



Final Technical Report

2011

**Sediment Toxicity Identification Evaluation: Advancing
Procedures for Extracting and Recovering Chemicals of
Concern from Sediment Interstitial Water**

June 2011



www.waterboards.ca.gov/swamp

**Sediment Toxicity Identification Evaluation: Advancing Procedures for
Extracting and Recovering Chemicals of Concern from Sediment Interstitial
Water**

FINAL REPORT

Authors: Bryn M. Phillips, Brian S. Anderson, Hector DeHaro, Jennifer P. Voorhees, Katie Siegler (University of California, Davis)

Project Director: Ron Tjeerdema (University of California, Davis)

Contract Manager: Stephanie Fong (Central Valley Water Quality Control Board)

June 23, 2011

Executive Summary

Hydrophobic contaminants discharged into aquatic environments quickly associate with suspended particulates and accumulate in depositional areas. Accumulated contaminants can be toxic to benthic organisms, and sediment toxicity is commonly observed in marine and freshwater habitats throughout California. Sediment toxicity identification evaluation (TIE) techniques are the primary tool used to identify specific chemicals responsible for toxicity. Current sediment TIE procedures are conducted on whole sediment or interstitial water, and can generally characterize the cause of toxicity (Phase I), but are less effective at identifying specific chemicals causing toxicity (Phase II). Most whole sediment treatments are Phase I procedures that characterize the cause of toxicity as metal, organic, ammonia, or a combination of these contaminants. Some whole sediment Phase II procedures have been developed but they are not used routinely, and recent research has demonstrated these procedures only qualitatively identify the cause of toxicity. Phase II procedures are better adapted to interstitial water because this matrix is easier to manipulate using standard TIE treatments.

Phase II treatments with interstitial water generally involve extracting the sample with some form of solid-phase extraction (SPE) medium to reduce chemical concentrations and toxicity. The extraction medium is then eluted with solvent, the solvent is reconstituted into an elution treatment, and the treatment is tested to determine if contaminants and toxicity can be recovered. Standard TIE procedures for water samples employ SPE columns for this procedure, but previous interstitial water TIE experiments with SPE columns have provided mixed results. Based in part on recommendations of national, state, and regional experts participating in a scientific working group convened by the San Francisco Estuary Institute (SFEI), this study was designed to evaluate batch extraction methods using XAD resin, re-examine SPE column extraction methods, and evaluate the use of different solvents for eluting the extraction media.

Three sets of experiments were conducted with natural fresh interstitial water spiked with the organophosphate pesticide chlorpyrifos, the pyrethroid pesticide bifenthrin, or a combination of the two pesticides. These pesticides have been shown to be responsible for sediment toxicity in California's central valley and central coast. The experiments were conducted iteratively based

on the results of the previous experiment. All toxicity tests were conducted with the amphipod *Hyalella azteca*. The first experiment evaluated batch extractions with Amberlite resin. In these extractions the resin was stirred with the interstitial water samples spiked with pesticide and allowed to freely interact. The extractions were conducted for four hours and 24 hours. Resin from both extraction treatments were then eluted with acetone 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 did not recover a significant amount of chemical.

The 24-hour batch extraction was repeated in the second experiment, but the resin was eluted with methylene chloride, a more polar solvent. Additional treatments in this experiment included two SPE columns (HLB and C18) 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 spiked chemical. Both column elutions recovered the same amount of toxicity in the bifenthrin and combination treatments, but the HLB column recovered a greater amount of toxicity in the chlorpyrifos treatment.

The third experiment focused exclusively on SPE columns, but varied the size (resin capacity) of the columns and the type of extraction solvent. All of the columns successfully reduced chemical concentrations and toxicity, but the HLB column and the 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 interstitial water TIEs, these columns worked well with pesticide-spiked fresh interstitial water. Results of these experiments demonstrate that Phase II sediment TIEs will provide better results when interstitial water is extracted using multiple types of solid-phase extraction columns (e.g., C18 and HLB). In addition, column elution efficiency is increased using methylene chloride.

Introduction

Many contaminants discharged to aquatic environments associate with particulate material and accumulate as bedded sediment in depositional areas of streams, lakes, estuaries, wetlands, and coastal waters. Accumulated contaminants can be toxic to benthic organisms, and sediment toxicity is commonly observed in marine and freshwater habitats throughout California.

Successful reduction of toxic sediments requires contaminant source control, which requires the identification of the chemical or chemicals causing the observed biological impacts. Chemicals of concern can sometimes be identified by comparing their measured concentrations in sediment with previously derived toxicity thresholds, such as median lethal concentrations (LC50s). This approach is limited, however, in sediments contaminated by multiple chemicals, as is frequently the case in watersheds supporting a variety of land use activities.

Sediment toxicity identification evaluation (TIE) techniques are the primary tool used to identify specific chemicals responsible for toxicity. TIE procedures are conducted using a phased approach. Phase I treatments characterize toxicity as being caused by one of three general categories of contaminants: organic chemicals, metals, or ammonia, or mixtures of chemicals. Phase II TIE treatments are designed to identify the specific contaminant(s) causing toxicity. More advanced Phase II procedures include procedures that isolate chemicals into various fractions that can be tested individually. These procedures would be used in conjunction with available LC50 values to interpret chemistry data. Phase III TIEs confirm the Phase I and II results. These phases are often conducted simultaneously to conserve effort and resources, and provide additional information. Whole sediment and interstitial water chemistry data are used to provide lines of evidence in this process.

Phase I whole sediment TIE treatments are well established (USEPA, 2007), and Phase II treatments have been evaluated (Anderson et al., 2007; Perron et al., 2009; Phillips et al., 2009a; Anderson et al., 2010), but not perfected. For instance, 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 reduce bioavailable contaminants in 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. In the case of whole sediment, resin addition methods used to date likely result in a nearly exhaustive removal of organic contaminants from the sediment, rather than removal of only the bioavailable fraction, because the resins are left in the sediment for the duration of the 10-day toxicity test. In most cases, elution of the resin after the exposure overestimates the bioavailable fraction of the sorbed contaminants (Phillips et al., 2009a), because the resin not only extracts 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). Eluting the resin at the termination of the exposure has proven to provide qualitative evidence for identifying the cause of toxicity.

This study follows consensus recommendations from national, state, and regional experts participating in a scientific working group convened by the San Francisco Estuary Institute (SFEI). The group included participants from UC Davis, UC Berkeley, SFEI, Southern California Coastal Water Research Project (SCCWRP), the U.S. Environmental Protection Agency, and others. The current project was designed to build upon whole sediment and interstitial water TIE improvements that were tested using the estuarine amphipod *Eohaustorius estuarius*. In that larger study, resin batch extractions of interstitial water spiked with the pyrethroid cyfluthrin were evaluated. Interstitial water toxicity caused by concentrations as high as 25 ng/L was reduced by extracting the samples for 24 hours using resin. However, Phase II resin elution procedures did not recover toxic concentrations of cyfluthrin.

Additional refinements of marine whole sediment TIE procedures were investigated in the SFEI study, and included the evaluation of solid-phase microextraction (SPME) fibers to quantify the

reduction of bioavailable chemicals after extraction resin was added to whole sediment samples. A single experiment was conducted with reference sediment spiked with cyfluthrin. 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 possible 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, this procedure could be further investigated with other chemicals in the context of conducting sediment TIEs (Phillips et al., 2011).

Because of the above-mentioned limitations on quantitative recovery of toxicants in whole sediment TIEs, there is increasing emphasis on improving Phase II identification procedures using sediment interstitial water. This was a specific recommendation of the SFEI TIE working group. Use of interstitial water in Phase II extraction and elution procedures provides more quantitative evidence of chemicals causing toxicity because equilibrium partitioning theory suggests this environmental compartment represents the bioavailable fraction of chemicals in sediment. If interstitial water toxicity is reduced or removed after treatment with an SPE column, toxicity can be ascribed to organic chemicals. If chemicals recovered from the column are toxic in the eluate treatment, this provides additional evidence that organic chemicals are responsible for toxicity. Concentrations of recovered chemicals provide a quantitative line of evidence of the cause of toxicity when they exceed known toxicity thresholds for the test organism.

The standard method for removing contaminants from interstitial water has historically involved SPE columns. However, extraction efficiency of these columns has been inconsistent with interstitial water, 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). This greatly limits the effectiveness of toxicant identification in sediments. This suggests a critical need to develop

more efficient procedures to extract and recover organic chemicals in sediment interstitial water, to improve the TIE Phase II toxicant identification process.

The objective of the current project was to improve upon the performance deficiencies of the SPE procedures currently used in interstitial water TIEs. Recent experiments using pesticide-contaminated water samples from the Santa Maria River watershed have demonstrated that the carbonaceous resin (Amberlite) used to extract chemicals from whole sediment is effective at removing contaminants and toxicity from water using batch extraction procedures (Phillips et al., 2010). These studies have also shown that toxic concentrations of chemicals could be recovered from the resin. The SFEI study on spiked estuarine sediments from San Francisco Bay also demonstrated a reduction of interstitial water toxicity with the addition of resin, but incomplete elution of the spiked cyfluthrin was observed (Phillips et al., 2011). The objectives of the current study were to evaluate batch extraction methods that might allow complete removal of organic contaminants and their toxicity from fresh interstitial water, and to maximize elution of the resin to allow complete recovery of the extracted chemicals. Additional experiments re-evaluated extraction and elution methods using different types of SPE columns in an attempt to reduce method variability. The study proceeded iteratively and built on the results of preceding SFEI TIE experiments.

Methods

Test Chemicals and Analysis

Laboratory water and natural interstitial water was spiked with two widely-used pesticides, the pyrethroid pesticide bifenthrin (Chem Service, West Chester, PA), and the organophosphate pesticide chlorpyrifos (Accustandard, New Haven, CT). These structurally distinct pesticides have been demonstrated to account for much of the sediment toxicity observed in Central Valley monitoring studies, and both pesticides often co-occur in toxic sediments. Although two pesticides are used as the model chemicals to spike the interstitial water, the goal of these experiments is to evaluate generic treatments that can be used routinely to investigate causes of sediment toxicity.

Laboratory water was used in the first experiment to determine the appropriate dosing concentration without the interference of dissolved organic carbon present in interstitial water. The laboratory water was natural well water from the Marine Pollution Studies Laboratory. Subsequent spiking experiments were conducted using interstitial water that was isolated from large quantities (>60 liters) of sediment collected from a reference site on the Carmel River. The spiking concentrations for the first experiments were 40 ng/L bifenthrin and 400 ng/L chlorpyrifos. These doses constituted approximately 4.3 and 4.7 toxic units, respectively, based on a 96-hour *Hyalella azteca* water-only LC50 value of 9.3 ng/L for bifenthrin (Anderson et al., 2006), and a ten-day *H. azteca* water-only LC50 value of 86 ng/L for chlorpyrifos (Phipps et al., 1995). Based on the results of the first experiment, the doses were increased by half for the second and third experiments to overcome possible reductions in pesticide bioavailability caused by dissolved organic carbon in the interstitial water. The dissolved organic matter in the interstitial water was not measured.

Enzyme-linked immunosorbent assays (ELISA, Strategic Diagnostics Inc, Newark, DE) were conducted on both chlorpyrifos treatments to measure chlorpyrifos concentrations in the baseline samples and after extractions and elutions. ELISA procedures followed those recommended by Sullivan and Goh (Sullivan and Goh, 2000). Readings were compared to a 5-point standard curve prepared using standards provided by the manufacturer. Accuracy was determined for each batch using external standards, and precision was determined with duplicate measurements. Samples were tested without dilution unless necessary. Lowest detectable concentrations for this procedure were 30 ng/L for diazinon and 50 ng/L for chlorpyrifos. Reporting limits were twice the lowest detectable concentrations. The final experiment also included analytical confirmation with liquid-liquid extraction followed by GC/MS. Chlorpyrifos was measured using EPA Method 625M and bifenthrin was measured using EPA Method 625M with negative chemical ionization (NCI).

Toxicity Testing and TIE Treatments

Water toxicity was assessed using 96 hour exposures with the amphipod *Hyalella azteca* (USEPA, 2002). Exposures were conducted in 50 mL beakers containing 15 mL of treated water

and five test organisms. All treatments consisted of five replicates. Water quality parameters including dissolved oxygen, pH, and conductivity were measured. Toxicity data were evaluated using separate-variance t-tests that compared the treatments to the baseline and the appropriate blanks.

Various treatments were used in each experiment of the study. As the study progressed, less effective treatments were replaced. Treatments in the first experiment included two batch extractions with Amberlite® XAD-4 resin (Rohm and Haas, Spring House, PA, USA) at different mixing times, and two types of solvent elution methods for each batch extraction. Batch extractions differ from column extractions in that the extraction medium (e.g., Amberlite resin beads) is placed loosely in the sample as opposed to being packed into a column and having the sample pass over it. The sample preparations are made prior to the addition of organisms. Resin beads were prepared for the batch extractions as per the manufacturer's instructions by soaking in methanol and then thoroughly rinsing with Nanopure® water. Spiked laboratory water was subjected to Amberlite extraction by spinning 100 mL of sample with 5g of prepared resin beads in flasks on a stirrer plate for two different times: 4 hours and 24 hours. The resin from each of the two extraction-time treatments was then sieved from the water, and each was eluted using two methods. The first method was a batch elution and involved transferring the resin to a 20 mL vial, adding 10 mL of acetone and stirring for one hour with a magnetic stirrer. The vial was then placed in a sonication bath for one hour, after which the resin was removed and the acetone was blown down to 1 mL with nitrogen. The second method was a column elution and involved placing the resin in a column and passing 10 mL of acetone through the column. The same aliquot of acetone was passed through the column four additional times before being blown down to 1 mL with nitrogen. The acetone aliquots were then added to 100 mL clean water to create eluate treatments for chemistry and toxicity testing. Standard TIE guidance recommends using methanol to elute extraction media, but we have determined that a stronger solvent is necessary to elute pyrethroids. Previous experiments with acetone determined that this solvent was not toxic to test organisms when added to water at less than one percent.

Batch elution procedures with methylene chloride were added in the second experiment to ascertain whether a more polar solvent would improve results in terms of the recovery of spiked

chemical and toxicity. This solvent is often used to elute SPE columns in analytical laboratories, but it is toxic to test organisms and had to be exchanged with acetone. Prior to incorporating these procedures, a small test was conducted to determine tolerance of amphipods to elution by methylene chloride with a subsequent solvent exchange. As part of this test, the solvents were passed through 5g Na₂SO₄ to remove residual water in the solvent. The Na₂SO₄ was prepared by rinsing the mass with clean methylene chloride. There was no toxicity in any of the treatments (data not shown), so methylene chloride elution and Na₂SO₄ water removal procedures were added to the second phase for comparison to acetone.

Additional treatments for the second experiment included two SPE columns: C18 (Varian Bond Elut®, 6 mL, 500 mg, Palo Alto, CA) and Oasis® HLB (Hydrophilic-Lipophilic Balance, 6 mL, 200 mg, Waters, Milford, MA, USA). The C18 column has worked well with chlorpyrifos extraction and recovery, but has not been adequately assessed for TIE applications with pyrethroids. The performance of the HLB column was re-examined for comparison to the C18 column. Column preparation was based on U.S. EPA guidance for the C18 column (USEPA, 1993), and manufacturer's instructions for the HLB column. The C18 column was prepared by pumping 15 mL of methanol followed by 15 mL of Nanopure at 5 mL per minute. The HLB column was prepared by pumping 3 mL of methanol followed by 5 mL of Nanopure at 1 mL/minute. Laboratory water was then passed through each column to provide a column blank for testing. The columns were re-prepared before pumping 120 mL of sample for extraction. The pumping rates for column loading were 5 mL/minute for the C18 column and 10 mL/minute for the HLB column. Both columns were eluted by pumping 12 mL of acetone through the column at 1 mL per minute. The acetone was collected from each column and blown down to 1.2 mL with nitrogen. The resulting solvent samples were added to 120 mL of water to prepare the eluate treatments.

Because of poor pesticide and toxicity recovery from batch extraction media, the third set of experiments emphasized extraction of interstitial water using SPE columns. These experiments included variations in SPE column size and extraction solvents. The HLB column treatment with acetone elution was repeated for reference, but two additional HLB column treatments and a C18 column treatment were introduced. In addition to acetone elution, a second HLB column

was eluted with methylene chloride. The C18 column was also eluted with methylene chloride. The third column treatment utilized a larger (1 gram) HLB column with methylene chloride elution.

Statistical differences between treatments were determined using separate-variance t-tests. Extraction treatments were compared with baseline to determine if toxicity was significantly reduced, and elution treatments were compared to extraction treatments to determine if significant toxicity was recovered. Both TIE treatments were also compared to treatment blanks.

Results and Discussion

Quality Assurance

Performance of individual toxicity controls and blanks will be discussed under the experiment headings below. Measurements of external standards accompanied each set of ELISA samples, and the recovery of these standards ranged from 106% to 113%. Duplicate samples had relative percent differences ranging from 1% to 7%. All GC/MS blanks were non-detect, and the recovery of standard reference materials ranged from 113% to 121%. No surrogate spikes were conducted.

Experiment 1 – 4-Hour vs. 24-Hour Batch Extractions and 1-Hour Batch Elution vs. Column Elution

Some mortality was observed in the baseline blank and the one-hour Amberlite batch elution blank in the first experiment (Table 1). These mortalities occurred suddenly on the fourth day of the exposure without obvious explanation. Although the mean percent survival is below what would normally be acceptable for a 96-hour acute test with this species, the goal of the experiment was to determine if there were significant differences in the reduction and recovery of toxicity in the TIE treatments. Statistical comparisons were conducted between the one-hour batch elution treatments and the corresponding blank to determine if the recovery of toxicity was significant. All water quality parameters were within acceptable ranges for the test organism.

All of the untreated bifenthrin and chlorpyrifos baseline samples were significantly toxic, but the percent survival in the baseline samples was higher than expected based on the nominal concentration of the baseline samples (Table 1). Although the individual spikes were both greater than four toxic units, neither caused complete mortality. Concentrations of chlorpyrifos were measured with ELISA on all treatments that contained the organophosphate. All results were non-detects or below the reporting limit of 100 ng/L (data not shown). Although it was not clear why the concentrations of chlorpyrifos were less than the target concentrations, the experiment was not repeated because significant toxicity was observed in the baseline samples that contained chlorpyrifos. This response was not significantly different from the baseline control, but it was low enough to evaluate the effectiveness of the TIE treatments.

All batch extraction treatments significantly reduced toxicity (>90% survival), and there were no differences between the 4-hour batch extractions and the 24-hour batch extractions. None of the column elutions recovered toxicity. The one-hour batch elutions that were prepared from the 4-hour batch extractions did not recover toxicity in the bifenthrin and chlorpyrifos tests. There was some toxicity in the one-hour batch elution in the combination experiment, but this result was not significantly different from the blank result. One-hour batch elutions from the bifenthrin and combination tests recovered significant toxicity, but this treatment did not recover toxicity in the chlorpyrifos test.

Results of the first experiment indicated that a 4-hour batch extraction was sufficient to remove spiked chemicals from water samples, but because the batch elution process from these extractions can take several hours to complete, we opted to perform the 24-hour batch extractions for the second phase. Using 24-hour extractions was a more conservative procedure for contaminant removal and allowed for additional time to conduct elution procedures. The loaded column elutions did not recover any toxicity in the first experiment, and were omitted in the second experiment. The batch elutions with sonication treatments had mixed results, but were repeated in the second experiment. In addition to the batch elutions with acetone, batch elutions with methylene chloride were added to the treatment list. Methylene chloride is toxic to amphipods, so a solvent exchange step was included following EPA procedures (USEPA, 1993).

In this step, the methylene chloride solvent was blown to dryness with nitrogen and reconstituted with acetone.

Table 1. Mean percent survival and standard deviation in treatments from the first experiment with Amberlite resin batch extractions and resin batch elutions with acetone. ELISA chlorpyrifos concentrations are not listed because all measurements were below reporting limits. * indicates significant removal of toxicity. ** indicates significant recovery of toxicity. *** indicates significant recovery of toxicity, but not significantly different from the blank.

Treatment	Bifenthrin		Chlorpyrifos		Combination		Controls	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Baseline	24	9	44	36	0	0	64	22
4-Hour Batch Extraction	100*	0	100*	0	96*	9	NA	NA
1-Hour Batch Elution	100	0	100	0	45***	34	NA	NA
Column Elution	72	18	100	0	100	0	NA	NA
24-Hour Batch Extraction	80*	14	92*	11	96*	9	88	11
1-Hour Batch Elution	40**	0	100	0	0**	0	63	19
Column Elution	96	9	80	20	96	9	84	17

Experiment 1 Conclusion: Batch extractions were effective at reducing toxicity. The column elutions of the batch extraction media were not effective and the batch elution results were variable.

Experiment 2 – Batch Extraction and Batch Elution with Methylene Chloride and Acetone, Column Extractions (C18 and HLB), and Column Elutions with Acetone

Nominal concentrations in this experiment were increased from 40 to 60 ng/L for bifenthrin, and from 400 to 600 ng/L for chlorpyrifos. Mean percent survival in all control treatments and blanks was greater than 96% (Table 2), and all water quality parameters were within acceptable ranges for the test organism. Three of the batch eluates with acetone had excessive amounts of solvent added to the treatments. There were problems getting the solvents to blow down to the required volume to prepare the eluate treatments. Instead of abandoning the treatments, the solvents were added and the volumes recorded. Only the chlorpyrifos batch elution had a greater amount of solvent than the batch elution blank, and was significantly toxic. The toxicity data were omitted from Table 2, but the chlorpyrifos data were retained in order to demonstrate the amount of chlorpyrifos recovered in the treatment.

The bifenthrin and chlorpyrifos doses were increased by half in this experiment in order to compensate for possible reduction in bioavailability caused by dissolved organic carbon in the interstitial water. The higher doses of pesticides caused complete mortality in the bifenthrin and combination baseline, and 12% survival in the chlorpyrifos baseline (Table 2). All of the 24-hour batch extractions significantly reduced toxicity, and although several batch elutions recovered concentrations of chlorpyrifos, none of them recovered toxicity that was significantly greater than the elution blank. The combination batch elution with methylene chloride recovered 483 ng/L chlorpyrifos (~5.6 toxic units), but did not cause significant toxicity. We hypothesize that the process of eluting the resin with solvent creates a byproduct that binds with the eluted chemical and reduces its bioavailability. This hypothesis was not investigated as part of the current study.

Both the C18 column and HLB column significantly reduced toxicity in all chemical treatments, and all but one of the column elution treatments recovered significant toxicity (Table 2). Overall, the HLB column performed better than the C18 column. Both of the column elution treatments in the bifenthrin test recovered about 30% of the toxicity. This was a significant amount compared to the blanks, but it was much less toxicity than the baseline. There were mixed results with the column recoveries in the chlorpyrifos test. The C18 column eluate recovered 257 ng/L chlorpyrifos, but there was no significant toxicity. The HLB column recovered 365 ng/L chlorpyrifos, and although this concentration is greater than four toxic units, the survival was only reduced to 38%. The column eluates in the combination test recovered approximately 70-90% of the chlorpyrifos in the baseline and caused complete mortality, whereas the column eluates in the chlorpyrifos treatment only recovered approximately 45-65% of the chlorpyrifos in the baseline. Although the method of elution was based on U.S. EPA TIE methods (USEPA, 1991) and column manufacturer recommendations, this range of recoveries was much lower than what is normally achieved in other research and commercial analytical labs. The majority of recovery from HLB columns has been documented from samples other than interstitial water. Solid-phase extraction of spiked interstitial water is more problematic because of the presence of dissolved organic carbon and other natural constituents in interstitial water.

Table 2. Mean percent survival (standard deviation) in treatments from the second experiment with resin batch extractions, batch elutions with acetone and methylene chloride (DCM), and two different column extractions and elutions. ELISA indicates the concentration of chlorpyrifos in ng/L. * indicates significant removal of toxicity. ** indicates significant recovery of toxicity. *** indicates significant recovery of toxicity, but not significantly different from the blank. NA indicates not analyzed.

Treatment	Bifenthrin	Chlorpyrifos		Combination		Controls
	Mean (SD)	Mean (SD)	ELISA (ng/L)	Mean (SD)	ELISA (ng/L)	Mean (SD)
Baseline	0 (0)	12 (18)	566	0 (0)	624	96 (9)
Resin – 24-Hour Batch Extraction	92 (11)*	92 (11)*	ND	92 (11)*	ND	96 (9)
1-Hour Batch Elution - Acetone	88 (18)	NA	255	96	<RL	96 (9)
1-Hour Batch Elution - DCM	80 (20)	100 (0)	140	64 (36)***	483	96 (9)
C18 Column Extraction	96 (9)*	96 (9)*	ND	100 (0)*	ND	96 (9)
C18 Column Elution	68 (11)**	80 (20)	257	0 (0)**	568	NA
HLB Column Extraction	100 (0)*	100 (0)*	ND	100 (0)*	ND	100 (0)
HLB Column Elution	64 (9)**	38 (27)**	365	0 (0)**	440	96 (9)

There were several instances of chlorpyrifos recovery (as measured by ELISA) where the concentrations were several times higher than the LC50 for *H. azteca*, but minimal toxicity was observed. The baseline chlorpyrifos sample contained 6.7 toxic units of the pesticide, but the survival was 12%. This is likely due to a reduction of chlorpyrifos bioavailability by the dissolved organic carbon (DOC) in the interstitial water, but many of the eluate treatments contained multiple toxic units of chlorpyrifos and did not demonstrate the expected toxicity. Eluate treatments were prepared with laboratory water containing low concentrations of DOC, so reduced bioavailability from the water should not have been an issue. The extraction media used in these experiments was designed for analytical work and not organism exposures. It is entirely possible that these media could be imparting something during the elution process that is reducing bioavailability, but this was not investigated as part of the current study.

Experiment 2 Conclusion: 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 spiked chemical. Both column elutions recovered the same amount of toxicity in the bifenthrin and combination treatments, but the HLB column recovered a greater amount of toxicity in the chlorpyrifos treatment.

Experiment 3 – Column Extractions (C18 and HLB) and Elutions with Acetone and Methylene Chloride

Nominal concentrations in this experiment were the same as those in the second experiment. Mean percent survival in all control treatments and blanks was greater than 88% with the exception of the HLB column elution blank with acetone (Table 3). The mean percent survival in this blank was 68% because of sudden mortality in two replicates on the last day of the exposure. As in the first experiment, the low survival in the blank does not preclude the data from interpretation because the goal is to determine significant differences among treatments. The analysis takes the control survival into consideration. All water quality parameters were within acceptable ranges for the test organism.

Baseline bifenthrin concentrations, as measured by GC/MS were non-detects. The baseline samples were prepared and stored in the same manner as all of the other samples. After much discussion with the analytical chemist, it was determined that the bifenthrin in these samples was lost at some point during the analytical procedure. Reasonable amounts of bifenthrin were recovered in the column elution samples, but without baseline concentrations it is impossible to determine the accuracy of the spiked concentrations or the percent recovery in the column elution treatments. Chlorpyrifos was spiked into the interstitial water at 600 ng/L. The baseline chlorpyrifos concentrations, as measured by GC/MS, were 496 ng/L and 650 ng/L (83% and 108% of nominal, respectively). The same samples as measured by ELISA were 562 ng/L and 665 ng/L (94% and 111% of nominal, respectively). Relative percent difference between the GC/MS measurements of chlorpyrifos and ELISA measurements were calculated. In all cases the ELISA measurements were greater than the GC/MS measurements (Table 3), and the relative percent differences ranged from 12% to 55%. In one case the GC/MS measured 72.3 ng/L chlorpyrifos, whereas the ELISA measured 463 ng/L. The analytical laboratory could provide no explanation for these results, but a recent study conducted with the Department of Pesticide Regulation revealed that there could be up to a 50% loss of spiked chlorpyrifos in as short a period as seven days, which is the recommended maximum holding time (Sue Peoples, DPR, Sacramento, CA). Baseline concentrations of chlorpyrifos, as measured by ELISA, were similar

in both experiments two and three, but the chlorpyrifos concentrations in the combination treatments were higher than those measured in the straight chlorpyrifos treatments. Similarly, the recovery concentrations in the eluates were universally higher in the combination treatments. It is not clear why this occurred, but the GC/MS measurements corroborate this result.

Survival in the baseline samples ranged from 8% for the combination treatment to 20% for the chlorpyrifos treatment. Lower rates of survival were expected based on the measured concentrations of spiked chemicals. The chemicals were measured as total concentrations, and it is apparent that a significant percentage of the spiked chemicals were not bioavailable. All of the column treatments successfully reduced toxicity and increased survival to greater than 92%, with the exception of the one-gram HLB column which increased survival to 84% (Table 3). None of the spiked chemicals were detected in the post-column extraction samples, indicating that all of the columns performed well (i.e., no column break-through).

The C18 column and HLB column elutions with methylene chloride both recovered significant toxicity in all three spiking regimes, and recovered a higher percentage of the spiked chemicals than the HLB column with acetone elution. Higher concentrations of bifenthrin were recovered by the methylene chloride elutions in the bifenthrin-only treatments, as opposed to the combination treatments, and higher concentrations of bifenthrin were recovered in the methylene chloride elutions of the HLB columns. In the chlorpyrifos only treatments, the HLB column eluate with acetone and the one-gram HLB column eluate with methylene chloride had a significant recovery of chlorpyrifos (multiple toxic units), but did not have a significant recovery of toxicity. Although the HLB column eluate with acetone performed well in the second experiment, and recovered a similar concentration of chlorpyrifos (ELISA), this treatment did not recover significant toxicity in the chlorpyrifos treatment.

The one-gram HLB column was introduced in this experiment to determine if a larger adsorption surface area would provide better extraction and elution. Standard chemical analysis procedures for solid-phase extraction generally incorporate smaller columns. The larger column reduced the toxicity of the bifenthrin and combination treatments, but did not reduce or recover toxicity as well in the chlorpyrifos treatment.

Table 3. Mean percent survival and standard deviation in treatments from the third experiment with column extractions and column elutions with acetone and methylene chloride (DCM). BIF and CHL indicate the GC/MS concentrations of bifenthrin and chlorpyrifos, respectively, in ng/L. ELISA indicates the concentration of chlorpyrifos in ng/L. * indicates significant removal of toxicity. ** indicates significant recovery of toxicity. *** indicates significant recovery of toxicity, but not significantly different from the blank. NA indicates not analyzed.

Treatment	Bifenthrin		Chlorpyrifos			Combination				Controls Mean (SD)
	Mean (SD)	BIF (ng/L)	Mean (SD)	CHL (ng/L)	ELISA (ng/L)	Mean (SD)	BIF (ng/L)	CHL (ng/L)	ELISA (ng/L)	
Baseline	15 (17)	ND	20 (14)	496	562	8 (11)	ND	650	665	100 (0)
C18 Extraction 1	100 (0)*	ND	100 (0)*	ND	ND	100 (0)*	ND	ND	ND	100 (0)
C18 Elution 1 - DCM	0 (0)**	43.8	36 (33)**	406	531	12 (11)**	12.3	565	672	88 (11)
HLB Extraction 1	100 (0)*	ND	93 (10)*	ND	ND	100 (0)*	ND	ND	ND	96 (9)
HLB Elution 1 Acetone	44 (22)***	24.9	92 (11)	203	356	32 (18)**	12.8	309	544	68 (36)
HLB Extraction 2	100 (0)*	ND	100 (0)*	ND	ND	100 (0)*	ND	ND	<RL	100 (0)
HLB Elution 2 - DCM	0 (0)**	48.8	76 (22)**	72.3	463	0 (0)**	20.1	473	659	100 (0)
HLB Extraction 3 (1g)	100 (0)*	NA	84 (17)*	NA	ND	92 (18)*	NA	NA	<RL	100 (0)
HLB Elution 3 - DCM	4 (9)**	NA	100 (0)	NA	470	8 (18)**	NA	NA	589	95 (10)

Experiment 3 Conclusion: All of the columns successfully reduced chemical concentrations and toxicity, but the HLB column and the C18 column eluates with methylene chloride produced the greatest recovery of toxicity and chemical.

Summary

All of the extraction treatments in the three experiments successfully removed toxicity from the spiked interstitial water samples. Mean percent survival in all but one of these extractions was greater than 90%. In the first experiment, there were no significant differences between 4-hour and 24-hour extractions with Amberlite resin, but there were differences between the two elution methods. The column elution did not recover toxicity in any of the treatments. The batch elution recovered a greater amount of toxicity from the 24-hour extraction resin, but did not recover toxicity in the chlorpyrifos treatment. It was assumed that the 24-hour extraction resin adsorbed a greater amount of the spiked chemicals, which were then carried through to the elution treatments.

The 24-hour batch extraction was repeated in the second experiment. It was conducted in duplicate with two different elution treatments. The acetone elution was not consistent with the results of the first experiment, and the methylene chloride elution only recovered toxicity in the combination treatment, although this recovery was not significantly different from the blank. The column elutions recovered the same amount of toxicity in the bifenthrin and combination treatments, but the HLB column recovered a greater amount of toxicity in the chlorpyrifos treatment. Although the acetone elutions from the columns were more successful than the batch elutions in this and the previous experiments, the recoveries of chlorpyrifos were variable.

Because there was no significant recovery in many of the elutions from the batch extractions, only column treatments were evaluated in the third experiment. The HLB column with acetone elution was repeated, but did not perform as well as it did in the second experiment. This treatment recovered a similar concentration of chlorpyrifos and some bifenthrin, but did not elicit the same amount of toxicity. This treatment also did not perform as well as the column elutions with methylene chloride. The C18 and HLB column extraction treatments performed similarly, but the methylene chloride elution of the C18 column generally had higher recoveries of toxicity and chlorpyrifos, but not bifenthrin. The HLB column elution with methylene chloride had the highest recovery of bifenthrin.

This study was originally designed to build on the results of a study conducted for SFEI and the San Francisco Bay Regional Monitoring Program. Improving interstitial water extraction methods was one recommendation of the TIE workgroup that was convened by SFEI. In a previous study for SFEI, experiments were conducted using batch extractions to reduce the toxicity of interstitial water to the estuarine amphipod *E. estuarius*. While these extractions successfully reduced toxicity, significant chemical concentrations could not be recovered in the elution process. The current study conducted three sets of experiments to determine the efficiency of batch extractions in a freshwater matrix, and to determine if SPE columns provided more effective Phase I and II TIE tools for application to interstitial water.

The results of these experiments suggest that when used in combination, HLB and C18 SPE columns provide a broader extraction and recovery of interstitial water contaminated with pesticide mixtures than batch extractions. The results also demonstrated that methylene chloride was a superior elution solvent for TIEs involving hydrophobic pesticides. Solvent exchange procedures where chemicals eluted with methylene chloride are transferred to acetone are applicable for TIEs using *H. azteca* (and the estuarine amphipod *E. estuarius*). The current study, along with the results of the SFEI study, are the first steps in understanding the use of alternate extraction media in interstitial water TIEs, but additional experiments with more complete analytical chemistry measurements are necessary to further evaluate the efficacy of extracting interstitial water with SPE columns. Recommended Phase I and II TIE procedures for freshwater and marine sediment TIEs could include a combination of SPE columns. In addition, SPE column elution for Phase II procedures could use methylene chloride to provide more efficient recovery of extracted organic chemicals.

References

- Anderson, B.S., Hunt, J.W., Phillips, B.M., Tjeerdema, R.S., 2007. Navigating the TMDL Process: Sediment Toxicity. Water Environment Research Foundation.
- Anderson, B.S., Phillips, B.M., Hunt, J.W., Clark, S.L., Voorhees, J.P., Tjeerdema, R.S., Casteline, J., Stewart, M., Crane, D., Mekebri, A., 2010. Evaluation of methods to determine causes of sediment toxicity in San Diego Bay, California, USA. *Ecotoxicol Environ Safety* 73, 534-540.
- Anderson, B.S., Phillips, B.M., Hunt, J.W., Connor, V., Richard, N., Tjeerdema, R.S., 2006. Identifying primary stressors impacting macroinvertebrates in the Salinas River (California, USA): Relative effects of pesticides and suspended particles. *Environmental Pollution* 141, 402-408.
- Cornelissen, G., Rigterink, H., ten Hulscher, D.E.M., Vrindi, B.A., van Noort, P.C.M., 2001. A simple Tenax extraction method to determine the availability of sediment-sorbed organic compounds. *Environ Toxicol Chem* 20, 706-711.
- Perron, M.M., Burgess, R.M., Ho, K.T., Pelletier, M.C., Friedman, C.L., Cantwell, M.G., Shine, J.P., 2009. Development and evaluation of reverse polyethylene samplers for marine phase II whole-sediment toxicity identification evaluations. *Environ Toxicol Chem* 28, 749-758.

Phillips, B.M., Anderson, B.A., Hunt, J.W., Siegler, K., Voorhees, J.P., McNeill, K., 2010. Santa Maria River Watershed and Oso Flaco Creek Watershed TMDL Monitoring Study – Final Report. Central Coast Regional Water Quality Control Board, San Luis Obispo, CA.

Phillips, B.M., Anderson, B.S., Hunt, J.W., Clark, S.L., Voorhees, J.P., Tjeerdema, R.S., Casteline, J., Stewart, M., 2009a. Evaluation of phase II sediment toxicity identification evaluation methods for freshwater whole sediment and interstitial water. *Chemosphere* 74, 648-653.

Phillips, B.M., Anderson, B.S., Lowe, S., 2011. RMP Sediment Study 2009-2010, Determining Causes of Sediment Toxicity in the San Francisco Estuary. Regional Monitoring Program for Water Quality in the San Francisco Estuary. Contribution No. 626. San Francisco Estuary Institute. Oakland, CA.

Phillips, B.M., Anderson, B.S., Lowe, S., Hunt, J.W., 2009b. RMP Sediment TIE Study 2007-2008: Using Toxicity Identification Evaluation (TIE) Methods to Investigate Causes of Sediment Toxicity to Amphipods. Regional Monitoring Program for Water Quality in the San Francisco Estuary Contribution No. 561. San Francisco Estuary Institute, Oakland, CA.

Phipps, G.L., Mattson, V.R., Ankley, G.T., 1995. The relative sensitivity of three benthic test species to ten chemicals. *Arch Environ Toxicol Chem* 28, 281-286.

Sullivan, J.J., Goh, K.S., 2000. Evaluation and validation of a commercial ELISA for diazinon in surface waters. *J Agric Food Chem* 48, 4071-4078.

USEPA, 1991. Methods for aquatic toxicity identification evaluations. Phase I Toxicity Characterization Procedures. EPA 600/6-91/003. Office of Research and Development. Washington, DC.

USEPA, 1993. Methods for aquatic toxicity identification evaluations. Phase II Toxicity Identification Procedures for Samples Exhibiting Acute and Chronic Toxicity. EPA 600/R-92/080. Office of Research and Development. Washington, D.C.

USEPA, 2002. Methods for measuring acute toxicity of effluents and receiving water to freshwater and marine organisms. EPA-821-R-02-021. Office of Research and Development, Washington, D.C.

USEPA, 2007. Sediment Toxicity Identification Evaluation (TIE) Phases I, II, and III Guidance Document. Draft. EPA 600-R07-080. Office of Research and Development, Atlantic Ecology Division. Narragansett, RI.