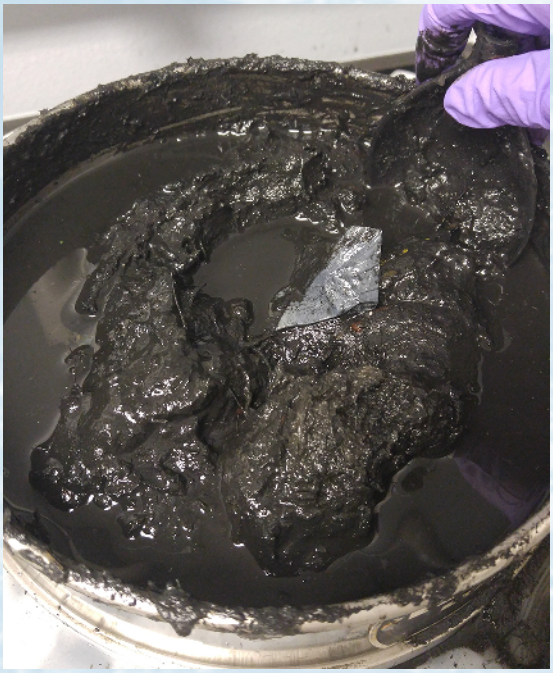
Spatial and Temporal Variability in Sediment Toxicity Identification Evaluations



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Southern California Coastal Water Research Project

SCCWRP Technical Report 1014

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Final Report

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EXECUTIVE SUMMARY

The State Water Resources Control Board Enclosed Bays and Estuaries Plan (EBE) specifies that for sites not achieving the SQO to protect aquatic life, the cause of the impacts should be determined through a process termed Stressor Identification (SI). SI often includes multiple components, including the review of existing data as a prelude to collecting new data. The most widely used and most effective method of SI is the Toxicity Identification Evaluation (TIE). The EBE Plan does not provide guidance regarding TIE study design or interpretation of the results, however. The objectives of this study were to: summarize information on TIE results variability and interpretation, investigate TIE variability for sediments in the Los Angeles Region, and provide recommendations regarding TIE study design and interpretation for use in future studies. This project included a literature review and field study to collect data to be used in design guidance. This report describes results from the field study and provides recommendations for the design of future TIE studies.

The field study was conducted in three stages: Stage I was a screening of several sites in the Los Angeles/Long Beach Harbor complex, seeking locations with sufficient toxicity to perform TIEs. This stage detected high levels of sediment toxicity in Consolidated Slip (Los Angeles Harbor) and ten stations within this site were selected for study in subsequent stages. In Stage II, comprehensive whole sediment and pore water TIE characterization tests were conducted at three stations within Consolidated Slip. In Stage III, whole sediment TIE characterization using a smaller number of targeted treatments was conducted at 10 stations. Concentration of trace organics and metals were measured in sediment samples from both stages.

Overall results from the TIEs were similar in Stages II and III, with a determination that sediment toxicity was caused by nonpolar organics, specifically pyrethroid pesticides and PAHs. These identifications were based on interpretation of the characterization results using standardized evaluation thresholds and Toxic Unit calculations based on sediment concentrations of pyrethroid and fipronil pesticides, as well as PAHs. Metals, chlordanes, DDTs, and PCBs, although present at high concentrations in Consolidated Slip, were ruled out as likely causes of toxicity.

Variable patterns in the TIE characterization results were present among the stations and sampling periods. Out of the 13 samples evaluated in both stages, the characterization results fell into three patterns: 1) seven stations where organic contaminants were identified, with pyrethroids probably accounting for most of the toxicity; 2) three stations where organics could not be identified as a cause, but pyrethroids were still indicated; and 3) three stations where organic contaminants were identified, but pyrethroids were not indicated as a cause. These patterns were further summarized into two categories: 10 stations where nonpolar organic chemicals were identified as a cause and three stations where the cause was not certain. None of these patterns was contradictory to the overall TIE conclusions for Consolidated Slip. However, use of a weight of evidence approach for data interpretation, consisting of TIE characterization, chemical analysis, and comparison to literature-based toxicity thresholds was essential for resolving inconsistencies in the results and improving confidence in data interpretation.

Statistical analyses of the results were conducted to estimate the probability of obtaining a successful TIE characterization result with different numbers of samples analyzed. Two scenarios of success were evaluated. The first scenario defined success as obtaining an effective

TIE characterization result for a majority of the tested samples. Under this scenario, analysis of seven samples yielded a 95% probability of success. The second scenario defined success as obtaining at least one effective TIE characterization outcome among the samples tested. For the second scenario, analysis of two samples resulted in a 95% probability of success, and analysis of five samples resulted in nearly 100% chance of success.

The overall TIE conclusion for Consolidated Slip was the same for each timepoint. The magnitude of toxicity, sediment chemical concentrations, and TIE characterization patterns were similar between sampling events. For this study, including multiple timepoints was not necessary to determine the cause of sediment toxicity.

Recommendations

The results from this study, combined with experience from other investigations, suggests several design principles that are likely to improve the success of sediment TIEs for stressor identification and increase confidence in results interpretation.

- Multiple stations should be evaluated for each TIE study site. Stations should be representative of the site characteristics.
- Preliminary toxicity screening should be conducted prior to selecting the final TIE stations and study design.
- TIEs should be conducted on a minimum of three spatially distributed stations, although site characteristics may warrant a larger number of stations.
- It is generally not necessary to conduct TIEs at more than one timepoint, unless supplemental information indicates that the cause of toxicity at the site is likely to vary seasonally.
- Analyses should always include whole sediment TIE analyses. Pore water TIEs can be used to support the conclusions of whole sediment TIEs, but should not be conducted in the absence of whole sediment TIEs.
- TIE characterization should include treatments or analyses diagnostic for the most prevalent causes of toxicity: nonpolar organics, metals, ammonia.
- Measure sediment contaminant concentrations in the TIE samples. Chemical analysis data is required to provide more specific identification of the cause of toxicity and to resolve variations in TIE characterization patterns.
- Stressor identification should be based on a weight of evidence interpretation of all results and utilize consistent criteria for interpretation of characterization results.

ACKNOWLEDGEMENTS

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INTRODUCTION

Toxicity tests are an important aspect of assessing sediment quality. However, it is not enough to just know that a location has sediments that are toxic to test organisms; it is even more important to know which chemicals are causing the toxicity so that the most effective management actions can be taken. To that end, Toxicity Identification Evaluation (TIE) methods were developed. A TIE includes a series of physical or chemical manipulations of the sample to alter the toxicity associated with various classes of pollutants (e.g. organic compounds) in conjunction with chemical analyses and data interpretation methods to then determine which chemical(s) in the sample are responsible for the observed toxicity. The scale and scope of a TIE may vary, depending upon the objectives of the study and characteristics of the study site. The methods used in a TIE are grouped into three categories: Characterization, including sample manipulations intended to identify the broad class of toxicant (e.g. metals or organics); Identification, including fractionation and chemical analysis of the sample to identify specific toxic constituents within a chemical class; and Confirmation, including subsequent sample analyses from the site to verify the accuracy of toxicant identification. Most TIEs include only the characterization and identification elements.

The first TIE methods were developed for freshwater aqueous samples (USEPA 1991) and were followed over a decade later by methods for sediments (USEPA 2007). Due to the greater complexity of the sediment matrix, there are fewer TIE treatments available for sediments than for aqueous samples. While the methods manuals gave detailed descriptions on how to perform the treatments, there was little information on study design and interpretation.

Sediment Quality Objectives (SQOs) are included in the State Water Resources Control Board Enclosed Bays and Estuaries Plan (EBE Plan, SWRCB 2009). The EBE Plan specifies that, for sites not achieving the SQO, the cause of the impacts should be determined through a process termed Stressor Identification (SI) as a component of developing regulatory or management actions. SI studies may include several types of analyses, such as review of existing data, TIE and enhanced chemical analysis, evaluation of contaminant bioavailability, and spiked sediment toxicity testing. SI should begin with a review of existing data before collecting new data or conducting additional laboratory studies, such as a TIE. Standardized laboratory methods for conducting a sediment TIE are available (USEPA 2007), but the EBE Plan does not provide guidance regarding study design or interpretation of the results. Because conducting SI may be an expensive undertaking, it is important to design studies that provide an accurate assessment while being cost effective.

The Water Boards funded this TIE variability study in 2015 to help address the lack of study design guidance. The objectives of the TIE study are to: summarize available information on sediment TIE variability and study design, document TIE variability for sediments in the Los Angeles Region, and provide recommendations regarding TIE study design and interpretation for use in future studies. This study consisted of two major elements: a literature review and a field study. The literature review determined that there was little information on TIE study design or variability available from the literature (Appendix A). The objectives of the field study were to measure temporal and spatial TIE variability at a field site, and then develop study design guidance for future sediment TIEs. This document presents results from both elements of the study.

Study Design

The field study was conducted in three stages (Figure 1). In Stage I, sediment samples were collected from three candidate sites within the greater Los Angeles/Long Beach Harbor complex to identify locations with sufficient toxicity for TIE testing (Figure 2, Table 1). Multiple sediment samples were collected and tested for toxicity from Consolidated Slip (CS), Port of Long Beach Channel 2 (CH2), and eastern San Pedro Bay (SPB). This testing found the highest levels of toxicity within Consolidated Slip and this site was selected for study in Stages II and III based on the high magnitude and apparent consistency of toxicity. Consolidated Slip is a semi-enclosed basin with the main source of contaminant input being Dominguez Channel entering at the northern end. There are numerous sources of potential contamination into Dominguez Channel including NPDES discharges, vessel hulls and discharges, stormwater discharges, and refineries (Anderson et al. 2007). A set of 10 stations within Consolidated Slip were selected using the Generalized Random Tessellated Stratified sampling design for site selection (Stevens 1997) (Figure 3, Table 2).

Each stage of the study had a different emphasis. The objective of Stage II was to conduct a thorough TIE at a few sites to identify the cause of toxicity. The station locations for Stage II were randomly selected and did not overlap the targeted locations examined in Stage I. Random station selection was used to ensure that the stations were representative of the Consolidated Slip site as a whole and that the locations were not influenced by assumptions regarding the source of cause of toxicity.

The objective of Stage III was to conduct more focused (targeted) TIEs at many sites within Consolidated Slip to investigate spatial variability in toxicity and chemistry. The same stations were sampled in both Stages II and III, which allowed for evaluation of temporal changes between the two sampling events. The number and type of toxicity and chemistry analyses varied in each stage. A summary of the testing activities is shown in Table 3.

The stations in Consolidated Slip were sampled in April (Stage II) and June 2017 (Stage III). Surface sediment was collected for initial toxicity testing, potential TIEs, and chemical analysis. Initial toxicity testing was conducted at both time points to determine which stations were suitable for TIE testing. Results from initial testing led to whole sediment and pore water TIEs being conducted on three stations from the April sampling and whole sediment TIEs on all 10 stations from the June sampling (Table 3). Pore water TIEs were not conducted in Stage III due to the low incidence and high variability of toxicity observed in previous tests.

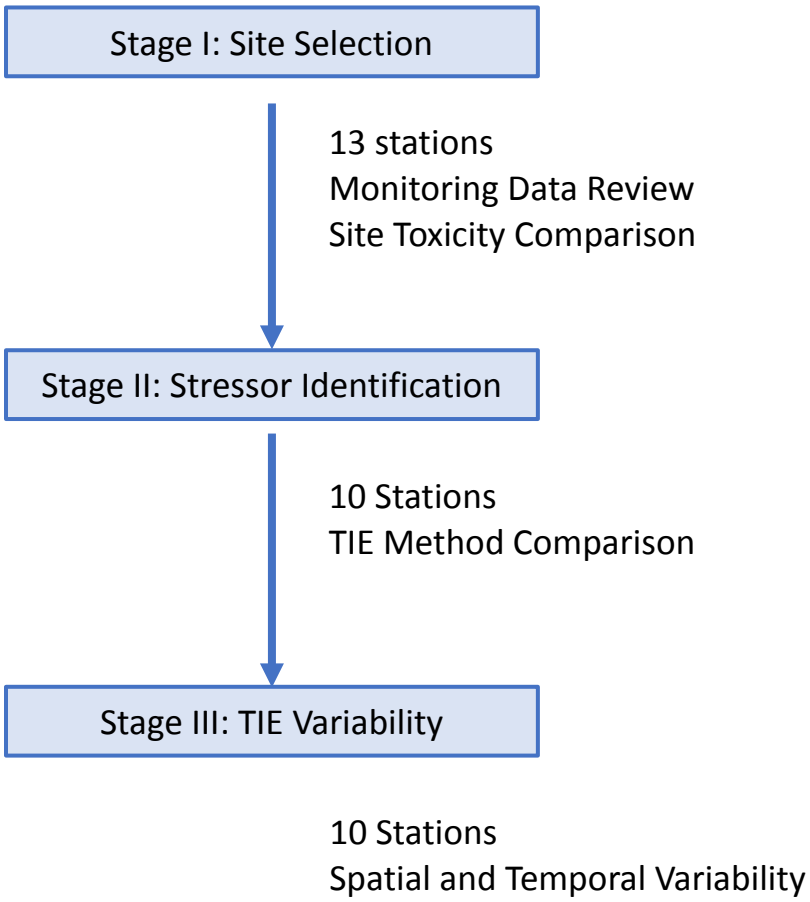

Figure 1. TIE Variability Study stages.

Figure 2. Map of station locations for initial toxicity survey in the Los Angeles/Long Beach Harbor complex.

Table 1. Coordinates for stations in the Los Angeles-Long Beach Harbor complex sampled for sediment toxicity screening.

Station	Latitude	Longitude
CH2-1	33.77202	-118.22016
CH2-2	33.77289	-118.21849
CH2-3	33.77400	-118.21554
CH2-4*	33.77525	-118.21278
CH2-5	33.77623	-118.21080
CS2	33.77615	-118.24349
CS3	33.77552	-118.24424
CS3	33.77543	-118.24420
CS4	33.77423	-118.24707
CS5	33.77300	-118.24879
CS6	33.77158	-118.24962
SPB1	33.74427	-118.16901
SPB2*	33.73900	-118.17132

*Nominal coordinates; actual coordinates lost.

Figure 3. Map of station locations within Consolidated Slip, Los Angeles Harbor.

Table 2. Station location coordinates within Consolidated Slip, Los Angeles Harbor.

Station	Longitude	Latitude
CS-T-01	-118.24515	33.77534
CS-T-02	-118.24852	33.77374
CS-T-03	-118.24825	33.77246
CS-T-05	-118.24390	33.77592
CS-T-06	-118.24714	33.77364
CS-T-07	-118.24924	33.77232
CS-T-09	-118.24439	33.77549
CS-T-10	-118.24716	33.77410
CS-T-11	-118.24920	33.77304
CS-T-12	-118.24551	33.77451

Table 3. Summary of analyses by study stage.

Stage	Analysis	Date	Number of Stations					
			Sediment Toxicity	Pore Water Toxicity	Sediment TIE	Pore Water TIE	Metals Chemistry	Organics Chemistry
II	Toxicity Screening	4/25/2017	10	0			3	10
II	TIE	5/16/2017			3	3		
III	Toxicity Screening	6/20/2017	10	10			3	10
III	TIE	7/18/2017			10	0		

FIELD AND LABORATORY METHODS

Sediment Sampling and Handling

Sediment samples were collected using a double Van Veen grab. The top 5 cm of sediment was removed and composited on board the vessel. Approximately 10 L of sediment were collected from each station and placed in multiple 2 L polyethylene jars. The samples were stored at 4°C until sieving and homogenization were completed.

Prior to toxicity testing, all sediment was removed from the polyethylene jars, passed through a 2 mm sieve without addition of water (i.e., press-sieved) to remove gravel, debris, and indigenous organisms. The sediment was then combined in a polycarbonate bucket, homogenized by hand using a stainless steel or plastic spoon, and returned to original storage containers. Most of the stations in Consolidated Slip contained a considerable amount of debris, such as trash and leaf litter. Up to 10% of the original sample volume was composed of debris for some stations. The sieving was conducted within five days of sediment collection and the toxicity screening tests for all three stages of the study were initiated within one week of sediment collection. Samples for chemical analysis were collected immediately after the sieving and homogenization; the samples were placed in 250 mL pre-cleaned glass jars and stored at -20°C until analysis.

Toxicity Testing

All toxicity tests were conducted using the amphipod *Eohaustorius estuarius* and standard 10-day survival test methods (USEPA 1994). Standard test conditions were used for the whole sediment tests, except a reduced volume was employed, using 250 ml beakers for test chambers instead of 1 L jars and 10 amphipods per replicate instead of 20. These changes were made to conserve the amount of sediment and laboratory space used for the large numbers of chambers needed for this project. This reduced volume has been used successfully in many previous studies by SCCWRP and other researchers (Ferretti et al. 2002). The sediment was added to the 50 ml mark (2 cm depth) in five replicate beakers for each station. The beakers were filled to the 200 ml mark with 32 psu seawater overlaying the sediment. The beakers were placed in a water bath at 15 °C and gentle aeration was added. The sediment was then allowed to equilibrate overnight.

Sediment pore water was separated from the particles by centrifugation at 3,000 x g for 30 min. Pore water testing was conducted in 20 ml glass vials with 10 ml of sample and five amphipods per replicate. Four replicates of pore water were tested in Stage II TIEs and three replicates were tested in Stage III screening. A reduced number of pore water replicates was tested in Stage III due to limitations in equipment and labor resources. Amphipods were added to the whole sediment and pore water test chambers after a 24-hour equilibration period.

A reference toxicant test was performed concurrently with the initial test samples in all three study stages. These tests were conducted using multiple concentrations of ammonia. The reference toxicant serves to verify that the sensitivity of test organisms is within normal bounds.

Water quality analyses were performed on overlying water at the beginning and end of each amphipod exposure. The measured constituents included temperature, pH, dissolved oxygen,

salinity, and total and un-ionized ammonia. Pore water temperature, ammonia and pH were measured at the beginning and/or end of each exposure, depending on the study stage.

TIE Characterization

Multiple treatments were applied to whole sediment and/or pore water, to identify the broad class of toxicants affecting amphipod survival (Table 4). In Stage II, TIEs were conducted on both whole sediment and pore water from three stations. Whole sediment TIEs were conducted on all 10 stations in Stage III. Pore water TIEs were not conducted in Stage III because initial screening results indicated a lack of sufficient toxicity (e.g., at least 30% reduction in survival and statistically significant difference relative to control). A reduced number of treatments were used in Stage III samples, which focused on the two major contaminant classes associated with sediment toxicity: non-polar organics and trace metals. Specific treatments for ammonia were not included because prior testing demonstrated that chemical measurements were sufficient to evaluate the influence of this parameter.

Whole sediment TIE methods followed those of USEPA (USEPA 2007). Addition of carbon was accomplished by mixing granular activated carbon (TOG 20x50, Calgon Corp.) at 15% by weight into sediment samples. This treatment sequesters nonpolar organic compounds, reducing their bioavailability. To sequester cationic metals, an exchange resin (SIR300, ResinTech) was added to sediment at 20% by weight. To remove ammonia from the sediment, zeolite (SIR600, ResinTech) was added at 20% by weight. An additional treatment in Stage II was a combination of carbon and cation exchange resin at 10% each by weight. This treatment was added to test for a combined effect of organics and metals in the sample. A dilution control was created by adding amphipod home sediment, defined as sediment typical of the estuarine amphipod collection site in Oregon. The relatively large particle size and extremely low total organic carbon content (99% sand, 0.09% TOC) of this sediment results in a relatively inert material that is unlikely to alter toxicant bioavailability. Home sediment was added to the samples at 20% by weight. This treatment was designed to control for the dilution effect of adding the carbon, resin, or zeolite. Treatment blanks were analyzed for carbon, resin, and zeolite treatments, which consisted of the treatment applied to home sediment. These blanks served to verify that the treatments themselves were not toxic to the amphipods.

Piperonyl butoxide (PBO) was added to the overlying water in the whole sediment treatments to a concentration of 400 µg/L. The PBO treatment affects the detoxification system of the amphipods thereby causing a decrease in toxicity in the presence of organophosphorus pesticides or an increase in toxicity in the presence of pyrethroid pesticides. Carboxylesterase (CEE) was added to the overlying water at a concentration of one unit per milliliter. This treatment breaks down pyrethroid pesticides, rendering them nontoxic. Bovine serum albumin (BSA) was added to the overlying water at the same concentration as CEE. This treatment acts as a control for the CEE treatment by distinguishing binding of chemicals to the enzyme from the actual breakdown of pyrethroids. The CEE and BSA treatments were added a day before animal addition and then every other day through the end of the exposure. Temperature reduction was not included in the TIEs because of the nonspecific nature of this treatment. Blanks for the PBO, CEE. And BSA treatments were also analyzed, which consisted of the treatment applied to home sediment.

The pore water treatments in Stage II were based on USEPA methods for marine waters (USEPA 1996). The CEE and BSA treatments were conducted at the same concentration and frequency as for the whole sediment. The PBO treatment was added to pore water at 200 µg/L. Ammonia

treatment was accomplished by passing the pore water sample through a column of zeolite at approximately 5 ml/min. To chelate cationic metals, EDTA was added to pore water to a concentration of 60 mg/L. Sodium thiosulfate was added at 50 mg/L to chemically reduce oxidizers, such as chlorine, and to reduce the toxicity of some metals such as copper. Solid phase extractions were performed on pore water to remove organic contaminants and metals. To remove organic chemicals, the water was passed through a C-18 column (Mega Bond Elut, 6 ml, Agilent). For removal of metals, a cation exchange column (LC-WCX, 3 ml, Supelco) was used. An additional sequential TIE treatment was included in Stage II, consisting of passing pore water first through the cation exchange column and then the C-18 column. This treatment was applied to identify the combined effects of metals and organic contaminants. The columns were not eluted and tested for toxicity, as these treatments were beyond the scope of the study. All the treatments types were also performed on a sample of laboratory seawater as a blank to ensure the treatments themselves were not causing toxicity.

Table 4. Treatments used for the whole sediment and pore water TIEs conducted in this study.

Treatment	Matrix	Purpose	Stage II	Stage III
Activated carbon	Sediment	Binding of organic contaminants	X	X
Cation exchange resin	Sediment	Binding of cationic metals	X	X
Dilution	Sediment	Control for sediment dilution caused by addition of carbon or cation exchange resin	X	X
Piperonyl butoxide (PBO)	Water/Sediment	Reduces toxicity of organophosphorus pesticides; increases toxicity of pyrethroid pesticides	X	X
Zeolite	Water/Sediment	Binding of ammonia and some metals	X	
Carboxylesterase	Water/Sediment	Breaks down pyrethroid pesticides	X	
Bovine Serum Albumin	Water/Sediment	Control for carboxylesterase addition	X	
EDTA	Water	Chelation of cationic metals (e.g. Zn, Cu)	X	
Sodium thiosulfate (STS)	Water	Reducing agent for oxidizers (e.g. chlorine); reduces toxicity of some metals	X	
C-18 column extraction	Water	Removal of non-polar organics	X	
Cation exchange column extraction	Water	Removal of cationic metals	X	

Chemical Analysis

All chemical analyses were performed by Physis Environmental Laboratories (Anaheim, CA). Organic chemical analysis was performed on all sediment samples from both sampling periods, and included measurement of Total Organic Carbon (TOC), particle size, and trace organics.

Sediment trace metals were measured on the three samples from the Stage II collection used for the TIE analysis. The Stage III samples from the same three stations were also analyzed for metals to make temporal comparisons.

Chlorinated pesticides, PCBs, and PAHs were measured using EPA Method 8270D, which includes final analysis on Gas Chromatograph with a Mass Spectrometer (GCMS). Pyrethroids and fipronils were measured using the same EPA method but with the GCMS in negative chemical ionization mode. Trace metals were analyzed by EPA Method 6020 which includes final analysis by Inductively Coupled Plasma – Mass Spectrometry (ICPMS). Total organic carbon was analyzed by EPA Method 9060. Sediment particle size was measured using Standard Methods SM2560 on a laser diffraction particle size analyzer.

Total sulfide concentration in pore water were measured by SCCWRP at all stations sampled in Stage III, using a modified methylene blue method (Kolthoff et al. 1969).

Data Analysis

Toxicity results were normalized to the negative control (home sediment) or appropriate TIE control or blank sample prior to statistical analysis. Statistically significant differences in survival were determined using an unequal variance t-test ($p \leq 0.05$). The charcoal, SIR300, combination of the two, and zeolite treatments were compared to the dilution control. The dilution control, CEE, BSA, and PBO treatments were compared to the baseline sample (untreated).

The magnitude and statistical significance of survival changes (relative to control or baseline) were compared to determine whether a given TIE treatment was effective. The treatment was classified as effective if it elicited a change of at least 20 percentage points in survival relative to its appropriate reference (dilution control or baseline). A treatment producing a survival change of 10-19 percentage points was also classified as effective, but only if the change was statistically significant. This change in survival had to be positive (i.e., increase in survival for treated sample) for all treatments except for PBO. PBO treatment can produce either an increase or decrease in survival, depending on the type of toxicant present (Table 4). A treatment that changed survival by less than 10 percentage points was deemed to be ineffective, regardless of whether it was significantly different. The 10% and 20% thresholds were selected based on the use of similar thresholds to classify toxicity in the State Water Board's sediment quality objectives program (SWRCB 2009).

A color code was assigned to each sediment sample, based on the dominant cause of toxicity indicated by the overall pattern of sediment treatments. A green color was assigned to samples where toxicity from nonpolar organics, dominated by pyrethroids, was indicated (e.g., charcoal and PBO treatments effective). Samples where pyrethroids were implicated as a potential cause of toxicity (PBO treatment effective), but the influence of nonpolar organics in general (including pyrethroids) was uncertain due to low effectiveness of the charcoal treatment, were assigned a blue color code. A yellow color code was assigned to samples where toxicity was associated with nonpolar organics, but the role of pyrethroids was uncertain (only charcoal treatment effective).

Correlations between chemical concentrations and amphipod survival (Phase III TIE method) were performed using a Spearman's rank correlation test in SigmaPlot 12.5. A toxic units (TU) approach was used to determine the potential for toxicity due to PAHs, pyrethroid pesticides and

fipronil. Toxic units for pyrethroids and fipronil were calculated as the organic carbon normalized concentration of a compound divided by the organic carbon normalized LC50 of that compound. The TUs for all detectable pyrethroid or fipronil compounds were summed. If the summed TUs for a station were greater than one, the potential for toxicity associated with pyrethroids or fipronil was present. Toxic units for PAHs were calculated by comparison to EPA water quality objectives (Final Acute Value) using the equilibrium sediment benchmark (ESB) approach (USEPA 2003). Toxic units (proportion of Final Acute Value) were calculated for each PAH measured and then summed and corrected for measurement of a subset of PAHs to determine the TU for total PAH.

A successful TIE characterization test was defined as being able to associate the cause of toxicity with either nonpolar organics, metals, or ammonia. The estimated probability of successfully characterizing the cause of toxicity at a single station was calculated as the number of successful characterization tests in this study divided by the total number of tests. The cumulative probability of attaining a successful TIE characterization at greater than two stations was calculated in Excel using the binomial distribution. The inputs for each binomial calculation included the total number of stations evaluated and the minimum number of stations required to have a successful characterization result, and the probability of success with only one sample.

RESULTS

All toxicity tests conducted during this project met control acceptability criteria. Reference toxicant tests indicated that the test organisms were within the normal range of sensitivity; all LC50 values were within two standard deviations of the historical mean. All water quality data were within normal ranges. The highest pore water un-ionized ammonia concentration in any of the toxicity exposures was 0.108 mg/L, far below the concentration expected to impact amphipod survival. The mean ammonia LC20 value (an indicator of the low-level toxicity threshold) from recent SCCWRP reference toxicant exposures is 0.725 mg/L, indicating that ammonia was unlikely a cause of toxicity in any of the tests. Lack of effectiveness of the zeolite TIE treatment confirmed that none of the toxicity in either sediment or pore water was likely to be the result of ammonia.

Stage I

Sediment toxicity was detected in sediment from all three sites investigated in Stage I. All but two of the stations sampled (CH2-1 and CH2-4) were found to have significantly less survival than the control (Figure 4). However, moderate to high toxicity (generally considered to be a mean survival of less than 70%) is usually required for a successful TIE. Only stations within Consolidated Slip had this level of toxicity. Therefore, Consolidated Slip was selected as the study site for Stages II and III.

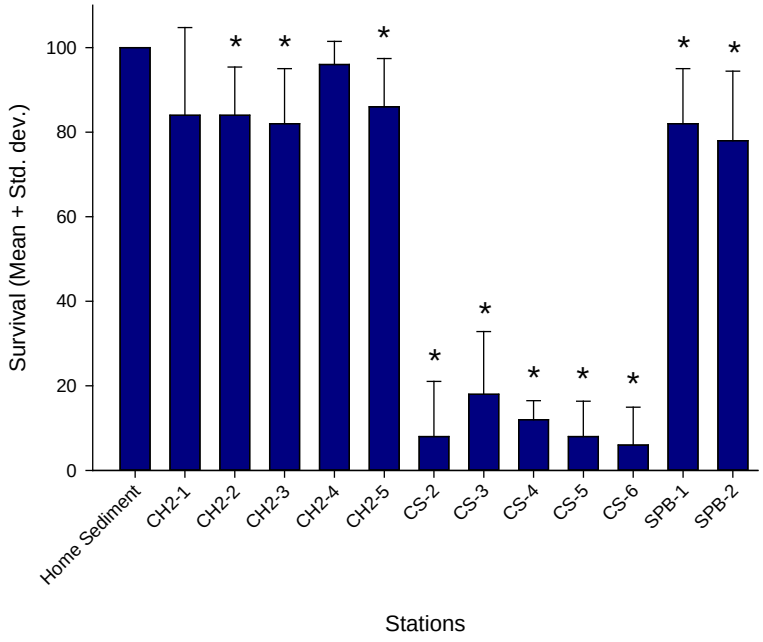

Figure 4. Results of screening sediment toxicity tests from the Stage I sampling in Los Angeles/Long Beach Harbor complex, using the amphipod *Eohaustorius estuarius* 10-day survival test. Asterisks indicate stations where mean percent survival was significantly lower than the control, p≤ 0.05.

Stage II Characterization

High toxicity was present in whole sediment from all but one of the stations sampled in Stage II, with less than 50% survival at most stations (Figure 5). Station T-1 was considerably less toxic than the rest. The reason for the lower toxicity at Station T-1 is not known at this time; contaminant concentrations and sediment geochemical characteristics (e.g., TOC and particle size) were similar among all stations. Based on a combination of toxicity magnitude and location, stations T-5, T-7, and T-9 were selected for whole sediment and pore water TIE characterization.

The whole sediment TIE characterization treatments for all three stations showed similar trends: the charcoal treatment reduced toxicity and the PBO treatment increased toxicity (Figures 6-8). Results for selected treatments that are indicative of the cause of toxicity are shown in the figures and tables. Results for all TIE treatments are presented in Appendix B. None of the remaining treatments consistently influenced toxicity among stations. Application of the treatment effectiveness criteria indicated a different, but related, likely cause of toxicity for each sample (Table 5). The PBO and charcoal treatments were both effective for Station T-5, resulting in a conclusion that nonpolar organics (likely pyrethroids) were the probable cause of toxicity (green code). Only the charcoal treatment was classified as effective for Station T-9, also indicating that nonpolar organics were the likely cause (influence of pyrethroids was uncertain). The characterization results for Station T-7 indicated that pyrethroids were probable contributors to the toxicity, but the influence of the larger category of nonpolar organics (which includes pyrethroids) was uncertain, leading to a blue color code (Table 5).

The Stage II pore water TIEs indicated toxicity in two of three untreated (baseline) samples: T-7 and T-9. However, the survival changes in the baseline and most TIE treatments had high between replicate variability and were not statistically significant (Figures 9-11). The only treatment that consistently influenced pore water toxicity was PBO, which increased toxicity as was seen in the whole sediment TIEs (Table 6). Other treatments had inconsistent effectiveness, including C-18 column extraction, cation exchange, sodium thiosulfate (STS), and carboxylesterase. Conflicting results were obtained among the treatments designed to characterize trace metal toxicity. The cation exchange resin and STS treatments reduced toxicity at stations T-7 and T-9, but EDTA, which should also remove the same cationic metals, was not effective. The influence of trace metals on pore water toxicity is uncertain, due to the lack of effectiveness of EDTA. EDTA is the most specific of these three treatments and generally highly effective when trace metal toxicity is present.

Organic chemical concentrations in the Stage II sediments were high at all stations (Table 7). Concentrations of DDTs, chlordanes, PAHs, and PCBs at each station exceeded the 95% upper confidence limit of the mean reported for estuary or port sediments from the 2013 Southern California Bight Regional Monitoring Survey, (Dodder et al. 2016). Pyrethroid concentrations were similar to those reported for estuaries in Bight'13. Concentrations of several trace metals were also high in the three Stage II samples analyzed, with cadmium, copper, zinc and lead levels exceeding Bight '13 survey values (Table 8). Mercury concentrations were similar to those reported for Bight'13. The TOC concentrations at all Consolidated Slip stations were also extremely high (6.1 – 7.7%), exceeding the Bight'13 Estuaries 95% upper confidence limit of

1.8% at every station (Table 9). Sediment particle size was similar among stations, with greater than 80% fines at most stations.

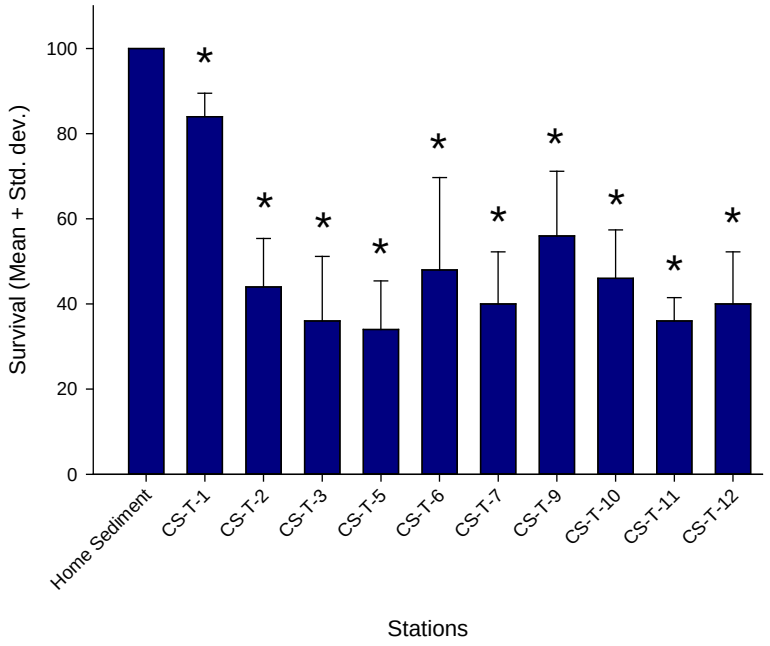

Figure 5. Results of initial sediment toxicity tests from the Stage II sampling in Consolidated Slip, using the amphipod *Eohaustorius estuarius* 10-day survival test. Asterisks indicate stations where mean percent survival was significantly lower than the control, p≤ 0.05.

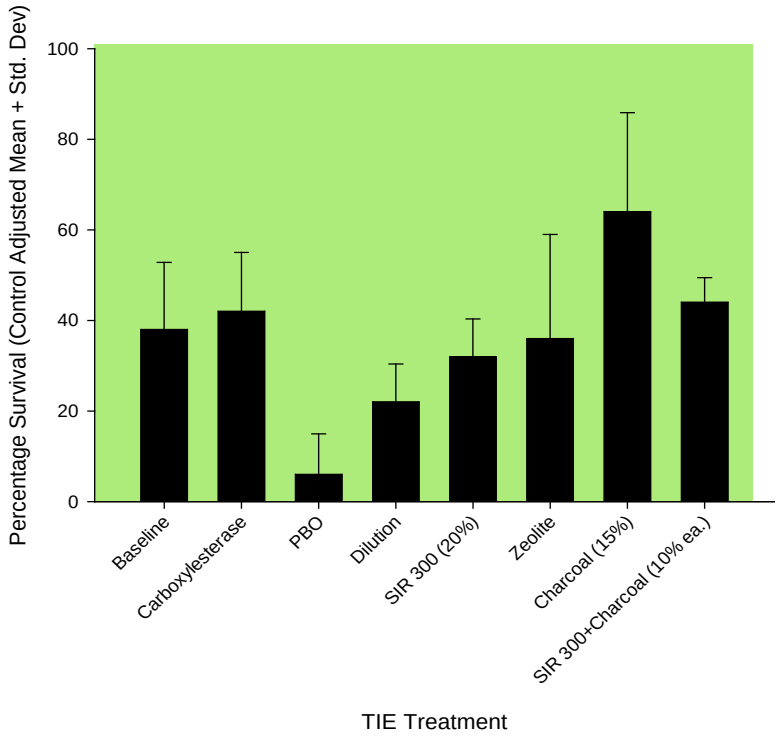

Figure 6. Amphipod survival results after whole sediment TIE treatments on Station CS-T-5 collected during Stage II.

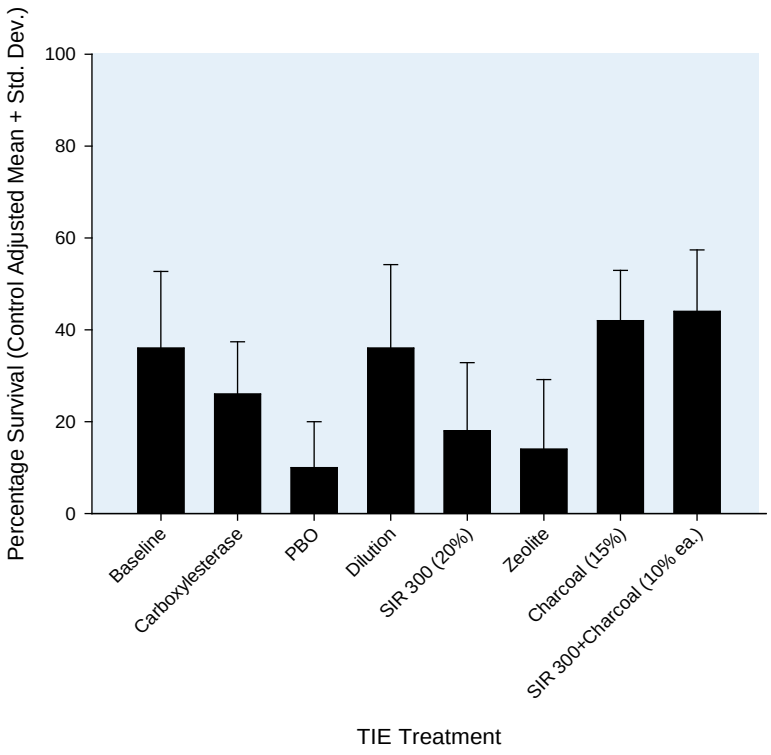

Figure 7 Amphipod survival results after whole sediment TIE treatments on Station CS-T-7 collected during Stage II.

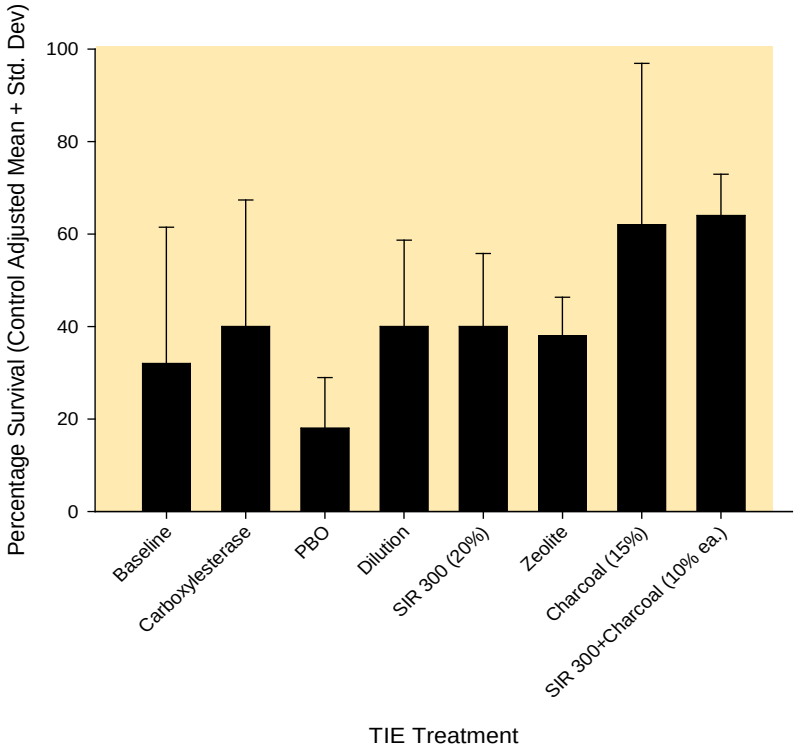

Figure 8. Amphipod survival results after whole sediment TIE treatments on Station CS-T-9 collected during Stage II.

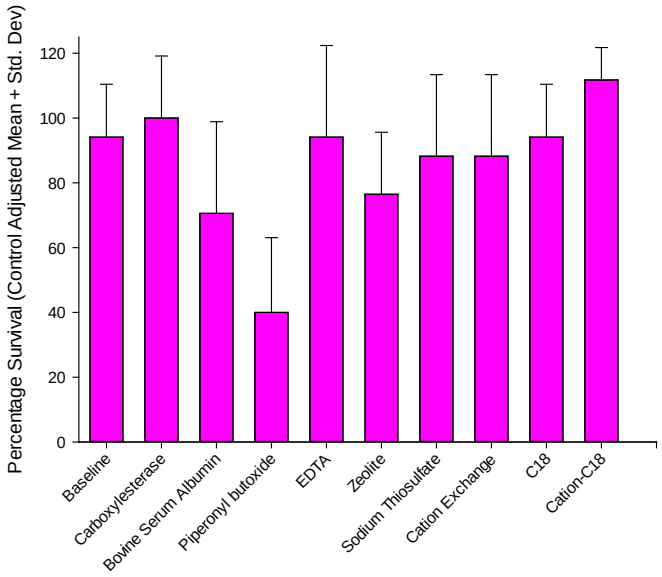

Figure 9. Amphipod survival results after pore water TIE treatments on Station CS-T-5 collected during Stage II.

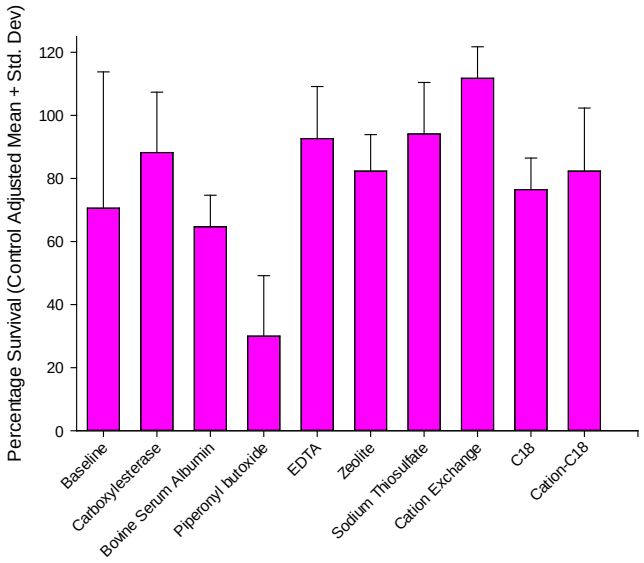

Figure 10. Amphipod survival results after pore water TIE treatments on Station CS-T-7 collected during Stage II.

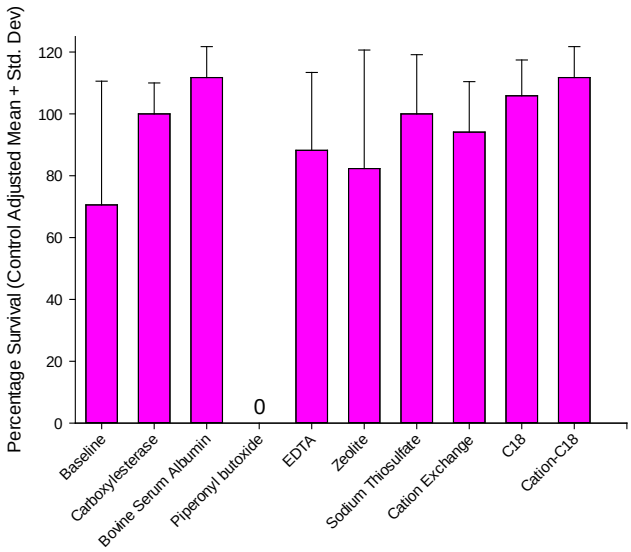

Figure 11. Amphipod survival results after pore water TIE treatments on Station CS-T-9 collected during Stage II.

Table 5. Effectiveness of TIE treatments on whole sediments from Stage II Consolidated Slip samples.

Test Stage	Station	Difference from baseline or dilution control					
		PBO	Charcoal (15%)	SIR 300 (20%)	SIR 300+Charcoal (10% ea.)	Zeolite	Carboxylesterase
II	5	-32[b]	42[ab]	10	22[b]	14	4
II	7	-26[ab]	6	-18	8	-22	-10
II	9	-14	22[b]	0	24[b]	-2	8

[a]Statistically significant t-test
[b]At or above the threshold of 20 percentage point difference from the baseline or dilution control

Table 6. Effectiveness of TIE treatments on pore water from Stage II Consolidated Slip samples.

Test Stage	Station	Difference from baseline							
		PBO	C-18	Cation Exchange	Cation Exchange then C-18	Zeolite	CEE	EDTA	STS
II	5	-60[ab]	0	-5	15	-15	5	0	-5
II	7	-45[b]	5	35[b]	10	10	15	19	20[b]
II	9	-60[ab]	30[b]	20[b]	35[b]	10	25[b]	15	25[b]

[a]Statistically significant t-test
[b]At or above the threshold of 20 percentage point difference from the baseline or dilution control

Table 7. Total sediment concentrations of organic compounds organized by class from Stage II sampling in Consolidated Slip. Concentrations are expressed in µg/kg dry weight. Results from the 2013 Southern California Bight Regional Survey Estuaries and Ports strata (95% Upper Confidence Limit of mean) are shown for comparison.

	T-1	T-2	T-3	T-5	T-6	T-7	T-9	T-10	T-11	T-12	B'13 Estuary	B'13 Port
DDTs	148	109	153	129	157	130	153	139	106	144	4	1
Chlordanes	23.1	20.2	36.8	22.6	26.3	23.7	28.9	27.8	19.3	22.8	0.6	0.1
Nonachlors	14.8	11.4	20.7	13.5	15.9	14.5	16.5	19.1	12.6	14.6	NA	NA
PAHs	8848	6687	7164	8667	8577	6611	8642	10003	5910	6770	530	560
PCBs	125	2130	158	112	223	92.4	105	80.7	1069	97.4	10	9
Pyrethroids	66.1	52.7	87.6	59.3	114	98.2	57.9	77.8	57.0	69.2	180	0.1
Fipronils	5.71	8.99	9.73	10.1	11.2	10.1	5.80	8.21	9.44	10.1	NA	NA

NA=Not available

Table 8. Selected sediment metals concentrations for both sampling stages in Consolidated Slip. Concentrations are expressed in mg/kg dry weight. Results from the 2013 Southern California Bight Regional Survey Estuaries and Ports strata (95% Upper Confidence Limit of mean) are shown for comparison.

	T-5	T-5	T-7	T-7	T-9	T-9	B'13 Estuary	B'13 Port
	Stage II	Stage III	Stage II	Stage III	Stage II	Stage III		
Cadmium	1.75	1.94	2.05	1.99	2.22	2.30	0.8	0.7
Copper	156	168	177	177	178	175	42	88
Zinc	700	749	799	784	725	778	120	170
Lead	101	104	111	111	125	115	21	36
Mercury	0.078	0.013	0.029	0.031	0.054	0.145	0.08	0.93

Table 9. Physical characteristics of sediment from Stage II sampling in Consolidated Slip. Concentrations expressed as percentage of dry weight.

	T-1	T-2	T-3	T-5	T-6	T-7	T-9	T-10	T-11	T-12
%Solids	38.4	36.9	41.5	37.7	35.1	38.7	39.1	33.6	35.3	39.6
%TOC	6.74	6.09	6.57	6.84	7.73	6.41	6.20	7.68	6.58	6.07
Grain Size										
%Sand	17.1	13.9	22.4	28.3	19.3	23.4	17.5	18.7	31.0	14.9
%Silt	61.5	65.2	60.5	55.5	62.5	58.0	60.6	61.5	53.4	64.8
%Clay	21.5	20.9	17.0	16.2	18.2	18.6	21.9	19.8	15.6	20.3

Stage III Characterization

All stations from the Stage III sampling had high whole sediment toxicity (Figure 12). Initial toxicity testing of pore water found insufficient toxicity to perform a TIE, with only station T-7 being significantly different from the control (Figure 13). Although exposure to pore water from station T-1 resulted in only 53% survival, the results were classified as insufficient for TIE due to high variability and lack of statistical significance relative to the control.

The Stage III TIE characterization results were similar to those in Stage II, with charcoal and PBO being the only effective treatments (Figure 14). Results for selected treatments are shown in the figure. Complete results are presented in Appendix B. There was a trend of increased survival with charcoal addition and reduced survival with PBO addition at each station, with these treatments classified as statistically effective in most cases (Table 10). The dilution control also substantially reduced toxicity for a few stations (T-5, T-10, and T-12), but not to the degree of the carbon treatment, indicating that nonpolar organic toxicants were the most likely cause of toxicity. The cation exchange resin treatment did not reduce toxicity at any station, indicating that exposure to sediment trace metals did not cause toxicity.

Application of the classification criteria to the characterization results indicated the same three related patterns of response as was observed in Stage II. Most samples were classified as having toxicity due to nonpolar organics, likely pyrethroids (green code, Table 10). There were also two instances where toxicity was associated with nonpolar organics, but the role of pyrethroids was less certain (yellow), as well as two instances where only pyrethroids were indicated as a potential cause of toxicity (blue).

Chemical concentrations in most Stage III sediment samples were similar to those from Stage II (Tables 7, 8, and 11). The one exception being station T-1, where all trace organic constituents except PCBs were lower in Stage III compared to Stage II. However, toxicity results for this station did not correspond to the change in chemical concentrations; T-1 had greater toxicity in Stage III compared to Stage II. The Stage III sediment physical characteristics were also similar compared to Stage II, with high TOC and fine-grained particle size (Table 12).

Sulfide concentrations in the Stage III pore water samples were highly variable and showed no correspondence with toxicity (Table 12). Concentrations in the initial pore water toxicity screening test varied by 84-fold, from barely detectable to high enough where toxicity could be expected (LC50 = 3.3 mg/L). However, pore water samples with the highest sulfide concentrations were not toxic. Sulfides in the sediment TIE pore water were high at the start of the test, but declined to being just above detection by the end of the exposure period.

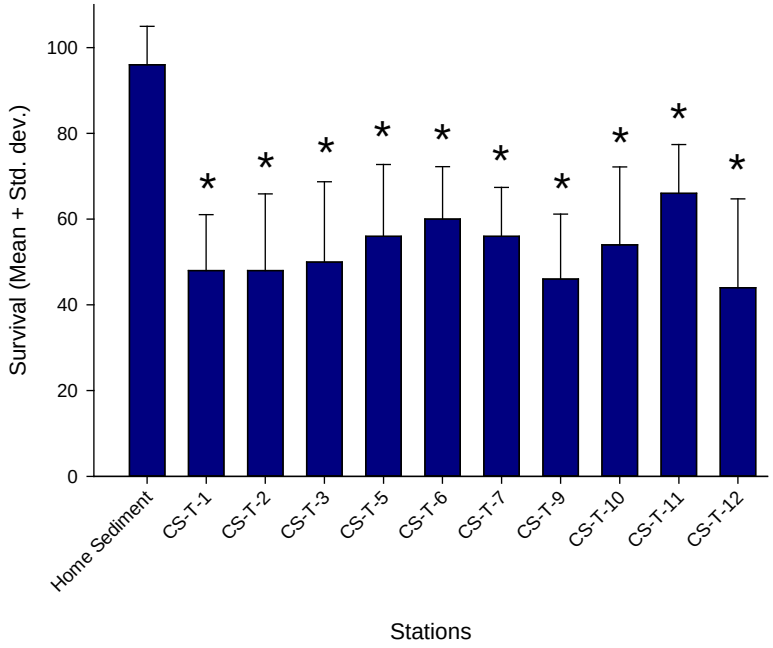

Figure 12. Results of initial sediment toxicity tests from the Stage III sampling in Consolidated Slip, using the amphipod *Eohaustorius estuarius* 10-day survival test. Asterisks indicate stations where survival was significantly lower than the control, p≤ 0.05.

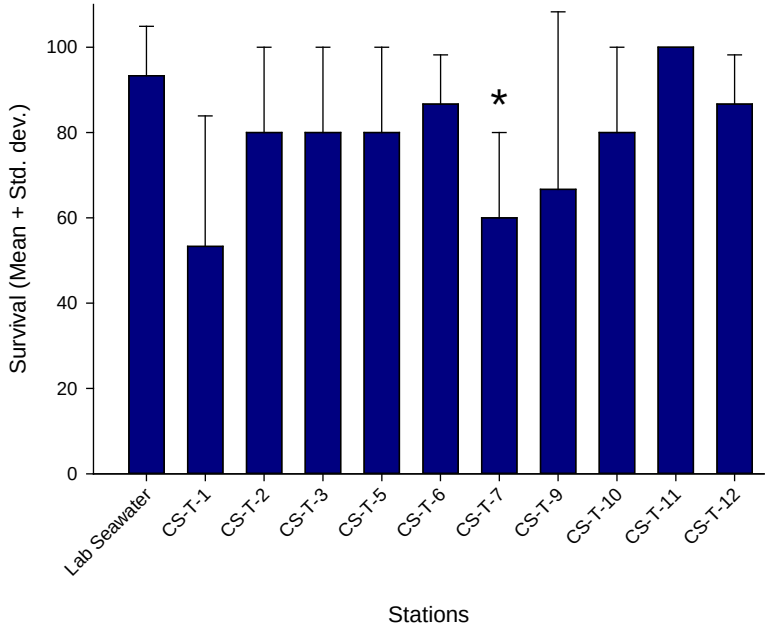

Figure 13. Results of initial pore water toxicity tests from the Stage III sampling in Consolidated Slip, using the amphipod *Eohaustorius estuarius* 10-day survival test. Asterisks indicate stations where survival was significantly lower than the control, p≤ 0.05.

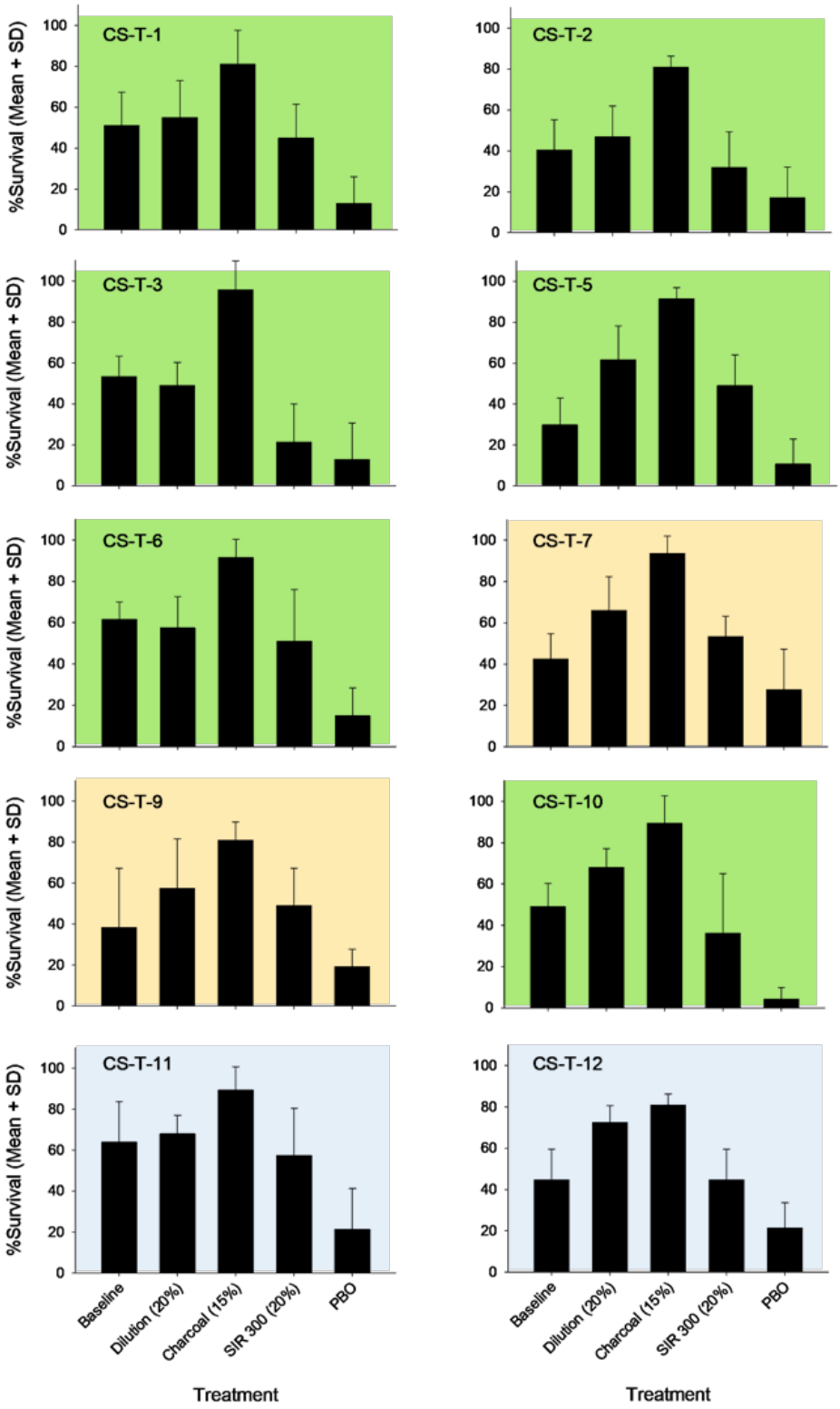

Figure 14. Amphipod survival results after whole sediment TIE treatments on samples collected during Stage III.

Table 10. Effectiveness of TIE treatments on whole sediments from Stage III Consolidated Slip samples.

		Difference from baseline or dilution control		
Test Stage	Station	PBO	Charcoal (15%)	SIR 300 (20%)
III	1	-36[ab]	24[ab]	-10
III	2	-22[ab]	32[ab]	-14
III	3	-38[ab]	44[ab]	-26
III	5	-18[a]	28[ab]	-12
III	6	-44[ab]	32[ab]	-6
III	7	-14	26[ab]	-12
III	9	-18	22[b]	-8
III	10	-42[ab]	20[ab]	-30
III	11	-40[ab]	14	-16
III	12	-22[ab]	8	-26

[a]Statistically significant t-test

[b]At or above the threshold of 20 percentage point difference from the baseline or dilution control

Table 11. Total sediment concentrations of organic compound classes from Stage III sampling in Consolidated Slip. Concentrations are expressed in µg/kg dry weight.

	T-1	T-2	T-3	T-5	T-6	T-7	T-9	T-10	T-11	T-12
DDTs	64.6	498	151	138	133	135	144	125	136	158
Chlordanes	13.4	25.8	24.9	24.8	24.7	26.8	28.3	21.5	26.1	27.4
Nonachlors	8.3	15.5	16.2	16.2	15.6	16.6	17.1	14.7	17.4	17.6
PAHs	3764	7581	7765	8645	6667	7691	7195	7864	8020	8083
PCBs	113	319	157	84.4	110	130	97.9	86.0	1372	106
Pyrethroids	30.7	82.3	103	93.0	70.8	85.3	124	118	89.5	69.4
Fipronils	4.96	8.82	9.72	7.35	10.5	9.30	8.88	9.67	10.1	11.1

Table 12. Physical characteristics of sediment and pore water sulfides from Stage III sampling in Consolidated Slip.

	T-1	T-2	T-3	T-5	T-6	T-7	T-9	T-10	T-11	T-12
%Solids	41.2	35.8	42.3	35.4	33.1	38.8	40.3	35.7	34.1	35.6
%TOC	6.26	7.52	6.16	7.86	7.47	6.75	7.97	6.82	8.12	7.17
Grain Size										
%Sand	22.2	19.2	18.0	18.0	17.1	22.0	22.6	21.1	16.1	18.0
%Silt	58.0	60.6	64.1	61.4	64.1	59.6	57.9	59.9	64.0	63.4
%Clay	19.8	20.3	17.8	20.6	18.7	18.4	19.5	19.1	19.9	18.7
Sulfides (mg/L)										
Pore water Screening	0.13	0.07	0.032	2.71	0.25	0.064	0.22	4.34	3.88	0.09
Sediment TIE T0	0.05	0.02	0.01	21.5	1.20	0.19	0.04	5.09	17.6	0.08
Sediment TIE TF	0.05	0.03	ND	0.02	ND	ND	ND	ND	0.01	0.05

TIE T0 = Start of TIE test; TIE TF = End of TIE test

Chemical Identification

Correlations with Toxicity

Spearman rank correlation analyses of the concentrations of sediment metals compared with amphipod survival found there to be no significant negative correlations, (Table 13; a negative correlation would indicate that survival decreases as metal concentration increases). A negative correlation coefficient (-0.213) was calculated for mercury, but this value is not statistically significant, indicating that the apparent correlation was likely due to chance and not indicative of a meaningful association between toxicity and contamination in Consolidated Slip.

For the organic constituents, only the summed concentration of fipronils had a significant negative correlation (Table 14). The negative correlation for chlordanes, although second highest in value (-0.116), was not statistically significant and not indicative of a meaningful relationship in Consolidated Slip.

None of the physical parameters had significant negative correlations (Table 15). Overall, the correlation analyses identified only variations in fipronil concentration as having a potentially meaningful association with sediment toxicity. The lack of significant correlations with other nonpolar organics, such as PAHs and pyrethroids, is likely due to the similarity of relative chemical concentrations (high at all stations) and toxicity (high at most stations) among stations; correlation methods are most effective at identifying probable causes of toxicity when a strong gradient of results is present.

Table 13. Spearman rank correlations of metal concentrations with amphipod survival from Stage II and III samples.

	Cd	Cu	Zn	Pb	Hg	Ni	As	Cr
r	0.577	0.941	0.638	0.698	-0.213	0.880	0.273	0.941
P Value	0.242	0.0167	0.175	0.136	0.658	0.0333	0.564	0.0167
N	6	6	6	6	6	6	6	6

Table 14. Spearman rank correlations of summed organic compound concentrations with amphipod survival from Stage II and III samples.

	ΣDDTs	ΣChlordane	ΣNonachlor	ΣPAHs	ΣPCBs	ΣPyreth.	ΣFipronils
r	-0.0491	-0.116	0.106	0.130	-0.0060	0.114	-0.587
P Value	0.831	0.621	0.649	0.581	0.977	0.626	0.0066
N	20	20	20	20	20	20	20

Table 15. Spearman rank correlations of ammonia, sulfide, and physical parameter concentrations with amphipod survival from Stage II and III samples.

	OW NH_3	PW NH_3	Sulfides	TOC	Fines
r	-0.268	-0.212	0.677	0.295	0.502
P Value	0.249	0.364	0.0290	0.203	0.0239
N	20	20	10	20	20

OW= Overlying water

PW= Pore water

Toxic Units

Calculations of toxic units for total pyrethroids, total fipronils and total PAHs indicated that pyrethroids likely accounted for most of the sediment toxicity in Consolidated Slip. Pyrethroid TUs were similar and above 1 for most stations, indicating that approximate 50% mortality of amphipods could be expected from this group of compounds (Tables 16 and 17). PAH TUs were also similar among all stations, ranging from 0.1 (Stage III station T-1) to 0.2 (Stage II station T-9). The PAH results indicate that this class of toxicants may be a contributor to the sediment toxicity observed in Consolidated Slip, but with a lesser influence than pyrethroids. The PAH TU calculations were based on acute toxicity thresholds derived from multiple species and so may not be an accurate predictor of the potential for acute toxicity to *Eohaustorius*. Additional confirmation studies are needed to determine the relative influence of PAHs of sediment toxicity in this study. Fipronil TUs ranged from 0.02 to 0.05 for all stations, indicating an insignificant role for this pesticide group in causing sediment toxicity in Consolidated Slip.

Correlation analysis based on TUs provided similar outcomes as analyses based on concentration: there was no significant correlation between toxicity and either pyrethroids or PAHs (Table 18). The fipronil TU values were significantly correlated with amphipod survival (Tables 18). The lack of correlation for pyrethroids and PAHs does not contradict the TU analyses described above; lack of a significant correlation was likely due to lack of a strong gradient in chemical concentrations as high contaminant levels were present throughout Consolidated slip.

Differences in the correlation and TU analyses illustrate the need to consider (and weight) multiple lines of evidence when trying to identify the cause of toxicity. Under ideal circumstances, the correlation and TU analyses would both show meaningful relationships with toxicity for the contaminant causing toxicity. In this study, the TU analyses for nonpolar organics (e.g., pyrethroids, fipronils, PAHs) are more reliable (and given more weight) than correlation analysis because concentration-based effect thresholds for amphipods are available and the influence of sediment characteristics on contaminant bioavailability is considered thorough TOC normalization. The lack of statistically significant correlations between pyrethroids or PAHs and toxicity is likely due to the relative similarity in toxicity response and chemical concentrations among most stations, relative to analytical variability. In such situations, it is difficult to statistically separate the toxicant response from measurement variability.

Table 16. Toxic units for selected chemicals from the Stage II sampling.

Station	ΣPyrethroids	ΣFipronils	ΣPAHs (ESB)	Total TUs
T-1	0.93	0.02	0.21	1.16
T-2	1.02	0.04	0.18	1.24
T-3	1.77	0.04	0.18	1.99
T-5	1.05	0.04	0.21	1.30
T-6	2.52	0.04	0.19	2.75
T-7	1.86	0.04	0.18	2.08
T-9	0.89	0.02	0.23	1.14
T-10	0.96	0.03	0.22	1.21
T-11	0.83	0.04	0.15	1.02
T-12	1.08	0.05	0.18	1.31

Table 17. Toxic units for selected chemicals from the Stage III sampling.

Station	ΣPyrethroids	ΣFipronils	ΣPAHs (ESB)	Total TUs
T-1	0.47	0.02	0.10	0.59
T-2	1.04	0.03	0.17	1.24
T-3	2.02	0.04	0.20	2.26
T-5	1.46	0.02	0.18	1.66
T-6	0.90	0.04	0.15	1.09
T-7	1.20	0.04	0.19	1.43
T-9	1.81	0.03	0.15	1.99
T-10	1.65	0.04	0.19	1.88
T-11	1.41	0.03	0.16	1.60
T-12	0.92	0.04	0.18	1.14

Table 18. Spearman correlations of amphipod survival with toxic units from Stage II and III samples.

	ΣPyrethroids	ΣFipronils	ΣPAHs
r	-0.063	-0.491	0.155
P Value	0.787	0.028	0.508
N	20	20	20

DISCUSSION

The multiple TIEs performed on sediment from Consolidated Slip were able to discern the chemicals likely causing toxicity for this site. Based on the effectiveness of the charcoal treatment, where 10 of 13 tests showed reduced toxicity, the cause of toxicity is likely to be nonpolar organic compounds (Table 19). More specifically, based on the increase in toxicity observed after the addition of PBO and the Toxic Unit calculations, pyrethroid pesticides are likely responsible for a substantial portion of the toxicity. Additionally, the ESB calculations for PAHs indicate that they are likely to account for some lesser portion of the toxicity.

Increasing use of the insecticide fipronil in urban and agricultural areas has heightened awareness of the potential contributions of this compound to sediment toxicity. There are currently no sediment TIE treatments specific for identifying fipronil (and its metabolites) as a cause of toxicity. However, the effectiveness of PBO as a synergist for Consolidated Slip toxicity, together with the low TUs associated with measured chemical concentrations, supports the conclusion that fipronil was not a likely cause of toxicity in this study. Studies on insects have demonstrated that PBO does not increase the toxicity of fipronil, as has been shown for pyrethroids (Khan et al, 2013), and marine amphipods are expected to react similarly to PBO addition. Additional studies are needed to increase the confidence in sediment effect thresholds for fipronil for marine benthic invertebrates and to provide more specific TIE tools.

The TIEs were also able to rule out some types of chemicals as a cause of toxicity. A consistent outcome of all the sediment TIE characterization tests was a lack of effectiveness of treatments designed to remove metals toxicity (Table 19). This, along with the low sensitivity of *E. estuarius* to metals (e.g., Cu LC50 > 1,000 mg/L; McPherson and Chapman 2000) and presence of plentiful metal-binding sulfides which would prevent metals from being bioavailable (Di Toro et al. 1992), indicates that metals were not a cause of the observed toxicity. While chlordanes, DDTs, and PCBs were at high concentrations relative to other southern California sites (Dodder et al. 2016), their concentrations were still orders of magnitude below where toxicity would be expected to occur (Appendix B, Murdoch et al. 1997, Greenstein et al. 2014).

While the weight of evidence from all TIE analyses indicated the cause of toxicity was the same among all stations and for both time periods, variation in the effectiveness of the TIE characterization treatments among stations was present. When only the characterization results were examined, without considering the chemical analysis results, three patterns were evident. Results for most stations (7 of 13) indicated that nonpolar organic contaminants, including pyrethroids, were the likely cause of toxicity. Two variations from this pattern were observed; the treatment to indicate pyrethroid toxicity did not meet effectiveness criteria for three stations and the treatment indicative of nonpolar organics was not classified as effective for three other stations. These variations in characterization pattern were primarily due to the lack of statistically significant changes in toxicity for some treatments. Each sediment sample had the same qualitative characterization pattern: less toxicity when bioavailability of nonpolar organics was reduced, exposure to pyrethroids present, and no toxicity associated with metals. The outcome of the toxic unit analyses for each sample was also the same (pyrethroids dominant cause of toxicity).

While toxicity from pyrethroids can be inferred by the PBO treatment, the PBO treatment alone cannot demonstrate that pyrethroids are the cause of toxicity. A concurrent reduction in toxicity following charcoal or carboxylesterase addition is also needed to demonstrate that pyrethroids

are a likely cause of toxicity. It is common to observe the PBO treatment reducing survival even when there is little or no toxicity in the untreated (baseline) sample (Greenstein et al. 2014). This is an indication of pyrethroids being present and bioavailable, but at a low enough concentration that the animals can detoxify them and prevent mortality. The addition of PBO inhibits the animal's ability to detoxify pyrethroids, thus reducing survival (Amweg and Weston 2007). Therefore, the 13 stations evaluated in this way can be lumped into two broader categories based on the TIE characterization treatment results: 10 samples for which nonpolar organic compounds were identified as the likely cause of toxicity and three stations (Station 7 in Stage II, and Stations 11 and 12 in Stage 3) for which no firm toxicity cause could be shown (only PBO treatment effective). Carboxylesterase addition was not effective in this study; the reason for its lack of effectiveness is not known.

Table 19. Number of stations where a sediment constituent was indicated by a TIE treatment or chemical analysis as causing toxicity versus the number of stations tested.

Constituent	Stage II	Stage III
Organics (charcoal treatment)	2/3	8/10
Metals (SIR-300)	0/3	0/10
Metals+organics (SIR-300+charcoal)	2/3	NA
Pyrethroids (Carboxylesterase)	0/3	NA
Pyrethroids (PBO)	2/3	8/10
Ammonia (Zeolite)	0/3	NA
Ammonia (PW measurement)	0/3	0/10
Sulfides (PW measurement)	NA	0/10

NA=Not analyzed

This pattern of some stations within a site having a cause identified and others not is not unusual (Anderson et al. 2007, Greenstein et al. 2014). The present study has demonstrated that effectiveness and precision of the TIE characterization treatments is variable, resulting in an inability to demonstrate a significant effect for some samples, even though chemical composition was similar. As long as there are enough stations within a site where a cause of toxicity is found and other sites do not identify a conflicting cause, then the weight of evidence can determine a cause for the site as a whole. When there are conflicting causes identified by the characterization treatments (e.g. some stations indicate organics and others indicate metals) it may be an indication of multiple sources of toxic chemicals. Availability of sediment chemistry data for each sample was essential in determining the cause of toxicity in Consolidated Slip.

The variation in TIE characterization results observed in this study can be used to estimate the probability of different study designs to yield unambiguous results, and support recommendations on the number of samples needed to have confidence in the TIE characterization results. For this study, 10 of 13 samples had a successful characterization of the cause of toxicity and this was used as the basis for the statistical analysis. Two sets (scenarios) of probabilities were calculated. In the first set, the probability of being able to characterize the cause of toxicity in a majority of the samples (defined as more than half of the stations) was calculated. For the second set, the probability of obtaining a successful characterization result for at least one station was calculated. For both sets, a successful characterization for Consolidated Slip was defined as indicating nonpolar organics to be the cause of toxicity. Results for both scenarios (described below) indicate that conducting TIE characterizations on at least three

stations will lead to a high chance of success and analysis of more stations will increase both the probability of success and level of confidence in the outcome. This recommendation is based on a typical study site size of several square kilometers or less. Much larger sites, or those with high spatial variability in toxicant sources, may require a larger number of TIE stations to provide assurance that the TIE results are representative of the entire site.

For the majority of samples scenario, TIE characterization of three samples resulted in an 87% probability of a successful outcome (Table 20); this probability increased to 95% when seven samples were analyzed. For the TIE characterization scenario where only one successful outcome is required, analysis of two samples yields a 95% probability of success and analysis of five samples results in nearly a 100% chance of success. It should be noted however, that the single outcome scenario may not result in a confident result in some situations. For example, if six samples are analyzed, but a successful characterization is obtained for only one sample, then the high frequency of inconclusive results will not generate much confidence that the characterization results are representative of the entire site.

For the two timepoints sampled, the overall conclusion as to the cause of toxicity at Consolidated Slip was the same. However, the TIE characterization pattern for individual stations varied slightly between events. For stations T-5 and T-9 the same characterization pattern was observed in Stages II and III. However, the characterization pattern for T-7 varied between timepoints; the PBO treatment was effective and charcoal treatment not effective in Stage II, whereas PBO was not effective but charcoal was effective in Stage III. This difference is relatively minor and does not affect the overall conclusions. Therefore, in this instance, sampling at multiple timepoints was probably unnecessary. However, if a site is known to have seasonal fluctuations in the type of toxicant inputs, then a temporal component to the study design may be warranted. In general, if resources are limited, it is more important to have greater spatial coverage than temporal information. Seasonal variations in the magnitude of toxicity may be present, such as that caused by pesticides in urban or agricultural runoff. Such variation may influence the feasibility of conducting a TIE by limiting the ability to identify effective treatments, but seasonal variation should not alter the conclusion regarding the cause of toxicity, unless the seasonal toxicity is masking substantial toxicity caused by other types of sediment toxicants present year-round at the site.

The present study found the pore water analyses to be of limited value for toxicant characterization. The efficacy of performing toxicity tests on pore water has been the subject of some debate (Chapman et al. 2002, Carr and Nipper 2003). For the Stage II sampling where TIEs were conducted on both whole sediment and pore water, the only treatment that agreed well between the two matrices was PBO, which reduced survival to some degree at all three stations. The pattern for T-9 pore water generally supported the conclusions for sediment with C-18, Cation Exchange plus C-18, PBO, and CEE treatments all indicating organic chemicals, and specifically pyrethroids, as the cause of toxicity. However, the cation exchange, EDTA, and STS treatments provided inconsistent and contradictory results. Difficulty in obtaining sufficient pore water for chemical analysis of organics further reduces the confidence in making decisions based on the characterization results. Since the act of collecting pore water changes its chemical nature (Chapman et al. 2002), the results should be viewed in context with supporting data to draw a conclusion. While pore water testing may be helpful in supporting whole sediment TIEs, it should not be conducted in the absence of whole sediment testing in an effort to reduce costs or

analysis time. Improved methods for pore water collection and testing, such as in situ test chambers, are needed to improve the reliability of TIEs with this matrix.

Table 20. Probability of successfully determining a cause of toxicity at a site depending on the number of stations on which TIEs are conducted.

No. of Samples	Probability of Drawing a Successful Conclusion a Majority of the Time	Probability of Drawing a Successful Conclusion One or More Times
1	0.769	0.769
2	0.591[a]	0.947
3	0.866	0.988
4	0.772	0.997
5	0.916	0.999
6	0.861	1.000
7	0.946	1.000
8	0.912	1.000
9	0.965	1.000
10	0.943	1.000

[a]Reduction in probability for even numbers of samples in this scenario is due to requirement that >50% of samples have a successful outcome. Meeting this rule requires proportionally more successful samples for scenarios for odd-numbered samples than for even-numbered ones.

CONCLUSIONS

- **TIEs were able to determine the likely cause of toxicity in Consolidated Slip**. The whole sediment testing identified pyrethroid pesticides, and to a lesser extent PAHs, as the most likely cause of toxicity. Metals, chlordanes, DDTs, and PCBs were ruled out as likely being responsible for toxicity.
- **Variability in TIE characterization results was commonly observed.** Even though the pattern of which TIE treatments were effective varied among Consolidated Slip stations, a weight of evidence approach was able to determine the cause of toxicity with confidence. Although three out of the 13 stations tested produced an uncertain characterization outcome, the remaining stations supported the overall conclusion and none of the sediment analyses suggested a contradictory toxicity cause.
- **Concurrent chemical analysis greatly increased confidence in TIE interpretation.** Measurement of chemical concentration and comparison to toxicity thresholds was essential for resolving uncertainty resulting from variable effectiveness of the characterization treatments.
- **There was little benefit to including a temporal component to the study.** Analysis of multiple sampling events did not provide additional information regarding the cause of toxicity in Consolidated Slip. While the patterns of TIE results for individual stations were not identical between timepoints, the overall conclusion remained the same. Both magnitude of toxicity and chemical concentrations were similar between timepoints. However, a temporal component could be beneficial in a system with seasonal variation in stressor type.
- **Pore water analysis was of limited value.** The pore water TIE results were inconsistent and contradictory in some cases. The potential introduction of artifacts due to handling methods and lack of chemical analysis data for results verification resulted in low confidence in interpretation of the pore water TIE results.

RECOMMENDATIONS

The results from this study, combined with experience from other investigations suggest several design principles that are likely to improve the success of sediment TIEs for stressor identification and increase confidence in results interpretation. These recommendations are illustrated in stepwise fashion in Figure 15 and described below.

1. **Identify site.** Prior assessment data, in addition to information about likely toxicant sources and seasonal inputs, should be consulted to identify the location and boundaries of the study site.
2. **Determine sampling station locations.** Station locations should be representative of site conditions. Analysis of more stations will increase both the probability of success and confidence in the interpretation. Spatially larger sites should have more TIE stations than smaller areas. It is usually not necessary to conduct TIEs at more than one timepoint. Neither the current study nor others in the literature provide examples where the cause of toxicity varies over a seasonal time scale. However, if a site in question has a known source of inputs that changes seasonally, then a temporal aspect to the study design may be warranted.
3. **Perform toxicity screening tests.** Preliminary screening tests should be conducted to verify that a sufficient magnitude of toxicity is present before investing substantial resources in TIE analyses.
4. **Select TIE stations.** Multiple stations should be evaluated. Results from this study suggest a minimum of three stations should be analyzed to increase the likelihood of successfully characterizing the cause of toxicity. Stations with at least 30% toxic effect are recommended for TIE analyses. A higher level of toxicity increases the likelihood that TIE characterization will be successful. A larger number of stations may need to be evaluated for sites having a lesser magnitude of toxicity or greater spatial variability.
5. **Give priority to whole sediment TIE characterization analyses.** TIE analyses conducted on whole sediment are generally more reliable and consistent, compared to pore water tests. For most situations, pore water TIE testing should only be conducted as a complement to whole sediment tests, not as a replacement. The initial TIE treatments or analyses performed should, at a minimum, include those that will reduce or identify toxicity from nonpolar organic compounds, metals, and ammonia. It is generally not recommended to perform initial TIEs focused on one class of compounds (e.g., metals), even when a cause is assumed *a priori*. It is often more cost effective and less time consuming to include other more focused treatments, such as PBO, and carboxylesterase during initial TIE testing rather than making it an iterative process.
6. **Conduct concurrent chemical analysis.** The sediment samples used for TIE characterization should also be analyzed for chemical composition. Comparison of concentrations to toxicity thresholds is one of the most effective steps for identifying the cause of toxicity beyond the general classes of nonpolar organics or metals. These data are also essential for resolving inconsistencies in characterization treatment effectiveness between samples.

7. **Stressor identification should be based on a weight of evidence.** Both TIE characterization and analytical chemistry results are needed to provide a confident identification of the cause of sediment toxicity. Both of these lines of evidence have advantages and limitations that result in a greater chance of TIE success when used together.

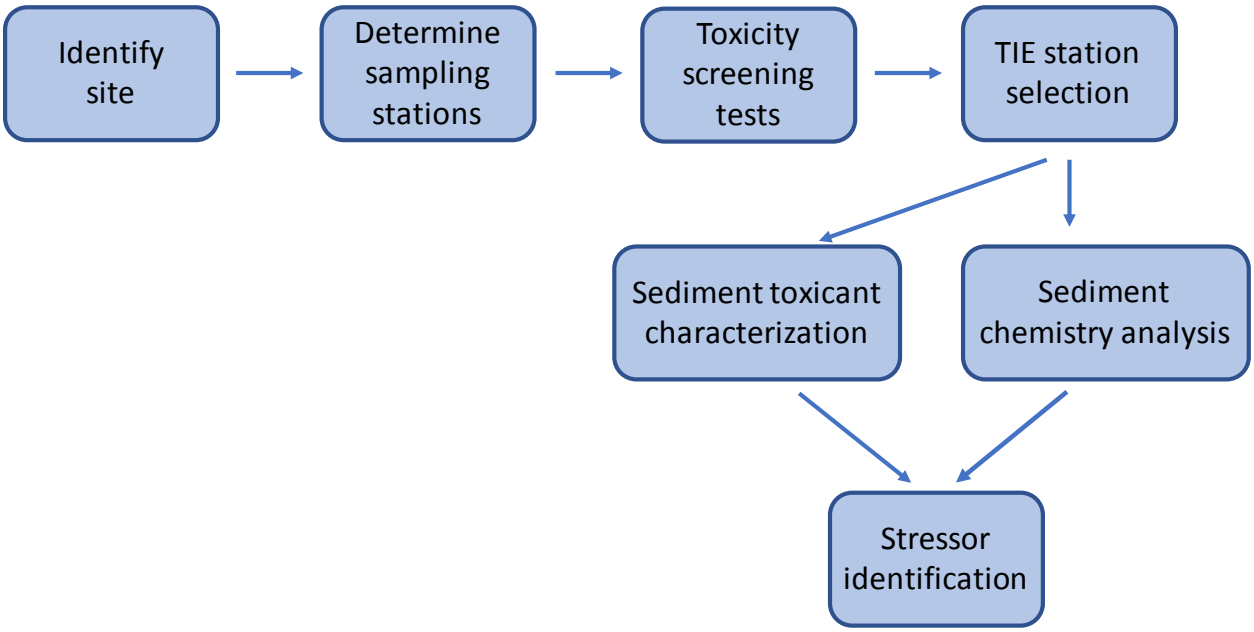

Figure 15. Recommended stressor identification study elements.

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APPENDIX A – REVIEW OF SEDIMENT TOXICITY IDENTIFICATION EVALUATION GUIDANCE AND VARIABILITY

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INTRODUCTION

Toxicity identification evaluations (TIEs) have been used for the past few decades to determine the chemical cause of toxicity in environmental samples (USEPA 1991). The foundation of conducting a TIE is to chemically or physically treat the samples so as to change the toxicity in a manner indicative of the presence of broad classes of chemicals (e.g. non-ionic organic chemicals). These manipulations are Phase I (Characterization) of a three-phase process to determine the cause of toxicity. In Phase II (Identification), targeted manipulations are made to narrow down the cause of toxicity to a specific class (e.g. pyrethroid pesticides) or even individual compounds (USEPA 1993a). In Phase III (Verification), methods such as statistical analysis and spiking of samples are used to verify the conclusions from the first two phases (USEPA 1993b).

The original TIE methodologies were designed for freshwater, aqueous samples and wastewater effluent. Methods for testing marine aqueous samples soon evolved (USEPA 1996). Finally, methods were developed for use in sediments (USEPA 2007). Due to the difference in the matrices between water and sediment, there are fewer TIE treatments available for sediment. However, the aqueous methodologies can be brought to bear on pore water extracted from whole sediment. Using pore water does have some unique challenges as its characteristics begin to change once it is removed from the sediment (Chapman et al. 2002, Carr and Nipper 2003).

The use of TIEs is recommended by the California Water Quality Control Plan (Plan) as part of the Sediment Quality Objectives (SQO) Program (SWRCB 2009). When sediments in a water body fail the SQO a stressor identification is required, the main component of which is a TIE. However, the Plan gives little guidance on the design components for conducting the TIE. As with any laboratory or field study, the quality of the results and confidence in conclusions can be greatly affected by the design. There are many design elements to consider when planning a TIE, which include: the test organism, which of the three phases are included, number of stations, time points, the degree of replication, and the methods of data analysis and interpretation. While each of these elements is important to the success of the study, many are not addressed in the primary guidance documents cited previously. Decisions regarding TIE study design are made on a project-specific basis, increasing the burden on regulatory agencies to evaluate the design and increasing the potential for reduced data comparability.

In an effort to provide guidance on TIE study design, a literature review was conducted focusing on three main goals. The first goal was to summarize what guidance is already available either in the scientific literature or from government agency documents. The search included guidance on design and interpretation, such as the number of stations, degree of replication, specific TIE treatment methods, and test matrix (i.e. pore water versus whole sediment). The second goal was to synthesize existing information regarding the spatial and temporal variability in TIE results. The synthesis results were used determine what study design elements most affect variability and how to best minimize it. The third goal was to identify important gaps in the knowledge of optimal TIE design. This information was used to identify future studies to fill in these gaps.

METHODS

The review included reports, manuals, and peer reviewed literature having to do with whole sediment and or pore water TIEs conducted in marine or freshwaters. The review did not include documents that focused on water column TIEs such as those on samples of effluent, rivers, lakes, and storm water. The review only included organism-based testing, not effects directed bioassays conducted using cell assays.

The literature search began with review of the USEPA guidance documents for conducting TIEs. These were used both as a direct source of information and their bibliographies were used as sources for other foundational literature. A search was conducted through the Web of Science online bibliography tool. Keyword phrases were used to search journal article titles, abstracts, and keywords. Articles from the search result were screened online for relevance. Those articles that appeared to contain relevant information were obtained either in electronic or hard copy form for further review. The bibliographies from each document were used to further expand the search.

A set of questions was developed to aid in the review. These questions were grouped under three broad categories:

- Study Design Guidance
    1. Are both whole sediment and pore water testing required?
    2. How many stations are needed to characterize the site (i.e. determine spatial variability)?
    3. How many results are needed to reach a conclusion?
    4. How should temporal variability be addressed (i.e. how many times does the site need to be sampled)?
    5. What phases of the TIE are required to reach a conclusion (are all three needed)?

- Characteristics of Results
    1. How many sediment samples were evaluated (different stations and different times)?
    2. Was presence of toxicity consistent within a given station?
    3. What stressors were identified in the study?
    4. Were the same stressors identified in each sample tested (different stations and different times)?
    5. Were both whole sediment and pore water investigated?
    6. Did whole sediment and pore water results agree for each sample?

- Data Interpretation
    1. What types of statistics were used to determine significance of the results?
    2. What approaches were used to communicate the results?

All possible questions were answered for each of the documents. An overall summary of the results from the documents was then developed based on the list of questions. This summary is the basis for the results presented below.

RESULTS

The literature search was successful in finding over 50 documents, each of which provided information related to a least one of the study questions. The results of the review are presented in four broad categories: 1) recommendations and guidance, including study design suggestions. The remaining three categories are based on how the studies in the literature were conducted: 2) design elements such as number of stations or samples, matrix considerations, and TIE phases used; 3) variability observed in the TIE results and conclusions; 4) means of data analysis and communication of results used in the studies.

Recommendations and Guidance

Due to the expense associated with conducting TIE testing, one of the most important factors in study design is the number of samples to test and their distribution is space and time. The primary guidance documents for TIEs, both aqueous and sediment, are largely silent on the issues of study design with regards to elements such as number of stations to screen, number of TIEs to perform, and how much information is needed to make a confident decision regarding the cause of toxicity (USEPA 1991, 1993a, 1993b, 1996, 2007). The sediment TIE document does suggest that a focus on spatial coverage of samples is usually more productive than a temporal focus, since bedded sediments do not change rapidly unless they are in a particularly dynamic environment, such as a river mouth (USEPA 2007). It is also recommended that reanalysis of sediment for initial toxicity be conducted if previous sampling has occurred greater than three years prior or if the site is particularly dynamic, before proceeding with TIEs (SAIC 2003).

One design element that does have some recommendations is the use of whole sediment versus pore water. The USEPA recommends performing TIEs on whichever matrix shows toxicity in initial screening, but also suggests that doing both matrices is preferred (USEPA 2007). Care must be taken when relating results from pore water back to the original sediments. Pore water is often extracted from anoxic sediment and then is in contact with air during the TIE testing resulting in a change of redox potential which may affect the bioavailability of contaminants relative to the whole sediment (Anderson et al. 2007, USEPA 2007). Other confounding factors that have been commonly found in pore water are ammonia, sulfide, pH, and dissolved oxygen (Carr and Nipper 2003). Finally, testing pore water eliminates the sediment particle ingestion exposure route that may be important to some organisms (Ho and Burgess 2009). For these reasons, it is recommended that pore water not be the only matrix tested in most sediment TIE studies.

There is some debate on the necessity of using all three TIE phases in all study designs. The USEPA guidance suggests that important information may be missed and spurious conclusions reached if all three phases are not employed (USEPA 2007). Other researchers have found success with a more focused approach with fewer treatments being a more cost-effective approach when much is already known about the study location (Weston et al. 2008, Weston and

Lydy 2010). In contrast, in an Australian study, a complete TIE led to the discovery of previously unknown sources of toxicity that were more easily remediated than upgrades to waste water treatment facilities that had previously been assumed to be the source of toxicity (Kellar et al. 2014). Using all three TIE phases is recommended in most situations, but they need not be conducted in a linear fashion (Ankley et al. 2011); elements of multiple phases can be done simultaneously.

There was no guidance in any of the documents regarding the amount of data needed to make a confident judgement as to the cause of toxicity. The literature contained studies with as little as one sample to tens of samples where a conclusion was drawn. No level of certainty in the conclusion was expressed based on the number of samples tested. A weight of evidence approach was often suggested which was not so much based on the number of samples tested as on the supporting evidence, such as chemistry, statistics, or proximity to a known source of contaminants (Hunt et al. 2001, Ho et al. 2012, Kellar et al. 2014).

Design Elements

As stated earlier, the number of stations where TIEs are conducted during a study is an important cost consideration and guidance on how many should be tested is limited. Information on the number of stations tested fell into two categories. The first category is the number of samples tested initially to determine if toxicity is present. The second is how many of these samples then have a TIE conducted on them. The number of stations tested varied widely, both in space and time. Many of the studies started out by testing multiple spatially distributed samples for initial toxicity, but then in most cases TIEs were only conducted on a few of these (Table A.1). Just under a quarter of the studies tested only one station initially, with about a third of studies testing more than ten. Some of the studies that used only one sample for both initial testing and TIEs did so due to the narrow focus of the project (Ho et al. 1997, Phillips et al. 2003). Some studies using few stations for testing indicated that earlier, either unpublished or previously published, work had been used to determine which stations to test (Burgess et al. 1993, Weston et al. 2008, Anderson et al. 2010, Phillips et al. 2010).

The number of samples on which TIEs were conducted was usually less than the number initially tested (Table A.1). While that reduction in numbers was often associated with lack of substantial toxicity in the initial samples, there were also cases where no reason was given for the reduction (i.e. samples found to be toxic did not have TIEs performed). More than 40% of the studies had TIEs performed on only one sample. While it was not often clearly stated, the implication for why few TIEs were conducted seemed to be cost. The study having the greatest number of TIEs performed did only a limited number of Phase I treatments (Poleza et al. 2014).

The distribution of TIE samples over multiple time points was fairly uncommon. Only 25% of the studies encountered in the literature included more than one time point. There were nine studies where TIEs were performed over two to four time points (Schubauer-Berigan and Ankley 1991, Sparks and Ross 1992, Boucher and Watzin 1999, Ho et al. 2002, Thomas et al. 2003, Kwok et al. 2005, Araujo et al. 2006, Weston et al. 2008, Greenstein et al. 2014). Another study conducted limited Phase I testing on six time points (Poleza et al. 2014).

Table A.1. Number of studies testing multiple samples for initial toxicity and TIEs.

	Number of Stations			
Analysis type	1	2-5	6-10	>10
Initial Toxicity	9[a]	8[b]	9[c]	12[d]
TIE	19[e]	18[f]	6[g]	2[h]

[a] (Schubauer-Berigan and Ankley 1991, Wenholz and Crunkilton 1995, Ho et al. 1997, Boucher and Watzin 1999, Araujo et al. 2006, Phillips et al. 2006, Ho et al. 2009, Anderson et al. 2010, Biales et al. 2013)

[b] (Burgess et al. 1993, Van Sprang et al. 1996, Van Sprang and Janssen 1997, Phillips et al. 2004, Hunt et al. 2008, Kay et al. 2008, Zhang et al. 2012, Matos et al. 2014)

[c] (Karuppiah and Gupta 1996, Anderson et al. 2006, Anderson et al. 2007, Bosch et al. 2009, Perron et al. 2010, Greenstein et al. 2014, Kellar et al. 2014, Yi et al. 2015, Campos et al. 2016)

[d] (Sparks and Ross 1992, Gupta and Karuppiah 1996a, 1996b, Carr et al. 2001a, Carr et al. 2001b, Hunt et al. 2001, Thomas et al. 2003, Weston et al. 2008, Mehler et al. 2010, Burgess et al. 2011, Poleza et al. 2014, Ke et al. 2015)

[e] (Schubauer-Berigan and Ankley 1991, Wenholz and Crunkilton 1995, Gupta and Karuppiah 1996a, 1996b, Ho et al. 1997, Boucher and Watzin 1999, Carr et al. 2001a, Phillips et al. 2003, Phillips et al. 2004, Kwok et al. 2005, Araujo et al. 2006, Phillips et al. 2006, Weston et al. 2008, Ho et al. 2009, Anderson et al. 2010, Perron et al. 2010, Biales et al. 2013, Matos et al. 2014, Campos et al. 2016)

[f] (Burgess et al. 1993, Karuppiah and Gupta 1996, Van Sprang et al. 1996, Van Sprang and Janssen 1997, Carr et al. 2001a, Hunt et al. 2001, Stronkhorst et al. 2003, Anderson et al. 2006, Anderson et al. 2008, Hunt et al. 2008, Kay et al. 2008, Bosch et al. 2009, Picone et al. 2009, Phillips et al. 2010, Ho et al. 2012, Zhang et al. 2012, Greenstein et al. 2014, Yi et al. 2015)

[g] (Sparks and Ross 1992, Anderson et al. 2007, Mehler et al. 2010, Burgess et al. 2011, Kellar et al. 2014, Ke et al. 2015)

[h] (Thomas et al. 2003, Poleza et al. 2014)

The matrix tested varied considerably between studies, with over half focusing on pore water only and the rest about evenly divided between whole sediment only and studies where both matrices were used (Table A.2). The older studies tended to be more likely to focus only on pore water, which makes sense because whole sediment TIE techniques were not widely available until a later date (USEPA 2007). An additional group of studies included sediment elutriate as a matrix, with two being elutriate only (Bosch et al. 2009, Poleza et al. 2014), one both elutriate and whole sediment (Burgess et al. 2011), and another a combination of elutriate and sediment-water interface sampling (Phillips et al. 2003). Two other studies tested both pore water and whole sediment initially, but did TIEs only on the pore water (Phillips et al. 2006, Kay et al. 2008).

Table A.2. Number of studies conducting TIEs on a specific matrix.

Matrix	Number of Studies
Pore water	20[a]
Whole Sediment	9[b]
Both	8[c]

[a] (Schubauer-Berigan and Ankley 1991, Sparks and Ross 1992, Wenholz and Crunkilton 1995, Gupta and Karuppiah 1996a, 1996b, Karuppiah and Gupta 1996, Van Sprang et al. 1996, Ho et al. 1997, Van Sprang and Janssen 1997, Boucher and Watzin 1999, Carr et al. 2001a, Carr et al. 2001b, Hunt et al. 2001, Stronkhorst et al. 2003, Thomas et al. 2003, Phillips et al. 2004, Kwok et al. 2005, Picone et al. 2009, Matos et al. 2014, Ke et al. 2015)

[b] (Stronkhorst et al. 2003, Weston et al. 2008, Ho et al. 2009, Mehler et al. 2010, Zhang et al. 2012, Biales et al. 2013, Kellar et al. 2014, Yi et al. 2015, Campos et al. 2016)

[c] (Burgess et al. 1993, Anderson et al. 2006, Anderson et al. 2007, Anderson et al. 2008, Hunt et al. 2008, Anderson et al. 2010, Phillips et al. 2010, Greenstein et al. 2014)

Another aspect of TIEs that was found to be highly variable in the literature was which TIE phases were employed. When classifying these results, two rules were applied: 1) to get credit for having performed a Phase I TIE, treatments had to be employed that were specific for both metals and organic compounds; 2) the use of any methodology that would be considered Phase II or Phase III would garner credit for those Phases (i.e. there were no minimum requirements for number of procedure conducted). In many of the studies, the authors did not specify which phases were included and the classification was based on their descriptions of methodologies. Most studies did more than just Phase I treatments (Table A.3). About half of the remaining studies used Phase I and II, with slightly less including Phase III. The remaining studies ("other" category) did either only partial Phase I (Poleza et al. 2014), partial Phase I and then elements of Phase II (Phillips et al. 2004, Weston et al. 2008), or a sequential Phase I (samples were subjected to one treatment, tested for toxicity, and then another treatment performed on the sample where the first treatment had been applied) followed by Phase II (Picone et al. 2009).

Table A.3. TIE phases employed by studies in the literature review.

TIE Phase(s)	Number of Studies
Full Phase I	3[a]
Phase I and II	20[b]
Phase I, II, and III	16[c]
Other	4[d]

[a] (Van Sprang et al. 1996, Stronkhorst et al. 2003, Biales et al. 2013)

[b] (Burgess et al. 1993, Wenholz and Crunkilton 1995, Gupta and Karuppiah 1996a, 1996b, Karuppiah and Gupta 1996, Carr et al. 2001a, Hunt et al. 2001, Thomas et al. 2003, Anderson et al. 2006, Araujo et al. 2006, Hunt et al. 2008, Kay et al. 2008, Mehler et al. 2010, Perron et al. 2010, Phillips et al. 2010, Zhang et al. 2012, Kellar et al. 2014, Matos et al. 2014, Ke et al. 2015, Yi et al. 2015, Campos et al. 2016)

[c] (Schubauer-Berigan and Ankley 1991, Sparks and Ross 1992, Ho et al. 1997, Van Sprang and Janssen 1997, Boucher and Watzin 1999, Carr et al. 2001b, Phillips et al. 2003, Kwok et al. 2005, Phillips et al. 2006, Anderson et al. 2007, Anderson et al. 2008, Bosch et al. 2009, Ho et al. 2009, Anderson et al. 2010, Burgess et al. 2011, Greenstein et al. 2014)

[d] (Phillips et al. 2004, Weston et al. 2008, Picone et al. 2009, Poleza et al. 2014)

Variability of TIE Results

The variability associated with TIE results was not discussed in the literature in quantifiable terms. No articles were found in which variability was the focus or in which the uncertainty of results were discussed or quantified. For studies where multiple samples were tested, either in space or time, similarities or differences in results were rarely compared statistically. When statistical results were presented, it was generally for initial toxicity and not for TIE results. In one study where multiple TIEs were performed both temporally and spatially, the results were expressed as the number of samples where each toxicant type was found to be the cause of toxicity (Poleza et al. 2014). When the study obtained different results between samples, the general approach was to attempt to explain the reasons why each conclusion might be correct, rather than to quantify the variability.

Of the studies where multiple samples were taken over time, toxicity was usually consistent throughout the study period. However, a few studies reported inconsistent toxicity between sampling events (Greenstein et al. 2014, Poleza et al. 2014, Campos et al. 2016). These tended to have taken place in dynamic environments, such as river or creek mouths where inconsistent results might be expected.

Another type of variability evaluated was consistency in the identification of the stressor among samples from different stations. For more than half of the investigations reviewed, the identification of the stressor responsible for toxicity was the same among multiple samples. Instances of a lack of agreement in results usually occurred when the study included large geographic areas or stations located in very different environments. For example, Anderson et al. (2007) tested samples from diverse parts of the country with the intention of identifying different types of stressors.

Consistency between TIE results for whole sediment and pore water for the studies was not always observed. About half of the studies found the same conclusion between the matrices (Burgess et al. 1993, Anderson et al. 2007, Hunt et al. 2008, Greenstein et al. 2014). In the case of Burgess, the agreement was that a chemical cause of toxicity could not be identified in either matrix. For some of the studies where there was not agreement, the whole sediment was found to be toxic and the pore water was not (Phillips et al. 2006, Anderson et al. 2010).

Few studies contained information regarding changes in the level of toxicity between initial testing of a sample and the TIE (Mehler et al. 2010, Perron et al. 2010). In both of these cases, a decrease in toxicity was observed between the initial sample and the TIE baseline. In a recent TIE at SCCWRP, a sample was found to have an increase in toxicity (SCCWRP, unpublished data).

In their review of the causes of sediment toxicity, Ho and Burgess (2013) found that either organics, metals, or ammonia alone was the source in nearly two-thirds of the cases for either whole sediment or pore water. Toxicity in most of the studies was caused by organics. The remaining causes were mostly combinations of the aforementioned classes of compounds, along with a few that involved sulfides or chlorine. The relative importance of organics vs. metals as a cause of toxicity varied for pore water and sediment. For pore water, 29, 19, and 6% of the samples were identified as toxic due to organics, metals, and ammonia, respectively. Toxicity in 25% of samples was due to a combination of organics and either metals or ammonia. For whole sediment, 70% of the samples were identified as toxic from organics and just 3% from metals,

with the rest being combinations. These results reinforce the recommendation from EPA 2007 that both matrices should be tested to achieve the most confident conclusion.

Data Analysis and Communication

The aspect of TIE literature that was fairly consistent was the way in which the Phase I results were analyzed and presented. Most of the data were analyzed with simple t-tests or ANOVAs, both to compare sample results to those of controls or reference samples, and to compare TIE treatments to the baseline samples. Several studies did toxicity testing on dilutions and then calculated the LC50 by probit or Spearman-Karber analysis. The LC50 data were then used to calculate toxic units (TUs) as part of Phase II, identification of toxicants. Literature LC50 values for individual compounds were then used to calculate TUs for comparison. Two investigations included more advanced statistics in Phase II, such as principal components analysis (PCA) to identify associations between chemicals and toxicity (Hunt et al. 2001, Campos et al. 2016).

Similar methods were used to present the TIE results. Data were most commonly presented as either tables of effects, bar graphs that compared results to either controls or baseline samples. The tables and graphs were often annotated to identify statistical differences. Other data presentation methods included stacked bar graphs of the TUs of individual chemicals, maps depicting areal extent or cause of toxicity, box and whisker plots, or scatterplots of toxicity results versus chemical concentration.

CONCLUSIONS AND RECOMMENDATIONS

The literature review found that while there is a great deal of guidance on how to conduct a TIE with regards to the laboratory methods, there is very little on how to design the study. Guidance is lacking for the number of samples needed to have confidence in a conclusion, and whether the samples should be distributed in space or time. No recommendations were available for determining at what point enough analysis has been done to reach a confident conclusion or how to quantify the level of confidence achieved. This lack of guidance is reflected in the TIEs that have been detailed in the literature. There is no consistent number of samples tested and no attempt at expressing or quantifying the level of confidence. Though there is a fairly strong recommendation from the EPA do all three TIE phases, this is often not followed.

Based on the results of the literature review, three recommendations are made for a field study to gain information regarding the variability of TIE results and support development of study design guidance:

- Measure small scale variability of toxicity and TIE results. Sample multiple stations over a fairly small area (hundreds of meters) to determine the variability in toxicity results among stations. These data can be used for power analyses to determine the number of stations needed to characterize the toxicity level. TIEs should be performed on any toxic samples to determine the consistency of the conclusions.
- Determine the level of effort within a TIE needed to get a confident result. For the TIE variability study described above, a high level of effort would be used (i.e. full application of all three TIE phases). The impact on the TIE conclusions from selectively

removing results for specific treatments would be compared to determine which TIE treatments were most important for reaching an accurate conclusion.

- Describe confidence levels expected for toxicity and TIE results. Use randomized station selection in the variability study described above to obtain toxicity and TIE results that are representative of the site characteristics. Statistical analysis of the results can be used to determine confidence intervals for the results.

Proposed Field Study

A study to implement the recommendations stated above should be conducted in an area with one primary source of toxicants and with a size of several hundred square meters. An area known to have multiple types of toxicants would be ideal so that there was a potential of obtaining different outcomes from the TIE analyses. Approximately 10 stations would be placed randomly within the site so that each station represented only a few hundred square meters. Sediment samples from each station would be screened for toxicity using *Eohaustorius estuarius* 10-day survival tests on whole sediment and pore water. Chemical analysis and TIEs would be conducted on any stations determined to be toxic in the initial testing. Full Phase I, II, and III treatments would be conducted.

Data analyses to answer the primary study questions would include calculation of area weighted survival with 95% confidence intervals. Similar analyses would be done on the results from each of the TIE treatments to determine the variability in outcomes. Finally, the predictive accuracy of TIE conclusions based on use of partial methods will be compared to determine which treatments have the greatest influence on the confidence in the study results.

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APPENDIX B – TOXICITY RESULTS SUMMARY

Table B.1. Survival results of Stage I testing of Los Angeles/Long Beach Harbor samples using the 10-day whole sediment protocol with *Eohaustorius estuarius.*

Sample	Mean	Standard Deviation	Number of Replicates
Home Sediment	100	0.0	5
CH2-1	84	20.7	5
CH2-2	84	11.4	5
CH2-3	82	13.0	5
CH2-4	96	5.5	5
CH2-5	86	11.4	5
CS-2	8	13.0	5
CS-3	18	14.8	5
CS-4	12	4.5	5
CS-5	8	8.4	5
CS-6	6	8.9	5
SPB-1	82	13.0	5
SPB-2	78	16.4	5

Table B.2. Survival results of Stage II initial testing of Consolidated Slip samples using the 10-day whole sediment protocol with *Eohaustorius estuarius.*

Sample	Mean	Standard Deviation	Number of Replicates
Home Sediment	100	0.0	5
CS-T-1	84	5.5	5
CS-T-2	44	11.4	5
CS-T-3	36	15.2	5
CS-T-5	34	11.4	5
CS-T-6	48	21.7	5
CS-T-7	40	12.2	5
CS-T-9	56	15.2	5
CS-T-10	46	11.4	5
CS-T-11	36	5.5	5
CS-T-12	40	12.2	5

Table B.3. Survival results of Stage II TIE testing of Consolidated Slip samples using the 10-day whole sediment protocol with *Eohaustorius estuarius.*

Sample	Mean	Standard Deviation	Number of Replicates
Home Sediment	100	0.0	5
CS-T-5 Baseline	38	14.8	5
CS-T-7 Baseline	36	16.7	5
CS-T-9 Baseline	32	29.5	5
CS-T-5 Dilution control	22	8.4	5
CS-T-7 Dilution control	36	18.2	5
CS-T-9 Dilution control	40	18.7	5
Carboxylesterase Blank	100	0.0	5
CS-T-5 Carboxylesterase	42	13.0	5
CS-T-7 Carboxylesterase	26	11.4	5
CS-T-9 Carboxylesterase	40	27.4	5
Bovine serum albumin blank	98	4.5	5
CS-T-5 BSA	28	13.0	5
CS-T-7 BSA	28	8.4	5
CS-T-9 BSA	34	15.2	5
Piperonyl butoxide Blank	98	4.5	5
CS-T-5 PBO	6	8.9	5
CS-T-7 PBO	10	10.0	5
CS-T-9 PBO	18	11.0	5
SIR 300 Blank	98	4.5	5
CS-T-5 SIR 300	32	8.4	5
CS-T-7 SIR 300	18	14.8	5
CS-T-9 SIR 300	40	15.8	5
Zeolite Blank	98	4.5	5
CS-T-5 Zeolite	36	23.0	5
CS-T-7 Zeolite	14	15.2	5
CS-T-9 Zeolite	38	8.4	5
Charcoal Blank	96	8.9	5
CS-T-5 Charcoal	64	21.9	5
CS-T-7 Charcoal	42	11.0	5
CS-T-9 Charcoal	62	34.9	5
SIR 300 + Charcoal Blank	98	4.5	5
CS-T-5 SIR 300 + Charcoal	44	5.5	5
CS-T-7 SIR 300 + Charcoal	44	13.4	5
CS-T-9 SIR 300 + Charcoal	64	8.9	5

Table B.4. Survival results of Stage II TIE testing of Consolidated Slip samples using the 10-day pore water protocol with *Eohaustorius estuarius.*

Sample	Mean	Standard Deviation	Number of Replicates
Laboratory Sea Water	85	19.1	4
CS-T-5 Baseline	80	16.3	4
CS-T-7 Baseline	60	43.2	4
CS-T-9 Baseline	60	40.0	4
Carboxylesterase Blank	90	11.5	4
CS-T-5 Carboxylesterase	85	19.1	4
CS-T-7 Carboxylesterase	75	19.1	4
CS-T-9 Carboxylesterase	85	10.0	4
Bovine serum albumin blank	80	16.3	4
CS-T-5 BSA	60	28.3	4
CS-T-7 BSA	55	10.0	4
CS-T-9 BSA	95	10.0	4
Piperonyl butoxide Blank	50	34.6	4
CS-T-5 PBO	20	23.1	4
CS-T-7 PBO	15	19.1	4
CS-T-9 PBO	0	0.0	4
EDTA Blank	80	16.3	4
CS-T-5 EDTA	80	28.3	4
CS-T-7 EDTA	79	16.5	4
CS-T-9 EDTA	75	25.2	4
Zeolite Column Blank	75	19.1	4
CS-T-5 Zeolite	65	19.1	4
CS-T-7 Zeolite	70	11.5	4
CS-T-9 Zeolite	70	38.3	4
Sodium Thiosulfate Blank	90	11.5	4
CS-T-5 STS	75	25.2	4
CS-T-7 STS	80	16.3	4
CS-T-9 STS	85	19.1	4
Cation Exchange Column Blank	90	11.5	4
CS-T-5 Cation Exchange	75	25.2	4
CS-T-7 Cation Exchange	95	10.0	4
CS-T-9 Cation Exchange	80	16.3	4
C18 Column Blank	90	11.5	4
CS-T-5 C18	80	16.3	4
CS-T-7 C18	65	10.0	4
CS-T-9 C18	90	11.5	4
CS-T-5 Cation→C18	95	10.0	4
CS-T-7 Cation→C18	70	20.0	4
CS-T-9 Cation→C18	95	10.0	4

Table B.5. Survival results of Stage III initial testing of Consolidated Slip samples using the 10-day whole sediment protocol with *Eohaustorius estuarius.*

Sample	Mean	Standard Deviation	Number of Replicates
Home Sediment	96	8.9	5
CS-T-1	48	13.0	5
CS-T-2	48	17.9	5
CS-T-3	50	18.7	5
CS-T-5	56	16.7	5
CS-T-6	60	12.2	5
CS-T-7	56	11.4	5
CS-T-9	46	15.2	5
CS-T-10	54	18.2	5
CS-T-11	66	11.4	5
CS-T-12	44	20.7	5

Table B.6. Survival results of Stage III initial testing of Consolidated Slip samples using the 10-day pore water protocol with *Eohaustorius estuarius*

Sample	Mean	Standard Deviation	Number of Replicates
Lab Seawater (32 ppt)	93	11.5	3
CS-T-1	53	30.6	3
CS-T-2	80	20.0	3
CS-T-3	80	20.0	3
CS-T-5	80	20.0	3
CS-T-6	87	11.5	3
CS-T-7	60	20.0	3
CS-T-9	67	41.6	3
CS-T-10	80	20.0	3
CS-T-11	100	0.0	3
CS-T-12	87	11.5	3

Table B.7. Survival results of Stage III TIE testing of Consolidated Slip samples using the 10-day whole sediment protocol with *Eohaustorius estuarius.*

Sample	Mean	Standard Deviation	Number of Replicates
Home Sediment	94	5.5	5
CS-T-1 Baseline	48	16.4	5
CS-T-2 Baseline	38	14.8	5
CS-T-3 Baseline	50	10.0	5
CS-T-5 Baseline	28	13.0	5
CS-T-6 Baseline	58	8.4	5
CS-T-7 Baseline	40	12.2	5
CS-T-9 Baseline	36	28.8	5
CS-T-10 Baseline	46	11.4	5
CS-T-11 Baseline	60	20.0	5
CS-T-12 Baseline	42	14.8	5
CS-T-1 Dilution control	52	17.9	5
CS-T-2 Dilution control	44	15.2	5
CS-T-3 Dilution control	46	11.4	5
CS-T-5 Dilution control	58	16.4	5
CS-T-6 Dilution control	54	15.2	5
CS-T-7 Dilution control	62	16.4	5
CS-T-9 Dilution control	54	24.1	5
CS-T-10 Dilution control	64	8.9	5
CS-T-11 Dilution control	70	18.7	5
CS-T-12 Dilution control	68	8.4	5
Piperonyl butoxide Blank	98	4.5	5
CS-T-1 PBO	12	13.0	5
CS-T-2 PBO	16	15.2	5
CS-T-3 PBO	12	17.9	5
CS-T-5 PBO	10	12.2	5
CS-T-6 PBO	14	13.4	5
CS-T-7 PBO	26	19.5	5
CS-T-9 PBO	18	8.4	5
CS-T-10 PBO	4	5.5	5
CS-T-11 PBO	20	20.0	5
CS-T-12 PBO	20	12.2	5
Charcoal Blank	100	0.0	5
CS-T-1 Charcoal	76	16.7	5
CS-T-2 Charcoal	76	5.5	5
CS-T-3 Charcoal	90	14.1	5
CS-T-5 Charcoal	86	5.5	5
CS-T-6 Charcoal	86	8.9	5
CS-T-7 Charcoal	88	8.4	5
CS-T-9 Charcoal	76	8.9	5
CS-T-10 Charcoal	84	13.4	5
CS-T-11 Charcoal	84	11.4	5
CS-T-12 Charcoal	76	5.5	5

Table B.7. Continued.

Sample	Mean	Standard Deviation	Number of Replicates
SIR 300 Blank	94	5.5	5
CS-T-1 SIR 300	42	16.4	5
CS-T-2 SIR 300	30	17.3	5
CS-T-3 SIR 300	20	18.7	5
CS-T-5 SIR 300	46	15.2	5
CS-T-6 SIR 300	48	24.9	5
CS-T-7 SIR 300	50	10.0	5
CS-T-9 SIR 300	46	18.2	5
CS-T-10 SIR 300	34	28.8	5
CS-T-11 SIR 300	54	23.0	5
CS-T-12 SIR 300	42	14.8	5

APPENDIX C – TOXIC UNIT CALCULATIONS FOR SEDIMENT

Table C.1. Acute toxicity values used for toxic unit calculations of pesticides and PCBs. All values are for various species of amphipod and are expressed on an organic carbon normalized basis.

Chemical	LC50 ug/g OC	Species	Source
Cyhalothrin, Total Lambda	0.22	*Hyalella azteca*	Amweg et al. 2005
Cyfluthrin	0.33	*Eohaustorius estuarius*	Greenstein et al. 2014
Cypermethrin	1.41	*Eohaustorius estuarius*	Anderson et al. 2008
Bifenthrin	1.05	*Eohaustorius estuarius*	Anderson et al. 2008
Esfenvalerate	1.55	*Hyalella azteca*	Amweg et al. 2005
Fipronil	2.58	*Eohaustorius estuarius*	SCCWRP, unpublished
Fipronil Sulfone	2.00	*Eohaustorius estuarius*	SCCWRP, unpublished
Fipronil Sulfide	5.39	*Eohaustorius estuarius*	SCCWRP, unpublished
DDT	266	*Eohaustorius estuarius*	Greenstein et al. 2014
DDE	>3050	*Eohaustorius estuarius*	Greenstein et al. 2014
PCBs	2600	*Rhepoxynius abrounius*	Swartz et al. 1988
Chlordane	>2120	*Eohaustorius estuarius*	Greenstein et al. 2014

Table C.2. Pyrethroid and fipronil toxic unit calculations for Stage II sediment samples.

Chemical	T-1 April	T-2 April	T-3 April	T-5 April	T-6 April	T-7 April	T-9 April	T-10 April	T-11 April	T-12 April
Pyrethroids ug/g OC										
Cyhalothrin, Total Lambda	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Cyfluthrin	ND	0.09	0.25	0.11	0.54	0.22	ND	ND	ND	ND
Danitol (Fenpropathrin)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Cypermethrin	ND	ND	0.05	ND	ND	0.25	ND	ND	ND	ND
Bifenthrin	0.98	0.77	1.03	0.76	0.91	1.06	0.93	1.01	0.87	1.14
Permethrin, cis-	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Permethrin, trans-	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Deltamethrin/Tralomethrin	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Esfenvalerate	ND	ND	ND	ND	0.02	ND	ND	ND	ND	ND
Pyrethroids TUs										
Cyhalothrin, Total Lambda	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Cyfluthrin	ND	0.28	0.75	0.32	1.64	0.68	ND	ND	ND	ND
Cypermethrin	ND	ND	0.04	ND	ND	0.18	ND	ND	ND	ND
Bifenthrin	0.93	0.73	0.99	0.73	0.87	1.01	0.89	0.96	0.83	1.08
Esfenvalerate	ND	ND	ND	ND	0.01	ND	ND	ND	ND	ND
Total TUs	0.93	1.02	1.77	1.05	2.52	1.86	0.89	0.96	0.83	1.08
Fipronils ug/g OC										
Fipronil (ug/g OC)	ND	0.01	0.01	0.01	ND	ND	ND	ND	ND	0.01
Fipronil Sulfone (ug/g OC)	0.02	0.03	0.03	0.03	0.04	0.04	0.01	0.03	0.03	0.04
Fipronil Sulfide (ug/g OC)	0.07	0.11	0.11	0.10	0.10	0.12	0.08	0.08	0.11	0.11
Fipronils TUs										
Fipronil	ND	0.00	0.00	0.00	ND	ND	ND	ND	ND	0.00
Fipronil Sulfone	0.00	0.00	0.00	0.00	0.01	0.01	0.00	0.00	0.00	0.01
Fipronil Sulfide	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Total TUs	0.01	0.02	0.02	0.02	0.02	0.02	0.01	0.01	0.02	0.02

Table C.3. Pyrethroid and fipronil toxic unit calculations for Stage III sediment samples.

Chemical	T-1 June	T-2 June	T-3 June	T-5 June	T-6 June	T-7 June	T-9 June	T-10 June	T-11 June	T-12 June
Pyrethroid ug/g OC										
Cyhalothrin, Total Lambda	ND	ND	ND	ND	ND	ND	0.26	ND	ND	ND
Cyfluthrin	ND	ND	0.23	0.16	ND	ND	ND	ND	0.17	ND
Danitol (Fenpropathrin)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Cypermethrin	ND	ND	0.14	ND	ND	ND	ND	ND	ND	ND
Bifenthrin	0.49	1.09	1.29	1.00	0.95	1.26	1.29	1.73	0.93	0.97
Permethrin, cis-	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Permethrin, trans-	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Deltamethrin/Tralomethrin	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Esfenvalerate	ND	ND	0.01	0.02	ND	ND	ND	ND	ND	ND
Pyrethroid TUs										
Cyhalothrin, Total Lambda	ND	ND	ND	ND	ND	ND	0.58	ND	ND	ND
Cyfluthrin	ND	ND	0.68	0.50	ND	ND	ND	ND	0.52	ND
Cypermethrin	ND	ND	0.10	ND	ND	ND	ND	ND	ND	ND
Bifenthrin	0.47	1.04	1.23	0.96	0.90	1.20	1.23	1.65	0.89	0.92
Esfenvalerate	ND	ND	0.01	0.01	ND	ND	ND	ND	ND	ND
Total TUs	0.47	1.04	2.02	1.46	0.90	1.20	1.81	1.65	1.41	0.92
Fipronil ug/g OC										
Fipronil	0.00	0.01	0.01	ND	0.01	ND	ND	0.01	ND	ND
Fipronil sulfone	0.02	0.03	0.04	0.02	0.03	0.04	0.03	0.04	0.03	0.04
Fipronil sulfide	0.05	0.08	0.10	0.07	0.10	0.10	0.08	0.10	0.09	0.11
Fipronil TUs										
Fipronil (TU)	0.00	0.00	0.00	ND	0.00	ND	ND	0.00	ND	ND
Fipronil Sulfone (TU)	0.01	0.02	0.02	0.01	0.02	0.02	0.01	0.02	0.02	0.02
Fipronil Sulfide (TU)	0.01	0.01	0.02	0.01	0.02	0.02	0.02	0.02	0.02	0.02
Total TUs	0.02	0.03	0.04	0.02	0.04	0.04	0.03	0.04	0.03	0.04

Table C.4. PAH acute toxic unit calculations for Stage II samples.

PAH	Acute ECP	T-1 April ug/g OC	T-1 April ESBTU	T-2 April ug/g OC	T-2 April ESBTU	T-3 April ug/g OC	T-3 April ESBTU	T-5 April ug/g OC	T-5 April ESBTU	T-6 April ug/g OC	T-6 April ESBTU
Acenaphthene	1021.000	0.484	0.000	0.332	0.000	0.368	0.000	0.569	0.001	0.360	0.000
Anthracene	1235.000	1.831	0.001	1.800	0.001	1.489	0.001	1.887	0.002	1.527	0.001
Phenanthrene	1241.000	7.295	0.006	4.755	0.004	4.607	0.004	8.569	0.007	6.517	0.005
Biphenyl		0.174		0.284		0.202		0.294		0.158	
Naphthalene	801.000	0.636	0.001	0.591	0.001	0.647	0.001	0.670	0.001	0.542	0.001
2,6-Dimethylnaphthalene	1068.000	0.561	0.001	0.793	0.001	0.890	0.001	0.985	0.001	0.784	0.001
Fluorene	1121.000	0.512	0.000	0.637	0.001	0.676	0.001	0.880	0.001	0.625	0.001
1-Methylnaphthalene	927.000	0.316	0.000	0.289	0.000	0.326	0.000	0.440	0.000	0.285	0.000
2-Methylnaphthalene	930.000	0.757	0.001	0.706	0.001	0.781	0.001	0.918	0.001	0.614	0.001
1-Methylphenanthrene	1394.000	0.834	0.001	0.951	0.001	1.105	0.001	1.404	0.001	1.493	0.001
Acenaphthylene	940.000	0.295	0.000	0.289	0.000	0.277	0.000	0.253	0.000	0.243	0.000
2,3,5-Trimethylnaphthalene	1215.000	0.364	0.000	1.135	0.001	1.553	0.001	1.231	0.001	1.488	0.001
Benz[a]anthracene	1749.000	9.098	0.005	7.808	0.004	7.651	0.004	9.051	0.005	6.691	0.004
Benzo[a]pyrene	2012.000	10.711	0.005	8.163	0.004	8.259	0.004	8.914	0.004	7.013	0.003
Benzo[e]pyrene	2012.000	11.062	0.005	8.992	0.004	8.982	0.004	9.345	0.005	7.853	0.004
Chrysene	1754.000	14.669	0.008	14.749	0.008	13.388	0.008	15.015	0.009	13.472	0.008
Dibenz[a,h]anthracene	2335.840	2.353	0.001	1.823	0.001	1.995	0.001	2.177	0.001	1.616	0.001
Fluoranthene	1472.000	12.450	0.008	11.071	0.008	10.373	0.007	15.298	0.010	15.417	0.010
Perylene	2012.000	3.139	0.002	2.502	0.001	2.254	0.001	2.380	0.001	1.995	0.001
Pyrene	1451.000	16.525	0.011	12.300	0.008	12.457	0.009	15.724	0.011	15.383	0.011
Benzo[k]fluoranthene	2038.000	9.004	0.004	7.355	0.004	6.887	0.003	7.096	0.003	6.295	0.003
Benzo[b]fluoranthene	2037.000	10.748	0.005	8.980	0.004	8.437	0.004	8.649	0.004	7.643	0.004
Benzo[g,h,i]perylene	2278.000	10.620	0.005	8.282	0.004	9.819	0.004	9.287	0.004	8.065	0.004
Indeno[1,2,3-c,d]pyrene	2319.200	6.837	0.003	5.218	0.002	5.623	0.002	5.681	0.002	4.884	0.004
ΣESBTUFAV			0.076		0.064		0.064		0.076		0.069
ΣESBTUFAV23			0.213		0.179		0.178		0.212		0.192

Table C.4. Continued.

PAH	T-7 April ug/g OC	T-7 April ESBTU	T-9 April ug/g OC	T-9 April ESBTU	T-10 April ug/g OC	T-10 April ESBTU	T-11 April ug/g OC	T-11 April ESBTU	T-12 April ug/g OC	T-12 April ESBTU
Acenaphthene	0.345	0.000	0.260	0.000	0.807	0.001	0.381	0.000	0.371	0.000
Anthracene	1.387	0.001	1.856	0.002	2.460	0.002	1.430	0.001	1.399	0.001
Phenanthrene	5.657	0.005	6.544	0.005	13.453	0.011	4.783	0.004	5.486	0.004
Biphenyl	0.178		0.161		0.189		0.137		0.262	
Naphthalene	0.649	0.001	0.663	0.001	0.669	0.001	0.407	0.001	0.641	0.001
2,6-Dimethylnaphthalene	0.800	0.001	0.684	0.001	0.753	0.001	0.736	0.001	0.965	0.001
Fluorene	0.641	0.001	0.413	0.000	1.107	0.001	0.672	0.001	0.682	0.001
1-Methylnaphthalene	0.301	0.000	0.361	0.000	0.383	0.000	0.240	0.000	0.662	0.001
2-Methylnaphthalene	0.668	0.001	0.894	0.001	0.781	0.001	0.527	0.001	0.853	0.001
1-Methylphenanthrene	1.131	0.001	0.948	0.001	1.382	0.001	1.163	0.001	0.850	0.001
Acenaphthylene	0.226	0.000	0.355	0.000	0.229	0.000	0.216	0.000	0.273	0.000
2,3,5-Trimethylnaphthalene	1.448	0.001	0.505	0.000	1.103	0.001	1.505	0.001	0.807	0.001
Benz[a]anthracene	7.048	0.004	9.553	0.005	9.215	0.005	6.087	0.003	7.387	0.004
Benzo[a]pyrene	7.092	0.004	10.789	0.005	8.371	0.004	6.347	0.003	7.852	0.004
Benzo[e]pyrene	7.955	0.004	11.539	0.006	9.022	0.004	7.036	0.003	9.091	0.005
Chrysene	12.814	0.007	19.953	0.011	15.313	0.009	11.067	0.006	13.598	0.008
Dibenz[a,h]anthracene	1.676	0.001	2.332	0.001	1.988	0.001	1.460	0.001	1.876	0.001
Fluoranthene	11.646	0.008	11.979	0.008	16.401	0.011	10.616	0.007	11.947	0.008
Perylene	2.012	0.001	3.016	0.001	2.246	0.001	1.717	0.001	2.707	0.001
Pyrene	12.326	0.008	18.598	0.013	15.250	0.011	11.147	0.008	12.498	0.009
Benzo[k]fluoranthene	6.315	0.003	8.929	0.004	6.900	0.003	5.193	0.003	7.229	0.004
Benzo[b]fluoranthene	7.618	0.004	10.869	0.005	8.449	0.004	6.438	0.003	8.852	0.004
Benzo[g,h,i]perylene	8.081	0.004	11.537	0.005	8.473	0.004	6.632	0.003	9.486	0.004
Indeno[1,2,3-c,d]pyrene	5.122	0.005	6.655	0.003	5.302	0.002	3.877	0.003	5.758	0.002
ΣESBTUFCV		0.063		0.081		0.079		0.055		0.065
ΣESBTUFCV23		0.177		0.226		0.222		0.155		0.183

Table C.5. PAH acute toxic unit calculations for Stage III samples.

PAH	T-1 June ug/g OC	T-1 June ESBTU	T-2 June ug/g OC	T-2 June ESBTU	T-3 June ug/g OC	T-3 June ESBTU	T-5 June ug/g OC	T-5 June ESBTU	T-6 June ug/g OC	T-6 June ESBTU
Acenaphthene	0.182	0.000	0.338	0.000	0.386	0.000	0.650	0.001	0.288	0.000
Anthracene	0.920	0.001	1.532	0.001	1.651	0.001	1.681	0.001	1.286	0.001
Phenanthrene	2.687	0.002	5.588	0.005	5.640	0.005	8.279	0.007	4.013	0.003
Biphenyl	0.093		0.180		0.209		0.146		0.134	
Naphthalene	0.249	0.000	0.491	0.001	0.841	0.001	0.538	0.001	0.435	0.001
2,6-Dimethylnaphthalene	0.380	0.000	0.492	0.000	0.825	0.001	0.560	0.001	0.703	0.001
Fluorene	0.351	0.000	0.503	0.000	0.714	0.001	0.902	0.001	0.561	0.001
1-Methylnaphthalene	0.126	0.000	0.238	0.000	0.333	0.000	0.262	0.000	0.224	0.000
2-Methylnaphthalene	0.331	0.000	0.555	0.001	0.860	0.001	0.587	0.001	0.527	0.001
1-Methylphenanthrene	0.567	0.000	0.847	0.001	1.003	0.001	1.087	0.001	0.959	0.001
Acenaphthylene	0.136	0.000	0.211	0.000	0.326	0.000	0.230	0.000	0.201	0.000
2,3,5-Trimethylnaphthalene	0.570	0.000	0.564	0.000	1.179	0.001	0.681	0.001	1.319	0.001
Benz[a]anthracene	3.883	0.002	6.585	0.004	8.062	0.005	7.240	0.004	5.681	0.003
Benzo[a]pyrene	4.267	0.002	7.090	0.004	9.547	0.005	7.562	0.004	6.475	0.003
Benzo[e]pyrene	4.823	0.002	8.049	0.004	10.445	0.005	8.080	0.004	7.431	0.004
Chrysene	6.949	0.004	11.775	0.007	14.151	0.008	12.938	0.007	10.289	0.006
Dibenz[a,h]anthracene	1.075	0.000	1.689	0.001	2.338	0.001	1.877	0.001	1.610	0.001
Fluoranthene	7.211	0.005	12.872	0.009	13.153	0.009	13.711	0.009	10.107	0.007
Perylene	1.217	0.001	2.069	0.001	2.955	0.001	2.000	0.001	1.766	0.001
Pyrene	7.933	0.005	13.491	0.009	15.414	0.011	14.364	0.010	11.843	0.008
Benzo[k]fluoranthene	3.850	0.002	5.940	0.003	7.875	0.004	6.302	0.003	5.015	0.002
Benzo[b]fluoranthene	4.570	0.002	7.366	0.004	9.703	0.005	7.318	0.004	6.554	0.003
Benzo[g,h,i]perylene	4.837	0.002	7.787	0.003	11.446	0.005	8.070	0.004	7.606	0.003
Indeno[1,2,3-c,d]pyrene	2.915	0.001	4.565	0.002	6.997	0.003	4.929	0.002	4.222	0.002
ΣESBTUFAV		0.035		0.059		0.073		0.066		0.053
ΣESBTUFAV23		0.099		0.166		0.205		0.184		0.147

Table C.5. Continued.

PAH	T-7 June ug/g OC	T-7 June ESBTU	T-9 June ug/g OC	T-9 June ESBTU	T-10 June ug/g OC	T-10 June ESBTU	T-11 June ug/g OC	T-11 June ESBTU	T-12 June ug/g OC	T-12 June ESBTU
Acenaphthene	0.501	0.000	0.289	0.000	0.377	0.000	0.502	0.000	0.431	0.000
Anthracene	1.924	0.002	1.276	0.001	1.730	0.001	1.727	0.001	1.481	0.001
Phenanthrene	6.276	0.005	3.844	0.003	5.597	0.005	4.616	0.004	5.202	0.004
Biphenyl	0.199		0.124		0.142		0.214		0.163	
Naphthalene	0.590	0.001	0.458	0.001	0.500	0.001	0.562	0.001	0.582	0.001
2,6-Dimethylnaphthalene	0.914	0.001	0.580	0.001	0.714	0.001	0.878	0.001	0.646	0.001
Fluorene	0.839	0.001	0.483	0.000	0.617	0.001	0.771	0.001	0.589	0.001
1-Methylnaphthalene	0.314	0.000	0.237	0.000	0.318	0.000	0.350	0.000	0.291	0.000
2-Methylnaphthalene	0.769	0.001	0.581	0.001	0.732	0.001	0.784	0.001	0.702	0.001
1-Methylphenanthrene	1.092	0.001	0.896	0.001	1.028	0.001	1.000	0.001	0.866	0.001
Acenaphthylene	0.247	0.000	0.201	0.000	0.199	0.000	0.244	0.000	0.286	0.000
2,3,5-Trimethylnaphthalene	1.548	0.001	0.841	0.001	1.051	0.001	1.552	0.001	0.809	0.001
Benz[a]anthracene	7.360	0.004	6.137	0.004	8.081	0.005	6.362	0.004	7.280	0.004
Benzo[a]pyrene	7.950	0.004	7.055	0.004	8.859	0.004	6.639	0.003	8.020	0.004
Benzo[e]pyrene	9.077	0.005	7.807	0.004	9.740	0.005	7.770	0.004	9.453	0.005
Chrysene	13.280	0.008	11.038	0.006	13.497	0.008	11.544	0.007	14.018	0.008
Dibenz[a,h]anthracene	1.944	0.001	1.789	0.001	2.081	0.001	1.581	0.001	1.932	0.001
Fluoranthene	13.333	0.009	9.435	0.006	12.913	0.009	11.969	0.008	12.583	0.009
Perylene	2.261	0.001	1.974	0.001	2.402	0.001	1.951	0.001	2.368	0.001
Pyrene	14.342	0.010	10.957	0.008	14.240	0.010	12.534	0.009	14.020	0.010
Benzo[k]fluoranthene	6.637	0.003	5.291	0.003	7.000	0.003	5.810	0.003	7.135	0.004
Benzo[b]fluoranthene	8.332	0.004	6.605	0.003	8.434	0.004	7.187	0.004	8.660	0.004
Benzo[g,h,i]perylene	8.876	0.004	7.992	0.004	9.459	0.004	7.642	0.003	9.498	0.004
Indeno[1,2,3-c,d]pyrene	5.335	0.002	4.384	0.002	5.595	0.002	4.580	0.002	5.720	0.002
ΣESBTUFAV		0.068		0.053		0.067		0.059		0.066
ΣESBTUFAV23		0.189		0.147		0.189		0.165		0.184