RMP Sediment Toxicity Study 2009-2010

Determining Causes of Sediment Toxicity in the San Francisco Estuary

Final Report

Regional Monitoring Program for Water Quality in the San Francisco Estuary

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Author: Bryn Phillips (UC-Davis, Marine Pollutions Studies Laboratory)
Author: Brian Anderson (UC-Davis, Marine Pollutions Studies Laboratory)
Project Director/Author: Sarah Lowe (San Francisco Estuary Institute)

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Executive Summary

The Five Year Plan developed by the San Francisco Estuary Institute's Exposure Effects Work Group (EEWG) for evaluating risks to benthic biota includes ongoing studies to determine causes of sediment toxicity in the San Francisco Estuary. Toxicity has been observed at a high proportion of Regional Monitoring Program (RMP) stations since the Status and Trends Program began in 1993. Effective management of factors contributing to toxicity depends on determining which specific pollutants or other factors are responsible. In addition, stressor identification is a key element of the State Water Resources Control Board's Sediment Quality Objectives (SQOs) program for enclosed bays and estuaries. While planned SQO assessments will provide information about sediment condition in the Estuary, they are not designed to identify causes of toxicity or associated benthic community impacts. The current study implements components of the EEWG Five Year Plan by (1) providing new thresholds of effects for key contaminants of concern to the Estuary, (2) further developing toxicity identification evaluation (TIE) methods to identify organic contaminant stressors in sediments, and (3) implementing a work group process to coordinate scientists around the state to develop and evaluate new TIE tools.

Median lethal concentrations (LC50s) are often used to interpret the relationship between chemical concentrations and organism response. Twenty sediment and water LC50s have been compiled for *Eohaustorius estuarius*, the amphipod species used for sediment toxicity assessments in the Estuary and the majority of these values have been generated as part of RMP research. Threshold concentrations are still lacking for the majority of contaminants commonly detected in sediments, and this highlights the limitation of using toxicity threshold values as the primary approach to determining causes of sediment toxicity. Despite this limitation, determining the LC50 values for key contaminants of concern in the Estuary aids the interpretation of routine monitoring data, as well as TIE studies. Expanding the database on toxic effects of sediment contaminants is also useful to resource managers throughout California as implementation of the State's SQO program progresses.

Whole sediment and water LC50 values for *E. estuarius* were developed for the legacy organochlorine pesticide trans-chlordane, the polycyclic aromatic hydrocarbon pyrene, and the pyrethroid pesticide cyfluthrin. Chlordane and pyrene were tested in whole sediment at

concentrations that were thousands of times higher than were environmentally relevant without causing toxicity. Final definitive LC50s based on measured sediment concentrations were >31,400 ng/g and >22,200 ng/g for trans-chlordane and pyrene, respectively. Although these two chemicals were tested at concentrations beyond their solubility in water, seven-day LC50s were determined. The seven-day water LC50s for trans-chlordane and pyrene were 224 µg/L and 36.9 µg/L, respectively. The whole sediment LC50 for cyfluthrin was based on corrected concentrations from two definitive tests. The mean ten-day LC50 was 3.23 ng/g, or 0.357 µg/g OC when normalized to total organic carbon content. Two definitive tests were also conducted with cyfluthrin in water. The first test was very sensitive and produced a four-day LC50 of <1.87 ng/L. The second test produced a four-day LC50 of 2.93 ng/L and a seven-day LC50 of 1.70 ng/L.

TIEs are the primary means to identify chemical stressors responsible for sediment toxicity. These procedures are conducted using a phased approach. Phase I treatments characterize toxicity caused by general categories of contaminants: organic chemicals, metals, or ammonia. Phase II TIE treatments are designed to identify the specific contaminant(s) causing toxicity. Phase II TIEs also utilize LC50 values to interpret chemistry data. Phase III TIEs confirm the Phase I and II results. Phase I treatments that reduce toxicity caused by organic chemicals include the addition of extraction media, such as carbonaceous resin or coconut charcoal, added directly to whole sediment, or passing interstitial water through a solid-phase extraction (SPE) column. These treatments remove bioavailable contaminants from the sample. When addition of these amendments or the use of SPE columns reduces whole sediment or interstitial water toxicity, the cause of toxicity is ascribed to organic compounds. Phase II TIE treatments include eluting sorbed chemicals from the media used for organic chemical extraction (e.g., carbonaceous resins, SPE columns). Eluate treatments are prepared by eluting the media with solvents and adding the solvent to clean water. These eluate treatments are then tested to determine whether the original chemicals and their toxicity were recovered. Whole sediment and interstitial water chemistry data are used to provide additional lines of evidence in this process.

Two experiments were conducted to further develop TIE methods. These were conducted on sediment spiked with cyfluthrin as part of the LC50 experiments. In the first experiment a resin

amendment was added to the spiked sediment to reduce toxicity caused by cyfluthrin, and solid-phase microextraction (SPME) fibers were used to quantify the reduction of bioavailable chemicals after extraction resin was added to whole sediment samples. Addition of extraction resin reduced toxicity during the course of the whole sediment exposure. At the termination of the exposure, interstitial water was extracted from the sediment and analyzed for cyfluthrin using SPME. It was hypothesized that the SPME fibers would detect differences in bioavailable cyfluthrin in exposures that had been conducted with and without the extraction resin. The fibers were analyzed after equilibrating with the interstitial water for seven days, but no cyfluthrin was detected. It was determined that the pyrethroid had degraded significantly during the 10-day exposure and 7-day equilibration. Although the SPME experiment was not able to measure the bioavailable fraction of cyfluthrin, other researchers have successfully used SPME to measure bioavailability. Further studies are needed to integrate this measurement into TIEs.

A second set of experiments were conducted to refine procedures used to extract contaminants from interstitial water, and therefore reduce toxicity. The standard method for removing contaminants from interstitial water has historically involved SPE columns. However, extraction efficiency of these columns when used in the TIE context with interstitial water has been inconsistent, particularly in sediments contaminated by pyrethroid pesticides. Several prior studies with SPE columns have resulted in incomplete removal of toxicity and incomplete return of toxicity when the columns are eluted with solvent (Phillips et al., 2009b). In the current study a batch extraction technique for removing cyfluthrin from interstitial water was evaluated. Extraction resin was added directly to interstitial water and equilibrated for 24 hours. Addition of resin reduced the toxicity of interstitial water spiked with cyfluthrin, but the eluate treatment prepared from the extraction resin did not recover the spiked chemical and was not toxic.

Aspects of the current project were continued with a project funded by the Central Valley Regional Water Quality Control Board (Region 5). In the Region 5 project the interstitial water experiments were repeated, but using the freshwater amphipod *Hyalella azteca*. Interstitial water from a reference site on the Carmel River was spiked with the organophosphate pesticide chlorpyrifos, the pyrethroid pesticide bifenthrin, or a combination of both. The goal of the Region 5 project was to optimize the batch extraction process and determine if elution of the

batch extraction media could be accomplished. Three experiments were conducted. In the first experiment the batch extractions were conducted for four hours and 24 hours. Resin from both extraction treatments were then eluted either by using a batch elution method, or by loading the resin in a column and passing solvent through it. The batch extractions were able to successfully reduce the concentration of the spiked chemical and the observed toxicity, but elutions of the resin were unable to recover a significant amount of chemical. The 24-hour batch extraction was repeated in the second experiment, and the resin was eluted with dichloromethane. Additional treatments in this experiment included two SPE columns with standard acetone elutions. All of the extraction media were successful at reducing the toxicity and concentrations of chemical, but the column eluates were more successful at recovering the toxicity and chemical. The third experiment focused exclusively on SPE columns and varied the size of the column and the extraction solvent. All of the columns successfully reduced chemical concentrations and toxicity, and the 200g HLB column and the 500g C18 column eluates with methylene chloride produced the greatest recovery of toxicity and chemical. Although SPE columns have had a variable performance in past studies with marine sediment TIEs, these columns worked well with pesticide-spiked fresh interstitial water. These results provide an additional useful tool for Phase II TIEs using interstitial water.

The last goal of the project was to conduct a Stressor Identification Workshop to facilitate information exchange between scientists researching methods to identify chemicals and other stressors responsible for sediment toxicity. The objectives were to identify existing and emerging TIE tools and other sublethal indicators of contaminant stress, determine the most important chemical and non-chemical factors responsible for sediment toxicity, and to identify data gaps and missing toxicological and analytical tools that may prove useful in future research. The workshop focused on stressors causing amphipod mortality. Scientists from SFEI, the US EPA, the State Water Resources Control Board, UC Davis, UC Berkeley, SCCWRP, the Central Valley Regional Water Quality Control Board, and private laboratories participated in the discussion. Discussion topics included an overview of sediment toxicity issues in San Francisco Bay, application of multiple approaches for stressor investigation in southern California sediments, analytical challenges associated with identifying chemicals responsible for sediment toxicity, non-anthropogenic chemicals and non-contaminant stressors and their role in amphipod

mortality, and genomic tools for identifying chemicals affecting *E. estuarius*. The workgroup listed stressors of concern and research needs to address each stressor, and created an updated sediment TIE flowchart. The priority for future research needs include determining the effects of fine-grained sediments and particle shape on the survival of *E. estuarius*, improving analytical methods and developing LC50 values for current-use pesticides, and improving interstitial water extraction and elution methods. The latter two research topics were partially addressed in the current study. The findings of the first workshop are summarized in a complete set of meeting notes and presentations that can be found at http://www.sfei.org/node/3117.

As an addendum to this project, data from recent comparisons between sediment tests with estuarine species and freshwater species are presented and discussed. The RMP typically uses *E. estuarius* to evaluate whole sediment toxicity, and *Mytilus galloprovincialis* to evaluate toxicity at the sediment-water interface. There has been concern that using these organisms to test primarily freshwater stations has not been environmentally relevant. During the 2009 and 2010 monitoring events, whole sediments from the San Joaquin and Sacramento Rivers and the upper estuary were also tested with the amphipod *Hyalella azteca* and the midge *Chironomus dilutus*. Sediment-water interface exposures were also conducted with the daphnid *Ceriodaphnia dubia*.

The RMP would like to monitor with ecologically relevant test species, while also trying to maintain a connection to the long-term data set. Historically, the program has used *E. estuarius* for whole sediment exposures, and *M. galloprovincialis* for elutriate exposures, and more recently, exposures at the sediment-water interface. The amphipod is tolerant to a wide range of salinities (0‰ to 34‰), but is generally tested at the brackish salinity of 20‰, and is considered a true estuarine organism. The bivalve has a low salinity tolerance of 25‰. The river stations clearly have low bottom salinities that are representative of freshwater habitat, and should be tested with freshwater species.

Introduction

The Five Year Plan developed by the San Francisco Estuary Institute's Exposure Effects Work Group (EEWG) for evaluating risks to benthic biota includes ongoing studies to determine causes of sediment toxicity in the San Francisco Estuary. Toxicity has been observed at a high proportion of Regional Monitoring Program (RMP) stations since the Status and Trends Program began in 1993. Effective management of factors contributing to toxicity depends on determining which specific pollutants or other factors are responsible. In addition, stressor identification is a key element of the State Water Resources Control Board's Sediment Quality Objectives (SQOs) program for enclosed bays and estuaries. While planned SQO assessments will provide information about sediment condition in the Estuary, they are not designed to identify causes of toxicity or associated benthic community impacts. The current study implements components of the EEWG Five Year Plan by (1) providing new thresholds of effects for key contaminants of concern to the Estuary, (2) further developing toxicity identification evaluation (TIE) methods to identify organic contaminant stressors in sediments, and (3) implementing a work group process to coordinate scientists around the state to develop and evaluate new TIE tools.

Evaluation of toxicity associated with whole sediment and interstitial water chemical concentrations can be constrained by a lack of information on the toxicity of certain chemicals to test organisms. Median lethal concentrations (LC50s) are often used to interpret the relationship between chemical concentrations and organism response. A number of LC50s exist for *E. estuarius*, the amphipod species used for sediment toxicity assessments in the Estuary (Table 1), but values are lacking for the majority of contaminants commonly detected in sediments. Determining the LC50 values for key contaminants of concern in the Estuary aids the interpretation of routine monitoring data, as well as TIE studies. Expanding the database on toxic effects of sediment contaminants is also useful to resource managers throughout California as implementation of the State's SQO program progresses.

TIEs are the primary means to identify chemical stressors responsible for sediment toxicity. These procedures are conducted using a phased approach. Phase I treatments characterize toxicity caused by general categories of contaminants: organic chemicals, metals, or ammonia. Phase II TIE treatments are designed to identify the specific contaminant(s) causing toxicity.

Phase II TIEs also utilize LC50 values to interpret chemistry data. Phase III TIEs confirm the Phase I and II results. Whole sediment and interstitial water chemistry data are used to provide additional lines of evidence in this process.

Table 1. Median lethal (LC50) concentrations for estuarine amphipods. *µg/g OC indicates organic carbon-corrected concentration.

Sediment Chemicals	**ng/g**	**ug/g OC***	**Endpoint**	**Species**	**Reference**
Pyrethroids					
Bifenthrin	8	1.025	LC50	*E. estuarius*	(Anderson et al., 2008)
Cypermethrin	11	1.41	LC50	*E. estuarius*	(Anderson et al., 2008)
Permethrin	140	17.95	LC50	*E. estuarius*	(Anderson et al., 2008)
Organochlorines					
DDT p,p'	49.5		LC50	*R. abronius*	(Word et al., 1987)
Total Chlordane	>49		NOEC	*E. estuarius*	(Stransky et al., 2006)
Total DDT	554	101	LC50	*E. estuarius*	(Weston, 1996)
Total DDT		2,500	LC50	*E. estuarius*	(Swartz et al., 1994)
Organophosphates					
Chlorpyrifos	103	13.21	LC50	*E. estuarius*	(Anderson et al., 2008)
PAHs					
Acenaphthene		2,577	LC50	*E. estuarius*	(USEPA, 2003)
Fluoranthene	85,300	10,935	LC50	*E. estuarius*	(Anderson et al., 2008)
Fluoranthene		3,533	LC50	*E. estuarius*	(DeWitt, 1989)
Phenanthrene		4,010	LC50	*E. estuarius*	(USEPA, 2003)
Total PAHs	10,750	1,800	LC50	*R. abronius*	(Swartz, 1999; Page et al., 2002)
PCBs					
Aroclor 1254	10800		LC50	*R. abronius*	(Swartz et al., 1988)
Metals	**ug/g**				
Cadmium	9810		LC50	*R. abronius*	(Mearns et al., 1986)
Copper	534		LC50	*E. estuarius*	(Anderson et al., 2008)
Mercury	13.1		LC50	*R. abronius*	(Swartz et al., 1988)
Zinc	276		LC50	*R. abronius*	(Swartz et al., 1988)
Water Chemicals			**Endpoint**		**Reference**
Unionized Ammonia	2.49		LC50	*E. estuarius*	(Kohn et al., 1994)
Pyrethroids	**ng/L**				
Cypermethrin	>1		LC50	*E. estuarius*	(Ernst et al., 2001) Product
Organochlorines	**µg/L**				
Chlordane (Total)	130		LC50	*E. estuarius*	(Stransky et al., 2006)
Organophosphates	**ng/L**				
Chlorpyrifos	529		LC50	*E. estuarius*	(Anderson et al., 2008)
PAHs	**µg/L**				
Fluoranthene	671		LC50	*E. estuarius*	(Phillips et al., 2009b)
Phenanthrene	158		LC50	*E. estuarius*	(Swartz et al., 1995)
Acenaphthene	708		LC50	*E. estuarius*	(Swartz et al., 1995)
Metals	**mg/L**				
Cadmium	5.1		LC50	*E. estuarius*	MPSL Unpublished
Copper	48.7		LC50	*E. estuarius*	(Phillips et al., 2009b)

Background

A recent TIE study in Mission Creek (Phillips et al., 2009b) used several procedures to investigate causes of sediment toxicity to *E. estuarius*. A combination of whole sediment and interstitial water TIE procedures provided lines of evidence to suggest that toxicity to amphipods was not caused by any single chemical constituent and was likely caused by a mixture of organic chemicals. While the treatments characterized the cause of toxicity, they did not identify the specific chemical(s) responsible for amphipod mortality.

The lack of conclusive TIE results was attributed to several factors. Evaluation of the Mission Creek whole sediment and interstitial water chemistry was constrained by a lack of information on the toxicity of specific chemicals to *E. estuarius*. As discussed above, determining sediment and water LC50s for these and other contaminants facilitates interpretation of chemical analyses conducted as part of future Status and Trends monitoring, as well as whole sediment and interstitial water TIEs conducted in the Estuary.

The results of the Mission Creek experiments also suggested that many of the Phase II TIE procedures require refinement. To date, Phase I whole sediment TIE treatments are well established (USEPA, 2007), and Phase II treatments have been evaluated but not perfected (Anderson et al., 2007b; Perron et al., 2009; Phillips et al., 2009a). Phase I treatments use extraction media in whole sediment and interstitial water to remove bioavailable contaminants from the sample. Phase II treatments generally involve eluting the extraction materials. Eluate treatments are prepared with the solvents used for elution, and tested to evaluate if toxicity can be recovered. The extraction materials used to remove organic chemicals in the Mission Creek TIEs were Amberlite® XAD-4 resin for the whole sediment TIEs and the Oasis HLB solid-phase extraction column (SPE, Hydrophilic-Lipophilic Balance®, 6 ml, 500 mg, Waters, Milford, MA, USA) for the interstitial water TIEs. In the case of whole sediment, resin addition likely resulted in an exhaustive removal of organic contaminants from the sediment, because the resins were left in the sediment for the duration of the 10 day toxicity test. Elution of the resin after the 10 day exposure overestimates the bioavailable fraction of the sorbed contaminants. This would occur because the resin would not only sorb the rapidly desorbing fraction in the sediment, which is thought to be the most bioavailable fraction, but also the slowly desorbing fraction (Cornelissen

et al., 2001). The presence of the resin essentially increases the gradient differential even though the system could reach equilibrium. Eluting the resin at the termination of the exposure has proven to only provide qualitative evidence for identifying the cause of toxicity (Phillips et al., 2010). It is necessary to accurately measure the bioavailable chemicals in whole sediment TIE treatments to improve Phase II identification of the cause of toxicity. In the case of the Mission Creek Phase II interstitial water TIE treatments, the solid-phase extraction and elution results were inconsistent. There was incomplete removal of toxicity when the chemicals in the interstitial water were extracted using HLB columns, and there was incomplete recovery of toxicity when the columns were eluted with solvent (acetone). The goals of the current experiments were to provide a better estimate of the bioavailable fractions of contaminants in the whole sediment exposures, and to overcome the performance variability of the solid-phase extraction procedures used in the interstitial water TIEs.

The current study used an integrated approach that combined dose-response experiments designed to establish chemical-specific LC50s with whole sediment and interstitial water TIE experiments. Chemical analysis of spiked sediments allowed for the calculation of LC50s based on measured concentrations, and provided confirmation of TIE treatment efficacy. In conjunction with ongoing collaborative research at the University of California Berkeley and the Southern California Coastal Water Research Project (SCCWRP), these experiments also provided amphipod samples from a number of dosing concentrations using three chemicals to allow development of a gene microarray for *E. estuarius* responding to specific chemicals of concern in the San Francisco Estuary. The goal of the latter research was to develop sublethal response data for *E. estuarius* for application to future TIEs in the Estuary and statewide.

Laboratory Methods

Reference Sediment Screening

Dose-response experiments to develop LC50s were designed to use reference sediments from the San Francisco Estuary. Reference sediment was collected from three locations in upper San Francisco Bay. Paradise Cove (37.8990, -122.4637) sediment was initially chosen because historical data show that it represents the average grain size and total organic carbon (TOC)

found in Estuary sediments. Past data also show that it was relatively uncontaminated, and was not toxic *E. estuarius* (Hunt et al., 2001a). Approximately 120 liters of fine-grained sediment was collected from Paradise Cove and a screening toxicity test was initiated on January 29, 2010. This sediment proved to be moderately toxic, so additional sediment was collected from two sites in San Pablo Bay: the RMP Status and Trends monitoring station SPB027 (38.06743, -122.46198) and Castro Cove (36.96160, -122.40656) on April 20, 2010. Station SPB027 was not toxic in previous RMP monitoring events, and while Castro Cove sediment has historically been toxic (Hunt et al., 2001b), a recent study demonstrated that this station was relatively uncontaminated (Phillips et al., 2009b). Neither of these sediments was toxic to *E. estuarius* and because survival was higher in the Castro Cove sediment (results below) this sediment was selected as the primary reference sediment for the spiked sediment toxicity tests. Interstitial water was later extracted from SPB027 sediment for additional interstitial water toxicity tests.

Dose Response Experiments

Chemicals

Dose-response experiments were conducted with the legacy organochlorine pesticide chlordane, one current-use pyrethroid pesticide (cyfluthrin), and the PAH pyrene. Experiments were conducted using spiked water and whole sediment to determine the toxicity of these chemicals to *E. estuarius*. These chemicals were chosen based on a review of sediment chemistry and coincident toxicity data from historical RMP monitoring. Chlordane was chosen because it has been linked to amphipod toxicity in RMP samples (Thompson et al., 1999) and comprised 60% of the total sediment quality guideline quotient value in the recent Mission Creek TIE study (Phillips et al., 2009b). Chlordane is commonly correlated with amphipod mortality in statewide and regional monitoring studies in California (Fairey et al., 1998; Anderson et al., 2001; Hunt et al., 2001b). Cyfluthrin is used extensively in structural pest management and was detected in one-half of toxic urban streams surveyed in California (Holmes et al., 2008). A number of pyrethroids including cyfluthrin were detected in sediment from East Bay and upper Delta stations (Weston et al., 2006), and pyrethroids were detected in the Sediment Quality Objectives Delta Study. The RMP has only recently started monitoring this class of pesticide. A recommendation to measure pyrethroids in environmental samples, determine their toxicity to standard test organisms, and develop TIE methods to identify their contribution to toxicity has

recently been made to the State Water Resources Control Board (TDC, 2008). Although median lethal concentrations for several pyrethroids have been developed for *E. estuarius*, a reliable LC50 for cyfluthrin has not. The Mission Creek chemistry results also suggested that PAHs contributed to the cause of toxicity of this sediment. Individual concentrations of fluoranthene and phenanthrene in this study were below published LC50 concentrations. The threshold for pyrene toxicity to *E. estuarius* has not been established and previous analyses of PAH mixtures in Estuary sediments have shown that fluoranthene and pyrene are usually the PAHs present at the highest concentrations. An evaluation of the likely contributions of PAHs to chronic toxicity of Estuary sediments concluded that the three PAHs most likely responsible for chronic toxicity are fluoranthene, pyrene, and dibenz(a,h)anthracene (Ross and Oros, 2006). Finally, evaluation of the Mission Creek sediment data showed that pyrene and fluoranthene were the two dominant PAHs.

Water and Sediment Spiking

Stock solutions for chlordane and pyrene were prepared by Accustandard (New Haven, CT, USA) at 25 mg/mL. Cyfluthrin stock solutions were prepared in acetone in the laboratory using 98% pure cyfluthrin solid obtained from Chem Service (West Chester, PA, USA). Secondary stocks were prepared in acetone from super stocks. For the dose-response experiments with water, contaminants were spiked into clean laboratory seawater adjusted to 20‰ with distilled water. This is the standard salinity used for testing *E. estuarius* for RMP monitoring. Water was spiked at a range of chemical concentrations on the day of test initiation and allowed to equilibrate for approximately one hour prior to exposing the amphipods.

Sediment was spiked in two or four-liter glass jars at a range of chemical concentrations. Sediment spiking methods were coordinated with SCCWRP to provide consistency between duplicate studies conducted at SCCWRP. Ten grams of silica sand was placed in each jar and secondary stock solution was added to the sand. The acetone was allowed to evaporate from the sand under a fume hood before the addition of wet reference sediment. Castro Cove sediment had a wet weight to volume ratio of 1.45g/mL (1 liter of wet sediment weighs 1450g). The moisture content was 54%, so one liter of wet sediment contained 667g of dry sediment. Various amounts of secondary stock solution and various volumes of sediment were added depending the

concentration and the amount of spiked sediment needed for testing. The jars were sealed and placed on a sediment roller (Wheaton Instruments, Millville, NJ, USA), and rolled overnight before being stored at 4ºC. Spiked sediments were rolled once per week for two hours and were equilibrated for thirty days (USEPA, 2001).

Toxicity Tests

All test organisms were wild-caught *E. estuarius* collected by Northwestern Aquatic Sciences (Newport, OR, USA). LC50 values were determined using water-only and whole sediment toxicity tests. This allowed the evaluation of the potential for toxicity in both whole sediment and interstitial water exposures. Each experiment consisted of a control and a minimum of five contaminant concentrations. Range-finder and definitive experiments were conducted for both water and sediment exposures (see Tables 2, 3, 6 and 7 for concentrations). The range-finder results were used to determine definitive test concentrations. Water tests were conducted in 250 mL glass beakers containing 100 mL spiked water. The tests were conducted for up to ten days and test solutions were renewed 50% every 48 hours. All water solutions were prepared with 20‰ water. All tests were accompanied by an un-spiked water control and an acetone blank that consisted of 20‰ water containing the same volume of acetone as the spiked water.

Range-finder sediment tests and the definitive tests for chlordane and pyrene were conducted in one-liter beakers containing 200 mL sediment (USEPA, 1994). The definitive tests with cyfluthrin were conducted using a small-volume TIE format in 250 mL beakers containing 50 mL sediment (Anderson et al., 2007b). The small volume arrangement allowed for use of TIE treatments in the second definitive test (see below). Range-finder tests consisted of three replicates and definitive tests consisted of five replicates. All sediment tests were accompanied by an un-spiked sample of Castro Cove sediment, a clean sediment control consisting of sand that was collected with the amphipods (home control), and an acetone blank containing the same volume of acetone as the spiked sediments.

Analytical confirmation of all definitive test concentrations was conducted using GC/MS. Samples were collected for analysis at the beginning of the exposures. Chlordane and pyrene were analyzed using EPA Method 625(M) and cyfluthrin was analyzed using GC/MS NCI.

Median lethal concentrations were calculated using Toxcalc® Software (McKinleyville CA). Range-finder LC50s were calculated based on nominal concentrations definitive LC50s were calculated based on measured concentrations. Median lethal concentrations were calculated for all range finder exposures regardless of control response. Definitive LC50s were calculated for all tests having control responses greater than 80% survival, but LC50s based on tests with control responses less than 90% survival are noted. Water LC50s were calculated for 4, 7 and 10-day survival data if the control response met the above criteria. Statistical comparisons between TIE treatments were conducted using separate-variance t-tests. A complete set of analytes including metals, PAHs, PCBs, organochlorine pesticides and pyrethroid pesticides were measured in the Castro Cove sediment to determine background concentrations. Total organic carbon (TOC) was measured in order to normalize sediment chemical concentrations to organic carbon, and grain size was measure to determine percent sand, silt and clay.

Sediment TIE Method Development

Whole Sediment TIEs

Carbonaceous resins are typically added to whole sediment in Phase I TIEs to reduce the bioavailability of organic contaminants. The resin interacts with the sediment throughout the exposure and maintains an exhaustive sink for bioavailable chemicals. In the case of acute amphipod tests, this results in an 11 day equilibration period which includes the 1 day pre-test mixing of the resin and test sediment and the 10 days of the toxicity test. As discussed above, isolating the resin from the sediment at the termination of the exposure, and eluting it with solvent for chemical analysis, can overestimate the bioavailable fraction of contaminant in the treatment (Phillips et al., 2009a). To determine which organic chemicals are contributing to toxicity in a TIE experiment, it is necessary to accurately measure the bioavailable concentrations in whole sediment with and without the resin. Recent research using solid-phase micro extraction (SPME) has demonstrated that this procedure provides a more accurate quantification of the bioavailable concentration of contaminants in sediments. We incorporated an equilibrium SPME procedure in the second definitive whole sediment cyfluthrin experiment to measure the bioavailable fractions with and without resin (Xu et al., 2007). This approach attempted to provide an accurate quantification of how well the Amberlite resin reduced the bioavailable fraction of cyfluthrin during the 11 day experiment.

Amberlite XAD-4 resin (Rohm and Haas, Spring House, PA, USA) was prepared by rinsing the beads thoroughly with Nanopure water in a 25 µm screen tube, and then submerging the beads in methanol for fifteen minutes to activate them. The resin beads were then rinsed again with Nanopure water. One part resin was added to nine parts spiked sediment (10% by weight). The sediment was rolled for 24 hours before being loaded into the exposure chambers. Toxicity of all cyfluthrin concentrations were tested with and without resin.

The SPME method used was an equilibrium procedure that required the fibers to interact with the samples for several days. This is in contrast to the non-equilibrium method which involves a short exposure of approximately 20-30 minutes immediately followed by direct injection of the fiber into a gas chromatograph. The non-equilibrium method uses a set of calibrators to create a standard curve that is used for calculating the sample concentrations. The equilibrium method uses a partition coefficient to determine sample concentration. Because we lacked the specific equipment needed for the non-equilibrium procedure, we performed the equilibrium method.

Polydimethylsiloxane (PDMS, 7 µm, obtained from Jay Gan at UC Riverside) SPME fibers were prepared by cutting to 1 cm lengths with a razor blade, rinsing with acetone and water, and them heating them in a muffle furnace to 260 ºC for 30 minutes. The equilibration and extraction procedures followed a combination of methods outlined by the Gan research group at UC Riverside (Svetlana Bondarenko, UC Riverside, personal communication; (Xu et al., 2007; Hunter et al., 2009)). Approximately 15 mL of interstitial water was extracted from extra exposure replicates at the termination of the cyfluthrin toxicity test exposure. Interstitial water samples were collected from every concentration of spiked sediment. Two fibers were placed in 20 mL vials containing the interstitial water. The vials were then placed on a shaker table for seven days to allow the bioavailable concentration of cyfluthrin in the interstitial water to equilibrate with the surface of the fiber. At the end of the equilibration period one set of fibers were wiped clean with a damp tissue and placed in a 350 µL GC vial. The fibers were extracted by adding 150 µL acetone, sonicating for five minutes in a sonication bath, adding an additional 100 µL of acetone, and sonicating for an additional five minutes. The contents of the vials were blown to dryness under nitrogen and the extract was reconstituted in 100 µL of hexane. The

hexane samples were then analyzed for cyfluthrin. The second set of fibers were wiped clean with tissue, placed in a 350 µL GC vial, and sent for extraction and duplicate analysis at University of California Riverside by Jay Gan. The bioavailable concentration of cyfluthrin (C_{free}) is calculated using the following equation:

$$C_{free} = C_{PDMS} / K_{PDMS}$$

C_{PDMS} is the concentration of cyfluthrin detected on the fiber and K_{PDMS} is the partition coefficient of cyfluthrin between water and the PDMS phase at equilibrium.

Interstitial Water TIEs

Interstitial water TIEs are most conclusive during Phase I when complete removal of toxicity is achieved using extraction media (e.g., carbonaceous resins or solid-phase extraction columns) and during Phase II when extracted chemicals are completely recovered using elution procedures. As discussed above, results of the Mission Creek interstitial water TIEs were somewhat inconclusive because of variability in the performance of the HLB SPE columns used to extract organic chemicals, and incomplete elution and detection of chemicals from the columns (Phillips et al., 2009b). Conversations with USGS chemists familiar with solid-phase extraction of sediment interstitial water have suggested that column effectiveness may be compromised when chemicals are associated with dissolved or colloidal organic matter (dissolved/colloidal organic matter, DOM/COM), because this interferes with their sorption to the column substrate. In addition, sorption to the column material may be limited by the gravity drip method currently used. Although this is a standard analytical procedure, it may not allow sufficient residence time to allow organic chemicals in the interstitial water to fully equilibrate with the column.

Based on discussions during toxicity work group (TWG) meetings, we proposed evaluating an alternative approach to extract organic chemicals from interstitial water. Instead of passing the interstitial water sample through a solid-phase extraction column, Amberlite resin was added directly to interstitial water samples and allowed to equilibrate for 24 hours prior to the beginning of the TIE toxicity test exposure (batch extraction). The idea was to provide a longer

residence time to facilitate more complete sorption of organic chemicals from interstitial water. Resin could then be removed from the interstitial water prior to the exposure and eluted to create a Phase II TIE treatment. The original intention was to conduct these experiments with interstitial water extracted from toxic concentrations of sediment that had been spiked with cyfluthrin, but these interstitial water samples proved to be only moderately toxic once extracted from the sediment. Additional interstitial water was spiked with cyfluthrin to provide media for the TIE evaluations. Because the supply of Castro Cove sediment had been exhausted during the spiking studies, we used interstitial water extracted from the additional San Pablo Bay reference sediment (SPB027). Interstitial water was extracted from SPB027 sediment using two methods. The first method involved stirring the buckets of sediment and allowing them to settle overnight. The overlying water was removed with a siphon the following day. Additional interstitial water was extracted with a refrigerated centrifuge (2500G, 4ºC).

The interstitial water was spiked with three concentrations of cyfluthrin (5, 10 and 25 ng/L). Amberlite resin for batch extraction was prepared as described above. Fifty-five grams of resin (wet weight) was added to 1100 mL of each concentration of spiked interstitial water. The Amberlite treatments were stirred overnight. The resin was separated from the samples by pouring it through a 25 µm screen. The isolated resin was eluted using three methods in sequence. First, the entire amount of resin was placed in a 60 mL polyethylene column and 50 mL of acetone was passed through the column at a rate of approximately 2 mL per minute. The resin was then placed in a 125 mL flask with an additional 50 mL of acetone and stirred for one hour. Lastly, the flask was placed in a sonication bath for one hour. The acetone was poured off of the resin and combined with the acetone from the column extraction. The acetone was reduced to approximately 5 mL with a stream of nitrogen and added to 1100 mL of clean water to create the eluate treatment. The three-step extraction process was designed for maximum recovery of cyfluthrin from the Amberlite resin.

Results and Discussion

Reference Sediment Screening

Mean percent survival in the Paradise Cove sediment was 67%, and the sediment was considered unsuitable for the spiking studies. Two additional sediment samples were collected in San Pablo Bay, and toxicity tests were initiated on April 23, 2010. Mean percent survival in Castro Cove and SPB027 sediments was 92% and 83%, respectively. Castro Cove was chosen for the whole-sediment spiking experiments, however, additional interstitial water from SPB027 was spiked with cyfluthrin for TIE methods development.

A complete set of chemical analyses were conducted on Castro Cove sediment by SCCWRP. These included metals, PAHs, pyrethroids, organochlorines, and PCBs. The sediment contained a number of metals and PAHs, but none were at concentrations high enough to affect *E. estuarius* (Table A1). Only low concentrations of two DDT metabolites were detected and no pyrethroid pesticides or PCBs were detected. The TOC was 0.9% and the particle distribution was approximately 25% sand and 75% fines.

Water LC50s

Two sets of range-finding experiments were conducted. The first experiment included all three chemicals, whereas the second experiment only tested chlordane and pyrene. Although *E. estuarius* is generally tested in water for only 96 hours in standard reference toxicant tests that accompany sediment tests, spiked water tests were conducted for up to ten days. In the case of the first range-finder, amphipod control survival decreased significantly after the fourth day, so 96 hour data are presented. In 96 hours there was a significant dose response with cyfluthrin (LC50 = 1.19 ng/L, nominal concentration), but tests with chlordane and pyrene did not produce significant dose responses with concentrations as high as 10 µg/L (Table 2).

Table 2. Mean percent survival (standard deviation) of *E. estuarius* at 96 hours in the first water range-finder exposures.

Chemical	Concentration	Mean	SD
Chlordane	0.1 µg/L	91	9
	0.5 µg/L	83	15
	1 µg/L	87	6
	5 µg/L	93	6
	10 µg/L	93	6
Pyrene	0.1 µg/L	97	6
	0.5 µg/L	77	12
	1 µg/L	87	15
	5 µg/L	93	6
	10 µg/L	83	21
Cyfluthrin	0.5 ng/L	83	15
	1 ng/L	57	15
	2 ng/L	3	6
	5 ng/L	0	0
	10 ng/L	0	0
Controls	Acetone Blank	87	15
	Water Control	77	6

Table 3. Mean percent survival (standard deviation) of *E. estuarius* at three durations in the second water range-finder exposures.

	Nominal	Day 4		Day 7		Day 10	
Chemical	Concentration	Mean	SD	Mean	SD	Mean	SD
Chlordane	5 µg/L	90	0	83	12	67	25
	10 µg/L	79	12	72	16	59	26
	50 µg/L	93	12	93	12	80	20
	100 µg/L	93	6	83	12	73	21
	200 µg/L	87	15	60	10	27	6
Pyrene	5 µg/L	90	0	87	6	57	21
	10 µg/L	97	6	93	6	84	12
	50 µg/L	90	10	67	25	20	20
	100 µg/L	60	10	0	0	0	0
	200 µg/L	60	20	0	0	0	0
Controls	Acetone Blank	87	6	83	12	67	25
	Water Control	90	10	90	10	73	31

Chlordane and pyrene were tested again in the second range-finder experiment. This test was conducted for 10 days, and data are presented for 4, 7 and 10 days. Survival in the water controls and acetone blanks were greater than 83% on Day 7, but by Day 10 the control survival was reduced to 67% and 73% for the acetone and water controls, respectively. Both chlordane and pyrene were tested at concentrations up to 200 µg/L, which was beyond the solubility of these chemicals in water (100 µg/L for chlordane and 90 µg/L for pyrene). As the stock was added to water, some precipitate formed on the surface. This precipitate was present during the

test, but did not seem to physically affect the organisms. The two tests produced significant dose responses. Chlordane was less toxic than pyrene and did not demonstrate a significant dose response until Day 10 (LC50 = 170 µg/L, nominal concentration, Table 3). Pyrene demonstrated toxicity as early as Day 4, but did not demonstrate a significant dose response until Day 5. The 7-day LC50 was 52.4 µg/L, and although there was some toxicity in the lowest concentration on Day 10, the 10-day LC50 was 29.6 µg/L (Table 3).

Although there was significant mortality at the lowest concentration of cyfluthrin, lower concentrations were not used in the definitive test because to do so would have placed the range of concentrations below the method detection limits of the analytical laboratory. The definitive test for chlordane was conducted using the upper three concentrations from the second range-finder experiment, and the pyrene test was conducted with the 10, 50 and 100 µg/L concentrations.

Single definitive tests were conducted with chlordane and pyrene. As in the second rangefinder test, chlordane was tested beyond its solubility in water. Measured chlordane concentrations ranged from 95% to 128% of nominal, and pyrene concentrations ranged from 75% to 125% of nominal. By Day 7 the survival in both controls was approximately 80%, but survival rates dropped to approximately 70% by Day 10. Unlike the range-finder test, significant mortality was observed in the highest concentration of chlordane on Day 6, and by Day 9 there was mortality in the other concentrations (Table 4). The seven LC50 based on measured concentrations of chlordane was 223 µg/L. Significant mortality was observed in the highest concentration of pyrene on Day 4, but survival in the highest concentration was not low enough to calculate an LC50. The seven-day LC50 based on measured concentrations of pyrene was 36.9 µg/L. In both cases toxicity increased with test duration. The ten-day LC50s were not reported because of low control survival.

The four-day LC50 based on measured concentrations of cyfluthrin was 2.93 ng/L (Table 5). A seven-day LC50 was also calculated using the survival in the acetone control as the base of the dose response curve. The seven-day LC50 was 1.70 ng/L. Ten-day LC50s were not calculated because of low survival in the acetone control. The upper three measured cyfluthrin

concentrations in both tests ranged from 81% to 144% of nominal, but the lower two measured concentrations were as high as 374% of nominal. It is unclear why the 0.5 and 1 ng/L concentrations appear to be over-spiked, but it is interesting to note that there were drastic differences in survival in the low concentrations between the two definitive tests.

Table 4. Mean percent survival (standard deviation) of *E. estuarius* at three durations in the first water definitive exposures.

	Nominal	Measured	Day 4		Day 7		Day 10	
Chemical	Concentration	Concentration	Mean	SD	Mean	SD	Mean	SD
Chlordane	50 µg/L	47.3 µg/L	98	4	86	9	32	30
	100 µg/L	110 µg/L	96	5	75	9	38	14
	200 µg/L	255 µg/L	86	5	36	21	8	13
Pyrene	10 µg/L	7.46 µg/L	88	8	70	10	58	16
	50 µg/L	40.7 µg/L	78	11	46	9	14	11
	100 µg/L	125 µg/L	70	19	2	4	0	0
Cyfluthrin	0.5 ng/L	1.87 ng/L	4	5	0	0	0	0
	1 ng/L	2.68 ng/L	12	13	2	4	0	0
	2 ng/L	2.87 ng/L	48	27	8	13	0	0
	5 ng/L	4.05 ng/L	36	26	4	9	0	0
	10 ng/L	9.08 ng/L	18	19	6	9	0	0
Controls	Acetone Blank	NA	88	8	79	3	71	14
	Water Control	NA	98	4	82	8	68	15

Table 5. Mean percent survival (standard deviation) of *E. estuarius* at three durations in the second water definitive exposure.

	Nominal	Measured	Day 4		Day 7		Day 10	
Chemical	Concentration	Concentration	Mean	SD	Mean	SD	Mean	SD
Cyfluthrin	0.5 ng/L	1.73	92	11	50	17	36	22
	1 ng/L	1.55	86	11	34	15	16	11
	2 ng/L	2.2	68	13	8	4	6	5
	5 ng/L	5.09	10	14	2	4	0	0
	10 ng/L	9.14	12	16	2	4	0	0
Controls	Acetone Blank	NA	94	5	66	18	32	24
	Water Control	NA	96	5	88	11	82	13

Sediment LC50s

Amphipod survival in the un-spiked Castro Cove sediment ranged from 85 to 92% in the sediment range-finder tests. Survival in the home sediment controls ranged from 88 to 93%. Survival in the acetone blanks was slightly lower with a range of 72 to 85%. The first range-finder test with cyfluthrin produced a significant dose response curve with a nominal LC50 of 2.23 ng/g (Table 6). No additional range-finder tests were conducted with cyfluthrin.

The first range-finder tests with chlordane and pyrene did not produce significant dose responses (Table 6). Although our highest spiked concentrations of these chemicals in the first range-finder test were well above Estuary concentrations, we conducted a second range-finder experiment with concentrations as high as 100,000 ng/g. The highest measured concentrations of trans-chlordane listed in the RMP database range up to 6.6 ng/g, and most of these measurements are from the southern sloughs. The highest measured concentrations of pyrene range up to 3,280 ng/g. The highest concentrations of pyrene were measured in the Central Bay portion of the Estuary. The concentration of trans-chlordane measured in sediment from Mission Creek, an area at the margin of the Estuary that is considered to be heavily impacted was 35.5 ng/g, and the concentration of pyrene was 1142 ng/g (Phillips et al., 2009b). Although high spiking concentrations were used to offset possible chemical loss during the spiking process, and to establish a threshold toxic effect concentration for each chemical, no toxicity was observed in sediments spiked with up to 100,000 ng/g pyrene or trans-chlordane (Table 7). The highest concentration of pyrene caused a slight effect, but the response was not significantly different from that of the acetone blank.

Table 6. Mean percent survival (standard deviation) of *E. estuarius* in the first sediment range-finder exposures.

Chemical	Concentration	Mean	SD
Chlordane	0 ng/g	85	5
	1,000 ng/g	80	5
	5,000 ng/g	85	5
	10,000 ng/g	90	0
	25,000 ng/g	83	7
	Acetone Blank	80	17
	Home Control	90	10
Pyrene	0 ng/g	85	5
	1,000 ng/g	87	8
	5,000 ng/g	90	13
	10,000 ng/g	83	6
	25,000 ng/g	82	14
	Acetone Blank	80	17
	Home Control	90	10
Cyfluthrin	0 ng/g	92	3
	0.1 ng/g	87	13
	0.5 ng/g	87	10
	1 ng/g	85	5
	5 ng/g	7	6
	10 ng/g	2	3
	Acetone Blank	85	9
	Home Control	93	8

Table 7. Mean percent survival (standard deviation) of *E. estuarius* in the second sediment range-finder exposures.

Chemical	Concentration	Mean	SD
Chlordane	0 ng/g	85	10
	50,000 ng/g	77	6
	100,000 ng/g	83	8
Pyrene	0 ng/g	85	10
	50,000 ng/g	80	0
	100,000 ng/g	68	6
	Acetone Blank	72	16
	Home Control	88	8

Although there was no significant toxicity observed in either the chlordane or the pyrene rangefinder tests, single definitive tests with analytical confirmation of spiked concentrations were conducted to establish definitive no observed effect concentrations (NOECs) for these chemicals. Sediments for these tests were spiked at concentrations up to 25,000 ng/g. These concentrations were lower than those in the second range-finder experiment, but still thousands of times higher than concentrations routinely measured in the Estuary. As expected, there was no significant toxicity observed in the definitive tests with chlordane and pyrene (Table 8). Control responses were all greater than 90% survival, and measured concentrations of chlordane and pyrene ranged from 80% to 141% of nominal concentrations.

Two definitive tests with cyfluthrin were conducted. After much discussion with the chemists responsible for measuring the pyrethroid, it was determined that the spiked chemical was lost somewhere in the analysis process. Two split samples were sent to the California Department of Fish and Game's Water Pollution Control Laboratory (WPCL) for analysis. WPCL recovered approximately 62% of the spiked cyfluthrin in the 50 ng/g sample (31.1 ng/g). No cyfluthrin was detected in the 5 ng/g sample. The LC50s were calculated based on nominal concentrations, but also presented based on the assumption of a 38% loss of cyfluthrin. Both definitive tests exhibited classic dose responses based on the spiked concentrations, and the LC50 concentrations were precise (4.89 ng/g and 5.51 ng/g, nominal). In previous threshold studies with spiked pyrethroids, measured concentrations were approximately 60% of the nominal concentrations (Anderson et al., 2008). Assuming a 38% loss in the current experiments, the

final LC50s were calculated to be 3.03 ng/g and 3.42 ng/g. These concentrations are comparable to *E. estuarius* cyfluthrin LC50 concentrations developed by SCCWRP (~2 ng/g).

Table 8. Mean percent survival (standard deviation) of *E. estuarius* in the first sediment definitive exposures. NA indicates not analyzed. NR indicates not reported.

Chemical	Nominal Concentration	Measured Concentration	Mean	SD
Chlordane	0 ng/g	NA	91	7
	5,000 ng/g	5,800 ng/g	89	5
	10,000 ng/g	9,840 ng/g	84	10
	25,000 ng/g	31,400 ng/g	88	8
	Acetone Blank	NA	90	8
	Home Control	NA	93	4
Pyrene	0 ng/g	NA	91	7
	5,000 ng/g	3,850 ng/g	89	5
	10,000 ng/g	7,090 ng/g	91	7
	25,000 ng/g	22,200 ng/g	89	4
	Acetone Blank	NA	90	8
	Home Control	NA	93	4
Cyfluthrin	0 ng/g	NA	94	9
	0.5 ng/g	NR	100	0
	1 ng/g	NR	94	9
	2.5 ng/g	NR	94	5
	5 ng/g	NR	50	21
	10 ng/g	NR	0	0
	25 ng/g	NR	2	4
	50 ng/g	NR	0	0
	Acetone Blank	NA	96	5
	Home Control	NA	98	4

The second definitive test incorporated whole sediment TIE treatments to reduce the bioavailability of cyfluthrin. Significant toxicity compared to the Home Control was observed in all of the concentrations greater than 2.5 ng/g (Table 9). Compared to the baseline treatments, toxicity in the 5 and 10 ng/g concentrations was significantly reduced by the addition of 10% Amberlite resin. The responses observed in the Amberlite treatments from these two concentrations were significantly higher than the responses in the corresponding dilution controls indicating that the resin had reduced toxicity be reducing bioavailable cyfluthrin, rather than through mere dilution. Survival in the un-spiked sediment, blanks and control were all greater than 90%.

The results of the Amberlite treatments demonstrate that addition of the resin significantly reduced cyfluthrin toxicity, but binding capacity of the resin was apparently overwhelmed by

high concentrations of the pyrethroid. Incorporating this treatment into a concentration series of spiked sediment, or a dilution series of ambient sediment, greatly increases the resolution of the TIE.

Table 9. Mean percent survival (standard deviation) of *E. estuarius* in the second sediment definitive exposure with cyfluthrin and TIE treatments. NA indicates not analyzed. NR indicates not reported.

		Baseline		Amberlite		Dilution	
Nominal Concentration	Measured Concentration	Mean	SD	Mean	SD	Mean	SD
0 ng/g	NA	98	4	94	9	NA	NA
0.5 ng/g	NR	94	5	100	0	98	4
1 ng/g	NR	94	5	98	4	100	0
2.5 ng/g	NR	92	8	96	5	100	0
5 ng/g	NR	52*	8	86*	9	70	16
10 ng/g	NR	16*	15	58*	16	8	8
25 ng/g	NR	0*	0	0	0	0	0
50 ng/g	NR	0*	0	0	0	0	0
Acetone Blank	NA	92	8	NA	NA	NA	NA
Home Control	NA	100	0	NA	NA	NA	NA
		*significant increase of toxicity compared to Home Control		*significant reduction of toxicity compared to corresponding Baseline Concentration			

LC50 Summary

Initial water-only range-finding tests with chlordane and pyrene used concentrations ranges well below the toxicity threshold for *E. estuarius*. The second range-finders with these chemicals produced dose responses, and LC50s were calculated based on nominal concentrations. The range-finding LC50s for all three chemicals were similar to LC50s calculated from measured concentrations in the definitive tests (Table 10). Chlordane in water was toxic to *E. estuarius*, but only at concentrations beyond its solubility. Amphipods were more sensitive to pyrene in water by approximately a factor of five, and were sensitive to cyfluthrin in the low ng/L range.

The only published LC50 values for trans-chlordane are for the bluegill fish (*Lepomis macrochirus*). These concentrations range from 50.5 to 210 µg/L (Mayer and Ellersick, 1986) and are within the same sensitivity range as *E. estuarius*. There is also one published *E. estuarius* LC50 for total chlordane that was in a range comparable to the LC50s derived in the

current study (130 µg/L, (Stransky et al., 2006)). There are a number of LC50s for pyrene that range from hundreds of ng/L to hundreds of µg/L for crustaceans. The freshwater amphipod *Hyalella azteca* has an LC50 value comparable to *E. estuarius* (77 µg/L, (Lee et al., 2001)). The water LC50 derived for cyfluthrin is also very similar to the LC50 for *H. azteca*. Weston and Jackson determined that the *H. azteca* LC50 was 2.3 ng/L, and the median effect concentration for causing amphipods to be moribund (EC50) was 1.9 ng/L (Weston and Jackson, 2009).

The water LC50 values for these chemicals were hundreds to thousands of time higher than water concentrations detected in the Estuary, yet these LC50s might be relevant for the evaluation of interstitial water concentrations under extreme contamination conditions. Interstitial water concentrations of chlordane and pyrene were not measured as part of the Mission Creek study, but they were measured as part of a larger TIE study of urban sediments (Anderson et al., 2007b). Interstitial water from stations representing the most toxic freshwater and marine sites of the country did not contain detectable concentrations of trans-chlordane. The interstitial water from these stations did, however, contain potentially toxic concentrations of pyrene.

Chlordane and pyrene did not cause toxicity in sediment, even when tested at concentrations tens to thousands of times higher than concentrations measured in Estuary sediments. The analytical confirmation of the spiked concentrations indicated that these chemicals were not lost during the spiking procedure, and the low TOC concentration of the Castro Cove sediment (0.9%) suggests that most of the spiked concentration was bioavailable. Previous correlative (Thompson et al., 1999) and modeling (Phillips et al., 2009b) studies have suggested that these chemicals have contributed to sediment toxicity, but based on the current sediment results, it is unlikely that these chemicals are significantly contributing to toxicity in Estuary sediments. In addition, though chlordane has often been significantly correlated with amphipod mortality in previous regional and statewide sediment assessment studies (Fairey et al., 1998; Anderson et al., 2001; Hunt et al., 2001b), results of the current spiking experiments confirm that this chemical is likely not contributing to sediment toxicity to *E. estuarius*.

Table 10. Summary of water and sediment LC50 values calculated from nominal concentrations in range-finder tests and measured concentrations in definitive tests. NA indicates not analyzed. NR indicates not reported because of blank survival <80%. * indicates blank survival ≥80%, but <90%. ** indicates LC50 calculation was conducted with acetone control response as the low end of the dose response curve. Numbers in parentheses are 95% confidence limits around the LC50.

	Units	Days	Range-Finder 1	Range-Finder 2	Definitive 1	Definitive 2
Water Tests						
Chlordane	µg/L	4	>10	>200	>255	NA
		7	NA	>200	224* (180-278)	NA
		10	NA	170 (145-200)	NR	NA
Pyrene	µg/L	4	>10	>200	>125	NA
		7	NA	52.4 (43.6-63.0)	36.9* (27.3-49.9)	NA
		10	NA	29.6 (24.0-36.5)	NR	NA
Cyfluthrin	ng/L	4	1.19 (1.05-1.36)	NA	<1.87	2.93 (2.66-3.33)
		7	NA	NA	NA	1.70** (1.64-1.76)
		10	NA	NA	NA	NA
Sediment Tests						
Chlordane	ng/g	10	>25,000	>100,000	>31,400	NA
	µg/g OC	10	NA	NA	>3,489	NA
Pyrene	ng/g	10	>25,000	>100,000	>22,200	NA
	µg/g OC	10	NA	NA	>2,467	NA
Cyfluthrin Nominal	ng/g	10	2.23 (1.98-2.50)	NA	4.89 (4.35-5.50)	5.51 (4.73-6.42)
	µg/g OC	10	0.248 (0.220-0.278)	NA	0.533 (0.483-0.611)	0.612 (0.526-0.713)
Cyfluthrin Corrected (62%)	ng/g	10	NA	NA	3.03 (2.70-3.41)	3.42 (2.93-3.98)
	µg/g OC	10	NA	NA	0.332 (1.67-2.11)	0.381 (0.326-0.442)

Cyfluthrin was toxic to *E. estuarius* at low ng/g concentrations. These results are comparable to the cyfluthrin LC50 for *H. azteca*. The cyfluthrin LC50 for *H. azteca* is 13.7 ng/g, and the organic carbon-corrected value is 1.08 µg/g OC (Amweg et al., 2005), whereas the mean *E. estuarius* LC50s based on nominal concentrations were 5.16 ng/g and 0.573 µg/g OC. If we assume a 40% loss of cyfluthrin in the spiking process, the expected LC50s would be approximately 3 ng/g, and would be comparable to cyfluthrin LC50 concentrations developed by SCCWRP (~2 ng/g).

TIEs and Solid-Phase Micro Extraction using Cyfluthrin-spiked sediment

The analysis of the SPME fibers was conducted by two different laboratories using different methods of extraction. One-centimeter fibers were placed in interstitial water that was isolated from untreated sediment and sediment that had been treated with Amberlite resin. The sediments were spiked with a range of cyfluthrin concentrations bracketing the LC50. The SPME fibers were allowed to come to equilibrium in the interstitial water before they were removed and extracted. One set was extracted by UC Davis researchers and analyzed by the Institute for Integrated Research in Materials, Environments and Society (IIRMES) and California State University Long Beach, and the other set of fibers was sent to Gan research group at UC Riverside for extraction and analysis.

Results from both laboratories indicated that the amount of cyfluthrin equilibrated with the fibers was undetectable. It is not clear why this occurred. Prior to the procedure we consulted with the Gan research group at UC Riverside to determine the optimal method for conducting the measurements of bioavailable cyfluthrin. These methods were implicitly followed and two fiber extraction procedures were employed in the event one provided less than optimal results.

It is possible that the range of concentrations in the spiked sediments (0.5 – 50 ng/g) was too low to be detected by the equilibration method that was used. Hunter et al. (2009) (Hunter et al., 2008) (Hunter et al., 2008) (Hunter et al., 2008) used the equilibration method for measuring the bioavailable fraction of several pyrethroids in sediments that had been spiked at a concentration of 200 ng/g. The equilibration method involves allowing the pyrethroid in the interstitial water sample to come to equilibrium with the surface of the fiber over several days. This period can be

shortened by agitating the samples, as in the current study. Other researchers have used the non-equilibration method, which involves immersing the fiber in the sample for approximately 30 minutes and then inserting the fiber directly into a gas chromatograph (GC) for analysis. This method has been used to analyze sediment concentrations of pyrethroids as low as 7.5 ng/g (Xu et al., 2007; Hunter et al., 2008), and might have been more advantageous for testing the lower concentrations used in the current study. Drawbacks of this method are that it can only be performed in the presence of a GC, samples have to be analyzed one at a time, and a range of calibrators have to be prepared and analyzed during the same event (Svetlana Bondarenko, UC Riverside, personal communication). These conditions did not exist at MPSL, so the equilibration method was used.

Although SPME fibers have not been specifically used in a TIE context, they have been used to measure bioavailable contaminants in whole sediment exposures, and to link measured concentrations with bioaccumulation and toxicity (Xu et al., 2007). Experiments measuring bioaccumulation in *Tubifex* worms demonstrated that SPME measurements provided an accurate prediction of chemical uptake by the test organisms (Conder and La Point, 2005). You et al. (You et al., 2006) reported that SPME fibers placed in sediments with *Lumbriculus variegatus* for the duration of their exposure accurately predicted the bioaccumulation of spiked contaminants by the organism. Xu et al. (2007) used SPME measurements in interstitial water to determine sediment LC50 values based on bioavailable concentrations of spiked pyrethroids. Although the current experiments were not successful in utilizing SPME as a TIE tool, further studies should be conducted using the non-equilibration method.

Interstitial Water Testing

All interstitial water control treatments had survival greater than 92%, but the measured cyfluthrin concentrations in the baseline treatments were well below nominal concentrations. Significant toxicity was observed in the baseline of all three cyfluthrin concentrations, indicating that there was enough cyfluthrin present to cause toxicity, but the analytical results were flawed by extensive holding time and improper storage. Samples were collected at the time of test initiation on December 2, 2010, but were not extracted until December 15. Samples were shipped to CRG Laboratories as per the contract, but were returned unopened. CRG

Laboratories had gone out of business and had not informed their clients. The samples had been warm for 48 hours. Arrangements were made to have the samples analyzed by Rich Gossett at IIRMES, and were re-chilled and shipped immediately. The sample holding time before extraction was seven days, but the samples were extracted thirteen days after preparation. We were informed by IIRMES that significant breakdown of cyfluthrin could have occurred during that time. Cyfluthrin was not detected in the 5 ng/L baseline, but 8.5 ng/L were detected in the 10 ng/L baseline, and 5.4 ng/L were detected in the 25 ng/L baseline (Table 11).

There was significant reduction of toxicity in all three Amberlite treatments (Table 11). Although three resin elution procedures were used in sequence, only the eluate treatment from the 25 ng/L cyfluthrin concentration demonstrated significant toxicity. There was complete mortality in the baseline of the highest concentration, but only 24% mortality in the eluate treatment (76% survival), indicating a fairly weak recovery of cyfluthrin from the Amberlite. It is impossible to discuss the reduction and recovery of cyfluthrin quantitatively because the analytical results were flawed. However, based on the toxicity results, it can be assumed that there was adequate reduction of cyfluthrin in the interstitial water, and less than adequate recover in the eluate treatments.

Table 11. Mean percent survival (standard deviation) of *E. estuarius* in interstitial water spiked with cyfluthrin (Baseline) and extracted with resin (Amberlite), and in an Eluate treatment prepared from the resin.

Nominal Concentration	Treatment	Cyfluthrin Concentration	Mean	SD
5 ng/L	Baseline	ND	68	23
	Amberlite	ND	92	18
	Eluate	ND	80	20
10 ng/L	Baseline	8.5 ng/L	12	18
	Amberlite	ND	92	11
	Eluate	ND	86	9
25 ng/L	Baseline	5.4 ng/L	0	0
	Amberlite	ND	96	9
	Eluate	ND	76	17
Control	Baseline	NA	96	9
	Amberlite	NA	92	11
	Eluate	NA	96	9

When these results are considered in the context of the goals of the Phase I and II TIE process, the resin treatments characterized the cause of toxicity (i.e., successful Phase I TIE objective),

but the elution treatments did not recover sufficient chemical for positive identification of cyfluthrin toxicity (unsuccessful Phase II TIE objective). Cyfluthrin was an appropriate model chemical for development of TIE procedures because it's highly toxic and extremely hydrophobic. Therefore, TIE procedures that work using cyfluthrin are likely to work using other organic chemicals. However, the properties that make pyrethroids good candidates for evaluating TIEs also make them problematic. The threshold for cyfluthrin toxicity to *E. estuarius* is close to the analytical method detection limit using GC/NCI (0.5 ng/g). It is not clear why the three step acetone elution procedures used in the current experiments did not recover more cyfluthrin from the Amberlite. Previous experiments with HLB SPE columns have shown up to 90% recovery of spiked pyrethroids using acetone, but the current experiments suggest that more polar solvents are required to fully elute pyrethroids from Amberlite. A side experiment was performed using hexane to extract Amberlite, but this solvent had a negative reaction with the resin and melted it.

Similar interstitial water experiments to those described above were performed for a separate State Water Board-funded project investigating sediment TIE methods in freshwater applications (Phillips et al., 2011). While this study also employed experiments with batch extractions and batch elutions, it also explored the efficiency of standard SPE columns. Three sets of experiments were conducted with natural fresh interstitial water spiked with the organophosphate pesticide chlorpyrifos and the pyrethroid pesticide bifenthrin. The experiments were conducted iteratively based on the results of the previous experiment. The first experiment evaluated batch extractions in a similar manner to those conducted in the current study, but with varying interaction times between the sample and the resin, and with varying elution methods. Regardless, the first experiment produced similar results. Batch extractions were able to successfully reduce the concentration of the spiked chemical and the observed toxicity, but elutions of the extraction medium (Amberlite) were unable to recover a significant amount of chemical.

The second experiment tested the batch extraction again, but eluted the resin with methylene chloride, a more polar solvent. Additional treatments in this experiment included two SPE columns with standard acetone elutions. All of the extraction media were successful at reducing

the toxicity and concentrations of chemical, but the column eluates were more successful at recovering the toxicity and chemical. The third experiment focused exclusively on SPE columns and varied the size of the column and the extraction solvent. All of the columns successfully reduced chemical concentrations and toxicity, and the 200g HLB column and the 500g C18 column eluates with methylene chloride produced the greatest recovery of toxicity and chemical. Although SPE columns have had a variable performance in past studies with marine sediment TIEs, these columns worked well with pesticide-spiked fresh interstitial water (Phillips et al., 2011). These results provide an important additional tool for Phase II TIEs using interstitial water.

Batch extraction and elution methods could still be optimized for both fresh and marine sediment TIEs, but it would require to exploring alternate extraction media to replace Amberlite, and alternate solvents for use in Phase II elution steps. For example, the XAD resin Tenax has been used to estimate chemical bioavailability in sediment toxicity research but has not been used in a TIE context. Tenax can be extracted with stronger solvents such as methylene chloride and may therefore allow greater recovery of chemicals. One drawback with Tenax is that this resin floats, and this characteristic makes it less amenable to exposures with organisms in TIE applications.

Stressor Identification Workshops

Background

Two SFEI workshops were planned to facilitate information exchange between scientists researching methods to identify chemicals and other stressors responsible for sediment toxicity. The first workshop was held in April 2010. The goals were to identify existing and emerging TIE tools and other sublethal indicators of contaminant stress, determine the most important chemical and non-chemical factors responsible for sediment toxicity, and to identify data gaps and missing toxicological and analytical tools that may prove useful in future research. The workshop focused on stressors causing amphipod mortality. Scientists from SFEI, the US EPA, the State Water Resources Control Board, UC Davis, UC Berkeley, SCCWRP, the Central Valley Regional Water Quality Control Board, the San Francisco Bay Regional Water Quality Control Board, and private laboratories participated in the discussion. The findings of the first

workshop are summarized below, but a complete set of meeting notes and presentations can be found at http://www.sfei.org/node/3117.

Overview of Sediment Toxicity Issues in San Francisco Bay (Brian Anderson, Bryn Phillips)

Amphipod mortality in the Estuary is generally moderate, with more toxicity occurring during the winter sampling events. Severe amphipod toxicity (<35% survival) is observed infrequently. There have been several correlation-type studies that examine the relationships among toxicity, selected contaminants, and physical factors, such as grain size (Thompson et al., 1999; Hunt et al., 2001b; Anderson et al., 2007a). There have also been a number of whole sediment, sediment elutriate, and interstitial water TIE studies conducted on Estuary sediments by the UC Davis research group. Approximately fifteen Elutriate TIEs and two interstitial water TIEs were conducted between 1996 and 2004 using the bivalve *Mytilus galloprovincialis*. The results of these studies indicated that metals, particularly copper, were the cause of toxicity (Hunt et al., 2001a; Phillips et al., 2003; Anderson et al., 2007a). Whole sediment TIEs were conducted on toxic sediments from Redwood Creek and Grizzly Bay. Treatments in these experiments utilized newly developed whole sediment methods including the addition of coconut charcoal to reduce the bioavailability of organic contaminants, and the addition of SIR-300 resin to reduce the bioavailability of metal contaminants (Hunt et al., 2005). The results of these initial whole sediment TIEs were inconclusive because the samples were moderately toxic and it was difficult to discern subtle difference in treatment responses. In addition to summarizing past TIE studies in the Estuary; detailed results of the Mission Creek TIE study (discussed above) were presented to the workshop attendees. These results provided the background information for afternoon discussions on future research needs for sediment TIEs and stressor-identification research in the San Francisco Estuary.

Application of Multiple Approaches for Stressor Investigation in Southern California Sediments (Steve Bay)

This presentation summarized a three year study at Ballona Creek in Southern California. This project used multiple study tasks to better understand the spatial and temporal extent of contamination in the creek and to further investigate tools for understanding the potential causes of toxicity. TIE method development at SCCWRP has included the use of pyrethroid-specific

TIE methods with some success. Their interpretation of TIE and chemistry results includes comparison to thresholds of effects (LC50s, ERMs, and SQGs), evaluating toxic units, and conducting statistical correlations. Based on the results of the Ballona Creek study, SCCWRP emphasized developing methods for better understanding the bio-available fraction of sediment contaminants using passive interstitial water samplers and SPME. The Ballona Creek TIE results also suggested there is a need to continue developing TIE methods for investigating causes of toxicity from emerging and legacy contaminants, and providing guidance for using and interpreting TIE results.

Analytical Challenges Associated with Identifying Chemicals Responsible for Sediment Toxicity (Kelly Smalling and Keith Maruya)

The first presentation discussed the fact that ambient samples contain mixtures of unmeasured compounds that may be contributing to toxicity, but lack toxicity information. Considering the short chemical analyte list of many toxicity studies, it is difficult to sort out which compounds are a priority for TIE studies (i.e., those chemicals that are likely toxic to lab organisms). It was suggested that researchers need to prioritize key emerging contaminants of concern by developing analytical chemistry methods and developing thresholds of effects (LC50s). A second approach suggested using existing Department of Pesticide Regulation databases to identify likely pesticides of concern.

Specific issues with the chemical analysis of pyrethroids were also discussed. USGS had recently participated in an inter-calibration study with variable results. It was suggested that the cause of the high variability was a lack of standard analysis methods for pyrethroids and lack of certified standards to evaluate accuracy. Another issue with pyrethroids is they are toxic to test organisms at concentrations very near the detection limits of most laboratories.

Keith Maruya presented the results of a study that used SPME fibers to extract the bioavailable fraction of organic compounds in both laboratory and environmental settings. The devices extract key contaminants of concern at levels comparable to bivalve bioaccumulation in controlled tests. A version of the laboratory methods was used in the current study.

The work group identified several groups that are working on the broader issue of emerging contaminants and concluded that coordination with these groups would be useful in developing a prioritized list of contaminants for future toxic effects studies. These groups include: The Pyrethroid Working Group, US EPA, RMP's Emerging Contaminants Workgroup, and other chemists that are developing low detection limits for pyrethroids.

Non-Anthropogenic Chemicals and Non-Contaminant Stressors and Their Role in Amphipod Mortality (Brian Anderson)

A list of potential non-anthropogenic stressors was created and discussed (see below). Some of these stressors can be controlled in the laboratory via the TIE process or by pre-treating the sample. The work group concluded that these stressors should be considered when interpreting TIE results with amphipods.

Ammonia and Sulfide

- Unionized Ammonia – can be treated with pH adjustment and aeration, or by zeolite.
- Hydrogen Sulfide – can be treated with aeration.

Other Toxins

- Phytotoxins – see below.
- Naturally occurring metals (Hg, Mn) – could possibly be treated with resins or via SPE.

Non-Traditional Contaminants

- Anions – can be treated with resin or SPE.
- Polar Organics – can be measured with LC/MS, but unsure of treatment.
- Oxidants – can be treated with sodium thiosulfate.

Test Organism Health

- Salinity Effects/Acclimation – *E. estuarius* has a wide salinity tolerance and the workshop attendees concluded this has a negligible effect on toxicity results if the acclimation guidance is followed.
- Seasonal Health – use of control chart data should indicate any seasonal effects.

Grain Size

- Percent Fines, Percent Clay, and Particle Shape – see below.

Regression analysis of amphipod survival versus percent fines and percent clay using RMP data suggest that amphipod survival in relatively uncontaminated sediments could be affected by grain size. Unpublished data from UC Davis also suggests there that particle size or shape could inhibit amphipod survival. US EPA researchers suggested one way to determine if toxicity was caused by contaminants versus grain size would be to test the sample side-by-side using *E. estuarius* and *Ampelisca abdita*. Since *A. abdita* prefers fine-grained sediments, this would provide additional information on whether chemicals were the cause of toxicity. It has been suggested that *A. abdita* are less sensitive than *E. estuarius* because they build tubes that could isolate them from potentially toxic interstitial water in sediment exposures. The US EPA Narragansett laboratory conducts *A. abdita* exposures using minimal sediment overlying water in the test beakers. This results in a greater exposure of the amphipod to sediment interstitial water because of the flux of contaminants into the overlying water.

Genomic Tools for Identifying Chemicals Affecting Eohaustorius estuarius (Chris Vulpe, Steve Bay)

The gene micro array is an emerging genomic tool for determining the effects of contaminants at a genetic level. An example was presented of micro array trials with daphnids. The Vulpe research team is completing a gene microarray for *E. estuarius*, and will be to begin diagnostic tests with contaminant-dosed samples from the current LC50 development studies conducted by UC Davis and SCCWRP. These results will be used to sort out gene expression signatures related to a toxic effect. Kay Ho (US EPA) mentioned that the EPA spent over two years working on a similar study with the amphipod *A. abdita*, and they were not able to produce clear stressor identification results using methods similar to those used at Berkeley. UC Berkeley researchers plan to exchange information with EPA to determine why there has been a delay in producing a usable method. Currently, UC Berkeley researchers are not far enough along in their development of an *E. estuarius* genomic tool to know if it will be able to indentify effects from specific contaminants or contaminant groups.

Table 12. Preliminary list of stressors of concern and summary of comments by the work group.

Stressor	Work Group comments
Unionized Ammonia	Have adequate tools to address unionized ammonia effects in toxicity tests.
Hydrogen Sulfide	Have adequate tools to address sulfide effects in toxicity tests.
Grain Size (Percent Fines, Percent Clay, or Particle Shape)	Need to determine the effects of grain size parameters on the survival of *E. estuarius*. We need to conduct side-by-side experiments with a species that is not sensitive to grain size effects (e.g. *Ampelisca*). We need to consult with researchers familiar with quantification of particle shape and sizes to determine if a laboratory experiments could be designed to address effects of clay. This should be done through the TWG process.
Physical toxicants (oils/smothering)	These stressors have not been addressed, but may be a factor in some Estuary sediments.
Phytotoxins	These stressors have not been addressed, but we need to determine if they are a factor in the Estuary. San Francisco Regional Board are to be consulted.
Metals	The group agreed that metals are unlikely to cause toxicity to *E. estuarius*. Bivalve embryos tend to be more sensitive to these compounds. We have adequate tools to address anion and cation effects in toxicity tests.
Pesticides	Pesticides are the most important group to prioritize for evaluation. Analytical methods, TIE methods and LC50 values need to be developed. Emphasis on emerging contaminants should focus on newly introduced pesticides.
Organochlorines	Based on established LC50 values (previous and current study) and sediment concentrations in the Estuary, it is unlikely this group of contaminants is contributing significantly to toxicity.
Organophosphates	These contaminants are less of an issue for Estuary sediments, but still need to be evaluated further.
Pyrethroids	These are important emerging pesticides. There are some established LC50s, but additional values are needed for a wider range of test species. There is a need to develop standard methods and establish standard reference materials for detection low-level concentrations in ambient samples.
Fungicides and Herbicides	The potential use and toxicity of these chemicals should be considered in the prioritization effort.
Other	Expand to include new contaminants such as fipronil and triclosan.
Polycyclic Aromatic Hydrocarbons	Specific PAHs and PAH mixtures remain a priority concern in sediment toxicity tests.
Polychlorinated Biphenyls	PCBs are unlikely to be toxic to sediment toxicity test organisms in acute exposures.
Personal Care Products	Worth adding to the list – and to prioritize similar to fungicides/herbicides
Polybrominated Diphenyl Ethers	PBDEs are unlikely to be toxic to sediment toxicity test organisms in acute exposures.
Mixtures	Mixtures were not discussed in detail but the work group agreed additive and synergistic effects are still a concern.

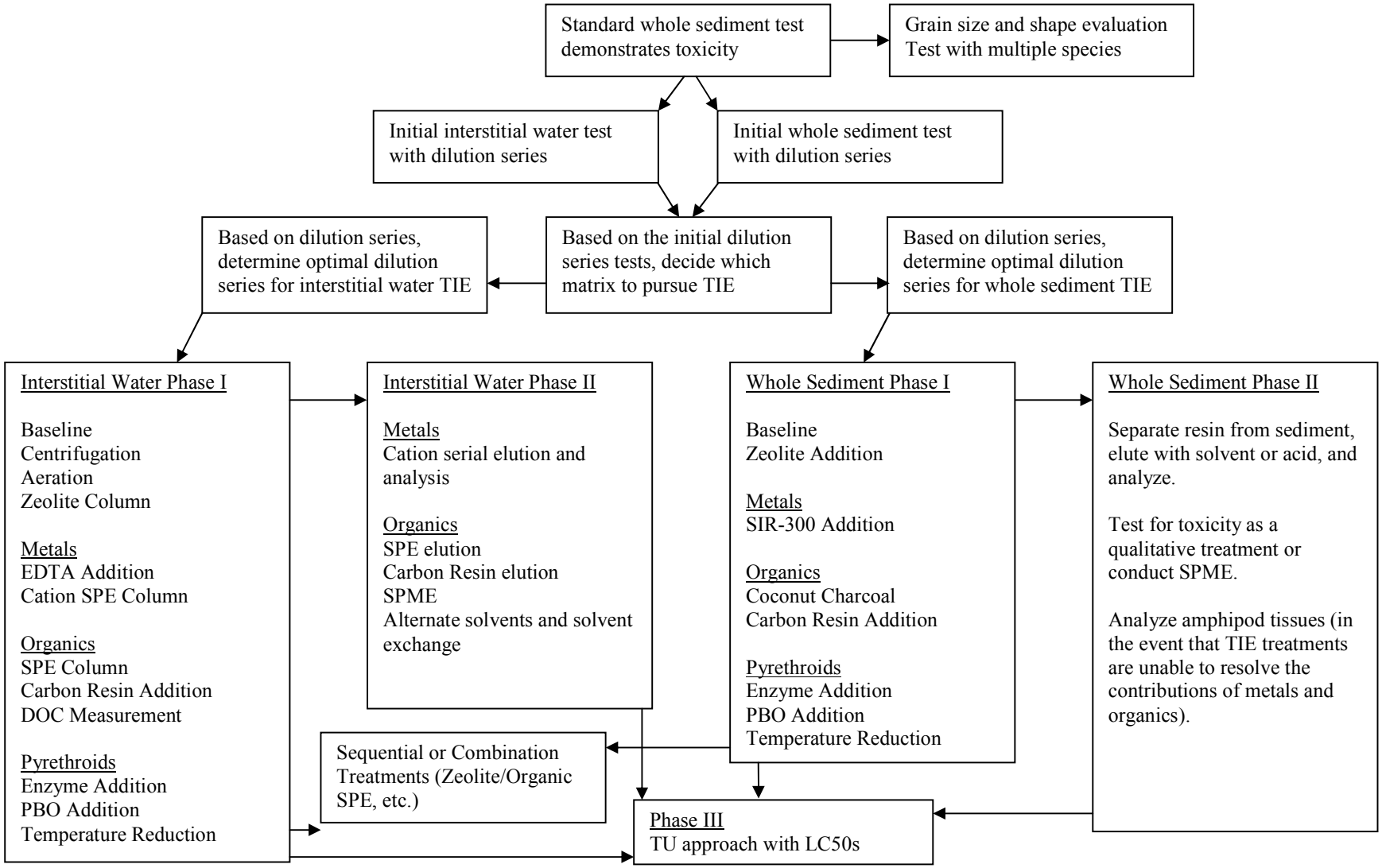

Figure 1. Flow chart of TIE methods.

Workshop Discussion

After the workshop presentations there was a group discussion that focused on three subject areas: 1) Creating a list of likely chemical and non-chemical stressors likely causing amphipod mortality (Table 12), 2) Creating a list of TIE tools and other methods to address these stressors (see Flowchart, Figure 1), and 3) Summarizing information or procedural deficiencies which should be addressed to improve TIEs.

The work group attendees concluded the flow chart would be useful as a basic decision tool. Several procedural comments from the work group were incorporated into the flow chart, while others were more general. All comments are listed below.

- The current SPE column of choice for the UC Davis research group is a hydrophilic-lipophilic balanced column (HLB). This column may not bind hydrophobic contaminants and may result in breakthrough. The C18 column may have stronger hydrophobic binding capabilities.
- Additional solvents need to be evaluated for the extraction of SPE columns. Stronger solvents could be used, but solvent exchange might be necessary.
- Although batch extractions and batch elutions were evaluated in the current study, these procedures might be inefficient.
- Sequential treatments were discussed but not included in the flow chart. It was suggested that less binding treatments be conducted first in the sequence.

The attendees agreed that it was important to recognize how well current TIE methods work to rule out groups of potential stressors (e.g., metals, ammonia, and sulfide). Chris Beegan from the State Water Board endorsed TIEs by stating help to put the sediment chemistry data in context may potentially save millions of dollars in unnecessary total maximum daily load (TMDL) listings by identifying toxicologically irrelevant chemicals. He encouraged moving forward with the development of existing sediment TIE methods.

The work group identified several research groups that are working on the broader issue of emerging contaminants, and thought that coordination with these researchers may be useful in

developing a prioritized list of contaminants to focus on in future toxic effects studies. These groups include: the Pyrethroid Working Group, the US EPA, RMP's Emerging Contaminants Workgroup, and the California State Department of Toxic Substance Control (DTSC).

The work group agreed that the issue of unmeasured contaminants is an ongoing concern. One approach to addressing the possibility that unknown or unidentified chemicals are contributing to sample toxicity would be through more thorough evaluation of chromatograms for non-targeted compounds. This could be conducted as part of the TIE process in close consultation between toxicologists and chemists. Oros et al. (Oros et al., 2003) identified a number of chemical classes in the waters of the Estuary that were previously not routinely measured. It was suggested that the University of Miami and Woods Hole Institute might have additional analytical capability that could help identify unknown chemicals detected in Estuary sediment. Quantitative structure-activity relationship (QSAR) tools can be used to evaluate the potential for toxicity based on chemical characteristics (US EPS QSAR Tool http://cfint.rtpnc.epa.gov/aster).

Future TIE Research Priorities

The following list of topics was compiled from comments by the work group.

Grain Size – Side-by-side experiment between *E. estuarius* and *A. abdita* in a small-volume exposure system would provide information on the sensitivity of *E. estuarius* to fine grained sediment. Additional experiments that include the quantification and possible manipulation of particle shape would also provide information on the effects of grain types.

Pesticides – These chemicals are the most important group to prioritize for evaluation potential for amphipod mortality. Analytical methods, TIE methods and LC50 values need to be developed for all pyrethroid pesticides, as well as emerging pesticides such as fipronil.

Method Development – Whether interstitial water samples are column extracted or batch extracted with resin, there needs to be additional method development for the solid-phase extraction media used in TIEs. Experiments with different columns and resins, as well as

different solvents and solvent exchange methods, need to be conducted with different chemicals to perfect the methods.

Summary of Findings from Comparative Tests with Freshwater and Estuarine Species

Background

During the last two years, the Status and Trends portion of the RMP has included tests with freshwater organisms at seven stations that are in low salinity locations. These stations include three historical sites (BF21, BG20 and BG30), as well as four rotating stations in Suisun Bay. In addition to tests with *E. estuarius*, tests with *H. azteca* and *Chironomus dilutus* were conducted in whole sediment. Sediment-water interface (SWI) tests with *M. galloprovincialis* were augmented with SWI tests with *Ceriodaphnia dubia*.

The RMP decided to test freshwater species at these stations because of historical data demonstrating the salinity regimes were closer to that of freshwater, than marine or even estuarine habitat. This is especially true in the winter months. Figures 2 and 3 depicts the average bottom salinity for the Sacramento and San Joaquin River stations and the Suisun Bay stations, respectively. Average bottom salinities at the river stations seldom exceed 5‰, and are clearly freshwater habitats. Average bottom salinities in Suisun Bay range from approximately 2‰ to approximately 14‰. During the rainy season the salinities in this bay are more representative of a freshwater environment, but during the dry season the salinities are more estuarine.

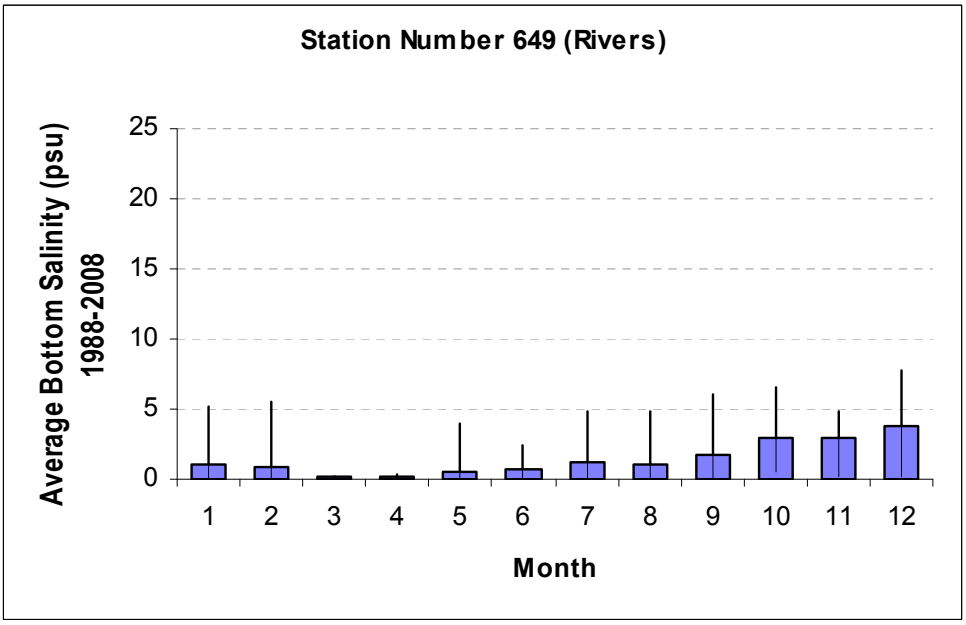

Figure 2. Average bottom salinities at Sacramento and San Joaquin River stations.

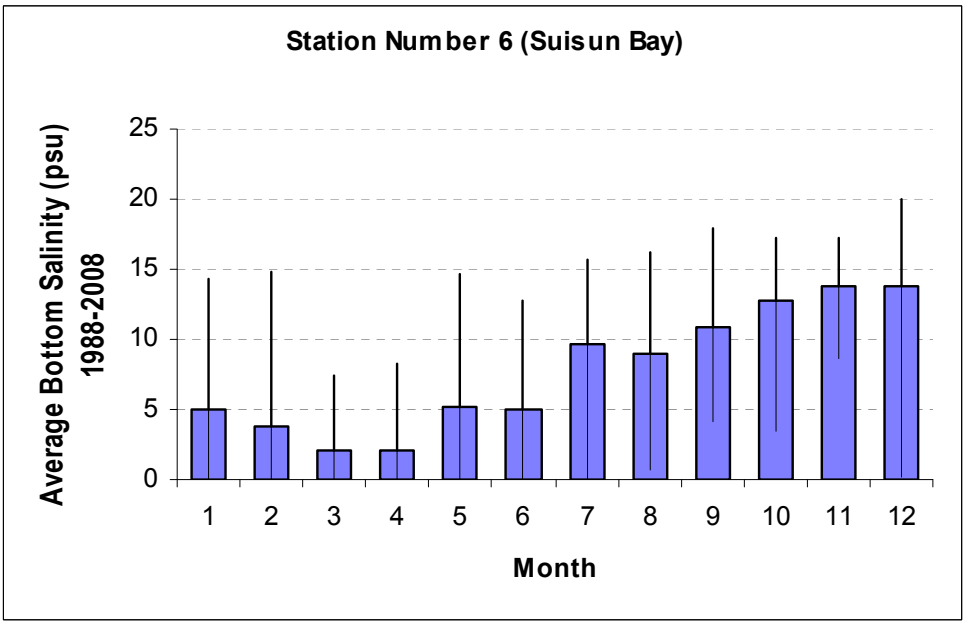

Figure 3. Average bottom salinities at Suisun Bay stations.

Tests with *E. estuarius* and *M. galloprovincialis* produced results that were similar to past sediment surveys. Fifty percent of the amphipod tests and 64% of the bivalve tests were significantly toxic (Table 13). The magnitude of toxicity in these samples was moderate, with survival ranging from 47% to 73% in the amphipod tests and 36% to 63% in the bivalve tests.

Only one of the fourteen samples was significantly toxic to *H. azteca*, and there was no toxicity to *Chironomus dilutus*. Three samples were toxic to *C. dubia* in 2009, but because the sediments were sampled in the summer and contained concentrations of salts that were greater than the organism tolerance, this was the likely cause of toxicity. High sample conductivity was not an issue in the 2010 tests, but low survival was still observed in five of the seven samples. Despite these low survival rates, there was no statistically significant toxicity to *C. dubia* because of high variability among the replicates. It is not clear what caused the high variability.

Table 13. Mean percent survival (standard deviation) of sediment tests with *H. azteca*, *C. dilutus*, and *C. dubia*. Shaded areas indicate sample mean was significantly different than control mean based on separate variance t-test (1-tailed, alpha = 0.01), and the difference between the mean control response and the mean sample response was greater than the 90th percentile minimum significant difference (MSD).

2009	*E. estuarius*		*M. galloprovincialis*		*H. azteca*		*C. dilutus*		*C. dubia*	
Station	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
BF21	53	15	56	7	89	8	81	21	20	24
BG20	89	6	47	19	85	12	86	12	100	0
BG30	81	7	36	12	95	5	94	9	64	43
SU016	55	6	59	12	70	21	89	6	28	39
SU073	59	11	63	8	63	14	80	11	4	9
SU085	87	12	76	10	60	27	89	14	56	46
SU090	90	12	74	7	66	24	86	11	76	26
Control	95	4	84	5	88	12	98	5	100	0

2010	*E. estuarius*		*M. galloprovincialis*		*H. azteca*		*C. dilutus*		*C. dubia*	
Station	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
BF21	47	8	53	16	90	8	96	5	56	52
BG20	90	9	36	27	99	4	95	5	44	46
BG30	87	6	63	13	93	9	91	10	44	41
SU060	73	11	39	13	95	8	89	11	56	43
SU073	61	8	91	12	93	14	90	12	80	28
SU084	54	13	86	23	94	7	98	5	92	11
SU109	92	8	63	21	96	5	90	9	20	28
Control	96	6	89	13	86	13	89	11	80	45

Recommendations

One goal of the RMP is to use ecologically relevant test species for monitoring, but the program is also trying to maintain a connection to a long-term data set. Historically, the program has used *E. estuarius* for whole sediment exposures, and *M. galloprovincialis* for elutriate exposures, and more recently, exposures at the sediment-water interface. The amphipod is tolerant to a wide range of salinities (0‰ to 34‰), but is generally tested at the brackish salinity of 20‰, and is considered a true estuarine organism. The bivalve has a low salinity tolerance of 25‰. The river stations clearly have low bottom salinities that are representative of freshwater habitat, and should be tested with freshwater species.

All of these organisms have varying sensitivities across a wide range of chemicals. Table 14 presents water LC50 values for four representative chemicals, but it should be noted that the durations of these exposures vary based on the different studies. The freshwater amphipod is more sensitive to chlorpyrifos and fluoranthene than *E. estuarius*, but they are somewhat comparable for copper and cyfluthrin. *Ceriodaphnia dubia* has comparable copper sensitivity to the bivalve, but is orders of magnitude more sensitive to chlorpyrifos.

Table 14. Median lethal concentrations (LC50s) in water for the three freshwater and two marine species for several chemicals commonly measured in the Estuary.

Species	Copper	Chlorpyrifos	Cyfluthrin	Fluoranthene
E. estuarius	49 mg/L (Phillips et al., 2009b) 10 Day	529 ng/L (Anderson et al., 2008) 4 Day	2.93 ng/L (current study) 4 Day	671 µg/L (Phillips et al., 2009b) 10 Day
M. galloprovincialis	7.8 µg/L (Phillips et al., 2003) 2 Day	4900 µg/L (Serrano et al., 1995) 2 Day		
H. azteca	35 µg/L (Phipps et al., 1995) 10 Day	86 ng/L (Phipps et al., 1995) 10 Day	2.3 ng/L (Weston and Jackson, 2009) - 10 Day	44.9 µg/L (Suedel et al., 1993) 10 Day
C. dilutus	54 µg/L (Phipps et al., 1995) 10 Day	70 ng/L (Phipps et al., 1995) 10 Day		31.9 µg/L (Suedel et al., 1993) 10 Day
C. dubia	15 µg/L (Schubauer-Berigan et al., 1993) - 2 Day	54 ng/L (Bailey et al., 1997) 4 Day	344 ng/L (Wheelock et al., 2004) 2 Day	45 µg/L (Oris et al., 1991) 2 Day

The authors recommend using *H. azteca* for exposures in whole sediment, and *C. dubia* for exposures at the sediment-water interface for monitoring the river stations. These organisms are more appropriate for the salinity regime of these stations. *Hyalella azteca* is generally more sensitive than *E. estuarius* and has comparable sensitivity to contaminants as *C. dilutus* (Table 14), but exposures with *H. azteca* generally provide more consistent results. Monitoring with *H. azteca* will also provide a link to monitoring in the river delta as part of the SQO program. *Ceriodaphnia dubia* is the primary water column test organism for fresh water, and is one leg of whole effluent toxicity three species testing. Tests with *C. dubia* at the sediment-water interface have demonstrated excessive variability in the past two RMP monitoring events, but this is a new application of this exposure system and variability will be surely reduced over time.

Based on the variable bottom salinities of Suisun Bay this area of the Estuary should be considered a transition zone between estuarine/marine habitat and the freshwater habitat. Although *H. azteca* can tolerate salinity up to 15‰, the salinity tolerance of *C. dubia* organisms is too low to test sediments from this area year round. For this reason, the authors recommend maintaining the use of *E. estuarius* for whole sediment testing and *M. galloprovincialis* for testing at the sediment-water interface.

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Table A1. Concentrations of chemicals detected in Castro Cove sediment. ND indicates not detected

Analyte	Concentration
Pyrethroids	**(ng/g)**
Allethrin	ND
Bifenthrin	ND
Cyfluthrin	ND
Cypermethrin	ND
Danitol	ND
Deltamethrin	ND
Esfenvalerate	ND
Fenvalerate	ND
Fluvalinate	ND
L-Cyhalothrin	ND
Permethrin	ND
Prallethrin	ND
Organochlorines	**(ng/g)**
2,4'-DDD	ND
2,4'-DDE	ND
2,4'-DDT	ND
4,4'-DDD	7.08
4,4'-DDE	ND
4,4'-DDT	7.55
Aldrin	ND
BHC-alpha	ND
BHC-beta	ND
BHC-delta	ND
BHC-gamma	ND
Chlordane-alpha	ND
Chlordane-gamma	ND
cis-Nonachlor	ND
Dacthal	ND
Dicofol (Kelthane)	ND
Dieldrin	ND
Endosulfan Sulfate	ND
Endosulfan-I	ND
Endosulfan-II	ND
Endrin	ND
Endrin Aldehyde	ND
Endrin Ketone	ND
Heptachlor	ND
Heptachlor Epoxide	ND
Methoxychlor	ND
Mirex	ND
Oxychlordane	ND
Perthane	ND
trans-Nonachlor	ND
PAHs	**(ng/g)**
1-Methylnaphthalene	ND
1-Methylphenanthrene	ND
2,3,5-Trimethylnaphthalene	ND
2,6-Dimethylnaphthalene	20.5

Analyte	Concentration
Metals	**(µg/g)**
Aluminum	15600
Antimony	0.375
Arsenic	8.32
Barium	41.0
Beryllium	0.370
Cadmium	0.425
Chromium	55.1
Cobalt	12.0
Copper	27.0
Iron	26300
Lead	13.8
Manganese	303
Mercury	0.220
Molybdenum	0.393
Nickel	51.4
Selenium	0.416
Silver	0.292
Strontium	38.2
Thallium	0.0980
Tin	1.55
Titanium (Ti)	485
Vanadium (V)	51.5
Zinc (Zn)	68.2
PCBs	**(ng/g)**
PCB003	ND
PCB018	ND
PCB028	ND
PCB031	ND
PCB033	ND
PCB037	ND
PCB044	ND
PCB049	ND
PCB052	ND
PCB056/060	ND
PCB066	ND
PCB070	ND
PCB074	ND
PCB077	ND
PCB081	ND
PCB087	ND
PCB095	ND
PCB097	ND
PCB099	ND
PCB101	ND
PCB105	ND
PCB110	ND
PCB114	ND
PCB119	ND
PCB123	ND

Analyte	Concentration	Analyte	Concentration
2-Methylnaphthalene	2.61	PCB126	ND
Acenaphthene	ND	PCB128	ND
Acenaphthylene	1.97	PCB138	ND
Anthracene	8.21	PCB141	ND
Benz[a]anthracene	34.5	PCB149	ND
Benzo[a]pyrene	31.2	PCB151	ND
Benzo[b]fluoranthene	31.6	PCB153	ND
Benzo[e]pyrene	28.1	PCB156	ND
Benzo[g,h,i]perylene	46.6	PCB157	ND
Benzo[k]fluoranthene	16.5	PCB158	ND
Biphenyl	4.80	PCB167	ND
Chrysene	35.1	PCB168/132	ND
Dibenz[a,h]anthracene	9.25	PCB169	ND
Dibenzothiophene	2.08	PCB170	ND
Fluoranthene	44.0	PCB174	ND
Fluorene	2.29	PCB177	ND
Indeno[1,2,3-c,d]pyrene	44.6	PCB183	ND
Naphthalene	4.29	PCB187	ND
Perylene	25.0	PCB189	ND
Phenanthrene	15.3	PCB194	ND
Pyrene	58.8	PCB195	ND
		PCB200	ND
Total Organic Carbon	0.9%	PCB201	ND
Sand	25.36%	PCB203	ND
Silt	57.20%	PCB206	ND
Clay	17.44%	PCB209	ND