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SUMMARY

ENVIRONMENT CANADA'S TOXICITY TEST

Environmental Canada performs Toxicity Testing for determining if a product could gain approval for use in Canada. The level that is considered toxic is 1,000 mg/L or less. A product that exceeds this level is deemed acceptable.

Oil Spill Eater II Concentrate, tested at 10,000 mg/L – which shows OSE II Concentrate is virtually non-toxic and far exceeds the level deemed to toxic by Environment Canada.

Rainbow Trout is one of the most sensitive fresh water organisms to test. OSE II proved that even with third party testing by a Foreign Government, OSE II is virtually non-toxic.

By: Steven R. Pedigo
Chairman/OSEI, Corp.



Environment Canada
Conservation and Protection

Emergencies Science Division
River Road Environmental Technology Centre
3439 River Road
Ottawa, Ontario K1A 0H3

May 17, 1993

4808-13-7

Steven R. Pedigo, Chairman,
OSEI Corporation
5545 Harvest Hill
Suite 1116
Dallas, TX 75230
U.S. A.

Dear Mr. Pedigo,

Thank-you for participating in the development of Environment Canada's draft guidelines for assessing the toxicity and effectiveness of oil spill bioremediation agents (OSBAs).

The Tier I toxicity testing is now complete. Our preliminary screening has indicated that the *Daphnia magna* test and the Microtox test were either insensitive or erratic. Therefore, we do not consider these particular tests useful for OSBA evaluation. Comments on the toxicity of your product will thus be limited to those obtained using the 96-hour Rainbow Trout acute lethality test. 'Oil Spill Eater II' had a rainbow trout 96-hour LC50 of greater than 10,000 mg of application solution per litre of water. There was, however, a 23% mean fish mortality at this concentration. Also note that between 24 and 96 hours of exposure to the product, sublethal effects were present. The fish were noted to surface, be on their side, turn dark, exhibit rapid breathing and no swimming. These sublethal effects should be of concern. The effectiveness test analyses are still being performed. You will be notified as soon as those results are available.

If your product meets both the effectiveness and toxicity criteria it will be placed on our Standard List of Oil Spill Bioremediation Agents. Placement on this list is not an indication that the product will be used in the event of an oil spill. The list and test results are public information. They may be provided to oil spill response personnel to enable them to make informed decisions.

Please take note that the placement of a product on our Standard List does not constitute an approval or certification or licensing of your product for use in Canada. Your product may be required to comply with the New Substances Notification Regulations (NSNR) for biotechnology products under the Canadian Environmental Protection Act (CEPA). For information on the draft regulations, please contact the Chief of the New Substances Division at (819) 997-4336 or at the following address: Chief, New Substances Division, CCB, Environment Canada, P.V.M. 14th Floor, Ottawa, Ontario, K1A 0H3, CANADA.

Sincerely,

Merv Fingas
Chief, Emergencies Science Division

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Canada

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ENVIRONMENT CANADA

TIER I TOXICITY TESTING

FOR EVALUATION OF DRAFT OSBA GUIDELINES

The testing was performed as follows. An application solution of the OSBA was prepared based on instructions provided by the manufacturer/supplier. The highest strength of solution tested was 10,000 mg of application solution per litre of water (approx. a 1:100 dilution). For products in which solids are normally added to the water, suspensions comprised of 10,000 mg of product/combined product per litre of water were prepared for use in the toxicity tests. (If several solids were to be added, they were combined in the appropriate ratio). This initial screening concentration was tested in triplicate. If this concentration was toxic to greater than 50% of the organisms, lower concentrations were tested. Sub-lethal effects on the behavior and/or appearance of the organisms were also made. The toxicity of the product in water was assessed using each of the following three biological test methods, developed and standardized by Environment Canada for these and other applications:

Environment Canada, 1990a. **Biological test method: acute lethality test using rainbow trout.** Environment Canada, Conservation and Protection, Ottawa, Ontario. Report EPS 1/RM/9, 51 pp.

Environment Canada, 1990b. **Biological test method: acute lethality test using *Daphnia* spp.** Environment Canada, Conservation and Protection, Ottawa, Ontario. Report EPS 1/RM/11, 57 pp.

Environment Canada, 1992. **Biological Test method: toxicity test using luminescent bacteria (*Photobacterium phosphoreum*).** Environment Canada, Conservation and Protection, Ottawa, Ontario. Report EPS 1/RM/24, 61 pp.

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TOXICITY TEST SUMMARY USING
CITGO GASOLINE, OIL SPILL EATER II
AND FATHEAD MINNOWS

To prove OIL SPILL EATER II rapidly detoxifies hydrocarbons once OSE II is applied, a Toxicity Test was set up with the Physical Engineer of the City of Plano, Texas.

One half gallon of gasoline was poured onto a concrete surface, where ½ gallon of OSE II (pre-diluted 100 to 1) was immediately applied. The treated gasoline was allowed to set for two (2) minutes at which time two (2) gallons of fresh water were used to wash this effluent into a catch basin. Approximately 1 ½ gallons were recovered and sent to Bio-Aquatic Laboratory.

Bio-Aquatic Laboratory performed a Static 48 Definitive Toxicity Test using Fathead Minnows (*Pimphales promelas*). The LC50 was 9,300 mg/L which is a relatively low toxicity level.

This test shows that OSE II when applied to a toxic constituent rapidly reduces toxicity. This detoxifying action of OSE II limits the toxicity of a spill to marine organisms, and will allow Mother Nature's Bacteria to rapidly attack this detoxified spill. The rapid detoxification of a spill shows that OSE II is a beneficial tool for first response cleanup for a spill. This test also shows that if OSE II is used to clean up a parking lot and washed into the storm drain there would be no adverse environmental impact.

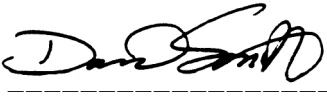
By: Steven R. Pedigo
Chairman/OSEI, Corp.

OSEI CORPORATION
OSE II/GASOLINE/WATER

Toxicity Test Report

DECEMBER 7, 1991

BIO-AQUATIC TESTING, INC.

Prepared by: 

David Smith,
Aquatic Toxicologist

BIO-AQUATIC TESTING, INC.

1555 Valwood Parkway, Ste. 100
Carrollton, Texas 75006
Tel: (214) 247-5928
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TOXICITY TEST REPORT – ACUTE

Client OSEI Corporation Laboratory I.D. BO-12-91-2239
Sample OSE II/Gasoline/Water Date December 7, 1991

Results: The 48-hour LC50 for *Pimephales promelas* exposed to a mixture of OSE II, gasoline, and water was 9,300 mg/L.

SAMPLE COLLECTION

Approximately one and a half gallons of runoff grab sample from an OSEI Corporation product demonstration was delivered to Bio-Aquatic Testing on December 5, 1991. The sample was manually collected by OSEI personnel. One toxicity test was requested: a static 48-hour definitive toxicity test using the fathead minnow (*Pimephales promelas*).

CHEMICAL MEASUREMENTS

The sample was analyzed for residual chlorine (EPA Method 330.1, Amperometric Titration Method) and was determined to contain <0.10 mg/L. Sample and laboratory dilution water pH, temperature, conductivity, hardness, alkalinity and D.O. were analyzed and recorded daily.

TEST PROCEDURES *Pimephales promelas*

The 48-hour fathead minnow larval survival test was initiated at 1450 hours, December 6, 1991. Five concentrations were established for testing (200 mg/L, 800 mg/L, 3,000 mg/L, 9,000 mg/L, and 30,000 mg/L) utilizing reconstituted distilled, deionized water as dilution water. The test was set up using distilled water rinsed 500 mL plastic cups as test chambers. Four replicate cups containing five organisms each in 250 mL of test solution were used per dilution. All organisms used were laboratory reared and less than 24 hours old at test initiation. The test was allowed to proceed for 48 hours during which mortality was recorded daily.

A control of four replicate chambers containing five organisms each in 100% synthetic laboratory water was conducted concurrently with the test. There was 100% survival in the control. Data on surviving organisms as well as water quality measurements were recorded on the data sheet. The test ended at 1450 hours, December 8, 1991. The acute toxicity data analysis program provided by the EPA was employed to determine the LC50 values.

LC50 RESULTS
Pimephales promelas

LC50 value calculated using the Binomial Method:

<u>CONC. (mg/L)</u>	<u># EXPOSED</u>	<u># DEAD</u>	<u>% DEAD</u>	<u>BINOMIAL %</u>
30,000	20	20	100	0.0001
9,000	20	6	30	5.7659
3,000	20	1	5	0.0020
800	20	0	0	0.0001
200	20	0	0	0.0001

The Binomial Test shows that 3,000 and 30,000 can be used as statistically sound conservative 95 percent confidence limits since the actual confidence level associated with these limits is 99.99791 percent.

An approximate LC50 for this set of data is 11,800 mg/L.

LC50 value calculated using the Trimmed Spearman-Kärber Method:

<u>Trim</u>	<u>Var. of Ln Est.</u>	<u>LC50</u>	<u>95% Conf. Limits</u>
0.00%	0.17396D-01	9,300 mg/L	7,100 to 12,100 mg/L

SUMMARY

The 48-hour LC50 for *Pimephales promelas* exposed to a mixture of OSE II, gasoline, and water was 9,300 mg/L.

BIO-AQUATIC TESTING, INC.

48 – HOUR <i>PIMEPHALES PROMELAS</i> ACUTE TOXICITY TEST

CLIENT	OSEI Corporation	BEGIN DATE	12/06/91
SAMPLE	OSE II, Gasoline, Water	END DATE	12/08/91
LAB ID #	BO-12-91-2239B	TEST ORGANISM	<i>Pimephales promelas</i>
DATE COLLECTED	12/05/91	TEST TEMPERATURE (°C)	25° ± 1
DATE RECEIVED	12/05/91	PHOTO PERIOD	16 hour light / 8 hour dark
SAMPLE TYPE	Grab	LIGHT INTENSITY	75 FT-C
TEST TYPE	Acute	ANALYST	W. Smith

SURVIVAL SUMMARY

% EFFLUENT CONC	NUMBER LIVE PER REP												x LIVE PER CONC x % Surv.
	START				24 HOURS				48 HOURS				
	a	b	c	d	a	b	c	d	a	b	c	d	
Control	5	5	5	5	5	5	5	5	5	5	5	5	100
200 mg/L	5	5	5	5	5	5	5	5	5	5	5	5	100
800 mg/L	5	5	5	5	5	5	5	5	5	5	5	5	100
3,000 mg/L	5	5	5	5	5	5	5	5	5	4	5	5	95
9,000 mg/L	5	5	5	5	3	3	5	5	3	1	5	5	70
30,000 mg/L	5	5	5	5	0	0	0	0	0	0	0	0	0

EFFLUENT MEASUREMENTS

D.O. @ 30,000 mg/L¹ 8.6/6.6
 pH @ 30,000¹ 8.3/8.4
 CONDUCTIVITY @ 30,000 (µMHOS) 500
 HARDNESS (mg/L as CaCO₃) 272.4 ALKALINITY (mg/L as CaCO₃) 625.0

DECHLORINATION

RESIDUAL Cl₂ (mg/L) <0.10 ANALYSIS METHOD Amperometric Titration Method (330.1)
 DECHLORINATION REAGENT Not Applicable

DILUTION WATER MEASUREMENTS

D.O. @ 100% (mg/L)¹ 8.6/6.9
 pH @ 100%¹ 8.4/8.3
 RECEIVING WATER DILUTION WATER Laboratory adjusted
 HARDNESS (mg/L as CaCO₃) 160.0 ALKALINITY (mg/L as CaCO₃) 107.0

¹ Recorded at the beginning and end of each 24-hour exposure period.

I. Summary

The acute toxicity of the dispersant – Batch #9820, No. 2 fuel oil, and a 1:10 mixture of dispersant and No. 2 fuel oil to *Artemia salina*, is described in this report. The test was conducted for corp for 48 hours during October 3 to 5, 1990, at the EnviroSystems Division of Resource Analysts, Inc. in Hampton, New Hampshire.

The test was performed under static conditions with five concentrations of each test substance and a dilution water control at a temperature of $20 \pm 1^{\circ}\text{C}$. The dilution water was sea water adjusted to a salinity of 20 parts per thousand. Aeration was not employed to maintain dissolved oxygen concentrations above an acceptable level. Nominal concentrations of all three test substances were: 0 mg/L (control), 10 mg/L, 25 mg/L, 40 mg/L, 60 mg/L and 100 mg/L. Nominal concentrations were used for all calculations.

Artemia salina used in the test were 24 hours old at the start of the test and they were all in good condition at the beginning of the study. *Exposure of Artemia salina* to the test substances resulted in the following 48 hours median lethal concentrations (LC50): dispersant 100 mg/L, No. 2 fuel oil – 12.6 mg/L (95% confidence interval = 10.0- 25.0 mg/L), and a 1:10 mixture of dispersant and No. 2 fuel oil-29.4 mg/L (95% confidence interval = 25.0 – 40.0 mg/L).