

SUMMARY
ENVIRONMENT CANADA'S TOXICITY TEST

Environmental Canada performs five (5) Toxicity Tests 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. The higher the number the less toxic.

Oil Spill Eater II Concentrate, tested at 10,000 mg/L – on Rainbow Trout (**Oncorhynchus mykiss**) which shows OSE II 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.

Environment Canada tested OSE II on water fleas (*Daphnia magna*) as well the LC 50 was > than 10,000 ppm million showing that OSE II would not be toxic to intertidal zone species.

The next three (3) test Environment Canada performed is interesting since it is tests to see if a product would adversely effect single celled bacteria living in intertidal zones. The reason it is interesting is the fact that Environment Canada performed the same efficacy test on OSE II as the US EPA established with NETAC to determine if products could remediate oil, so a product could then be placed on the US EPA National contingency Plan approved list. This test also determined the number of bacteria OSE II/a product could colonize/enhance/grow as well. If a product enhances or grows bacteria then there is little chance it will be toxic to bacteria, so to perform a bacteria toxicity test is interesting. Environment Canada's test was performed on bacteria photobacterium phosphoreum for .5 (30 minutes), the LC 50 for this time was 5209 mg/l for .25 (15 minutes) which had an LC 50 of 5474 mg/l and .083 (4.98 minutes) which had an LC 50 of 7952 mg/l. These varied timed toxicity test further shows OSE II is non toxic to even single celled bacteria, therefore the likely hood of being toxic to any species would be minimal, since single celled bacteria are more susceptible to toxins than larger species.

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.121

Environment Canada
Conservation and PotetionEmergencies Science Division
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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, Environmental Canada, P.V.M. 14th Floor, Ottawa, Ontario, K1A 0H3, CANADA.

Sincerely,
Merv Fingas
Chief, Emergencies Science Division

**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:

Oil Properties
Brochure
Spilltox

Chemical Synonyms PPA Instruments Tanker Spills

Spills

Spilltox

[[ETC](#) > [Databases](#) > [Spills](#) > **Spilltox**]

Environmental Technology Centre

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OILSPILL EATER II

Aliases

OSEII

Species Latin Name
Test Length (h)

Test Endpoint

Qualifier

Toxicity Value
Units of Measurement

Daphnia magna

48

LC50

>

10000

mg/L

Oncorhynchus mykiss

96

LC50

>

10000

mg/L

Photobacterium phosphoreum

.5

IC50

=

5109

mg/L

Photobacterium phosphoreum

.25

IC50

=

5474
mg/L

Photobacterium phosphoreum

.083
IC50

=

7952
mg/L

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.

May 17, 1993123 OIL SPILL EATER INTERNATIONAL, CORP.