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California Regional Water Quality Control Board
Central Valley Region

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Toxicity Identification Evaluation Results
Region 5 - Station 541STC516
Surface Water Ambient Monitoring Program

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Introduction

A sediment sample collected from Region 5 as part of the Surface Water Ambient Monitoring Program (SWAMP) was tested for toxicity to *Hyalella azteca* using established testing protocols. Because the sample was significantly toxic to the test organism, a toxicity identification evaluation (TIE) was conducted. TIEs are designed to proceed in three phases. The purpose of a Phase 1 TIE is to characterized the cause of toxicity. Information from the Phase 1 characterization may then be used in subsecuent Phase 2 (identification) and Phase 3 (confirmation) TIEs. Based on the results of initial toxicity tests, two Phase 1 TIEs were conducted to investigate the causes of toxicity. This report presents the data obtained from these TIEs, 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

Sediment samples were collected on May 29 and September 11, 2002 under the supervision of Jay Rowan (Central Valley Regional Water Quality Control Board). Samples were transported on ice and in the dark to the Marine Pollution Studies Laboratory at Granite Canyon for initial toxicity testing, which began within 14 days of collection (see attached copy of chain of custody form). Pore water was extracted from the first sample on June 6, and an initial test initiated on June 7. After the termination of the initial test, the first TIE was initiated on June 14. Pore water was extracted from the second sample

on September 12, and initial tests with sediment and pore water were initiated on September 13. The pore water TIE was initiated on September 16.

TIE Methods

The following Phase 1 TIE treatments were performed on a dilution series of each sample (US EPA 1991). Sample concentrations in the first TIE were 0 (treatment blank), 10, 25, 50, and 100%. Concentrations in the second TIE were 0 (blank), 1, 5, 10, and 50%. 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.
- Centrifugation - Used to determine whether toxicants are associated with particles. Also used as a pretreatment step for the column treatments. Because TIEs were conducted on pore water that was extracted via centrifugation, this treatment served as the Baseline.
- Aeration - Samples are aerated to determine if their toxicity is due to volatile compounds or surfactants.
- 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.
- STS (Sodium Thiosulfate) - Addition of STS, a reducing agent, to a sample containing oxidants (chlorine, bromine), results in a reduction reaction that may decrease sample toxicity. STS is also a chelating agent for some cationic metals.
- pH Shift - Changes in pH can affect solubility, polarity, volatility, stability and speciation of a compound, thereby affecting its bioavailability and toxicity. Shifting pH is designed to determine how much sample toxicity can be attributed to volatile, sublateable or oxidizeable compounds. Shifts in pH can also be combined with Aeration or solid-phase extraction with the C8 Column.
- 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.
- Oasis Column – Another type of solid-phase extraction column designed to remove non-polar organic compounds from the sample. This column has been shown to remove pyrethroid pesticides.
- 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.

In addition to the standard Phase 1 treatments, two experimental treatments were used to characterize pyrethroid toxicity. The temperature treatment mimics the Baseline treatment but the organisms are exposed at 15°C instead of 23°C. Type 1 pyrethroid pesticides are known to be more toxic at colder temperatures. Because Type 1 compounds are among the more commonly used pyrethroids, toxicity at colder temperatures may help characterize this class of pesticides as the cause of toxicity. The Enzyme treatment uses a pyrethroid-binding enzyme to reduce the bioavailability of the pesticides.

Exposures were conducted in 20 mL glass scintillation vials (5 to 10 replicates) containing 15 mL treated sample and one amphipod. 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). The California Department of Fish and Game Nimbus Laboratory conducted pesticide analyses under the supervision of Dave Crane. Pyrethroids (EPA Method 1660), organochlorines (EPA Method 8081), and organophosphates (EPA Method 8141A) were analyzed in sediment and porewater.

Data Interpretation

Treatment blanks were evaluated to determine if sample manipulations added toxic artifacts. Treatment data were then compared to one another using the toxic unit approach. Toxic units (TU) were calculated by dividing 100 by the LC50 calculated from each treatment dilution series. More toxic units indicate a more toxic sample.

Results and Discussion

Hyalella TIE 6/14/02

The initial test was significantly toxic to *Hyalella* in a 96-hour acute exposure (Table 1). Toxic units were *not calculated as part of the initial test because there was no dilution series.*

Several Phase 1 treatments reduced toxicity. All of the pH treatments reduced toxicity to some extent, but the pH 3 Shift reduced toxicity the most (7.9 TU to 3.5 TU). Shifting pH can reduce toxicity by converting ionic compounds to more volatile or precipitant forms, and can affect the ionization state of polar toxicants, thus making them more or less volatile (US EPA 1988). It is not clear what compounds were being affected by the pH shifts. The Column treatments also reduced toxicity, but the pH 3 Column

was the most successful. Because the pH 3 Column reduced toxicity to the same level as the pH 3 Shift alone, this reduction could be attributed to the pH shift. Some toxicity was added back in both Column Eluates. PBO increased toxicity by a factor of 2.5, suggesting the presence of pyrethroid pesticides. Organophosphate pesticides were below the threshold values for *Hyalella*, and water quality parameters were all within the tolerance limits of the organism (Unionized Ammonia LC50 = 4.7 mg/L, Table 2).

Table 1. Mean percent survival of *Hyalella*, concentrations of organophosphate pesticides, and toxic units from Phase 1 TIE treatments conducted on 5/14/02.

Treatment	Toxic Units	Percent Sample 0%	10%	25%	50%	100%	Chlorpyrifos (µg/L)	Diazinon (µg/L)
Initial Test	NA	100				0	0.056	0.047
Baseline	7.9	100	60	20	0	0	NA	NA
EDTA	6.3	100	80	20	0	0	NA	NA
STS	11.5	100	40	20	0	0	NA	NA
pH 3 Shift	3.5	100	80	60	0	0	NA	NA
pH 11 Shift	5.0	100	80	40	0	20	NA	NA
pH 3 Aeration	5.1	100	80	40	0	0	NA	NA
pH Ambient Aeration	5.4	100	100	20	0	0	NA	NA
pH 11 Aeration	4.1	100	100	40	20	20	NA	NA
pH 3 C8 Column	3.7	100	80	60	20	20	NA	NA
pH 3 C8 Eluate	1.4	100	80	100	100	0	NA	NA
pH Ambient C8 Column	4.8	100	80	40	20	0	NA	NA
pH Ambient C8 Eluate	1.9	100	80	100	60	0	NA	NA
Oasis Column	4.6	100	100	40	0	0	NA	NA
PBO	20.0	100	0	0	0	0	NA	NA

Table 2. Water quality measurements for Phase 1 TIE treatments conducted on 5/14/02.

Treatment	Water Quality Parameter pH	Dissolved Oxygen (mg/L)	Conductivity (µS/cm)	Total Ammonia (mg/L)	Un-ionized Ammonia (mg/L)
Baseline	7.64	3.11	1657	3.3	0.076
EDTA	7.61	3.99	1666	3.3	0.065
STS	7.77	2.42	1740	3.4	0.096
pH 3 Shift	7.56	8.37	2590	3.6	0.067
pH 11 Shift	7.71	8.05	2980	3.1	0.076
pH 3 Aeration	7.74	8.55	2260	4.0	0.106
pH Ambient Aeration	8.52	5.50	1662	3.3	0.463
pH 11 Aeration	7.68	8.40	2920	3.0	0.069
pH 3 C8 Column	7.61	8.46	2540	4.0	0.079
pH 3 C8 Eluate	8.14	7.43	700	ND	ND
pH Ambient C8 Column	7.90	5.82	1691	2.7	0.102
pH Ambient C8 Eluate	8.18	7.50	690	0.2	0.006
Oasis Column	8.12	6.80	1915	3.4	0.092
PBO	7.84	3.10	1683	3.3	0.047

The initial test was significantly toxic to *Hyalella* in a second 96-hour acute exposure (Table 3). Toxic units were calculated as part of the initial test because there was a dilution series. *Hyalella* exposed in sediment also demonstrated complete mortality. The pH 3 Shift slightly reduced toxicity in the second Phase 1 TIE, but no other treatments were successful. The Baseline 15°C and PBO treatments both increased toxicity, again suggesting the presence of pyrethroids. The Pyrethroid Enzyme mitigated toxicity up to 48 hours (data not shown), but because the enzyme breaks down, 96-hour toxicity was not reduced. Future use of this experimental treatment will include daily renewals.

Pesticide concentrations in pore water and sediment are summarized in Table 4. Concentrations of the organochlorines are below threshold values for *Hyalella*, and organophosphates were not detected. The concentration of the pyrethroid bifenthrin might be affecting the test organisms. We were unable to find published *Hyalella* LC50 values for bifenthrin, but the pore water concentration in this sample was similar to published LC50 values for a variety of other aquatic bioassay organisms (Table 5).

Water quality parameters were all within the tolerance limits of the organism (Table 6). The ammonia concentration in the Enzyme treatment was elevated because the enzyme is prepared with ammonium sulfate. The concentration of unionized ammonia was still below the *Hyalella* threshold (Unionized Ammonia LC50 = 4.7 mg/L).

Table 3. Mean percent survival of *Hyalella*, concentrations of organophosphate pesticides, and toxic units from Phase 1 TIE treatments conducted on 9/16/02.

Treatment	Toxic Units	Percent Sample 0%	10%	25%	50%	100%	Chlorpyrifos (µg/L)	Diazinon (µg/L)
Initial Test	20.0	100	0	0	0	0	ND	0.089
		0%	1%	5%	10%	50%		
Baseline 23°C	17.9	100	100	60	20	0	NA	NA
Baseline 15°C	203.0	100	0	0	0	0	NA	NA
pH 3 Shift	13.9	100	100	100	0	10	NA	NA
pH 11 Shift	15.9	100	100	60	30	0	NA	NA
pH Ambient C8 Column	45.5	80	60	0	0	0	NA	NA
pH Ambient C8 Eluate	12.8	100	90	90	20	0	NA	NA
PBO	76.9	100	70	0	0	0	NA	NA
Enzyme	25.0	100	100	40	10	0	NA	NA

Table 4. Select pesticide concentrations from sediment and pore water tested on 9/16/02.

	Pore Water Concentration (µg/L)	Method Blank (µg/L)	Laboratory Spike (% Recovery)	Sediment Concentration (ng/g dry wt.)	Method Blank (µg/L)	Laboratory Spike (% Recovery)
Pyrethroids						
Bifenthrin	0.048	ND	93.5	43.2	ND	93.5
Permethrin	ND	ND	NA	20.4	ND	NA
Organochlorines						
DDE, o,p'	0.003	ND	94.5	1.37	ND	94.0
DDE, p,p'	0.056	ND	100	39.5	ND	97.0
DDT, o,p'	ND	ND	99.0	1.28	ND	106
DDT, p,p'	0.014	ND	100	14.7	ND	104
Dieldrin	0.004	ND	95.0	1.54	ND	102
Endosulfan I	0.003	ND	100	0.78	ND	85.6
Endosulfan sulfate	ND	ND	105	7.63	ND	90.0
HCH, alpha	0.004	ND	100	ND	ND	93.2
Mirex	0.056	ND	94.1	0.56	ND	91.3
Oxadiazone	0.023	ND	93.3	ND	ND	33.3

Table 5. Bifenthrin LC50 values for various aquatic organisms.

Organism	Test Duration	LC50 (µg/L)	Reference
Ceriodaphnia dubia	48-hour	0.078	CDFG – ATL Report P2161-2 (1999)
Daphnia magna	48-hour	0.320	Mokry and Hoagland (1990)
Pimephales promelas	96-hour	0.780	CDFG – ATL Report P2161-1 (1999)
Americamysis bahia	96-hour	0.004	Office of Pesticide Programs (2000)
Onchorhynchus mykiss	96-hour	0.150	Office of Pesticide Programs (2000)

Table 6. Water quality measurements for Phase 1 TIE treatments conducted on 9/16/02.

	Water Quality Parameter				
Treatment	pH	Dissolved Oxygen (mg/L)	Conductivity (µS/cm)	Total Ammonia (mg/L)	Un-ionized Ammonia (mg/L)
Baseline 23°C	8.07	8.16	1148	0.6	0.033
Baseline 25°C	8.07	8.16	1148	0.6	0.033
pH 3 Shift	8.11	8.06	1559	1.0	0.060
pH 11 Shift	8.24	6.12	1485	1.1	0.087
pH Ambient C8 Column	8.15	8.44	1127	0.7	0.046
pH Ambient C8 Eluate	8.35	8.50	640	ND	ND
PBO	8.08	7.93	1102	0.8	0.045
Enzyme	7.94	8.08	1221	23.5	0.968

Conclusion

Results from both sediment pore water TIEs were consistent. Toxicity was increased by adding PBO and by decreasing test temperature. Both lines of evidence suggest pyrethroid pesticides. Chemical analysis indicated sample concentrations of bifenthrin above toxicity thresholds for mysids. Bifenthrin toxicity to *Hyalella* is unknown.

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