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Acute Toxicity of ENVIROKLEAN® PLUS to the Mysid (*Mysidopsis bahia*) and Inland Silverside (*Menidia beryllina*)

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STATEMENT OF COMPLIANCE

The test procedures, original records and report for this study comply with the general requirements of ASTM E 729-88a and PBS&J Standard Operating Procedures. The report is an accurate reflection of the original data. Original data from this study are archived at the PBS&J Environmental Toxicology Laboratory in Houston, TX.

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1.0 INTRODUCTION

An understanding of the toxicity of chemical products and of product components is essential for protection of the marine environment. Marine fish and invertebrates, including the inland silverside (*Menidia beryllina*) and the mysid (*Mysidopsis bahia*), may be exposed to man-made chemicals during manufacture, use or disposal. Knowledge of product toxicity can facilitate reasonable use decisions and attainment of a balance between product toxicity and product performance characteristics. Moreover, testing organisms at sensitive or critical stages in their life cycle allows establishment of relatively safe environmental concentrations.

The toxicity of **ENVIROKLEAN® PLUS**, a readily biodegradable cleaning product for removal of marine growth, calcium deposits, rust and heavy scale, was evaluated in static, acute exposures using juvenile inland silversides and mysids. Both organisms are recognized by regulatory agencies as representative and sensitive marine organisms and are often used as screening tools for comparing the relative toxicities of chemicals. The tests were conducted in general accordance with ASTM E 729-88a, "Standard Guide for Conducting Acute Toxicity Tests with Fishes, Macroinvertebrates, and Amphibians." Test concentrations were chosen using the results of preliminary range-finding tests and were based on the amount of product added to reconstituted sea water (vol/vol basis). Testing was conducted at the PBS&J Environmental Toxicology Laboratory in Houston, Texas. The data and final report are archived by PBS&J.

2.0 MATERIALS AND METHODS

2.1 Test Substance

The test substance, Enviroklean® Plus, was received from The Astonishing Products Company Houston, Texas on 4 August 2000. Approximately 1 gallon of the test substance (a clear pale-yellow liquid; pH <1) was packaged in a linear polyethylene bottle. The test substance was stored in a laboratory cabinet at room temperature.

2.2 Test Organisms

Species:	*Mysidopsis bahia*	*Menidia beryllina*
Source:	PBS&J laboratory cultures	PBS&J laboratory cultures
Age:	3 days old	13 days old
Feeding:	newly-hatched (< 24 hour old) brine shrimp; at set-up, twice on day 1, and once on day 2	newly-hatched (< 24 hour old) brine shrimp prior to test set-up; no feeding during test

2.3 Dilution Water

Water used to prepare the test solutions was ~25‰ salinity (S) synthetic sea water prepared from a commercial sea salt (Hawaiian MarineMix®) and deionized water. The sea water was vigorously aerated and aged prior to use.

2.4 Exposure Vessels

Exposure vessels were disposable, plastic (polyethylene) beakers. Exposure vessels used for the mysid tests were 250 mL nominal volume and contained approximately 100 mL of test solution. Exposure vessels used in fish assays were 800 mL nominal volume and contained about 300 mL of test solution. Each beaker was labeled with test identification, test species, exposure concentration, and replicate number.

2.5 Test Conditions

Test conditions were maintained by placing the exposure vessels, in random order, on racks within a testing room equipped with automatic temperature and photoperiod controls. For these tests, temperature was established at 20 ± 1 EC and photoperiod was 16L:8D hours.

2.6 Test Solution Preparation

2.6.1 Range Finder Tests

A 1% stock solution was prepared by placing 10 mL of Enviroklean® Plus into a 1-L graduated cylinder; the cylinder was filled to the 1-L mark with reconstituted sea water and mixed by gently pouring the contents back and forth from the cylinder into a disposable beaker. The pH of the stock solution was adjusted to about 7.5 by addition of 6N sodium hydroxide (NaOH).

A 100-mL aliquot of the stock solution was removed and reserved for use in preparing the next lower concentration; the remainder was divided into two cups each for the mysid and inland silverside range-finding tests. The reserved portion was returned to the 1-L cylinder and diluted with reconstituted sea water to the one-liter mark, producing a 0.1% solution of the test material. This ten percent serial dilution process was repeated twice again to produce test concentrations of 0.01% and 0.001% test material.

The temperature of the test solutions was adjusted to the required range by placing the exposure vessels in the testing room for equilibration before the animals were introduced. Water quality parameters (temperature, dissolved oxygen, pH and salinity) were measured and the solutions were partitioned into test vessels (2 replicates/treatment); approximately 300 mL of solution were added to each beaker for the inland silverside test and about 100 mL were added to each beaker for the mysid test.

2.6.2 Definitive Tests

A 1% working stock solution was prepared for the definitive tests by measuring 200 mL of the test material into a 2-L graduated cylinder; the cylinder was filled to the 2-L mark with reconstituted sea water and the contents were poured into a 10-L cubitainer. Next, four additional 2-L portions of reconstituted sea water were added to the cubitainer and the solution was mixed thoroughly. Finally, the stock solution pH was adjusted from 1.2 to about 7.5 by addition of 6N NaOH.

Test concentrations and volumes of stock solution and diluent used for the mysid and inland silverside definitive tests are summarized below. Reconstituted sea water was used as the diluent and control in both definitive tests.

Test concentration (%)	Stock Solution (mL)	Diluent (mL)
1.0	4000	0
0.56	2240	1760
0.31	1240	2760
0.18	720	3280
0.10	400	3600
0.056	224	3776
0.031	124	3876
(Control) 0	0	4000

Test solution temperature was adjusted to the required range by placing the exposure vessels in the testing room for equilibration. Water quality parameters (temperature, dissolved oxygen, pH and salinity) were measured and the solutions were partitioned into test vessels (5 replicates/per treatment); approximately 300 mL of solution were added to each beaker for the inland silverside test and about 100 mL were added to each beaker for the mysid test.

2.7 Test Initiation

The range finder and definitive tests were initiated by adding ten test organisms to each test vessel. Mysids were transferred from a culture bowl to the test vessels with a small piece of nylon (Nitex®) net. Inland silversides were transferred into test vessels with a wide-bore, glass pipette. Once the animals had been distributed, the number of organisms in each exposure vessel was verified.

2.8 Test Monitoring/Termination

The number of surviving organisms in each exposure vessel was determined and recorded daily during the tests. The concentration of dissolved oxygen, temperature, pH and salinity were measured at the beginning and end of both the range finding and definitive tests. Both tests were terminated (final survival counted) 48±1 hours after introduction of the test organisms.

2.9 Statistical Analysis

Survival data were analyzed by the Trimmed Spearman-Karber procedure (a non-parametric test) using software distributed by the U.S. EPA. This linear-regression model provides a median lethal concentration (LC_{50}) estimate and 95% confidence interval for data which demonstrates a dose response.

3.0 RESULTS

Copies of laboratory notebooks containing original data records are presented in Appendix A.

3.1 Preliminary Range Finding Tests

3.1.1 Mysid test

Mysid survival in the range finder test control treatments (Table 1) was acceptable at 24 and 48 hours exposure (100% survival). Complete mortality was observed after 24 hours exposure to the 1% test concentration, however, all specimens survived the lower concentrations during the same exposure interval. After 48 hours exposure, partial mortalities were observed in the 0.001% and 0.1% concentrations; no mortality occurred in the 0.01% solution. Based upon these results, seven concentrations in a geometrical progression covering the range from 1% to 0.031% (a 56% dilution series) were chosen for the definitive test.

3.1.2 Inland silverside test

Fish survival in the range finder test controls (Table 2) was acceptable after 24 and 48 hours exposure (100% survival). All fish exposed to the 1% solution died within 24 hours. In the 0.1% treatment, 95% and 90% survival was observed after 24 and 48 hours exposure, respectively. Fish exposed to the 0.01% and 0.001% solutions survived through the end of the test. Based upon these results, seven test concentrations in a geometrical progression covering the range from 1% to 0.031% (a 56% dilution series) were chosen for the definitive test.

3.2 Definitive Test Conditions

The physical test conditions (dissolved oxygen, pH, salinity, and temperature) monitored at the beginning and end of the definitive tests were within acceptable ranges (Tables 3 and 4 and Appendix A). Dissolved oxygen concentrations trended downward slightly from initial levels which

were near or slightly above saturation (~7.7 mg/L @25 ‰ S and 20 EC) to end-of-test levels which were about 89% to 96% saturation. The pH of test solutions varied only slightly; however, there was a general trend toward lower pH values at the end of both tests. Test solution salinity ranged from 26 ‰ in the control to 29 and 30 ‰ in the mysid and fish tests, respectively, at test initiation; salinity was not measured at the end of the tests. Temperature remained constant at 20EC in both tests.

3.3 Organism Response

Statistical analyses of test organism survival data are presented in Appendix B.

3.3.2 Mysid test

Mysid survival was 100% in the control treatments and in the two lowest test treatments (0.031% and 0.056%) at the end of the 48-hr test (Table 5). Total mortality was observed in the three highest Enviroklean® Plus treatment levels (0.31%, 0.56% and 1%) after 24 hours exposure. Partial mortalities were recorded in the two middle treatment levels (0.1% and 0.18%) after 24 and 48 hours exposure. A concentration-related dose response was evident at both time periods. The 24 and 48 hr median lethal concentrations of Enviroklean® Plus to *Mysidopsis bahia* are estimated to be 0.18% (95% Cl: 0.17-0.20%) and 0.13 % (95% Cl: 0.12-0.14%), respectively.

3.3.3 Inland silverside test

Inland silverside survival was 100% and 96% in the control treatments at 24 and 48 hours, respectively (Table 6). Total mortality was observed in the four highest test concentrations (0.18%, 0.31%, 0.56% and 1%) after 24 hr exposure. Partial mortalities were observed in the three lower treatment levels at both exposure durations, and a concentration related dose response was apparent. The 24 and 48 hr median lethal concentrations of Enviroklean® Plus to *Menidia beryllina* are estimated to be 0.09% (95% Cl: 0.08-0.10%) and 0.08% (95% Cl: 0.07-0.09%), respectively.

4.0 CONCLUSIONS

The median lethal concentration of Enviroklean® Plus (LC_{50}, the concentration expected to cause mortality to half of an exposed population within a specified time period) to the mysid, *Mysidopsis bahia*, is 0.16% (~1600 ppm) for a 24 hr exposure and 0.13% (~1300 ppm) for a 48 hr exposure. The LC_{50} to the inland silverside, *Menidia beryllina*, is 0.09% (~900 ppm) for a 24 hr exposure and 0.08% (~800 ppm) for a 48 hr exposure. Accordingly, this product should be used with reasonable care to prevent un-controlled losses to the marine environment.

Under normal use conditions, Enviroklean® Plus is contained in small containers of less than five gallons capacity. The material is applied as a thin film to surfaces requiring rust or stain removal and, after an appropriate contact time, is flushed away with copious amounts of water. Very little impact is anticipated from the small amounts of Enviroklean® Plus introduced to the environment under typical use conditions. However, loss of Enviroklean® Plus to the environment resulting from an accidental spill, because of the instantaneous introduction of a moderately-large volume, poses a substantially greater, although finite, risk.

Environmental risk may be estimated in a number of ways. One useful estimate, particularly for substances exhibiting acute toxicity, is the volume of water required to dilute a given substance to a concentration equivalent to a defined level of activity (the latter usually expressed as a LC_{50}, or other point estimate of toxicity). For this approach, a large dilution volume equates to great risk. For Enviroklean® Plus, using a 48-hr LC_{50} value of ~800 ppm (from the fish test), a maximum spill of 5 gallons, and the density (1.12), the volume of water required to dilute the product to the median toxicity level is approximately 26.5 m^3; this is a relatively small volume considering the scale of the environment in which the product is commonly used. Bioaccumulation potential, a measure of the degree for a chemical to become concentrated into an organism tissues, is another often used estimate of environmental risk. Neither principal Enviroklean® Plus constituent, hydrogen and chloride ions (when completely dissociated), is considered a bioaccumulating chemical; therefore, there is little or no risk to marine organisms.

5.0 REFERENCES

ASTM E 729-88a. "Standard Guide for Conducting Acute Toxicity Tests with Fishes, Macroinvertebrates and Amphibians." American Society for Testing and Materials, Philadelphia, PA. 1989.

Table 1. Percent survival of *Mysidopsis bahia* in a range-finding exposure to Enviroklean® Plus

Test Concentration (%)	Test Hour		
	0	24	48
Control	100	100	100
0.001	100	100	95
0.01	100	100	100
0.1	100	100	90
1	100	0	0

Table 2. Percent survival of *Menidia beryllina* in a range-finding exposure to Enviroklean® Plus

Test Concentration (%)	Test Hour		
	0	24	48
Control	100	100	100
0.001	100	100	100
0.01	100	100	100
0.1	100	95	90
1	100	0	0

Table 3. Water quality parameters measured during the definitive exposure of *Mysidopsis bahia* to Enviroklean® Plus

		Test Hour[1]	
Parameter	Treatment	0	48
Dissolved Oxygen (mg/L)	Control	7.7	6.9
	1%	7.7	NM[2](7.0)[3]
pH	Control	7.9	7.5
	1%	7.7	NM(7.6)
Temperature (°C)	Control	20	20
	1%	20	NM(20)
Salinity (‰)	Control	26	NM
	1%	30	NM

[1] Water quality parameters were measured at time zero prior to the introduction of the test organisms and at the end of the test

[2] NM = not measured

[3] Values in parentheses represent lowest measurements from treatments with live organisms at the end of the test.

Table 4. Water quality parameters measured during the definitive exposure of *Menidia beryllina* to Enviroklean® Plus

Parameter	Treatment	Test Hour[1] 0	Test Hour[1] 48
Dissolved Oxygen (mg/L)	Control	7.8	7.0
	1%	7.9	NM[2](7.4)[3]
pH	Control	7.9	7.6
	1%	7.8	NM(7.7)
Temperature (°C)	Control	20	20
	1%	20	NM(20)
Salinity (‰)	Control	26	NM
	1%	29	NM

[1] Water quality parameters were measured at time zero prior to the introduction of the test organisms and at the end of the test

[2] NM = not measured

[3] Values in parentheses represent lowest measurements from treatments with live organisms at the end of the test.

Table 5. Percent survival of *Mysidopsis bahia* in a definitive static, acute toxicity test of Enviroklean® Plus

Test Concentration (%)	Test Hour		
	0	24	48
Control	100	100	100
0.031	100	100	100
0.056	100	100	100
0.1	100	98	82
0.18	100	58	10
0.31	100	0	0
0.56	100	0	0
1.0	100	0	0
	LC_{50}:	**0.18%**	**0.13%**
	95% Cl:	**0.17-0.20%**	**0.12-0.14%**

Table 6. Percent survival of *Menidia beryllina* in a definitive static, acute toxicity test of Enviroklean® Plus

Test Concentration (%)	Test Hour		
	0	24	48
Control	100	100	96
0.031	100	90	90
0.056	100	100	90
0.1	100	34	18
0.18	100	0	0
0.31	100	0	0
0.56	100	0	0
1.0	100	0	0
	LC_{50}:	**0.09%**	**0.08%**
	95% CI:	**0.08-0.10%**	**0.07-0.09%**

APPENDIX A. TEST DATA

APPENDIX B. STATISTICAL ANALYSES