Toxicity Identification Evaluation Results
Region 5 – Combined Stations 03-ICARR-014 and 03-ICARR-015
Surface Water Ambient Monitoring Program

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Introduction

Two sediment samples were submitted as part of the Surface Water Ambient Monitoring Program (SWAMP) for combined toxicity identification (TIE) analysis. Prior to submittal, solid-phase toxicity tests were performed by Pacific Eco Risk (Martinez, CA), and significant toxicity to *Hyalella azteca* was observed. Upon receipt, the sediments were combined and homogenized. After interstitial water was extracted, a TIE with *Hyalella azteca* was conducted using established testing protocols. TIEs are designed to proceed in three phases. The purpose of a Phase 1 TIE is to characterize the cause of toxicity. Information from the Phase 1 characterization may then be used in subsequent Phase 2 (identification) and Phase 3 (confirmation) TIEs. Based on the results of initial toxicity tests, an abbreviated Phase 1 TIE was conducted to investigate the causes of toxicity. The TIE did not utilize the normal suite of treatments because of minimal sample availability. This report presents the data obtained from the TIE, including the mean percent survival of amphipods after exposure to various TIE treatments, water quality measurements of test solutions, and copies of the original data sheets and quality assurance forms.

Methods

Sample Handling

The sediment samples were collected on September 13, 2004, under the supervision of Jay Rowan (Central Valley Regional Water Quality Control Board). After initial solid phase testing at Pacific Ecorisk, the samples were transported on ice and in the dark to the Marine Pollution Studies Laboratory at Granite

Canyon for initial interstitial water toxicity testing. Interstitial water was extracted from the sample on October 27, and an initial test started on October 28, 2004. After the termination of the initial test, the TIE was initiated on November 5, 2004.

TIE Methods

The following Phase 1 TIE treatments were performed on a dilution series of each sample (US EPA 1991). Sample concentrations in the initial test were 0 (treatment blank), 25, and 100%. The treatment blank was control water that underwent the same manipulation as the sample.

Treatments:

• Baseline - Toxicity test on un-manipulated sample. Concentrations were chosen to bracket the effect concentration of the sample and might differ from initial test.

• C8 Column - The C8 Column is designed to remove non-polar organic compounds from the sample. In the manipulation, reverse phase liquid chromatography is applied to extract nonionic organic toxicants from the aqueous sample. Column can be eluted with methanol and resulting eluate tested to determine if substances removed by the column are indeed toxic.

• EDTA (Disodium Ethylenediaminetetraacetic acid) - EDTA is an organic chelating agent that preferentially binds with divalent metals, such as copper, nickel, lead, zinc, cadmium, mercury, and other transition metals to form non-toxic complexes. It will not complex with anionic forms of metals such as selenids, chromates and hydrochromates.

• PBO (Piperonyl Butoxide) - PBO is a metabolic inhibitor that removes the toxicity associated with metabolically activated pesticides such as diazinon and chlorpyrifos. An increase of toxicity with the PBO treatments can indicate the presence of non-metabolically activated compounds such as pyrethroid pesticides.

• Carboxylesterase Enzyme - Porcine carboxylesterase was added to the sample to break down suspected pyrethroid pesticides (Wheelock et al. 2004).

Exposures were conducted in 20 mL glass scintillation vials (3 replicates) containing 15 mL treated sample and five amphipods. Acute exposures were conducted for 96 hours, following US EPA 1993.

Physical and Chemical Measurements

Water quality parameters of dissolved oxygen, pH and conductivity were measured using a Hach SensION© selective ion meter with appropriate electrodes; and ammonia was measured using a Hach 2010 spectrophotometer. Temperature was measured using a continuously recording thermograph and thermometer. Concentrations of the organophosphate pesticides chlorpyrifos and diazinon were measured using enzyme-linked immunosorbent assays (ELISA, Strategic Diagnostics Inc, Newark, DE). Water quality parameters were only measured on 100% Baseline sample because of lack of sample.

Data Interpretation

Treatment blanks were evaluated to determine if sample manipulations added toxic artifacts. Treatment data were then compared to one another based on organism response.

Results and Discussion

The initial test was significantly toxic to *H. azteca* at the 10% concentration in a 96-hour acute exposure (LC50 = 5.8%, Table 1). There was no toxicity observed in the treatment blanks. Water quality parameters were all within the tolerance limits of the test organism. Concentrations of chlorpyrifos and diazinon were below detection limits (Table 1).

At 96 hours the Baseline treatment had complete mortality in both the 25% and 100% concentrations. The only treatment that reduced toxicity was the Enzyme, indicating the cause of toxicity was a pyrethroid pesticide, or a combination of pyrethroids (Table 1). Additional evidence for pyrethroid toxicity is in the results of the PBO treatment. At 48 hours the toxicity of the PBO treatment was higher than Baseline indicating the signal was being increased by the addition of PBO. The C8 Column did not reduce toxicity, but it did bind some non-polar organic contaminants because the Column Eluate returned significant toxicity to clean dilution water. The sediment and the methanol extract from the C8 Column were analyzed for pyrethroid pesticides by the Water Pollution Control Laboratory (Rancho Cordova, CA).

The sediment contained cyfluthrin, esfenvalerate/fenvalerate, and lambda cyhalothrin (Table 2). The concentration of lambda cyhalothrin was more than four times the mean sediment LC50 reported by Amweg et al. (In Press). Although the methanol extract from the column eluate treatment returned toxicity to clean water, chemical analysis of the extract did not find any pyrethroid pesticides (Table 2).

Because of the minimal sample provided, additional sediment chemistry could not be conducted, yet additional analysis of the methanol extract is still feasible providing the chemistry laboratory has leftover extract. The high concentration of lambda cyhalothrin strongly suggests the cause of toxicity to be a pyrethroid, but additional factors could be contributing to toxicity.

References

Amweg, EL, Weston, DP, Ureda, NM. In Press. Use and toxicity of pyrethroid pesticides in the Central Valley, CA, USA. *Environ Toxicol Chem*.

U.S. Environmental Protection Agency. 1991. Methods for Aquatic Toxicity Identification Evaluations. Phase I Toxicity Characterization Procedures. Second Edition. EPA 600/6-91/003. Office of Research and Development. Washington, DC.

U.S. Environmental Protection Agency. 1993. Methods for measuring acute toxicity of effluents and receiving water to freshwater and marine organisms, 4th edition. EPA 600/4-91/002. Technical Report. Washington, DC.

Wheelock CE, Miller JL, Miller MJ, Gee SJ, Shan G, Hammock BD. 2004. Development of toxicity identification evaluation procedures for pyrethroid detection using esterase activity. *Environ Toxicol Chem*. 23: 2699-2708.

Table 1. Mean percent survival of *H. azteca* and concentrations of organophosphate pesticides from Phase 1 TIE treatments conducted on 11/5/04. NA indicates not analyzed. ND indicates not detected.

	Percent Sample					Chlorpyrifos	Diazinon
Treatment	0%	10%	25%	50%	100%	(μg/L)	(μg/L)
Initial Test	100	13	7	0	0	ND	ND
48-Hour Results							
Baseline	100		73		0	NA	NA
C8 Column	100		33		0	NA	NA
C8 Eluate	100		60		73	NA	NA
EDTA	100		53		7	NA	NA
PBO	100		27		0	NA	NA
Enzyme	100		100		93	NA	NA
96-Hour Results							
Baseline	100		0		0	NA	NA
C8 Column	100		0		0	NA	NA
C8 Eluate	100		27		0	NA	NA
EDTA	100		0		0	NA	NA
PBO	100		0		0	NA	NA
Enzyme	100		100		73	NA	NA

Table 2. Pyrethroid concentrations in sediment and methanol extract, and mean sediment LC50 values from Amweg et al. (In Press). ND indicates not detected. NR indicates not reported.

Pyrethroid	Sediment ng/g dry wt.	Methanol Extract	Sediment LC50 ng/g dry wt.
Bifenthrin	ND	ND	4.4
Cyfluthrin	3.21	ND	14.2
Cypermethrin	ND	ND	NR
Esfenvalerate/Fenvalerate	5.47	ND	42.2
Lambda (Cyhalothrin)	25.2	ND	5.8
Permethrin	ND	ND	206.3