RMP Sediment TIE Study 2007-2008: Using Toxicity Identification Evaluation (TIE) Methods to Investigate Causes of Sediment Toxicity to Amphipods

by

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Executive Summary

More than 25 years of monitoring studies have shown widespread sediment toxicity in the San Francisco Estuary. While toxicity is generally persistent, patterns of sediment toxicity demonstrate temporal and spatial variability, with greater toxicity observed in samples collected during the rainy winter season at sites near the mouths of tributaries. Samples collected as part of the Regional Monitoring Program's Status and Trends program consistently show significant but moderate levels of toxicity at many stations.

A number of studies have shown that amphipod mortality in laboratory tests is correlated with several metal and organic chemical constituents in the Estuary's sediments. These studies have also demonstrated significant correlations between amphipod mortality and chemical mixtures. While correlative studies are useful, they do not provide direct evidence of the causes of sediment toxicity. Understanding the cause(s) of sediment toxicity is one of the primary goals of the RMP because management of contaminants entering the Estuary is most efficient when it targets the key chemicals responsible for biological impacts.

One approach for determining the cause(s) of toxicity is the process known as Toxicity Identification Evaluation (TIE), which involves a series of procedures designed to decrease, increase, or transform the bioavailable fractions of sediment contaminants to assess their contributions to sample toxicity. TIEs have been used to show that divalent cations (e.g., copper) were responsible for toxicity to bivalves in sediment samples from the Estuary. To date, however, TIE methods have not been sufficiently developed to determine the causes of sediment toxicity to amphipods.

With the recent development of new sediment TIE procedures, the RMP funded a TIE study designed to investigate causes of sediment toxicity in two samples collected during the rainy season from stations located near the margins of the Estuary. Based on historical data, twelve samples from previously toxic stations were selected for a screening survey, from which two stations were to be selected for TIEs. Toxicity of these sediments was tested with the amphipod *Eohaustorius estuarius*, the primary species used in the Status and Trends program, as well as the benchmark species used in the State of California's Sediment Quality Objectives program. Fourteen samples from twelve stations were surveyed, but only Mission Creek sediment was sufficiently toxic for use in a TIE (<50% survival). One station had poor sediment quality and could not be tested, and a thirteenth station was added in an effort to find another

significantly toxic station for TIE development. The reason for the lack of significant toxicity in the remaining stations is not clear, but underscores the temporal variability of sediment toxicity in this system.

A combination of whole-sediment and interstitial water TIE procedures was used to provide a weight of evidence to determine the cause(s) of sediment toxicity in the Mission Creek sample. The lines of evidence suggest that toxicity to amphipods in this sample was caused by a mixture of organic chemicals. The lines of evidence can be summarized as follows:

1. Whole sediment and interstitial water toxicity was significantly reduced by procedures designed to reduce the bioavailability of organic compounds.
2. Toxicity was returned when the organic compounds were eluted from the TIE media and added to clean water.
3. Similar procedures targeting divalent cations did not remove or recover toxicity, indicating that toxicity was not caused by metals.
4. Treatments for ammonia showed that while ammonia was elevated in some matrices, reducing the toxic unionized ammonia fraction did not remove sample toxicity.

These results provide the first successful characterization of the causes of toxicity to amphipods in sediment samples from the Estuary. While some of the TIE procedures produced conflicting results, these lines of evidence, combined with the results of chemical analysis, indicate that toxicity of the Mission Creek sediment was caused by mixtures of organic chemicals, including PAHs and pesticides that occurred at toxic concentrations in the solid-phase sample, TIE media eluates, and interstitial water. These direct lines of evidence for toxicity due to organic chemical mixtures are supported by the solid-phase chemistry data from the sample, which, when compared to specific organism thresholds and sediment quality guideline values derived from correlations using large data sets, produced a sum sediment quality guideline quotient value (SQGQ) that indicates the sediment contained elevated concentrations of a mixture of chemicals.

This TIE investigation was constrained by factors related to chemical procedures and gaps in the available literature, including: poor detection of organic chemicals in eluates of the solid-phase extraction columns used in the interstitial water tests; a lack of literature toxicity values for some of the chemicals present in the samples (particularly when chemicals are present as mixtures); and the likely presence of unmeasured contaminants.

These results provide evidence of organic chemical toxicity to amphipods in Mission Creek sediment and suggest several areas of future research. Additional work on TIE method development is necessary to understand the relationships between contaminant concentrations present in whole sediment, sediment interstitial water, and eluates of carbonaceous resins and other media used in the TIE process. It is also necessary to continue to develop toxicity information for single chemicals of concern identified in these matrices and for chemicals occurring in mixtures, particularly for amphipods exposed to whole sediment and in interstitial water. The goal of this research is to develop tools that will identify the cause of sediment toxicity in Mission Creek, and other Estuary sediments, so that sources of these chemicals (or toxic agents) can be determined. The eventual goal is to reduce loadings of toxic chemicals.

Continued refinement of these methods should allow better resolution of the causes of sediment toxicity in highly to moderately toxic sediments. With increased understanding of the causes of sediment toxicity, these results may be used to design future studies to develop and validate sediment quality objectives for the San Francisco Estuary, and to investigate relationships between amphipod mortality in laboratory tests and contaminant impacts on estuarine benthic community structure.

Introduction

The San Francisco Estuary Regional Monitoring Program for Water Quality (RMP) Status and Trends Program has been monitoring sediment toxicity in the estuary since 1993. Until 1999 toxicity tests were conducted in winter and summer with amphipods (*Eohaustorius estuarius*) exposed to whole sediment and larval mussels (*Mytilus galloprovincialis*) exposed to sediment elutriate. The twice-yearly sampling events focused on stations that ranged along the central axis of the Bay. A review of data collected between 1991 and 1999 showed consistent toxicity in both seasons sampled using bivalve elutriate tests (31% samples toxic in winter vs. 33% toxic in summer) and greater winter-season toxicity in sediment tests with amphipods (51% samples toxic in winter vs. 16% toxic in summer). Because greater sediment toxicity was observed in winter months, contaminants were thought to enter the system via stormwater from tributaries (Anderson *et al.*, 2007a). Regardless of season, the majority of samples that were identified as toxic had survival greater than 50%, demonstrating moderate toxicity. Between 2000 and 2001, the RMP changed the frequency of sampling, and toxicity testing was conducted in the summer only. In 2002 the RMP changed the sample design to include a Generalized Random-tessellation Stratified (GRTS) Design, while maintaining several historic sampling stations to monitor trends over time. The new spatially balanced sampling design allows the program to better characterize the chemical and toxicological condition of the Estuary in the dry season. Within the new sample design approximately 18% of the summer samples tested with amphipods have been toxic. Although these samples were presumably collected closer to potential sources of pollutants (i.e. runoff from urban creeks), the magnitude of toxicity has remained moderate, with fewer than 13% of the toxic samples having amphipod survival less than 50%.

The RMP has also conducted water column toxicity testing, and historical results show that aquatic toxicity in the estuary's tributaries declined between 1996 and 2001 (www.sfei.org/rmp/index.html). This is thought to be due to decreased use of organophosphate pesticides, coinciding with the increased use of more hydrophobic pyrethroid pesticides (Amweg *et al.*, 2005). Pyrethroid pesticides are transported with particles and could be conveyed into the estuary with suspended sediment (Liu *et al.*, 2004). One hypothesis is that instances of sediment toxicity in tributaries may be increasing with the increased use of pyrethroid pesticides, and recent studies of urban creeks in the Estuary's watersheds have shown toxic concentrations of

these pesticides in sediments at some stations (Amweg *et al.*, 2006; Lowe *et al.*, 2007; Holmes *et al.*, 2008). Other studies in the Estuary have shown correlations between amphipod mortality and a number of organic chemicals (Thompson *et al.*, 1999; Hunt *et al.*, 2001; Anderson *et al.*, 2007a). In addition, amphipod mortality has been correlated with chemical mixtures (Thompson *et al.*, 1999; Hunt *et al.*, 2001; Anderson *et al.*, 2007a).

Identifying the causes of persistent sediment toxicity in the Estuary is an objective of the RMP, and one approach to achieve this objective is to employ toxicity identification evaluation methods (TIEs). TIEs involve a series of procedures designed to decrease, increase, or transform the bioavailable fractions of sediment contaminants to assess their contributions to sample toxicity (USEPA, 1991, 1993b, a). The U.S. EPA and others have developed sediment TIE procedures designed to proceed in three phases (Ankley *et al.*, 1991; USEPA, 2007). Phase I manipulations characterize the classes of chemicals causing toxicity and typically differentiate between toxicity caused by organic chemicals, metals, or ammonia. Phase II TIE manipulations identify the cause of toxicity, and Phase III TIEs are designed to confirm the chemical(s) identified in Phase II. Sediment TIEs are conducted using both the aqueous matrix (sediment interstitial water) and the whole sediment matrix. While the growing literature has demonstrated the efficacy of TIE procedures for identifying causes of toxicity in effluents and receiving waters (Norberg-King *et al.*, 2005), their application to sediments is less well developed. The Water Environment Research Foundation (WERF) initiated a project in 2005 to evaluate sediment TIE methods for application in Total Maximum Daily Load (TMDL) studies (Anderson *et al.*, 2007b). A number of solid-phase and interstitial water TIE procedures were developed as part of this project, and these procedures were used as the framework for the current RMP TIE project.

Previous TIE studies conducted as part of the RMP and the Bay Protection and Toxic Cleanup Program identified a cause of toxicity to larval mussels tested in sediment elutriate (Phillips *et al.*, 2003) and larval sea urchins tested in interstitial water (Hunt *et al.*, 2001), but only one study has characterized a cause of toxicity of whole sediment to amphipods (Hunt *et al.*, 2005). Causes of amphipod mortality have proven to be difficult to determine because of limitations of the solid-phase TIE procedures, the complexity of contaminant mixtures in the Estuary's sediments, and because of the relatively low magnitude of sediment toxicity observed at RMP Status and Trends stations in the summer sampling period. The RMP Toxicity Workgroup held a series of meetings (between November, 2006 and March, 2007) to devise an

approach for determining the cause of sediment toxicity in the Estuary. Compiling information from previous studies in the Estuary and its watersheds, the strategy emphasized conducting TIEs at stations which had previously been identified as being highly toxic. When subjected to the various TIE procedures, highly toxic sediments allow greater resolution of differences among treatments, giving a better chance of successfully identifying the toxic constituents. Because the historical evidence has shown that greater toxicity occurs in the rainy winter season at stations near the mouths of major stormwater tributaries, these stations were selected for this project. Emphasis was placed on whole sediment toxicity using the amphipod *E. estuarius*, because this is one of the tests employed by the RMP and a benchmark test in the State Water Resources Control Board's Sediment Quality Objectives program (Greenstein *et al.*, 2008).

The goal of the current study was to use standardized and newly-developed sediment TIE methods to determine the causes of wet-season sediment toxicity at two stations located at the margins of the estuary near the mouths of major stormwater tributaries. Information gathered from this study is intended to improve and refine the TIE methodology for the long-term goal of using TIEs to address potential causes of the observed, persistent sediment toxicity in the San Francisco Estuary as monitored by the Regional Monitoring Program's Status and Trends study.

Methods

Site Selection

Site selection criteria were based on current knowledge of land use, conceptual models of sediment transport, previous monitoring results (Table 1), and best professional judgment. Previous monitoring results included both freshwater and estuarine studies. Study results from freshwater sites were given a lower weight in the development of the final list. The current study focuses on the San Francisco Estuary margins within the tidal prism. The RMP Toxicity Workgroup reviewed summary documentation outlining the site selection criteria and toxicity results from several Bay Area studies, and developed the following site selection criteria: 1) stations must be located within the estuarine regions of the Estuary-margin, 2) there must be evidence of a high magnitude of sediment toxicity to amphipods from previous studies (>50% mortality), 3) there must be evidence of persistent toxicity over time (>2 toxic samples if sampled more than once), and 4) the stations must have similar sediment quality characteristics as those generally found in the Estuary. These criteria were used to prioritize target sampling

locations for the current study. Stations that are estuarine and showed promise for possible future gradient studies were also prioritized based on best professional judgment of the Toxicity Workgroup.

Table 1. San Francisco Estuary sediment toxicity studies reviewed during the site selection process.

Study	Citation
Regional Monitoring Program	
CEP – Analysis of Bay Area Urban Creeks	(Ruby, 2005)
Surface Water Ambient Monitoring Program	(SFBRWQCB, 2007)
Bay Protection and Toxic Cleanup Program	(Hunt *et al.*, 2001)
UC Berkeley Pyrethroid study in Urban Creeks	(Amweg *et al.*, 2006)
PRISM –tributary study of sediment toxicity and contaminants	(Lowe *et al.*, 2007)
SWAMP Statewide Assessment of Urban Pyrethroids	(Holmes *et al.*, 2008)
Western Environmental Monitoring and Assessment Program	(Phillips and Anderson, 2003)
Alameda Naval Air Station – Seaplane Lagoon	Data from Sediment Quality Objectives Database (www.swrcb.ca.gov/water_issues/programs/bptcp/sediment.shtml)
San Francisco Airport Sediment Characterization	
Clipper Yacht Harbor	
Vallejo Ferry Terminal	

Table 2. Prioritized list of sampling locations for the RMP Sediment TIE Study.

Location	Region	Priority	Additional Rationale
Rheem Creek	San Pablo Bay (east)	High	Persistent toxicity; proximate to Estuary
Mission Creek	Central Bay (west)	High	BPTCP hotspot
San Leandro Bay	Central Bay (east)	High	BPTCP Hotspot
Fruitvale	Central Bay (east)	High	BPTCP indicated elevated chemistry, toxicity and possible degraded benthos at these sites in the Inner Harbor
Islais Creek	Central Bay (west)	Medium - High	BPTCP Hotspot
San Mateo Creek	South Bay	Medium - High	Toxic more than once (20-40% survival (CN)); proximate to Estuary
SF Airport	Central Bay (west)	Medium	Toxicity between 20-40% survival (CN) at many sites in June-2000
Dumbarton Bridge	Lower South Bay	Medium - High	Seems to have persistent toxicity and is estuarine
Suisun Slough	Suisun Bay/Grizzly Bay	Low - Medium	Located in largely freshwater region of Estuary. Toxicity persistence unknown; not proximate to Estuary.
Castro Cove	San Pablo Bay (east)	Low	Complicated by historic discharge
Kirker Creek	Suisun Bay	Low - Medium	Located in largely freshwater region of Estuary. Toxicity persistence unknown.
Corte Madera Marsh	Central Bay (north)	Low	Small amount of data but has been toxic to FW species

Figure 1. Sampling locations for the RMP Sediment TIE Study.

Twelve sites were prioritized for screening, and divided into groups of four for collection during three sampling events (Table 2, Figure 1). In considering site selection criterion number four (above), the RMP Toxicity Workgroup decided that the eastern region of Suisun Bay (e.g., stations closer to the Delta such as Kirker Creek and Pacheco Creek) was a lower priority since that region's salinity is less representative of the Estuary at large. The RMP Toxicity Workgroup also decided that while Islais Creek was a good candidate station in terms of consistently high toxicity, it may also have elevated hydrogen sulfide and/or TOC, and these may confound the TIE. This station was therefore given a lower priority. Based on the criteria, the RMP Toxicity Workgroup ranked stations in San Leandro Bay higher for this project.

Once two stations produced adequate toxicity for TIE analysis, no further samples were to be collected. During the first sampling event in 2007 sediment from Mission Creek and San

Leandro Bay were successfully collected, but the substrate at Fruitvale was dominated by sponges and tunicates and therefore was inappropriate for collection and testing. Sediment from Rheem Creek and San Mateo Creek was also collected and tested. The last sample collected as part of the 2007 sampling season was Islais Creek. Of these samples, only Mission Creek produced an adequate toxicity signal to support a TIE (<50% survival). Sampling resumed in the winter of 2008 to find a second station for a TIE. Sediment from San Mateo Creek and Islais Creek were re-sampled in January after the winter storms. Sediment from the Dumbarton Bridge and San Francisco Airport sites were also collected in January. Three of these four stations were significantly toxic but did not have a high enough magnitude of toxicity for a TIE, so a final sampling event was scheduled. Sediment from Kirker Creek, Suisun Bay, Castro Cove, and Corte Madera was collected in March 2008. No significant toxicity signals were observed with these samples. Although all of the stations on the priority list had been sampled, and two of them had been sampled twice, only Mission Creek sediment was sufficiently toxic to warrant a TIE. In a final effort to find an additional sediment sample for TIE, Redwood Creek was sampled in April 2008, but no toxicity was observed.

Sample Collection

Sediment was collected using either a Van Veen grab deployed from the research vessel Questuary (Mission Creek, Fruitvale, San Leandro Bay), or using a petite Ponar grab sampler deployed from a 14 ft inflatable (all other samples except San Mateo Creek). San Mateo Creek was sampled by hand with a 7 cm polycarbonate core sampler. The top 5 cm of bedded sediment was removed from the grab or core and placed in a 20-liter bucket lined with a polyethylene bag. Twenty liters of sediment were collected from each site. The RMP Status and Trends program's sample collection, storage, and handling protocols were followed, as appropriate (Lowe *et al.*, 1999). The sediment was transported to the laboratory and stored at 4ºC. The contents of the sample buckets were homogenized prior to toxicity testing or sub-sampling for analysis.

Toxicity Testing

Whole sediment and interstitial water screening tests were performed using *Eohaustorius estuarius*. Standard whole sediment methods followed U.S. EPA protocols for the 10-day amphipod survival test (USEPA, 1994a). Interstitial water was extracted from the sediment via

refrigerated centrifuge (2500 G, 4ºC.). Interstitial water tests were conducted in five replicate 20 mL scintillation vials containing 10 mL of sample and a single organism. Screening tests of whole sediment and interstitial water were conducted with a dilution series consisting of 0% (control), 10%, 25%, 50%, and 100% (undiluted sample). Whole sediment was diluted on a wet weight basis with amphipod collection site sediment (Northwestern Aquatic Sciences, Newport, OR, USA). Samples were considered toxic if, 1) the sample response was significantly different from the control response based on a separate variance t-test (alpha = 0.01), and 2) the difference between the sample response and the control response was greater than 18.8% (Lowe *et al.*, 1999). Interstitial water was diluted with 20‰ water prepared with ambient seawater and distilled water. Screening tests were conducted with a dilution series to determine the magnitude of toxicity of both whole sediment and interstitial water. Median lethal concentrations (LC50s) were calculated based on the proportion survival in each concentration using Trimmed Spearman-Karber analysis (Hamilton *et al.*, 1977). Toxic units (TUs) were then calculated by dividing 100 by the LC50.

Toxicity Identification Evaluation

Phase I and II TIE treatments were conducted on both whole sediment and interstitial water matrices. Treatments are described in Anderson *et al.* (Anderson *et al.*, 2007b) and are based on U.S. Environmental Protection Agency (USEPA, 2007). Whole sediment TIE treatments were conducted once, and two TIEs were conducted on interstitial water. Whole sediment TIEs consisted of five replicate 250 mL beakers containing 50 mL sediment and approximately 200 mL overlying water and five amphipods. Interstitial water TIEs followed the methods of the screening tests.

Phase I whole sediment TIE treatments included 10% percent SIR-300 addition, 10% Amberlite® addition, and 10% powdered coconut charcoal addition (PCC). SIR-300 (ResinTech, West Berlin, NJ, USA), is a macroporous weak acid cation exchange resin which has chelating properties for metal ions, and is used to reduce bioavailability of cationic metals (Burgess *et al.*, 2000). Amberlite XAD-4 (Rohm and Haas, Spring House, PA, USA) is a carbonaceous resin used to reduce bioavailability of non-polar organic chemicals (Kosian *et al.*, 1999). Resins were thoroughly rinsed with Nanopure water, and one part resin was added to nine parts sediment (by wet weight) for a final concentration of 10%. PCC is pyrolized,

activated coconut husk that has been ground to <45 μm (90-96%, Calgon Carbon, Pittsburgh, PA, U.S.;(Ho *et al.*, 2004)) and is added to sediment to reduce the bioavailability of organic contaminants. Phase II treatments included the separation and elution of the Amberlite and SIR-300 resins with acetone and 1N HCl, respectively (Anderson *et al.*, 2007b; Phillips *et al.*, 2009). The resin eluate treatments were created by adding a portion of the solvent or acid to laboratory dilution water and testing it using water-only exposures of amphipods. Survival results in the resin eluate treatments can provide a qualitative line of evidence for the cause of toxicity.

Phase I and II interstitial water TIE treatments were based on the results of the whole sediment TIE. Whole sediment toxicity was reduced by the addition of amendments that reduce the bioavailability of organic contaminants, therefore the focus of the interstitial water TIE was on treatments that reduced organic toxicity. There was also an elevated concentration of ammonia in the interstitial water, so additional treatments and combinations were used to reduce toxicity caused by ammonia. Ammonia reduction treatments included passing the sample through a zeolite column and air stripping. Zeolite is an inorganic-based ion exchange resin that preferentially removes ammonia from water. Air stripping removes ammonia by increasing the pH of the sample to 10 to increase the concentration of unionized ammonia and then volatilizing the unionized ammonia by stirring the sample for four hours. The pH is adjusted back to the ambient sample pH before testing. Passing the interstitial water through a solid phase extraction (SPE) column reduced organic toxicity (Oasis Hydrophilic-Lipophilic Balance® [HLB] 6 mL, 500 mg, Waters, Milford, MA, USA). The HLB columns were also eluted as a Phase II procedure. Eluate treatments were prepared in the same way as the Amberlite eluate by eluting the column with acetone. Solvent fractions were added to water to create an eluate treatment for testing.

In addition to these methods, both matrices underwent recently developed treatments for the characterization and identification of pyrethroid pesticide toxicity. The addition of carboxylesterase enzyme (Sigma-Aldrich, St. Louis, MO, USA) to the overlying water of a sediment exposure, and to interstitial water, hydrolyzes ester-containing compounds, such as pyrethroid pesticides to their corresponding acid and alcohol, which are generally not toxic (Wheelock *et al.*, 2004). A bovine serum albumin (BSA) protein-addition control was conducted with this treatment to account for reduction of contaminant bioavailability due to complexation by the enzyme addition. Piperonyl butoxide (PBO, Sigma-Aldrich, St. Louis, MO, USA) is a

metabolic inhibitor used to block the metabolic activation of acetylcholinesterase-inhibiting organophosphate pesticides (Ankley *et al.*, 1991). It is also a potent synergist of pyrethroid toxicity, because it inhibits their metabolism (Kakko *et al.*, 2000; Ware and Whitacre, 2004). PBO was added to overlying and interstitial water to reduce toxicity caused by organophosphate pesticides and increase toxicity caused by pyrethroids.

The acceptability of each TIE treatment was evaluated by checking for adequate amphipod survival in each whole sediment or interstitial water treatment blank. Then the results of each individual TIE treatment were compared to the baseline result (= untreated sample) using a separate variance t-test (alpha = 0.05). Interstitial water treatments were compared to baseline using TUs (see above). Comparing TUs among the treatments provided better resolution than simply comparing single concentrations from the various dilution series.

Chemistry

Chemical analyses were conducted on the baseline sediment and interstitial water samples, and TIE extracts. Ammonia concentrations were measured in sediment overlying water and interstitial water using a spectrophotometric salicylate method (Hach Company, Loveland, CO, USA). Grain size and total organic carbon (TOC) were measured on the majority of the screening samples using ASTM D 422 (ASTM, 2007) and U.S. EPA Method 9060 (USEPA, 1994b), respectively. The following classes of chemicals were measured in the TIE sediment: polycyclic aromatic hydrocarbons (EPA Method 8270), organophosphate pesticides (EPA Method 8141), organochlorine pesticides (EPA Method 8081), polychlorinated biphenyls and polybrominated diphenyl ethers (EPA Method 8082)(USEPA, 1994b), pyrethroid pesticides (EPA Method 1660)(USEPA, 1993c), fipronil (EPA Method 619), and metals (EPA Method 6020)(USEPA, 1990). TIE extracts were analyzed for the same classes of chemicals except metals, and TIE interstitial water was analyzed for PAHs. As part of the Phase III TIE process, contaminant concentrations in sediment, interstitial water and eluate treatments were evaluated against known guideline values and toxicity thresholds.

Dose Response Experiments

Water only dose response experiments were conducted with amphipods exposed to copper and fluoranthene to establish LC50s for these chemicals because toxicity thresholds for

these chemicals are not currently available in the literature. The LC50s were then compared to concentrations of these chemicals measured in sediment interstitial water and TIE eluate treatments to determine whether they were present at toxic concentrations. After conducting a rangefinder test with fluoranthene, three definitive tests were conducted. Samples from the third definitive test were analyzed using GC-MS in order to calculate an LC50 based on measured concentrations. Two definitive tests were conducted with copper, and two concentrations were analyzed from each test to confirm the accuracy of the copper spikes. Two copper LC50s were calculated based on nominal concentrations.

Results

Screening

Whole sediment and interstitial water screening tests were conducted on a total of fourteen samples from 12 sites. Only three sediments samples caused significant toxicity to *E. estuarius*, and although it was not significantly toxic, only Mission Creek sediment had low enough survival (<50%) to warrant a TIE based on the criteria (Table 3). The mean survival of amphipods in Mission Creek sediment was 48%, but because of high variability, this response was not significantly different from the control ($p = 0.011$). Mission Creek sediment contained approximately 1 toxic unit (TU) and the interstitial water contained 3.6 TUs. A whole-sediment TIE was initiated on May 4, 2007 and the first interstitial water TIE was initiated on June 1, 2007. Islais Creek was sampled in the summer of 2007, but was not toxic. Sampling resumed in 2008 with repeat sampling of San Mateo and Islais Creeks along with the Dumbarton Bridge and Airport stations. Significant toxicity was observed in Islais Creek (64%), Dumbarton (70%), and Airport (76%). Although the result from the Islais Creek test did not meet the TIE criterion, a whole-sediment TIE was attempted, but the baseline toxicity signal was weak and variable (mean survival = 68%, and standard deviation = 23%) and the treatments were not effective at reducing toxicity. None of the other samples were significantly toxic. The final four samples on the priority list were also not significantly toxic. Because past data has shown significant toxicity in the Estuary adjacent to Redwood Creek, this creek was sampled at the Highway 101 crossing in a final effort to locate a second significantly toxic sample that met the criteria for a TIE. Sediment from Redwood Creek was not toxic, but undiluted interstitial water was significantly toxic. Total organic carbon (TOC) and grain size analysis were performed on nine of these

samples. TOC ranged from 0.55% at Kirker Creek to 6.4% at Mission Creek, and the percent fines (<62.5 µm) ranged from 48.5% at Kirker Creek to 99.7% at Suisun Bay. The percentage of fines at Mission Creek was 54.2%.

Mission Creek TIE

Initial Tests

The whole sediment and interstitial water screening tests for Mission Creek served as the initial tests for the TIE and were conducted on April 13, 2007. The dilution series of the initial whole sediment test with Mission Creek did not produce a steep dose response. Survival in the undiluted samples was 48%, or approximately 1 TU (Table 3). Dissolved oxygen (DO) and unionized ammonia concentrations in the overlying water were within acceptable ranges during the tests (DO = 8.30 mg/L and unionized ammonia = 0.30 mg/L). A greater magnitude of toxicity was observed in the initial interstitial water test with no survival in the undiluted sample, accounting for 3.6 TUs overall. The DO in the initial interstitial water test was very low, and the sample required aeration before testing. The DO dropped again during the exposure, and could have contributed to the observed mortality. The concentration of unionized ammonia in this sample was 0.018 mg/L, well below the toxicity threshold for *E. estuarius* of 2.40 mg/L (MPSL, unpublished data). The concentration of hydrogen sulfide before aeration was 1.26 mg/L, but was reduced to 0.28 mg/L by the aeration process. The latter concentration was still greater than the *E. estuarius* LC50 of 0.20 mg/L (Knezovich *et al.*, 1996). A second initial interstitial water test was conducted on May 2, 2007, but not before the sample was bubbled for several hours with oxygen. The DO in the second interstitial water sample was <1 mg/L before aeration, and increased to 6.09 mg/L after aeration. After 24 hours storage, the DO was 5.63 mg/L and the test was initiated. The concentration of unionized ammonia in the interstitial water had increased to 2.47 mg/L since the first interstitial water test (19 days), which is sufficiently high to contribute to toxicity, but the hydrogen sulfide concentration in this sample was 0.09 mg/L and was less than the LC50. Dissolved oxygen and unionized ammonia concentrations were monitored daily during the second interstitial water test. The unionized ammonia decreased steadily during the 10-day exposure, and the DO decreased to <1 mg/L after two days, but increased to >7 mg/L during the remainder of the exposure.

Table 3. Mean percent survival and standard deviation of *Eohaustorius estuarius* in sediment and interstitial water from twelve San Francisco Estuary stations screened for toxicity in a series of dilutions. Toxic units were calculated by dividing 100 by the percent dilution series LC50. Controls = 0% dilution. * = significantly toxic.

	Rheem		Mission		San Leandro		San Mateo 1		Islais 1		San Mateo 2		Islais 2		Airport		Dumbarton		Kirker		Suisun		Castro		Corte Madera		Redwood	
Sample Date	4/4/07		4/5/07		4/5/07		4/18/07		6/28/07		1/17/08		2/5/08		1/17/08		2/5/08		3/14/08		3/14/08		3/18/08		3/18/08		4/23/08	
Test Date	4/13/07		4/13/07		4/13/07		4/27/07		7/6/07		1/25/08		1/25/08		1/25/08		1/25/08		3/21/08		3/21/08		3/21/08		3/21/08		4/25/08	
Sediment	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
0% Control	92	11	96	9	92	18	100	0	100	0	96	9	100	0	96	9	100	0	92	11	92	11	92	11	92	11	92	11
10%	88	11	68	18	92	11	92	11	96	9	84	17	100	0	80	14	92	11	96	9	96	9	96	9	92	11	92	11
25%	100	0	80	14	92	11	80	20	96	9	84	9	96	9	80	14	100	0	92	11	92	11	96	9	84	9	80	24
50%	100	0	80	20	80	20	96	9	96	9	88	11	92	11	84	17	96	9	96	9	88	11	96	9	96	9	76	17
100%	88	18	48	30	76	26	88	18	88	11	96	9	64*	9	76*	9	70*	12	92	11	76	17	88	18	94	17	80	14
Toxic Unit	<1		~1		<1		<1		<1		<1		<1		<1		<1		<1		<1		<1		<1		<1	
Interstitial Water	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
0% Control	100	0	80	45	80	45	100	0	80	45	100	0	100	0	100	0	100	0	80	45	100	0	80	45	100	0	100	0
10%	100	0	80	45	100	0	80	45	100	0	80	45	100	0	60	55	100	0	100	0	100	0	100	0	100	0	80	45
25%	80	45	40	55	60	55	80	45	100	0	100	0	100	0	60	55	80	45	60	55	100	0	80	45	100	0	100	0
50%	80	45	20	45	80	45	40	55	80	45	80	45	100	0	80	45	100	0	100	0	80	45	80	45	80	45	100	0
100%	80	45	0*	0	60	55	60	55	80	45	80	45	100	0	60	55	100	0	100	0	100	0	60	55	80	45	20*	45
Toxic Unit	<1		3.6		<1		<1		<1		<1		<1		<1		<1		<1		<1		<1		<1		1.3	

Whole Sediment TIE

The whole sediment TIE was conducted on May 4, 2007. Survival in all treatment blanks was greater than 88% indicating that there were no adverse effects from the TIE treatments. The dilution blank was significantly toxic indicating that the addition of 10% TIE amendments did not significantly dilute the sediment. Survival in the baseline sample (the untreated Mission Creek sediment) was 20%, but the addition of Amberlite significantly increased it to 76%, and the addition of PCC increased it to 88% (Table 4). Reduction of toxicity by Amberlite and PCC characterize the cause of toxicity as due to an organic chemical. Toxicity was not reduced by the addition of SIR-300, indicating that divalent cationic metals were not contributing to toxicity. The concentration of unionized ammonia in the sediment overlying water was higher than that of the initial tests (0.378 mg/L vs. 0.223 mg/L), but was below the whole-sediment unionized ammonia toxicity threshold (0.8 mg/L, (USEPA, 1994a). Addition of carboxylesterase enzyme to the sediment overlying water did not increase survival, and the addition of PBO to the overlying water did not increase toxicity, thus there was no evidence that pyrethroid pesticides were contributing to toxicity.

Table 4. Mean percent survival (SD) of amphipods in Phase I and II whole sediment TIE treatments. * indicates significant difference from Baseline.

Treatment	Sediment	Phase I Mean (SD)	Phase II Mean (SD)
Baseline	Mission Creek	20 (20)	
	Control	88 (18)	
SIR-300 (10%)	Mission Creek	32 (41)	80 (45)
	Control	88 (11)	80 (45)
Amberlite (10%)	Mission Creek	76 (26)*	0 (0)
	Control	100 (0)	100 (0)
Powdered Coconut Charcoal (10%)	Mission Creek	88 (18)*	
	Control	92 (11)	
Enzyme	Mission Creek	8 (18)	
	Control	88 (11)	
Bovine Serum Albumin (BSA)	Mission Creek	0 (0)	
	Control	96 (9)	
Piperonyl Butoxide (PBO)	Mission Creek	4 (9)	
	Control	96 (9)	
Dilution Blank	Mission Creek	28 (30)	

The Amberlite and SIR-300 resins were separated from the test sediment at the termination of the exposure and eluted with acetone and hydrochloric acid, respectively (Phase II TIE procedure). The solvent and acid were used to prepare eluate treatments that are used to

further characterize, and possibly identify, the cause of toxicity. Complete mortality was observed in the Amberlite eluate, further indicating that organic chemical(s) were contributing to toxicity, whereas the survival in the SIR-300 eluate treatment was 80%, further indicating that divalent metals did not play a role in toxicity (Table 4).

Interstitial Water TIEs

Based on the results of the whole sediment TIE, the interstitial water TIEs focused on ammonia and organic contaminants as the cause of toxicity. The first interstitial water TIE was conducted on June 1, 2007. The sample was pre-treated with oxygen to increase the DO and equilibrated for 48 hours before the TIE was initiated. Dissolved oxygen was monitored during the 10-day exposure and remained at acceptable concentrations. Survival in all treatment blanks was greater than 80% except for the two treatments that included zeolite. These treatments had blank survivals values of 60%, however, data from these treatments were still evaluated because survival in the remaining interstitial water dilutions from these treatments was not significantly different from the negative control. Complete mortality was observed in the undiluted baseline interstitial water (2.9 TU, Table 5). The concentration of unionized ammonia had increased from 0.018 mg/L in the initial interstitial water test to 2.47 mg/L in the second test. The interstitial water unionized ammonia concentration in the baseline of the TIE was 2.52 mg/L and was higher than the LC50 (2.40 mg/L, MPSL unpublished data). The zeolite column and air stripping treatments both reduced the unionized ammonia below the LC50. Treatment with the zeolite column reduced the toxicity to 1.3 TU, while air stripping did not reduce toxicity. Increased reduction of toxicity with the zeolite column beyond that caused by ammonia could be partially due to sorption of other contaminants on the column. Passing the sample through the HLB column did not reduce toxicity in the post-column sample (PCS), but the HLB eluate was also toxic, indicating toxic organic contaminants were removed from the sample onto the column. The unionized ammonia concentration in the post-HLB column sample was 2.07 mg/L, and could have contributed to the observed toxicity. It is also possible that the organic contaminants were not sufficiently bound by the HLB column and there was breakthrough into the post column treatment. There was no reduction of toxicity with carboxylesterase, indicating that pyrethroids did not contribute to toxicity.

Table 5. Toxic unit (TU) and ammonia results for the first interstitial water TIE. PCS indicates post-column sample.

Treatment	Dilution Toxic Units	Ammonia (mg/L) Total	Unionized
Baseline	2.9	65.6	2.52
Zeolite	1.3	12.9	0.34
Air Strip	2.9	32.2	0.93
HLB PCS	2.8	62.9	2.07
HLB Eluate	1.5		
HLB Zeolite PCS	1.8	17.2	0.49
HLB Zeolite Eluate	2.8		
Air Strip HLB PCS	1.4	26.6	0.98
Air Strip HLB Eluate	<1		
Carboxylesterase	2.8	50.9	1.79
Bovine Serum Albumin (BSA)	3.3	61	2.10

Partial reduction of toxicity with the zeolite column, and the presence of toxicity in the HLB eluate characterize the cause of toxicity as a combination of unionized ammonia and organic contaminant(s). Samples that have evidence of mixtures of toxic concentrations of unionized ammonia and organic compounds require steps to separate these constituents. To do this, treatments are conducted sequentially to remove each class of contaminant. One sample was first passed through the HLB column to remove organic contaminants, and then subjected to the zeolite treatment to reduce ammonia. Another sub-sample underwent the air stripping treatment before being passed through the HLB column. The sequential treatments both reduced toxicity, but did not completely remove it. Unionized ammonia concentrations were below the LC50, so it was assumed that there was some contaminant breakthrough in the HLB column. Column breakthrough can occur when the binding capacity of the column is overwhelmed by the amount of contaminants in the sample.

The HLB column eluate treatments provide further characterization of the chemicals responsible for toxicity. In this procedure, contaminants that are bound to the column are eluted with solvent and mixed with water to create the eluate treatment to which the amphipods are exposed. Reduction of toxicity with the column and return of toxicity with the eluate indicate that organic chemical(s) that are bound to the column are causing toxicity. In the Phase II TIE,

chemical analysis of the eluate is then used to identify the contaminant causing toxicity. After eluting the HLB columns with acetone, both the individual HLB eluate and the HLB-zeolite eluate contained 1.5 TU (Table 5), indicating that an organic contaminant (or mixture) was eluted from the column. The air stripping-HLB eluate was not toxic. In the case of this sequential treatment, the ammonia removal step was performed prior to treatment with the HLB column. It is possible that the pH adjustment procedure used in the air stripping treatment altered the chemistry of the organics in the sample and reduced their toxicity.

The second interstitial water TIE was designed to address ammonia toxicity and to reduce toxicity caused by organics with two additional HLB column treatments. Zeolite was used as a post-HLB column treatment to remove ammonia remaining after HLB treatment. The first treatment was identical to the HLB column in the first interstitial water TIE. The second HLB treatment utilized two HLB columns in sequence, and the third utilized three HLB columns in sequence. The assumption was that the load of organic chemicals present in the Mission Creek interstitial water overwhelmed the capacity of individual HLB columns. The sequential HLB treatments were intended to increase the HLB binding capacity to reduce organic chemical loading to below toxic thresholds. All of the columns within each treatment were eluted and the solvent fractions were combined to provide a concentrated eluate treatment. Because of the small volumes of interstitial water remaining, treatments were only conducted on undiluted interstitial water, and therefore, TUs were not calculated. Median lethal times (LT50s) were calculated to depict the number of days it took for half of the organisms to die in a given treatment. The concentration of total ammonia in the interstitial water was over twice that of the previous TIE and the unionized ammonia concentration was 50% higher (Table 6). Sample dissolved oxygen was increased and maintained using the same procedure described above. Neither the individual HLB column nor the zeolite treatment reduced toxicity, although the zeolite reduced the concentration of unionized ammonia below the LC50. These results characterize the cause of toxicity as a combination of ammonia and organic chemical and were consistent with the first interstitial water TIE. The sequential HLB column and zeolite treatments were not able to remove toxicity, but increasing the number of columns the sample passed through increased the LT50. This result indicated that additional organic contaminants were removed with the additional HLB columns and this increased the time it took for mortality to occur (i.e., toxicity decreased). Although it appears organic contaminants were removed with

the HLB columns, only the second of the three column eluate treatments was toxic. It is not clear why the other two eluates were not toxic.

Table 6. Percent survival and ammonia results for the second interstitial water TIE. PCS indicates post-column sample. LT50 indicates medial lethal time in days.

	Percent	Ammonia (mg/L)		
Treatment	Survival	Total	Unionized	LT50
Baseline	0	132.4	3.64	0.50
HLB PCS	0	127.4	6.98	0.50
HLB Eluate	80	NA		>10
Zeolite PCS	0	15.2	0.68	2.08
HLB Zeolite 1 PCS	0	21.8	0.90	2.13
HLB Zeolite 1 Eluate	100	NA		>10
HLB Zeolite 2 PCS	0	28.4	1.12	2.25
HLB Zeolite 2 Eluate	0	NA		3.50
HLB Zeolite 3 PCS	0	27.7	0.42	3.75
HLB Zeolite 3 Eluate	100	NA		>10

Sediment Chemistry

The whole sediment and interstitial water TIEs characterized the cause of toxicity as a combination of ammonia and organic contaminants. Ammonia concentrations measured in the overlying water of the whole sediment initial test and TIE were not high enough to contribute significantly to toxicity, but increasing concentrations of interstitial water ammonia contributed to the toxicity of that matrix. The interstitial water unionized ammonia concentrations were reduced to non-toxic concentrations using zeolite and air stripping, but toxicity was not completely removed. These results indicate that an organic contaminant contributed to toxicity. The concentrations of contaminants in the whole sediment, interstitial water, and in the Amberlite and HLB eluate treatments were used to provide additional evidence for and against the likely causes of toxicity in the sample.

There are few sediment toxicity threshold values available specifically for *E. estuarius*. In the absence of organism-specific thresholds, LC50s for other amphipods or guideline values [e.g., ERMs, (Long *et al.*, 1995)] were used to evaluate sediment contaminant concentrations. Of the metals measured, the concentrations of cadmium and copper did not exceed LC50 values

for *Rhepoxynius abronius* and *E. estuarius*, respectively, but the concentration of zinc did exceed the *R. abronius* LC50. Only the sediment concentrations of lead, nickel and zinc exceeded their respective ERM values. Although the concentration of zinc was higher than the *R. abronius* LC50 and ERM, the whole sediment TIE results did not suggest metals were the cause of toxicity (Table A1, Appendix). Of the other analyte groups evaluated, no chemicals were present at sufficiently high concentrations to account for the observed toxicity.

Two pyrethroid pesticides were detected in the sediment, but the concentrations were well below published LC50 values. The organophosphate pesticide chlorpyrifos was detected at greater than half its LC50 value and could have contributed to toxicity, but when expressed in terms of organic carbon content the concentration is only 7% of the organic carbon normalized LC50 for *E. estuarius*. The sediment contained a large amount of leafy material that contributed to the organic carbon content. While organic carbon can affect the bioavailability of sediment contaminants, the type of organic carbon also plays a role. Leaf litter is less likely to bind hydrophobic organic contaminants than humified plant materials. Therefore, the toxicity of chlorpyrifos might be underestimated by the organic carbon normalized LC50 (Gunnarson *et al.*, 1999). Concentrations of organochlorine pesticides and polychlorinated biphenyls were also below guideline values. There are no sediment LC50s or guideline values for any of the PBDE congeners; therefore we were unable to evaluate their sediment concentrations directly. Most studies of PDBE emphasize their potential to bioaccumulate rather than cause acute toxicity. One reported PDBE toxicity study with the freshwater oligochaete, *Lumbriculus variegates,* used sediment spiked with as much as 1600 ng/g total PDBE (Ciparis and Hale, 2005). No other relevant acute toxicity data were available for PBDEs. Although sensitivity of *L. variegatus* is not directly comparable to *E. estuarius*, because the former species is notoriously insensitive to sediment contaminants, the total concentration of PBDE in the Mission Creek sediment was 136.7 ng/g and it is unlikely this concentration was acutely toxic to *E. estuarius*. However, since PBDEs are emerging chemicals of concern in the Estuary, dose-response data for selected compounds from this class of chemicals would be helpful to conclusively demonstrate their potential for toxicity to RMP test organisms.

Individually, the sediment concentrations of PAHs were below guideline values (Table A1). The concentration of fluoranthene was compared to the LC50 value previously generated at MPSL (85,300 ng/g) and was well below this concentration (Anderson *et al.*, 2008). The

concentration of phenanthrene was also below the LC50 value for *R. abronius* (Swartz *et al.*, 1989). Although the PAH concentrations were generally low, the frequency of PAH detections in this sample suggested that a mixture of these chemicals could have contributed to toxicity. Whole sediment PAHs were summed and evaluated using sediment quality guidelines (Long *et al.*, 1995), the organic-carbon normalized Threshold Effect Concentration (TEC) derived by Swartz (Swartz, 1999), and the total PAH LC50 value developed for the marine amphipod *R. abronius* (Page *et al.*, 2002). In addition to these threshold concentrations, several models were used to evaluate whole sediment PAH toxicity. The number of toxic units contributed by PAHs was calculated based on the target lipid model ((Di Toro, 2000); Joy McGrath, HydroQual Inc., Mahwah, NJ, U.S., personal communication), and the equilibrium partitioning sediment benchmark model (USEPA, 2003).

The concentrations for low molecular weight PAHs, high molecular weight PAHs, and total PAHs were below the ERM values, and the total organic-carbon corrected TEC (Table 7). While the calculations for these guidelines only use PAH parent compounds, the total PAH calculation Page *et al.* (2002) used to establish an LC50 for *Rhepoxynius abronius* includes many of the alkylated compounds. The concentration of total PAH in the Mission Creek sediment was 1.6 times that of the *R. abronius* LC50. The target lipid model also takes into account the concentrations of the alkylated compounds, but this model predicted that PAHs would contribute only a small portion of the observed whole sediment and interstitial water toxicity (0.040 TU). The U.S. EPA equilibrium partitioning sediment benchmark model also predicted minimal toxicity due to sediment PAHs (0.326 TU, Table 8). The various lines of evidence from the whole sediment chemical analysis are discussed below.

Chemical mixtures were also evaluated using a sediment quality guideline quotient [SQGQ (Fairey *et al.*, 2001)]. SQGQs are calculated by dividing the concentrations of various chemicals and chemical classes by their individual guidelines, and then summing the quotients. Fairey *et al.* (2001) evaluated a number of quotient values and the SQGQ derived by these authors provided the strongest correlation with amphipod mortality in laboratory tests of field samples. Using this method, we calculated a SQGQ value of 21.3 for the Mission Creek sediment. This quotient was largely driven by total chlordane, which accounted for approximately 70% of the total quotient value (without chlordane the quotient was 6.9). A mixture with a quotient greater than 3.5 would be predicted to cause significant toxicity (Fairey

et al., 2001). There is no toxicity threshold for total chlordane, but Stransky *et al.* (Stransky *et al.*, 2006) observed no effect at 49 ng/g, the highest concentration tested. The concentration of chlordane in Mission Creek sediment was 86 ng/g.

Table 7. Summation and evaluation of PAH concentrations. LMW indicates low molecular weight and HMW indicates high molecular weight. ERM indicates effect range median. TEC indicates threshold effect concentration. OC indicates organic carbon. TU indicates toxic units. ESB indicates equilibrium partitioning sediment benchmark.

Summation	Threshold	Concentration	Reference
Sediment LMW ERM (ng/g)	3160	988	(Long *et al.*, 1995)
Sediment HMW ERM (ng/g)	9600	5225	(Long *et al.*, 1995)
Sediment Total ERM (ng/g)	44792	6213	(Long *et al.*, 1995)
Sediment TEC (µg/g oc)	290	97.1	(Swartz, 1999)
R. abronius Sediment LC50 (ng/g)	10750	17298	(Page *et al.*, 2002)
Target Lipid Model Sediment (TU)	>1	0.040	(Di Toro, 2000)
Target Lipid Model Interstitial (TU)	>1	0.029	(Di Toro, 2000)
ESB Toxic Units (TU)	>1	0.326	(USEPA, 2003)
ESB Lipid Conc. Range (mmol/g lipid)	15-75	18.7	(Hawthorne *et al.*, 2007)

Interstitial Water and Eluate Chemistry

Chemicals measured in the TIE eluates and interstitial water were compared to water-only LC50 values for *E. estuarius* as part of the second phase of the TIE (identification). As described above, the Amberlite eluate was prepared by sieving the resin from the whole sediment at the end of the exposure, eluting a portion of the resin with acetone, and combining the acetone with water. HLB eluates were prepared in a similar manner in the interstitial water TIE. Table A2 (Appendix) summarizes the chemical concentrations in the eluate treatments. The concentration of cypermethrin was not within the range of *E. estuarius* LC50s reported by Ernst *et al.* (2001), but the concentrations of bifenthrin and permethrin were greater than the *Hyalella azteca* LC50s reported by Anderson *et al.* (2006). *H. azteca* have been shown to have similar sensitivities to some pyrethroid pesticides as *E. estuarius* (Amweg *et al.*, 2005; Anderson *et al.*, 2008). However, it is unlikely that pyrethroids contributed significantly to toxicity of the Mission Creek sediment because although they were detected, their whole-sediment concentrations were below the *E. estuarius* toxicity thresholds. Recent studies have demonstrated that chemicals measured in the Amberlite eluate can overestimate the bioavailable

concentration of chemical present in the original sediment because, during the TIE, the resin is in contact with the sediment for approximately twelve days. During this time, the equilibrium of the sediment is affected by the presence of the resin. Data suggest the resin sorbs chemicals in the sediment interstitial water driving the chemical equilibrium and concentrating compounds in the eluate (Phillips *et al.*, 2009). Similarly, the concentration of fipronil in the Amberlite eluate was in the range of toxicity for some invertebrate test species, but because no fipronil was detected in the sediment, it is unlikely this chemical contributed to toxicity. It is likely the pyrethroids and fipronil were present in the Mission Creek sediment but their concentrations were below their respective analytical detection limits. The concentration of chlorpyrifos in the sediment was approximately half the LC50, but chlorpyrifos was not detected in the eluate treatments. The Amberlite eluate concentration of Aroclor 1254 was well below the LC50 for *Ampelisca abdita* (Ho *et al.*, 1997). All other above-mentioned chemical classes were not detected in the HLB column eluate and were not analyzed in the interstitial water.

As was the case with the sediment, PAHs were the most commonly detected analyte in the Amberlite eluate and in the interstitial water. However, no PAHs were detected in the HLB column eluate (Table 9, Appendix). Published LC50 values for acenaphthene and phenanthrene (Swartz *et al.*, 1995) and the *E. estuarius* LC50 for fluoranthene (current study) were used to determine whether these compounds were present at toxic concentrations in Mission Creek samples. Individual measured concentrations in the Amberlite eluate and interstitial water were well below the LC50s. There are no threshold values for summed PAHs in a water matrix. The target lipid model was used to evaluate total PAHs in interstitial water (Di Toro, 2000). Based on an assumed dissolved organic carbon concentration of 50 mg/L, the model calculated the contribution of PAHs to interstitial water toxicity to be 0.029 TU (Table 8). This value was similar to the number of TUs predicted from the whole sediment PAH concentrations using the same model and based on the measured sediment TOC (0.040 TU). Because the model calculates LC50 values for *E. estuarius*, the measured interstitial water concentrations can also be compared directly to these values, and TUs can be calculated and summed. The results of a direct comparison that does not take organic carbon concentration into account suggested that PAHs contributed approximately one TU to the overall toxicity. The target lipid model was also used to calculate the contribution of PAHs to toxicity based on the Amberlite eluate concentrations. The total PAH in the Amberlite eluate contributed approximately 14 TU to the

eluate toxicity. As discussed above, concentrations of PAHs in the Amberlite eluate likely over-estimate their concentrations in the interstitial water because the Amberlite influences the chemical equilibrium during the exposure (Phillips *et al.*, 2009). It is not known how much these concentrations might have been overestimated in the current study.

The equilibrium partitioning model was used to predict the lipid concentration of total PAHs in the amphipods based on the interstitial water concentrations (USEPA, 2003; Hawthorne *et al.*, 2007). This concentration can be used to predict toxicity based on known toxic responses to a range of concentrations. Hawthorne *et al.* (2007) predicted a range of lipid-associated PAH concentrations that corresponded to the survival of the freshwater amphipod *H. azteca*. Their concentrations ranged from 15 mmol/g lipid (85% survival) to 75 mmol/g lipid (15% survival). Assuming *H. azteca* and *E. estuarius* respond similarly to PAHs, the predicted lipid-associated PAH concentration of 18 mmol/g lipid in *E. estuarius* was at the low end of the toxicity range.

Weight of Evidence

Percent survival in the initial test with Mission Creek sediment was 48% and the survival in the baseline (untreated sample) of the whole sediment TIE was 20%. The total ammonia concentration in the overlying water of the TIE baseline had increased twofold from that of the initial test. A similar increase in ammonia concentrations were observed among the four interstitial water tests. While ammonia probably did not significantly contribute to toxicity in the initial tests, it became a factor in subsequent tests, particularly the interstitial TIEs. The overlying water unionized ammonia concentration in the whole sediment TIE was below the toxicity threshold, but toxicity was still observed in the baseline, and was removed by treatments that reduce the bioavailability of organic contaminants. There were no lines of evidence suggesting metals were responsible for Mission Creek sediment toxicity.

Review of the organic chemistry data did not provide conclusive evidence of the organic chemical(s) causing toxicity, but did suggest that PAHs played a role. The lines of evidence for PAH toxicity include the total PAH concentration exceeding the *R. abronius* LC50, the sum of the interstitial water toxic units based on the individual LC50s using the target lipid model, and the sum of the predicted lipid concentrations based on the equilibrium partitioning sediment benchmark model falling within the range predicted to be toxic to *H. azteca*. There are other established ways to interpret the data to suggest that PAHs played only a partial role in toxicity

of this sediment. The concentrations of high and low molecular weight PAHs and total PAHs were well below ERM concentrations, and the total organic-carbon corrected concentration was below the threshold effect concentration of Swartz *et al.* (1999). The target lipid model and equilibrium partitioning sediment benchmark toxic unit model also predicted that PAHs would only make a small contribution to toxicity.

Dose Response Experiments

All controls in the dose response experiments had greater than 90% survival. Two concentrations of copper were measured in each definitive test and demonstrated that the relative percent difference between the measured and nominal concentrations was <1.3%. The calculated LC50s based on nominal concentrations were 38.7 mg/L and 58.6 mg/L (mean = 48.7 ± 14 SD mg/L). The fluoranthene rangefinder test produced a nominal LC50 of 482 µg/L, and the first two definitive tests produced nominal LC50s of 793 and 852 µg/L. Using the measured concentrations in the third definitive test produced an LC50 of 671 µg/L. The water only copper LC50 demonstrates *E. estuarius* is very tolerant of copper, and this observation supports evidence against metal toxicity in the Mission Creek sample. The fluoranthene LC50 was used to evaluate toxicity of this PAH in the Mission Creek interstitial water and also indicates toxicity was not caused by this compound.

Discussion

In the current study, fourteen samples were collected based on the results of previous toxicity studies, including two samples at San Mateo Creek, but only one of the fourteen samples was sufficiently toxic to justify conducting a TIE (<50% survival). Mission Creek did not meet the criterion for significant difference from the control ($p < 0.01$) because of high variability among the replicate samples, but did have the highest magnitude of toxicity and was therefore the most likely candidate for a TIE. Three other samples were significantly toxic, but ranged from 64% to 76% survival and did not have a strong enough response to warrant a TIE. Given the preponderance of evidence that these sites are among the most toxic in the Estuary, the lack of toxicity in these samples was striking. The majority of the samples were collected within a short period following storm events when elevated toxicity was expected. The current results demonstrate the temporally variable nature of sediment toxicity in the margins of the Estuary.

Given that some of these sites had demonstrated consistent toxicity in previous studies, it is not clear whether the current lack of toxicity represents a temporary change in the chemistry of these sediments, or a more permanent reduction in chemical contamination at these sites. This can only be answered by conducting additional seasonal investigations at these sites.

Although the survival in the initial test for Mission Creek was 48%, baseline survival in the subsequent whole sediment TIE was 20% with less between-replicate variability. This higher magnitude of toxicity provided greater resolution for the TIE treatments. The concentration of ammonia increased with every test conducted, and it appeared that ammonia contributed to this increase in toxicity. The whole sediment TIE characterized organic contaminants as another cause of sediment toxicity. Toxicity in the Phase II Amberlite eluate further characterized the cause as organic, but analysis of the resin eluate did not positively identify the class of organics causing toxicity.

While several lines of evidence suggest PAHs are at least partially responsible, the lack of conclusive evidence for the cause of toxicity in Mission Creek could be due to several possibilities. One is that the TIE and analytical chemical methods require further refinement. The TIE methods used in this study indicated that the cause of toxicity in the complex sediment mixture as due to organic chemicals, but the methods did not conclusively identify the specific compound(s) responsible for toxicity. The results suggest that further development of the Phase II procedures is necessary. In the whole sediment TIEs the Amberlite eluate is used as a Phase II treatment. This treatment is currently considered to provide a qualitative rather than a quantitative line of evidence because the relationship between contaminant concentrations in the whole sediment, the interstitial water, and the Amberlite eluate are not completely understood (Anderson *et al.*, 2007). Evidence suggests that the resin can act like a sink for sediment chemicals, driving the equilibrium of chemicals from the sediment to the resin. When the resin is eluted, the bioavailable concentration of contaminants (i.e., the concentration present in the untreated sediment's interstitial water) may be overestimated (Phillips *et al.*, 2009). Determining the optimal mass of resin used, the most appropriate equilibration time for resin exposure, and the optimum resin type will improve the use of carbonaceous resins in whole sediment TIEs, and make the results of Phase II elution steps in the TIE more quantitative.

Solvent fractionation of resin eluates using high pressure liquid chromatography (HPLC) has also been employed as a method to separate compounds having variable solubilities

(USEPA, 1993a) in Phase II TIE development using resins. The HPLC method fractionates the resin solvent into thirty sub-samples that can be individually tested and analyzed. Less polar contaminants appear in the first fractions while more polar contaminants elute in the later fractions. Once toxic fractions are identified, they can be chemically analyzed. This method for eluate fractionation provided mixed results, but refinement of this method may allow separation of chemicals when they are present in complex mixture (Anderson *et al.*, 2007b).

Further development of the Phase II interstitial water procedures is necessary. Significant toxicity was observed in only half of the HLB column eluate treatments indicating that the columns were not eluting consistently. In addition to the columns that were prepared for toxicity testing, a column was prepared for chemical analysis. Based on the fact that there were no detected chemicals in the column eluates, and the inconsistent toxicity observed in some of the other eluate treatments, it is possible that contaminants were not completely eluted from the columns, or were lost during cleanup steps prior to the chromatographic analyses. The columns are currently eluted using the method employed by USGS (Kelly Smalling, USGS Sacramento, CA, personal communication), so it is unlikely that the method alone is to blame for inconsistent elution. Given that there was incomplete removal of toxicity with the HLB column, it is also possible that the flow rate of interstitial water through the column did not provide sufficient contact time for removal of all contaminants for the water. Additional studies in this area should include testing additional methods for removing contaminants from interstitial water matrix to allow sufficient equilibration between interstitial water and the extraction media to maximize extraction of chemicals. In addition, all steps used in the analytical procedures should be reviewed to make sure they are appropriate for the chemical classes present in interstitial waters.

Another possible reason for lack of conclusive confirmation of the cause of toxicity is that toxicity may have been due to unmeasured chemicals. TIEs were originally developed to determine the cause of toxicity in municipal and industrial effluents. In a toxic effluent sample there are usually a minimal number of contaminants responsible for toxicity. Ambient sediments usually contain highly complex mixtures of chemicals, and there can be multiple contaminants and breakdown products contributing to toxicity. There are millions of registered organic contaminants, but monitoring programs only focus on the contaminants with regulatory benchmarks (Hoenicke *et al.*, 2007). It is time-consuming and expensive to monitor these additional chemicals, so toxicity testing is used to determine their potential impact by measuring

the bioavailable fraction with the health of a test organism. Once an impact on the organism is observed it is the goal of the TIE to identify the cause. Because of the unknown toxicity of many of the chemicals in sediments, it is sometimes difficult to progress beyond the characterization phase of the TIE. While efforts are being made to define the priorities for routine monitoring (Oros *et al.*, 2003; Hoenicke *et al.*, 2007), relative toxicities of emergent chemicals (e.g., pesticides, PBDEs) must also be determined using spiking studies.

A final possibility is that toxicity of this sample was due to a mixture of chemicals. As mentioned above, several studies have suggested that chemical mixtures in San Francisco Estuary sediments are strongly correlated with amphipod mortality in laboratory toxicity tests (e.g., Thompson *et al.*, 1999, Hunt *et al.*, 2001). The calculated SQGQ for Mission Creek sediment was 21.3, but 70% of this quotient was contributed by chlordane. Without chlordane the SQGQ was 6.9 and demonstrates a strong chemical mixture. The total chlordane concentration was approximately 14 times the ERM, and was about twice the highest concentration tested by Stransky *et al.* (2006) in which no effect was observed. Thompson *et al.* (1999) observed a significant relationship between chlordane concentrations and toxicity in RMP sediment from the North Bay, but because there is not a definitive *E. estuarius* toxicity threshold for chlordane, it is difficult to link the observed toxicity in Mission Creek sediment to this chemical.

Future TIE studies should proceed in several directions. First, there is a need to build a database of toxicity thresholds for estuarine species for selected current and emerging contaminants. This should include emerging pesticides such fipronil, selected PAHs, legacy pesticides such as chlordane, and other newly identified contaminants of concern. There is also a need for additional TIE method development to improve the efficacy of the Phase II Amberlite resin treatment, and interstitial water extraction and elution methods. These research needs can be met more efficiently using integrated studies that combine dose response experiments using spiked sediments with whole sediment and interstitial water method development experiments. Chemical analysis of spiked sediments will allow for the calculation of LC50s based on measured concentrations, and will provide confirmation of chemical removal and mass balance relationships necessary to assess efficacy of the TIE methods.

Recent results have suggested that the 10d Amberlite equilibration provides an exhaustive treatment. Over this equilibration period, the resin adsorbs the bioavailable fraction

of organic chemicals from the interstitial water then continues to drive the equilibrium between sediment and interstitial water as it sorbs the slowly desorbing fraction of residual chemical from the sediment via the interstitial water. The elution of the Amberlite resin at the termination of the 10d exposure likely provides an overestimation of the bioavailable fraction of chemical in the sediment (Phillips *et al.*, 2009). Measuring the rapidly desorbing fraction of chemical in sediments may provide a better estimate of the bioavailable fraction (Cornelissen *et al.*, 2001; Leppanen *et al.*, 2003). Although the optimal exposure duration is dependent on the chemicals present and the exposure scenario, employment of a short-term Amberlite treatment, in addition to the exhaustive Amberlite treatment, could provide an estimate of bioavailability. Rather than eluting the Amberlite from the exhaustive treatment, the Amberlite from a 24-hour exposure will be eluted, tested and analyzed. Analysis of chemicals in the interstitial water using solid-phase micro-extraction (SPME) may also be used to compare the bioavailable fraction of chemical in this matrix, to the concentration of chemical eluted after the 24h Amberlite equilibration. For interstitial water, the addition of Amberlite directly to the sample will provide an exhaustive treatment that can be separated and eluted as a Phase II treatment. This treatment will hopefully overcome variables associated with adsorption and elution of chemicals that have been observed in the process of extracting interstitial water with the HLB column. Elution of chemical from the interstitial water Amberlite treatment may also be compared to the SPME and whole-sediment Amberlite eluates.

Results from the current study and past RMP status and trends monitoring has shown that many sediments in the Estuary are characterized as being contaminated with complex chemical mixtures resulting in a low but significant magnitude of toxicity. TIE methods developed to date are not sufficiently robust to resolve toxicity of weakly or moderately toxic sediments. To improve TIE resolution of moderately toxic sediments, new approaches for measuring sublethal effects in standard test organisms might be incorporated into the TIE process. One example of sublethal indicators of toxicity uses gene microarrays (Larkin *et al.*, 2007). Analyses of surviving amphipods using gene microarrays after exposure to moderately toxic sediments might provide more sensitive endpoints that can be indicative of exposures to specific classes of chemicals. Researchers at UC Berkeley, in collaboration with the Southern California Coastal Water Research Project are in the preliminary stages of developing a gene microarray using *E. estuarius*. Surviving amphipods from the spiking and TIE development experiments described

above can be used to provide animals for development of microarray endpoints that are indicators of response to specific classes of chemicals. These endpoints can then be applied to sediment TIEs to provide more sensitive tools for assessing toxicity of moderately toxic sediments.

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Appendix - Analytical Chemistry Results

Table A1. Concentrations of detected chemicals in Mission Creek sediment. Detected concentrations were compared to evaluation concentrations including LC50s (medial lethal concentration) and NOECs (no observed effect concentration) for *E. estuarius* or alternate species, and sediment quality guideline values such as ERMs (effects range median concentration) (Long *et al.*, 1995)and PECs (probable effects concentration) (Macdonald *et al.*, 2000). SQG = sediment quality guideline. OC = organic carbon. DNQ = detected not quantified. Italicized PAHs refer to alkylated compounds.

Analyte	Sediment Conc.	Evaluation Conc.	Evaluation Type	Reference	SQG Conc. and Type
Total Organic Carbon (%TOC)	6.4				
Metals (µg/g)					
Aluminum	67500				
Antimony	3.55				25 ERM
Arsenic	9.14				70 ERM
Barium	148				
Beryllium	0.210				
Cadmium	1.85	9.81	*R. Abronius* LC50	(Mearns *et al.*, 1986)	9.6 ERM
Chromium	70.8				370 ERM
Cobalt	8.82				
Copper	173	534	LC50	(Anderson *et al.*, 2008)	270 ERM
Iron	103				
Lead	251				218 ERM
Manganese	171				
Mercury	DNQ	13.1	*R. Abronius* LC50	(Swartz *et al.*, 1988)	
Molybdenum	7.80				
Nickel	58.7				51.6 ERM
Selenium	1.47				
Silver	0.680				3.7 ERM
Strontium	58.5				
Thallium	0.210				
Tin	15.7				
Titanium	593				
Vanadium	57.7				
Zinc	472	276	*R. Abronius* LC50	(Swartz *et al.*, 1988)	410 ERM
Pyrethroid Pesticides (ng/g)					
Cyfluthrin	DNQ	13.7	*H. azteca* LC50	(Amweg *et al.*, 2005)	
Esfenvalerate/Fenvalerate	4.11	41.8	*H. azteca* LC50	(Amweg *et al.*, 2005)	
Esfenvalerate/Fenvalerate µg/g oc	0.064 ug/g oc	1.54 ug/g oc	*H. azteca* LC50	(Amweg *et al.*, 2005)	
Permethrin	8.26	140	LC50	(Anderson *et al.*, 2008)	
Organophosphates (ng/g)					
Chlorpyrifos	64.7	103	LC50	(Anderson *et al.*,	

Analyte	Sediment Conc.	Evaluation Conc.	Evaluation Type	Reference	SQG Conc. and Type
				2008)	
Organochlorines (ng/g)					
Chlordane, cis-	29.8				
Chlordane, trans-	35.5				
DDD(o,p')	8.18				
DDD(p,p')	67.3				
DDE(p,p')	14.2				27 ERM
DDT(o,p')	DNQ				
DDT(p,p')	DNQ	49.5	*R. abronius* LC50	(Word *et al.*, 1987)	
Dieldrin	3.78				8 ERM
Nonachlor, cis-	5.88				
Nonachlor, trans-	14.8				
Total Chlordane	85.98	>49	NOEC	(Stransky *et al.*, 2006)	6 ERM
Total DDT	89.68	554	LC50	(Weston, 1996)	46.1 ERM
Total DDT ug/g oc	1.40 ug/g oc	2500 ug/g oc	LC50	(Swartz *et al.*, 1994)	
Polychlorinated Biphenyls (ng/g)					
PCB 008	DNQ				
PCB 018*	1.18				
PCB 028*	3.37				
PCB 031	2.69				
PCB 033	1.78				
PCB 044*	7.67				
PCB 049	8.98				
PCB 052*	16.4				
PCB 056	2.78				
PCB 060	1.36				
PCB 064	1.41				
PCB 066*	7.71				
PCB 070	13.8				
PCB 074	4.04				
PCB 077	1.25				
PCB 087	14.4				
PCB 095	25.9				
PCB 097	10.8				
PCB 099	17.7				
PCB 101*	42.8				
PCB 105*	13.3				
PCB 110	40.4				
PCB 114	0.665				
PCB 118*	34.5				
PCB 126	DNQ				
PCB 128*	9.43				
PCB 137	2.29				
PCB 138*	47.9				
PCB 141	11.3				
PCB 146	4.45				
PCB 149	44.6				
PCB 151	10.9				
PCB 153*	58.9				

Analyte	Sediment Conc.	Evaluation Conc.	Evaluation Type	Reference	SQG Conc. and Type
PCB 156	5.35				
PCB 157	1.02				
PCB 158	7.43				
PCB 170*	12.7				
PCB 174	14.6				
PCB 177	8.06				
PCB 180*	31.4				
PCB 183	8.42				
PCB 187*	19.2				
PCB 189	0.530				
PCB 194	6.79				
PCB 195*	2.71				
PCB 198/199	0.982				
PCB 200	1.11				
PCB 201	8.03				
PCB 203	9.04				
PCB 206*	2.98				
PCB 209*	0.996				
PCB AROCLOR 1248	72.0				
PCB AROCLOR 1254	490	10800	*R. Abronius* LC50	(Swartz *et al.*, 1988)	
PCB AROCLOR 1260	300				
Total PCBs (*18 Congeners)	313				400 PEC
Polybrominated Diphenyl Ethers (ng/g)					
PBDE 047	43.3	NONE			
PBDE 085	3.57				
PBDE 099	63.6				
PBDE 100	12.6				
PBDE 153	7.17				
PBDE 154	6.49				
Polycyclic Aromatic Hydrocarbons (ng/g)					
Naphthalene	95.7				2100 ERM
Methylnaphthalene, 2-	103				670 ERM
Methylnaphthalene, 1-	47.9				
Dimethylnaphthalene, 2,6-	160				
Trimethylnaphthalene, 2,3,5-	14.2				
Naphthalenes, C1 -	157				
Naphthalenes, C2 -	492				
Naphthalenes, C3 -	247				
Naphthalenes, C4 -	120				
Biphenyl	28.1				
Acenaphthylene	37.4				640 ERM
Acenaphthene	35.6				500 ERM
Fluorene	58.7				540 ERM
Methylfluorene, 1-	34.5				
Fluorenes, C1 -	95.6				
Fluorenes, C2 -	238				
Fluorenes, C3 -	516				
Dibenzothiophene	36.0				
Methyldibenzothiophene, 4-	37.7				
Dibenzothiophenes, C1 -	90.4				

Analyte	Sediment Conc.	Evaluation Conc.	Evaluation Type	Reference	SQG Conc. and Type
Dibenzothiophenes, C2 -	337				
Dibenzothiophenes, C3 -	386				
Phenanthrene	585	3680	*R. abronius* LC50	(Swartz *et al.*, 1989)	1500 ERM
Methylphenanthrene, 1-	98.8				
Dimethylphenanthrene, 3,6-	70.2				
Phenanthrene/Anthracene, C1 -	729				
Phenanthrene/Anthracene, C2 -	818				
Phenanthrene/Anthracene, C3 -	712				
Phenanthrene/Anthracene, C4 -	507				
Anthracene	175				1100 ERM
Fluoranthene	1150	85300	LC50	(Anderson *et al.*, 2008)	
Methylfluoranthene, 2-	136				
Fluoranthene/Pyrenes, C1 -	1400				
Pyrene	1142				2600 ERM
Benz(a)anthracene	536				1600 ERM
Chrysene	562				2800 ERM
Chrysenes, C1 -	890				
Chrysenes, C2 -	766				
Chrysenes, C3 -	422				
Benzo(b)fluoranthene	931				
Benzo(k)fluoranthene	247				
Benzo(e)pyrene	620				
Benzo(a)pyrene	658				1600 ERM
Perylene	170				
Indeno(1,2,3-c,d)pyrene	573				
Dibenz(a,h)anthracene	224				260 ERM
Benzo(g,h,i)perylene	512				

Table A2. Concentrations of detected chemicals in the Amberlite eluate treatment, interstitial water, and HLB column eluate treatment. Measured concentrations were compared to published LC50 (median lethal concentration) values. NA indicates not analyzed, ND indicates not detected, and <RL indicates below reporting limit.

Analyte	Amberlite Eluate Concentration (ug/L)	Interstitial Water Concentration (ug/L)	HLB Column Eluate Concentration (ug/L)	LC50 (ug/L)	Reference
Pyrethroids					
Bifenthrin	0.063	NA	ND	0.0093	(Anderson *et al.*, 2006)
Cyfluthrin	0.068	NA	ND		
Cypermethrin	0.160	NA	ND	1-3.6	(Ernst *et al.*, 2001)
Esfenvalerate/Fenvalerate	0.085	NA	ND		
Lambda-cyhalothrin	0.455	NA	ND		
Permethrin	0.953	NA	ND	0.0211	(Anderson *et al.*, 2006)
Fipronil	22.7	NA	ND	6.8[1]	(Chandler *et al.*, 2004)
Polychlorinated Biphenyls					
PCB 049	0.020	NA	ND		
PCB 070	0.037	NA	ND		
PCB 097	0.086	NA	ND		
PCB 101	0.037	NA	ND		
PCB 110	0.043	NA	ND		
PCB 149	0.028	NA	ND		
PCB 180	0.016	NA	ND		
PCB 198/199	0.091	NA	ND		
PCB AROCLOR 1254	0.300	NA	ND	40[2]	(Ho *et al.*, 1997)
Polycyclic Aromatic Hydrocarbons					
Naphthalene	5024.8	0.00905	ND		
Methylnaphthalene, 2-	1.8	0.00668	ND		
Methylnaphthalene, 1-	1.1	<RL	ND		
Dimethylnaphthalene, 2,6-	3.5	0.0443	ND		
Trimethylnaphthalene, 2,3,5-	1.1	<RL	ND		
Naphthalenes, C1 -	2.9	0.00867	ND		
Naphthalenes, C2 -	14.2	0.0780	ND		
Naphthalenes, C3 -	28.1	0.196	ND		
Naphthalenes, C4 -	14.9	0.279	ND		
Biphenyl	4.6	0.0226	ND		
Acenaphthylene	<RL	<RL	ND		
Acenaphthene	18.3	0.0243	ND	708	(Swartz *et al.*, 1995)
Fluorene	7.1	0.0228	ND		
Methylfluorene, 1-	3.1	<RL	ND		
Fluorenes, C1 -	10.3	0.0653	ND		
Fluorenes, C2 -	20.7	0.297	ND		
Fluorenes, C3 -	43.6	0.774	ND		
Dibenzothiophene	3.4	0.0165	ND		
Methyldibenzothiophene, 4-	3.6	0.0230	ND		

Analyte	Amberlite Eluate Concentration (ug/L)	Interstitial Water Concentration (ug/L)	HLB Column Eluate Concentration (ug/L)	LC50 (ug/L)	Reference
Dibenzothiophenes, C1 -	7.6	0.0604	ND		
Dibenzothiophenes, C2 -	15.6	0.324	ND		
Dibenzothiophenes, C3 -	10.8	0.432	ND		
Phenanthrene	20.4	0.0702	ND	158	(Swartz *et al.*, 1995)
Methylphenanthrene, 1-	5.5	0.0267	ND		
Dimethylphenanthrene, 3,6-	<RL	0.0401	ND		
Phenanthrene/Anthracene, C1 -	37.6	0.156	ND		
Phenanthrene/Anthracene, C2 -	15.5	2.38	ND		
Phenanthrene/Anthracene, C3 -	25.4	1.39	ND		
Phenanthrene/Anthracene, C4 -	11.2	0.973	ND		
Anthracene	11.6	0.0523	ND		
Fluoranthene	34.1	0.295	ND	671	MPSL (current study)
Methylfluoranthene, 2-	2.8	0.0464	ND		
Fluoranthene/Pyrenes, C1 -	30.0	0.539	ND		
Pyrene	14.9	0.222	ND		
Benz(a)anthracene	4.4	0.0822	ND		
Chrysene	3.5	0.110	ND		
Chrysenes, C1 -	7.3	0.293	ND		
Chrysenes, C2 -	5.2	0.564	ND		
Chrysenes, C3 -	1.8	0.233	ND		
Benzo(b)fluoranthene	5.0	0.101	ND		
Benzo(k)fluoranthene	1.3	0.0279	ND		
Benzo(e)pyrene	13.5	0.0939	ND		
Benzo(a)pyrene	1.4	0.0559	ND		
Perylene	0.7	0.0207	ND		
Indeno(1,2,3-c,d)pyrene	0.6	0.0747	ND		
Dibenz(a,h)anthracene	<RL	0.0386	ND		
Benzo(g,h,i)perylene	1.0	0.106	ND		

[1] LC50 for *Amphiascus tenuiremis*, [2] LC50 for *Ampelisca abdita*