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Fresh Water Marine Toxicity Test Summary  
South Korea (Minnows)

The OSEI Corporation performed a toxicity test for the Korean Government approval process involving minnows (*Pimephales promelas*). The toxicity test was a 24 hour acute toxicity test. The LC50 value for this test was 707.11 mg/l at a 20% concentration, which is the concentration the Korean government test required. If you extrapolate the test value, had the test been performed at the OSE II application concentration of 2% instead of 20%, then the LC50 would have been over 1337.11 mg/l which proves OSE II to be virtually non toxic. There are several government agencies around the world that try to force specific tests to be performed at a single concentration without allowing for the application rate of a product. So while they come up with a value at a certain concentration it may, or may not be applicable to every product, which is why we point out the extrapolation calculation for OSE II at the recommended application rate.

Steven Pedigo  
Chairman/CEO OSEI Corporation

OIL SPILL EATER II (2%)  
ACUTE PRODUCT TEST

June 2008

24-Hour Acute Toxicity Test Results

*Pimephales promelas*

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ACUTE LC50 PRODUCT REPORT

Client . . . . . OSEI, Corporation                      Project No. . . . . OS457  
Sample . . . . . Oil Spill Eater II                      Test Date . . . . . June 2008

Results:

24-hr. *P. Promelas* LC50:                      5,856.34 mg/L  
95% Upper Confidence Limits:                      6,265.67 mg/L  
95% Lower Confidence Limits:                      5,473.76 mg/L

INTRODUCTION

A product identified as Oil Spill Eater II, Concentrate was delivered to Huthur and Associates, Inc. on June 26, 2008. One acute toxicity test was conducted: a static acute 24-hour definitive toxicity test using *Pimephales promelas* (fathead minnow). Test procedures followed recommended methods contained in "Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms, Fifth Edition", EPA-821-R-02-012, October 2004.

*P. promelas* are a freshwater aquatic indicator organism frequently used to evaluate the potential toxicity of a compound or an effluent. The acute toxicity of a compound or effluent is generally measured using a multi-concentration, or definitive test, consisting of a control water and a minimum of five increasing concentrations of product added to control water. The test is designed to provide dose-response information, expressed as the concentration that is lethal to 50% of the test organisms (LC50).

SAMPLE PREPARATION

Oil Spill Eater II was initially prepared for definitive testing by adding the product to distilled, deionized water at a ratio of 50 parts water to 1 part product (2% concentration; stock solution). Seven test concentrations of stock solution were prepared in distilled, deionized water reconstituted to 104 mg/L as CaCO<sub>3</sub>. The seven concentrations were 250, 500, 1000, 2000, 4000, 8000 and 16,000 mg/L. Dissolved oxygen, pH and conductivity were measured in each concentration prior to test initiation and at 24-hours. The test was conducted at 25°C in a photoperiod of 16 hours light and 8 hours dark.

TEST DESIGN  
*Pimephales promelas*

The definitive *Pimephales promelas* test was conducted in 300 mL beakers containing 250 mL of test solution. The test was initiated June 28, 2008. Ten *P. promelas* larvae were added to each of two replicate beakers per concentration. Larvae originated from laboratory cultures and were 48-hours old at test initiation. Larvae were fed *Artemia* nauplii prior to test initiation.

A control of two replicate beakers containing ten *P. promelas* larvae each in laboratory water was conducted concurrently with the test. Survival data were statistically analyzed using the Trimmed Spearman-Kärber point estimate test to determine the LC50.

**RESULTS**  
*Pimephales promelas*

The following LC50 value was determined for Oil Spill Eater II (2%):

24-Hour Definitive Test				
Conc. (mg/L)	# exposed	# alive	#dead	% survival
Control	20	20	0	100.0
250	20	20	0	100.0
500	20	20	0	100.0
1000	20	20	0	100.0
2000	20	20	0	100.0
4000	20	20	0	100.0
8000	20	1	19	5.0
16000	20	0	20	0.0
Percent Spearman-Kärber Trim:			0.00%	
Estimated LC50 (mg/L):			5,856.34	
95% Lower C.L. (mg/L):			5,473.76	
95% Upper C.L. (mg/L):			6,265.67	

The pH in all solutions was within the organism's tolerance range.

**DISCUSSION AND CONCLUSIONS**

One LC50 determination was made for Oil Spill Eater II tested at a 2% concentration: 24-hour *Pimephales promelas* LC50: 5,856.34 mg/L. The acute test was conducted from June 28, 2008 to June 29, 2008.

**24-HOUR PIMEPHALES PROMELAS SURVIVAL**

CLIENT: OSE - 2<sup>g</sup>

PROJECT #: 05457

CONC.	NUMBER ORGANISMS, 0 HRS		NUMBER ORGANISMS, 24 HRS	
	A	B	A	B
<i>Cov</i>	10	10	10	10
<i>250 mg/L</i>	10	10	10	10
<i>500</i>	10	10	10	10
<i>1000</i>	10	10	10	10
<i>2000</i>	10	10	10	10
<i>4000</i>	10	10	10	10
<i>8000</i>	10	10	1 <sub>g</sub>	0 <sub>10</sub>
<i>16,000</i>	10	10	0 <sub>10</sub>	0 <sub>10</sub>
DATE/TIME	<i>mm</i>		<i>mm</i>	
TECHNICIAN	<i>6/28/08</i>	<i>1430</i>	<i>6/29/08</i>	<i>1430</i>



DATE: JUNE 200 TEST NUMBER: 1 DURATION: 24 H  
 TOXICANT : OSE II  
 SPECIES: P. PROMELAS

RAW DATA:	Concentration (MG/L)	Number Exposed	Mortalities
	.00	20	0
	1000.00	20	0
	2000.00	20	0
	4000.00	20	0
	8000.00	20	19
	*****	20	20
	16000.00 $\bar{X}$		

SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES: LC50: 5856.34  
 95% LOWER CONFIDENCE: 5473.76  
 95% UPPER CONFIDENCE: 6265.67

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