in February, a reduced number of manipulations were tested. In the Baseline test, toxicity of *H. azteca* exposed to the 75 percent sample concentration and undiluted storm water (96 and 100% survival, respectively) was significantly

lower than that in the initial toxicity test (62.5 and 10% survival, respectively). This indicates that during the time elapsed between the initial and baseline toxicity tests, the causative agent in the storm water sample had degraded. As a consequence, it was not possible to see a reduction in toxicity in the Filtration and Carboxyl esterase treatments. However, the PBO treatment led to increased toxicity in the undiluted storm water sample, which was consistent with the results of TIE tests performed in October and December. The repeated potentiation of toxicity using PBO in all recent TIEs provides strong evidence that pyrethroids are the causative agents, because pyrethroid toxicity is well-known to increase following the addition of PBO (Budavari, 1989).

### 3.2 CHEMICAL ANALYSES OF CHOLLAS CREEK STORM WATER

Below is a summary of chemical analyses performed on Chollas Creek storm water samples during the 2006-2007 monitoring season (Table 6). Briefly, with the exception of diazinon and malathion, all organophosphate pesticides were below the detection limit. Diazinon was found at a concentration of 0.100 µg/L in the October storm event, which exceeded the water quality objective of 0.08 µg/L. Malathion was found at concentrations of 0.949, 0.270, and 0.095 µg/L for the October, December, and February storm events, respectively. Only the October storm event exceeded the water quality objective of 0.430 µg/L. All dissolved metals analyzed were found at concentrations below their water quality objectives, with the exception of copper and lead. Dissolved copper was found at concentrations ranging from 0.007 to 0.014 mg/L across all storm events, all which exceeded its corresponding water quality objective. In the October storm event, dissolved lead was found at a concentration of 0.004 mg/L, which exceeded its water quality objective. Total copper (0.040-0.115 mg/L), lead (0.034-0.072 mg/L), and zinc (0.233-0.659 mg/L) were found across all storm events at concentrations that exceeded their water quality objectives. Total cadmium only exceeded its water quality objective in the October and December storm events at concentrations of 0.003 and 0.007 mg/L, respectively. Significant levels of pyrethroids were detected in the Chollas Creek storm water samples. Bifenthrin (0.057-0.398 µg/L), cyfluthrin (0.165-0.354 µg/L), and cypermethrin (0.116-0.451 µg/L) were found across all storm events. Esfenvalerate and fenvalerate were detected in the February storm event at concentrations of 0.005 and 0.003 µg/L, respectively. L-cyhalothrin was detected in the October and February storm events at concentrations of 0.025 and 0.031 µg/L, respectively. Prallethrin was detected in the December and February storm event at concentrations of 0.289 and 0.011 µg/L, respectively.

**Table 6. Chemical Analyses of Chollas Creek Storm water Samples Collected During the 2006-2007 Monitoring Season**

Analyte	UNITS	10/14/2006	12/10/2006	2/19/2007
General Chemistry				
Ammonia As N	mg/L	1.64	2.12	1.53
Un-ionized Ammonia as N	µg/L	23.9	<b>64.1</b>	<b>87.7</b>
Biochemical Oxygen Demand	mg/L	17.3	25.2	<b>31.2</b>
Chemical Oxygen Demand	mg/L	<b>447</b>	<b>266</b>	94
Dissolved Organic Carbon	mg/L	47.9	29.7	9.67
Dissolved Phosphorus	mg/L	0.88	0.64	0.32
Total Hardness	mg CaCO <sub>3</sub> /L	89	101	60
Nitrate As N	mg/L	2.4	0.27	<0.05
Nitrite As N	mg/L	0.1	0.06	0.07
Surfactants (MBAS)	mg/L	0.5	<0.5	<0.5
Total Dissolved Solids	mg/L	210	210	104
Total Kjeldahl Nitrogen	mg/L	4.2	4.5	3.7
Total Organic Carbon	mg/L	64	33.3	11.3
Total Phosphorus	mg/L	1.22	1.24	0.61
Total Suspended Solids	mg/L	<b>438</b>	<b>418</b>	<b>239</b>
Turbidity	NTU	<b>168</b>	<b>129</b>	<b>123</b>
Total Metals				
Antimony	mg/L	0.005	0.005	0.004
Arsenic	mg/L	0.012	<0.001	0.002
Cadmium	mg/L	<b>0.003</b>	<b>0.007</b>	<0.001
Chromium	mg/L	0.011	0.02	0.011
Copper	mg/L	<b>0.071</b>	<b>0.115</b>	<b>0.04</b>
Lead	mg/L	<b>0.072</b>	<b>0.071</b>	<b>0.034</b>
Nickel	mg/L	0.017	0.021	0.009
Selenium	mg/L	<0.004	<0.004	<0.004
Zinc	mg/L	<b>0.515</b>	<b>0.659</b>	<b>0.233</b>
Dissolved Metals				
Antimony	mg/L	0.002	<0.002	0.003
Arsenic	mg/L	<0.001	<0.001	<0.001
Cadmium	mg/L	<0.001	<0.001	<0.001
Chromium	mg/L	<0.005	<0.005	<0.005
Copper	mg/L	<b>0.014</b>	<b>0.014</b>	<b>0.007</b>
Lead	mg/L	<b>0.004</b>	0.002	<0.001
Nickel	mg/L	0.007	0.006	0.002
Selenium	mg/L	<0.004	<0.004	<0.004
Zinc	mg/L	0.092	0.072	0.021
Organophosphorus Pesticides				
Bolstar (Sulprofos)	ug/L	<0.004	<0.004	<0.004
Chlorpyrifos	ug/L	<0.002	<0.002	<0.002
Demeton	ug/L	<0.002	<0.002	<0.002
Diazinon	ug/L	<b>0.100</b>	<0.004	<0.004
Dichlorvos	ug/L	<0.006	<0.006	<0.006
Dimethoate	ug/L	<0.006	<0.006	<0.006
Disulfoton	ug/L	<0.002	<0.002	<0.002

Analyte	UNITS	10/14/2006	12/10/2006	2/19/2007
Ethoprop (Ethoprofos)	ug/L	<0.002	<0.002	<0.002
Fenchlorphos (Ronnell)	ug/L	<0.004	<0.004	<0.004
Fensulfothion	ug/L	<0.002	<0.002	<0.002
Fenthion	ug/L	<0.004	<0.004	<0.004
Malathion	ug/L	<b>0.949</b>	<b>0.270</b>	<b>0.095</b>
Merphos	ug/L	<0.002	<0.002	<0.002
Methyl Parathion	ug/L	<0.002	<0.002	<0.002
Mevinphos (Phosdrin)	ug/L	<0.01	<0.016	<0.016
Phorate	ug/L	<0.01	<0.012	<0.012
Tetrachlorvinphos (Stirofos)	ug/L	<0.004	<0.004	<0.004
Tokuthion	ug/L	<0.006	<0.006	<0.006
Trichloronate	ug/L	<0.002	<0.002	<0.002
Pyrethroids				
Allethrin	ug/L	<0.002	<0.002	<0.002
Bifenthrin	ug/L	0.090	0.057	0.398
Cyfluthrin	ug/L	0.191	0.354	0.165
Cypermethrin	ug/L	0.131	0.451	0.116
Danitol	ug/L	<0.002	<0.002	<0.002
Deltamethrin	ug/L	<0.002	<0.002	<0.002
Esfenvalerate	ug/L	<0.002	<0.002	0.005
Fenvalerate	ug/L	<0.002	<0.002	0.003
L-Cyhalothrin	ug/L	0.025	<0.002	0.031
Permethrin	ug/L	<0.002	<0.002	<0.002
Prallethrin	ug/L	<0.002	0.289	0.011

Shaded and **Bold** values indicated exceedance of water quality objective based on water hardness values.

#### 4. DISCUSSION

TIE test results provide strong evidence that pyrethroids are the causative agent of toxicity in the Chollas Creek storm water samples collected during the 2006-2007 monitoring season. In TIEs performed on samples collected in October, December, and February, PBO treatments led to increased toxicity in the diluted and undiluted storm water sample. The repeated potentiation of toxicity using PBO in all TIEs performed on the 2006-2007 Chollas Creek storm water samples suggests that pyrethroids may be the causative agents, because PBO is known to potentiate pyrethroid toxicity through its inhibition of the cytochrome P450 monooxygenase system (Budavari, 1989). A carboxyl esterase treatment was tested as an additional method for determining whether pyrethroids were the causative agents of toxicity. The carboxyl esterase enzyme caused a significant reduction in toxicity in the storm water samples exhibiting toxicity (i.e., October and December samples), indicating that the chemicals causing toxicity in the storm water sample were enzymatically degraded by carboxyl esterase. Because carboxyl esterase enzymes have a strong affinity for pyrethroids and are known to metabolize pyrethroids to less toxic forms (Wheelock et al., 2004), these results support the idea that pyrethroids were the causative agent of toxicity in the Chollas Creek storm water samples.

TIE tests also indicate that the causative agents of toxicity in storm water had similar physicochemical properties to those of pyrethroids. Filtration of the storm water sample significantly reduced the toxicity to *H. azteca* in the 100 percent storm water samples from October and December, indicating that the causative agents of toxicity were highly bound to particulates in the storm water sample. It is well known that pyrethroids are insoluble in water but soluble in solvents (have high  $K_{ow}$ s), have low vapor pressures (indicating low volatility), and have high adsorption coefficients, indicating their tendency to adsorb to particulates (Kidd and James, 1991). In the 100 percent storm water samples from February, a reduction in toxicity was not observed in the Filtration treatment because the causative agent of toxicity had degraded in the storm water sample and toxicity initially present in toxicity tests was not observed in the Baseline treatment of the TIE.

TIE tests also indicate that the causative agents of toxicity in the Chollas Creek storm water samples did not share similar physicochemical properties to those of many other classes of chemicals. The lack of toxicity reduction in the EDTA, STS, graduated pH, and aeration tests in TIEs performed on the October storm water sample indicates that the causative agent was likely not a metal, an oxidative chemical, a pH-sensitive chemical, or a volatile chemical or surfactant, respectively. A slight toxicity reduction was observed in the aeration tests in TIEs performed on the December storm water sample. This indicates that a volatile chemical or surfactant may have slightly contributed to toxicity in this storm water sample; however there is little evidence that any of these chemicals were a major cause of toxicity.

In addition to TIE tests, chemical analyses of Chollas Creek storm water samples indicate that pyrethroids are likely the primary causative agents of toxicity. Pyrethroids including bifenthrin were measured in Chollas Creek storm water samples at levels that exceeded aqueous 96-hour  $LC_{50}$  values for *H. azteca* of 0.0093 ng/L (Anderson et al., 2006). A number of other pyrethroids were detected in storm water samples including cyfluthrin, cypermethrin, esfenvalerate, fenvalerate, L-cyhalothrin, and prallethrin. While the aqueous 96 hour  $LC_{50}$  values for *H. azteca* exposed to these pyrethroids are currently unknown, concentrations of cyfluthrin, cypermethrin, L-cyhalothrin, and prallethrin in Chollas Creek storm water samples were comparable to those bifenthrin, suggesting that they may have also contributed to the toxicity observed in the Chollas Creek samples.

Other chemicals were detected in the Chollas Creek storm water sample; however, there is little evidence that any of these chemicals were a major cause of toxicity. All organophosphate pesticides were below their detection limits, with the exception of diazinon and malathion. Malathion may have contributed to the toxicity observed in the October storm event because the concentration detected exceeded the water quality objectives; however this concentration was significantly lower than the LC<sub>50</sub> for *H. azteca* (80.3 µg/L, Weston Bioassay Lab data). Diazinon also may have contributed to the toxicity observed in the October storm event because the concentration detected exceeded the water quality objectives; however this concentration was significantly lower than the LC<sub>50</sub> for *H. azteca* (6.51 µg/L). It is possible that metals may have contributed to the toxicity observed in the Chollas Creek storm water sample because concentrations of total cadmium, copper, lead, and zinc were above their respective water quality objectives storm water samples; however, concentrations of dissolved cadmium and zinc were below their water quality objectives. Because dissolved forms of metals are more bioavailable to organisms, it is unlikely that cadmium and zinc were responsible for the observed toxicity in Chollas Creek storm water samples. Dissolved copper concentrations were below the LC<sub>50</sub> for *H. azteca* in either soft water (LC<sub>50</sub> = 0.056 mg/L; Borgmann et al., 2005), or hard water (LC<sub>50</sub> = 0.249 mg/L, Weston Bioassay Lab data), further indicating that copper was not associated with the observed toxicity in storm water samples. Dissolved lead concentrations were below the LC<sub>50</sub> for *H. azteca* in soft water (LC<sub>50</sub> = 0.0048 mg/L; Borgmann et al., 2005). Concentrations of dissolved lead only exceeded the water quality objectives in the October storm event, and toxicity was observed in all three storm events, indicating that lead was most likely not associated with the observed toxicity in storm water samples.

Pesticide runoff into Chollas Creek is not surprising, because pesticides including diazinon have been measured in Chollas Creek storm water for a number of years (Weston 2005). However, diazinon concentrations in Chollas Creek that exceed the water quality objectives have diminished significantly over the last five years, in conjunction with reduced toxicity to the test species *C. dubia*. Nevertheless, the frequency of toxicity tests demonstrating persistent toxicity to *H. azteca* continues to be evident (i.e., more than 50% of the toxicity tests conducted to date have a NOEC of less than 100%), while no additional pesticides until now have been linked to this persistent toxicity.

An examination of TIE results conducted on Chollas Creek storm water, together with information on pyrethroid use in California further indicates the likelihood of pyrethroids as the causative agents of toxicity. In 1999, Chollas Creek storm water caused significant mortality to *C. dubia* and TIEs provided evidence that organophosphates were the causative agents of toxicity (Schiff et al., 2000). During subsequent monitoring periods for the San Diego County Municipal Storm water Copermittees (2000 to 2005), high frequency COCs included turbidity, diazinon, total and dissolved copper, and total zinc were measured in Chollas Creek storm water samples. However, diazinon use and levels in storm water diminished significantly during this time period, first dropping approximately 10 fold from 2001 to 2002, and then to below detection limits by 2005, likely due to the recent removal of pesticide formulations in the U.S. containing chlorpyrifos and diazinon. In contrast, pyrethroid use in California has increased over the same time period, and is used residentially in insecticides that previously had organophosphates such as diazinon and chlorpyrifos as the active ingredients (California Department of Pesticide Regulation, 2004). While TIEs performed in recent years on Chollas Creek storm water samples were inconclusive, a re-analysis of TIE data from 2002-2005 demonstrated that pyrethroids may have been the causative agent of toxicity in these samples. Specifically, similar to the present study, PBO strongly enhanced the toxicity observed in the TIEs performed on samples collected in 2002-2003, indicating that pyrethroids were a possible cause of toxicity. Moreover, similar to the TIE performed on the October Chollas Creek storm

water sample in 2005, samples in TIEs performed in 2002 to the spring of 2005 demonstrated decreased toxicity after a short holding time in the laboratory. These results indicate the causative agent was pyrethroids because like pyrethroids, the causative agent of toxicity was strongly adsorbing to the plastic cubitainers in which the samples were held (Wheelock et al., 2005). Finally, results of TIE tests conducted on storm water samples collected from Chollas Creek during the 2005-2006 monitoring season provided strong evidence that pyrethroids were the causative agents of toxicity. TIE tests including the PBO and the carboxyl esterase tests, indicated that pyrethroids were a likely causative agent. Results of filtration tests also provided evidence of pyrethroids, suggesting that the causative agents were bound to particulates. Chemical analyses of Chollas Creek storm water samples indicated that pyrethroids were present. Pyrethroids including bifenthrin and permethrin were measured in Chollas Creek storm water samples at levels that exceeded aqueous bifenthrin and permethrin 96-hour LC<sub>50</sub>s for *H. azteca*. These results indicated that bifenthrin and permethrin were likely contributors to the toxicity observed in the Chollas Creek storm water samples during the 2005-2006 monitoring season.

Results of this study provide strong evidence that pyrethroids are the causative agent of toxicity in Chollas Creek storm water samples collected as part of the 2006-2007 monitoring period for the County of San Diego and copermittees. TIE tests indicated that the causative agent(s) of toxicity shared all of the physicochemical properties of pyrethroids, and primarily lacked properties that characterize other classes of chemicals. Chemistry results demonstrated the presence of pyrethroids including bifenthrin which measured at concentrations in Chollas Creek storm water samples that exceed the LC<sub>50</sub> concentrations for *H. azteca*. Finally, increased use of pyrethroids, as demonstrated by the data presented by the California Department of Pesticide Regulation, to replace the organophosphates previously used in residential insecticides also indicate the likelihood that pyrethroids are the causative agents in these storm water samples.

## 5. REFERENCES

- Anderson, B.S., Phillips, B.M., Hunt, J.W., Connor, V., Richard, N., and R.S. Tjeerdema. 2006. Identifying primary stressors impacting macroinvertebrates in the Salinas River (California, USA): relative effects of pesticides and suspended particles. *Environmental Pollution*, 141:402-408.
- Borgmann, U., Couillard, Y., Doyle, P., and D.G. Dixon. 2005. Toxicity of sixty-three metals and metalloids to *Hyalella azteca* at two levels of water hardness. *Environmental Toxicology and Chemistry*, 24:641-652.
- Budavari, S., (ed.). 1989. *The Merck Index*. Merck & Co. Rahway, NJ, USA.
- California Department of Pesticide Regulation, 2004. <http://www.cdpr.ca.gov/>. Sacramento, California.
- Kidd, H. and D.R. James, (Eds.). 1991. *The Agrochemicals Handbook*, Third Edition. Royal Society of Chemistry Information Services, Cambridge, UK, pp. 2-13.
- MEC – Weston. 2004. *Watershed Data Assessment Framework*.
- Schiff, K.C., Bay, S.M., and C. Stransky. 2000. Characterization of storm water toxicants from an urban watershed to freshwater and marine organisms. Southern California Coastal Water Research Project. 1999-2000 Annual Report.
- United States Environmental Protection Agency (USEPA). 1991. *Methods for Aquatic Toxicity Identification Evaluations. Phase I Toxicity Characterization Procedures*. EPA/600/6-91/003. EPA Office of Research and Development. Second Edition. February
- United States Environmental Protection Agency (USEPA). 1992. *Toxicity Identification Evaluation. Characterization of Chronically Toxic Effluents, Phase I*. EPA/600/6-91/005F. EPA Office of Research and Development. May.
- United States Environmental Protection Agency (USEPA). 1993a. *Methods for Aquatic Toxicity Identification Evaluations. Phase II Toxicity Characterization Procedures for Samples Exhibiting Acute and Chronic Toxicity* EPA/600/R-92/080. EPA Office of Research and Development. September.
- United States Environmental Protection Agency (USEPA). 1993b. *Methods for Aquatic Toxicity Identification Evaluations. Phase III Toxicity Characterization Procedures for Samples Exhibiting Acute and Chronic Toxicity* EPA/600/R-92/081. EPA Office of Research and Development. September.
- United States Environmental Protection Agency (USEPA). 2000. *Methods for Measuring the Toxicity and Bioaccumulation of Sediment-associated Contaminants with Freshwater Invertebrates*. EPA/600/R-99/064. EPA Office of Water. March.
- Weston Solutions, Inc. 2005. *San Diego County Municipal Copermittees 2004-2005 Urban Runoff Monitoring. Final Report*. December.

- Wheelock, C.E., Miller, J.L., Miller, M.J., Gee, S.J., Shan, G., and B. Hammock. 2004. Development of toxicity identification evaluation procedures for pyrethroid detection using esterase activity. *Environmental Toxicology and Chemistry*, 23:2699–2708.
- Wheelock, C.E., Miller, J.L., Miller, M.J., Phillips, B.M., Gee, S.J., Tjeerdema, R.S., and B.D. Hammock. 2005. Influence of container adsorption upon observed pyrethroid toxicity to *Ceriodaphnia dubia* and *Hyalella azteca*. *Aquatic Toxicology*, 74:47-52.