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Date June 30, 2008

Toxicity Test Summary for a Ceriodaphnia Dubia  
Fresh Water Flea

The OSEI Corporation performed a toxicity test for a land, water, and airborne based species a Ceriodaphnia Dubia (water flea). The estimated LC 50 for this species even at a higher concentration 20%, than OSE II is applied was 2199.62 which shows that OSE II is also virtually non toxic to bugs as well. The extrapolated value for the LC 50 at OSE II normal application rate of 2% would have been over 4000 mg/l, which shows OSE II is virtually non toxic to water fleas.

Steven Pedigo  
Chairman/ CEO OSEI Corporation

**OIL SPILL EATER II (2%)  
ACUTE PRODUCT TEST**

June 2008

24-Hour Acute Toxicity Test Results

*Ceriodaphnia dubia*

Prepared for:

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ACUTE LC50 PRODUCT REPORT

Client . . . . . OSEI, Corporation Project No. . . . . OS457
Sample . . . . . 2% Oil Spill Eater II Test Date . . . . . June 2008

Results:

24-hr. C. dubia LC50: > 16,000.00 mg/L
95% Upper Confidence Limits: N/A
95% Lower Confidence Limits: N/A

INTRODUCTION

A product identified as Oil Spill Eater II, Concentrate was delivered to Huth and Associates, Inc. on June 26, 2008. One acute toxicity test was conducted: a static acute 24-hour definitive toxicity test using Ceriodaphnia dubia (water flea). 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.

C. dubia 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 CaCO3. 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 Ceriodaphnia dubia

The definitive Ceriodaphnia dubia test was conducted in 25 mL beakers containing 15 mL of test solution. The test was initiated June 28, 2008. Five C. dubia neonates were added to each of four replicate beakers per concentration. Neonates originated from laboratory cultures and were 24-hours old at test initiation. Neonates were fed Selenastrum capricornutum prior to test initiation.

A control of four replicate beakers containing five *C. dubia* 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**  
*Ceriodaphnia dubia*

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	19	1	95.0
8000	20	20	0	100.0
16000	20	17	3	85.0
Percent Spearman-Kärber Trim:			0.00%	
<b>Estimated LC50 (mg/L):</b>			<b>&gt; 16,000.00</b>	
95% Lower C.L. (mg/L):			N/A	
95% Upper C.L. (mg/L):			N/A	

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 *Ceriodaphnia dubia* LC50: >16,000.00 mg/L. The acute test was conducted from June 28, 2008 to June 29, 2008.



24-HOUR CERIODAPHNIA DUBIA SURVIVAL

CLIENT: OSE 2%

PROJECT #: OS457

CONC.	NUMBER ORGANISMS, 0 HRS				NUMBER ORGANISMS, 24 HRS			
	A	B	C	D	A	B	C	D
CON	5	5	5	5	5	5	5	5
250 mg/L	5	5	5	5	5	5	5	5
500	5	5	5	5	5	5	5	5
1000	5	5	5	5	5	5	5	5
2000	5	5	5	5	5	5	5	5
4000	5	5	5	5	5	5	5	4
8000	5	5	5	5	5	5	5	5
16,000	5	5	5	5	4	4	5	4
DATE/TIME	6/28/08 1245				6/29/08 1245			
TECHNICIAN	MM				MM			





**ACUTE REFERENCE TOXICANT TEST RESULTS**

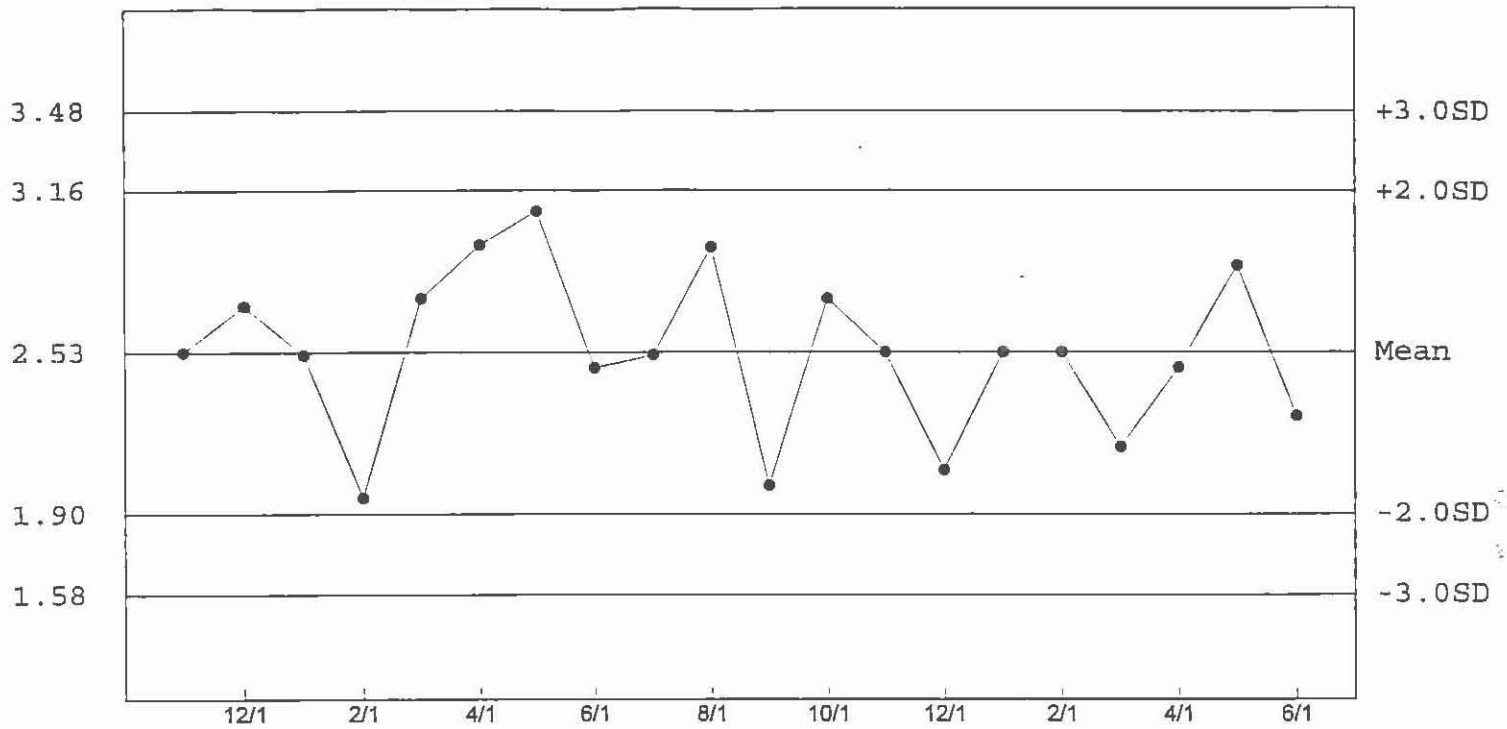
SPECIES: *Ceriodaphnia dubia*  
 CHEMICAL: Sodium Chloride  
 DURATION: 48-Hours  
 TEST NUMBER: 6  
 TEST DATE: June 2008  
 STATISTICAL METHOD: Spearman-Karber

CONCENTRATION (g/L)	NUMBER EXPOSED	NUMBER DEAD
1.0	10	0
1.5	10	0
2.0	10	0
2.5	10	9
3.0	10	10
4.0	10	10

LC50	95% LOWER CONFIDENCE LIMITS	95% UPPER CONFIDENCE LIMITS
2.28 g/L	2.20 g/L	2.37 g/L

Ref. Toxicant Sodium chloride g/L

Ceriodaphnia dubia LC50



n= 20 Mean= 2.53 SD= 0.32 CV= 12.49% Min= 1.96 Max= 3.08